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SUMMARY

FRUIT GROWING

VITICULTURE AND OENOLOGY

VEGETABLE GROWING

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE

MISCELLANEOUS

FRUIT GROWING


EX·TERRA·AVRVM

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IMPACT OF NITROGEN FERTILIZATION ON GROWTH AND PHOTOSYNTHETIC ACTIVITY OF WALNUT PLANTING MATERIAL (*JUGLANS REGIA* L.), CULTIVATED IN CONTAINERS

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Abstract

The object of the experiment was the walnut cultivar Izvor 10, grafted on a walnut rootstock (Juglans regia L.). The plants were propagated by the "Hot Callus" method and grown in containers (50 l) with peat-pearlite mixture (2:1). The impact of nitrogen fertilization on the growth and the physiological characteristics of young walnut plants was studied. Variants of the experiment were: Control (not-fertilized), Variant II - 2 g N / container and Variant III - 4 g N container. The height of the fertilized plants varied from 86 to 107 cm and the stem diameter - from 12.76 to 13.61 mm, while the control plants reached average values of 49.33 cm in height and 10.53 mm in stem diameter and the differences were statistically proven. It was found that fertilization with ammonium nitrate (NH_4NO_3), in the range of 2 - 4g N/container contributes to a more efficient development and structuring of the photosynthetic apparatus, which, on the other hand, is a prerequisite for more intensive photoassimilation and biomass accumulation. It was concluded that fertilization is mandatory for the production of walnut planting material in containers.

Key words*: walnut, pot cultivation, fertilization, vegetative characteristics, photosynthesis.*

INTRODUCTION

In the world of fruit growing the conventional production of planting material in the field is mainly applied which requires a lot of manual labour and is dependent on climatic and soil conditions. This is a prerequisite for the search of alternative approaches. One such approach is container cultivation, which has become increasingly popular in recent years. Its advantages are the easier to maintain the conditions of the cultivation, such as pH of the nutrient substrate, water and nutrient requirements, diseases and pests (Ruter, 1993). Plants grown in containers have a higher fine root mass than field-grown plants (Gilman & Beeson, 1996) and show much less stress when planted in the orchard (Harris & Gilman, 1993).

Fertilization is one of the most important practices for the quality of container grown plants, because they are grown in a limited nutritional volume which prevents their growth (Landis, 1989). According to Oliet et. al. (2004) fertilization can boost the growth of plants, improve their nutrient supply and increase the resistance to water stress, low temperatures and diseases.

Nitrogen is the most important nutrient in fertilizer programs because plants usually need more nitrogen during intense growth than other nutrients and is a key element in the applied fertilizers.

Often fertilizers used in plant nurseries with container cultivation exceed the required rates for optimal growth (Maust & Williamson, 1991).

Improving the efficiency of fertilizer application is one of the ways of reducing production costs and obtaining plants suitable for growing fruit orchards.

The aim of the present study was to investigate the impact of nitrogen fertilization on the growth and the physiological characteristics of the walnut plants grown in containers.

MATERIALS AND METHODS

The study was conducted in 2017 at the Fruit Growing Institute - Plovdiv, Bulgaria under the conditions of pot experiment. The object of the study was the walnut cultivar Izvor 10, grafted

on a walnut rootstock (*Juglans regia* L.). The plants were propagated by the "Hot Callus" method. The successfully grafted experimental plants in the winter months of January and February, 2017 were planted in March in plastic containers (3 l) with peat-perlite mixture (2:1; pH in the range of 5.5 to 6.5; N:P_2O_5:K_2O (14:16:18) and adapted to light in an experimental plot covered with an 80% shading net. Two weeks later, the already adapted plants were transferred to larger containers (50 l) and the 80% shading net was replaced with a 50% shading. The experiment was based on three variants in ten replications, each plant was considered a separate replicate.

Ammonium nitrate (NH_4NO_3) fertilizer was applied on the peat surface three times every twenty days, with the first application being made in mid-July. The following variants were thus formed:

I - Control (not fertilized);

II - 2 g N/container;

III - 4 g N/container.

The soil moisture in the containers was maintained to a field capacity, with the number of watering complied with the specific temperature conditions and the amount of precipitated rainfall.

Analysis of growth

At the end of the vegetation, the following parameters were taken into account: plant height (cm), stem diameter (mm), number of complex leaves. The leaves, stem and roots were separated and a specific fresh mass of the relevant botanical organs (g), leaf area (cm^2), root system volume (cm^3) were determined. The dry mass of the leaves, stems and roots was determined after drying at 80 °C to constant mass. The relative proportion of the individual botanical organs to the dry mass of the whole plant was determined as follows:

(Dry leaf mass/Dry mass of the whole plant) x 100 (%) (Leaf weight ratio, LWR)

The relative proportion of stems, roots and leaf stalks was calculated similarly.

The leaf area was measured by scanning the leaves and analysing the resulting images with specialized software (Gao et al., 2011).

The volume of the root system was measured by the Burdett method (1979).

Photosynthetic pigments

The content of chlorophyll (a, b, a+b) and carotenoids was determined spectrophotometrically in 95% ethyl alcohol extract (Skazkin et al., 1958).

Gas-exchange analyzes

Gas-exchange analysis was performed on the youngest fully developed leaves of 3 randomly selected plants of the respective variant. Measurements were taken with a LCpro + portable gasometer system (ADC, UK) on a sunny day at a light intensity of about 850 μmol m^{-2} s^{-1} PPFD and a temperature of 25 °C. Net photosynthesis rate (A, μmol CO_2 m^{-2} s^{-1}), transpiration intensity (E, mmol H_2O m^{-2} s^{-1}), stomatal conductivity (gs, mol $m^{-2}s^{-1}$) were determined.

Chlorophyll fluorescence

Chlorophyll fluorescence analysis was performed on the youngest fully developed leaves of 5 representative plants of the respective variant. The basic parameters of rapid chlorophyll fluorescence (JIP test) were taken with a HandyPEA portable system (Hansatech Instruments, UK). The leaves were dark adapted for 40 minutes with special clips. The main parameters of chlorophyll fluorescence were measured - minimal (F0), maximal (Fm), and variable (Fv) fluorescence, Fv/Fm, as well as HandyPEA-specific indicators - Performance index (PI_{ABS}) on an absorption basis and total PI (PItotal) measuring the performance up to the PSI end electron acceptors (Goltsev et al., 2010).

Statistical processing of data

The results obtained are subjected to mathematical analysis using the method developed by David B. Duncan (Duncan, 1955).

RESULTS AND DISCUSSIONS

The data presented in Table 1 shows the growth characteristics of walnut plants being influenced by the used nitrogen fertilizer rates. The fertilized variants (var. II and var. III) had higher values in all measured parameters compared to

the control (var. I), with the differences being statistically proven. The obtained results show that there are no significant differences between the fertilized variants (var. II and var. III) in the individual growth parameters.

Table 1. Influence of the nitrogen fertilization on the growth characteristics of walnut plants cultivar Izvor 10, cultivated in containers

Variants	Plant height (cm)	Stem diameter (mm)	Number of complex leaves	Leaf area (cm^2)	Volume of root system (cm^3)
I (Control)	49.33 b	10.53 b	17.00 b	4199.58 b	166.67 b
II	86.00 a	12.76 ab	24.33 a	11435.42 a	266.67 ab
III	107.00 a	13.61 a	26.00 a	13796.24 a	300.00 a

The nourished plants (var. II and var. III) had a height of 86 to 107 cm, and those of the control variant had lower average height values (49.33 cm) (Figure 1).

Figure 1. Appearance of the experimental plants at the end of the vegetation

The average stem diameter of the control plants was 10.53 mm. The fertilized plants (var. II and var. III) had higher average values for stem diameter - from 12.76 to 13.61 mm. The differences are statistically proven. As the fertilizer increased, the number of complex leaves, leaf area and volume of the root system increased significantly, with values at these growth parameters averaging 0.5 to more than 3 times higher than those of the control plants (var. I). Nourished plants (var. II and var. III) had higher average number of complex leaves, 24 to 26, and those of the control variant were characterized by lower average number (17).

The most significant increase was observed in the leaf area and the volume of the root system. The plants of fertilized variants (var. II and var. III) had leaf area from 11435.42 to 13796.24 cm^2, and those of the control variant were characterized by lower average values (4199.58 cm^2) (Figure 2).

Figure 2. Root system of the plants

The data shows that fertilization affects both the aboveground part of the plants and the root system. The average values of the volume of the root system of the nourished plants (var. II - 266.67 cm^3 and var. III - 300 cm^3) are higher than the non-nourished plants of var. I (166.67 cm^3). A number of authors point to a positive correlation between the volume of the root system and the subsequent crop development under field conditions, with higher root system planting material having higher survival rates (Rose et al. 1991a, 1991b, 1992, 1997; Jacobs et al., 2005).

Both fertilizer rates 2 g N/container and 4 g N/container are found to be effective and stimulate the growth of walnut plants.

Nourished plants (var. II and var. III) had higher average values of fresh and dry mass (leaves, stems, leaf stalks and roots), compared to control plants (var. I) (Table 2).

Table 2. Fertilization impact with ammonium nitrate (NH_4NO_3) on plants biomass

Variants	I (Control)	II	III
Fresh mass/leaves (g)	85.75 b	203.84 a	246.91 a
Fresh mass/stems (g)	55.10 b	110.41 ab	154.84 a
Fresh mass/roots (g)	243.82 a	309.52 a	314.60 a
Fresh mass/leaf stalks (g)	29.69 b	73.49 a	91.15 a
Dry mass/leaves (g)	28.76 b	73.81 a	87.20 a
Dry mass/stems (g)	26.73 b	44.20 ab	59.83 a
Dry mass/roots (g)	108.27 a	122.84 a	111.34 a
Dry mass/leaf stalks (g)	8.57 b	21.72 a	26.19 a

The fresh and dry mass of the leaves of the plants from the fertilized variants was twice as

high (203.84-246.91 g) as those of the control plants (85.75 g). The differences are statistically proven.

As the fertilizer rate increases, the average values of fresh and dry root mass also increases, but the differences being non-significant compared to the control.

The content of photosynthetic pigments in the leaves is an important indicator of the photosynthetic competence of the plants. The results obtained for the photosynthetic pigments in the leaves (mg/g fresh weight) are presented in Table 3.

Table 3. Content of photosynthetic pigments in leaves (mg/g fresh weight) of walnut plants with different doses of nitrogen

Variants	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a + b (mg/g)	Carotenoids (mg/g)
I (Control)	1.22 c	0.53 a	1.75 b	1.43 ab
II	1.34 b	0.57 a	1.90 b	1.38 b
III	1.51 a	0.60 a	2.12 a	1.47 a

No symptoms of chlorosis were observed and the content of chlorophyll a was expected to be significantly higher than that of chlorophyll b for all variants tested. The concentration of chlorophyll a showed a considerable variation in the fertilization applied. As the fertilizer rate of nitrogen increased, the content of chlorophyll a also increased, and differences were statistically proven. There was a tendency towards an increase in chlorophyll b content with the increase of introduced nitrogen, but no statistically proven difference between the variants. The content of total chlorophyll (a + b) was the highest at the higher nitrogen fertilizer rate, and the difference with the control and the lower nitrogen dose is statistically proven. Nitrogen is a structural element of the chlorophyll and protein molecules and thus affects the formation of chloroplasts and the accumulation of chlorophyll in them (Tucker, 2004). Therefore, nitrogen fertilization has a direct effect on the chlorophyll content of the leaves of walnut plants. From the obtained results we can conclude that the high fertilizer rate has a favorable effect on the content of the plastid pigments in the leaves, which in turn is a prerequisite for their good photosynthetic competence. Although the content of photosynthetic pigments is not the only indicator for photosynthesis of plants, their increase can be considered as an expression of better structuring of the photosynthetic apparatus under conditions of improved nutrition.

The results of leaf gas exchange of walnut plants indicate that the increasing nitrogen dose had no significant effect on the rate of net photosynthesis (A) (Table 4). The significantly larger leaf area of the nourished plants results in an increased photoassimilation and significantly greater biomass accumulation.

Table 4. Effect of fertilization on transpiration intensity - /E/ (mmol H_2O $m^{-2}s^{-1}$), stomatal conductance - /gs/ (mol $m^{-2}s^{-1}$) and photosynthesis rate - /A/ (µmol CO_2 m^{-2} s^{-1}) at 850 µmol m^{-2} s^{-1} PPFD

Variants	Photosynthesis rate /A/ (µmol CO_2 $m^{-2}s^{-1}$)	Intensity of transpiration /E/ (mmol H_2O $m^{-2}s^{-1}$)	Stomatal conductance /gs/ (mol $m^{-2}s^{-1}$)
I (Control)	5.34 a	0.42 a	0.11 a
II	5.41 a	0.19 b	0.04 b
III	4.85 a	0.23 b	0.06 ab

Along with the intensity of photosynthesis, another indicator of the functional activity of the photosynthetic apparatus of plants is chlorophyll fluorescence. The analysis of the induction curves of rapid chlorophyll fluorescence (OJIP test) links the structure and functionality of the photosynthetic apparatus and allows rapid assessment of plant viability, especially in stress conditions (Strasser et al., 2000, 2004). In the three variants studied, the rapid chlorophyll fluorescence curves have a typical OJIP shape from F0 to Fm level with clearly separated J and I phases (Figure 3), indicating that the walnut plants included in the experiment are photosynthetically active (Yusuf et al., 2010).

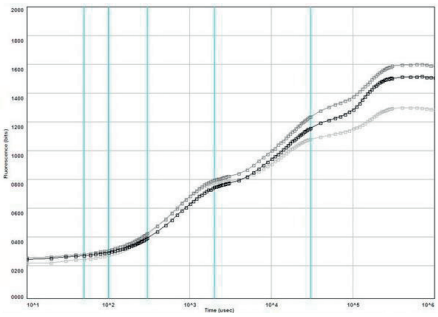

Figure 3. Induction curves of rapid chlorophyll fluorescence (OJIP test); (**XXX**) Control without fertilization; (**XXX**) variant II (2 g N / container); (**XXX**) variant III (4 g N / container)

The minimal (F_0) and maximal (F_m) fluorescence of the control plants was the lowest (Table 5), and the difference was statistically proven for F_0. At F_m, the values of the nourished plants are higher, but the difference is significant only in variant II. Lower F_m values may indicate that the photosynthetic object is in a state of stress and not all electron acceptors in PS II can be completely reduced. Maximal fluorescence is a complex parameter that is determined by a number of factors but also depends on the chlorophyll content of the tissues examined. Indeed, the lower Fm values in the control plants correspond to the measured lower content of total chlorophyll a and total chlorophyll in these plants (Tables 3 and 5).

Table 5. Basic parameters of chlorophyll fluorescence (JIP test)

Variants/Parameters	I (Control)	II	III
T for Fm	567 a	667 a	633 a
F0	242 b	283 a	276 a
Fm	1311 b	1610 a	1525 ab
Fv	1069 b	1326 a	1249 ab
F0/Fm	0.185 a	0.176 a	0.182 a
Fv/Fm	0.815 a	0.824 a	0.818 a
Fv/Fo	4.41 a	4.68 a	4.51 a
phi(E0)	0.39 b	0.49 a	0.50 a
psi(E0)	0.48 b	0.60 a	0.61 a
delta(R0)	0.42 a	0.45 a	0.46 a
PI abs	2.85 b	5.41 a	5.97 a
PI total	2.02 b	4.49 a	5.21 a

Despite fluctuations in the initial, maximum, and variable fluorescence, the quantum yield (Yield = F_v/F_m), reflecting the potential photochemical activity of PS II, ranges from 0.815-0.824 and corresponds to normal (0.750-0.830) in healthy, unstressed leaves (Bolhar-Nordenkampf and Oquist, 1993). This indicates that in all three variants studied, a normally developed photosynthetic apparatus was functioning. This is confirmed by the slight differences in the measured values of the rate of net photosynthesis. However, a more in-depth analysis of the parameters of the JIP test revealed some characteristic features of the potential of the photosynthetic apparatus in fertilized and control (unfertilized plants).

Characteristic differences between plants grown with and without N-fertilization were reported in the other three important parameters of the JIP test - psi (Eo) (ψEo), the performance index (PI abs) and the total performance index (PItotal). For plants cultivated on a nitrogen-enriched substrate, these parameters are higher and differences are statistically significant. ψEo reflects the probability of electron transport outside Q_A. The performance index (PI abs) shows the functional activity of the FS II relative to the energy absorbed, and the total performance index (PItotal) reflects the functional activity of the PS II, PS I and the electron transport chain between them. PI_{total} is closely related to overall plant growth and survival under stress and is considered to be a very sensitive indicator of the JIP test. The higher PItotal of the plants in the fertilized variants clearly shows the effectiveness of the applied treatment. Plant nutrition contributes to the more active development and structuring of the photosynthetic apparatus, which in turn is a prerequisite for more intensive photoassimilation and biomass accumulation (Table 2). No plants of the control variant reached the required size. The results obtained showed that both nitrogen norms (variant II and variant III) could well be used for production of planting material suitable for planting in orchards. Furthermore, in container grown walnut plants, fertilization should be a mandatory practice.

CONCLUSIONS

The fertilization with ammonium nitrate (NH_4NO_3) has a significant effect on the growth, the development of the photosynthetic apparatus and the construction of the biomass of the walnut plants grown in containers. Nutrition induces stronger growth compared to the control.

Both fertilizer rates 2 g N/container and 4 g N/container are effective, stimulate plant growth and are suitable for the production of walnut trees for planting in fruit orchards.

In container production of walnut plants, nutrition should be a mandatory practice.

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FIRST YEAR REACTION OF SOME EARLY HIGHBUSH BLUEBERRY VARIETIES GROWN IN CONTAINERS TO ORGANIC FERTILIZERS AND PEST CONTROL

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Abstract

In the light of current trends for enlarging the growing possibilities of blueberry plants, we were focusing in the present work on the innovative systems designed for higher productions and in the same time organically managed to accomplish the sustainable goals. Three northern highbush blueberry varieties were subject of the trial set up in early 2019: 'Early blue', 'Duke' and 'Hannah's choice'. The two years old planting material was moved under the plastic solar in containers of 65 liters with different substrate composition. A fertilization scheme using only organic commercial products was applied and the reaction of the plants was evaluated. 'Hannah's choice' was the most vigorous variety according to the total annual growths per plant. Several organic products to control pests were tested too, and the most efficient one to aphids was Chrisopa. In order to define a tailored organic technology for such a crop system, the results need further validation.

Key words*: Vaccinium corymbosum L., pots, growing substrates, organic. greenhouse.*

INTRODUCTION

Blueberries are nowadays worldwide considered as one of the healthy fruits (Lobos, 2015) and with high economic potential. Therefore, the crop catches the attention of many investors. The expansion of highbush blueberry in new areas of growing has to follow the genetic pattern of the varieties and also the environmental challenges in fully accordance with the market and consumers need.

Many countries such as Netherlands or Belgium has short harvest period and solutions for extending harvest period are expected by all the growers. In this regard, there are few solutions considering protected crops as a reliable response to this. Under the rain cover, the harvest can be delayed for about 2 to 3 weeks, but the plastic tunnels can advance 5-6 weeks prior to the open field early production (Bal, 1997).

Some economic studies were also been done in order to evaluate the opportunity of such investment (Asanica, 2018; Julian, 2011). This is not an easy decision due to a higher investment rate but became more actual in the context of land use efficiency and climate change. To better control the substrate, environmental factors and the harvest time, growing blueberries in containers (Asanica, 2019) has a high chance to foster the common goals of the future urban horticulture.

The water use efficiency inside the greenhouse and in open field is another issue to be addressed in the near future (Nicola, 2020).

Heiberg and Lunde (2006) studied the effect of growth media on highbush blueberries grown in pots and conclude that in the two years of experiment there was no significant differences between the substrate's mixtures used upon the plant height and fruit yield.

Producing blueberries in containers offers the advantage of a better pH control, drainage, organic mater and avoid the shortings of the open field crop (Kingstin et al., 2017).

In Mississippi, using high tunnels for blueberry container crops advanced blueberry production up to 5 weeks (Li and Bi, 2019) with an evident increase of market price. One interesting mention of the same authors concern the delay of the first harvest in the case of using organic fertilizers possible due to the low rate for nitrogen release.

Since more and more growers and consumers are focus on producing high quality organic fruits (Strik et al., 2016) with premium prices (DeVetter et al., 2015), we are setting up an

experiment combining more elements that we consider proper to be approached together in the near future: blueberry crop in containers, under the plastic and in organic system. The goal is to find the best solution for growing organic blueberries in the urban or peri-urban areas and in an economic range of profitability for the growers. One immediate aim is to see the optimum technological measures to foster the growths of the young blueberry plants in order to shorten the preharvest period.

MATERIALS AND METHODS

The experiment was established in 22nd of April, 2019 and designed as follows:
Size of the solar: 6 m x 18 m.
Solar cover: Ginegar Suncover Nectarin of 150 µ (87% light transmission, 35% light diffusion, 85% termicity); UV trans 300-380 nm - 45%.
Biological material: three blueberry early varieties ('Duke', 'Early blue' and 'Hannah's choice').
Blueberry pots (containers): 65 litres.
Growing substrate: different share of acid peat Kekkila FBM525 Vaccinium and pine grinded bark.
Mulch: Agrotextile under the pots.
Irrigation: drip line with stakes emitters of 6 LPH (3 stakes/pot).
Nutrition: organic fertilizers (New Logic, Bioact Veg foliar and soil application); Trianum-V (Koppert) in pot only at the planting time.
Fertilizers were applied regularly starting from the planting day (22nd of April) with Protamin (100 g/pot) in the upper part of the substrate and Trianum-V 1 g/10 plants (23rd of April, 2019) by watering. New Logic has been applied three times on substrate (3rd of May, 22nd of July and 5th of August) in a dose of 3 ml/pot/application (0.6%) and three times foliar application (15th of July, 18th of July and 23th of July) in 1% dose, respectively 2 ml/plant/application.
Bioact Veg has been used seven times for fertilize the plants on substrate with 3 ml/pot/application (11th of May, 20th of June, 29th of June, 25th of July, 13rd of August, 20th of August) and seven times as foliar sprays with 2 ml/pot/application (20th of June, 11st of July, 18th of July, 23rd of July, 22nd of August, 10th of September).
The experimental scheme is presented in the Table 1 and the module design in the Figure 1.

Table 1. Experimental variants constituted by location, substrate mixture and fertilization scheme application

Variant	Type	Substrate	Foliar fertilization	Substrate fertilization
V1	solar	Peat 90% + Pine bark 10%	New Logic	New Logic + BioactVeg
V2	solar	Peat 90% + Pine bark 10%	BioactVeg	New Logic + BioactVeg
V3	solar	Peat 75% + Pine bark 25%	New Logic	New Logic + BioactVeg
V4	solar	Peat 75% + Pine bark 25%	BioactVeg	New Logic + BioactVeg
V5	solar	Peat 50% + Pine bark 50%	New Logic	New Logic + BioactVeg
V6	solar	Peat 50% + Pine bark 50%	BioactVeg	New Logic + BioactVeg
V7	open field	Peat 90% + Pine bark 10%	New Logic	New Logic + BioactVeg
V8	open field	Peat 90% + Pine bark 10%	BioactVeg	New Logic + BioactVeg

Phyto protection: ecological products (Laser 240SC, Garex B, Prev-Am., Deffort) and natural predators (*Chrysopa*, *Chrysoperla carnea*). The insects were lunched inside the solar on 10th of August and 31st of August when the aphids were present indoor on the top of the shoots.
The predators were released in the proximity of the aphids (on the attacked shoots leaves), on the top of the pot substrate (base of the plant) and suspended in the small boxes hanging very close to the plants.

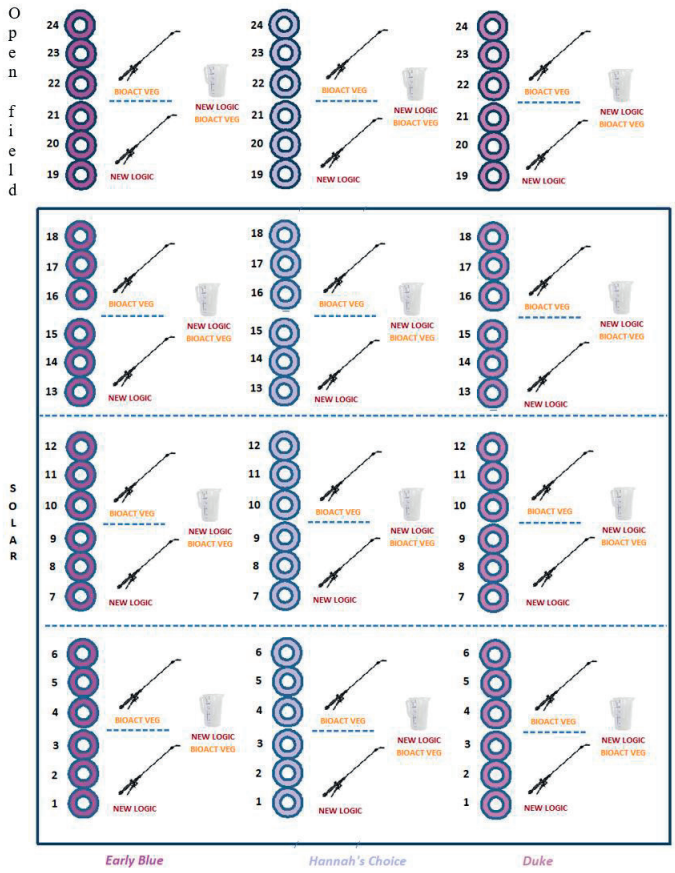


Figure 1. Experimental model for blueberry fertilization

RESULTS AND DISCUSSIONS

The matrix of factors involved in the research brings evident influences in the vegetative growths of the blueberry plants.

The Protamin and Trianum-V products applied at the beginning of poticulture assured a good start of the growing season and reduced the number of died plants after planting.

From the data gathered in 2019 and analyzed, it results that the growth vigour of 'Hannah's choice' variety is much higher that the other two varieties 'Duke' and 'Early blue'.

The total growths/plant indicates 'Hannah's choice' with the most productive vegetation volume (Figures 2 and 3).

The yearly growth sum is about two times more than 'Early blue' and four times more than 'Duke' (Figure 4).

Figure 2. Evolution of blueberry plant growths in 2019 in relation with variety and fertilization scheme applied (left - June, 2019 and right - September, 2019)

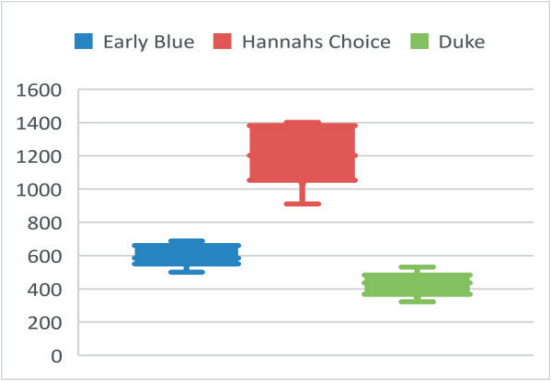
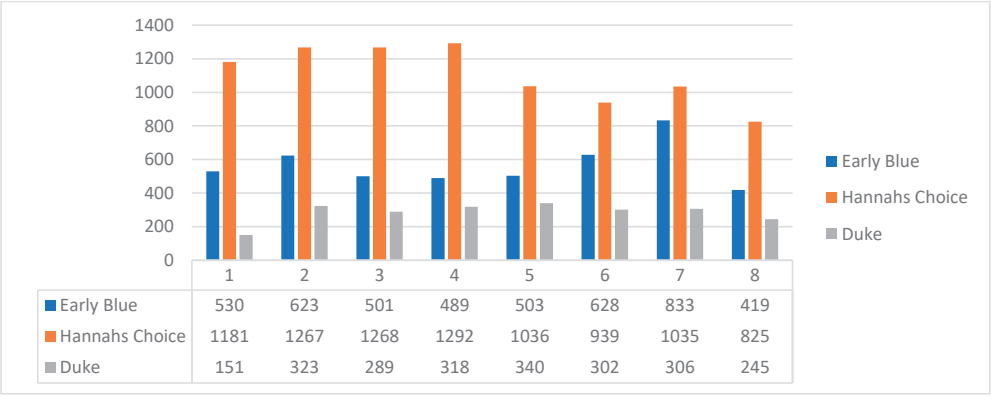


Figure 3. Vigour of blueberry varieties



	1	2	3	4	5	6	7	8
Early Blue	530	623	501	489	503	628	833	419
Hannahs Choice	1181	1267	1268	1292	1036	939	1035	825
Duke	151	323	289	318	340	302	306	245

Figure 4. Annual blueberry varieties growth sum (cm)

The tallest plants and with greater growth sum overpass 12 m of annual growths at 'Hannah's choice' and remains only at maximum 8.3 m at 'Early blue' and 3.4 m at 'Duke'.

The variability in growths is of 467 cm for 'Hannah's choice', 414 cm at 'Early blue' and 172 cm at 'Duke'.

The growing conditions have more impact in the total values of plant growths also for Hannah's choice mainly. The plastic cover enhanced the total growths with 25% (Figure 5). For the other two variants these conditions were not effective in due time.

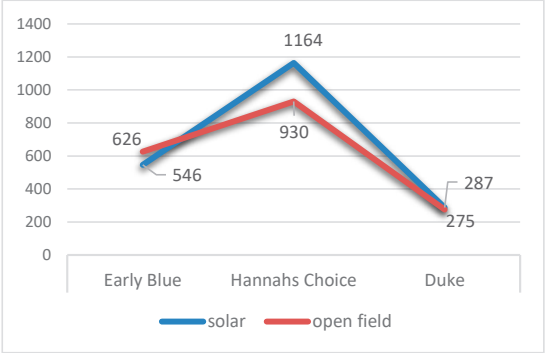


Figure 5. Plants growth under the plastic and open field

In terms of substrate and fertilization scheme applied upon the plant heigh, it was remarked that independent of blueberry variety (Figure 6), the presence of 90% of peat and 10% pine bark plus application of Bioact Veg sprays and New Logic and Bioact Veg applied directly in the substrate contributed to a better growth of the plants.

In contrast, the equal parts of the peat and pine bark in the pots and the fertilization with New logic foliar plus both products in the substrate generated lower growths at the end of the 2019 season.

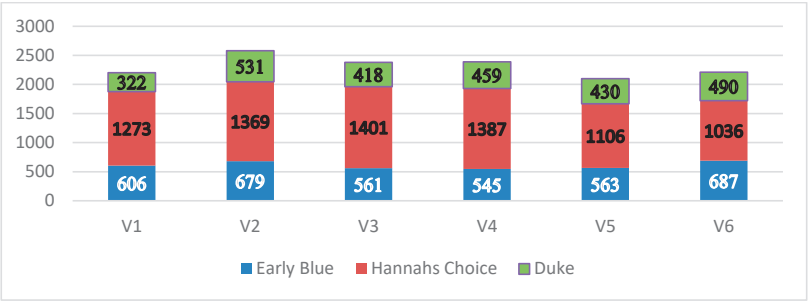


Figure 6. The influence of substrate and fertilization in the total growth of the blueberry varieties

Each variety reacted different at the fertilization and substrate composition (Figure 7). We can observe the similar trendline at the 'Duke' and 'Early Blue' varieties and while 'Hannah's choice' behaved better in a rich peat substrate and indoor.

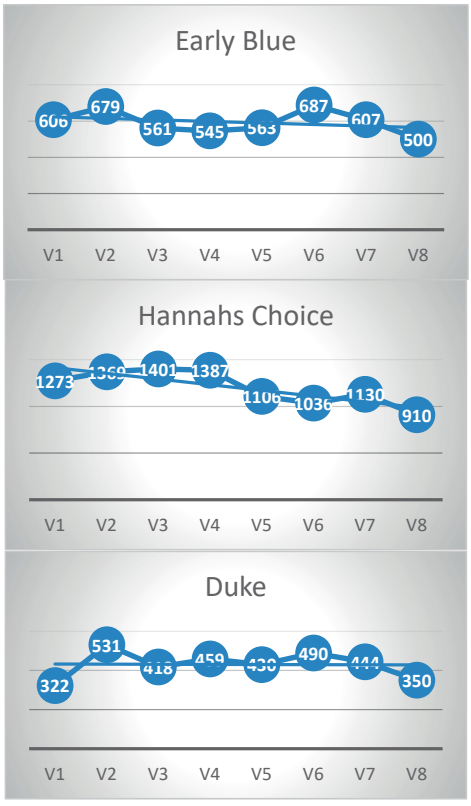


Figure 7. Variety growths according to the fertilization and substrate variant

Except 'Hannah's choice' where New logic increased the plant height, Bioact Veg had the same influence on the "Early blue' and 'Duke' growth (Figure 8).

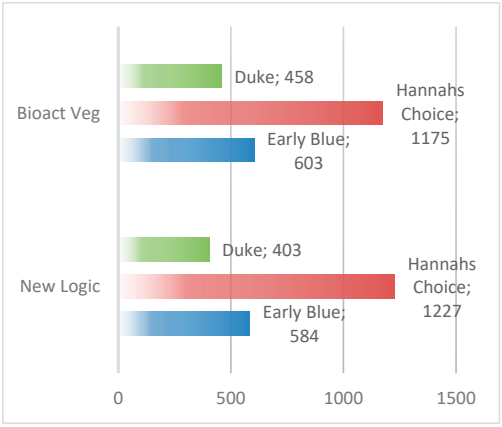


Figure 8. Influence of product fertilization on the total plant height (2019)

During the growing season, inside and in open field plants were not affected by diseases. The only key problem were aphids.

In August, the young shoots were severe attacked by the insects and couple of products were tested in this regard to assess the efficacity of them (Table 2).

Table 2. Efficacity of some organic products in the blueberry pest control

Product	Date of application	Dose	Efficacity
Laser 240 SC	May, 28 2019	6 ml/10 l water	Very good
GAREX B	July, 17 2019	10 ml/10 l water	Medium
PREV-AM	July, 18 2019	60 ml ml/10 l water	Medium
DEFFORT	July, 25 2019	30 ml/10 l water	Medium
GAREX B	July, 30 2019	60 ml/10 l water	Medium
DEFFORT + PREV-AM	July, 31 2019	25 ml DEF + 60 ml PREV/ 10 l water	
Chyisopa	August, 10 2019	*Chrysoperla carnea*	Excellent
PREV-AM + DEFFORT	August, 26 2019	PREV-AM 40 ml + DEF 30 ml/10 l water	
PREV-AM + LASER	August, 29 2019	PREV-AM 40 ml + LASER 5 ml/10 l water	
Chrisopa	August, 31 2019	*Chrysoperla carnea*	Very good

At the beginning, Laser was very efficient in combat the aphids but soon after another wave of aphids attacked the blueberry shoots and the repetition of the spray with Laser this time was not efficient as first application.
The other products such as Garex B, Prev-Am, Deffort had more repellent effect rather to fight against the aphids.
The natural predators *Chrysoperla carnea* proved a high efficiency and eliminated the aphids. At the second lunch in the end of August, 2019 the number of predators decreased and therefore to continue fight against aphids it was necessary to release more adults.

CONCLUSIONS

The fertilization strategy has to follow the environmental conditions and variety need.
Most vigorous variety was 'Hannah's choice' which grew higher.
Under the plastic, during one season for 2 out of three young blueberry varieties the influence was not enough expressed in terms of total growths.
More peat in the substrate proved to be in favour of supporting vegetative grows for the blueberry plants.
To protect the vegetative growths of the blueberry plants, two methods proved their efficiency: Laser spraying one time and *Chrysopa* as a natural predator product against aphids.

ACKNOWLEDGEMENTS

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RESPONSE OF POTTED RASPBERRIES AND BLACKBERRY VARIETIES TO ORGANIC TECHNOLOGY MEASURES IN HIGH TUNNEL SYSTEM

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Abstract

Nowadays, raspberry and blackberry are one of the most desired fruits for fresh consumption even beyond the ripening season. To meet the market need and support farmers with new growing solutions and organic measures applied in the orchards, in early 2019, we set out couple of experiments to demonstrate the viability of such innovative crop systems. Two new raspberry varieties 'Polonez' and 'Poemat' and one blackberry variety 'Navaho' were subject of pot system in high tunnel protected area with Nectarine plastic film. Substrates were composed by different share of organic peat, Biohumus and mycorrhiza. In each pot of 20 litres volume were planted two root cubes and a trellis system was provided. A mix of organic fertilizers were applied during the vegetation period and the plants response was evaluated in terms of growth and fruiting capacity. By the end of the year, 'Poemat' was picked for 5 times and 'Polonez' for one time more. No fruits available at 'Navaho' in the first year. The total number of harvested fruits were higher at 'Polonez' but the size was lower comparative to 'Poemat'. Longer canes were measured at 'Polonez' but 'Poemat' assembled more growths in terms of total annual growths due to a better ramification can. The Biohumus positively affect the growths of the plants independent of the fertilization scheme.

Key words*: Rubus idaeus, primocane, Rubus fruticosus, substrates, organic, fertilizers.*

INTRODUCTION

The demand for organic berry fruits is worldwide increasing and the profitability is higher by extending the harvest out of regular season (Rom et al., 2010).

More research is conducted to control on command the flowering and fruiting in berry crops (Strik, 2012) manipulating the crop for year-round production.

Raspberry performance in tunnels is well known (Demchak, K., 2009) and is more productive than in the open field and the fruits are bigger (Wien and Pritts, 2009).

In the northern countries raspberries cultivation under the plastic is the only way to achieve profitable yields due to the climate constraints (Svensson, 2016). Even for the blackberry in the cold regions (Hanson, 2012).

One solution to boost cane growth, flowering and fruiting before fall frost is to cover the row early in the Spring and unveil later on or to use high tunnels (Lewandowski et al., 2015).

Another approach is to protect plants from frost in the months of the year while canes are still late fruiting in order to extend the harvest as much as possible (Oliviera et al. 1996).

Raspberry pot culture in high tunnels can yield better than in-ground soil production (Qiu et al., 2016). Cultivating in pots, we can manage the timing of fruit production by keeping plants in cold storage until the desired production time similar with strawberries (Pritts, 2008). Another big advantage of using berry pot culture is that we can avoid the soil borne diseases and pathogens and control efficiently the risk of spreading the infection (Asanica, 2019).

Shorter canes are subject to less productive floricanes (Hanson et al., 2019) but primocane-fruiting varieties can be grown for several years in pots with great success.

In organic system, several key diseases, pests and disorders are in the eye of the researchers. Duringthe vegetation season, plants are frequently attacked by aphids. Therefore, beside common methods to fight against insects in ecological manner is the breeding method (Dossett and Kempler, 2016).

In the modern culture, raspberries easily adapt to fertirigation and substrate growing conditions (Qiu et al., 2017) but the regime and the

technological measures are subject to more conducted research in this topic.
The aim of the present work is to find the optimum way for growing organic raspberries and blackberry varieties in an appropriate and suitable technological manner for controlled climate and growing conditions starting from substrate composition till the fertigation application scheme. The adaptation in such growing system of some brand-new Polish raspberry varieties is a subgoal of the current research.

MATERIALS AND METHODS

The experiment was established in 22nd of April, 2019 and designed as follows:
Size of the solar: 6 m x 18 m.
Solar cover: Ginegar Suncover Nectarin of 150µ (87% light transmission, 35% light diffusion, 85% termicity); UV trans 300-380 nm - 45%.
Biological material: two raspberry varieties ('Polonez' and 'Poemat') and one blackberry variety ('Navaho').
Growing pots (containers): 20 litres.
Growing substrate: different share of organic peat Kekkila OPM525 and biohumus.
Mulch: Agrotextile under the pots.
Irrigation: drip line with stakes emitters of 6 LPH (2 stakes/pot)/
Nutrition: organic fertilizers (New Logic, Bioact Veg for soil application; Lumbreco for foliar and substrate application); mycorrhiza Glomus (Aegys Sym) and Trianum-V (Koppert) in pot only at the planting time.
Fertilizers were applied regularly starting from the planting day (22nd of April) with mycorrhiza (1 cup/pot) in the upper part of the substrate and Trianum-V 1 g/10 plants (23rd of April, 2019) by watering.
New Logic (0.6%) has been applied three times on substrate (3rd of May, 29th of May and 5th of August) in a dose of 3 ml/pot/application.
Bioact Veg has been used eight times to fertilize the plants on substrate with 3ml/pot/application (11th of May, 28th of May, 13rd of June, 29th of June, 18th of July, 25th of July, 13rd of August, 16th of September).
Lumbreco (2%) was used to fertilize by five times the substrate of the pots with 10 ml/pot/application on the following dates: 25th of April, 3rd of June, 11th of July, 23th of July, 26th of August). Other five application were made with Lumbreco (0.6%) by spraying on leaves with 0.33 ml/plant/application (6th of May, 20th of June, 15th of July, 22nd of August, 9th of September).
The experimental scheme is presented for the raspberry and the blackberry in the Table 1 and the module design in the Figure 1.

Table 1. Experimental variants constituted by location, substrate mixture and fertilization scheme application

Variant	Type	Substrate	Foliar fertilization	Substrate fertilization
V1	solar	peat 100%	Lumbreco	New Logic
V2	solar	peat 100%	-	Bioact Veg + Lumbreco
V3	solar	peat 75% + Biohumus 25%	Lumbreco	New Logic
V4	solar	peat 75% + Biohumus 25%	-	Bioact Veg + Lumbreco
V5	solar	peat 50% + Biohumus 50%	Lumbreco	New Logic
V6	solar	peat 50% + Biohumus 50%	-	Bioact Veg + Lumbreco
V7	solar	peat 75% + Biohumus 25% & mycorrhiza	Lumbreco	New Logic
V8	solar	peat 75% + Biohumus 25% & mycorrhiza	-	Bioact Veg + Lumbreco
V9	solar	peat 100% & mycorrhiza	Lumbreco	New Logic
V10	solar	peat 100% & mycorrhiza	-	Bioact Veg + Lumbreco
V11	open field	peat 75% + Biohumus 25% & mycorrhiza	Lumbreco	New Logic
V12	open field	peat 75% + Biohumus 25% & mycorrhiza	-	Bioact Veg + Lumbreco
V13	open field	peat 100% & mycorrhiza	Lumbreco	New Logic
V14	open field	peat 100% & mycorrhiza	-	Bioact Veg + Lumbreco

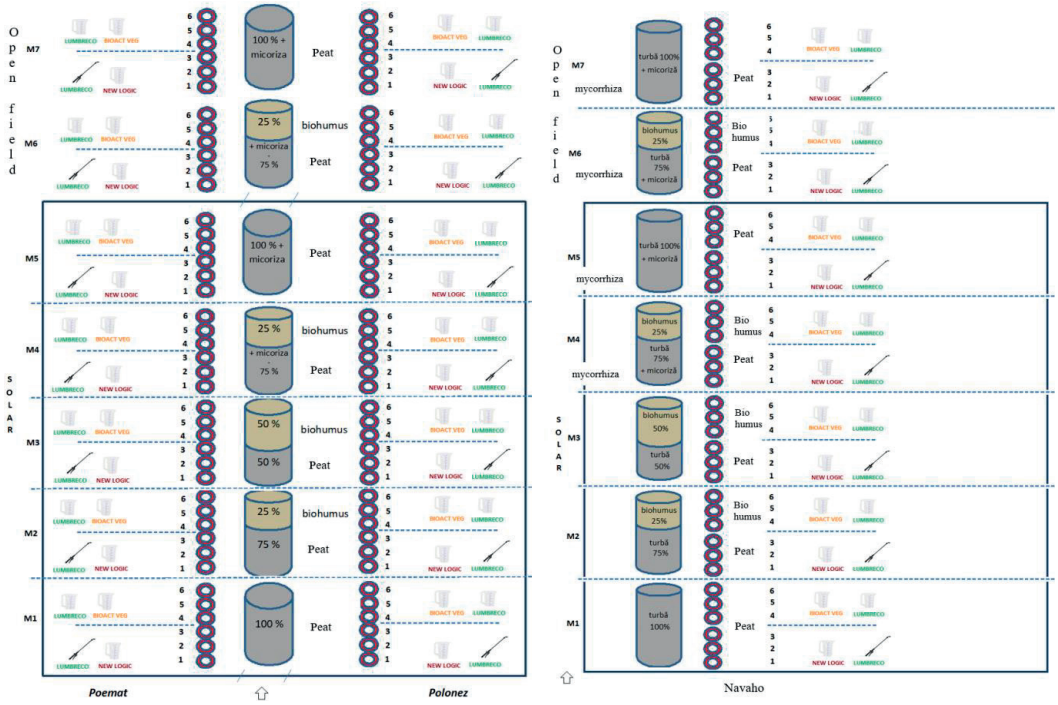

Figure 1. Experimental model for raspberry and blackberry fertilization

RESULTS AND DISCUSSIONS

The experiments started in 22th of April, 2019 and first organic products were applied. For instance, Trianum-V (1 g/10 plants) and 1 cup of Aegys mycorrhiza were distributed.

For raspberries we remarked wide differences regarding the height of the canes. The variants with the Bioact Veg and Lumbreco applied in the substrate increased the growths comparing to New Logic applied in substrate and with foliar Lumbreco sprays.

The average values independent of the other experimental factors indicate a higher vigour with 8% of 'Polonez' than 'Poemat' translated in height of the plants (Figures 2 and 3).

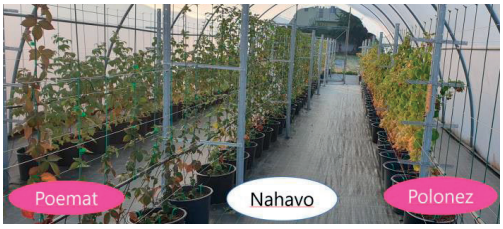

Figure 2. Evolution of raspberry and blackberry plant growths in relation with variety and fertilization scheme applied (October, 2019)

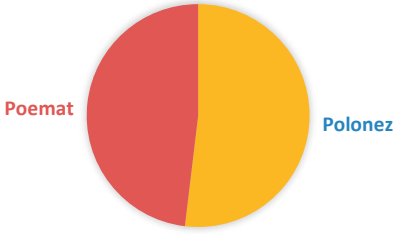

Figure 3. Vigour of raspberry varieties

One big difference between the two raspberry varieties is the ramification capacity. 'Poemat' is more spreading than 'Polonez' and exceed the 'Poemat' growths in terms of total annual growths / plant. From this point of view, the growing potential of 'Poemat' is in average with 38.63% higher than 'Polonez'.

Presence of biohumus in the substrate highly influenced the plants development regardless the variety features and the fertilization scheme (Figure 4).

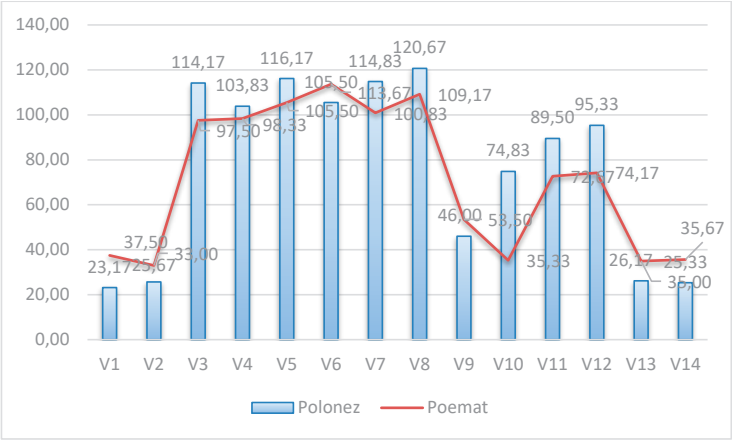

Figure 4 Influence of biohumus in the total height of the raspberries canes (cm)

The cumulative factors effect upon the height of the blackberry canes shows at the end of the growing season a large variation in these biometric values. Thus, the lowest plants were ones planted in the substrate containing only organic peat (65 cm) while the variants with biohumus added in different shares increased the total annual growth sum. The blackberry canes exceed 300 cm in height (Figure 5).

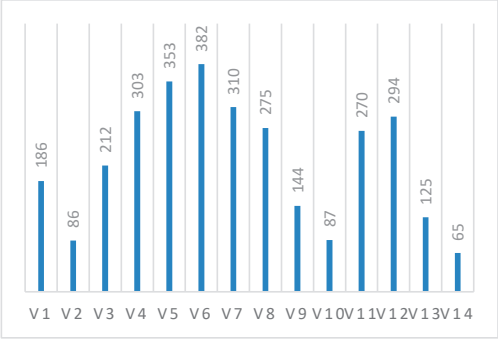

Figure 5. Fertilization scheme and substrate composition influence on 'Navaho' blackberry height

Regarding the blackberry fertilization scheme, the differences found were not significantly higher but a slight positive influence on plant growth was noted when applying the New Logic product in combination with leaf Lumbreco application (plants grew on average 228.57 cm) compared to BioactVeg with radicular Lumbreco application (213.14 cm).

First year for blackberry did not bring fruits on lateral shoots but for both raspberry varieties the production was quite good, proving the precocity of the Polish cultivars.

'Polonez' remarked with a higher yield. In average, it was picked 105 fruits/plant comparing to 23 fruits/plant at 'Poemat'.

The harvest period was marked by seven picks with the following dates: 20th of August, 30th of August, 20th of September, 27th of September, 3rd of October, 15th of October and 23rd of October.

Counting the number of fruits harvested at 'Poemat' (4617 fruits) and relating them to the yield (4.62 kg) results in an average weight of a fruit of 2.4 g. The Polish variety surpassed the sister variety with a number of fruits harvested in 2019 of 7848 pics, respectively 7.85 kg, but the size of the fruit was slightly smaller, of about 2.0 g (Figure 6).

The number of raspberry fruits were directly influenced by the substrate composition and fertilization model (Table 2).

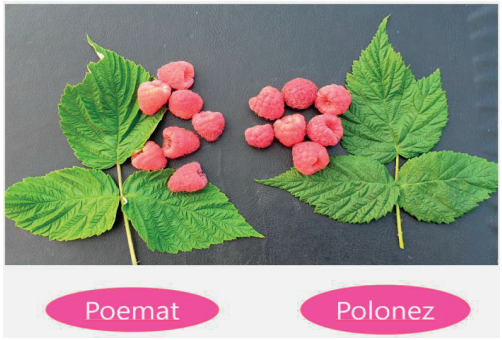

Figure 6. Size and appearance of the Polish raspberry varieties

Table 2. Number of fruits picked in the first year of raspberries planting under the influence of different fertilization schemes and substate composition

Variant	Type	Substrate composition	Application method of the fertilizer		No of fruits	
			Foliar	Substrate	'Polonez'	'Poemat'
V1	solar	peat 100%	Lumbreco	New Logic	0.00	0.00
V2	solar	peat 100%	-	Bioact Veg + Lumbreco	0.00	1.00
V3	solar	peat 75% + Biohumus 25%	Lumbreco	New Logic	195.83	44.00
V4	solar	peat 75% + Biohumus 25%	-	Bioact Veg + Lumbreco	203.83	59.83
V5	solar	peat 50% + Biohumus 50%	Lumbreco	New Logic	223.00	47.00
V6	solar	peat 50% + Biohumus 50%	-	Bioact Veg + Lumbreco	227.00	26.00
V7	solar	peat 75% + Biohumus 25% & mycorrhiza	Lumbreco	New Logic	231.00	48.83
V8	solar	peat 75% + Biohumus 25% & mycorrhiza	-	Bioact Veg + Lumbreco	260.83	71.67
V9	solar	peat 100% & mycorrhiza	Lumbreco	New Logic	33.17	3.50
V10	solar	peat 100% & mycorrhiza	-	Bioact Veg + Lumbreco	44.50	4.67
V11	open field	peat 75% + Biohumus 25% & mycorrhiza	Lumbreco	New Logic	24.33	9.50
V12	open field	peat 75% + Biohumus 25% & mycorrhiza	-	Bioact Veg + Lumbreco	29.00	9.50
V13	open field	peat 100% & mycorrhiza	Lumbreco	New Logic	0.00	0.67
V14	open field	peat 100% & mycorrhiza	-	Bioact Veg + Lumbreco	0.00	0.50
Average					*105.18*	*23.33*

The highest number of fruits picked at 'Polonez' was on 15th of October (Figures 7 and 9) while the pick of the harvest at 'Poemat' was 30th of August (Figure 7).

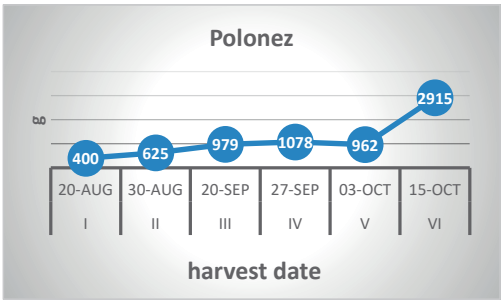

Figure 7. Dynamic of harvest at 'Polonez' variety

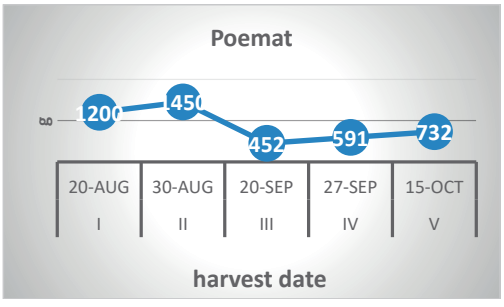

Figure 8. Dynamic of harvest at 'Poemat' variety

Figure 9. Late ripening of the 'Polonez' fruits (15.10.2019)

The mycorrhization of the substrate in some variants did not showed an improvement for fructification, neither for growths but the effect should be investigated on longer term.

Lumbreco with Bioact Veg were more efficient in raspberry productivity when it was applied directly in substrate with about 9% increase of production than fertilization scheme based on New Logic in soil and foliar Lumbreco.

During the growing season, inside and in open field plants were affected by aphids which has been controlled efficiently with Laser and Chrysopa Koppert natural predators.

CONCLUSIONS

Longer canes were measured at 'Polonez' but 'Poemat' summed more growths in terms of total annual growths due to a better ramification can.
The total number of harvested fruits were higher at 'Polonez' but the size was lower comparative to 'Poemat'.
The harvest pick at 'Poemat' is in the end of August while at 'Polonez' is in late Autumn.
Biohumus positively affect the growths of the plants independent of the fertilization scheme.
New Logic product in combination with leaf Lumbreco application had a good influence on blackberry growths. No fruits were harvested in the first year from 'Navaho'.

ACKNOWLEDGEMENTS

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EFFECTS OF DIFFERENT CULTURE MEDIA AND PLANT GROWTH REGULATORS ON MICROPROPAGATION OF 'GISELA 5' CHERRY ROOTSTOCK

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Abstract

We evaluated the influence of various culture media and plant growth regulators (PGR) upon axillary shoot proliferation rates and shoot lengths obtained in the in vitro multiplication stage in cherry rootstock 'Gisela 5'. The treatments consisted of the use of three basal media: Driver and Kuniyuki (DKW), Murashige and Skoog (MS), Woody Plant Medium (WPM) as well as three plant growth regulators (PGR) in various concentrations and combinations: N^6-benzyladenine (BA at 0.3 and 0.5 mg/l), DL-Dihydrozeatin (DHZ at 1.0 and 2.0 mg/l) and N-benzyl-9-(tetrahydropyranyl)-adenine (BPA at 1.0 mg/l). The results show that the type of basal media and PGRs influenced the quantity as well as the quality of axillary shoot development. In the presence of BA at 0.3 and 0.5 mg/l in DKW media the proliferation rates (PR) were superior to those in MS and WPM media, both at 0.3 mg/l BA (PR = 6.66 ± 0.65) and 0.5 mg/l BA (PR = 8.93 ± 0.86) in the aforementioned DKW treatments. The DKW culture medium supplemented with 1 mg/l indole-3-butyric acid (IBA) provided the highest in vitro rooting percentage (94.74%).

Key words*: cherry, benzyladenine, dihydrozeatin, micropropagation, shoot culture.*

INTRODUCTION

'Gisela 5' (*P. cerasus* × *P. canescens*) is known as the most popular clone of the Gisela rootstocks in Germany. One of its great advantages is that in contrast to standard *P. avium* rootstocks, it significantly reduces the vigor of sweet cherry trees and they can begin to flower and fruit in the second year in the nursery and achieve full cropping in the 5th year. The propagation and marketing of this rootstock is handled by a consortium of German rootstock nurseries Deutscher Baumschulen GmbH (http://www.cdb-rootstocks.com/en/cdb---consortium.html) (Franken-Bembenek, 2005). Since classical propagation methods of this rootstock are inefficient (Bošnjak and Kereša, 2012), several micropropagation protocols have already been developed as viable alternatives for its propagation. *In vitro* cultures of 'Gisela 5' are not dependent on season, provide clean, disease and virus-free planting material (Sharma et al., 2017). Previous reports show that the most commonly used basal medium for *in vitro* propagation of 'Gisela 5' rootstocks was MS (Murashige and Skoog, 1962) (Ružić et al., 2000; Vujović et al., 2012; Clapa et al., 2013; Xu et al., 2015; Thakur et al., 2016; Sharma et al. 2017; Tariverdi et al. 2017;). Other scientific reports show that the less tested *in vitro* culture media for 'Gisela 5' were QL (Quoirin and Lepoivre medium) (Mihovilović Bošnjak and Kereša, 2012) or DKW (Driver and Kuniyuki Juglans medium) and WPM (McCown Woody Plant medium) (Fallahpour et al., 2015; Ozudogru et al., 2017). Furthermore, N^6-benzyladenine (BA) is the most frequently used cytokinin in the initiation and multiplication stage of 'Gisela 5' cherry rootstock in various concentrations and/or combinations (Buyukdemirci, 2008; Fidanci et al., 2008; Bošnjak et al., 2012; Xu et al., 2015; Thakur et al., 2016).

Other studies show that the in vitro rooting percentage ranged from 14-93.7% when 0.5, 1 or 2 mg/l indole-3-butyric acid (IBA) was added to the culture media in the in vitro rooting stage (Fallahpour et al., 2015). Furthermore, concentrations of 0.25 and 0.5 mg/l α-naphthaleneacetic acid (NAA) led to rooting percentages of 64 and 72%.

Several investigations have been carried out regarding other aspects of micropropagation of 'Gisela 5' cherry rootstock such as in vitro conservation (Ružić et al., 2015), the effects of multiple subculturing (Vujović et al., 2012), effects of gas-tight and gas-permeable culture containers and different sucrose concentrations, as well as sucrose and mannitol combinations applied in tissue culture (Ozudogru et al., 2017). Therefore, the main aim of this research was to test different culture media and plant growth regulators such as DL-Dihydrozeatin (DHZ) and N-benzyl-9-(tetrahydropyranyl)-adenine (BPA) in micropropagation processes of 'Gisela 5' cherry rootstock, which to the best of our knowledge had not be used until now neither for 'Gisela 5' tissue cultures nor for other rootstock.

MATERIALS AND METHODS

Explant preparation and tissue culture initiation

For tissue culture initiation herbaceous shoots were used obtained from the forced twigs in laboratory conditions in March, 2018. The shoots were fragmented and internodes were eliminated followed by washing the plant material in running tap water and then rinsed with deionised water. Then the shoot fragments were sterilised with ACE 20% for 20 minutes and rinsed repeatedly with deionised water (5 rinses) in laminar flow hood. The axillary and terminal buds were excised aseptically from the nodal segments and then inoculated directly onto the MS (Murashige & Skoog, 1962) culture media in glass tubes (Table 1). The media was supplemented with 0.5 mg/l BA and solidified with cu 4 g/l Plant agar. After 8 weeks, shoot regeneration percentage was calculated, which represented the number of inoculated buds with successful shoot regeneration. For *in vitro* culture stabilisation 1.5-2 cm length shoot fragments with 3-4 buds obtained in the initiation stage were inoculated on the same above mentioned culture media. Hereafter, for all the experimental treatments 720 ml glass jars were used with crew lids with an antibacterial filter to ensure gas exchange with the atmosphere. 100 ml of culture medium was dispersed/culture vessel and each vessel contained 5 nodal segments which were introduced in the culture media in slanted position. The duration of the in vitro cycles was 6 weeks.

Effect of various culture media and plant growth regulators (PGR) on in vitro multiplication

The influence of various culture media and plant growth regulators (PGR) upon axillary shoot proliferation rates and shoot lengths were also evaluated in this study. The treatments consisted of the use of three different basal media (Table 1): Driver and Kuniyuki (DKW) (Driver and Kuniyuki, 1984), Murashige and Skoog, 1962 (MS), Woody Plant Medium (WPM) (Lloyd and McCown, 1980) as well as three plant growth regulators (PGRs) in various concentrations and combinations: N^6-benzyladenine (BA at 0.3 and 0.5 mg/l), DL-Dihydrozeatin (DHZ at 1.0 and 2.0 mg/l) and N-benzyl-9-(tetrahydropyranyl)-adenine (BPA at 1.0 mg/l).

Table 1. Chemical composition of the basal media used for the micropropagation of 'Gisela 5' cherry rootstock

	Concentrations		
Composition	MS	DKW	WPM
Macro-elements	MS	DKW	WPM
Micro-elements	MS	DKW	WPM
FeNaEDTA	36.7 mg/l	44.63 mg/l	36.7 mg/l
Myo-inositol	100 mg/l	100 mg/l	100 mg/l
Vitamin B1	2 mg/l	2 mg/l	1 mg/l
Vitamin B6	1 mg/l	-	0.5 mg/l
Acid nicotinic	1 mg/l	1 mg/l	0.5 mg/l
Glycine	-	2 mg/l	-
Sugar	30 g/l	30 g/l	30 g/l
Plant Agar	4 g/l	4 g/l	4 g/l
pH = 5.8			

Rooting and acclimatization

In vitro rooting was carried out on DKW basal media without PGRs or supplemented with IBA and gelled with Plant Agar. Thus, the following treatments (variants) were established:

V1 - DKW + 4 g/l Plant agar

V2 - DKW+ 0.5 mg /l BA + 4 g/l Plant agar

V3 - DKW+ 1 mg/l BA + 4 g/l Plant agar

The young shoots (1.5 month old) were inoculated onto the culture media (15 shoots/vessel in three repetitions). After 6 weeks of culturing shoot length, number of shoots, length of roots, number of roots and the maximum length of the roots were recorded.

Ex vitro rooting and acclimatization

The unrooted shoots taken from the multiplication stage were rooted and hardened in ex vitro conditions in floating perlite, while the *in vitro* rooted shoots were hardened in floating hydroculture (Clapa et al., 2013). After 30 days the rooting and plant survival percentages were calculated based on the data recorded from 45 shoots from three repetitions (15 shoots/repetition).

Data Analysis

To analyse the data, ANOVA analysis was performed first to check the differences among the means. When the null hypothesis was rejected, Tukey's HSD test ($P \leq 0.05$) was performed to determine the means that are significantly different from each other. Values shown are means ± SE. In addition, Pearson's correlation was assessed to check the relationships between the mean length of the shoots, number of shoots and the length of the roots developed in the *ex vitro* rooting stage of the 'Gisela 5' cherry rootstock.

RESULTS AND DISCUSSIONS

For the initiation of 'Gisela 5' cherry rootstock the tissue culture were established in March, 2018, using apical buds and nodal shoot fragments as results of forcing of the annual twigs in laboratory conditions. Thus, the in vitro culture could be established earlier, and the young shoots used for initiation were free of any type of diseases. For this reason, there were no shoot infections observed in the initiation stage of the in vitro culture as shown in Figure 1.

Figure 1. *In vitro* culture initiation of 'Gisela 5' cherry rootstock

In this manner, the initiation percentage recorded was 86.25%. In contrast, other studies show that when using 'Gisela 5' plant material obtained from greenhouse can considerably increase the risk of infections and can lead to a contamination percentage of the explants of 71.7%, and an initiation success of only 28.3% as reported by Vujović et al. (2012).

The influence of the culture medium and BA concentration on the multiplication rate of 'Gisela 5' cherry rootstock

Our results show that all the treatments had different effects on the development of 'Gisela 5' cherry rootstock in terms of number of shoots/explant/vessel and shoot length recorded in the multiplication stage as presented in Table 2. It was also observed that both the basal media and BA concentrations influenced the proliferation capacity of 'Gisela 5' cherry rootstock. The highest numbers of shoots/vessel and shoots/explant were obtained on the basal media supplemented with 0.5 mg/l BA. Thus, the highest number of shoots/vessel (42.33 ± 2.59) and shoots/explant (8.93 ± 0.86) were recorded on DKW + 0.5 mg/l BA + 4 g/l Plant agar (Figure 2) followed by 39.66 ± 2.73 shoots/vessel and 7.93 ± 0.57 shoots/explant on MS + 0.5 mg/l BA + 4 g/l Plant agar. Between these two no statistically significant differences were recorded. On the WPM + 0.5 mg/l BA + 4 g/l Plant agar medium the mean number of shoots/vessel recorded was 35.66 ± 2.87 and 7.13±0.98 shoots/explant which were significantly lower than those recorded on DKW+0.5 mg/l BA: 42.33 ± 2.59 and 8.93±0.86.

Lower number of shoots were recorded on the media supplemented with 0.3 mg/l BA but also in this case basal media DKW and MS stimulated the most the development of the

shoots/vessel (33.33 ± 1.67 and 30.33 ± 0.83), but no statistically significant differences were observed between these and those obtained on WPM + 0.3 mg/l BA (Figure 5).

Our findings reveal that the highest average lengths of the shoots were obtained on DKW+0.3 mg/l BA, reaching 3.28 ± 0.31 cm, followed by those developed on DKW + 0.5 mg/l BA with an average of 3.01 ± 0.26 cm. Statistically significant differences had been determined between these two and the rest of the media used for propagation. Therefore, the shoots developed the most on the DKW basal medium. Depending on the concentration of BA in the basal medium, the longest shoots were developed on the media supplemented with 0.3 mg/l BA, on all the three basal media (Table 2).

Table 2. The influence of culture media and BA concentrations on the in vitro multiplication of 'Gisela 5' cherry rootstock

Variant/Treatment	Average number of shoots/vessel	Average number of shoots/explant	Average length of shoots
WPM+0.3 mg/l BA	22.00±1.36 ^{e*}	4.40±0.63 ^{e}	2.41±0.22 ^{b}
WPM+0.5 mg/l BA	35.66±2.87 ^{bc}	7.13±0.98^{bc}	2.32±0.19 ^{b}
MS+0.3 mg/l BA	30.33±0.83 ^{d}	6.06±0.50 ^{d}	2.46±0.19 ^{b}
MS+0.5 mg/l BA	39.66±2.73 ^{ab}	7.93±0.57 ^{ab}	2.17±0.19 ^{b}
DKW+0.3 mg/l BA	33.33±1.67 ^{cd}	6.66±0.65 ^{cd}	3.28±0.31 ^{a}
DKW+0.5 mg/l BA	42.33±2.59 ^{a}	8.93±0.86 ^{a}	3.01±0.26 ^{a}

*The values shown are means ± SE. Different lowercase letters indicate significant differences between the means among the treatments according to Tukey's HSD test (p≤ 0.05).

Figure 2. 'Gisela 5' cherry rootstock in vitro multiplication stage on DKW culture medium supplemented with 0.5 mg/l BA

Both the number of shoots/explant and length of the shoots reached much higher values than those reported by other in all types of culture media used for this experiment (D Ružić et al., 2000; Sharma et al., 2017; Thakur et al., 2016; Vujović et al., 2012).

Effect of various plant growth regulators (PGRs) on the in vitro multiplication of 'Gisela 5' cherry rootstock

Cytokinins such as DHZ and BPA, tested for the first time in 'Gisela 5' cherry rootstock tissue culture proved to be less efficient than BA in terms of proliferation rate. The combination of 1 mg/l BPA + 1 mg/l DHZ generated longer shoots than any of the BA (Figures 3 and 5).

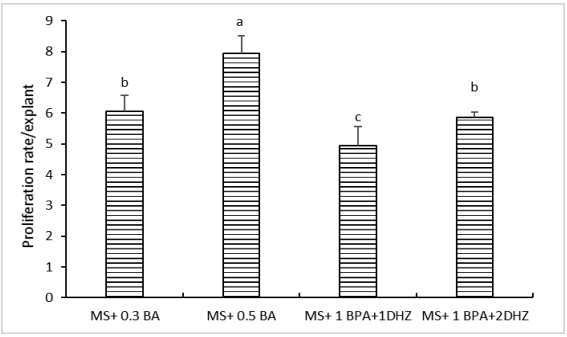

Figure 3. The influence of the Murashige and Skoog 1962 (MS) media supplemented with N6-benzyladenine (BA at 0.3 and 0.5 mg/l), DL-Dihydrozeatin (DHZ at 1.0 and 2.0 mg/l) and N-benzyl-9-(tetrahydropyranyl)-adenine (BPA at 1.0 mg/l) on in vitro the proliferation rate of 'Gisela 5' cherry rootstock. The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test (p≤ 0.05)

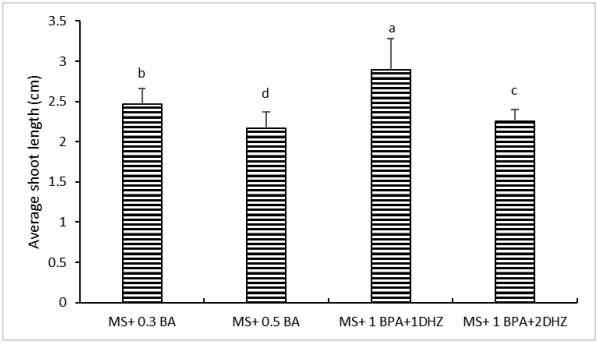

Figure 4. The influence of Murashige and Skoog 1962 (MS) supplemented with N6-benzyladenine (BA at 0.3 and 0.5 mg/l), DL-Dihydrozeatin (DHZ at 1.0 and 2.0 mg/l) and N-benzyl-9-(tetrahydropyranyl)-adenine (BPA at 1.0 mg/l) on shoot length in the *in vitro* multiplication stage of a 'Gisela 5' cherry rootstock. The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

Figure 5. *In vitro* shoot regeneration of 'Gisela 5' cherry rootstock on different culture media: Murashige and Skoog (MS), Woody Plant Medium (WPM, Lloyd & McCown) and Driver and Kuniyuki (DKW) supplemented with 0.3 mg/l BA and 0.5 mg/l B.

Regarding shoot length, the combination of MS + 1 BPA + 1 DHZ generated the longest shoots (2.89 ± 0.39 cm) as compared to 2.46 ± 0.19 cm developed on MS + 0.5 mg/l BA. The average length of the shoots developed on MS supplemented with these cytokinins were much greater than those obtained on the same basal medium but supplemented with BA, Kin, TDZ and gibberellin GA3 combinations ranging between 0.75-2.25 cm (Thakur et al., 2016).

Rooting and acclimatization

Our results show that for in vitro rooting DKW basal medium proved to be the most suitable for the multiplication stage of 'Gisela 5' cherry rootstock. Based on our findings, the use of newly developed, full length shoots excised from the young plantlets obtained during the 1-1.5-months- in vitro culture is recommended due to the crucial role of the apical bud (Clapa et al., 2013). It was also observed that, shoots from the media with no PCRs added did not emerged any roots. The highest rooting percentage (94.74%), though, was recorded in shoots regenerated on DKW + 1 mg/l IBA medium, followed by 74.36% on the medium supplemented with 0.5 mg/l IBA (Figure 6). Our study provides further evidence for the effectiveness of WPM medium containing 2 mg/l IBA for rooting (93.7%) as compared to MS (53.1%) or DKW (14.0%) which showed much lower rooting percentages (Fallahpour et al., 2015). Similar results have been found by Xu et al. (2015), who used MS, 1/2 MS, 1/4 MS and 1/8 MS supplemented with 2 mg/l IBA and recorded low rooting percentages such as 77.5, 82.5, 87.5 and 77.5% as compared to WPM (Xu et al., 2015). Other low rooting percentages were also reported by Fidanci et al. (2008) when MS supplemented with 0.5 mg/l and 1 mg/l IBA generating rooting percentages of 67 and 89%, which were 8.67 and 27.74% lower than the rooting percentage obtained on DKW. The in vitro rooted shoots were acclimatised in floating hydroculture and the survival rate recorded after 30 day of hardening was 49.96%. The unrooted shoots were subjected simultaneously to ex vitro rooting and hardening in floating perlite + 1 mg/l IBA. The rooting percentage and survival rate recorded was 96.15%. The hardened shoots (plantlets) had an average length of 3.67 ± 0.23 cm, and an average number of 6.13 ± 0.64 roots/shoot, and the maximum average length of the roots recorded was 5.83 ± 0.47 cm.

These results show a good positive correlation between the measured parameters (Figure 7). Namely, the longest the shoots were the longest and highest the roots and their number were.

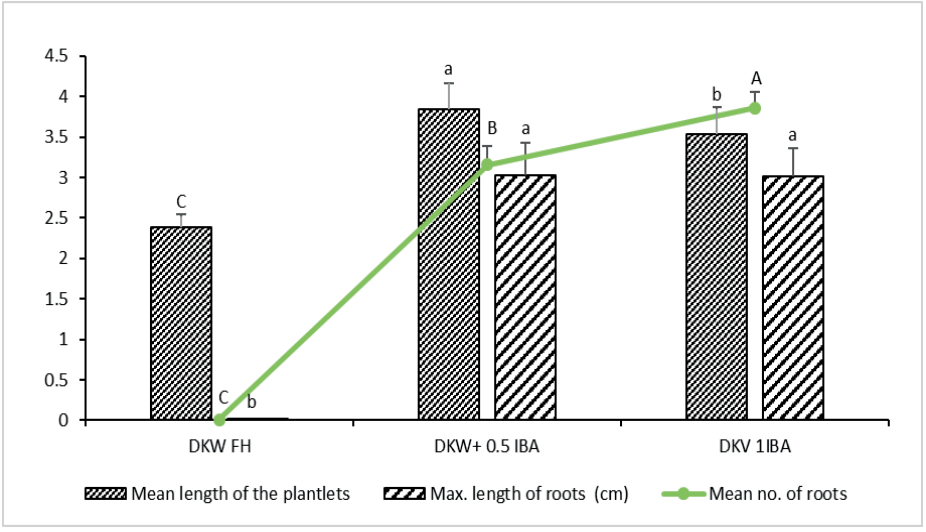

Figure 6. In vitro rooting of 'Gisela 5' on Driver and Kuniyuki (DKW) medium without PGRs and supplemented with 0.5 and 1 mg/l IB

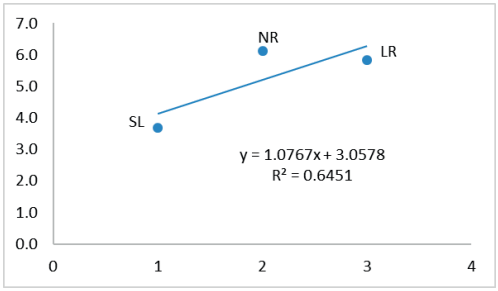

Figure 7. Correlation between the average length of shoots (SL), number of roots (NR) and the length of the roots of due to *ex vitro* rooting of 'Gisela 5' cherry rootstock

CONCLUSIONS

Our work led us to conclude that for the in vitro initiation of 'Gisela 5' cherry rootstock in spring, young, fresh and herbaceous shoots are the most suitable to be used as results of forcing the annually harvested twigs from orchard. The forcing of the harvested twigs can successfully be carried out in laboratory conditions with natural light and constant temperature. After sterilisation, shoot fragments with axillary or apical buds were the most effective to be used on MS and DKW media supplemented with 0.5 mg/l BA and gelled with Plant agar. Regarding the *in vitro* multiplication, the findings of our study indicate that MS and DKW media supplemented with 0.3-0.5 mg/l BAP and solidified with 4 g/l Plant Agar were the most adequate for this stage. The results of this research point towards the idea that 'Gisela 5' cherry rootstock can simultaneously be rooted and hardened ex vitro in floating perlite with 1 mg/l IBA to increase the economic efficiency of the production chain by eliminating the rooting stage of the regenerated plantlets. As mentioned before, in this case the rooting percentage and survival rate of the micropropagated plants can reach over 95%.

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BEHAVIOUR OF SOME PLUM CULTIVARS UNDER ECOLOGICAL CONDITIONS FROM ARGES AREA

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Abstract

In last year's, as a result of the biological material exchange with different fruit growing institutes from Europe, a large number of plum varieties have been introduced into the culture. The aim of this study was to evaluate the behaviour of some plum varieties with different origins (Belarus, Russia, Estonia and USA) in the climatic conditions of Maracineni, Arges area. In 2016-2018 periods, at 13 plum varieties ('Kometa', 'Vilnor', 'Kadri', 'Nesmeyana', 'Lama', 'Soneyka', 'Dalikatnaya', 'Asloda', 'Okskaya', 'Wanette', 'Mirnaya', 'Vengherka Kaukaskaya', 'Vengherka Belaruskaya') were evaluated phenological traits (flowering and ripening time) and some fruits characteristics (shape, colour, weight, soluble solids content, titratable acidity and firmness). The most plum varieties studied were characterized by early flowering (about 7 days earlier than most plum varieties cultivated in Romania) and early ripening (the first two decade of July). The skin color varied from yellow ('Soneyka' cv) to blue ('Vilnor', 'Kadri', 'Okskaya', 'Wanette', 'Vengherka Kaukaskaya', 'Vengherka Belaruskaya' cvs.). The 'Lama' variety was noted by skin and flesh red of fruits. The average fruit weight ranged from 22.21 g ('Kometa' cv.) to 50.41 g ('Vengherska Beloruskaya' cv.). The following varieties were noted for large fruits (over 40 g): 'Lama', 'Dalikatnaya', 'Asaloda', 'Okskaya', 'Mirnaya', 'Vengherka Kaukaskaya' and 'Vengherka Belaruskaya'. The 'Soneyka' and 'Vengherka Kaukaskaya' were clearly differentiated by firmness (64.0 N, respectively 56.06 N), while the 'Wanette' and 'Mirnaya' cvs. were differentiated by the high content in soluble solids (19.25% respectively 18.75% Brix).

Key words*: plum, cultivars, phenological traits, fruits characteristics.*

INTRODUCTION

Plum is the most important species in Romania. The average annual production of 481.278 t in the period 2015-2017 ranks Romania on the second place in the word, after China (FAOSTAT 2019). Although most of the production is intended for processing, the interest for the consumption of fresh fruits is constantly increasing (Milatovic et al., 2018). Therefore, the improvement of the plum assortment was a primary concern in Romania, and implicitly at the Research Institute for Fruit Growing Pitesti-Maracineni. This is why, at RIFG Pitesti Maracineni, besides the activity of creating new varieties another objective is to introduce the new foreign cultivars and their study in Romanian climatic and soil conditions in order to choice and spread the better cultivars in the region of Arges district (Butac, 2015). For this purpose, in the period 2010-2014 in our institute were carried out 4 bilateral projects with the Institute for Fruit Growing Minsk, Belarus, occasion with which there were made reciprocal exchanges of biological material. Thus, 13 plum varieties of different origins (Belarus, Russia and the Baltic countries) were evaluated in a field trial, from a phenological and qualitative point of view.

MATERIALS AND METHODS

The studies were carried out in a field trial located at the RIFG Pitesti Maracineni, Genetics and Breeding Laboratory on a number of 13 plum varieties, which have not been cultivated in

Romania, obtained following a bilateral scientific program with Institute for Fruit Growing Minsk - Belarus carried out during the period 2010-2014 (Table 1). The trees, grafted on 'Myrobalan' rootstock were planted in the spring of 2015, at a distance of 4 x 3 m, under non-irrigation conditions.

At these varieties the following traits were evaluated:

- phenological traits - flowering and ripening time - were appreciated by noting the calendar date;
- fruit color appreciated visually;
- fruit weight was recorded with a balance in g/fruit;
- soluble solid contents were measured with a portable refractometer, in % Brix;
- fruit firmness was measured with non-destructive penetrometer Qualitest HPE equipped with a plunger of diameter 0.10 cm^2;
- fruit content in malic acid of fruits were measured using the device Minititrator and pH meter for fruit juice - Hanna Instrument 84532. Titratable acidity is expressed as g/100 g fresh matter.

Data were analyzed statistically using Duncan's multiple range test - $P \leq 0.05$.

Table 1. Biological material studied

No	Varieties	Origin	Type of species	Year of registered in Belarus
1	Lama*	Belarus, Hybrid 9-250 (*P. cerasifera* var. *Pissardii*) open pollination	Diploid	2003
2	Asaloda*	Belarus, hybrid (*P. cerasifera* ×*P. ussuriensis*) × Puteshestvennitsa	Diploid	2003
3	Dalikatnaya*	Belarus, Evrasia 21 × d'Agen	Hexaploid	2005
4	Soneyka*	Belarus, Mara open pollination	Diploid	2015
5	Vengherka Belaruskaya*	Belarus, Stanley × Dalikatnaya	Hexaploid	2015
6	Vilnor**	Estonia, Wilhelmina Späth × Noarootsi Punane	Hexaploid	1989
7	Kadri**	Estonia, Latvijas Dzelteno Olplumi × Suhkruploom	Hexaploid	1994
8	Kometa (Kometa kubanskaya)**	Russia, Skoroplodnaia × Pionerka	Diploid	1999
9	Mirnaya*	Russia, Skorospelka Krasnaya × Reine Claude de Bavay	Hexaploid	2013
10	Vengherka Kaukaskaya*	Russia, Reine Claude d'Althan × Sochinskaya yubileinaya	Hexaploid	-
11	Nesmeyana*	Russia, Kometa kubanskaya open pollination	Diploid	2005
12	Okskaya**	Russia, Severyanka × Record	Hexaploid	-
13	Wanette	USA, *P. americana*×*P. ussuriensis or P. salicina*	Diploid	-

*Kazlouskaya et.al., 2015; **Janes and Pae, 1998; Karklins et al., 2007.

RESULTS AND DISCUSSIONS

Phenological traits

Flowering time. Usually in Romania plum blooms in mid-April depending on weather conditions. Average flowering time of studied cultivars was in the first decade of April (Table 2). The earliest start of flowering was recorded at the 'Dalikatnaya', 'Soneyka', 'Kometa', 'Nesmeyana' cvs. (April, 1) and the latest at the 'Kadri' cv. (April, 7). The average difference between cultivars with earliest and latest flowering was seven days. The earliest flowering was in 2017 (average March, 28) and the latest flowering was in 2019 (Average April, 11). The average difference between years with earliest and latest flowering was 14 days much bigger than difference between cultivars. We can see that diploid cultivars blossomed earlier than hexaploid cultivars. This characteristic exposing them to the risk of late spring frosts which usually appear till middle of April.

Ripening time. Most of the plum varieties studied were very early (starting July 1 to 31), with the exception of the 'Vengherka Belaruskaya', 'Vengherka Kaukaskaya' and 'Mirnaya' varieties that matured on August 15.

Cv. 'Dalikatnaya' was studied also in Serbia where the same results were obtained (Milatovic et al., 2018).

Table 2. Phenological characteristics of plum cultivars (average, 2017-2019)

No.	Varieties	Flowering time	Abundance of flowering (0-5 scale)	Ripening time
1	Lama	3 April	2	13 July
2	Asaloda	4 April	5	13 July
3	Dalikatnaya	1 April	5	13 July
4	Soneyka	1 April	5	15 July
5	Vengherska Belaruskaya	5 April	4	15 August
6	Vilnor	4 April	4	1 July
7	Kadri	7 April	3	1 July
8	Kometa	1 April	5	1 July
9	Mirnaya	5 April	4	15 August
10	Vengherka Kaukaskaya	5 April	3	15 August
11	Nesmeyana	1 April	5	1 July
12	Okskaya	5 April	2	15 July
13	Wanette	5 April	3	31 July
	Amplitude	***01 -14.04***	***2-5***	***1.07-15.08***

Physical fruits characteristics

Fruit shape of plum varieties studied varied from spherical (Lama, Dalikatnaya, Vilnor, Kometa, Mirnaya), ovate (Asaloda, Soneyka, Kadri, Okskaya, Wanette) to elongated spherical (Vengherka Belaruskaya, Vengherka Kaukaskaya, Nesmeyana) (Table 3).

Skin colour varied from yellow ('Soneyka') to blue ('Vilnor', 'Kadri', 'Okskaya', 'Wanette', 'Vengherka Kaukaskaya', 'Vengherka Belaruskaya'). The 'Lama' variety was noted by skin and flesh red of fruits (Table 3).

Stone adherence. 6 varieties are freestone, 5 are semi-adherent, and only 2 have pulp-adherent (Table 3).

Table 3. Fruits characteristics: shape, colour, stone adherence

No.	Varieties	Shape	Colour	Stone adherence
1	Lama	Spherical	Reddish	Freestone
2	Asaloda	Ovate	Reddish	Semi-adherent
3	Dalikatnaya	Spherical	Red violet	Freestone
4	Soneyka	Ovate	Yellow	Semi-adherent
5	Vengherka Belaruskaya	Elongated Spherical	Blue	Semi-adherent
6	Vilnor	Spherical	Blue	Clingstone
7	Kadri	Ovate	Blue	Freestone
8	Kometa	Spherical	Reddish	Semi-adherent
9	Mirnaya	Spherical	Reddish	Freestone
10	Vengherka Kaukaskaya	Elongated Spherical	Blue	Freestone
11	Nesmeyana	Elongated Spherical	Reddish	Semi-adherent
12	Okskaya	Ovate	Blue	Clingstone
13	Wanette	Ovate	Blue	Freestone

Fruits weight. An important role in marketing for plum varieties designated for fresh consumption has fruit size. The largest fruits were recorded 'Vengherka Belaruskaya' (50.41 g) and 'Lama' (47.98 g), values which differ very significantly and significantly from the other varieties studied. At early varieties, the fruit weight not exceed 31 grams ('Kometa' - 22.21 g, 'Vinor' - 25.3 g, 'Nesmeyana' - 31.3 g), while at later varieties (e.g. 'Vengherka Belaruskaya') the fruit weight reaches 50.41 grams (Table 4).

Flesh firmness. Firmness is an important factor in stone fruits often related to taste and shelf life, and firmness assessment is widely used both in the marketing chain to judge overall fruit quality and by researchers in variety testing and programs including fruit quality (Sekse and Wermund, 2010). Generally, flesh firmness decreases during the maturation and ripening. Early season plum varieties are usually less firm at the minimum maturity time than late season varieties (Crisosto, 1994). The varieties with the highest flesh firmness were 'Vengherka Belaruskaya' (46.83 N), 'Lama' (47.98 N), 'Vengherka Kaukaskaya' (56.06 N) and 'Soneyka' (67.00 N) (Table 4).

Fruit soluble solids content increases with maturity and ripening and could be a good quality

index. The soluble solids content ranged from 11.08 % at 'Kometa' cv. to 19.25 % at 'Wanette' cv. The varieties with the highest soluble solids content were 'Dalikatnaya' (15.20%), 'Okskaya' (16.53%), 'Kadri' (16.83%), 'Mirnaya' (18.75%) and 'Wanette' (19.25%) (Table 4).

Titratable acidity of plums varied between 0.11g malic acid at 'Soneyka' cv. and 0.59 g malic acid at 'Okskaya' cv. It is observed that the acid content has a correlation with the skin color: fruits with light color have a low acid content, while fruits with dark color have a high acid content.

Minas et al. (2015) has found that plums were harvested at 27-35 N flesh firmness, SSC was 11.1-19.7% and TA varied from 0.30 to 1.60%.

Table 4. Fruits characteristics of plum varieties studied (2017-2018)

No.	Variety	Fruit weight (g)	Firmness (N or HPE units)	Soluble solid contents - SST (% Brix)	Titratable acidity - TA (Acid malic %)
1	Lama	47.98 **a**	49.65 **b**	13.21 **de**	0.29 **cd**
2	Asaloda	40.25 **b**	30.45 **cd**	12.80 **e**	0.47 **b**
3	Dalikatnaya	41.31 **b**	31.00 **cd**	15.20 **bc**	0.36 **c**
4	Soneyka	32.85 **c**	67.00 **a**	13.95 **cde**	0.11 **g**
5	Vengherka Belaruskaya	50.41 **a**	46.83 **b**	13.63 **de**	0.22 **def**
6	Vilnor	25.33 **d**	19.50 **e**	14.61 **cd**	0.52 **ab**
7	Kadri	30.06 **c**	37.15 **c**	16.83 **b**	0.28 **cd**
8	Kometa	22.21 **e**	30.01 **cd**	11.08 **f**	0.12 **g**
9	Mirnaya	40.68 **b**	23.41 **de**	18.75 **a**	0.28 **de**
10	Vengherka Kaukaskaya	41.93 **b**	56.06 **b**	14.55 **cd**	0.13 **fg**
11	Nesmeyana	31.93 **c**	26.93 **cde**	11.20 **f**	0.15 **efg**
12	Okskaya	40.95 **b**	25.05 **de**	16.53 **b**	0.59 **a**
13	Wanette	24.95 **de**	30.26 **cd**	19.25 **a**	0.34 **c**

Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P>0.05).

Figure 1. Pictures with some cultivars studied (a - Okskaya; b - Lama; c -Asaloda; d - Dalikatnaya; e - Soneyka)

CONCLUSIONS

The most plum varieties studied were characterized by early flowering (about 7 days earlier than most plum varieties cultivated in Romania) and early ripening (the first two decade of July).

The skin color varied from yellow ('Soneyka' cv) to blue ('Vilnor', 'Kadri', 'Oksana', 'Wanette', 'Vengherka Kaukaskaya', 'Vengherka Belaruskaya' cvs.). The 'Lama' variety was noted by skin and flesh red of fruits.

The average fruit weight ranged from 22.21 g ('Kometa' cv.) to 50.41 g ('Vengherka Belaruskaya' cv.).

The following varieties were noted for large fruits (over 40 g): 'Lama', 'Dalikatnaya', 'Asaloda', 'Okskaya', 'Mirnaya', 'Vengherka Kaukaskaya' and 'Vengherka Belaruskaya'.

The 'Soneyka' and 'Vengherka Kaukaskaya' were clearly differentiated by firmness (64.0 N, respectively 56.06 N), while the 'Wanette' and 'Mirnaya' cvs. were differentiated by the high content in soluble solids (19.25%, respectively 18.75% Brix).

The studied plums varieties had physical and chemical properties which make them suitable to be grown in Romania. A few cultivars might be less accepted for fresh consumption due to their low soluble solids content, but they can compensate easily by the early fruit ripening. Although it is not an option for the current Romanian market, red or yellow plums are an important source of genes and diversity while waiting for a change in the taste of the Romanian consumer.

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STUDIES REGARDING THE INFLUENCE OF POSTHARVEST INTERVENTION UPON THE QUALITY AND THE STORAGE PERIOD OF SOME APRICOT AND PEACH CULTIVARS

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Abstract

Apricots and peaches are perishable fruits which require great care both at harvesting and during storage. In order to reduce losses and prolong their storage period, intervention during the post harvesting period is advisable through adequate techniques. The 'Dacia' and 'Olimp' apricot varieties have been taken under study, as well as the 'Nabby', 'Royal Glory', 'Raluca', 'Filip', and 'Sweet Dreams' peach varieties. These were harvested from the experimental field of the Faculty of Horticulture of University of Agronomic Sciences and Veterinary Medicine Bucharest. The preservation variants were: V1 - AN = the normal atmosphere typical to a storage space, the ambient temperature=24^{0}C and the relative humidity = 64%; V2 - AF = low temperature conditions (1-2^{0}C) and the relative humidity = 80-85%; V3 - AM = modified atmosphere resulted by applying a plastic pellicle (LDPE 5228 type) of 15 µm over the preserving containers, at the 1-2^{0}C temperature and 85-90% relative humidity. The assessment of the main physic-chemical characteristics of the fruit, as well as of the weight losses and the qualitative depreciation (attack by Monilinia sp.) during the preservation period was performed both at the time of the harvesting and after period of preservation. The optimal duration of fruits storage in modified atmosphere conditions was: 14-20 days for apricots and 10-14 days for peaches. The total losses recorded during storage were lower fruits harvested at the advanced ripening stage and stored in modified atmosphere conditions.

Key words*: harvesting time, parameters, physic - chemical, postharvest, storage period.*

INTRODUCTION

Apricots and peaches have outstanding organoleptic qualities and contain high amounts of fiber and vitamins, making it ideal in the human diet. Consequently, these versatile fruits are consumed in fresh, canned, dried and other processed forma (Southwick, 2003).

The apricot and peach fruits pose a serious challenge during their utilization due to the high level of perishability and to the very high temperatures during the harvesting time (Balan, et al., 2008). At present, in the varietal conveyer of these fruit tree species in Romania, in some climatic conditions, many deficient or high productions are reported. Therefore, maintaining the fruit quality for a certain period of time in order to diversify the fruit sort out of season and to stimulate the export are very timely problems (Chira et al., 2018).

The peach cultivars present on the market place are often judged flavorless and appear to lack the strong "peach flavor" expected by consumers, even when harvested an optimum maturity stage (Chira, 2008). The improvement of peach quality represents a crucial aspect for promoting consumption, prompting breeders toward the selection of novel and more flavorful cultivars that can develop flavors before the onset of the softening process (Cirilli et al., 2016).

From this point of view, using the selective plastic in making the physiological wrappers may be considered a good biotechnology that intervenes in the post harvesting phase autonomously or combined with preservation techniques that are already established (Hitka, 2011).

MATERIALS AND METHODS

The experimental work was performed on fruits of the well-known apricot cultivars: 'Dacia' and 'Olimp' (Figures 6, 7) and on the new peach cultivars: 'Nabby', 'Royal Glory', 'Raluca', 'Filip', and 'Sweet Dreams' (Figures 1-5). New commercial cultivars are distinguished by their fruit large size (Stănică et al., 2011). Attractive colour of the skin, intense blush and very firm,

slow softening flesh that facilitates shipping and handling. In many cases, however the improvement of the technological characteristics occurs in flavour detriment (taste and aroma) causing consumer dissatisfaction: consumers are attracted by the aesthetic features but are disappointed by the poor eating quality (Piagnani et al., 2013). The trees from which the fruits were sampled belong to a micro orchard from University of Agronomic Sciences and Veterinary Medicine Bucharest, Faculty of Horticulture, the system of experiment being the randomized block.

Figure 1. The 'Nabby' cultivar
Source: own photo

Figure 2. The 'Royal Glory' cultivar
Source: own photo

Figure 3. The 'Raluca' cultivar
Source: own photo

Figure 4. The 'Filip' cultivar
Source: own photo

Figure 5. The 'Sweet Dreams' cultivar
Source: own photo

Figure 6. The 'Olimp' cultivar
Source: Apricot tree and apricot fruits book

Figure 7. The 'Dacia' cultivar
Source: Apricot tree and apricot fruits book

Fruits of every species were harvested at the ripening stage (one cultivar), at the advanced ripening stage (the other cultivar), than were brought to the laboratory for physic-chemical analysis and preservation. These have been distributed in three variants, each in three repetitions, with a quantity of 2 kg for each repeat (Chira et al., 2014).

The preservation variants were:

V1 - AN = normal atmosphere typical to a storage space, ambient temperature = 24°C and relative humidity = 64%;

V2 - AF = low temperature conditions = 1-2°C and relative humidity = 80-85%;

V3 - AM = modified atmosphere resulted by applying a plastic pellicle (LDPE 5228 type) of 15µm over the preserving containers, at 1-2°C and 85-90% relative humidity.

The evaluation of the physic-chemical parameters of the fruit was performed both at the time of harvesting and after the period of preservation. In addition, the weight losses and the quantitative depreciation (attack by *Monilinia* sp.) during the preservation period were also assessed (Chira et al., 2017).

The physic-chemical analysis methods used in the experiment were as follow: the fruit firmness was determined by using an Effegi penetrometer with 8 mm piston diameter; the total soluble solids content was determined by using the refractometer method; the total titratable acidity was determined by titration with NaOH and the ascorbic acid content was determined by iodometric method.

RESULTS AND DISCUSSIONS

The physic-chemical fruit parameters at the harvest time

By comparatively analysing the level of some essential components of fruit quality an evolutional transformation of the fruit size could be observed, as well as that of the sugar content, of the titratable acidity, of the ascorbic acid and the pulp firmness (Table 1), from the ripening stage to the advanced ripening one.

Limiting our comments only to the total soluble solids / titratable acidity ratio, that is a synthetic indicative of the quality; one can see that its value is increasing despite of the cultivar or species, from the ripening towards the advanced ripening stage. For example, from 6.7 at the 'Olimp' cultivar to 8.2 at the 'Dacia' cultivar for apricots, respectively from 9.8 to 19.7 for peaches cultivars.

The evolution of the physic-chemical parameters of the fruit during their storage period

The obtained results are presented in Table 2, showing that:.

The pulp firmness continuously decreased during the storage due to the solubilisation of the pectic substances, but with different values depending of the storage conditions. For example: the 'Dacia' cultivar firmness decreased from 2.0 kgf/cm^2 at the harvest time, to 0.9 kgf/cm^2 at V1, respectively to 1.0 kgf/cm^2 at V2 and V3 at the end of storage period. The total soluble solids recorded an increase despite the cultivar and the storage variant, but with higher values in the fruit stored in normal atmosphere (V1) due to the effect of the high temperature, and lesser values in the fruit stored in modified atmosphere (V3), due to the slowing-down of the metabolic process under the influence of the low temperature and of the increased concentration of CO_2 (5%, measured by using an gas analyser) from storage containers. For example: at the 'Dacia' cultivar, these values increase from 14.0% at picking to 16.5% V1; 15.6% V2 and 15.0% V3 after storage period. The titratable acidity had lower values in comparison with the ones at harvest times with differences between cultivars and according to the storage conditions. The lowest values have been recorded in the fruit stored in normal atmosphere (V1), due to the intense oxidation of the organic substances at the high temperature, while in the cold and in the modified atmosphere, the determined values were higher. For example: at the 'Dacia' cultivar values ranged from 1.7% at picking, to 1.2% (V1); 1.3% (V2) and 1.5% (V3), respectively.

The values of the total soluble solids/titratable acidity were higher in the fruit harvested at an advanced ripening stage despite of the species. For every cultivar, according to the storage conditions, the values were a little higher for the fruit stored in normal atmosphere, comparing to the ones stored in low temperature conditions or in modified atmosphere. That leads us to suggest that the modified atmosphere, by reducing the metabolic processes and prolonging the fruit lifetime is recommended to be used for the products that reach toward the maximum of their organoleptic qualities (the consuming maturity). For example: for the 'Dacia' cultivar these values increase from 8.2 at picking to 13.8 (V1), 10.0 (V2 and V3) after the storage period.

Table 1. The physic-chemical fruit parameters at the harvest time

Cultivar	The phase of the harvest	Medium weight (g)	The firmness (kgf/ 0.5 cm^2)	Asorbic acid (mg/100 g F.W.)	Total soluble solids (%)	Titratable acidity (% malic acid)	Total soluble solids/Titratable acidity
'DACIA' apricot	Advanced ripening	52.5	2.0	7.04	14.0	1.70	8.2
'OLIMP' apricot	Ripening	46.0	3.0	4.67	11.8	1.76	6.7
'NABBY' peach	Advanced ripening	71.0	2.5	5.42	11.8	0.88	13.4
'ROYAL GLORY' peach	Ripening	66.0	3.8	3.52	9.6	0.98	9.8
'RALUCA' peach	Advanced ripening	62.4	3.5	5.42	11.8	0.60	19.7
'FILIP' flat peach	Ripening	60.2	3.4	3.52	10.0	0.76	13.2
'SWEET DREAMS' flat peach	Ripening	61.5	3.2	3.85	10.8	0.82	13.1

Table 2. The physic-chemical fruits parameters at the end of the storage period

Species	Cultivar	Storage variant	The firmness (kgf/0.5cm^2)	Ascorbic acid (mg/100 g F.W.)	Total soluble solids (%)	Titratable acidity (% malic acid)	Total soluble solids/ Titratable acidity
Apricot	'DACIA'	V1	0.9	5.40	16.5	1.2	13.8
		V2	1.0	5.72	15.6	1.3	10.0
		V3	1.0	6.16	15.0	1.5	10.0
Apricot	'OLIMP'	V1	1.2	4.12	13.8	1.4	9.8
		V2	1.3	4.32	13.4	1.5	8.9
		V3	1.3	4.44	12.6	1.6	7.9
Peach	'NABBY'	V1	0.9	3.52	12.2	0.78	15.6
		V2	1.0	3.52	13.0	0.84	15.5
		V3	1.0	4.16	13.4	1.80	15.5
Peach	'ROYAL GLORY'	V1	1.2	3.52	10.9	0.78	13.7
		V2	1.4	3.52	11.0	0.87	12.6
		V3	1.4	4.16	10.2	0.75	13.6
Peach	'RALUCA'	V1	1.0	2.64	12.6	0.72	17.5
		V2	1.5	2.64	12.4	0.69	17.8
		V3	1.2	3.52	12.9	0.69	17.7
Flat peach	'FILIP'	V1	1.0	2.64	10.6	0.72	14.7
		V2	1.8	3.52	10.8	0.70	15.4
		V3	1.4	3.52	10.5	0.75	14.0
Flat peach	'SWEET DREAMS'	V1	0.9	2.68	10.5	0.73	14.3
		V2	1.8	3.48	10.8	0.72	15.0
		V3	1.5	3.50	10.8	0.75	14.4

The capacity for fruit temporary storage

The optimal duration of fruit storage varied for every species according to the moment of fruit harvesting (Table 3), which highlights that the fruit harvested at the ripening phase had a longer storage period. The organoleptic quality of the fruit was inferior comparing to the fruit harvested at the advanced ripening stage as reported in Tables 1 and 2. The storage conditions influenced the storage duration to a great extent, as follows:

4-8 days for apricots in normal atmosphere (V1),

3-4 days for peaches in normal atmosphere (V1),

5 days for plate peaches in normal atmosphere (V1),

14-20 days for apricots in modified atmosphere (V3),
10-13 days for peaches in modified atmosphere (V3),
14 days for plate peaches in modified atmosphere (V3).

The total losses recorded (Table 3) during storage were higher for all species, in the cultivars for which the fruit have been harvested at ripening stage, and the storage conditions influenced a lot the level of these losses, that were high in normal atmosphere (V1) and 2-3 times lower in modified atmosphere conditions (V3). This fact reveals the efficiency of this type of wrapping that represents a means of reducing the weight losses due to the lower fruits transpiration, under the 85. 90% relative humidity value, measured inside the package unit.

Table 3. The fruits storage capacity under different conditions

Species	Cultivar	Storage conditions	Storage duration (days)	Weight losses (%)	Quality losses (%)	Total losses (%)
Apricot	'DACIA'	V1	4	8.9	1.7	10.6
		V2	10	5.9	0	5.9
		V3	14	5.6	0	5.6
Apricot	'OLIMP'	V1	8	15.3	6.4	21.7
		V2	14	7.1	6.2	13.3
		V3	20	3.7	0	3.7
Peach	'NABBY'	V1	4	7.9	0	7.9
		V2	7	6.7	0	6.7
		V3	10	3.2	0	3.2
Peach	'ROYAL GLORY'	V1	4	7.9	0	7.9
		V2	9	6.9	0	6.9
		V3	13	4.6	0	4.6
Peach	'RALUCA'	V1	4	8.5	6.0	14.5
		V2	10	12.2	0	12.2
		V3	12	8.4	0	8.4
Flat peach	'FILIP'	V1	5	8.8	2.4	11.2
		V2	10	8.7	0	8.7
		V3	14	3.5	0	3.5
Flat peach	'SWEET DREAMS'	V1	5	8.9	2.2	11.1
		V2	11	7.5	0	7.5
		V3	14	3.8	0	3.8

CONCLUSIONS

The fruit belonging to the apricot and peach cultivars evolve toward maturity after harvest, achieving superior qualitative levels when the fruits are harvested at an advanced ripening phase;

The fruit qualitative levels at the end of the storage period were higher for the fruit maintained in normal atmosphere (ambient), and a little lower for the fruit stored in modified atmosphere (physiological wrappers);

It is recommended that the storage of the apricot and peach fruits should be done in modified atmosphere conditions, providing that the fruit are harvested at a stage closer to the full maturity, when the organoleptic qualities have maximal levels;

It is recommended to organize commercial units based on physiological wrappers that avoid the repeated handling and therefore fruit depreciation, that will come into contact with the ambient environment only at the final stage of the commercial circuit;

The optimal duration of fruit storage in modified atmosphere conditions were:

- 14-20 days for apricots,
- 10-14 days for peaches.

The total losses recorded during storage were lower in the fruit harvested at the advanced ripening stage and stored in modified atmosphere.

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BIOMETRICAL ASPECTS OF SOME PEACH TREE (*PERSICA VULGARIS* L.) FRUIT VARIETIES GRAFTED ON DIFFERENT ROOTSTOCKS IN THE CONDITIONS OF LUGOJ NURSERY, TIMIȘ COUNTY

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Abstract

The aim of this research was to determine the behavior of some peach varieties from other countries when grafted on rootstocks currently used in our country, in order to find variants that are suitable for cultivating this species in Banat region conditions and in the context of climate changes that have been influencing the fruiting of peach trees in recent years. The fruit weight and the fruit size index were determined. The study was conducted during the year 2017 and 2018. In 2017, the fruit weight had values between 57.31 g in the 'Gold Dust' variety and 147.0 g in the 'Elbertina' variety and the size index had values between 44.0 in 'Gold Dust' variety and 63.45 in 'Desert Gold' variety. In 2018, the fruit weight had values between 85.32 g in the DS 62-193 genotype and 149. 83g in the Piroș Magdalena variety and the size index had values between 47.33 in 'Early Red' variety and 65.99 in 'Desert Gold' variety.

Key words*: peach tree, variety, rootstock, fruit weight, size index.*

INTRODUCTION

Finding the appropriate rootstock-variety combinations is very important to any orchard. A good rootstock x variety interaction leads to high fruit yield and increased fruit quality.

While the tree vigor, shoot growth and canopy density are strongly influenced by the rootstock, these rootstock effects are not separable from the effects of light environment with in the canopy (Hrotkó K., 2013)

Our research results showed how the interaction between rootstock and variety influenced the biometric aspects of the fruits. In the past years studies on this subject were made and the results showed how fruit aspects are influenced by the interaction between rootstock and variety. We mention other researchers that studied the influence of rootstock and variety on the quality of peach fruits: Fideghelli C. and A. Nicotra, 2002; Layne D.R. et al., 2002; Venig Aurora, 2007; Mathias, C. et al., 2008; Carmen Martinez-Ballesta, M. et al., 2010; Pal M. et al., 2017. The quality of the fruits expressed by their size, appearance and taste, is very important prerequisite for their sale. A review of the fruits should include mechanical, sensorial, chemical and microbiological control, as well as remarks for the variety (Stamatovska V. et al., 2017).

Bussi, C. et al., 2011, registered values of the peach fruit weight between 114.00 g and 134.00 g on different variety x rootstock combinations. According to Andreiaș A., 2011, the same cultivar can have different fruit weight when grafted on different rootstocks, for example the weight of the fruits of 'Redhaven' variety grafted on 5 different rootstocks ranged between 142.5 g and 151.6 g while the fruits of 'Springcrest' variety ranged from 71.0 g to 75.2 g.

Others recorded the average fruit weight of the varieties grafted on different rootstocks between 241.1 g and 296.0 g (Jimenez Sergio et al., 2011), 76 g-320 g for one cultivar and 70 g-296 g for another cultivar (Basile, B. et al., 2007).

Gavăț C. et al. (2016) considered that the average fruit weight was 75 g ('Springold') and 220 g ('Flacăra clon 1'), the fruits´ destination being for fresh consumption and processing.

According to Orazem Primoz et al., 2011, significant interaction between rootstock and

cultivar on fruit weight was evident and could have been affected by different crop load among rootstocks.

MATERIALS AND METHODS

The biological material consisted of 14 peach genotypes which were less common in our country, gathered from different Earth's regions and grafted at Lugoj nursery using rootstocks that are still commonly used in Romania, especially in the western part of the country, namely, the wax cherry and Oradea. The trees were planted in the experimental field in the autumn of 2015 and by the time the study was conducted the trees were in the first years of fruiting. The planting distances were 4 x 5 m, the trees were fan shaped and the growing technology used was the one that is currently used for peach trees. The experience was the multifactorial type, the factor *a* being represented by the genotype (variety) and the factor *b* by rootstocks. The data collected were calculated and interpreted statistically using the analysis of variance, respectively the Duncan test, and the control being chosen the average of the experience. The research methods regarding the biometric characteristics of the fruit in the studied varieties and genotypes, grafted on the two chosen rootstocks are according to literature.

Thus:

The fruit height (mm) was determined by measuring with the electronic caliper a number of 25 fruits from each variant, the measurement being made when the fruits reached the technological maturity.

The fruit diameter (mm) was determined by measuring with the electronic chisel a number of 25 fruits reached the technological maturity, of each variant. The measurement was made on all fruits on two diameters.

The fruit weight (g) was determined by weighing 25 fruits on a WPS - C2 model analytical balance, the results being expressed in grams with two decimals, for each analyzed variant.

The size index was calculated by the formula IM = D + d + H/3, as an arithmetic mean of the diameters and height of the fruit.

RESULTS AND DISCUSSIONS

The combined effect of genotype and rootstock on peach fruit weight character (2017)

The fruit weight (mass) represents one of the most variable size characters in one and the same variety, depending on the age of the tree, the climatic conditions and the applied agrotechnics. Drăgănescu E. (2006) classifies peach varieties according to their large diameter and average weight as follows: small fruits, diameter below 4.5 cm and average weight below 75.0 g; medium fruit, diameter between 4.5-6.5 cm and average weight between 65-80 g; large fruits, diameter between 6.5-8.5 cm and average weight between 120-200 g.

Chira L., V. Chereji, M. Roman (2008), classifies peaches according to their average weight as follows: small (under 100 g), medium (100-175 g), large (175-250 g), very large (over 250 g).

Table 1. The interaction betwen genotype and rootstock on peach fruits weight in 2017

Factor interaction	*Fruits weight* (g)	*Relative values* (%)	*Difference* (g)	*Significance*
a_1 x b_1 – 'Gold Dust' x Wax cherry	54.53ab	60.8	-35.12	000
a_2 x b_1 – Piroş Magdalena x Wax cherry	112.71ij	125.7	23.06	**
a_3 x b_1 – 'Desert Gold' x Wax cherry	140.90k	157.2	51.25	***
a_4 x b_1 – DS 62-193 x Wax cherry	60.00abc	66.9	-29.65	000
a_5 x b_1 – 'Elbertina' x Wax cherry	136.90k	152.7	47.25	***
a_6 x b_1 – 'Poli' x Wax cherry	73.73cde	82.2	-15.92	-
a_7 x b_1 – 'Tokinostate' x Wax cherry	51.00a	56.9	-38.65	000
a_8 x b_1 – GDRT x Wax cherry	72.00cde	80.3	-17.65	0
a_9 x b_1 – 'Maria Bianca' x Wax cherry	94.72fgh	105.7	5.07	-
a_{10} x b_1 – DR 32-15 x Wax cherry	105.30hij	117.5	15.65	-
a_{11} x b_1 – 'Maria Delicia' x Wax cherry	82.17	91.7	-7.48	-
a_{12} x b_1 – HB 19-9 x Wax cherry	108.17hij	120.7	18.52	*
a_{13} x b_1 – 'Early Red' x Wax cherry	52.00a	58.0	-37.65	000
a_{14} x b_1 – 'Tebana' x Wax cherry	111.02ij	123.8	21.37	*
Media / *Average* a x b_1	89.65	100.0	0.00	Mt / *Control*

Factor interaction	*Fruits weight* (g)	*Relative values* (%)	*Difference* (g)	*Significance*
a_1 x b_2 – 'Gold Dust' x Oradea	60.09abc	58.1	-43.41	000
a_2 x b_2 – Piroş Magdalena x Oradea	121.02j	116.9	17.52	*
a_3 x b_2 – 'Desert Gold' x Oradea	148.90kl	143.9	45.40	***
a_4 x b_2 – DS 62-193 x Oradea	68.85bcd	66.5	-34.65	000
a_5 x b_2 – 'Elbertina' x Oradea	157.10l	151.8	53.60	***
a_6 x b_2 – 'Poli' x Oradea	88.30efg	85.3	-15.20	-
a_7 x b_2 – 'Tokinostate' x Oradea	80.58def	77.9	-22.92	00
a_8 x b_2 – GDRT x Oradea	79.02def	76.3	-24.48	00
a_9 x b_2 – 'Maria Bianca' x Oradea	136.80k	132.2	33.30	***
a_{10} x b_2 – DR 32-15 x Oradea	113.06ij	109.2	9.56	-
a_{11} x b_2 – 'Maria Delicia' x Oradea	93.90fgh	90.7	-9.60	-
a_{12} x b_2 – HB 19-9 x Oradea	101.10ghi	97.7	-2.40	-
a_{13} x b_2 – 'Early Red' x Oradea	58.50abc	56.5	-45.00	000
a_{14} x b_2 – 'Tebana' x Oradea	141.80kl	137.0	38.30	***
Media / *Average* a x b_2	103.50	100.0	0.00	Mt / *Control*

DL $_{(p\ 5\%)}$ *LSD* $_{(p\ 5\%)}$ = 16.13 g DL $_{(p\ 1\%)}$ *LSD* $_{(p\ 1\%)}$ = 21.75 g DL $_{(p\ 0,1\%)}$ *LSD* $_{(p\ 0,1\%)}$ = 28.98 g

Regarding the genotype-rootstock interaction (Table 1) on the fruit weight character, it was found that the lowest value was registered for the combinations: 'Tokinostate' x Wax cherry, 'Early Red' x Wax cherry, 'Gold Dust' x Wax cherry, DS 62-193 x Wax cherry, 'Early Red' x Oradea, 'Gold Dust' x Oradea and DS 62-193 x Oradea all being very significantly negative compared to the average of the experience. The 'Gold Dust' and 'Early Red' varieties obtained low weight fruit regardless of the used rootstock.

At the opposite end, the highest values of fruit weight were recorded in the following combinations: 'Elbertina' x Oradea, 'Desert Gold' x Oradea, 'Tebana' x Oradea, 'Desert Gold' x Wax cherry, 'Elbertina' x Wax cherry and 'Maria Bianca' x Oradea, all these are very significantly positive compared to the average of the experience. 'Desert Gold' and 'Elbertina' varieties obtained the heaviest fruits regardless of the used rootstock.

The combined effect of genotype and rootstock on peach fruit weight character (2018)

In 2018 (Table 2), the genotype-rootstock interaction on fruit weight character led to the following low value combinations: 'Gold Dust' x Wax cherry, DS 62-193 x Wax cherry, 'Early Red' x Wax cherry, 'Tokinostate' x Wax cherry, HB 19-9 x Oradea, DS 62-193 x Oradea, DR 32-15 x Oradea and 'Early Red' x Oradea, all being very negative compared to the average of the experience. 'Early Red' variety and DS 62-193 genotype obtained low weight fruit regardless of the used rootstock.

Table 2. The interaction betwen genotype and rootstock on peach fruits weight in 2018

Factor interaction	*Fruit weight* (g)	*Relative values* (%)	*Difference* (g)	*Significance*
a_1 x b_1 – 'Gold Dust' x Wax cherry	63.13a	62.2	-38.33	000
a_2 x b_1 – Piroş Magdalena x Wax cherry	119.68fgh	11.0	18.22	***
a_3 x b_1 – 'Desert Gold' x Wax cherry	149.25m	147.1	47.79	***
a_4 x b_1 – DS 62-193 x Wax cherry	65.05a	64.1	-36.41	000
a_5 x b_1 – 'Elbertina' x Wax cherry	141.67klm	139.6	40.21	***
a_6 x b_1 – 'Poli' x Wax cherry	87.67b	86.4	-13.79	00
a_7 x b_1 – 'Tokinostate' x Wax cherry	68.62a	67.6	-32.84	000
a_8 x b_1 – GDRT x Wax cherry	87.95b	86.7	-13.51	00
a_9 x b_1 – 'Maria Bianca' x Wax cherry	111.28ef	10.7	9.82	*
a_{10} x b_1 – DR 32-15 x Wax cherry	120.77fgh	119.0	19.31	***
a_{11} x b_1 – 'Maria Delicia' x Wax cherry	93.91bc	92.6	-7.55	-
a_{12} x b_1 – HB 19-9 x Wax cherry	117.52fg	115.8	16.06	***
a_{13} x b_1 – 'Early Red' x Wax cherry	66.53a	65.6	34.93	000
a_{14} x b_1 – 'Tebana' x Wax cherry	127.42hij	125.6	25.96	***
Media / *Average* a x b_1	101.46	100.0	0.00	Mt / *Control*

Factor interaction	*Fruit weight* (g)	*Relative values* (%)	*Difference* (g)	*Significance*
a_1 x b_2 – 'Gold Dust' x Oradea	136.37jkl	96.3	-5.29	-
a_2 x b_2 – Piroş Magdalena x Oradea	179.97o	127.0	38.31	***
a_3 x b_2 – 'Desert Gold' x Oradea	150.00m	105.9	8.34	-
a_4 x b_2 – DS 62-193 x Oradea	105.58de	74.5	-36.07	000
a_5 x b_2 – 'Elbertina' x Oradea	130.67ij	92.2	-10.99	0
a_6 x b_2 – 'Poli' x Oradea	132.87ijk	93.8	-8.79	0
a_7 x b_2 – 'Tokinostate' x Oradea	183.67o	129.7	42.01	***
a_8 x b_2 – GDRT x Oradea	170.46n	120.3	28.81	***
a_9 x b_2 – 'Maria Bianca' x Oradea	168.46n	118.9	26.80	***
a_{10} x b_2 – DR 32-15 x Oradea	113.33ef	80.0	-28.32	000
a_{11} x b_2 – 'Maria Delicia' x Oradea	147.06m	103.8	5.41	-
a_{12} x b_2 – HB 19-9 x Oradea	98.50cd	69.5	-43.16	000
a_{13} x b_2 – 'Early Red' x Oradea	123.67ghi	87.3	-17.99	000
a_{14} x b_2 – 'Tebana' x Oradea	142.60lm	100.7	0.94	-
Media / *Average* a x b_2	141.66	100.0	0.00	Mt / *Control*

DL $_{(p\ 5\%)}$ *LSD* $_{(p\ 5\%)}$ = 8.68 g DL $_{(p\ 1\%)}$ *LSD* $_{(p\ 1\%)}$ = 11.70 g DL $_{(p\ 0,1\%)}$ *LSD* $_{(p\ 0,1\%)}$ = 15.58 g

At the opposite end, the highest values of fruit weight were recorded in the following combinations: 'Tokinostate' x Oradea, Pyros Magdalena x Oradea, GDRT x Oradea, 'Maria Bianca' x Oradea, 'Desert Gold' x Wax cherry, 'Elbertina' x Wax cherry, 'Tebana' x Wax cherry, DR 32-15 x Wax cherry, HB 19-9 x Wax cherry, Piroş Magdalena x Wax cherry, all of which are very significantly positive face to control. The only variety that recorded a large fruit weight regardless of rootstock was Piroş Magdalena, the other varieties recording small fruits on Wax cherry and large on Oradea or vice versa (exp. 'Tokinostate' and HB 19-9).

The use of the Oradea rootstock in the production of biological material has resulted in obtaining fruits with an average weight of over 100 grams for most peach genotypes experienced in 2018, but nevertheless the variability of the genotypes in terms of the analyzed morphological character is medium to low.

Results concerning the influence of variety (genotype) and rootstock on fruit index size (2017)

Regarding the classification by size classes that lead to a proper appreciation of the fruits, the researchers created several scales to classify the fruits by quality classes. Thus, Mitre V. (2008) classifies the fruits of the varieties according to their size into three groups, as follows:

- extra fruits- with a diameter over 56 mm;
- first class fruits - with a diameter between 51-55 mm;
- second class fruits - with a diameter of less than 50 mm.

For a proper classification of the fruits from our experience, the table 3 comes with an average of the diameter of the peaches obtained using the two rootstocks, in the pedoclimatic conditions of the Lugoj nursery where the experience was located.

According to this classification, from Table 3 it can be observed that the studied varieties and genotypes, regardless of the rootstock used, obtained fruits that fall into the first two quality groups, extra and first class.

Table 3. The medium values of peach fruit diameter in varieties studied grafted on two rootstocks studied

Variety/ genotype	*Fruit diameter (mm) in 2017 (average)*	
	wax cherry	Oradea
'Gold Dust'	44.33	49.67
Piroş Magdalena	56.00	61.33
'Desert Gold'	62.00	67.00
DS 62-193	54.67	61.33
'Elbertina'	59.33	68.00
'Poli'	49.67	54.33
'Tokinostate'	51.00	56.33
GDRT	47.33	52.00
'Maria Bianca'	53.33	56.67
DR 32-15	57.00	60.33
'Maria Delicia'	51.	55.0
HB 19-9	55.66	62.0
'Early Red'	45.33	50.66
'Tebana'	58.33	61.66

When using Wax cherry as rootstocks, it was found that 7 of the 14 studied varieties and genotypes were able to fit in the extra fruit group ('Desert Gold', 'Elbertina', 'Tebana', DR 32-15, Pyros Magdalena, HB 19-9 and DS 62-193), 4 of them obtained first- class fruits

('Poli', 'Tokinostate', 'Maria Bianca' and 'Maria Delicia'), and four, second-class fruits ('Gold Dust', GDRT, 'Early Red' and Tebana).

When using the Oradea rootstock, 10 of the studied varieties and genotypes recorded extra quality fruits - the same as in the case of the Wax cherry to which were added those that were in the first class, while only 3 varieties were classified as first class exceeding 51 mm ('Gold Dust', GDRT and 'Early Red').

Previous studies conducted on five of the 11 varieties we addressed in this research (Iordănescu O.A. et al., 2014, 2016; Costea V., 2016) have led to a classification under these classes, the 'Elberina' variety being the only one that has obtained fruits with an average diameter of 56.16 mm. In this context, the pedoclimatic conditions of the Lugoj town, Timiș County has positively impacted the quality of the obtained fruits, at least in terms of their external features.

Regarding the combined effect of the variety (genotype) and the rootstock (Table 4) on the fruit size index, 5 combinations / Wax cherry and 6 combinations / Oradea led to low values of the indicator, all being very significantly negative, while 5 combinations on both Wax cherry and Oradea led to high values of the size index, being very significantly positive face to control (Figure 1). 'Gold Dust' and 'Early Red' varieties put their mark on the combination variety-rootstocks, the values being similar regardless the used rootstock (low values).

Table 4. The interaction betwen genotype and rootstock on fruit index size in 2017

Factor interaction	*Fruit´s index size*	*Relative values* (%)	*Difference*	*Significance*
a_1 x b_1 – 'Gold Dust' x Wax cherry	41.88a	82.4	-8.94	000
a_2 x b_1 – Piroș Magdalena x Wax cherry	53.78h	105.8	2.96	***
a_3 x b_1 – 'Desert Gold' x Wax cherry	61.11m	120.2	10.29	***
a_4 x b_1 – DS 62-193 x Wax cherry	51.78g	101.9	0.96	-
a_5 x b_1 – 'Elbertina' x Wax cherry	56.99k	112.1	6.17	***
a_6 x b_1 – 'Poli' x Wax cherry	48.22de	94.9	-2.60	000
a_7 x b_1 – 'Tokinostate' x Wax cherry	47.11cd	92.7	-3.71	000
a_8 x b_1 – GDRT x Wax cherry	45.44b	89.4	-5.38	000
a_9 x b_1 – 'Maria Bianca' x Wax cherry	50.55f	99.5	-0.27	-
a_{10} x b_1 – DR 32-15 x Wax cherry	55.55ij	109.3	4.73	***
a_{11} x b_1 – 'Maria Delicia' x Wax cherry	49.22e	96.9	-1.60	0
a_{12} x b_1 – HB 19-9 x Wax cherry	52.10g	102.5	1.28	-
a_{13} x b_1 – 'Early Red' x Wax cherry	41.99a	82.6	-8.83	000
a_{14} x b_1 – 'Tebana' x Wax cherry	55.77ijk	109.7	4.95	***
Media / *Average* a x b_1	50.82	100.0	0.00	Mt / *Control*
a_1 x b_2 – 'Gold Dust' x Oradea	46.11bc	83.3	-9.24	000
a_2 x b_2 – Piroș Magdalena x Oradea	60.99m	110.2	5.64	***
a_3 x b_2 – 'Desert Gold' x Oradea	65.78o	118.8	10.43	***
a_4 x b_2 – DS 62-193 x Oradea	56.22jk	101.6	0.87	-
a_5 x b_2 – 'Elbertina' x Oradea	64.44n	116.4	9.09	***
a_6 x b_2 – 'Poli' x Oradea	51.77g	93.5	-3.58	000
a_7 x b_2 – 'Tokinostate' x Oradea	51.89g	93.7	-3.46	000
a_8 x b_2 – GDRT x Oradea	48.78e	88.1	-6.57	000
a_9 x b_2 – 'Maria Bianca' x Oradea	54.77hi	98.9	-0.58	-
a_{10} x b_2 – DR 32-15 x Oradea	58.77l	106.2	3.42	***
a_{11} x b_2 – 'Maria Delicia' x Oradea	52.33g	94.5	-3.02	000
a_{12} x b_2 – HB 19-9 x Oradea	56.33jk	101.8	0.98	-
a_{13} x b_2 – 'Early Red' x Oradea	46.66bc	84.3	-8.69	000
a_{14} x b_2 – 'Tebana' x Oradea	60.10m	108.6	4.75	***
Media / *Average* a x b_2	55.35	100.0	0.00	Mt / *Control*

DL $_{(p\ 5\%)}$ *LSD* $_{(p\ 5\%)}$ = 1.29 DL $_{(p\ 1\%)}$ *LSD* $_{(p\ 1\%)}$ = 1.74 DL $_{(p\ 0,1\%)}$ *LSD* $_{(p\ 0,1\%)}$ = 2.32

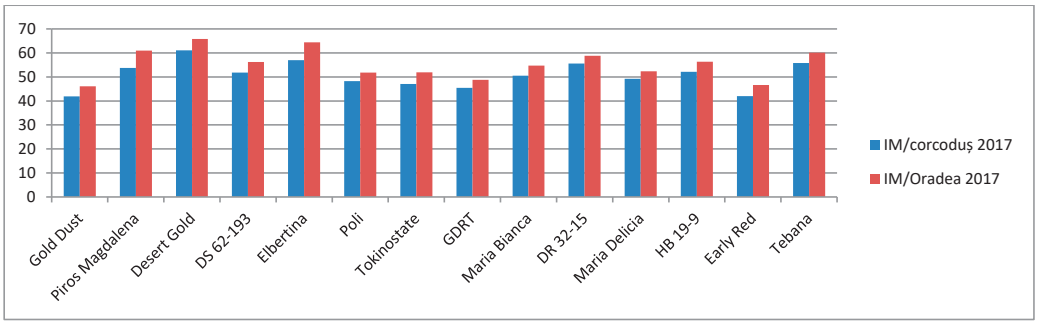

Figure 1. Fruit index size in 2017

Results concerning the influence of variety (genotype) and rootstock on fruit index size (2018)

In Table 5 are presented the medium values of peach fruit diameter, with a limit of variation between 56.66 mm in 'Gold Dust'/ wax cherry and 72.00 mm in 'Elbertina'/Oradea.

Table 5. The medium values of peach fruit diameter in the studied varieties grafted on two rootstocks

Variety/genotype	*Fruit diameter (mm) in 2018 (average)*	
	wax cherry	Oradea
'Gold Dust'	46.66	55.00
Piros Magdalena	57.67	66.33
'Desert Gold'	65.33	70.33
DS 62-193	57.33	66.33
'Elbertina'	64.00	72.00
'Poli'	53.00	60.00
'Tokinostate'	53.67	62.33
GDRT	50.67	57.33
'Maria Bianca'	55.67	61.00
DR 32-15	59.67	64.67
'Maria Delicia'	55.33	58.33
HB 19-9	57.66	66.0

The combined influence of the variety/rootstock combination on the value of the size index in 2018 led to very significantly negative values for 3 combinations/Wax cherry and 3 combinations/Oradea, the genetic imprint of the variety being observed, 'Gold Dust', 'Early Red' and GDRT recording the lowest values on both rootstocks.

The combinations that led to high values of the size index aimed the 'Desert Gold', 'Elbertina' and 'Tebana' varieties, all combinations being very significantly positive face to control.

The results obtained with the Duncan test (Table 6), for the year 2018, group the combinations variety - rootstock according to the size index value, as follows:

- small fruit varieties: 'Gold Dust' x Wax cherry, 'Early Red' x Wax cherry, GDRT x Wax cherry, 'Gold Dust' x Oradea, 'Early Red' x Oradea;
- small to medium fruit varieties: 'Tokinostate' x Wax cherry, 'Gold Dust' x Oradea, 'Poli' x Wax cherry, GDRT x Oradea;
- medium fruit varieties: 'Maria Bianca' x Wax cherry, 'Maria Delicia' x Wax cherry, DS 62-193 x Wax cherry, Piroș Magdalena x Wax cherry, HB 19-9 x Wax cherry, 'Tokinostate' x Oradea, 'Poli' x Oradea;
- supra-medium fruit varieties: 'Maria Delicia' x Oradea, 'Maria Bianca' x Oradea, DR 32-15 x Wax cherry, DS 62-193 x Oradea, 'Tebana' x Wax cherry, HB 19-9 x Oradea, 'Elbertina' x Wax cherry;
- large and very large fruit varieties: 'Desert Gold' x Wax cherry, Piroș Magdalena x Oradea, 'Tebana' x Oradea, 'Desert Gold' x Oradea, 'Elbertina' x Oradea.

In 2018, the values of the fruit index size (Figure 2) in the studied peach varieties and genotypes recorded higher values compared to the previous year, Oradea rootstock being the one that led to obtaining the highest values. Regarding the genetic imprint of the variety, 'Elbertina' variety slightly exceeded the value of 'Desert Gold' variety, followed by 'Tebana' and 'Piroș Magdalena' but also by the genotype DR 32-15 which exceeded the value of 60 mm.

From the graphs we can see an increase of the size index 2018 compared to 2017, maintaining the upward evolution of the variety/rootstock combination, Oradea proving to be a good alternative for the experimentation conditions.

Table 6. The interaction between genotype and rootstock on fruits size index in 2018

Factor interaction	*Fruit index size*	*Relative values* (%)	*Difference*	*Significance*
a_1 x b_1 – 'Gold Dust' x Wax cherry	44.55a	82.5	-9.48	000
a_2 x b_1 – Piroş Magdalena x Wax cherry	55.11fghi	102.0	1.08	-
a_3 x b_1 – 'Desert Gold' x Wax cherry	63.99n	118.4	9.96	***
a_4 x b_1 – DS 62-193 x Wax cherry	54.55fgh	101.0	0.52	-
a_5 x b_1 – 'Elbertina' x Wax cherry	61.77lmn	114.3	7.75	***
a_6 x b_1 – 'Poli' x Wax cherry	50.78cde	94.0	-3.25	0
a_7 x b_1 – 'Tokinostate' x Wax cherry	50.11cd	92.7	-3.92	00
a_8 x b_1 – GDRT x Wax cherry	48.00bc	88.8	-6.03	000
a_9 x b_1 – 'Maria Bianca' x Wax cherry	53.77efg	99.5	-0.26	-
a_{10} x b_1 – DR 32-15 x Wax cherry	58.89jkl	109.0	4.86	***
a_{11} x b_1 – 'Maria Delicia' x Wax cherry	53.99efg	99.9	-0.04	-
a_{12} x b_1 – HB 19-9 x Wax cherry	55.32fghi	102.4	1.29	-
a_{13} x b_1 – 'Early Red' x Wax cherry	45,11ab	83.5	-8.92	000
a_{14} x b_1 – 'Tebana' x Wax cherry	60.44klm	111.9	6.41	***
Average a x b_1	54.03	100.0	0.00	Mt / *Control*
a_1 x b_2 – 'Gold Dust' x Oradea	50.18cd	84.4	-9.28	000
a_2 x b_2 – Piroş Magdalena x Oradea	64.55n	108.6	5.09	***
a_3 x b_2 – 'Desert Gold' x Oradea	67.99o	114.3	8.53	***
a_4 x b_2 – DS 62-193 x Oradea	60.22klm	101.3	0.76	-
a_5 x b_2 – 'Elbertina' x Oradea	68.44o	115.1	8.98	***
a_6 x b_2 – 'Poli' x Oradea	56.89ghij	95.7	-2.57	-
a_7 x b_2 – 'Tokinostate' x Oradea	56.66ghij	95.3	-2.80	0
a_8 x b_2 – GDRT x Oradea	53.11def	89.3	-6.35	000
a_9 x b_2 – 'Maria Bianca' x Oradea	58.22ijk	97.9	-1.24	-
a_{10} x b_2 – DR 32-15 x Oradea	63.31mn	106.5	3.85	**
a_{11} x b_2 – 'Maria Delicia' x Oradea	57.88hijk	97.3	-1.58	-
a_{12} x b_2 – HB 19-9 x Oradea	60.66klm	102.0	1.20	-
a_{13} x b_2 – 'Early Red' x Oradea	49.55c	83.3	-9.91	000
a_{14} x b_2 – 'Tebana' x Oradea	64.77n	108.9	5.31	***
Average a x b_2	59.46	100.0	0.00	Mt / *Control*

DL $_{(p\ 5\%)}$ *LSD* $_{(p\ 5\%)}$ = 2.68 DL $_{(p\ 1\%)}$ *LSD* $_{(p\ 1\%)}$ = 3.61 DL $_{(p\ 0,1\%)}$ *LSD* $_{(p\ 0,1\%)}$ = 4.80

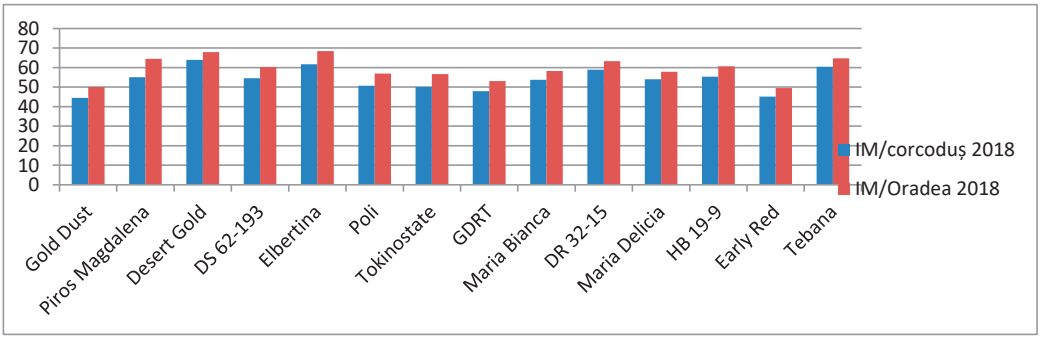

Figure 2. The fruit index size in 2018

CONCLUSIONS

Fruit weight is an important parameter, being closely correlated with the fruit size index. Following this parameter, based on the data obtained and depending on the variety (genotype) - rootstock combination, peach fruits can be grouped:

- low weight: 'Early Red' x Wax cherry, 'Early Red' x Oradea, 'Gold Dust' x Wax cherry, 'Gold Dust' x Oradea, DS 62-193 x Wax cherry, DS 62-193 x Oradea, 'Poli' x Wax cherry, 'Poli' x Oradea , GDRT x Wax cherry, GDRT x Oradea, 'Maria Delicia' x Wax cherry;
- with average weight: 'Maria Bianca' x Wax cherry, DR 32-15 x Wax cherry, DR 32-15 x Oradea, HB 19-9 x Oradea, HB 19-9 x Wax cherry, Piroș Magdalena x Wax cherry;
- heavy weight: 'Elbertina' x Wax cherry, 'Elbertina' x Oradea, 'Desert Gold' x Wax cherry, 'Desert Gold' x Oradea, 'Tebana' x Oradea, 'Maria Bianca' x Oradea, GDRT x Oradea, 'Piroș Magdalena' x Oradea, 'Tokinostate' x Oradea.

Based the size index, the fruits of the peach varieties (genotypes), depending on the variety - rootstock combination, can be grouped into:

- varieties with small fruits: 'Gold Dust' x Wax cherry, 'Gold Dust' x Wax cherry, 'Early Red' x Wax cherry, 'Early Red' x Oradea, GDRT x Wax cherry, GDRT x Oradea, 'Maria Delicia' x Wax cherry, 'Maria Bianca' x Wax cherry, Poly x Wax cherry;
- medium fruit varieties: 'Poli' x Oradea, DS 62-193 x Oradea, DS 62-193 x Wax cherry, HB 19-9 x Wax cherry, HB 19-9 x Oradea;
- varieties with large fruits 'Tebana' x Oradea, Piroș Magdalena x Oradea, 'Desert Gold' x Oradea, 'Elbertina' x Oradea.

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THE INFLUENCE OF TEMPERATURE ON PHENOLOGY OF ORNAMENTAL WOODY SPECIES IN URBAN ENVIRONMENT

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Abstract

The main aim of this paper is to establish how urban environment conditions do influence the development of shrub ornamental plants in terms of their phenology. Spring season phenology of six species of ornamental shrubs and four of trees from an urban area of Muntenia, Romania, was analyzed under the influence of temperature. The analyses were made using correlations and the cold hours accumulated by species were calculated until the flowering bud breaking. The results obtained show that temperature significantly influences the onset of spring phenological phases and their duration. The research will be going on focusing on changes that occur in phenology of species from one year to another, under the influence of variable climatic conditions from year to year, in order to highlight the effect of climate changes on urban phenology.

Key words*: phenology, temperature, phenophase duration, global thermic balance, cold hours.*

INTRODUCTION

Climate lays conditions on the living environment of world life, being a major factor in the development and spread of vegetation. During a season, depending on occurring phenophase, different environmental factors can affect the triggering and evolution of the plant growth. Among many environmental factors that affect the development and implicitly of plant phenology, temperature is probably the most important in the case of the budding, budding and flowering in the temperate climate (Glover, 2007; Tooke & Battey, 2010; Heide, 2011; Cosmulescu et al., 2010a, Birsanu & Cosmulescu, 2017; Cosmulescu & Calusaru, 2020).

The second factor affecting the phenophases is the photoperiod, which has implications especially for flowering. Glover (2007) considers that flowering is more important during the day, then temperature and less water stress. This is explained by the fact that there is an optimal time for flowering, which differs in each species, depending on numerous factors and formed as a result of a selection process (Thomas & Vince-Prue, 1997; Glover, 2007).

The city is a complex phenomenon whose physical dimension can change the local climate. The shape and manner of layout of the buildings, the materials from which they are made, the degree of waterproofing of soil, the characteristics and dimensions of paved surfaces in the squares and on streets are elements that affect the city climate.

The most well-known climatic phenomenon generated by geometry and "consistency" of urban environment is the island of urban heat. The phenomenon especially affects the air temperature and urban surfaces in the urban climate compared to the surrounding areas (Gartland, 2012; Costache & Rădutoiu, 2007). The most well-known effect of the urban heat island on the development of plants is the onset of some phenophases, especially in spring season (Lakatos & Gulyás, 2003; Cosmulescu et al., 2010b; Cosmulescu & Gruia, 2016; Costache & Radutoiu, 2006).

The impact of some environmental factors on phenophases has led to the development of urban phenology. The researchers believe that the effects generated by urban heat island especially on temperatures, offer the possibility to study the impact of climate change on plants

development. This is because current temperatures in urban areas are considered representative of what will happen in the future and in rural areas (Neil & Wu, 2006; Jochner & Menzel, 2015). It can be said that temperature plays the most important role in weeding, as demonstrated by testing several phenological models (Fu et al., 2012).

Sparks & Menzel (2002) present phenology as the ideal way to demonstrate the effects of global warming on the living world. Phenological changes are a large part of all evidence that species respond to climate change (Walther et al., 2002). Due to the comprehensive biological record, plants have become a model group in detecting the impact of climate change, including the impact on phenology (Stefanescu et al., 2003; Dell et al., 2005; Menzel et al., 2006).

However, understanding and predicting these changes remains rather a challenge. In the context of climate change but also of challenges related to pollution in urban environment, ornamental vegetation is one of the factors that can improve the environmental quality but also the ornamental quality of urban spaces and can provide an environment for a quality life for the citizens of cities (Radutoiu & Stefanescu, 2017).

The main aim of this paper is to establish how urban environment conditions influence the development of ornamental plants through their phenology. Phenological data applied in landscaping could lead to optimization of expenses with the maintenance of green areas, to the avoidance of some choices of dendrological-floricultural material that is unsuitable to the place and purpose, and to obtaining of urban green spaces of quality and more diverse, with reduced efforts expenses. The choice of shrub and tree species as the subject of the research was due to the fact that they are vital elements in the composition of green spaces.

MATERIALS AND METHODS

The research area, Găești town, is located in South-West of Dîmbovița County, Muntenia, Romania, at $44^{0}50'$ north latitude, $25^{0}19'$ east longitude and 190.62 m altitude. Located at the contact between the Romanian Plain and Cândești Piedmont, between the valleys of Argeș and Potopu rivers, it has a temperate continental climate of transition characterized by hot, dry summers and winters with average temperatures below 0^{0}C. From this area, 6 species of ornamental shrubs were selected for the study (*Forsythia x intermedia, Mahonia aquifolium, Spiraea x vanhouttei, Albizia julibrissin, Syringa vulgaris, Chaenomeles japonica*) and 4 ornamental trees (*Aesculus hippocastanum, Prunus cerasifera, Catalpa* sp., *Tilia* sp.). Phenophases of the spring season, from the beginning of vegetation period until the end of flowering were recorded using BBCH-scales by observations on leafing and flowering in 2018-2019, at an interval of 2-4 days, calculating the number of days between different phenophases.

To highlight the influence of temperature on phenology, climate data from Tîrgoviște Meteorological Station, 27 km from the research area, were used. The global thermal balance resulting from the summation of average daily temperature over the time interval was calculated. With this help can be highlighted the amounts of temperature degrees until the triggering of a certain phenophase, or in other words allowing to appreciate what amount of degrees is needed for a particular species to go through a certain phenophase (for flowering, for ripening fruit, etc.).

The accumulated cold hours from November 1 until flowering bud burst were calculated according to the method used by Cosmulescu & Ionescu (2018). The relationships between temperature and phenology were investigated using correlations.

RESULTS AND DISCUSSIONS

Phenological changes are the clearest manifestations of climate warming. Understanding long-term trends, as well as the mechanisms underlying divergent responses, is an essential part of managing and mitigating the effects of climate changes through phenology. From a phenological point of view, the main reaction to these changes is considered to be the increase of vegetation season length (Robeson, 2002), respectively, an earlier occurrence of spring phenophases and a delay of autumn ones.

Chart 1 presents the phenogram of spring phenology, respectively, bud-breaking and flowering of the 10 species under study. The moment of blossoming bud-breaking varies considerably. The earliest blossoming bud-breaking occurred in *Chaenomeles* (February 28) while the latest in *A. julibrissin* (June 10). The timing of phenological stages (phenophases) varies according to species, age, local climate and many biotic and abiotic conditions (Denny et al., 2014; White et al., 1997). The shortest duration from bud-breaking to the end of blossoming was in *P. cerasifera* (March 15 - April 15).

Table 1 shows the duration, in days, of different phenophasesfor the 10 species. The fewest days from November 1 to vegetative bud-breaking (107 days) passed in *M. aquifolium*, while the highest number of days (176 days) in *A. julibrissin*. The number of days from 1 November to blossoming bud-breaking was 128 days in *Forsythia intermedia* and 211 in *Catalpa* sp. From blossoming bud-breaking to flowering, the time duration was between 2 days in *Tillia* sp. and *S. vulgaris* and 15 days in *Catalpa* sp. The duration of flowering phenophase was between 9 days in *S. vanhouthei* and 53 days in *Tillia* sp. and *Chaenomeles*. In literature there are many studies related to phenology. Defila & Clot (2001), reported a growth trend of vegetation season period by 13.3-day in chestnut (*Aesculus hippocastanum*). Also, analyzing 100 species, Abu-Asab et al. (2001) found in 89 of them a significant trend towards occurrence of the first flowering by 2.4 days earlier.

The same authors demonstrate the higher variability of spring phenophases, compared to the autumn ones, similarly to the results presented by Roetzer et al. (2000). The time and duration of flowering phenophase are influenced by temperature (Birsanu Ionescu & Cosmulescu, 2017; Cosmulescu et al., 2015). Pálešová & Snopková (2010) in central Slovakia confirmed that higher temperatures recorded in the last decades have a significant influence on spring phenophases development. Thus, the higher air temperature in the spring months triggers the onset of spring phenological phases.

The same trend was observed in Suffolk, UK (Sparks et al., 2006) and in Europe (Ahas et al., 2002). Each fruit tree species has a specific requirement regarding the cold which refers to the hours accumulated below the cooling temperature threshold, hours that are important for leaving the dormant state. The maximal biological effect of an hour of cold is obtained, according to the specialized literature, between 0 and 7°C. The cold requirement is the result of long-term climate adaptation of tree genotypes in different regions. Instead, it limits the climatic distribution of genotypes of fruit trees in temperate areas (Sherman & Beckman, 2003). The need for cold is the main factor that determines the flowering time (Egea et al., 2003; Ruiz et al., 2007), which is an important agronomic feature for seeds and fruits development in species of fruit trees in temperate areas. Fan et al. (2010) report in *Prunus* genus a need for cold hours between 320 and 1049 hours in 2008, and between 294 and 970 hours in 2009, in genotypes analyzed within the Southern Fruit and Tree Nut Research Laboratory (Byron, GA, USA).

Chart 1. Phenogram of blossoming bud-breaking - the end of flowering

Month	Feb	Mar			Apr					May				June				July	
Species / Day	28	8	11	15	15	20	22	25	28	2	12	20	30	4	10	21	24	11	24
Forsythia intermedia																			
Mahonia aquifolium																			
Spiraea vanhouttei																			
Syringa vulgaris																			
Albizzia julibrissin																			
Tillia sp.																			
Aesculus hippocastanum																			
Catalpa sp.																			
Prunus cerasifera																			
Chaenomeles japonica																			

Table 1. Duration(days) of different vegetation phenophases

Species/Phenophases	1 November-vegetative bud-breaking	1 November-blossoming bud-breaking	Bud-breaking - flowering	Beginning-end of flowering
Forsythia intermedia	112	128	3	50
Mahonia aquifolium	107	131	8	26
Spiraea vanhouttei	135	173	3	9
Syringa vulgaris	135	171	2	28
Albizzia julibrissin	176	222	6	40
Tillia sp.	112	183	2	53
Aesculus hippocastanum	152	179	6	50
Catalpa sp.	138	211	15	39
Prunus cerasifera	117	132	7	29
Chaenomeles japonica	112	120	5	53

The number of cold hours accumulated by the 10 species from November 1 to blossoming bud-breaking (Table 2) varies between 1632 hours in *Chaenomeles*, 1680 in *F. intermedia* and *M. aquifolium*, respectively, 1704 hours in the rest of the species, although they live under the same climatic conditions. The results are in accordance with the literature that states that each species has a certain requirement regarding the cold and only after accumulating the necessary cold hours it leaves the dormant state (Cosmulescu & Birsanu, 2018). Vegetation phenophases are influenced and are triggered when a certain amount of temperature is accumulated. The timing of flowering phenophase depends on the cultivar's requirements for heat. Citadin et al. (2001) consider that the requirement for heat is another factor that determines the bud-breaking and flowering of temperate species.

Table 2. Hours of cold accumulated from November 1 to blossoming bud-breaking

Species / Phenophases	Date of blossoming bud-breaking	Days of cold	Hours of cold
Forsythia intermedia	08 March	70	1680
Mahonia aquifolium	11 March	70	1680
Spiraea vanhouttei	22 April	71	1704
Syringa vulgaris	20 April	71	1704
Albizzia julibrissin	10 June	71	1704
Tillia sp.	02 May	71	1704
Aesculus hippocastanum	28 April	71	1704
Catalpa sp.	30 May	71	1704
Prunus cerasifera	12 March	71	1704
Chaenomeles japonica	28 February	68	1632

Table 3 presents the average temperature of flowering period and the global thermal balance in analyzed species. The average temperature during the flowering period ranged from 9.69^0C in *Prunus* to 22.85^0C in *Catalpa* sp. The sum of average temperature degrees from January 1 at the beginning of flowering phenophase ranged from 153^0C in *Chaenomeles* to 1601^0C in *Alizzia*. The findings of Zverko et al. (2014) indicate that the increase of temperature has a significant influence on the earlier occurrence of spring phenological phases in the species analyzed in the Boky Nature Reserve of Slovakia (*Crataegus monogyna*, *Corrylus avellana*, *Prunus spinosa*, *Cornus mas*). Long-term systematic monitoring of phenophases offers the possibility to estimate changes in the onset or the end of phenophases, which allows to evaluating the influence of climate changes on nature. Several variables involved in spring phenology are modified in urban areas. Air temperature appears to be the main climatic factor in plant phenology (Roetzer et al., 2000; Defila & Clot, 2001; Menzel et al., 2006; Defila & Jeanneret, 2007; Luo et al., 2007). Mimet et al. (2009) found an early onset of flowering in the town, confirming the previous results from European cities (Roetzer et al., 2000) and from China (Lu et al., 2006). Mimet

et al. (2009) combined experimental and observational methodology to provide a better and deeper view on climatic habitat in an urban context, and it shows that the town influences the phenology of plants by reducing daily temperature variation and by increasing minimum temperature.

Table 3. Average temperature in flowering period and global thermal balance (BTG) for flowering phenophase

Species	Beginning of flowering	End of flowering	Average t in flowering period (˚C)	BTG (˚C) for flowering phenophase
Forsythia intermedia	10 March	28 April	10.31	222
Mahonia aquifolium	18 March	12 May	11.45	301
Spiraea vanhouttei	24 April	04 June	16.81	657
Syringa vulgaris	21 April	20 May	15.03	623
Albizzia julibrissin	15 June	24 July	22.50	1601
Tillia sp.	03 May	24 June	19.51	796
Aesculus hippocastanum	03.May	21 June	19.24	796
Catalpa sp.	13 June	11 July	22.85	1556
Prunus cerasifera	15 March	15 April	9.69	259
Chaenomeles japonica	04 March	25 April	9.92	153

Table 4 shows the maximum, minimum and average temperatures over 2018-2019 in Găești area, and Table 5 shows the correlations between the temperature factor and duration of different spring phenophases. Positive correlations were calculated between the average temperature of flowering period and the number of days from November 1 to blossoming bud-breaking (r = 0.98), the average temperature in flowering period and the number of days from November 1 to vegetative bud-breaking (r = 0.74), the average temperature in the flowering period and the number of days from bud-breaking to flowering (r = 0.31), between the global thermal balance and the number of days from 1 November to blossoming bud-breaking (r = 0.97), in vegetative bud-breaking (r = 0.79), between the global thermal balance and the number of days from bud-breaking to flowering (r = 0.46), between the maximum temperature of the November-July period and the number of days from bud-breaking to flowering (r = 0.57), between the average temperature and the number of days from bud-breaking to flowering (r = 0.60). And the duration of flowering phenophase is influenced by maximum, minimum and average temperatures of November - July period (r = 0.44; r = 0.42; r = 0.44).

Table 4. Minimum, average and maximum temperature over period November 2018 - July 2019 in Găești town area

Month	Maximum temperature	Minimum temperature	Average temperature
November	8.13	2.4	5.17
December	3.07	-2.8	0.17
January	1.63	-4.43	-1.4
February	9.07	-1.18	3.86
March	16.33	2.47	9.2
April	16.6	5.83	11.23
May	22.63	11.37	16.9
June	28.97	17.9	22.87
July	28.67	16.67	22.13
Average	**15.01**	**5.36**	**10.01**

Table 5. Correlations existing between different phenophases

	No of days from November 1 - vegetative bud-breaking	No of days from November 1 – blossoming bud-breaking	No of days bud-breaking - flowering	No of days beginning – end of flowering	Hours of cold	Average t in flowering period (˚C)	BTG (˚C) for flowering phenophase
No of days from November 1–vegetative bud-breaking	1						
No of days from November 1 –blossoming bud-breaking	0.80	1					
No of days bud-breaking – flowering	0.16	0.28	1				
No of days beginning – end of flowering	-0.07	-0.02	-0.02	1			
Hours of cold	-0.07	-0.02	-0.02	1	1		
Average t in flowering period (˚C)	0.74	0.98	0.31	0.07	0.07	1	
BTG (˚C) for flowering phenophase	0.79	0.97	0.46	0.03	0.03	0.94	1
T MAX November – July	0.24	0.33	0.57	0.44	0.44	0.33	0.40
T MIN November – July	0.06	0.17	0.63	0.42	0.42	0.20	0.27
T MED November – July	0.16	0.26	0.60	0.44	0.44	0.28	0.35

CONCLUSIONS

This case study shows that temperature significantly influences the onset of spring phenological phases, their duration. The results regarding the cold hours do confirm that each species has a certain requirement regarding the cold and only after accumulating the necessary cold hours it leaves the dormant state, having in view that the 10 species analyzed, although they live under the same climatic conditions, they needed a variable number of hours of cold to trigger bud-breaking. The research will continue focused on the aspect of changes that occur in phenology of species from year to year, under the influence of variable climatic conditions from year to year in order to highlight the effect of climate changes on urban phenology.

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VARIABILITY OF PHYSICAL-CHEMICAL CHARACTERISTICS IN MEDLAR GENOTYPES (*MESPILUS GERMANICA* L.) DEPENDING ON CLIMATIC YEAR

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Abstract

This study was performed to determine some physical-chemical characteristics of 5 medlar genotypes, selected from the South-Western area of Romania, identified in both spontaneous and cultivated flora. During the 3 years of study the physical-chemical characteristics of fruits showed significant differences both between genotypes and between years (the average weight of fruits, the diameter of fruits and the volume of fruits varied between 3.08 g-36.68 g, 17.98 mm-44.15 mm, respectively, 3.92 cm³ - 37.18 cm³). The soluble dry matter has recorded values between 9.5% -26.6%, the total dry substance was between 24.96% - 44.97%, and titratable acidity was between 1.60 and 7.03 g malic acid/100 g fresh substance. This variability can be exploited to select valuable medlar genotypes (Mespilus germanica L.) for preservation and use in culture.

Key words*: genotype, Mespilus germanica L., morphological characteristics, variability.*

INTRODUCTION

The medlar (*Mespilus germanica* L.) is part of the *Rosaceae* family and is native to the eastern Mediterranean. It was cultivated about three thousand years ago in the Caspian Sea region of northern Iran (Velickovic et al., 2013).
The interest for it gradually disappeared, and later it was replaced by other, more productive cultures. Nowadays, medlar is cultivated quite rarely, mainly in botanical gardens or in private gardens (Cosmulescu et al., 2020).
The fruits are astringent and hard to harvest. They can be consumed fresh because they are high in potassium (Glew et al., 2003a) and amino acids (Glew et al., 2003b). Medlar fruits are used as a treatment for constipation, as a diuretic and for treating kidney stones and bladder (Baird and Thieret, 1989).
It is a healthy fruit, with phytochemicals including antioxidants (Ayaz at al., 2008; Selcuk and Erkan, 2015). Medlar is not resistant to temperature and has a much higher resistance against pests. Recently, more attention has been paid to morphological and biochemical properties of different medlar genotypes (Scrieciu and Cosmulescu, 2019; Cosmulescu et al., 2019; 2020). Some researchers have studied the spreading area of medlar (Yilmaz et al., Realcioglu, 2013), the phenological stages (Atay, 2013), but also the characteristics of pollen (Cavusoglu and Sulusoglu, 2013).
The fruit is medium in size (20-30 mm in diameter), hemispherical, flattened at the tip, chestnut-red colour, with a very large calyx cavity, the cup shape, on the side of which there are inserted large, lanceolated and persistent sepals. The fruit epidermis is thick, and the pulp is firstly whitish-yellowish, hard, sour and astringent; at maturity, as a result of fermentation, it becomes brown, soft (like a paste), acid-sweet, with a fine, specific, pleasant aroma. The seeds, in number of 5, have an irregular, woody coating (Dirr, 1990). Fruits are harvested at the end of October - beginning of November, after the fall of autumn hoarfrost. Being hard when they are harvested, the fruits are easily carried. In warehouse they should be placed on a layer of

straw. After 3-4 weeks of storage, their pulp becomes soft and edible (Baird and Thieret, 1989). There are not enough studies on chemical and nutritional composition of medlar fruits (Aydin and Kadioglu, 2001). The aim of this study was to determine genetic diversity, based on physical-chemical characteristics of fruits, in medlar (*Mespilus germanica* L.) from the South-Western area of Romania, found in both spontaneous and cultivated flora. This variability can be exploited to select valuable genotypes for conservation and use in culture.

MATERIALS AND METHODS

The study was carried out on five genotypes of medlar (*Mespilus germanica* L.) selected from different areas (Dolj, Gorj, Caraș-Severin, Teleorman), being identified in both spontaneous and cultivated flora. The fruits were harvested during autumn season (production of 2017, 2018, 2019) and carried to laboratory for biometric measurements and chemical analyses. Starting with the name of locality where they were identified, the biotypes were coded as follows: C1 (Craiova), N1 (Nanov), M1 (Mătăsari), T1 (Turnu Ruieni), E1 (Ezeriș). The determinations were performed on 50 fruits (randomly collected) from each identified genotype.

Physical characteristics

Biometric measurements were made, following: the weight of fruits was determined using the precision balance; the diameter and the height of fruits were used the electronic cube with ultrafast digital display and accuracy of 0.01 mm; volume (V; cm^3) and fruit density (ρ; g/cm^3) was determined using the liquid displacement method in a graduated cylinder, where 100 ml of water was added and then the fruit, thus determining the volume of fruits, and density was calculated using the formula $\rho = m/V$, where m = mass and V = volume. The shape index (If) was calculated according to the method presented by Ionică et al. (2018) and Cosmulescu (2013): If = H/D, where H = fruit height; D = fruit diameter.

Chemical characteristics

Total acidity was determined using the method described by Ionică (2014), by titrating a fruit extract, obtained by boiling and filtering and neutralization with NaOH, and the results obtained were expressed in ml of 0.1 N/100 g NaOH fresh substance. Total dry matter (SUT) was determined using the method based on water evaporation from the analytical average sample, using the oven, at temperatures of 85-105°C, the results being expressed as a percentage. The soluble dry matter (SUS) was determined with refractometer, the final result being expressed as a percentage. The data obtained were statistically processed using the Excel descriptive statistics program (StatPoint Technologies, Warrenton, VA, USA).

RESULTS AND DISCUSSIONS

The results regarding the variability of fruit characteristics for the studied genotypes are presented in Tables 1 and 2. Regarding the average weight of medlar fruits, there were big differences both between genotypes and within genotype from year to year. Thus, in 2017 the average fruit weight was between 3.15-36.68 g, in 2018 between 7.5-32.65 g, respectively, 3.08-27.20 g in 2019. The highest weight was observed in T1 genotype (36.68 g) in 2017, followed by C1 genotype (32.65-27.20 g), which recorded the highest average values for 2018 and 2019.

Medlar genotypes studied differ from each other in fruit weight. Aygun and Tasci (2013) reported that the average weight of medlar fruits in genotypes grown in Ordu region ranged from 6.32 g to 36.42 g. Similarly, Ozkan et al. (1997) and Bostan et al. (2002, 2007) reported that the average fruit weight was 16.51-32.98 g and 9.46-40.80 g, respectively. Previous studies by Ylmaz et al. (2016) showed that the average fruit weight was between 17.71-32.46 g in 2011 and 15.99-37.54 g in 2012 in genotypes grown in Tokat province.

Table 1 Morphologic characteristics of medlar fruits identified (2017-2019)

Genotype	Descriptive statistics	Fruit weight (g)	Fruit diameter (mm)	Fruit height (mm)	Fruit volume (cm^3)	Fruit density (g/cm^3)	Shape index (mm)
C1	Mean±SD Min./max CV%	27.13±5.55 21.55/32.65 20.45	38.67±2.78 36.54/41.82 7.19	34.96±1.40 33.62/36.43 4.03	27.63±4.74 22.48/31.82 17.16	0.98±0.04 0.95/1.03 4.23	0.90±0.08 0.83/0.99 8.94
N1	*Mean±SD* *Min./max* *CV%*	*21.16±1.45* *19.49/22.14* *6.87*	*36.22±0.68* *35.6/36.96* *1.89*	*33.94±0.26* *33.76/34.25* *0.79*	*20.50±1.30* *19.05/21.56* *6.34*	*1.03±0.01* *1.02/1.04* *1.11*	*0.93±0.01* *0.91/0.94* *1.86*
M1	Mean±SD Min./max CV%	4.57±2.53 3.08/7.5 55.32	20.24±3.53 17.98/24.31 17.45	20.06±0.81 19.13/20.66 4.07	5.04±1.55 3.92/6.82 30.92	0.89±0.18 0.76/1.1 20.31	1.00±0.19 0.78/1.14 19.60
T1	Mean±SD Min./max CV%	27.16±9.19 18.32/36.68 33.86	39.11±4.86 34.44/44.15 12.43	31.81±1.07 30.59/32.63 3.39	27.14±9.69 17.84/37.18 35.70	1.00±0.02 0.98/1.03 2.49	0.93±0.30 0.73/1.28 32.70
E1	Mean±SD Min./max CV%	20.21±1.46 18.68/21.61 7.27	32.8±1.42 31.47/34.31 4.35	35.50±1.08 34.36/36.52 3.05	20.61±1.64 19.08/22.35 7.97	0.98±0.01 0.96/0.99 1.76	1.07±0.04 1.03/1.11 3.86

*values include mean of the 3 years (2017, 2018, 2019) for each separate characteristic in selected genotypes

The fruit diameter is considered a very important quality element for medlar fruit, thus, the smallest value of fruit diameter over the three years of study was recorded in M1 genotype (18.43 mm, 24.31 mm, respectively 17.98 mm), and the highest value of fruit diameter was in T1 genotype (44.15 mm) in 2017 and in C1 genotype (41.82 mm, 40.24 mm) in 2018 and 2019. The values of variation coefficient, for this characteristic, were between 1.89% (N1) and 17.45% (M1) the variability being high. The results are in agreement with those obtained by Aygun and Tascı (2013) but also with Bostan (2002, 2007), studies that showed that fruit diameter was 23.10-42.65 mm, 31.52-42.44 mm, respectively 14.96-35.68 mm. Studies by Ylmaz et al. (2016) on physical characteristics of fruit showed that fruit diameter was significantly influenced by genotype and less by environmental conditions, recording values between 21.07-41.05 mm in 2011 and 17.49-43.63 mm. The average volume of medlar fruits has varied in very wide range, between 4.38 cm^3 (M1) and 37.18 cm^3 (T1), resulting in a difference of 8.48 times between the two genotypes in 2017, between 6.82 cm^3 (M1) and 31.82 cm^3 (C1), with a difference of 4.66 times between the two genotypes in 2018 and between 3.92 cm^3 (M1) and 28.60 cm^3 (C1), with a difference of 7.29 times between the two genotypes in 2019. Similar studies by Haciseferogullari et al. (2005) showed that fruit volume in genotypes from Eğirdir area, Turkey, was higher (13.7 cm^3) compared to the results obtained in M1 genotype in the present paper. For fruit density (ρ), the average value was between 0.76 (M1) - 1.04 g/cm^3 (N1) in 2017, between 0.96 (E1) - 1.10 g/cm^3 (M1) in 2018 and between 0.82 (M1) - 1.03 g/cm^3 (T1) in 2019. The variation coefficient recorded values between 1.11% in N1 genotype and 20.31% in M1 genotype. In order to determine the fruit shape, the shape index was calculated, ranging from 0.89 (C1) - 1.07 (M1, T1, E1) in 2017, between 0.78 (M1) - 0.99 (E1) in 2018, and in 2019 between 0.91 (C1) - 1.13 (M1 and T1). There is a high variability of all fruit characteristics both between genotypes and between climatic years. Thus, the average fruit height recorded the lowest value in M1 genotype in all three years of study (20.39 mm, 19.13 mm, 20.66 mm), and the highest value was obtained in E1 genotype (34.36 mm, 35.64 mm, 36.52 mm). The variation limits for the average fruit height were between 14.93 mm (M1) in 2018 and 41.43 mm (E1) in 2019. The highest value of variation coefficient for the average fruit height (4.07%) was calculated in M1 genotype. Šebek et al. (2019) have reported that the average height of medlar fruits, in 'Royal' medlar cultivar in the town of Bijelo Polje, had higher values (38.4 mm), compared to the results obtained in the present paper. Chemical characteristics of medlar fruits were recorded in Table 2.

Table 2 Chemical characteristics of medlar fruits (*Mespilus germanica* L.) mean of years 2017-2019

Statistical analysis/Genotype		SUT (%)	SUS (%)	TA (ml NaOH 0.1 u/100 g) g acid malic/100 g sp
Mean ± SD Minimum/Maximum CV%	M1	39.486 ± 4.79 36.11/44.97 12.13	16.83 ± 5.00 13/22.5 29.75	3.88 ± 2.74 2.01/7.03 70.71
	N1	29.770 ± 1.76 27.94/31.46 5.92	16.86 ± 8.09 11/26.1 47.98	4.01 ± 1.45 2.34/5.0 36.27
	C1	27.673 ± 3.51 24.96/31.64 12.69	16.26 ± 2.40 14.5/19 14.75	2.87 ± 1.21 1.6/4.02 42.25
	E1	27.676 ± 0.83 26.83/28.49 3.00	18.60 ± 8.16 9.5/25.3 43.91	3.45 ± 0.50 3.01/4.0 14.56
	T1	30.303 ± 2.80 27.08/32.18 9.25	21.20 ± 6.15 14.5/26.6 29.02	3.45 ± 1.27 2.0/4.35 36.80

*SUT = total dry substance; SUS = dry soluble substance; TA = titrable acidity; sp = fresh substance.

Total dry matter ranged from 27.08% (T1) to 36.11% (M1) in 2017, between 26.42% (C1) and 44.97% (M1) in 2018, and in 2019 values between 24.96% (C1) and 37.38% (M1) were registered. The variation coefficient was between 3.00% in E1 genotype and 12.69% (C1), representing a high variability.

The results of this research related to dry substance for the selected genotypes showed values close to medlar genotypes in Tokat province, where total dry substance was determined between 27.34-44.11%, in the paper done by Yilmaz et al. (2016). Regarding soluble dry matter, it ranged between 9.5% in E1 genotype, in 2017 and 26.6% in T1 genotype in 2019. Similar research by Durul et al. (2016) show that the soluble dry matter (SUS) values of medlar fruits grown in different agro-climatic regions of Turkey (Kocaeli province) were between 16.4 and 22.2%. The soluble dry matter content varies between 17.0 and 24.0%, for the selected medlar genotypes from Turkey (Tonya district of Trabzon province), by Ylmaz (2015).

The results of this study on soluble dry matter showed similar results with these studies. Titrable acidity recorded values between 2.01 (M1) and 4.69 g malic acid/100 g sp (N1) in 2017, between 2.34 (N1) and 7.03 g malic acid/100 g sp (M1) in 2018, and in in 2019 the titrable acidity was between 1.60 (C1) and 5.00 g malic acid/100 g sp (N1). According to studies by Ylmaz et al. (2016), showed that titrable acidity was between 4.25 and 8.94% in medlar genotypes in 2011-2012.

The values recorded for total dry matter, the titratable acidity and soluble dry matter of medlar fruits may be the result of different genetically based characteristics, but also the effect on agro-ecological conditions of culture.

CONCLUSIONS

The high variability of characteristics of medlar genotypes analyzed, offers the possibility of selecting the genotypes with superior characteristics, adapted to climatic conditions, which can be used for introduction into the culture and for development of new cultivars. This study shows that T1, C1 and N1 genotypes are promising in terms of characteristics evaluated in development of new cultivars.

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FRUIT-BEARING POTENTIAL MODELING OF THE FLORICANE RASPBERRY CV. WILLAMETTE

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Abstract

During the 3-year period (2014-2016) modeling of the fruit-bearing potential of the cultivar, Willamette was conducted with the aim of defining the optimal load of the mixed buds per meter of the hedgerow. Modeling was conducted by a reduction in the fruit-bearing potential of 160 mixed buds, corresponding to a load of canes in the ordinary production practice. The fruit-bearing potential was reduced to 120 (medium potential) and 90 buds (low potential). Lower intensity reduction in a number of mixed buds per meter of hedgerow and selection of quality buds enables an increase in yield for 31.7%. Reduction in a number of mixed buds for about 50% in comparison to ordinary production practice led to a 14.6% decrease in total yield, during the three-year period. The increase in the number of buds per meter of hedgerow to a certain number may affect the increase and continuity of the yield. An increase in fruit-bearing potential outside of the range of the optimum determined by this research (120 mixed buds) may prove counterproductive and lead to a decrease in yield.

Key words*: hedgerow, cane, mixed bud, fruit, yield, quality.*

INTRODUCTION

Raspberry production plays an important role in both agricultural production and rural development in Bosnia and Herzegovina. Frozen raspberry export for the period 2014-2018. had a share from 52.0 to 66.6% in the total value of the fruit and vegetables exports from Bosnia and Herzegovina (Zivotic et al., 2018). New varieties (Sava, 2013) and growing technology (Asanica, 2019) are of crucial importance for efficiency in the raspberry production. Floricane type cultivar Willamette dominates in the countries' raspberry production with share 80% of the total production. Willamete is high-yield characteristics cultivar, resistant to the most significant raspberry diseases and pests and suitable for cultivation in the different local agro ecological conditions (Velickovic et al., 2004; Stanisavljevic et al., 2004; Kempler et al., 2005; Eyduran et al., 2006; Kulina et al., 2012; Fotiric-Aksic et al., 2012; Milivojevic et al., 2012; Poledica et al., 2012; Alibabic et al., 2018). Willamette is cultivated in hedgerow system, patented in Serbia during 1970s (Glisic et al., 2009; Leposavic et al., 2013). Lack of knowledge considering growth biology and development of the floricane raspberry cultivars (Micic et al., 2015) and desire to realize maximal yields often result in an application of the inadequate pomotechnical treatments in commercial plantations. To achieve high yields, producers often leave the maximal number of year-old shoots for fruiting in the next season. Large number of aboveground shoots in one season influences extremely dense plant set per meter of hedgerow, thus resulting in formation of a large number of small fruit. A large number of canes

in the fruiting phase (two-years-old) result in negative competition with the formation of new year-old shoots for the next season. In such conditions shoots cannot reach desired height and diameter which certainly affects the number of mixed buds and the stage of their differentiation. The aim of this research was to define optimal number of mixed buds and density of aboveground shoots for the cultivar Willamette by modeling the fruit-bearing potential through difference in number of mixed buds per meter of hedgerow.

MATERIALS AND METHODS

The experiment was conducted in the commercial orchard owned in the village Borkovac, municipality Bratunac (eastern Bosnia and Herzegovina) during the period 2014-2016. The orchard is situated at plateau of the local river, at the altitude of 216 m (Lat: 44°11'9.37" N; Long: 19°18'29.62" E). The orchard was established in 2008. The spacing was 0.25 m in row and 2 m between rows. The orchard was planted exclusively with cultivar Willamette. Training system was vertical trellis - hedgerow growing system with two wires placed at 120 cm and 180 cm above the ground. Modeling of the fruit-bearing potential was done by reduction in number of mixed buds per meter of hedgerow in comparison to the ordinary production practice. The control was a load of 160 mixed buds per meter of hedgerow i.e. the density of 8 canes per meter of hedgerow, each with 20 mixed buds (8 × 20), corresponding to the load of canes in the ordinary production practice – indicated k_{160} in the tables. Treatments of the reduction of the fruit-bearing potential were done by reduction in number of canes per meter of hedgerow and by the reduction in number of buds per cane. Two different treatments were applied: reduction to 120 mixed buds per meter of hedgerow by retaining 8 canes with 15 buds per each cane - 8 × 15 (treatment 1 - medium fruit-bearing potential, indicated as t_{120}); reduction to 90 buds per meter of hedgerow by retaining 6 canes with 15 buds per each cane (treatment 2 - low fruit-bearing potential, indicated as t_{90}). Primary sampling unit was 1 meter of hedgerow. Each treatment was laid out in a randomized block design in 5 replications (total of 15 meters of hedgerow) at different positions in the orchard. Number of fruiting laterals and number of fruits per meter of hedgerow, percentage of activated buds (%), number of fruiting laterals per cane, fruit weight, fruit length, fruit width, fruit shape index, yield per cane and yield per unit area were determined. The statistical analysis was performed using Statgraphics Centurion. Obtained results were subjected to analysis of variance (ANOVA) according to a factorial design, where the sources of variation were year and treatment, and their interaction. Comparison of means was performed by the Duncan test ($\alpha = 0.05$). The results are presented as the mean value ± standard error of mean (SEM).

RESULTS AND DISCUSSIONS

Applied treatments had a statistically highly significant influence on the average percentage of activated mixed buds, number of fruiting laterals and number of fruits per meter of hedgerow (Table 1). In the control treatment, there was 15 to 20% less activated buds compared to t_1 and t_2, while less significant difference was observed between t_1 and t_2. Reduction in number of mixed buds per meter of hedgerow, during all three years of research induced statistically higher number of fruiting laterals of the control canes compared to canes with reduced bud number. Slightly larger number of fruiting laterals was observed within the control canes, even though in some seasons (2015 and to some extent 2014) the difference was not statistically significant. Number of fruits per meter of hedgerow was significantly higher in the seasons 2014 and 2015, compared to the season 2016.

Analysis of the number of fruits implies significant differences between applied treatments. Highest number of fruits per cane was recorded for the treatment $t_{2\text{-}120}$. The research demonstrated that the control canes bearing the highest number of mixed buds, in absolute quantity, also developing the highest number of fruiting laterals compared to treatments where the number of mixed buds was reduced. However, for the control canes, in average 15 to 20% of the retained buds never were activated and did not produce fruiting laterals.

Table 1. The influence of year and treatment on percentage of activated buds (%), the number of fruiting laterals per meter of hedgerow, the number of fruits per meter of hedgerow, the number of fruiting laterals per cane and number of fruits per cane

	2014	2015	2016
	$\overline{X}$ ± SEM		
	percentage of activated buds (%)		
$t_{1\text{-}90}$	90.6^{a} ± 1.47	95.5^{a} ± 1.22	100.0^{a} ± 0.0
$t_{2\text{-}120}$	97.3^{a} ± 1.33	95.3^{a} ± 1.19	98.8^{a} ± 0.82
$k_{\text{-}160}$	79.6^{b} ± 2.99	80.9^{b} ± 2.73	81.6^{b} ± 2.78
average	89.2 ± 2.23	90.6 ± 2.08	93.5 ± 2.42
	number of fruiting laterals (m^{-1})		
$t_{1\text{-}90}$	81.6^{c} ± 1.33	86.0^{b} ± 1.09	90.0^{c} ± 0.00
$t_{2\text{-}120}$	116.8^{b} ± 1.59	114.4^{a} ± 1.44	118.6^{b} ± 0.98
$k_{\text{-}160}$	135.2^{a} ± 9.57	122.4^{a} ± 5.19	145.8^{a} ± 9.60
average	111.2 ± 1.06	107.6 ± 4.5	112.3 ± 6.78
	number of fruits (m^{-1})		
$t_{1\text{-}90}$	1206.6^{b} ± 73.41	1219.0^{b} ± 52.27	1067.6^{c} ± 59.43
$t_{2\text{-}120}$	1724.8^{a} ± 72.30	1717.8^{a} ± 79.40	1658.0^{a} ± 113.66
$k_{\text{-}160}$	1570.0^{a} ± 130.84	1629.8^{a} ± 97.27	1336.8^{b} ± 61.81
average	1500.5 ± 77.53	1522.2 ± 71.68	1354.1 ± 78.04
	number of fruiting laterals per cane		
$t_{1\text{-}90}$	13.6	14.3	15.0
$t_{2\text{-}120}$	14.6	14.3	14.8
$k_{\text{-}160}$	16.9	15.3	18.2
average	15.0	14.6	16.0
	number of fruits per cane		
$t_{1\text{-}90}$	201.1	203.2	177.9
$t_{2\text{-}120}$	215.6	214.7	207.3
$k_{\text{-}160}$	196.3	203.7	167.1
average	204.3	207.2	184.1

$^{a, b, c}$ means followed by different letter within the particular year are significantly different (Duncan, α=0.05)

The number of fruiting laterals per cane was in the range from 13.6 to 18.2. These results are in accordance with the other research (Poledica et al., 2012), where the number of fruiting laterals was in the range of 11.2 to 18.6 depending on the applied treatments and in the range from 7.4 to 23.4 (Fotiric-Aksic et al., 2012). It should be noted that the number of fruiting laterals, during this research, was very uniform for the $t_{2\text{-}120}$, and with significant differences among $t_{1\text{-}90}$ and $k_{\text{-}160}$. The number of fruits per meter of hedgerow was significantly influenced by applied treatments. This confirms the assertion that the number of developed fruiting laterals per cane does not necessarily imply large number of fruits. It could be said that projected – moderate reduction in number of mixed buds per meter of hedgerow ($t_{2\text{-}120}$) provides good balance between vegetative and generative growth, as well as continuity of high fruit number. The reduction of higher intensity ($t_{1\text{-}90}$) decreases fruit-bearing potential. High fruit-bearing potential ($k_{\text{-}160}$) may support the occurrence of extremely high yields in one season, resulting in extremely low yields in the next season. The analysis of the number of fruiting laterals and fruits per cane implies the validity of moderate reduction of mixed buds per cane. Control canes had higher number of fruiting laterals compared to canes in treatments $t_{1\text{-}90}$ and $t_{2\text{-}120}$. Taking into account that the control canes had an average of 30% more retained buds per cane observed difference in number of fruiting laterals is negligible, except for the season 2016 when it was only slightly expressed. The number of fruits per cane was both, the highest and most constant through the seasons for the treatment with moderate reduction of the fruit-bearing potential ($t_{2\text{-}120}$). Slightly lower number of fruits per cane (161.3) was determined in research conducted in Serbia (Milivojevic et al., 2012). Significant variations of the number of fruits in relation to applied treatment in a range from 147.0 to 218.0 and 128.5 to 226.1 were observed by other authors as well (Glisic et al., 2009; Poledica et al., 2012; Alibabic et al., 2018). Yield per cane and per unit area is to

a large degree specific to the variety of the raspberry, because it depends on the number of fruits and on average fruit weight as well.

The largest fruit size, regardless of the year of research (Table 2), was recorded for the treatment $t_{2\text{-}120}$.

Table 2. Values of the average fruit weight (g), fruit length (mm), fruit width (mm) and fruit shape index

	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit shape index (length/width)
Year (Y)	***	***	***	*
Treatment (T)	***	***	***	ns
Y×T	***	***	*	*
Year		$\overline{X} \pm$ SD		
2014	$5.0^{a} \pm 0.15$	$23.9^{b} \pm 0.31$	$22.3^{b} \pm 0.30$	$1.07^{a} \pm 0.006$
2015	$4.7^{b} \pm 0.08$	$25.2^{a} \pm 0.21$	$24.2^{a} \pm 0.28$	$1.05^{b} \pm 0.009$
2016	$3.4^{c} \pm 0.04$	$20.7^{c} \pm 0.15$	$19.5^{c} \pm 0.11$	$1.07^{a} \pm 0.007$
Treatment				
$t_{1\text{-}90}$	$4.3^{b} \pm 0.12$	$22.8^{b} \pm 0.33$	$21.9^{b} \pm 0.32$	$1.05^{b} \pm 0.0074$
$t_{2\text{-}120}$	$4.4^{a} \pm 0.15$	$23.5^{a} \pm 0.35$	$22.0^{a} \pm 0.38$	$1.06^{ab} \pm 0.0088$
$k_{\text{-}160}$	$4.0^{c} \pm 0.10$	$22.9^{b} \pm 0.28$	$21.4^{b} \pm 0.28$	$1.07^{a} \pm 0.0065$

$^{a, b, c}$means followed by different letter within the particular year are significantly different (Duncan, α=0.05)

Fruits of the largest size, with the largest fruit length and width, were recorded for the treatment $t_{2\text{-}120}$, during all years of research. Research done in Serbia (Milivojevic et al., 2012) determined slightly lower values of the fruit weight (2.9 g in average) which is in accordance with the other researchers (Alibabic et al., 2018). There are some findings (Kempler et al., 2005) stated the fruit weight was ranging from 3.2 to 3.7 g, with the ascertainment that growing conditions and applied treatments significantly affect fruit characteristics. Higher values of fruit weight (3.3-3.4 g) were recorded in the traditional raspberry growing area in Serbia (Velickovic et al., 2004). Previous data about fruit weight within the same region in which this research was conducted in Bosnia and Herzegovina (Kulina et al., 2012) determined the average fruit weight of 3.51 g. Very large fruits with average fruit weight of 4.72 g were observed by some other researchers (Stanisavljevic et al., 2004; Poledica et al., 2012). Results obtained on fruit length and width are in accordance with the results obtained by other authors (Milivojevic et al., 2012; Alibabic et al., 2018). Most authors agree that fruit shape index for the cultivar Willamette is slightly larger than 1, which is in accordance with the results obtained in this research. Tendency of linear decrease in yield was observed for applied treatments during all three years of research, while the control treatment showed uneven variations (Table 3).

Table 3. The influence of year and treatment on average yield

	Average yield per cane (g)			Average and total yield (t) per unit area (1 ha)				Difference in yield for t_1 and t_2 compared to control (%)
	2014	2015	2016	2014	2015	2016	Total	
$t_{1\text{-}90}$	1102.0	918.3	599.6	21.2	17.6	11.5	50.3	- 14.6
$t_{2\text{-}120}$	1263.4	1062.9	702.6	32.3	27.2	17.9	77.5	+ 31.6
$k_{\text{-}160}$	790.9	951.4	558.1	20.3	24.4	14.3	58.9	0.0

Yield per meter of hedgerow was highest for the treatment $t_{2\text{-}120}$ in all seasons. Exceptionally high yields in 2014 and 2015 were conditioned not only by the large number of fruits, but by large fruit size as well. Number of fruits per cane and average fruit weight were used to calculate yield per cane. Highest average yield per cane was recorded for the treatment $t_{2\text{-}120}$ during all years of research, while the lowest yield was recorded for the control treatment in 2014 and for the treatment $t_{1\text{-}90}$ in 2015. Raspberry average yield in available literature is most often under the real value of raspberry fruit-bearing potential. In the research conducted in Turkey (Eyduran et al., 2006) was recorded very low yields per cane (96.0 g)

which could be explained by unfavourable climatic conditions and/or the lack of adequate pomotechnical and agrotechnical treatments. Researchers from Serbia recorded significantly higher yields (Leposavic et al., 2013) as well as in Bosnia and Herzegovina (Kulina et al., 2012) where determined the average yield of 20100.0 kg/ha. Achieved yields within this research suggest that the optimal modeling of the fruit-bearing potential could provide for profitable, and in certain conditions extremely profitable yields. Lower intensity of the reduction in number of mixed buds per meter of hedgerow $t_{2\text{-}120}$ and selection of quality buds enables increase in yield for 31.7%. This is important, considering the attitude of most of the producers and some of the experts that increase in number of mixed buds per meter of hedgerow (via increase in retained canes and buds they bear) necessarily leads to increase in yield. Reduction in number of mixed buds for about 50.0% ($t_{2\text{-}120}$) in comparison to ordinary production practice led to decrease in total yield for 14.6%, during the three-year period. The abovementioned imposes the necessity of additional analysis of the production in conditions of the reduced fruit-bearing potential by the ease of application of standard pomotechnical and agrotechnical procedures in orchards with lower vegetative mass. Could the decrease in yield of 14.6% be compensated by more efficient pesticide application, less necessity for manual labor during dormancy, more cost effective agrotechnical procedures (fertilization and pesticide application) and picking? These are questions that should be additionally answered in the next period, so the more complete assessment of the efficacy of this treatment could be provided.

CONCLUSIONS

Obtained results clearly demonstrate that the increase in number of buds per meter of hedgerow to a certain number may affect increase and continuity of the yield. Increase in fruit-bearing potential outside of the range of the optimum determined by this research (120 mixed buds per meter of hedgerow) may prove counterproductive and lead to decrease in yield. This occurs as a result of the creation of unfavorable, competitive relations between one and two-year aboveground shoots. Large number of mixed buds per meter of hedgerow is not a guarantee of high yield. Increase in number of canes per meter of hedgerow implies increase in application of the agrotechnical procedures, namely fertilization as well as manual labor during pruning and picking and more difficult (less efficient) application of pesticides. Such procedures have to be taken into consideration as to achieve the objective evaluation of the applied treatments of the reduction of the fruit-bearing potential and its impact on the cultivation of the Willamette variety in local production.

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EVALUATION OF SOME NUTRITIONAL PROPERTIES OF CHINESE JUJUBE (*ZIZIPHUS JUJUBA* MILL.) FRUITS ORGANICALY PRODUCED IN BUCHAREST AREA

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Abstract

Chinese jujube is one of the most commonly used fruit in Chinese herbal medicine, being considered by the researchers and nutritionists a super fruit in terms of nutrition. In Romania, more than 20 genotypes were introduced from China, in the Experimental Field of the Faculty of Horticulture within the UASVM Bucharest. This study aims to present several biochemical characteristics of ten Chinese jujube genotypes. Fresh fruits were harvested at the beginning of September until October. After morphological measurements, fruits were stored at 2-3 ° C and 90-95% relative humidity. For analysis hydro alcoholic extracts using 1:10 ratio was used. The extracts were subjected to spectrophotometric analysis. Using Folin - Ciocâlteu method, the total phenolic content of the extracts was determined. The flavonoid content was identified using an adapted method based on rutin equivalent. The free radical scavenging activity of the extracts was determined using 2,2 diphenyl-1-picrylhydrazyl radical. The obtained results confirm the data presented by the researches on the similar genotypes and can be an important aspect in promoting the cultivation and consumption of these fruits in more regions of our country.

Key words*: Chinese jujube, flavonoid content, free radical scavenging activity, phenolic content, spectrophotometry.*

INTRODUCTION

Chinese jujube (*Ziziphus jujube* Mill.) is one of the most commonly used fruit in Chinese herbal medicine, being considered by the researchers and nutritionists a super fruit in terms of nutrition. The fruits represent a good source of minerals (phosphorus, potassium, calcium and manganese) and antioxidant compounds, having antiproliferative, antitumoral, anti-inflammatory properties and protective effect against myocardial injury. Jujube, with numerous pharmacological properties, can be consider a very important source of nutraceuticals (Cheng et al., 2012; Wang et al., 2016; Wojdylo et al., 2019; Song et al., 2019; Chen et al., 2019; Shahrajabian et al., 2019). Chinese jujube's fruits are used from fresh or dehydrated until processed in the form of syrups, marmalades, sweets, flour, cider, etc. The food value of Chinese jujube is high especially due to the high content of soluble dry matter exceeding the value of 30%, as the total carbohydrate content is 27%. The fruit's acidity varies between 0.3 and 1.0%. It has a very high content of ascorbic acid (vitamin C) depending on the variety, with values of 330-880 mg/100 g fresh weight. The content of vitamin P exceeds 1000 mg/100 g fresh weight. Chinese jujube (*Ziziphus jujuba* Mill., sin. *Ziziphus sativa* Gaertn.) is part of the *Rhamnaceae* family (Ciocârlan, 2000). Originally from China, it has been cultivated since the earliest times and expanded to Asia Minor, Europe and America. As the name mentions, Chinese jujube is the national fruit in China (Liu et al., 2014), the high valuable production being obtained in Gobi Desert. Knowing their value, China cultivated these fruit plants since more than 4300 years. Today, there are more than 20 million farmers producing jujube, 2 million ha cultivated, 6.24 mil. tons produced/year, income 100 billion RMB (about 12.85 billion euro). This crop had an essential role in the program to eradicate poverty in northern China's mountain areas. There is a particular interest in Europe towards diversifying fresh and processed fruits offer,

including the introduction of new species with high nutraceutical value. In the same time, many agricultural areas in Europe are faced with very serious problems of desertification, water shortages and saltiness, pressure of diseases or pests to traditional fruit species. In this context, the introduction of Chinese jujube crop, characterized by a very high resistance to drought and high to salty soils, as well as a high capacity to capitalize on poor soils and a high tolerance to diseases and pests (Chireceanu et al., 2013; Mardare et al., 2016; Ciceoi et al., 2017), can be a life-saving solution.

In our country, jujube trees can be found on the right bank of Danube in Ostrov area, Constanța County, without knowing their origin. The locals call the "olive tree of Dobrogea" and the existence of abundantly producing fruit trees, perfectly adapted to the local conditions, is known (Stănică, 2009; 2016). In Jurilovca, Doloşman Cape, near the ruins of the Argamum Greek colony, another population of wild Chinese jujube (*Ziziphus acido-jujuba*) (Wang et al., 2019), which grows as bush tree, was identified (Stănică and Vasile, 2008).

In Romania, more than 20 genotypes were introduced from China, in the Experimental Field of the Faculty of Horticulture from UASVM Bucharest. Researches started in 1996, following a cooperation project between the USAMV of Bucharest and Shanxi Academy of Agricultural and Forestry Sciences and Taigu Fruit Research Institute, when few genotypes of jujube have been introduced in Romania, at the Faculty of Horticulture. The collection was completed in May-June 1998, when few other varieties were grafted on a Romanian rootstock. Other new varieties were introduced after 2016, within the scientific cooperation between Hebei Agricultural University from Baoding and USAMV of Bucharest. In January 2018, was inaugurated the China-Romania Joint Jujube Key Research Laboratory with the same partner (Stănică, 2019).

In the Faculty of Horticulture collection, the genotypes recorded a fruit weight between 5.89 g (R3P10 selection) to 28.57 g (Cheng Tuo Zao). Most of the analyzed genotypes had the soluble solid content higher than 30% Brix. Fruit content in minerals varied between 0.16% and 3.38% with an average of 1.78%. The ascorbic acid content varied between 110.0 mg/100 g fw and 1020.0 mg/100 g fw (R1P11 selection) with an average of 306.1 mg/100 g fw. Fruit acidity, expressed as malic acid, varied from 0.16% to 0.82% with an average of 0.36% (Stănică, 2000; Stănică and Vasile, 2008; Dicianu et al., 2017).

The polyphenol content changes during fruit ripening. Thus, the highest content of polyphenols is found in the fruit at the beginning of its formation and decreases with its maturation, all this time playing the role of protection against pathogens and pests (Shi et al., 2018; Wang et al., 2016).

This study aims to present several biochemical characteristics of the fruits of Chinese jujube from Faculty of Horticulture collection, like total polyphenol, respectively flavonoid content and antioxidant capacity, in order to complete the fruit germplasm biochemical description.

MATERIALS AND METHODS

Ten genotypes from Experimental Field of the Faculty of Horticulture of USAMV of Bucharest were studied: R1P2, R1P7, R1P10 (Hu Ping Zao*), R2P7, R3P2, R3P3 (crack resistant), R3P4 (Hu Ping Zao*), R3P6, R3P10 (Taigu) and Dong Zao (Figure 1). Two clones of Hu Ping Zao (R2P8 and R3P4) and one genotype R3P8 were analyzed in dehydrated form (*clones of different origins) verifying whether or not this method of preservation influences the quality parameters of the fruit.

Fresh fruits were harvested at the beginning of September until October, 2017. After morphological measurements, fruits were stored at 2-3°C and 90-95% relative humidity. Part of them were dehydrated using an Excalibur dehydrator for 20 hours at 45°C.

The physio-chemical analyses were performed in the Researcher Centre for Study of Food Quality and Agricultural Products, USAMV of Bucharest.

Sample Extraction

The jujube fruits (1g) were extracted with 10 mL of ethanolic solution 50%, 30 minutes in ultrasound water bath. The samples were filtrate and after that analyzed.

Determination of polyphenols, flavonoids and antioxidant activity

Total polyphenol content (TPC) was determined spectrophotometrically with the Folin - Ciocalteu method after Skupień (2006), Khanizadeh et al. (2008), Delian et al. (2011), Mureşan et al. (2014) and Bezdadea Cătuneanu et al. (2017), with some modifications. The results expressed as mg gallic acid equivalent (GAE)/g of fresh weight (mg GAE/g FW).
Total flavonoids content (TFC) was determined spectrophotometrically using a method adapted after Zilić et al. (2011) and Shen et al. (2016). The results were expressed as mg rutin equivalent/g of fresh weight.

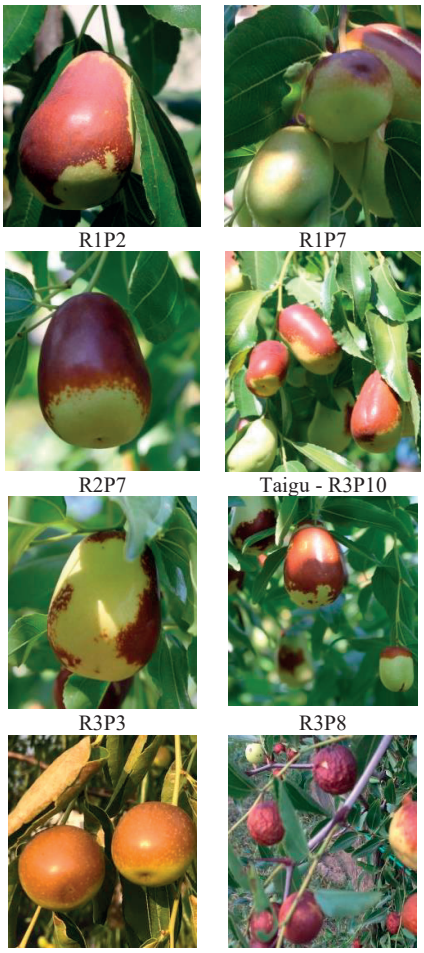

Figure 1. Chinese jujube genotypes

Free radical was determined using DPPH scavenging activity method, after Khanizadeh et al. (2008), Mureşan et al. (2014), Tudor et al. (2015) and Drogoudi et al. (2016). The absorbance of the samples was measured at 515 nm using ethanol as blank. The results were expressed as rate of inhibition and calculated using the formula: $I\% = [(A_0 - A_s)/A_0)] \times 100$, where A_0 was the absorbance of control (DPPH solution), and A_s was the absorbance of the test sample at 30 min.

RESULTS AND DISCUSSIONS

The obtained results showed that genotype influenced the composition of bioactive compounds, similar with Cosmulescu et al. (2018). Of the 10 fresh fruit samples selected, the highest total flavonoid content had the genotype R3P2 (2.03 mg RE/g FW), and the lowest flavonoid content has the R1P2 genotype (0.68 mg RE/g FW).

Flavonoid content of jujube fruits compared with other fruits is equal and higher that jackfruit pulp extracts with 1.20 mg of RE/g FW, found by Basu et al. (2016).
These results are lower than those obtained by Chen et al. (2018) studying *Ziziphus jujuba* cv. Dazao, *Ziziphus jujuba* cv. Junzao, and *Ziziphus jujuba* cv. Huizao cultivars.
On the other hand, the content in biologically active compounds such as polyphenols, flavonoids and antiradical activity, even antioxidant, are strongly influenced by the nature of the extraction solvent. In this regard, it has been shown that ethyl acetate and chloroform are extraction solvents of these compounds from jujube, but it seems strongly influence antioxidant activity (Al-Saeedi et al., 2016). Also, in this sense, the extraction method and ratio are extremely important. Thus, extraction methods as ultra-high-pressure extraction (UHPE) can obtain higher concentrations of total flavonoids and stronger DPPH radical-scavenging activity (Zhang et al., 2019).
In dehydrated fruits the highest total flavonoid content had the genotype R2P8 (1.77 mg RE/g FW) and the lowest genotype of Hu Ping Zao* - R3P4 (1.71 mg RE/g FW) (Figure 2).

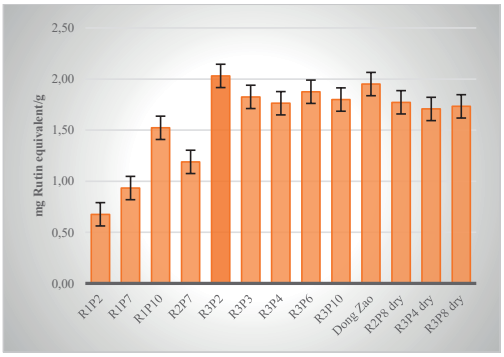

Figure 2. Total flavonoid content in Chinese jujube genotypes

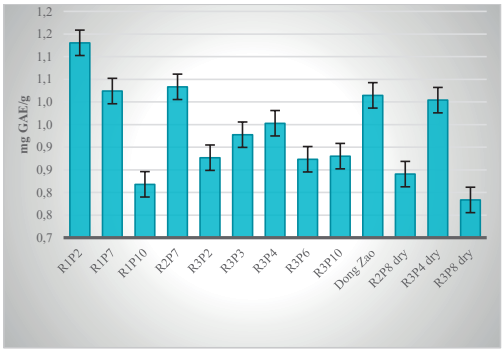

Figure 3. Total polyphenol content in Chinese jujube genotypes

Total flavonoid content in Hu Ping Zao* - R3P4 genotype was higher in the dehydrated samples compared with the fresh ones. The results are similar to those found by Najjaa et al. (2020) (172.07 ± 24.84 mg catechin equivalents (CE)/100 g powder).

Non-significant differences were between fresh samples of Hu Ping Zao clones.

The highest amount of total polyphenols content was found in the R1P2 genotype (1.13 mg GAE/g FW) and the smallest quantity in the Hu Ping Zao* - R1P10 genotype (0.82 mg GAE/g FW). The total content of polyphenol in the dehydrated fruits in the largest quantity was found in the Hu Ping Zao* - R3P4 genotype (1.00 mg GAE/g FW) and the lowest amount in the R3P8 genotype (0.78 mg GAE/g FW) (Figure 3).

These results are lower than those obtained by Koley et al. (2016) studying *Ziziphus jujuba* cutivars: Chuhara, Mundia, Thornless, Jogia, Gola, Kaithali, Umran, Seb, ZG-3, Sonaur-5, Rashmi and Elaichi.

Chinese jujube fruits TPC content it is comparable to those reported for highbush blueberry pulp cultivars that varied between 0.476-2.770 mg/g results obtained by Ribera et al. (2010) and to Perez-Jimenez et al. (2010) for apple 1.35 mg/g, peach 0.59 mg/g, redcurrant 0.43 mg/g.

Similar with flavonoid content, in the dehydrated fruits total polyphenolic content was higher than in fresh ones. Significant differences were between TPC in fresh samples of Hu Ping Zao clones and in dry ones also.

The highest inhibition capacity (I% inhibition) had the Dong Zao genotype and the lowest inhibition capacity had the Taigu - R3P10 genotype (Figure 4). The results from this study were similar with Azizi et al. (2016) who found inhibition capacities between 70.69% and 93.93% inhibition in jujube accessions. Kamiloglu et al. (2009) presented a series of Turkish jujube genotypes with inhibition capacities for dried fruits between 82% and 99%. Dong Zao extract showed the highest inhibition activity among all the cultivars, similar with Xue et al. (2009), justified by the its highest TPC.

No significant differences were noticed between antioxidant capacity values Hu Ping Zao clones.

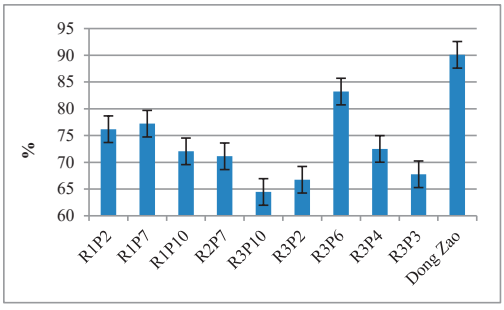

Figure 4. Antioxidant capacity of Chinese jujube genotypes

CONCLUSIONS

Analysis of the biochemical composition of Chinese jujube fruits highlighted the richness of flavonoids, polyphenols and antioxidant capacity.

Among the genotypes studied, Dong Zao cultivated in Bucharest area, known as the best quality fruit for fresh consumption, recorded significant higher values for biochemical parameters than most of the analyzed genotypes.

Regarding the storage of biologically active compounds, this study has shown that the method of preservation by drying can be an effective method of maintaining the nutritional properties of these fruits by giving the consumer the opportunity to consume them in the off-season.

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AGROBIOLOGICAL ASSESSMENT OF APPLICATION OPPORTUNITIES OF NATURAL HUMATES AND PYROLYSIS RESIDUE IN THE PRODUCTION OF APPLE ROOTSTOCKS

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Abstract

Two products are applied as supplements in soil - natural humates and pyrolisis residue - during shoot production of apple rootstock MM106 in stool bed.
Chemical analysis of the experimental supplements shows high levels of organic substances, as well as a supply of macro and micro elements.
Some growth manifestations of shoots are analysed. There are several combinations of shoots: covered by soil with supplements in different doses and without supplements. The improved values of some of the monitored growth indicators are observed in the combinations with soil with supplements, especially the ones with a higher supplement dose. Among the monitored growth indicators, improved plant development is achieved in respect to length, thickness, feathering and rooting which aligns with the standard norms.

Key words*: apple rootstocks, natural humates, pyrolysis residue.*

INTRODUCTION

Adequate supply of nutritional substances, water and air in soil are important determinants for achieving high productivity of root shoots in stool bed plants participating in the development of apple clonal rootstocks (Stamatov et al., 1982; Stojanowska, 1987; Licznar and Licznar, 2004).

There are different methods for improving the performance of productivity determinants. Dobrevska et al., (2015a), Dobrevska et al., (2015b), Popova et al. (2014) experiment with organic supplements in stool bed and find positive results regarding soil substrate and rootstock quality.

Popova et al. (2016) and Dobrevska et al. (unpublished) apply moisture absorbent in the soil covering layer of apple stool bed and report improved values regarding growth and soil indicators in respect to the combinations containing moisture absorbent in different doses. They also show an improved ecological assessment of future soil and conduct an economic evaluation of results when the supplement is applied.

In the context of intensive fruit growing, the effective production process is based on the use of high-quality seedlings. In this respect, it is not only necessary to supply air and water in soil, but also deliver sufficient organic substances due to their important role in the process of apple rootstock production in stool bed. Nowadays, there are various alternative methods to improve organic substance supply in soil which slowly and surely become popular in different fields of agriculture. One of them is the application of natural humates containing some moisture-absorbing crystals and pressed organic substances, the use of which dramatically reduces the demand for water and fertilizers (http://www.terawet.com). Another alternative approach is pyrolisis residue which is derived from the use of biogenic fuels for greenhouse heating. Pyrolisis residue has not been studied before in the field of fruit growing and it deserves research attention (Brezin et al., 2013).

The latter explains the main goal of the study. It accomplishment requires an evaluation of the influence of natural humates and pyrolisis residue as a supplement in the covering soil layer in a stool bed of the MM106 rootstock.

To what extent, the application of natural humates and pyrolisis residue are likely to alter soil conditions and lead to the production of

high-quality shoots from the apple rootstock MM106 - this is the main goal of the current study.

MATERIALS AND METHODS

The stool bed plant is developed from root shoots of the MM106 rootstock.
The experiment is conducted with four replicates for each combination by following the block method of Fisher (Zapryanov and Marinkov, 1978).
Plants are cultivated according to conventional stool bed technology (Trachev et al., 1975). The method includes plant protection measures and multiple soil treatments. The latter are performed with specialized equipment for shallow treatments and soil covering in orchards (Todorov, 1966; Todorov et al., 1974; Trachev et al., 1975). The inter-row strips are managed with the black fallow system. Having said this, there were several shallow inter-row soil treatments leading to improved nutrition supply, water and air regime, as well as, destruction of weeds. There was also a deep inter-row autumn soil ploughing at a depth of 18-20 cm. During the vegetation period, three additional soil covering procedures were performed on the basis of the experimental plants; thereby, contributing to their better rooting.
The supplement of humate tablets (HT) and pyrolisis residue (PR) are delivered to soil at the end of the winter dormancy period. Three combinations were created during the HT experiment: 1st combination - no natural humates; 2nd combination - natural humates equal to 25 kg/dka and 3rd combination - natural humates equal to 50 kg/dka. Three combinations were created during the PR experiment: 1st combination - no pyrolisis residue; 2nd combination - pyrolisis residue equal to 250 kg/dka and 3rd combination - pyrolisis residue equal to 500 kg/dka.
Chemical analysis of the applied supplements – HT and PR - was applied. Findings indicated:
1. Organic substance, % - BSS ISO 14235:2002;
2. Total N, mg/kg - BSS EN 13654-1:2004;
3. Total P, mg/kg - BSS EN 13650:2003;
4. Total K, mg/kg - BSS EN 13650:2003.

Chemical analysis of soil was conducted, as well. The accessed results were recorded at the end of the initial vegetation phase, as well as in the beginning of plants winter dormancy.
Two indicators were monitored, as follows:
1. pH - potentiometric in water solution;
2. Organic substance, % - according to Turin's method
After the end of the vegetation period, the following growth indicators were analyzed in respect to the experimental shoots:
1. Average number of shoots per plant;
2. Average length of shoot, cm;
3. Average thickness of shoot, mm;
4. Average number of feathers per shoot, units;
5. Average length of feathers, cm;
6. Average number of roots per shoot, units;
7. Leaf area, cm^2.
The data from the monitored indicators are statistically processed by following the analysis of variance (ANOVA) method.

RESULTS AND DISCUSSIONS

Chemical analysis shows improved levels of organic substances in both products (Table 1). In the case of humate tablets, organic substances account for 64.54%, whereas their share reaches 78.43% when pyrolisis residue is taken into consideration. The organic substance in both products has a different origin (Filcheva, 2007; Yaneva et al., 2013). In humate tablets, it is plant-based. Hence, humic acids account for 39.03%. They quickly bind with Mg and Ca in soil and develop humates which are easily absorbed by plants. The organic substance in pyrolisis residue contains fulvic acids which are a product of wood. They are very mobile and can be easily washed away from soil (Yaneva et al., 2013). Perhaps, that fact positively influenced the improved performance of experimental plants during the initial year of observations in the combination with humate tablets, despite the larger content of organic substance in pyrolisis residue (Tables 4 and 5).
The chemical analyses of humate tablets showed content of Ca, Mf, Fe and Mn. Respectively, they achieved values of 1.13%, 0.22%, 0.90% and 0.026%. N, P and K are present in both products, as well.

Table 1. Chemical analysis of applied supplements

Name of the characteristic	Humate tablets	Pyrolysis residue
Organic substance, %	64.54	78.43
Total N, mg/kg	2.61	0.29
Total P, mg/kg	0.06	0.16
Total K, mg/kg	2.51	0.25

The conducted analysis of soil with added humate tablets in the covering layer into doses of 25 kg/dka and 50 kg/dka, the organic substance, which enters soil, is 16.135 kg/dka and 32.270 kg/dka, respectively. In the combination with pyrolisis residue into doses of 250 kg/dka and 500 kg/dka, the organic substance is 196.075 kg/dka and 39.150 kg/dka, respectively.

Soil analysis results are presented in Tables 2 and 3. Assessments are performed at the end of the initial vegetation phase and at the beginning of winter dormancy.

The measured pH level in the covering layer in respect to the different combinations determines soil reaction as neutral or slightly alkaline (7.0-7.7).

Table 2. Soil physicochemical properties during initial growth

Samples	pH (H_2O)	Organic substance, %
Soil with humate tablets - 50 kg/dka	7.7	3.79
Soil with humate tablets - 25 kg/dka	7.1	3.03
Soil free of supplements	7.3	2.44
Soil with pyrolisis residue - 500 kg/dka	7.5	3.90
Soil with pyrolisis residue - 250 kg/dka	7.0	3.42
Soil free of supplements	7.2	2.39

Table 3. Physicochemical properties of soil during initial winter dormancy

Samples	pH (H_2O)	Organic substance, %
Soil with humate tablets - 50 kg/dka	7.6	2.49
Soil with humate tablets - 25 kg/dka	7.0	2.11
Soil free of supplements	7.0	1.87
Soil with pyrolisis residue - 500 kg/dka	7.2	2.93
Soil with pyrolisis residue - 250 kg/dka	7.0	2.79
Soil free of supplements	7.1	1.75

In relation to organic substance, the first measurement of organic substance in soils with humate tablets into a dose of 50 kg/dka is 1.3% higher in comparison to the value from the second measurement. The introduction of 32.270 kg/dka (3.79%) of organic matter and a measured residue of 21.201 kg/dka (2.49%) leads to a difference of organic matter equal to 11.069 kg/dka (1.3%). In the case with a dose of 25 kg/dka, the measured organic content is 0.92% higher than the second measurement. With other words, the introduction of organic content equal to 16.135 kg/dka (3.03%) and measured residual of 11.236 kg/dka (2.11%) leads to a difference of 4.899 kg/dka. There is a difference in soil without supplements - 0.57% better values during the first measurement (Tables 2 and 3). Similar tendency, although weaker, is noted during the second experiment with pyrolisis residue supplement. The differences in this case are the following: with a dose of 500 kg/dka - 0.97% better values during the first measurement. Or, when introducing 390 kg/dka of organic matter and measured residual of 293 kg/dka (2.93%), the difference of organic substance is 97 kg/dka (0.97%). Under the scenario with a dose of 250 kg/dka, there are 0.63% more organic substances during the first measurement. The introduction of 195 kg/dka (3.42%) and measured residual of measured residual of 35.921 kg/dka (0.63%) leads to a difference of organic substance content equal to 159.079 kg/dka (2.79). The difference in soils without supplements is 0.64% in favor of the first measurement.

Among other things, all differences in soil samples are due to the absorbed organic substances by plants during the vegetation period.

The monitoring of the vegetative manifestations of experimental plants shows no differences between combinations with respect to the average number of shoots per plant. Findings refer to the first experiment where humate tablets are used into two doses as a supplement in the soil covering layer (Table 4). When the “average length of shoots” indicator is taken into consideration, there is a significant difference between the control and the combination which contains a soil supplement of 50 kg/dka. The supplemented combination has the highest value (Table 4).

Table 4. Growth manifestations of plants cultivated in soil with humate tablets (HT) and with no supplements.

Indicators / Samples	Number of shoots per plant	Shoot length, cm	Average thickness of shoots, mm	Number of feathers per shoot	Feather length, cm	Average number of roots per shoot	Leaf area, cm^2
Soil with humate tablets - 50 kg/dka	17,33	95,38	8,64	5,69	12,90	43,99	17,42
Soil with humate tablets - 25 kg/dka	17,00	82,69	8,38	5,93	6,49	42,33	17,34
Soil without supplements	16,32	69,37	8,09	5,43	4,21	31,18	16,11
GD 5%	2,09	25,98	0,52	0,52	8,67	10,55	1,99
1%	3,31	39,62	1,31	0,75	13,13	15,98	4,04
0,1%	5,26	64,38	2,11	1,28	21,11	25,68	5,11

Table 5. Growth manifestations of plants cultivated in soil with pyrolisis residue supplement and with no supplement.

Indicators / Samples	Number of shoots per plant	Shoot length, cm	Average thickness of shoots, mm	Number of feathers per shoot	Feather length, cm	Average number of roots per shoot	Leaf area, cm^2
Soil with pyrolisis residue –500 kg/dka	17,88	85,49	8,40	4,98	7,92	37,29	16,12
Soil with pyrolisis residue –250 kg/dka	16,88	83,19	8,11	4,59	8,75	37,90	16,44
Soil without supplements	16,00	79,35	8,05	4,45	4,83	34,52	16,01
GD 5%	3,23	22,28	0,32	0,55	12,86	12,49	2,29
1%	4,71	31,22	1,29	0,73	19,48	18,93	4,64
0,1%	6,26	60,38	2,01	1,17	31,31	30,43	5,91

All plants were categorized as first-class quality because the measured thickness covered the required standards (Table 4). Statistically significant differences are shown between the combinations with a higher dose of humate tablets and those with no supplements.

Rootstock feathering at a specific height has a positive effect on their overall development. All shoots have branches without statistically significant differences. In respect to length, however, the combination with the highest content of humate tablets in soil is shown to have the longest branches. Respectively, the shortest branches are found in the combination without supplements (Table 4).

Good rooting is a major quality indicator of shoots. It is shown that the highest number of roots per shoots is observed in the combination with the highest content of humate tablets. Respectively, the lowest number of roots per shoot is found in the combination without supplements (Table 4).

The good development of leaf mass, including crown area, is an important indicator for the quality of rootstock photosynthesis process. There are no statistically significant differences in the leaf area of the different combinations (Table 4).

During the second experiment of using pyrolysis residue into two doses as a supplement in soil covering layer, there are no statistically significant differences among the combinations in relation to the indicators of average number of shoots per plant and average shoot length. Nevertheless, the thickest root shoots are shown in the combination with the highest supplement quantity, whereas the thinnest ones take place in the combination without supplements (Table 5).

All shoots have similar feathering without proved differences in respect to number and length of feathers (Table 5).

Identical results are found in respect to the indicators of “average number of roots” and “leaf area”, as well.

CONCLUSIONS

The study of soil content and development of apple clonal rootstocks in stool bed with different content of humate tablets and pyrolysis residue supplements suggests:

1. High levels of organic substances, macro and micro elements during the conducted chemical analysis of experimental supplements;
2. Improved values of some of the monitored growth indicators in soil combinations containing supplements, especially those with an increased amount of supplements. Among the monitored growth indicators, improved plant development is achieved in respect to length, thickness, feathering and rooting which aligns with the standard norms.

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CHANGES IN CONVENTIONAL AGROTECHNOLOGY IN THE GROW OF APPLE ROOTSTOCKS

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Abstract

The growth manifestations of shoots, which are derived from MM106 rootstock in stoolbed, are studied. Plants are covered by soil containing moisture-absorbing polymer in two dozes - 1,500 kg/dka and 3,500 kg/dka. These two combinations are also compared with the performance of plants which are developed in soil without moisture absorbent. Improved values in some growth indicators are monitored among the combinations with moisture-absorbing polymer and, in particular, with the ones with higher polymer content. Among all vegetative indicators, it is important to note the improved feather shoot formation and rooting among rootstocks developed in soil with moisture absorbent doze of 3,500 kg/dka.

Key words*: apple, MM106 rootstock, moisture absorbent polymer, stoolbed.*

INTRODUCTION

The high quality of rootstocks, which are derived in stoolbed, inevitably contributes to the success of the production of apple trees. Apple clonal rootstocks are produced by covering a vertical shoot which leads to the development of additional rooted shoots.

Studying the method of production in stoolbed has been developed for decades (Trachev et al.; 1975; Gryazev, 1979; Mitov et al., 1979; Andreev, 1979; Koval, 1980; Samus, 1983; Verobyev, 1985; Vehov and Retinskaya, 1988; Quamme and Brownlee, 1990; Karpenchuk, 1993; Pepelyankov and Dobrevska, 1995; Dobrevska and Tabakov, 2002; Lipa and Lipicki, 2006; Dobrevska, 2010; Lipa, 2012; Dobrevska, 2013).

Rooting plays an important role in the process of shoot production in stoolbed. The presence of favourable soil indicators has a key role in the process and their optimisation depends on some conditions. Dobrevska et al. (2015a) and Dobrevska et al. (2015b) study the growth manifestations of M9 and MM106 rootstocks developed in a stoolbed with soils which are enriched by organic additions derived from different types of forest wood varieties. Popova et al. (2014) also use a stoolbed. They examine the impact of the same organic additions on some soil indicators influencing the higher production rate of shoots. These additions increase the organic composition and humidity of soil in important phenophase of plant development, as well as, increase plants' quality (Popova et al., 2014; Dobrevska et al., 2015a; Dobrevska et al., 2015b).

Recently, some unconventional products, such as soil mixtures, have been introduced in agriculture. These are some soil moisture superabsorbents which are used in the cultivation of different crops in agriculture. There have been insufficient studies exploring the influence of soil moisture absorbents in horticulture. Popova et al. (2016) study the impact of moisture absorbent on soil characteristics in a stoolbed of apple rootstocks. They find improved values in some soil indicators.

The purpose of the current experiment is to determine the influence of soil moisture absorbent on growth manifestations and quality of apple clonal rootstock MM106 in stoolbed.

MATERIALS AND METHODS

The root shoots of the experimental stoolbed plant are derived by somatic organogenesis of leaf explants (Dobrevska, 2008).

The experiment is structured according to the block method of Fisher (Zapryanov and Marinkov, 1978). It consists of four replicates for each combination.

After planting, the plants were cultivated according to the conventional stoolbed technology with multiple soil treatments (Trachev et al., 1975). Specialised equipment for orchards is used for the soil treatment procedures, such as fruit disc harrows and cultivators. Universal or specialised tractors were used as energy source (Todorov, 1966; Todorov et al., 1974; Trachev et al., 1975). The most suitable system for stoolbed soil maintenance refers to the so-called black fallow system. As a result, there were 5-7 shallow inter-row soil treatments leading to preservation of its fertility, water and air regime, as well as, destruction of weed vegetation. There was also a deep inter-row autumn soil ploughing at a depth of 18-20 cm. During the vegetation period, three additional soil covering procedures were performed on the basis of the experimental plants, contributing to their better rooting. The moisture-absorbing polymer was introduced in two dozes - 1,500 kg/dka and 3,500 kg/dka - at the beginning of the vegetation period at the base of the root shoots during the initial covering procedure when the average plants' height was 15-20 cm (Todorov et al., 1974).

At the end of the vegetative period, shoots' growth manifestations are analysed in soil with no moisture-absorbing polymer, as well as, in soils with two different dozes of moisture-absorbing polymer - 1,500 kg/dka and 3,500 kg/dka.

The following growth indicators are monitored and reported:

1. Average number of shoots per plant, units;
2. Average length of shoot, cm;
3. Average thickness of shoot, mm;
4. Average number of feathers per shoot, units;
5. Average length of feathers, cm;
6. Average number of roots per shoot, units;
7. Leaf area, cm^2, A = k.1.b, where:

k - coefficient (in the case of apple - 0.69);
l - leaf length;
b - leaf width.

The data from the monitored indicators are statistically processed by following the analysis of variance (ANOVA) method.

RESULTS AND DISCUSSIONS

In respect to average number of shoots per plant and average shoot length, there are no statistically significant differences among the different combinations (Table 1).

There are no statistical significant differences in respect to average shoot thickness (Table 2). According to these indicators, all studied shoots fit within the established first-quality standard norms (Tables 1 and 2).

Table 1. Growth indicators of root shoots

Indicators/ Soil substrate	Number of shoots per plant	Shoot length, cm	Number of feathers per shoot	Feather length, cm
With 3,500 kg/dka moisture-absorbing polymer	16.00	94.40	7.84	8.65
With 1,500 kg/dka moisture-absorbing polymer	16.00	92.69	7.68	6.72
No moisture-absorbing polymer	15.75	90.10	7.08	6.65
GD - 5% - 1% - 0.1%	 2.14 3.24 5.21	 26.87 40.71 65.44	 0.51 0.77 1.24	 1.33 2.02 3.24

Table 2. Growth indicators of root shoots

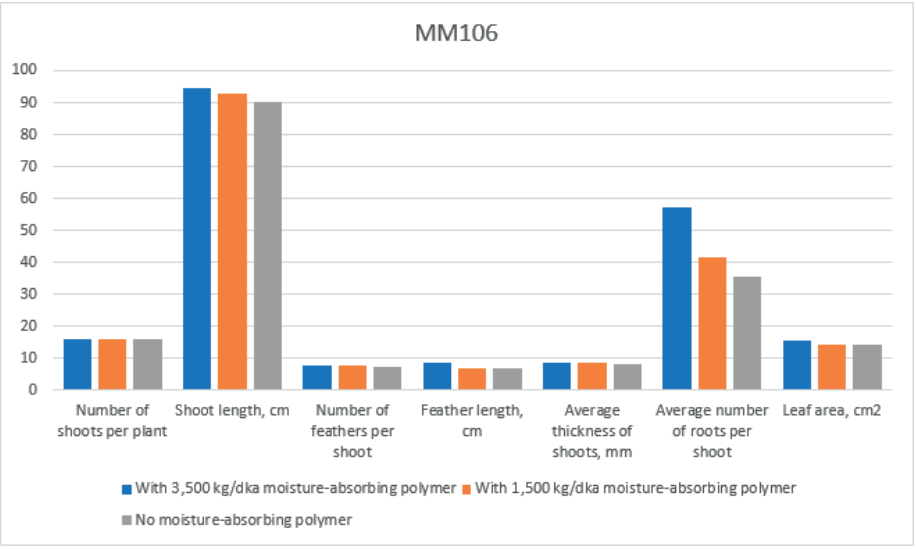
Indicators/ Soil substrate	Average thickness of shoots, mm	Average number of roots per shoot	Leaf area, cm^2
With 3,500 kg/dka moisture-absorbing polymer	8.51	57.24	15.62
With 1,500 kg/dka moisture-absorbing polymer	8.49	41.52	14.29
No moisture-absorbing polymer	8.11	35.35	14.10
GD - 5% - 1% - 0.1%	0.84 1.,28 2.05	12.00 18.91 30.40	2.06 4.14 5.01

It is proven that the combination with the highest content of moisture absorbent leads to the highest number and longest feathers, whereas the combination with no moisture

absorbent has the least and the shortest feathers. The combination with a lower moisture absorbent quantity occupies the middle position in terms of feathers' length (Table 1). The formation of rootstock feathers at an appropriate height has a favourable effect on the overall physiological development of shoots. Therefore, the accessed results suggest that the use of soil moisture absorber has a positive effect on the volume of plants' photosynthetic green mass, although there are no statistically significant differences in terms of total leaf mass of the individual combinations (Table 2).

Rooting is a very important factor in the production of high-quality shoots. The results of our analysis show that the highest number of roots is formed in the combination with the highest amount of soil moisture absorbent, followed by the combination with lower soil moisture absorbent. Finally, the least number of root shoots occurs in the combination without any moisture absorbent (Table 2).

The information on Figure 1 provides a very good description of the above-mentioned interpretation of the presented and statistically processed results.



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CONCLUSIONS

Findings from the examination of apple clonal rootstocks MM106, which are derived from somatic organogenesis of leaf explants in stoolbed with different content of soil moisture absorbent, show that the combinations with moisture absorbent additions in soils demonstrate considerably better values in some of the monitored growth indicators.

These indicators are particularly improved in the cases when higher amounts of additions are present. The increased length and number of feathers, as well as, the better rooting of shoots are among the most indicative growth manifestations. This supports the production of first-class orchard trees material from apple rootstocks when using soil moisture absorbent in stoolbed.

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OPTIMISATION OF AGROTECHNOLOGY IN THE PRODUCTION OF APPLE ROOTSTOCKS

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Abstract

The study investigated some growth manifestations of root shoots derived from M9 rootstock in stoolbed. The plants were covered with soil without a moisture-absorbing polymer and with soil using a different amount of moisture-absorbing polymer - 1,500 kg/dka and 3,500 kg/dka. Improved values in some of the studied growth indicators are observed among the combinations with soil containing moisture-absorbing polymer, especially the ones with higher content. In respect to the studied vegetative parameters, very good rooting of rootstocks is achieved when applying moisture absorbent in a dose of 3,500 kg/dka.

Key words*: apple, M9 rootstock, moisture absorbent polymer, stoolbe.*

INTRODUCTION

The recently introduced intensification in the field of fruit growing requires the production of high quality apple rootstocks in stoolbed.

The traditional way to produce apple clonal rootstocks is by covering of a vertical shoots getting new extra rooted shoots, originating from adventive root buds. This method has been developed for decades (Trachev et al., 1975; Andreen, 1979; Gryazev, 1979; Mitov et al., 1979; Koval, 1980; Samus, 1983; Verobyov, 1985; Vehov and Retinskaya, 1988; Quamme and Brownlee, 1990; Karpenchuk, 1993; Pepelyankov and Dobrevska, 1995; Dobrevska and Tabakov, 2002; Lipa and Lipicki, 2006; Dobrevska, 2010; Lipa, 2012; Dobrevska, 2013).

Good rooting is considered as an important criterion for the production of high-quality shoots. In addition to the type of planting material, a set of soil indicators also tends to influence shoots' development process. Their optimisation depends on various conditions. Dobrevska et al. (2015a) and Dobrevska et al. (2015b) examine the growth manifestations of M9 and MM106 rootstocks which are developed in soils with different content of organic additions (pine wood chips in two varieties) in stoolbed. Similarly, Popova et al. (2014) examine the effects of the wood chips on some soil characteristics which positively influence the production of first-class quality apple clonal shoots. In addition to the above-mentioned soil additions, which improve the organic content and temporary moisture (in the most important periods of shoots' growth phenophase) of the covering soil layer and lead to a higher productivity level, some unorthodox methods, such as moisture superabsorbent, have been implemented in cultivating different crops in many agriculture sectors (Popova et al., 2014; Dobrevska et al., 2015a; Dobrevska et al., 2015b).

So far, experimental studies on the effect of moisture absorbent on plants in horticulture have been almost never done. Popova et al., (2016) investigate the influence of moisture-absorbing polymer on some soil characteristics in a stoolbed of apple rootstocks and show an improvement in some indicators.

The idea of our experiment is to explore the influence of moisture-absorbing polymer on growth manifestations and quality of root shoots derived from M9 rootstock in stoolbed.

MATERIALS AND METHODS

The experimental stoolbed plant is developed from root shoots derived by somatic organogenesis of leaf explants (Dobrevska, 2008).

The experiment was conducted by following the block method of Fisher (Zapryanov and

Marinkov, 1978) with four replicates for each combination.

After planting, the plants were cultivated according to the conventional stoolbed technology (Trachev et al., 1975). The method includes, among other things, multiple soil treatments performed with specialised equipment for orchards - fruit disc harrows and cultivators. Universal or specialised tractors were used as energy source (Todorov, 1966; Todorov et al., 1974; Trachev et al., 1975). The most suitable technological solution for stoolbed soil maintenance, which is also the most typical in our country, refers to the so-called black fallow system. As a result, there were 5-7 shallow inter-row soil treatments leading to preservation of its fertility, water and air regime, as well as, destruction of weed vegetation. There was also a deep inter-row autumn soil ploughing at a depth of 18-20 cm. During the vegetation period, three additional soil covering procedures were performed on the basis of the experimental plants, contributing to their better rooting. The moisture-absorbing polymer was introduced in two dozes - 1,500 kg/dka and 3,500 kg/dka - at the beginning of the vegetation period at the base of the root shoots during the initial covering procedure at a plant height of 15-20 cm (Todorov et al., 1974).

With the purpose of determining the effects of the applied mixture component on plants' performance at the end of the vegetative period, their growth manifestations were analysed in three soil types: with no moisture-absorbing polymer and with moisture-absorbing polymer in two dozes - 1,500 kg/dka and 3,500 kg/dka.

The following growth indicators are monitored and reported:

1. Average number of shoots per plant, units;
2. Average length of shoot, cm;
3. Average thickness of shoot, mm;
4. Average number of feathers per shoot, units;
5. Average length of feathers, cm;
6. Average number of roots per shoot, units;
7. Leaf area, cm^2 (Lazarov, 1965) - $A = k.1.b$, where:

K - coefficient (in the case of apple - 0.69);
L - leaf length;
b - leaf width.

The data from the monitored indicators are statistically processed by following the analysis of variance (ANOVA) method.

RESULTS AND DISCUSSIONS

In respect to average number of shoots per plant and average shoot length, there are no statistically significant differences among the different combinations (Table 1).

Table 1. Growth indicators of root shoots

Indicators / Soil substrate	Number of shoots per plant	Shoot length, cm	Number of feathers per shoot	Feather length, cm
With 3,500 kg/dka moisture-absorbing polymer	9.81	58.77	4.63	12.15
With 1,500 kg/dka moisture-absorbing polymer	9.47	56.21	4.17	10.24
No moisture-absorbing polymer	8.52	55.78	4.01	8.76
GD - 5%	2.11	25.78	0.43	1.24
GD - 1%	3.21	39.62	0.69	1.93
GD - 0.1%	5.18	64.35	1.16	3.15

Nevertheless, given the range of accessed results, it can be noted that the thickest root shoots are reported in the combination with the highest values of moisture-absorbing polymer, whereas the thinnest ones occur in the combination without moisture absorbent (Table 2).

Feathering of rootstocks at a certain height has a beneficial effect on their overall development. The reported differences in respect to the number and length of feathers are statistically significant in all three combinations.

A higher number and longer feathers are observed in the combinations containing moisture absorbent.

Respectively, the highest growth parameters are achieved in the instances where the maximum content of moisture-absorbing polymer is applied (Table 1).

Table 2. Growth indicators of root shoots

Indicators / Soil substrate	Average thickness of shoots, mm	Average number of roots per shoot	Leaf area, cm^2
With 3,500 kg/dka moisture-absorbing polymer	11.93	31.05	17.82
With 1,500 kg/dka moisture-absorbing polymer	11.45	21.12	16.54
No moisture-absorbing polymer	11.15	19.78	14.57
GD - 5%	0.69	9.75	2.37
GD - 1%	1.13	16.66	2.69
GD - 0.1%	1.90	28.15	4.61

Rooting constitutes a very important indicator of the quality of shoots.

The experiment results unambiguously show that the highest number of root shoots is formed in the combination with the highest content of moisture absorbent, followed by the combination with the reduced amount of moisture absorbent. Finally, the least number of root shoots occurs in the combination without any moisture absorbent (Table 2).

An important factor for effective photosynthetic activity of rootstocks is the good health of their leaf mass and total leaf area. Findings show that the largest and the smallest leaf areas are reported in the combinations with the highest quantity of moisture-absorbing polymer and with no polymer, respectively (Table 2). Figure 1 provides a very good visualization of the above-mentioned interpretation of the presented and statistically processed results.

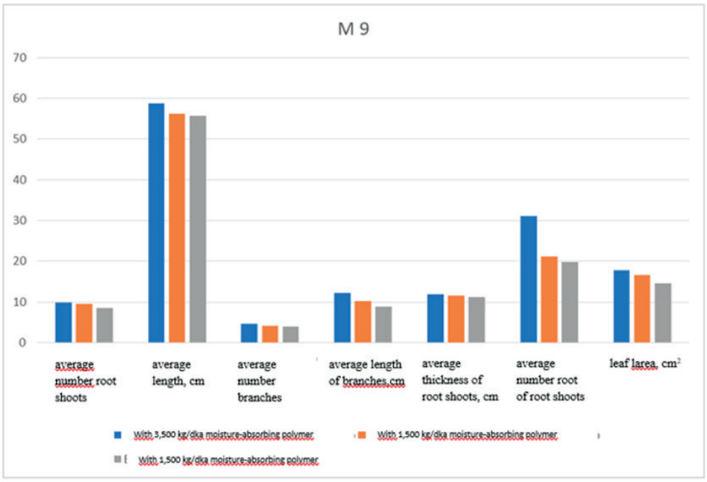

Figure 1. Exemple text to describe to figure above that should be replacement with your information

CONCLUSIONS

The performed examination of the development of apple clonal rootstocks M9, which are derived from somatic organogenesis in stoolbed with different content of soil moisture absorbent, shows that better values of some of the observed growth parameters occur in the soil combinations where moisture absorbent is present.

This is particularly visible in the case with the highest moisture-absorbent content. The better rooting is one of the most notable expressions when all vegetative growth indicators are taken into consideration.

The use of moisture absorbent is a critically important factor for the production of premium quality orchard trees of apple rootstocks in stoolbed.

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RESEARCH REGARDING THE INFLUENCE OF ROOTSTOCK ON THE PRODUCTION AND FRUIT QUALITY FOR THE PINOVA APPLE VARIETY

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Abstract

The research was carried out in the period 2016-2018, in an experimental plantation established in 2015, with the Pinova variety in several graft combinations, directly on the B9, M9, MM 106 and Pi 80 rootstocks, and with B9 as intermediary on the A2 and MM106 rootstocks. The soil was maintained worked along the row for most variants, for 2 variants with grafting intermediary the soil was kept grassed, and for other 2 variants the intermediary was buried at planting. The rootstock influenced fruit production and fruit quality differently. The highest average production in the first years of fructification, of over 26 t/ha, were obtained for the rootstocks B9, M9 and combinations with intermediary of B9/MM106. The largest fruit were obtained from trees grafted with intermediary on A2 and worked soil, over 169 g and about 78 mm in diameter. A higher amount of soluble dry matter was recorded for the fruit obtained from grafted trees from variants V2, V5 and V8, while higher carbohydrate content was recorded for the variants V2, V8 and V1. The best firmness had the fruit of the V8 variant, while higher content in anthocyanins had the fruit of the V8 and V7 variants.

Key words*: grafting, quality, production.*

INTRODUCTION

Apple culture is one of the most important cultures globally, while in Romania it occupies the first place. Fruits are capitalized as fresh or processed under different forms: jam, marmalade, compote, juice, dehydrated etc. Fruit quality depends on several factors, such as: age of the trees, rootstock used, using grafting intermediaries, intensity of pruning, management of soil (Zho et al., 1983; Lord et al., 1985; Dudu et al., 2015; Asanica et al., 2013; D` Abrosca et al., 2017; Iordănescu et al., 2018). Due to a relatively high consumption of workforce with pruning and harvesting, attempts are made regarding the reduction of the size of the trees and the increase of number of trees per unit of area (Hrutko, 2013). The intensification implies the existence of small rootstocks on one side, but also ensuring a good anchoring in the soil of the root system on the other hand. The small rootstocks frequently used in the modern fruit growing (M9, M27, B9) have a superficial rooting and require a support system the increases the production costs, but also imply a very careful irrigation and fertilization in order to ensure the water and nutrition necessary for the trees (Zhou et al., 2016). Using a grafting intermediary implies increasing the time needed to obtain trees by one year, and for this attempts are made to find solutions for producing trees in two years (Baciu et al., 2008). The grafting combinations used until now did not result in a tree close to the ideal tree, which is why research is still conducted to simplify the culture technology and to decrease the costs, but with maintaining a high fructification potential and a good commercial quality of the fruit. In order to ensure a better anchoring of the trees and to benefit from the soil resources, but in the same time to maintain the trees small, several grafting combinations have been tested, with low vigor intermediary on vigorous rootstocks (Bărăscu et al., 2017). It was observed that the intermediary

used for grafting positively influences fruit quality (Vercammen et al., 2007; Zho et al., 1983; Samad et al., 1999), productivity (Rufato et al., 2006; Samad et al., 1999; Filho et al., 2019), increases the ramification capacity for young trees (Bărăscu et al., 2017), decreases tree vigor expressed through height and diameter of the trunk (Tojuko et al., 2007; Di Vaio et al., 2009; Oliviera et al., 2019; Karlidag et al., 2014), but also tree precocity (Webster et al., 1995). Research conducted by Samad, McNeil and Khan using as intermediary the M9 rootstock showed a reduction of tree size by 20% while the production increased by 30% (Samad et al., 1999). The length of the used intermediary influences the vigor, the elements being inversely proportionally correlated. A longer intermediary determined a better ramification capacity and a good precocity (Karlidag et al., 2014).

MATERIALS AND METHODS

The experiment was conducted during 2016-2018, within the Vâlcea plant nursery, situated in the northern part of the Vâlcea County, in the Mihăiești locality. The soil was typical preluvosoil, with medium texture and weakly alkaline reaction on the surface and weakly acid in the root growth area. The supply with mineral elements was medium. The plantation was established in 2015, with the Pinova variety, grafted into many combinations as it follows:

V1 - Pinova/B9/A2, grassed soil
V2 - Pinova/B9/A2, worked soil
V3 - Pinova /B9/A2 intermediary buried, grassed soil
V4 - Pinova/B9/MM106, grassed soil
V5 - Pinova/B9/MM106, worked soil
V6 - Pinova/B9/MM106 intermediary buried, worked soil
V7 - Pinova/B9
V8 - Pinova/M9
V9 - Pinova/MM106
V10 - Pinova /Pi 80

The technology applied in the orchard was specific for an intensive apple plantation, while the soil was maintained grassed for the majority of the variants, as shown in the experimental scheme. During fruit harvesting, the production per tree was recorded, the production per unit of area was calculated and measurements were made regarding fruit quality. For analysis purposes, 10 fruit from each part of the crown were harvested, while the physical-chemical measurements were made based on average samples resulted from these fruits.

Physical-chemical analysis

Determinations regarding fruit quality were made at the Research Center for Studies of Food Quality and Agricultural Products - University of Agronomic Sciences and Veterinary Medicine of Bucharest, and consisted of physical analysis: average weight, caliber and firmness, and also biochemical analysis: soluble dry matter, titrable acidity, carbohydrates and anthocyanins.

Average fruit weight was measured using the balance with two decimals, WTB 2000.

Fruit caliber was determined using the caliper, two readings being made for each fruit on two different directions.

The ***dry matter and water content*** of the samples were determined by oven drying for 24 hours at 105°C using a UN110 Memmert oven, method used also by Delian (2011), Mureşan (2014), Ticha (2015). To determine the fruit firmness an electronic penetrometer TR was used, and the results were expressed in kg/cm^2 (Chen, 2015).

Carbohydrates were determined from apple juice (Mureşan, 2014; Oltenacu, 2015), with refractive device Kruss DR301-95 (% Brix).

Titratable acidity calculation was done using the formula: $\frac{F \times C \times a \times b \times 100}{b \times c}$, where F is the factor NaOH solution 0.1 N (1,002), C = coefficient of correction for citric acid (0.0064), a = quantity of 0.1 N NaOH titrated, b = volume of the extraction solution, c = mass of the sample. For titration with 0.1 N NaOH the automatic titrator TitroLine easy was used. The results were expressed in g citric acid/100 g.

Total anthocyanins content was measured with spectrophotometric absorbance at wavelength λ = 540 nm (Bărăscu et al., 2016), after an adapted method. The extracts were filtered under vacuum and completed up to 50 ml volume. The results were calculated using the formula: Total anthocyanins = DO_{540} x F, where DO_{540} is absorbance at wavelength λ = 540 nm and factor F = 11.16. The total anthocyanins content was expressed in mg/100 g in fresh weight. The

interpretation of the results was made through variant analysis.

RESULTS AND DISCUSSIONS

The production capacity of the Pinova variety was very good, starting with the second year in the orchard the trees fructified and the average production for the first 3 years was over 9 kg/tree, with a maximum value of 12.7 kg/tree for the variant V5 (Table 1).

Using the MM106 rootstock with M9 intermediary led to very good results for the variants with normal tree planting (V4 and V5), with the partial burring of the intermediary slightly decreased the production (V6).

The heaviest fruit weight was obtained when using the vigorous A2 rootstock with B9 as intermediary, the fruit having values over 180 g. A positive influence over fruit size was also recorded for the MM 106 rootstock, with or without intermediary, the values being over 170 g, compared to the fruit obtained for the M9 grafting combinations, where fruit weight was under 140 g.

The fruit caliber was correlated with the average weight, the correlation coefficient being r^2 = 0.68 (Figure 1).

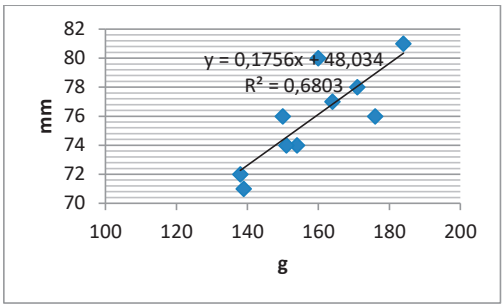

Figure 1. Correlation between the average weight and the diameter of the fruit

Table 1. The production capacity for the Pinova variety grafted on different rootstocks

Variant	Production (kg/tree)		Production (t/ha)		Average weight (g)		Caliber (mm)	
	Media	± St. Dev.	Media	± St. Dev.	Media	± St. Dev.	Media	± St. Dev.
1	9.0	0.24	20.0^{000}	2.15	150.62***	13.57	76.25***	4.10
2	9.5	0.51	21.1^{000}	3.16	138.34 N	25.26	72.50 N	4.65
3	11.6	0.23	25.7^{000}	4.25	184.49***	16.74	81.00***	3.35
4	12.3	0.72	27.3***	4.29	176.50***	22.66	75.84***	5.80
5	12.7	0.15	28.2***	3.78	160.71***	37.77	80.50***	6.95
6	11.9	0.16	26.4 N	3.87	154.68***	24.38	74.34**	4.45
7	12.0	0.11	26.6 N	2.98	164.14***	17.22	76.67***	5.32
8	11.9	0.14	26.4 C	3.64	139.14 C	10.44	71.15 C	4.75
9	10.0	0.09	22.2 ^{000}	2.24	171.58***	30.95	78.00***	6.20
10	11.3	0.12	25.1000	3.12	151.47***	9.91	74.00**	4.60
DL 5%			0.26		2.02		1.82	
DL 1%			0.37		2.91		2.61	
DL 0.1%			0.55		4.28		3.84	

The water content of the fruit was slightly influenced by the grafting combination, the values obtained being between 79.04 and 83.4%.

A higher value was obtained for V4, the variant with large fruit, which showed that growth was determined through a better fruit hydration (Table 2).

The total dry matter depended on the water content, the correlation between the two parameters being a negative one, with a coefficient value of r^2 = - 0.49 (Figure 2).

The fruit mineral content, expressed through the ash content, did not have a strong correlation with the dry matter, the coefficient being 0.47.

Fruit firmness was influenced by the grafting combination, the firmest fruit were obtained from the trees grafted on the M9 rootstock, the value measured being 9.24 kgf/cm^2, while the lowest recorded value for the trees grafted on MM106 with B9 intermediary, of 7.22 kgf/cm^2.

Titrable acidity was slightly influenced by the grafting combination, a higher value being recorded for the fruit obtained from trees grafted

on MM 106 with intermediary M9, while the lowest value was recorded for the fruit obtained from trees grafted on A2 with intermediary B9 (Table 3).

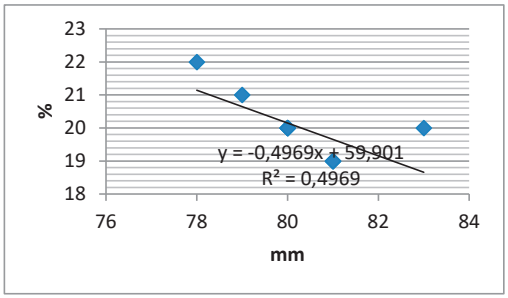

Figure 2. Correlation between total dry matter content and fruit diameter

The carbohydrates content had similar values amongst the variants, the deviation being under 1.5%.

The content in anthocyanins was heavily influenced by the rootstock or the grafting combination. Thus, the highest content was recorded for the fruit obtained from the trees grafted on B9, 2.16 mg/100 g, followed by the rootstock Pi 80, with a content of 1.72 mg/100 g. A good accumulation of anthocyanins was also determined by the rootstock MM 111, especially the variants V4 and V5 with over 1.48 mg/100 g, compared to only 0.98 mg/100 g for the fruit obtained from the trees grafted on the rootstock M9, the rootstock most used in super intensive plantations.

Table 2. Influence of the grafting combination on some physical indicators of the fruit

Variant	Water content (%)	Total dry matter (%)	Ash content (%)		Fruit firmness (kgf/cm^2)	
			Media	± St. Dev.	Media	± St. Dev.
1	80.22	19.78	0.26	0.01	7.98^{000}	0.52
2	78.25	21.75	0.44	0.02	8.2^{000}	0.56
3	81.40	18.60	0.28	0.01	7.47^{000}	0.78
4	83.48	16.52	0.20	0.01	7.22^{000}	0.58
5	79.04	20.96	0.24	0.02	7.86^{000}	0.43
6	81.33	18.67	0.28	0.01	8.13^{000}	0.82
7	80.34	19.66	0.27	0.01	8.03^{000}	1.1
8	79.53	20.47	0.21	0.01	9.24 C	0.67
9	80.69	19.31	0.37	0.02	7.93^{000}	0.75
10	80.14	19.86	0.38	0.02	7.99^{000}	0.69

DL5%=0.18 kgf/cm^2, DL 1%=0.26 kgf/cm^2, DL 0.1%=0.39 kgf/cm^2

Table 3. Influence of the grafting combination on some biochemical indicators of the fruit

Variant	Titrable acidity (g malic acid/100 g m.v.)		Carbohydrates (Brix %)		Anthocyanins (mg/100 g)	
	Media	**± St. Dev.**	**Media**	**± St. Dev.**	**Media**	**± St. Dev.**
1	0.32	0.0019	13.17	0.995	1.14	0.092
2	0.44	0.0019	13.02	1.052	1.04	0.018
3	0.43	0.0038	13.44	1.270	1.00	0.077
4	0.45	0.0009	13.52	0.815	1.58	0.005
5	0.50	0.0009	14.35	0.992	1.48	0.091
6	0.45	0.0009	13.17	0.863	1.17	0.078
7	0.41	0.0009	13.50	0.863	2.16	0.037
8	0.43	0.0009	14.34	1.441	0.98	0.209
9	0.48	0.0009	13.83	1.015	1.35	0.024
10	0.39	0.0009	13.22	1.220	1.72	0.076

CONCLUSIONS

The present study confirms the statements from the specialty literature regarding the possibility to improve fruit quality by using grafting intermediary. Using the rootstock B9 in combination the with vigorous rootstocks A2 and MM 111 led to an increase in fruit size, a slightly increased content in soluble dry matter, a better firmness and fruit coloring compared to the rootstock M9.

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CONTENT OF MACRO, MICROELEMENTS AND PIGMENTS IN LEAVES OF 'TEGERA' AND 'ELENA' PLUM CULTIVARS IN DIFFERENT FERTILIZATION VARIANTS

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Abstract

At the Research Institute of Mountain Stockbreeding and Agriculture in Troyan, Bulgaria, in the period 2017-2018, the influence of different types of fertilization (conventional and organic) on the content of the basic nutrients (macro and microelements), as well as some pigments in the leaves of 'Tegera' and 'Elena' plum cultivars was studied. The established parameters and the dynamics of changes in the values of nutrients and pigments composition in the leaf samples, before and after the harvesting of fruits, significantly determine some of the characteristics of the physiology and genotypic specificity of the cultivars. It has been reported that the amount of pigment content of the fertilizer variants of 'Tegera' cultivar decreased after harvest and increased in the control trees. For 'Elena', after the harvest, the leaf mass in the conventional fertilization variant increased its percentage of nitrogen and phosphorus.

Key words*: plum, fertilization, leaves, nutrients, pigments.*

INTRODUCTION

It is well known that fertilizers are needed to improve vegetative activity and increase the productivity of fruit trees. Fertilization is one of the main agrotechnical events that increase the yield per unit area (Georgiev et al., 2019). In order to determine the optimal fertilizer rates/doses, it is important to identify first the nutritional needs of trees through plant and soil analysis.

Leaves are the main and most commonly used plant organ, so, analysing and determining their chemical composition give an idea of the degree of supply of plants with nutrients (Volodarsky, 1971; Guo-yi et al., 2015). According to Gorbanov (2018), foliar nourishment gives good results in a number of vegetable and fruit crops. To affect plants, leaf fertilizers must be easily absorbed, transported quickly, and easily release their ions (Larue et al., 1989).

The use of fertilizers for the purpose of improving the quality and yield has an effect on the pigments, macro and microelements contained in the leaf matter (Yancheva, 2002). Iron (Fe) complexes with protein forming important enzymes in plants are linked to chloroplasts, which play an essential role in chlorophyll synthesis. Chlorophyll is the main green photosynthetic pigment found in plants through which they absorb the light energy (Kiang et al., 2007). The macronutrients, such as potassium (K) and magnesium (Mg) are involved in the photosynthesis process and in a number of plant functions (Tränkner et al., 2018). This requires monitoring the nutrient content of the foliage of fruit species.

The aim of the present study is to evaluate of the content of the basic nutrients (macro and microelements), as well as some pigments in the leaf mass of four fertilization variants analyzed before and after harvesting of fruits of 'Tegera' and 'Elena' plum cultivars.

MATERIALS AND METHODS

Location of the experiment

The experiment was conducted in 2017-2018 at the collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture - Troyan, Bulgaria.

Biological material
The plantation with both plums cultivars was created in the spring of 2001.
The trees were planted in trenches with organic stockpile fertilizing (130 kg/1 linear meter). Planting distances are 4/2.5 m.
The row spacing is grassed with tall fescue, and the intra row spacing is maintained in black fallow.
During the vegetation season, the necessary mowing in the interrows and digging in the intra rows were carried out, as well as other agrotechnical events such as pruning, plant protection etc.

The experimentally variants:
- **Variant I** - biofertilisation - including the following fertilizers: Agriful (soil) - 5 l/da, Tecamin Flower (foliar) - 0.3%, Tecnocel Amino Ca (foliar) - 0.4%;
- **Variant II –** conventional fertilisation - Yara Mila Complex (soil) - 0.500 kg/tree, YaraVita Frutrel (foliar) - 0.500 ml/da, Yara Vita Universal Bio (foliar) - 0.500 ml/da, ammonium nitrate - 0.200 g/tree;
- **Variant III** - organic fertilisation (granulated chicken manure - 0.500 kg/tree);
- **Variant IV** - Control (without fertilisation).

Fertilization periods:
- **Agriful** - applied five times from the beginning of vegetation over a period of 15-20 days;
- **Tecamin Flower** - 2 applications. Applied before blossoming and during the fruit-set formation;
- **Tecnocel Amino Ca** - 2 applications. Applied after blossoming and a month before harvesting;
- **Yara Mila Complex** - 1 application in 2018;
- **Ammonium nitrate** - 1 application in 2017;
- **YaraVita Frutrel** - four applications: at the phase of winter buds, at the phase of white button, at the fruit-set formation and a month after the harvest;
- **Yara Vita Universal Bio** - 3 applications: before and after blossoming, and after harvest;
- **Granulated chicken manure** - one application in 2018.

Figure 1. Plum plantation with 'Tegera' and 'Elena' cultivars

The analysed parameters leaf samples were taken seven days before ripening of the fruit and immediately after harvest (following day).
- plant analysis
  – In 2017 years Macro- and micronutrients were determined by atomic absorption spectrophotometer. Sample burned in muffle furnaces and dissolution in 20% HCl (Mincheva and Brashnarova, 1975). In 2018 there were determined only macronutrients (N, P, K).
  – Total nitrogen-Kjeldahl method by digestion with concentrated H_2SO_4 and 30% H_2O_2.
- chlorophyll "a" (mg/g FW), chlorophyll "b" (mg/g FW) and β-carotene (mg/g FW) were determined by spectrophotometric analysis. Extraction of plant pigments was performed with 85% acetone.

Statistical analysis
The following statistical analyzes were used to process the data obtained from the experimental work of the examined varieties:
variational analysis, one-way ANOVA and correlation analysis.

RESULTS AND DISCUSSIONS

The experimental data in the 2017 years are presented in Table 1.

Table 1. Content of chlorophyllian pigments and β-carotene, micro and macroelements in the leaves of 'Tegera' and 'Elena' plum cultivars for 2017

Indicators	N	P	K	Ca	Mg	Zn	Cu	Mn	Fe	Chloro phyll "a"	Chloro phyll "b"	β-carote ne
	%				mg/kg FW					mg/g FW		
before fruit ripening												
'Tegera'												
I Biofertilizer	1.36	0.28	2.8	1.21	0.32	35	5	28	98	2.29	1.74	1.14
II Conventional	1.08	0.25	2.6	1.26	0.30	23	12	26	91	1.96	1.02	1.01
III Chicken manure	1.20	0.29	3.1	1.36	0.28	18	12	31	109	2.03	1.05	1.07
IV Control	1.09	0.29	2.9	1.24	0.29	19	8	32	88	1.86	1.07	0.97
St. error	0.06	0.01	0.10	0.03	0.01	3.90	1.70	1.37	4.66	0.09	0.17	0.03
St. Dev.	0.13	0.02	0.20	0.06	0.01	7.80	3.40	2.75	9.32	0.18	0.34	0.07
CV %	11.01	6.82	7.30	5.12	5.74	32.86	36.79	9.40	9.66	9.02	28.46	7.07
'Elena'												
I Biofertilizer	0.63	0.23	2.7	1.04	0.29	12	5	33	98	1.26	0.62	0.68
II Conventional	0.56	0.25	2.9	0.95	0.26	14	12	18	91	1.77	0.83	0.91
III Chicken manure	1.25	0.25	2.5	1.00	0.26	13	12	41	109	1.74	0.99	0.91
IV Control	1.16	0.30	2.8	1.15	0.26	11	8	13	88	1.37	0.88	0.71
St. error	0.17	0.01	0.08	0.04	0.01	0.64	1.70	6.49	4.66	0.12	0.07	0.06
St. Dev.	0.35	0.02	0.17	0.08	0.01	1.29	3.40	12.99	9.32	0.25	0.15	0.12
CV %	39.47	11.59	6.26	8.21	5.60	10.32	36.79	49.51	9.66	16.82	18.69	15.54
after fruit harvesting												
'Tegera'												
I Biofertilizer	0.70	0.25	3.0	2.72	0.36	30	16	25	115	1.63	0.80	0.87
II Conventional	0.90	0.20	2.6	3.22	0.37	39	7	21	97	1.89	0.96	0.99
III Chicken manure	1.12	0.33	3.3	3.10	0.36	28	21	15	171	1.51	0.77	0.80
IV Control	1.16	0.32	1.8	3.02	0.41	19	8	47	132	2.11	1.55	1.07
St. error	0.10	0.03	0.32	0.10	0.01	4.10	3.34	6.97	15.79	0.13	0.18	0.06
St. Dev.	0.21	0.06	0.65	0.21	0.02	8.20	6.68	13.95	31.58	0.26	0.36	0.12
CV %	21.98	22.31	24.29	7.06	6.34	28.29	51.41	51.67	24.53	15.04	35.59	12.93
'Elena'												
I Biofertilizer	0.53	0.42	3.4	2.24	0.37	14	39	15	133	0.92	0.72	0.55
IIConventional	0.69	0.20	3.8	2.06	0.33	22	17	7	104	1.04	0.61	0.58
III Chicken manure	0.76	0.29	3.1	2.43	0.28	14	42	18	105	1.48	0.78	0.79
IV Control	0.55	0.39	3.3	1.96	0.31	14	16	7	102	0.71	0.43	0.42
St. error	0.05	0.05	0.14	0.10	0.01	2	6.95	2.80	7.35	0.16	0.07	0.07
St. Dev.	0.11	0.10	0.29	0.20	0.03	4	13.91	5.61	14.71	0.32	0.15	0.15
CV %	17.52	30.82	8.65	9.53	11.70	25	48.82	47.82	13.26	31.32	24.20	26.20

The results from 2017 with respect to the basic nutrients in the leaves before fruit ripening determine that nitrogen was in the range of 0.56% (conventional fertilization of 'Elena' cultivar) to 1.36% (biofertilization of 'Tegera'). The variation coefficient of that element in the cultivars before ripening is from low to medium and after ripening from medium to high. Decreases in that macroelement were also reported in 2018 after fruit harvesting (Table 2). According to Dinkova (2009), the optimal levels of nitrogen and phosphorus in the leaves should be at the intervals: for N (2.5-3.2%) and P_2O_5 (0.38-0.48%). Similar is Kvong (1973) opinion that nitrogen reserves in leaves should be 2.1%. The inverse dependence was found in the phosphorus content. After fruit harvesting in 2017, the amount of the element increased in 'Tegera' up to 0.32-0.33% in the chicken manure variants and the control. Phosphorus content in 'Elena' cultivar was increased to 0.42% in the variant with application of biofertilizers.

Table 2. Content of chlorophyllian pigments, β-carotene and macroelements in leaves of 'Tegera' and 'Elena' cultivars for 2018

Indicators	N	P	K	Chlorophyll "a"	Chlorophyll "b"	β-carotene
	%			mg/g FW		
before fruit ripening						
'Tegera'						
I Biofertilizer	1.10	0.13	2.0	1.35	0.79	0.73
II Conventional	0.97	0.08	1.4	1.07	0.74	0.62
III Chicken manure	0.95	0.10	1.6	1.32	0.89	0.72
IV Control	0.89	0.12	1.5	1.19	0.72	0.67
St. error	0.04	0.01	0.26	0.06	0.03	0.02
St. Dev.	0.08	0.02	0.13	0.12	0.07	0.05
CV %	9.04	20.62	16.18	10.44	9.67	7.39
'Elena'						
I Biofertilizer	0.85	0.39	2.7	1.26	0.73	1.59
II Conventional	0.56	0.31	3.1	1.03	0.51	1.38
III Chicken manure	0.76	0.54	3.4	1.08	0.51	1.34
IV Control	0.90	0.55	2.6	1.01	0.45	1.32
St. error	0.07	0.05	0.18	0.05	0.06	0.06
St. Dev.	0.14	0.11	0.36	0.11	0.12	0.12
CV %	19.54	26.21	12.53	10.39	22.41	8.82
after fruit harvesting						
'Tegera'						
I Biofertilizer	0.90	0.74	4.3	1.26	0.67	0.70
II Conventional	0.69	0.53	3.8	0.96	0.65	0.54
III Chicken manure	0.73	0.84	4.3	1.25	0.67	0.69
IV Control	0.83	0.65	3.8	1.62	1.21	0.86
St. error	0.04	0.06	0.14	0.13	0.13	0.06
St. Dev.	0.09	0.13	0.28	0.27	0.27	0.13
CV %	12.10	19.11	7.12	21.23	34.18	18.74
'Elena'						
I Biofertilizer	0.74	0.48	2.6	1.25	0.48	1.55
II Conventional	0.57	0.36	2.4	1.05	0.35	1.25
III Chicken manure	0.76	0.55	3.4	1.11	1.26	1.33
IV Control	0.74	0.62	3.1	0.71	0.28	0.93
St. error	0.04	0.05	0.22	0.11	0.22	0.12
St. Dev.	0.08	0.11	0.45	0.22	0.45	0.25
CV %	12.64	22.06	15.90	22.25	76.39	20.30

The analysis conducted in 2018 reports an increase in the amount of phosphorus in all variants for both plum cultivars. A slight difference was found in the potassium content in the second experimental year among the different cultivars and variants, before and after fruit ripening. Its amount for 2017 was in the range of 1.8% (nontreated control 'Tegera', after fruit harvesting) to 3.8% (conventional fertilization of 'Elena', after fruit ripening). The lowest potassium content was registered in 2017 in all variants of fertilization before fruit ripening of 'Elena'.

Calcium and manganese content increased after fruit harvesting in the year 2017. Calcium content before ripening was about 1 mg/kg FW and after harvest about 2-3 mg/kg FW for both cultivars in the four variants. The analysis shows that the microelements content, such as zinc and copper increased significantly after the harvest. Zinc content was higher in the conventional fertilizer variant for all tested plum cultivars, and copper content reached 42 mg/kg FW in 'Elena' cultivar. Manganese content over the experimental period varied significantly from 7 to 41 mg/kg FW. The iron content also increased in the leaves after fruit harvesting, most strongly in 'Tegera' ranging from 88 mg/kg FW to 171 mg/kg FW.

Chlorophyll "a" in 2017 ranged from 1.37 mg/g FW ('Elena') to 2.29 mg/g FW ('Tegera'). After fruit harvesting, its content decreased to 0.92 mg/g FW (in the biofertilization variant, 'Elena' cultivar).

In the following year, lower chlorophyll "a" content was again reported after harvesting. Chlorophyll "b" pigment had similar values throughout the experimental period, before and after fruit ripening.

The green pigments chlorophyll "a", chlorophyll "b", and β-carotene in 2017 and 2018, before ripening and after harvesting, had higher values for 'Tegera' in comparison with 'Elena'.

Table 3. Correlation dependences between chlorophyllian pigments, β-carotene and basic macroelements in the leaves analyzed before and after fruit harvesting of 'Tegera' and 'Elena', average for the period 2017-2018

Cultivar		N, %	P, %	K, %	Chlorophyll "a", mg/g FW	Chlorophyll "b", mg/g FW	β-carotene, mg/g FW
'Tegera'	**before harvesting**						
	N, %	1.00					
	P, %	0.37	1.00				
	K, %	-0.40	0.67	1.00			
	Chlorophyll "a", mg/g FW	0.96	0.53	-0.27	1.00		
	Chlorophyll "b", mg/g FW	0.98	0.51	-0.23	0.95	1.00	
	β-carotene, mg/g FW	0.92	0.53	-0.27	0.99	0.90	1.00
	after harvesting						
	N, %	1.00					
	P, %	0.52	1.00				
	K, %	-0.75	0.02	1.00			
	Chlorophyll "a", mg/g FW mg/gFW	0.71	-0.05	-1.00	1.00		
	Chlorophyll "b", mg/g FW mg/gFW	0.74	-0.10	-0.99	0.99	1.00	
	β-carotene, mg/g FW	0.70	-0.05	-1.00	1.00	0.98	1.00
'Elena'	**before harvesting**						
	N, %	1.00					
	P, %	0.98	1.00				
	K, %	-0.36	-0.34	1.00			
	Chlorophyll "a", mg/g FW mg/gFW	-0.36	-0.40	0.95	1.00		
	Chlorophyll "b", mg/g FW mg/gFW	0.45	0.34	0.47	0.61	1.00	
	β-carotene, mg/g FW	-0.69	-0.79	0.58	0.76	0.30	1.00
	after harvesting						
	N, %	1.00					
	P, %	0.13	1.00				
	K, %	0.25	-0.34	1.00			
	Chlorophyll "a", mg/g FW mg/gFW	0.65	-0.40	-0.17	1.00		
	Chlorophyll "b", mg/g FW mg/gFW	0.91	-0.04	-0.04	0.90	1.00	
	β-carotene, mg/g FW	0.43	-0.31	-0.48	0.94	0.77	1.00

The correlation dependences were made between the pigment's composition and the main macroelements in the leaves of 'Tegera' and 'Elena' plum cultivars before and after harvesting (Table 3). They showed that nitrogen in the leaves, before and after ripening of 'Tegera', correlated positively with pigments, such as chlorophyll "a", chlorophyll "b" and β-carotene. After fruit harvesting of 'Elena', a high variation coefficient was reported between nitrogen and chlorophyll "b" (r = 0.91).

CONCLUSIONS

A comparative study was conducted on the effect of different types of fertilization (conventional and organic) on the content of basic nutrients (macro and microelements) chlorophyllian pigments and β-carotene in the leaves of 'Tegera' and 'Elena' plum cultivars. It was found that the values of macroelements and pigment composition in the leaf samples are variable during pre- and post-harvesting periods

for the individual elements and to a large extent determined some characteristics of the physiology and genotypic specificity of the cultivars.

It was found that the amount of chlorophyllian pigments content in the fertilizing variants with 'Tegera' cultivars decreased after harvesting and increased for control trees. Nitrogen and phosphorus increased their content in the leaves of 'Elena' after fruit harvesting in the variant with conventional fertilization.

'Elena' showed a decrease in the values of the pigment content in the leaves after the harvest of the fruit with some exceptions, with a slight increase in the amount of chlorophyll "b" from the 2017 version of biofertilization and significantly larger differences in the same pigment from the chicken manure variant for 2018 (before-0.51 and after fruit harvesting -1.26).

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KIWIFRUIT *(ACTINIDIA* SPP.) PHENOLOGICAL GROWTH STAGES IN SOUTHERN ROMANIAN CLIMATE ACCORDING TO THE BBCH SCALE

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Abstract

The aim of this paper is to present the phenological growth stages of two kiwifruit cultivars (Hayward and Bruno) and some Romanian intra and interspecific Actinidia hybrids. The experimental field was established in 2000. The plants were grown on a T-bar trellis system, under an organic orchard management. The inter row surface was covered with a mixture of perennial grasses and along the row, the soil was kept clean. Drip irrigation and micro spray irrigation system was provided. The phenological growth stages were described in the environmental conditions of Romanian plain (Bucharest area), according to the BBCH General Scale (Meier, 2001) and the nomenclature that has been used for Actinidia deliciosa 'Hayward' (Salinero et al., 2009). Data were recorded during two consecutive growing seasons (2018-2019). Kiwi is a relative new fruit specie in Romania and the descriptions of principal growth stages for bud, leaf and shoot development, inflorescence emergence, flowering, fruit development, fruit maturity and plant senescence can improve some horticultural practices and operations on kiwifruit orchard management (such as pruning, girdling, pollination techniques, frost protection, fertilization, irrigation etc.).

Key words*: phenology, hybrids, A. arguta, A. chinensis, A. deliciosa.*

INTRODUCTION

Domesticated from wild populations located on Yangtze River basin from China, kiwifruit is a recently developed crop, due to its nutritional properties, high vitamin C content, as well as its taste and flavour (Biao et. al., 2018; Litz 2005; Yang, 2010; Young et al., 1995).

Actinidia genus belongs to the family *Actinidiaceae* and according to the latest revision (Huang et al., 2007) has over 75 species and about 125 known taxa worldwide. Current commercial cultivation is almost entirely based on *A. deliciosa* and *A. chinensis* (Huang, 2016; Zhang et al., 2010). Lesser extent, in colder regions, *A. arguta* commercial potential started to be recognised, in the early 20th century (Ferguson and Huang, 2007).

Kiwifruit is widely distributed in Asia ranging from the tropics (latitude 0°) to cold temperate regions (50°N) (Huang et al., 2007). According with Cui (1993), *Actinidia* species are found from India to Japan, and from Siberia to Indonesia. In different climates and geographical environments, *Actinidia* species exhibit tremendous biological variation (Huang et al., 2007).

The study of periodic biological events was called phenology (Hernández et al., 2014). Throughout the time a large number of studies are reported in the literature concerning descriptions of principal growth stages in different horticultural crop (Aydin et al., 2019; Bratu et al., 2019; Mușat et al., 2019; Panchev et al., 2019; Stănică, 2019a; Stănică, 2019b; Stroe et Cojanu, 2019).

In 1945, using a combination of letters and numbers, Fleckinger defined 'phenological stages' (Fleckinger, 1948). Adopting the same codes, Zadoks et al. (1974) published the first decimal code to standardise the description for the growth stages of different crops. Based upon these descriptions of cereals (Zadokset al.,1974), a uniform decimal code, known as the BBCH - scale (Biologische Bundesanstalt, Bundessortenamt, Chemische Industrie), was proposed by Lancashire et al. (1991) and Bleiholder et al. (1991). Hack et al. (1992) and Hess et al. (1997) proposed a more advanced scale, the extended BBCH and later, the

'BBCH-Monograph' (representing a group of 27 crops and weeds) was published (Meier, 1997). BBCH-scale (Meier, 2001) it is used now by many researchers for describing the growth stages of different fruit trees (Hernández et al., 2014).

For kiwifruit, first phenological growth stages according BBCH scale, have been described by Salinero et al. (2009), for *Actinidia deliciosa* 'Hayward', in Pontevedra region, from northern-vest of Spain. Important contributions to the study of the phenology of *Actinidia deliciosa* 'Hayward' were made also by Brundell (1975a; 1975b). He proposed six stages for bud development after winter dormancy, and six stages for the development of flower buds until full bloom. To describe these stages, he used the initials of a few words that briefly described each stage (for example 'bb' for bud burst). Regarding fruit development, Hopping (1976) established a growth curve divided on three stages (namely I, II and III), based on fruit weight and growth rate. Later, Beever and Hopkirk (1990) revised the characteristics of Hayward fruit development and physiology, but without presenting a phenological scale.

In Romania kiwifruit research and culture started in 1993 (Peticilă et al., 2002; Stănică, 2009). The first orchards with *Actinidia deliciosa* were planted at Ostrov (Constanța County), on the border of the Danube River (Stănică & Cepoiu, 1996; Stănică, 2009). The most important studies were conducted in a common Italian-Romanian kiwifruit breeding program, initiated at the Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest (Stănică & Zuccherelli, 2007; Stănică & Zuccherelli, 2009).

Taking into consideration that *Actinidia* is a new fruit specie in Romania, the descriptions of principal growth stages can improve some horticultural practices and operations on kiwifruit orchard management (such as pruning, girdling, pollination techniques, frost protection, fertilization, irrigation or pest control).

In this context, the aim of this research was to define the phenological stages of two kiwifruit varieties ('Hayward' and 'Bruno') and some Romanian intra and interspecific *Actinidia* hybrids, in Bucharest area. This study can improve the cultivation of this new crop in Romania, can contribute in Romanian breeding program and also in zoning of the main *Actinidia* species on our country climatic conditions.

MATERIALS AND METHODS

The study was conducted during two consecutive growing seasons (2018 and 2019), in the Experimental Field at the Faculty of Horticulture, within the University of Agronomic Sciences and Veterinary Medicine of Bucharest, for two kiwifruit varieties and eight Romanian intra and interspecific *Actinidia* hybrids. The plant material is presented in Table 1.

Table 1. Plant material description

Variety/Hybrid		Species
Hayward		*A. deliciosa*
Bruno		*A. deliciosa*
R0P10		*A. chinensis* intraspecific hybrid
R0P13		*A. deliciosa* and *A. chinensis* interspecific hybrid
R1P8		*A. deliciosa* and *A. chinensis* interspecific hybrid
R1P9		*A. deliciosa* and *A. chinensis* interspecific hybrid
R1P12		*A. deliciosa* and *A. chinensis* interspecific hybrid
R2P6		*A. chinensis* and *A. arguta* interspecific hybrid
R8P1		*A. arguta* intraspecific hybrid
R10P20		*A. arguta* intraspecific hybrid

The climate in the experimental area is typically temperate-continental, with cold winter and warm, sometimes torrid, summer, with frequent droughts (Asănică, 2010).

The annual mean temperature is between 10.5ºC in the peripheral areas, and 12ºC in the center, caused by the high concentration of constructions, the street traffic and the industrial activities. The annual precipitation between 550 and 600 mm was recorded, mostly falling between May and July. The dominant air

circulation is east and northeast during the winter and from west to the rest of the year, with the maximum wind speed of 3.5-4 m/sec (Asănică, 2010; Asănică, 2011).
The plants were grown in a preluvosoil, on a T-bar trellis system, under an organic orchard management. The inter row surface was covered with a mixture of perennial grasses and along the row, the soil was kept clean. Drip irrigation and micro spray irrigation system was provided.
The phenological growth stages were described in the environmental conditions of Romanian Plain, Vlăsiei Plain subdivision, between winter dormancy and leaf fall, according to the BBCH General Scale (Meier, 2001) and the nomenclature that has been used for *Actinidia deliciosa* 'Hayward' (Salinero et al., 2009). The extended BBCH scale considered 10 principal growth stages, numbered from 0 to 9. This study handled 8 of the 10 principal stages - bud, leaf and shoot development, inflorescence emergence, flowering, fruit development, fruit maturity and plant senescence, described in Table 2.

RESULTS AND DISCUSSIONS

The evolution of growth stages, according to BBCH scale (Meier, 2001), provides an accurate description of kiwifruit plants phenology. The scale is based on a two-digit code where the first digit describes the principal growth stages such as bud development, leaf development, flowering etc., while the second digit gives a more precise timing event of the principal stage. Some of the primary and secondary phenological growth stages of kiwifruit according to BBCH scale (described in Table 2), are represented with photographs for the most cultivated *Actinidia* species - *A. deliciosa* (Figure 1), *A. chinensis* (Figure 2) and *A. arguta* (Figure 3).
During the experimental period, for a better description of the climatic conditions of Bucharest area were noted in Table 3 - the minimum, maximum and mean temperature registered on 2018 and 2019 for every month. Also, was noted the number of days of precipitation and total mm, per every month. These data are very important for correlating the environmental conditions with the phenological growth stage and the main horticultural practices for orchard management.
The lowest temperatures were recorded mainly in winter period, but also in late autumn, -12°C in November 2018 and early spring, -22°C in March 2018.
The kiwifruit species stayed in dormancy until March, when the mean temperature registered are higher than 3.5°C.
Higher temperature differences between night and day were recorded in the months February to April and September to November, in every year. When warmer temperatures in late winter encourage early bud break, these young buds are highly frost - susceptible (Debersaques et al., 2019).
The highest temperatures, between 33-36°C, were recorded in the summer and autumn begging. For reduce drought effect, that can cause considerable damage on kiwifruit orchards, it is necessary to improve atmospheric humidity with sprinkler irrigation.
Annual rainfall averaged in 2018 was 661.1 mm, respectively 636.2 mm in 2019. The rainy period was as usual in this area, occurring during end of spring and middle of summer (Table 3).
The optimal amount of precipitation for kiwifruit is between 1200-1500 mm/year, eventually distributed over the growing season (Hennion, 2003; Debersaques et al., 2019). Thus, water supply by irrigation was provided.
Actinidia cultivation requires a wide range of horticultural practices such as pruning, girdling, frost protection, pollination techniques, flower and fruit thinning, fertilization, irrigation or pest control and harvest (Salinero et al., 2009). To properly manage kiwifruit orchards, an accurate tree phenology must be scheduled.
The phenophases from bud development to fruit maturity and senescence were presented in Table 4, for two kiwifruit varieties ('Hayward' and 'Bruno') and eight Romanian intra and interspecific *Actinidia* hybrids (R0P10, R0P13, R1P8, R1P9, R1P12, R2P6, R8P1, R10P20), in two observational years (2018 and 2019). In Table 5 the main operations on kiwifruit orchard management were presented.

Table 2. Proposal of principal kiwifruit phenological growth stages adapted according to the BBCH scale

BBCH code	Growth stage description
	Principal growth stage 0: Bud development
00	Dormant buds grown in the previous crop-year are completely closed. A small ostiole (less than 2 mm in diameter) is visible (Figures 1, 2, 3).
01	Beginning of bud swelling; scales just visible (Figures 1, 2, 3). For *A. deliciosa* and *A. chinensis* they are covered by white trichomes.
03	End of bud swelling; scales protruded through the corky tissue of the stem. For *A. deliciosa* and *A. chinensis*, scales are densely covered by brown trichomes on their abaxial surface (Figures 1, 2); for *A. arguta* scales tips joined apically (Figure 3).
07	Beginning of bud burst. For *A. deliciosa* and *A. chinensis*, leaf and inflorescence buds enclosed by scales covered by brown trichomes (Figures 1, 2); for *A. arguta* scales tips dispersed along bud axis (Figure 3).
09	Scales separate and green leaf tips are visible. For *A. deliciosa* and *A. chinensis* they are covered by brown trichomes (Figures 1, 2); for *A. arguta* they are covered abaxially by white ones (Figure 3).
	Principal growth stage 1: Leaf development
10	The bud develops into an open cluster containing a few visible leaves (Figures 1, 2, 3).
11	Visible leaves unfolded and start spreading away from the shoot (Figures 1, 2, 3).
12-18	Two to eight or more leaves unfolded, but not yet at full size (Figures 1, 2, 3).
19	First leaves completely developed (Figures 1, 2, 3).
	Principal growth stage 3: Shoot development
31	Shoots reach about 10% of final length.
32	Shoots reach about 20% of final length.
35	Shoots reach about 50% of final length.
39	Shoots reach about 90% of final length.
	Principal growth stage 5: Inflorescence emergence
51	Inflorescence bud swelling (flowers borne as singlets or triplets in the leaf axils). Buds closed, with no peduncle, greenish sepals visible covered by trichomes (Figures 1, 2, 3).
53	Flower buds growing, they still closed, reddish peduncles elongating (Figures 1, 2, 3).
55	Sepals begin to separate. A white-greenish corolla starts to be visible, reddish peduncles continue to elongate (Figures 1, 2, 3).
56	Sepals continue to separate, and peduncles to elongate and thicken. Corolla clearly visible, longer than calyx, changes colour from white-greenish to white (Figures 1, 2, 3).
57	Corolla at balloon stage, first flowers with white petals forming a hollow ball. One of the petals separates from the rest (Figures 1, 2, 3).
59	Several petals separate, pistils still not visible longer than calyx (Figures 1, 2, 3).
	Principal growth stage 6: Flowering
60	First flowers open, corolla bell-shaped, pistil visible (Figures 1, 2, 3).
61	Beginning of flowering: 10% of flowers open.
65	Full flowering: at least 50% of flowers open (Figures 1, 2, 3).
67	First petals fading or falling. Some pistils still fertile (Figures 1, 2, 3).
68	Most petals dry or fallen. All pistils dry and no longer functional.
69	End of flowering, fruit set visible (Figures 1, 2, 3).
	Principal growth stage 7: Fruit development
71	Fruit about 10% of final size, showing the typical characteristic of the cultivar (Figures 1, 2, 3).
73	Fruit about 30% of final size (Figures 1, 2, 3).
75	Fruit about 50% of final size.
79	Fruit about 90% of final size: fruit suitable for commercial picking (Figures 1, 2, 3).
	Principal growth stage 8: Maturity of fruit
81	Seeds reach their full size, harden and change colour from white to brown, progressing through tan to dark brown.
85	Fruit ripe for commercial picking, solids content higher than 6.2%. Seed colour becomes black. Fruit at physiological maturity (still not suitable for consumption), begins to soften (Figures 1, 3).
89	Fruit fully ripe for consumption: fruit has typical taste, flavor and firmness. Soluble solids about 14–16% (Figure 2).
	Principal growth stage 9: Senescence. Beginning of dormancy
91	Shoot growth complete; foliage fully dark green.
93	Beginning of senescence of old leaves; leaves fall (Figures 1, 2).
97	All leaves fallen. Winter rest period (Figures 1, 2, 3).

(According with Meier, 2001; Salinero et al., 2009; Labeke et al., 2015)

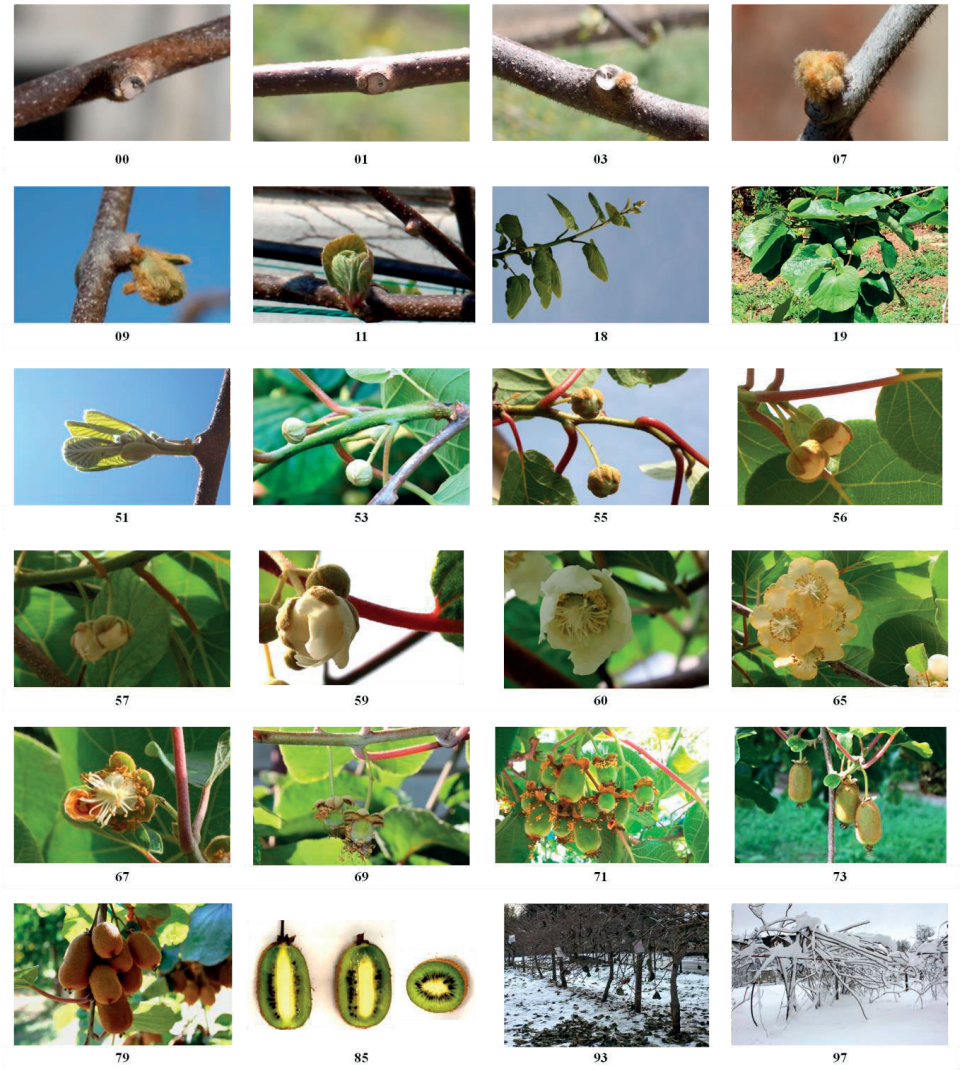

Figure 1. Some phenological growth stages of 'Bruno' variety (*A. deliciosa*), according to BBCH scale

The different stages of bud development mostly took place in the beginning to mid-March, for all three studied species, when the mean temperature registered was higher than 3.5°C.

Leaf development and emergence/development of the inflorescences occurred from the last weeks of March till beginning April.

Full flowering ensued from early May for *A. chinensis* and *A. arguta* through middle of May, for *A. deliciosa*, when the mean temperature registered was higher than 15°C.

Phenological stage and temperature monitoring are very important, because cultivars that have a lower basal temperature for bud and flower development might be more susceptible to spring frost especially if no frost protection is present (Labeke et al., 2015).

According Spano et al. (1997), temperature initiates all biological processes that result in the occurrence of a certain phenological stage and also, temperature affects the morphological and quality characteristics of fruits.

Fruit maturity has been reached in the first weeks of September for *A. arguta*, at the last weeks of October for *A. chinensis* and at the beginning of November for *A. deliciosa*.

Figure 2. Some phenological growth stages of R0P10 intraspecific hybrid (*A. chinensis*), according to BBCH scale

Studying the hybrids of the different species of *Actinidia*, it is clear that differences between the developments of the phenological stages exist.

The growing season for kiwifruit is long: up to 240 days. This begins with vine pruning in winter, which follows the previous year's harvest (Baker et al., 2018). During the winter months (December to February) the vines lay dormant, allowing growers the opportunity to remove last season's fruiting canes and to select and tie down new canes which form the foundations for new growth (Baker et al., 2018).

According to Costa et al. (1996), in established kiwifruit vines, pruning and girdling practices are carried out to renew fruiting wood, to achieve a good balance between vegetative growth and fruit production, and to improve light interception and air penetration through the canopy. Each year the winter pruning is initiated at the end of autumn, when all leaves are fallen (BBCH stage 97), and is continued meanwhile vines remain dormant (stage 00).

Figure 3. Some phenological growth stages of R8P1 intraspecific hybrid (*A. arguta*), according to BBCH scale

Springtime (March to May) sees the kiwifruit vines begin to grow again. New shoots appear on the canes along with the first flower buds.

Especially important for kiwifruit production is the recognition of phenological stages during the development of the floral bud, because they are key for flower and fruit thinning and to increase the success of pollination (Salinero et al., 2009).

Flower and fruit thinning are done to reduce excessive fruit load, thus diminishing the competence among fruits for carbohydrates and obtaining a final higher fruit size, and to eliminate lateral and misshapen fruits (Salinero et al., 2009). Considering the BBCH scale, flower thinning should be done from stage 55 to stage 60, whereas fruit thinning is advised from stage 71 to stage 73 (Salinero et al., 2009). These operations that increase fruits quality are achieved especially for *A. deliciosa* and *A. chinensis*.

During the spring–summer period, fertirrigation is commonly applied at orchards to provide necessary nutrients, macro and microelements like N, P, K, Ca, Mg etc. (Salinero et al., 2007; Salinero et al., 2009).

Table 3. Monthly maximum, minimum and mean air temperatures (°C); total precipitation (mm) and days per month, for 2018 and 2019 growing seasons

Month	2018					2019				
	Temperature (°C)			Total precipitation		Temperature (°C)			Total precipitation	
	Max.	Min.	Mean	mm	days	Max.	Min.	Mean	mm	days
Jan	14	-11	1.01±6.21*	46.6	10	9	-16	-1.69±5.30*	57.3	16
Feb	17	-13	1.08±6.18*	94.3	19	19	-8	3.42±7.26*	7.6	4
Mar	24	-22	3.59±8.56*	68.8	15	25	-4	8.74±8.70*	33.3	5
Apr	30	0	15.43±8.83*	4.9	4	26	-2	10.96±7.35*	75.3	12
May	31	6	18.62±8.01*	7.1	8	28	4	16.59±7.32*	153.3	16
Jun	34	9	21.91±7.55*	166.5	16	33	12	22.75±7.22*	72.9	10
Jul	32	11	22.53±6.75*	90.2	17	36	10	22.10±8.11*	70.7	9
Aug	35	12	23.62±8.30*	8.7	2	36	10	23.38±9.13*	24.1	5
Sep	34	-1	18.68±9.10*	33.6	5	34	2	18.80±9.35*	8.2	4
Oct	28	0	13.45±8.39*	15	4	30	1	13.32±8.33*	44.1	10
Nov	20	-12	4.83±6.83*	65.6	11	25	-2	9.61±7.14*	76.7	15
Dec	10	-13	-0.51±5.10*	59.8	4	17	-7	3.71±6.22*	12.7	10
	Temperature average 12.07 °C			**Total precipitation 661.1 mm/year**		**Temperature average 12.69 °C**			**Total precipitation 636.2 mm/year**	

* Standard deviation

Table 4. Comparison of phenological stages between *A. deliciosa*, *A. chinensis* and *A. arguta* Romanian hybrids, in two growing seasons (2018-2019)

BBCH CODE	Bud development (01)	Leaf development (11)	Inflorescence emergence (51)	Flowering (61)	Fruit development (71)	Fruit maturity (85)	Senescence (93)
Variety			**2018**				
			Actinidia chinensis				
R0P10	06.03	17.03	27.03	03.05	22.05	24.10	19.11
			Actinidia deliciosa				
Hayward	08.03	24.03	07.04	18.05	04.06	07.11	29.11
Bruno	06.03	21.03	05.04	13.05	28.05	07.11	25.11
			***A. chinensis* and *A. deliciosa* interspecific hybrid**				
R0P13	08.03	24.03	07.04	13.05	28.05	07.11	25.11
R1P8	08.03	24.03	07.04	15.05	04.06	07.11	29.11
R1P9	08.03	24.03	07.04	15.05	04.06	07.11	29.11
R1P12	08.03	24.03	07.04	15.05	04.06	02.11	29.11
			***A. chinensis* and *A. arguta* interspecific hybrid**				
R2P6	06.03	17.03	01.04	13.05	25.05	24.10	19.11
			Actinidia arguta				
R8P1	04.03	20.03	01.04	08.05	19.05	18.09	12.11
R10P20	04.03	20.03	01.04	08.05	19.05	18.09	12.11
Variety			**2019**				
			Actinidia chinensis				
R0P10	04.03	22.03	01.04	09.05	28.05	25.10	21.11
			Actinidia deliciosa				
Hayward	06.03	28.03	12.04	25.05	10.06	08.11	03.12
Bruno	04.03	25.03	09.04	20.05	05.06	08.11	30.11
			***A. chinensis* and *A. deliciosa* interspecific hybrid**				
R0P13	06.03	28.03	12.04	20.05	05.06	05.11	30.11
R1P8	06.03	28.03	12.04	23.05	10.06	05.11	03.12
R1P9	06.03	28.03	12.04	23.05	10.06	05.11	03.12
R1P12	06.03	28.03	12.04	23.05	10.06	05.11	03.12
			***A. chinensis* and *A. arguta* interspecific hybrid**				
R2P6	04.03	22.03	05.04	20.05	01.06	25.10	21.11
			Actinidia arguta				
R8P1	02.03	25.03	05.04	16.05	25.05	17.09	21.11
R10P20	02.03	25.03	05.04	16.05	25.05	17.09	21.11

Table 5. Kiwifruit growth stages and the main orchard management practices on a growing cycle

Season	Winter			Spring			Summer				Autum	
	DEC	JAN	FEB	MAR	APRIL	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV
	Dormant			Budbreak		Flowering	Fruit set	Fruit growth				Leaf fall
	Winter pruning			Budbreak sprays		Pollination	Male pruning	Canopy management, thinning and girdling			Harvest	

During flowering, in May, in commercial orchards natural (introduction of bee hives) and/or artificial (hand and machine pollination) systems of pollination can be apply (Salinero et al., 2009). The bee hives must be moved into the orchard when 10–20% of flowers are open (Clinch, 1990). The introduction of bee hives should be done at BBCH stage 61, and hand and machine pollination from BBCH stage 65 to stage 67 (Salinero et al., 2009).

As summer starts, kiwifruit vines undergo tremendous growth and growers frequently prune the vines to direct growth and manage the canopy (the canes can sometimes reach up to 5-6 meters in length during the growing process) (Baker et al., 2018). The fruit grow quickly, and crop volume can be estimated. Growers selectively thin kiwifruit to optimize fruit size and taste (generally the less there are, the larger and tastier they grow) (Baker et al., 2018).

Summer pruning is carried out during the growing season in the spring-summer period, and should be done at BBCH stages 18 and 19 in spring, to remove upright growing suckers, and during the summer, starting immediately after fruit set (stage 69) until stage 73, to cut growing ends of fruiting canes, what will result in larger fruit. The summer pruning sometimes is done until few days before harvest (stage 85), to cut growing ends, to prevent tangling, and twisted and tangled ends of all shoots (Salinero et al., 2009).

Girdling is performed only in some kiwifruit orchards. It must be done on 1-year-old wood (parent canes), supporting seasons floral shoot, and is usually carried out after fruit set until 2–4 weeks afterwards (therefore starting after stage 69 until 73) (Salinero et al., 2009).

In last weeks of summer and beginning of autumn, the kiwiberry, respectively kiwifruit, are tested for ripeness. When they pass a certain criterion for quality (BBCH stage 85), the fruits are carefully picked.

CONCLUSIONS

Kiwifruit has certain requirements as well as all fruit species, regarding temperature, humidity, wind, soil etc. Besides of these, commercial crops require significant management practices to be productive. Vine training, pruning, pollination, shelter from the wind, pest and disease control among other things all have a significant impact on the profitability and productivity of the crop. These horticultural practices impact the size and the dry matter of fruit and also the market acceptance.

The phenological enlargement of kiwifruit could improve the quality of fruits by providing information about evolution of the varieties and local hybrids under the environmental conditions of Southern Romanian. An accurate understanding of kiwifruit plant phenological stages it is essential for an appropriate orchard management.

In conclusion, this study can improve the cultivation of this new crop in Romania, can contribute in Romanian breeding program and also in zoning of the main *Actinidia* species on our country climatic conditions.

Further observation needs to be done, because the effect of climate and especially the temperature on seasonal variation requires longer observation periods than presented in the present study. To obtain more accurate results, continuing research is proposed for more years.

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STORAGE LIFE UNDER CONTROLLED ATMOSPHERE OF JONATHAN AND BELLE OF BOSKOOP APPLES, TRADITIONALLY PRODUCED IN BRAN-ZĂRNEȘTI AREA

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Abstract

This study is part of the extended research focused on the identification of local fruit varieties, on their nutraceutical properties in order to be promoted on the market both as fresh fruits or processed products with added value. Among the South-East Transylvanian fruits, the old apple varieties Jonathan and Belle of Boskoop are cultivated in traditional orchards of Bran-Zărnești area. This article aims to present the results of a detailed analysis of the physical and biochemical transformations undergone in controlled atmosphere storage by the two apple varieties. Fruits were harvested from a traditional orchard from 40-50 years old trees, planted at 15 x 15 meters. The soil was maintained covered with multispecies grass and the vegetation was mown twice a year for hay production. Fruits were stored in controlled atmosphere cells of 1m^3 capacity and determinations were done on monthly bases. Dry matter content, total soluble sugars and total acidity were significantly different at the two varieties. Similar values for the ascorbic acid content were registered. Belle of Boskoop had a low storage life and correlations between parameters were detailed.

Key words: *M. domestica, total soluble sugar, dry matter, ascorbic acid, total acidity*.

INTRODUCTION

Healthy food is more and more promoted as an answer to the massively increasing number of illnesses, particularly digestive, that are very difficult to treat. In this context, the nutritive value of some natural products like fruits is considered for use. Fruits represent the essential component of the daily diet helping to balance the nutritive value and eliminating the monotony of meat-and cereal-based diets.

As they stimulate the secretion of digestive juices and have a distinct nutritive and gustative value, fruits are the food that must not be absent from the everyday diet. Carrying essential mineral salts, fruits contribute to the neutralization of the body's acidity, which can occur because of the excessive intake of animal products explosively present in the population's daily menu (Râpeanu, 2010; Ticha et al., 2015).

Research focuses as much as possible on native fruits, which are within the population's reach and should replace a large part of its animal proteic diet.

Apples prevail in the researched area (Bran-Zărnești). These are grown in traditional orchards with 40-50-year trees. The orchards floor is maintained with a grass cover, the vegetation being mowed for hay twice a year. The most representative local apple cultivars are Jonathan, Boskoop Gel and Boiken.

The qualitative appreciation of a cultivar is based on its content in water, total sugars, minerals, ascorbic acid, pectic substances as well as the fruits' physical characteristics: size (weight, height, diameter), the proportion between the edible and waste parts. Firmness is influenced by fruit size during the post-harvest period (Saei et al., 2011).

It is known that drying is the oldest and most healthy method of preserving fruits and vegetables, more suitable than cold room storage (Bujdei et al., 2019). During dehydration, the fruits undergo certain transformations, such as structure transformation; volume reduction; turbulence

by losing water from the structure; color transformations (color change depending on the oxidative process, time and duration of drying); flavor transformations (the warm air entails some of the aromas resulting in their diminution); loss of some vitamins; increases of the energy value of dried products.
The aim of this study was to present the results of an analysis of the physical and biochemical transformations undergone in controlled atmosphere storage and respectively through drying by the two apple cultivars.

MATERIALS AND METHODS

Jonathan cultivar, created in 1880 at Ulster, USA, has medium-sized fruits, red color skin and white-yellowish color pulp. The cultivar is very sensitive to mildew (Cimpoieș et al., 2001). It is one of the most appreciated apple cultivars for fresh consumption in Romania (Hoza, 2000; Ghena and Braniște, 2003; Stănică and Braniște, 2011).
Belle of Boskoop (sin. Reinette von Monfort), created in the Netherlands, has large-sized fruits and green-yellowish color of skin. The cultivar is scab sensitive (Braniște and Uncheașu, 2011).
For this study, Jonathan and Belle of Boskoop cultivars fruits were harvested from a traditional orchard from Brașov county, Sub Măgura area, Predeluț village, Bran commune, Nicolae Boș Street. The trees were 40-50 years old, planted at 15 x 15 meters. The soil in the orchard was maintained covered with multispecies grass and the vegetation was mown twice a year for hay production.
After harvesting, the fruit samples were kept in controlled atmosphere cells at 2°C temperature, 90% humidity, 2% oxygen and 3% CO_2.
The average fruit weight, diameter and firmness determined with a digital penetrometer were registered monthly.
The water and dry substance content were determined through oven drying at 105°C, 24 hours, following the sample's weight loss through water evaporation, the total dry extract being derived by deduction (100-water).
The total titratable acidity in the analyzed sample was determined through the titration of the fruit extract with NaOH 0.1N in the presence of phenolphthalein as an indicator.
The ascorbic acid (vitamin C) was determined through the iodometric method by titration with potassium iodate.
The sugar concentration was determined with the help of the ABBE-PULFRICH refractometer.
Obtaining apple chips by hot air drying
Apples from Jonathan and Boskoop cultivars were used, after being washed and sliced. Immediately after slicing, the slices of about 0.5 cm thick, after been immersed in a lemon juice mixed with water to avoid oxidation, were placed in the dryer, in layers, on trays. The sliced fruits were kept for one, two and three hours respectively at 80°C. After each hour of drying, the humidity was determined. The same parameters were determined for 67°C and three, six and nine hours.
Data statistical analysis was performed with Excel (MS Office). For correlation between two data sets Excel statistical functions with a significance level $p < 0.05$ were used.

RESULTS AND DISCUSSIONS

Belle of Boskoop had a higher average fruit weight (162.3 g) comparing with Jonathan's cultivar. At the initial moment of the research, the average flesh firmness was 4.0 kg f/cm^2 at Belle of Boskoop comparing with 3.5 kg f/cm^2 at Jonathan (Figure 1).

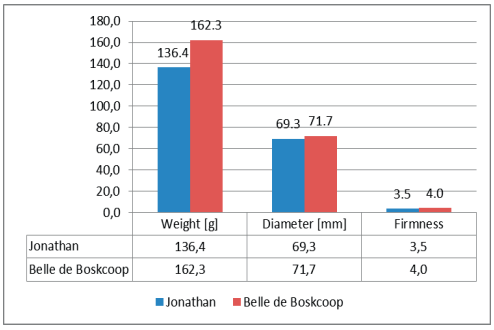

Figure 1. Apples physical parameters

Ancient apple cultivars were studies also by Iordănescu et al. (2019) in the Banat region. Jonathan cultivar in the Bran area presented higher weight compared with the Banat region (93.65 g). Oltenacu and Oltenacu (2013) presented higher physical parameters for Jonathan cultivar cultivated in the Ilfov area (171.05 g/fruit harvested at 3.47 kg f/cm^2). In Table 1 are presented data about the fruits' weight modification and the waste occurring during the controlled atmosphere storage period, October 2016 - January 2017.

Table 1. The behavior of the Jonathan and Belle of Boskoop cultivars grown in traditional orchards in controlled atmosphere storage

Cultivar	October	November	Waste	Weight loss
Jonathan	9415.78 g	9225 g	190.78	-
Boskoop	9181.65 g	8890 g	186.65	105
	December		**Waste**	**Weight loss**
Jonathan	8867.83 g		357.017	-
Boskoop	8763.68 g		126.32	-
	January		**Waste**	**Weight loss**
Jonathan	8438.83 g		429	-
Boskoop	8442.08 g		321.6	-

The weight variation in the four months period beginning with October, when they were harvested, until processed as chips was mostly due to damages. Similar results regarding fruit depreciation were observed at Ivan et al. (2019), where organic apples, after four months of storage at 1°C and 90% humidity, were affected by postharvest diseases, predominantly caused by fungal pathogens. Paul and Pandey (2014) presented an estimation for apple postharvest losses to 14% in developing countries. For both analyzed cultivars total losses were significantly lower than other similar researches with different storage conditions (Oltenacu and Oltenacu, 2013) where average total losses were at 16.33% compared with 9.18%. At Jonathan cultivar, total losses were 19.28% compared with 10.37% and damage losses 11.87% compared with 10.37%. Comparable results for the organic apple in a modified atmosphere were obtained by Chira et al. (2014).

Table 2 presents the fruit biochemical characteristics of the studied apple cultivars.

Table 2. The fruit biochemical composition of the Jonathan and Boskoop cultivars.

The apple cultivar	Water %	Total dry substance %	Sugar concentration %	Total acidity %	Ascorbic acid %
Jonathan	80.89	19.11	16.00	0.1009	97.20
Boskoop	89.22	10.78	11.00	0.2570	93.10

The obtained results showed that water, the fruits' main component, varied according to the cultivar, a higher content of water, 89.22%, being recorded at Boskoop cultivar.

The results of water content were similar with Bezdadea-Cătuneanu et al. (2019) for some cultivars of organic apples at the initial moment of storage (79.52%-87.25%) and similar for some cultivars studied by Bujdei et al. (2019) were water content for organic apple cultivars varied from 69.07% to 84.98%. Chira (2008) estimated an apple water content between 83-89%.

For Jonathan cultivar, Mureșan et al. (2014) registered significantly differences for water content (85.34%), total acidity (0.29%) and total soluble content (23.25% Brix) in the Reghin area. Similar, Oltenacu and Oltenacu (2013) for the Ilfov area.

Dry substance determination was an important analysis, which revealed apple quality. The fruit of the Johnathan cultivar was the richest in dry substance (19.11%), while of the Boskoop cultivar had a lower content (10.78%).

The total titratable acidity was recorded in a higher percentage, double even, with the Boskoop cultivar, being 0.2570% as compared to Jonathan's 0.1009%.

As for the sugar content, there were also notable differences, as the above table showed, the Johnathan apples being sweeter than the Boskoop ones. The ascorbic acid content was on about the same level: 93.1 and 97.2 (Figure 2).

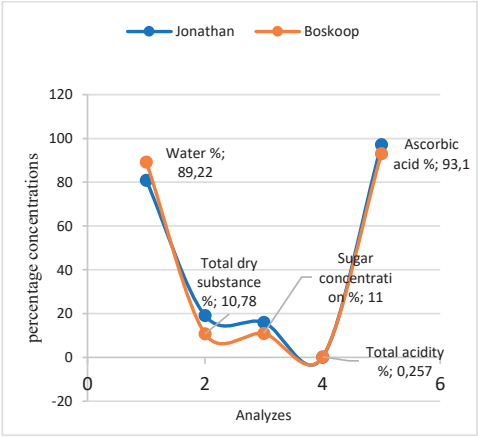

Figure 2. Structural analysis of Jonathan and Boskoop fruits

Obtaining apple chips by hot air drying

In order to determine the optimum time for apple drying at 80°C, after each hour the apple humidity was measured (Table 3).

Table 3. Determination of apple chips humidity

Time h	Temperature ^{0}C	Jonathan u %	Boskoop u %
1	80	16.271	17.024
2	80	14.494	15.671
3	80	12.744	14.194

For drying at 67° C, different time periods were necessary (Table 4) in order to obtain de necessary humidity.

Table 4. Determination of apple chips humidity after drying for a period of 3, 6 and 9 hours

Time h	Temperature ^{0}C	Jonathan u %	Boskoop u %
3	67	14.131	14.924
6	67	13.264	13.261
9	67	11.864	12.984

The apple chips humidities are represented after dehydration in different periods of time. At 80° C (Figure 3), Jonathan chips were more dehydrated than those in Boskoop apples, which leads us to conclude that the chips in Jonathan are drier, have a lower percentage of moisture and thus become more crunchy.

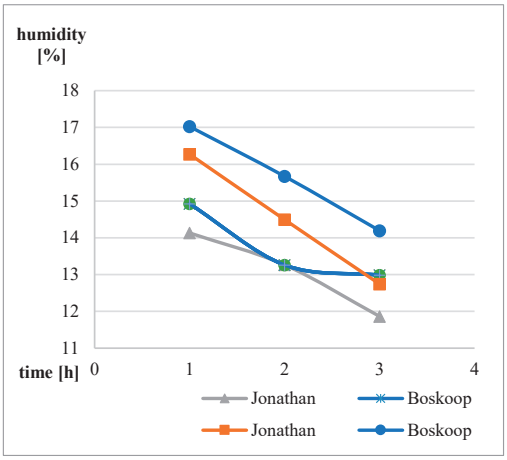

Figure 3. Variation of apple chips humidity on drying at 67^{0}C and 80^{0}C

Drying at 67^{0}C, after six hours was obtained for these apple cultivar samples the same value of the humidity (13.26%).

Different parameters after drying could be obtained according to apple cultivars, being important in the production process. Moura et al. (2005) presented also the differences in water loss during dehydration between apple cultivars according to several time units.

Determination of vitamin C content in dried chips

Ascorbic acid (vitamin C) content in the dried apple chips was lower than in fresh products due to the used temperature (Tables 5 and 6).

Table 5. Vitamin C content in dried apple chips at 80^{0}C

Time (h)	Temperature ^{0}C	Dry sample mass	Jonathan mg C vitamin	Boscoop mg C vitamin
1	80	100	61.070	58.163
2	80	100	54.963	52.347
3	80	100	48.856	46.530

Table 6. Vitamin C content in dried apple chips at 67^0C

Time (h)	Temperature ^{0}C	Dry sample mass	Jonathan mg C vitamin	Boscoop mg C vitamin
3	67	100	58.463	55.063
6	67	100	53.201	50.107
9	67	100	49.693	46.804

From the experimental data obtained from the samples of chips at a lower temperature, the quantitative values of vitamin C are higher for Jonathan cultivar at the same humidity and similar at Belle of Boskoop.

CONCLUSIONS

Jonathan and Belle of Boskoop, apple cultivars produced in a traditional organic orchard in Bran (Brașov) area, presented good post-harvest qualities.

For apple drying, the working temperature should be between 67^0C and 70^0C, in order to maintain their quality parameters, ascorbic acid presented a higher value at similar dry matter content and cultivar.

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PRELIMINARY RESULTS REGARDING THE POSTHARVEST PATHOLOGY OF PAWPAW (*ASIMINA TRILOBA* DUNAL) FRUITS

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Abstract

*Asimina triloba, the pawpaw, is one of the few fruit tree species native to the United States. The plant is native to eastern North America, growing spontaneously from the Gulf of Mexico till the Great Lakes. The tree produces large fruits, yellow-green colour, sweet and flavored. In Romania, the first plants were introduced in Transylvania in 1926 and have been grown locally, remaining unknown to the rest of the country. Starting with the year 2000, within the Faculty of Horticulture from Bucharest, there were cultivated several varieties and hybrids of pawpaw, in order to determine the suitability of this species for the establishment of new plantations in Romania. The fruits were harvested manually and stored in controlled atmosphere rooms with 1.5% CO_2, at 4°C temperature and 90% relative humidity. During the 2 months storage period were identified several pathogens affecting the pawpaw fruit. The micromycetes identification was carried out by isolation and successive subculture on PDA medium culture (Potato Dextrose Agar), followed by incubation at 22°C in the thermostated chambe*r.

Key words*: Asimina triloba, storage pathogens, postharvest.*

INTRODUCTION

The fruits are rich in active ingredients, vitamins and minerals. The fruits have an excellent exotic taste, that evolves during the storage from the taste of vanilla cream at crème brûlée, and finally, to chocolate cream taste.

Asimina triloba (pawpaw L. Dunal), is part of the family *Annonaceae* being known in North America - its area of origin, as Pawpaw (Stanica, F. et al., 2008). The genus includes the largest edible fruits originating in North America (Darrow, 1975). It is a large, thin, leathery fruit with two rows of almond-sized seeds. Its shape can vary from oval to elongated, peanut-shaped and can vary between 3 and 6 mm in length (McGrath M., 1994). Of the approximately 2300 species and 130 genera in the *Annonaceae* family, only the genus *Asimina* grows in temperate climates. All other genera of the family *Annonaceae* grow in the tropics. In south-eastern Florida and Georgia there are eight species of the genus *Asimina*: *Asimina angustifolia* Raf. - *Pawpaw* with narrow leaves, *Asimina incana* (W. Bartram) - *Pawpaw* with wool, *Asimina* obovata (Willd.) Nash - *Pawpaw* with large flowers, *Asimina parviflora* (Michx.) Dunal- *Pawpaw* with small flowers, *Asimina pygmea* (W. Bartram) Dunal - *Pawpaw* dwarf, *Asimina reticulata* Shuttlw. ex Chapman- Netted *Pawpaw*, *Asimina tetramera* Small - *Pawpaw* with four petals, Asimina triloba (L.) Dunal - Common *Pawpaw* (Callaway, 1993).

Pawpaw grows at the edge of forests and is noted for its high frost resistance, surviving winters at -25...-30°C, however, it is grown as an orchard crop in several states, including Alabama, California, Maryland, Michigan, Missouri., North Carolina, Kentucky, West Virginia, and Ohio. It has also been planted in Italy, China, Japan, Israel, Belgium, Portugal and Romania (Brannan, R. G et al., 2015). In Romania, it was introduced around 1900 in Pianu Nou, Alba County, by an emigrant returning from America. From there, some plants arrived in the locality of Geoagiu, where they are still found in the present of some locals (Stănică, F. et al., 2004).

In 2000, at the Faculty of Horticulture in Bucharest, 7 varieties and 3 hybrids of *Asimina triloba* (L.) Dunal imported from Italy were

introduced, with which three collections were established. One collection exists within the Bucharest Faculty of Horticulture, and the other two collections are in private gardens in Argeş and Ilfov counties (Stănică F., 2002; Stănică F. et al., 2004; Stănică F., 2012). Today, the northern banana is one of the most exotic plants that have been acclimatized in Romania with a huge potential on the domestic market, especially due to the special taste of vanilla and chocolate. *Pawpaw* fruits have a special taste, are rich in nutrients, dietary fiber, protein, minerals and vitamin C, they can be used as ornamental and medicinal species (Layne, D.R., 1996; Picchioni, G. A et al., 2004; Pomper, K.W., et al., 2002; Pomper and Layne, 2005).

The harvest season for pawpaw fruit is from mid-August to the end of September. A color change often occurs later in the ripening period. On the other hand, a decrease in fruit firmness is relatively obvious and a detectable indicator of maturity (Pomper et al., 2008). It is also recommended to harvest the fruits during the early ripening period, in order to increase the storage time (Archbold et al., 2003; Pomper & Layne, 2005; Szilagyi B. A., 2015).

In Bucharest, the fruits of *Asimina triloba* (L.) Dunal ripen only at the beginning of October (Cepoiu, N. et al., 2003).

During ripening, the loss of firmness is extremely fast, the fruits soften quickly, at ambient temperature and this can be an obstacle in the development of a wider development market (Galii et al., 2008).

Monilinia spp is the plant pathogen responsible for the occurrence of grey mold and fruit rot in stone fruits species, and it is present in all cultivated areas (Cristea S. et al., 2017)

Agricultural production is vulnerable to contamination and infection with various microorganisms during storage and the safety of agri-food products can be achieved by maintaining climatic factors in the stored areas, thus limiting the population level of contaminating microorganisms. The most common genera of fungi identified in storage room are *Aspergillus*, *Penicillium* and *Fusarium* (Dudoiu, R. et al, 2016)

Micromycetes' development on storage room is conditioned by temperature and atmospheric humidity present in stored areas and by its fluctuations in time (Cristea S., 2004)

The results showed that Romanian natural conditions are suitable for growing this species as one of the most exotic plants that were ever acclimated in Romania. The pawpaw has an huge potential on the domestic market.

MATERIALS AND METHODS

The present paper presents the results of research conducted between 2016-2017, a period in which no phytosanytary treatements have been applied, the plants proving their suitability to the organic production systems

Research has sought to identify the presence of mycoflora on the fruits of *Asimina triloba* (L.). The biological material consisted of samples of pawpaw fruit, stored in the room with a controlled atmosphere, from which samples were taken for analysis. The fruits were harvested by hand and stored in rooms with a controlled atmosphere with 1.5% CO_2 at 4°C and a relative humidity of 90%.

The samples were taken immediately after collection and stored for 60 days. The batch sampling was performed on three levels, respectively the base, the middle and the upper surface, then a sample of 100 fruits was constituted (Chira L., 2008).

PDA culture medium was used to isolate and identify microorganisms associated with the disintegration of pawpaw fruits.

The fruits were washed from the ground with tap water and finally rinsed with sterile water. No pesticides have been used as they can also affect pathogenic fungi. Since the aim was to isolate the fungi that spore on the surface, the introduction of the samples in the humid chamber was preceded by a day or two (Severin, V. et al., 2009).

Segments of 1-2 mm of tissue from the edge of the affected area are passed, with the help of a sterile scalpel and a repeating needle, in Petri dishes with a diameter of 70 mm, on the PDA culture medium. The vessels were incubated at 22°C for 9 days. Observations were made at 3, 6 and 9 days. The Euromex stereo microscope and the Euromex Ox Range microscope were used to identify fungi, based on the scientific literature (Raicu C., 1978; Hulea A., et al., 1986; Hulea A., 1969).

RESULTS AND DISCUSSIONS

Studying the spectrum of pathogens on the fruits of the "northern banana" it was found that the mycoflora present in the analyzed samples was composed of fungal species belonging to the genera *Alternaria* spp., *Verticillium* spp., *Penicillium* spp., *Fusarium* spp. and *Monilinia* spp.

The analysis of the 2016 samples shows that the fruits from the studied genotypes showed fructifications of the micromycetes *Alternaria* spp., *Verticillium* spp., *Penicillium* spp., *Fusarium* spp. and *Monilinia* spp.

Table 1. Mycoflora detected on pawpaw fruits

Genotypes	The pathogen agent				
	Alternaria spp.	*Verticillium* spp.	*Monilinia* spp.	*Penicillium* spp.	*Fusarium* spp.
R1P2	+	+	+	-	-
R1P8	-	-	-	+	-
R2P1	-	-	+	+	+
R2P3	+	+	+	+	+
10344	-	-	+	+	-
10836	-	-	+	+	+

In the samples of pawpaw fruits, from the 2016 harvest, micromycetes belonging to the genera *Alternaria* spp., *Verticillium* spp., *Penicillium* spp., *Fusarium* spp. and *Monilinia* spp. When analyzed genotype R2P3i was identified and detected the fungus *Alternaria* spp., who was present also on the skin of genotype R1P2, R2P3.

The pathogen *Penicillium* spp. was identified on the genotypes R1P8, R2P1, R2P3, 10344, 10836. The fungus *Fusarium* spp. was present on the genotypes R2P1, R2P3, 10836.

Micromycetes of the genus *Monilinia* spp. Were identified on genotypes R1P2, R2P1, R2P3, 10344 and 10836.

Observations made on the incidence of mycoflora detected on “northern banana” fruits in 2016 (Table 2) show that the micromycete *Alternaria* spp., present on genotypes R1P2, R2P3, had the highest frequency value, in the variety R1P2 with F = 70%.

Pathogens of the genus *Monilinia* spp, showed the highest incidence values in the variety R2P3 with 66%, 10344 (56%) and R2P1 with 45%. The lowest incidence value was noted in the R1P2 genotype (20%).

In the analyzed samples, the micromycete *Penicillium* spp. Was identified on the genotypes R1P8, R2P1, 10836, 10344, R2P3, with frequency values of 100%, 48%, 44% and F = 17%, respectively.

Table 2. Incidence of mycoflora detected on pawpaw fruits expressed as a percentage (2016)

Genotypes	The pathogen agent (after 9 days)				
	Alternaria spp.	*Verticillium* spp.	*Monilinia* spp.	*Penicillium* spp.	*Fusarium* spp.
R1P2	70	10	20	-	-
R1P8	-	-	-	100	-
R2P1	-	-	45	20	35
R2P3	10	4	66	17	3
10344	-	-	56	44	-
10836	-	-	35	48	7

The results showed that *Alternaria* spp., *Verticillium* spp., *Penicillium* spp., *Fusarium* spp. and *Monilinia* spp. were found with pawpaw fruits studied. All five isolated organisms were confirmed to be pathogenic on pawpaw fruits, but in different percentages. Gupta and Pathak (1986) previously reported that pathogens were responsible for post-harvest pawpaw losses in southwestern Nigeria.

CONCLUSIONS

Research has shown that fruit rot and the development of pathogens depend on various factors. The optimum temperature range for the development of pathogenic fungi was 22°C.

Isolation of these pathogens confirmed the studies of Baiyewu and Amusa (1999), Baiyewu et al. (2007), Gupta and Pathak (1986) which showed pathogenic fungi cause significant losses in pawpaw fruits at post-harvest.

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MODELLING THE CUMULATIVE EFFECTS OF MICROCLIMATE IN AN INTENSIVE APPLE ORCHARD BASED ON MICROMETEOROLOGICAL MEASUREMENTS

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Abstract

The study of specific microclimate in apple fruit orchards is very important, thus micrometeorological measurements give valuable information on how the plants will react on different weather changes. Modelling the variations of environmental factors in apple orchard, specialist can give directions on critical periods and points when is an urgent demand of technological intervention (irrigation and other orchard management). In the plant-air interaction system critical parameters such as air maximum and minimum temperature, relative humidity, rainfall directly influences the crop physiological responses. A series of micrometeorological parameters were evaluated in an intensive apple orchard in Northern Transylvania, Bistrita fruit region from Romania with the objective of defining the interactions between trees and the aerial environment. An important objective was the study of daily meteorological observations in the flowering period in spring with implications on floral development and specific summer drought periods.

Key words*: micrometeorology, environment, plant-air system, flowering, drought period.*

INTRODUCTION

Micrometeorology deals with measurements and observations in small scale and time, smaller than 1 km and occur at the bottom of the atmospheric layer close to the earth surface. It shows primary interactions of low exchange processes between plants, water, land atmosphere, radiant energy. Microclimatology and agrometeorology measurements thus give valuable information on plant-microclimate interaction. In fruit growing is essential the study of these parameters including air temperature (minimum, maximum), air relative humidity, rainfall, sunshine hours and solar irradiance, wind. Knowledge of these factors are crucial in plant protection (Cristian M.F., 2019), irrigation scheduling, water uptake, evapotranspiraton, breeding, physiology. The Intergovernmental Panel on Climate Change-IPCC Special Report on Global Warming showed an increase of 1.5°C in Europe, for Romania it is estimated an increase of 0.5-1.5°C also, for the period 2020-2029. Several researchers studied the effect of temperatures in tree phenology in Europe (Chmielewski, 2001, 2002, 2005), the negative influence of drought periods (Mateescu, 2012; Sandu, 2010) and the effect of water deficit (Paltineanu et al., 2008, 2011) in Romania. Objective of the present study was the accurate modelling of the main micrometeorological factors like average, minimum, maximum temperatures, relative humidity, rainfall and those impact in the last three years (2017-2019) in an intensive apple orchard in Bistrita fruit region, Northern Transylvania, Romania.

MATERIALS AND METHODS

The micrometeorological observations were effectuated at FRDS Bistrita, in an intensive apple orchard planted with Romanian bred - cultivars Auriu de Bistrita, Aura, Generos grafted on M26 and M9 rootstock. Main micrometeorological parameters were registered by Adcon Telemetry weather station. The orchard was planted on a clay-loamy site, well drained, with specific thermal and rainfall conditions. The microclimatological data acquisition was effectuated at 2.0 m height, experimental period was the last 3 years (2017-2019). Data were registered every 15 minutes, downloaded, respectively analysed by MS Office Excel package.

RESULTS AND DISCUSSIONS

The aim of the study was the presentation of daily, monthly and yearly fluctuations of main micrometeorological parameters, these factors being characteristic to local conditions of Bistrita fruit production region from Northern Transylvania, Romania, which influenced the physiology of apple orchards between 2017-2019. Global climatological parameters showed, that the yearly average values had a clear increasing tendency (Fig. 1), when average values of the experimental years (10.8°C) were compared with the 9.6°C multiannual reference temperature (1993-2019). The calculated difference temperature was 1.2°C (Table 1) in agreement with the IPCC modelling for Europe and implicitly for Romania.

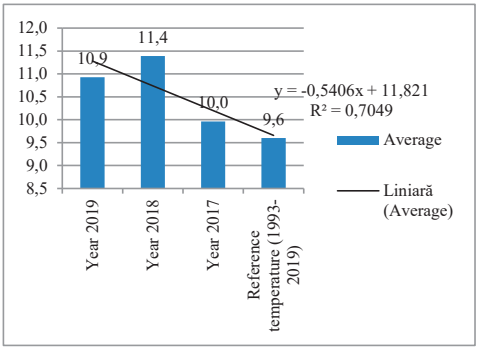

Figure 1. Average temperatures registered at micrometerological weather station FRDS Bistrita

Average minimum temperatures showed fluctuations in the last 3 years, the calculated difference was 1.3°C (Fig.2).

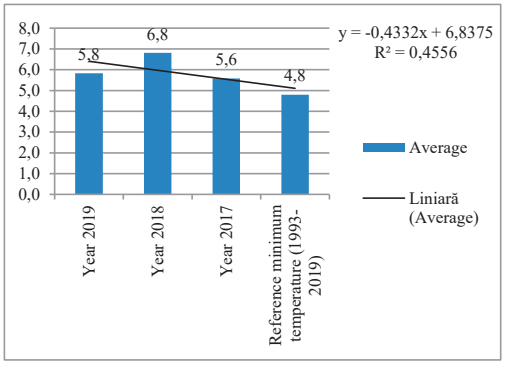

Figure 2. Average minimum temperatures registered at micrometerological weather station FRDS Bistrita

We can observe that the minimum temperatures in 2017 and 2019 had close values, excepting year 2018 which had a higher average minimum temperature value (6.8°C).

In the analysis of maximum temperatures (Fig. 3) it is shown a linear tendency of increasing of temperatures from 15.4°C to 17.1°C between 2017-2018, respectively from 15.4°C to 16.9°C in 2019. There are observed great fluctuations between the experimental years regarding the average maximum temperature parameter.

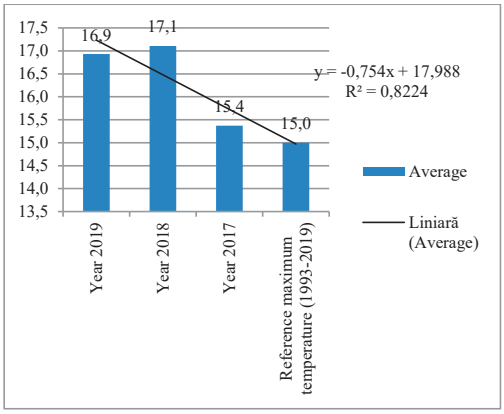

Figure 3. Average maximum temperatures registered at micrometerological weather station FRDS Bistrita

However, when analysing average maximum temperatures in the studied interval (1993-2019), one can observe in overall, that there are differences of 1.5°C.

Relative humidity fluctuations (Fig. 4) showed relatively close values, average being 73.9%.

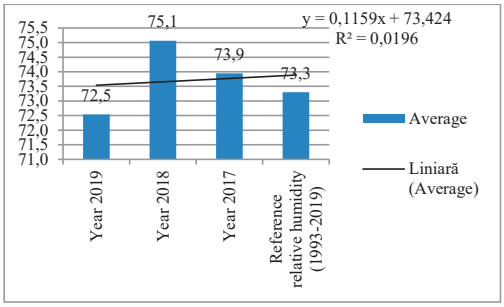

Figure 4. Relative humidity registered at micrometerological weather station FRDS Bistrita

Calculated relative humidity difference (Table 4) between reference relative humidity interval and the studied period was slight, namely 0.6%. One of the most important micrometeorological

factor is the rainfall for the life of a fruit tree. Measurements showed (Fig. 5) a decreased tendency of rainfall when compared with the multiannual reference interval (756.9 mm).

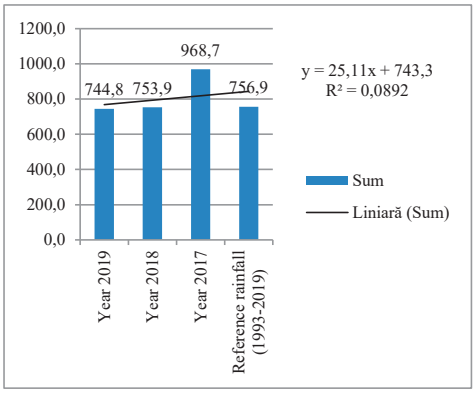

Figure 5. Rainfall registered at micrometerological weather station FRDS Bistrita

One can observe however, a great rainfall quantity in 2017 (968.7 mm) which decreased in time to 744.8 mm in 2019, thus influencing dramatically the apple orchard. The drought period in summer influenced negatively the yield, the fruits weight and diameter. Monthly average temperatures (Fig. 6) showed in 2018 a slight increase, during April-June, but in 2017 and 2019 were registered close values regarding thermal fluctuations.

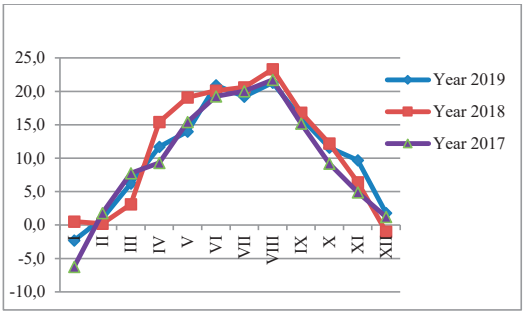

Figure 6. Average monthly temperatures registered at micrometerological weather station FRDS Bistrita

Monthly minimum temperatures (Fig. 7) showed also greater values during April-August in 2018, the graph showing clearly the increasing tendency.

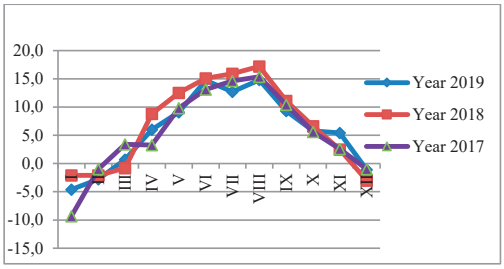

Figure 7. Average monthly minimum temperatures registered at micrometerological weather station FRDS Bistrita

The average monthly maximum temperatures (Fig. 8) in 2018 showed also a slight increase during April-June, being above the values from 2017-2018.

Figure 8. Average monthly maximum temperatures registered at micrometerological weather station FRDS Bistrita

Relative humidity fluctuations (Fig. 9) appeared in months April and May 2019, when compared with 2018-2017 period, showing higher values in the flowering period. Oppositely, during 2018, lower relative humidity values were registered in the same period, having a slight negative effect on the flowering of fruit trees.

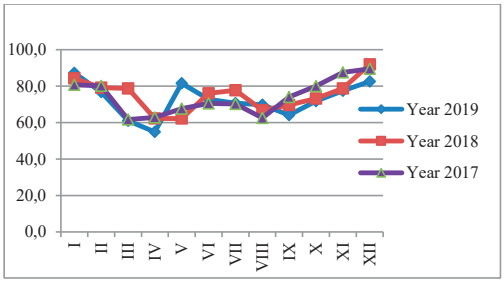

Figure 9. Average monthly relative humidity fluctuations registered at micrometerological weather station FRDS Bistrita

The registered rainfall (Fig. 10) showed also strong monthly variations, critical periods were June-July in 2019, causing drought in the most important physiological period of the year, namely the preparing of floral bud anthesis for the following year. The fruit growing during the same period (June-July) of summer was affected by the severe drought. Low rainfall values were registered also in September-October causing severe drought, falling of fruits before harvest and again a lesser yield.

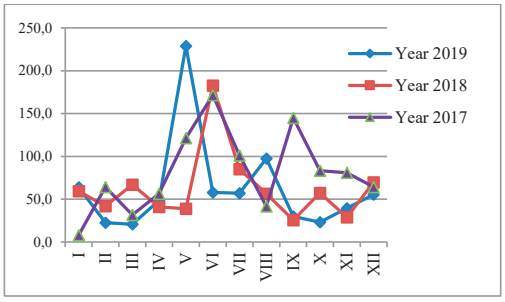

Figure 10. Average monthly rainfall fluctuations registered at micrometerological weather station FRDS Bistrita

Heavy rainfall occurred in May 2019, the soil was fully saturated with precipitations, hail event was also registered in July 2019.

The study of absolute minimum (Fig.11) temperature values showed low values in 2017 during winter (-19.1°C) and a severe decrease of temperatures in March 2018 (-14°C), April 2017 (-4.0°C). Absolute minimum temperatures showed a relative constancy in April-May 2019 registering temperatures just slightly above 0°C.

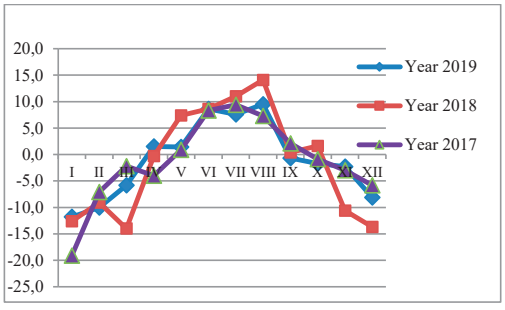

Figure 11. Absolute minimum temperatures registered at micrometerological weather station FRDS Bistrita

Absolute maximum values showed clearly higher values in 2018 during April-May and June (Fig. 12).

Figure 12. Absolute maximum temperatures registered at micrometerological weather station FRDS Bistrita

Analysing fig. 13 one can observe that in the most important period of flowering, generally lower maximum temperatures were observed in 2019 when compared with 2018, starting from 09 March until 12 May, the past year being colder in the sprig period.

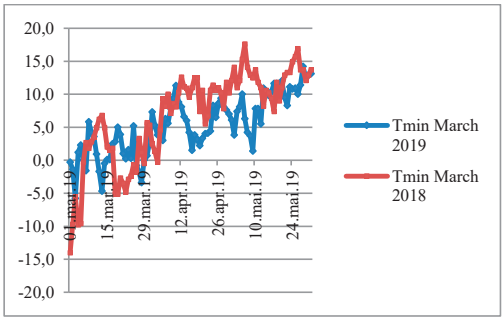

Figure 13. Daily minimum and maximum temperatures in May registered at micrometerological weather station FRDS Bistrita

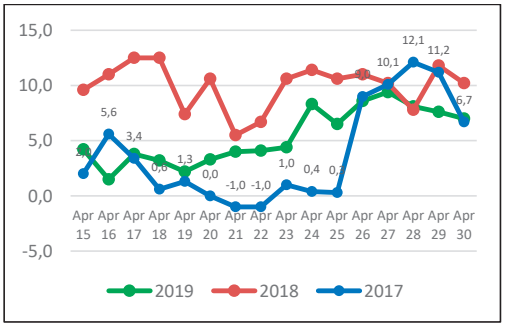

Figure 14. Micrometeorological conditions in the flowering period (April 2017-2019) at FRDS Bistrita

Focusing on the flowering period (Fig.14) of apple cultivars one can observe that climate conditions were not optimum in 2017 (18-25 April), several days the meteorological weather station registered very low values between -1.0 and 1.3°C. Thus floral development was affected (Table 1) in 2017, with negative implications on fruit quality and quantity. Flowering period was longer with 1-2 days in 2019 when compared with 2018 at Auriu de Bistrita cultivar (Table 1).

Studying the rainfall amount per month (Fig. 15) in the active vegetation period, we can observe that in August before harvesting the fruit yield in September, generally very low amount of precipitation was registered in 2017 and 2018, implications were crucial on fruit diameter, fruits were smaller and were not so crisp and turgescent (Table 1).

Fig. 16 shows the sum of rainfall in the active vegetation period, in 2018 were just 458.7 mm registered when compared with 2017 and 2019 period (523.7 mm in 2017 and 510.4 mm in 2019).

Table. 1 Flowering and floral development of some experimental cultivars during 2017-2019 at SCDP Bistrita

Cultivar/ Rootstock	Flowering period (start date) BBCH 57	Flowering period (end date) BCH 69	Floral development	Fruit diameter (mm)
Auriu de Bistrita-2019	18.04.19	02.05.19	Abundant flowering	92
Auriu de Bistrita-2018	16.04.18	30.04.18	abundant flowering	84
Auriu de Bistrita-2017	17.04.17	01.05.17	slightly affected	95
Aura-2019	18.04.19	02.05.19	Abundant flowering	84
Aura-2018	16.04.18	30.04.18	Abundant flowering	78
Aura-2017	17.04.17	01.05.17	slightly affected	82
Generos-2019	19.04.19	03.05.19	Abundant flowering	83
Generos-2018	17.04.19	01.05.19	Abundant flowering	82
Generos-2017	18.04.17	02.05.17	slightly affected	79

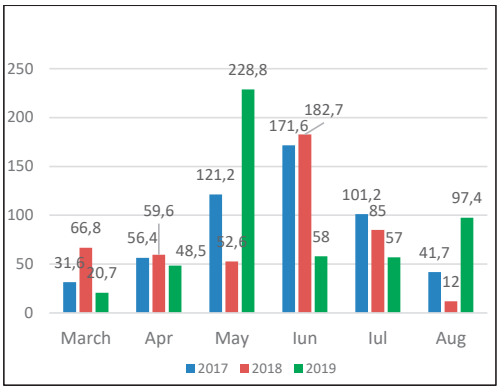

Figure 15. Rainfall in the active vegetation period during 2017-2019 at FRDS Bistrita

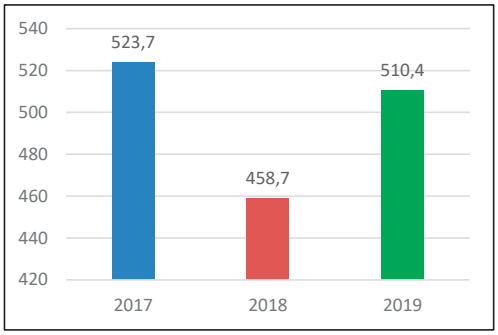

Figure 16. Sum of rainfall in the active vegetation period during 2017-2019

CONCLUSIONS

Investigations were effectuated in Northern Transylvania, Bistrita fruit region at Fruit Research and Development Station Bistrita during 2017-2019. Temperature (average, minimum, maximum, absolut minimum, absolut maximum), relative humidity, rainfall were registered in an intensive apple orchard at 2.0 m height.

The micrometeorological parameters together, cumulatively influenced the physiology of apple orchard, causing different variations in yield, diameter of fruits, weight of the fruits. The research showed an overall tendency of average temperature increasing in the last 3 years (+1,2℃) when compared with the multiannual reference interval, according to IPCC simulation. We confirm that the IPCC modelling was correct, indeed the temperature increasing is real and shows a linear tendency, also at the maximum temperatures (+1.5℃).

Low minimum temperatures were observed in the flowering period in 2017, thus yield was affected by meteorological conditions. Temperatures fluctuated between -1.0 and 1.3°C in the flowering period in April, floral development was affected, thus pistils and anthers of flowers. In the colder flowering period bees also have not searched the flowers for pollination. This process begun again just after 26.04.2017 when temperatures increased above 12°C and meteorological conditions were better. At the other hand rainfall measurements showed low precipitation level in June and July in the study period, especially in 2018 and 2019, when drought conducted to curling of leaves and the trees suffered of water deficit. Fruit diameter was greatly affected in 2018, the low amount of precipitation in August conducted to smaller fruits due to lack of water, fruits were not crisp thus quality of apples were affected also. Thus, researches show the urgent necessity of irrigation in fruit orchards in the summer period also in Northern Transylvania, Romania.

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PRELIMINARY RESULTS ON THE EFFICACY OF SOME ORGANIC INSECTICIDES AGAINST APHIDS TO EUROPEAN PLUM

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Abstract

The use of ecological treatments in the prevention and control of diseases and pests for fruit trees is increasing. Thus, more and more orchards are established on an ecological system in order to obtain crops with no pesticide residues after harvesting. There are few organic insecticides on the market and their effectiveness is not fully known. This situation creates difficulties for those who choose such crops, assuming practically the risk of potential inefficient control of the main diseases and pests. In this experiment, the effectiveness of several organic insecticides was studied against one of the main plum pests, the aphids respectively. The tests were performed both in the field and under laboratory conditions, on shoots heavily populated by aphids. The treatments were applied by spraying the whole surface of the shoots. The preliminary results obtained after 48 hours highlited two organic insecticides, Ovipron Top and Prev-Am, that had an efficiency of over 90%, and 70%, respectively at the concentrations recommended by the manufacturer.

Key words: *organic insecticides, aphids, prevention and control, plum*.

INTRODUCTION

The necessity to use eco-treatments instead of those of chemical synthesis is due to an ever-increasing demand of developing farmers, which want to return to eco fruit products with no pesticide residues and chemical fertilizers (Maxim, 2008; Neelesh and Attika, 2015). This change is felt globally from the traditionally developed countries in agriculture to the developing states that are trying to align with the current market demands, the consumer being the main segment in this change (Theodosiou et al., 2014; Manoj, 2017; Lacey et al., 2015).

The organic insecticides against diseases and pests, which are currently on the market, are limited in number and their effectiveness is not yet fully known. This could generate some concerns for those who want to practice organic crops, assuming the potential risks for an inefficient control of main diseases and pests to plum.

In Romania, the expansion of new organic orchards took place after the implementation of measure 4.1a "Investments in fruits trees orchards" within the National Programme of the Rural Development, 2014-2020. Establishment of ecological orchards with non-refundable funds from the European Union by the mentioned programme received higher score, and therefore become very attractive to farmers. The problem that arises is, however, the chance of success of these orchards, or rather, how much of the forecasted output will be able to be realized so that the consumer will benefit from organic fruits from the allocated funds.

The plum is still the dominant fruit species in Romania (FAOSTAT, 2018), and part of the production would be desirable to be obtained organically.

In this context, controlling the main diseases and pests in the orchards remainned problematic, since limited information about the effectiveness of organic insecticides are available. This thing leads to the need to test organic insecticides in order to establish their effectiveness against the main diseases and pests.

It is known that the aphids are one of the main factors that affect the development and production of plum trees (Rakauskas, 2015; Țucă, 2013; Lenteren et al., 2006). Treatments against aphids are applied for a dual purpose: first, they are designed to reduce aphid populations to decrease the direct damage due to their feeding on young shoots and leaves; secondly, treatments with insecticides are applied to reduce the potential spread of the most detrimental viral pathogen - *Plum pox virus* (PPV), by decreasing aphid vector populations (Zagrai et al., 2020).

In this study, we assessed the potential use of some organic insecticides against aphids, in order to provide information for practical application.

MATERIALS AND METHODS

The experiment was organised in micro variants in two distinct tests, on plum: one carried out under laboratory conditions, and the other in the orchard. A comparison of organic insecticides in relation to those of chemical synthesis used as controls against aphids was made. The organic insecticides tested in the experience were: Konflic, Laser 240 SC, Prev-Am, Canelys, Oleorgan, Algasil, Ovipron Top, Deffort and BactoSpeine DF. All products used are approved for organic farming.

Konflic (0.3%) it's a contact insecticide based on 50% Potassium Salt from vegetable oil extract and 50% bitter Quassia extract. It is a natural product used to control and reduce the population of pests (*Trialeurodes vaporariorum*, *Thripida* sp., *Aphidoidea* sp.) from horticultural crops.

Laser 240 SC (Spinosad 240 g/l) (0.06%) new family of biological insecticides, naturally includes insecticides derived from metabolites of living organisms. It do not act systemically (it is not translated by xylem or phloem), but they penetrate the leaf blade and are in translation. It is recommended against the orders: Lepidoptera, Diptera, Hymenoptera, Coleoptera, Thysanoptera, Isoptera.

Prev-Am (Orange oil 60 g/l) (0.8%) is a contact product with a physical mode of action, with insecticidal and fungicidal effect. The product is in the category of essential oils, natural and volatile. It acts on the layers of insect protection and the external mycelium to the mushroom.

Canelys (Cinnamon extract 700 g/l) (0.3%) is a contact insecticide based on cinnamon tree extract (*Cinnamomum zaylanicum*). It has a proven effect on stimulating the self-defence reactions of plants in flour and other fungi but also in control of *Acari* spp. found in vegetable crops, flowers, fruit trees and vines.

Oleorgan (0.3%) (Neem extract 400 g/l) is an insecticide based on vegetable oils that can be used to control pests in vegetable crops, flowers, fruit trees and vines. The insecticide also has a repellent effect against pests. It's recommended for effective fighting of: *Trialeurodes vaporariorum*, *Thripida* spp., *Aphidoidea* spp. etc.

Algasil (Algae extract plus K_2O 90 g/l and SiO_2 200 g/1) (0.5%) is a multi-activity self-defence stimulator and inducer (fungicide, insecticide, acaricide). It contains algae extract (*Ascophyllum nodosum*) plus silicon and potassium, which exerts a stimulating effect on crops. It amplifies the natural resistance mechanisms of the plants in the presence of pests such as *Acari* spp. or other insects with soft body.

Ovipron (Top Paraffinic oil 96.5 g/kg) (2.5%) is a highly refined, paraffinic mineral oil with insecticidal action, being also an excellent adjuvant for other contact plant protection products. Insecticidal action is mainly manifested by asphyxiation, covering the body of the insects with a fine film, which penetrates through the trachea channels, blocking the breathing, thus causing death.

Deffort (Fabaceae family extract 8 g/l) (0.3%), an organic insecticides with insecticidal effect, increases the self-defence capacity of the plant. It has a repellent effect that ensures that the plant tissues are not attractive to pests. Even egg laying is avoided. The product has in its composition plant extract from the Fabaceae family and microelements as physiological activators. Contains alkaloids that cause insecticidal activity.

BactoSpeine DF (0.1%) insecticide, the latest generation that acts on the target larvae of pests as soon as they are consumed from the treated plants. The product is a combination of toxic protein crystals and a spore. It contains: 54% *Bacillus thuringiensis*, *Kurstaki* subsp. ABTS

351. It acts on pests by degrading the intestinal walls, the larvae can no longer feed and die within 24 to 72 hours.

The chemical synthesis products used in relation to the ecological ones were: Calypso (Thiacloprid 480 g/l) (0.02%), Mospilan (Acetamiprid 200 g/kg) (0.02%), Actara (Thiamethoxam 250 g/kg) (0.01%), Movento (Spirotetramat 100 g/l) (0.19%), Zeon Karate (Lambda-cyhalothrin 50 g/l) (0.015%).

During the first test carried out under laboratory conditions, 42 plum shoots from a "Stanley" plum tree fully populated by aphids (*Hyalopterus pruni*) were collected. In choosing the shoots, it was taken into account that they have approximately the same size and a similar number of aphids. The shoots were then suddenly distributed in 14 glass containers (three shoots each), in water (Figure 1).

Figure 1. The type of variants distribution (original)

Nine of those were used for testing organic insecticides, while five for chemical synthesis products, used as controls. Each variant was labelled and then the shoots were sprayed with the mentioned products. The concentrations used were those recommended by the manufacturer.

The observations were made at two days, considering the fact that most of the organic insecticides have a contact action and their effect is visible according to the manufacture after 24-48 hours.

The second test was performed in the orchard, where two trees of Stanley variety, strongly populated by aphids, were selected for spraying. These trees were not treated before from the beginning of the vegetation period until the moment of spraying. Organic insecticides were tested on one of the trees, and the products of chemical synthesis, used as controls, were tested on the other tree. Thus, nine variants were prepared on the tree on which the organic insecticides were applied, corresponding to the nine organic insecticides. For each variant, six shoots with a similar size, placed on the same branch from different parts of the crown, were labelled. After the clear delimitation for each variant, shoots were treated by spraying with organic insecticides, depending on the variant previously established. To reduce the risk of accidentally spraying another variant, a cardboard panel was used for protection. In a similar way, five variants were prepared on the control tree, where the chemical insecticides were applied.

The observations were made at two days, taking into account the fact that most of the organic insecticides have a contact effect and the results should be visible after 24-48 hours from application. Thus, the rate of mortality was determined after 24, and 48 hours respectively from the spraying time by counting the aphids on infested leaves.

RESULTS AND DISCUSSIONS

In the first experiment, performed under laboratory conditions, the results revealed that after 24 hours from the treatments, the highest rate of mortality after spraying with organic insecticides, was recorded at Ovipron Top, with a rate of mortality of 70%. Spraying with Prev-Am produced a rate of mortality that was reach to 40%, while the treatments with the rest of the organic insecticides determined a rate of mortality between 3-10% (Figure 2).

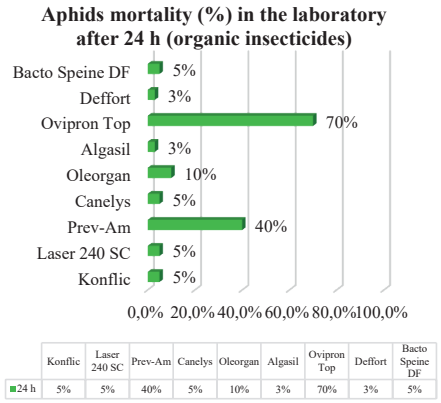
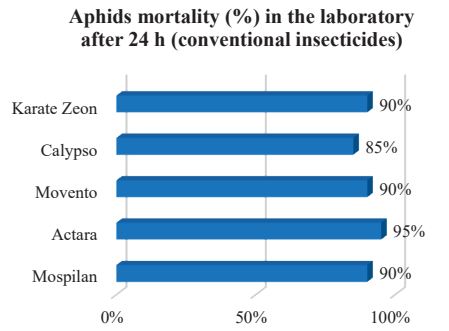


Figure 2. Rate of aphids mortality on variants with organic insecticides (after 24 hours) under laboratory conditions

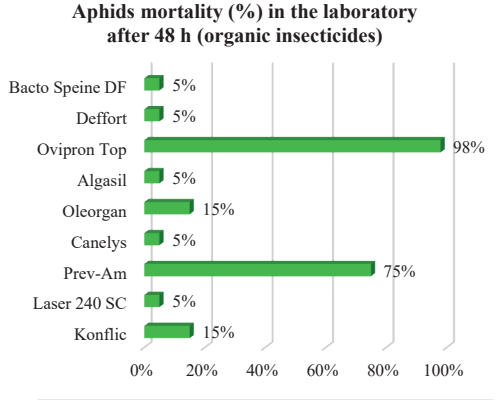
In the same experiment, application of chemical insecticides produced a mortality of over 80% in the first 24 hours (Figure 3).



	Mospilan	Actara	Movento	Calypso	Karate Zeon
24 h	90%	95%	90%	85%	90%

Figure 3. Rate of aphids mortality on variants with chemical products (after 24 hours) under laboratory conditions

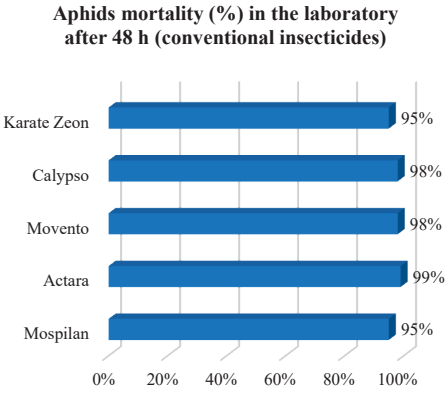
The results obtained after 48 hours for the organic insecticides generally showed a slightly increasing rate of mortality in comparison with those recorded after 24 hours. However, treatments with Ovipron Top led to a high rate of mortality (98%), comparable with that recorded on chemical variants. Also, treatments with Prev-Am reach to 60%. Treatments with the other organic insecticides determined a rate of mortality between 3-15%, being thus unsatisfactory (Figure 4).



	Konflic	Laser 240 SC	Prev-Am	Canelys	Oleorgan	Algasil	Ovipron Top	Deffort	Bacto Speine DF
48 h	15%	5%	75%	5%	15%	5%	98%	5%	5%

Figure 4. Rate of aphids mortality on variants with organic insecticides (after 48 hours) in laboratory conditions

In the case of chemical synthesis products, their effectiveness reaches at almost 100% mortality after 48 hours from spraying (Figure 5).

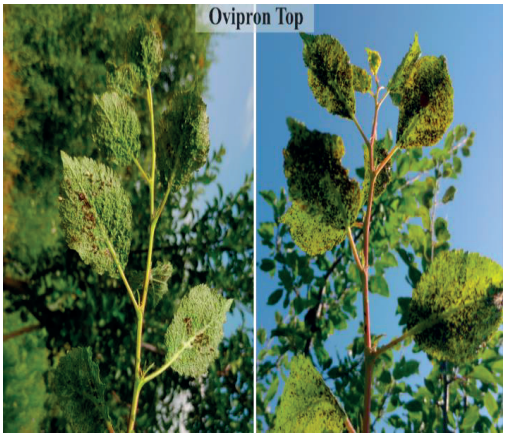


	Mospilan	Actara	Movento	Calypso	Karate Zeon
48 h	95%	99%	98%	98%	95%

Figure 5. Rate of aphids mortality on variants with chemical products (after 48 hours) in the laboratory conditions

For the second experiment undertaken in the orchard, the results are largely preserved similar to the data from the first test, under laboratory conditions.

Thus, the highest rate of mortality was determined by spraying with Ovipron Top, with 95% mortality after 24 hours, and 98% mortality after 48 hours (Figure 6).

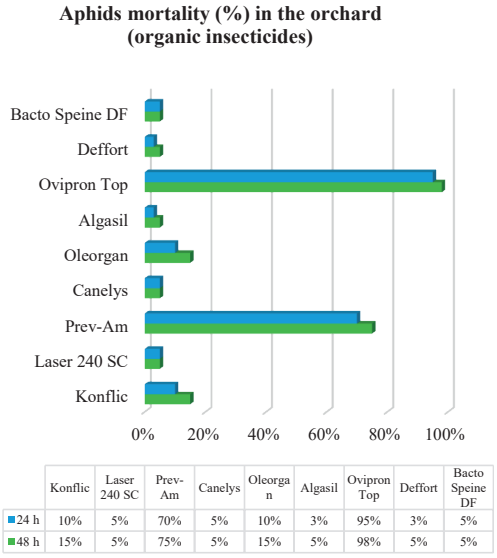


a b

Figure 6. Phytosanitary status before (a) and after (b) application the treatment with Ovipron Top

Spraying with Prev-Am also produced a good rate of mortality that reach to 70% after 24

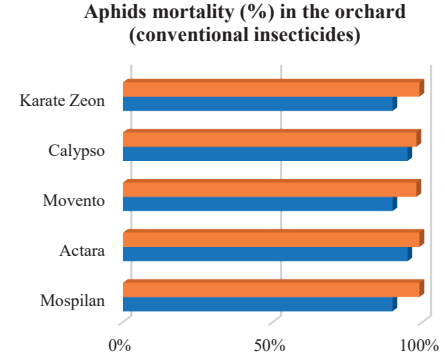
hours from application, and 75% after 48 hours (Figure 7).



	Konflic	Laser 240 SC	Prev-Am	Canelys	Oleorgan	Algasil	Ovipron Top	Deffort	Bacto Speine DF
24 h	10%	5%	70%	5%	10%	3%	95%	3%	5%
48 h	15%	5%	75%	5%	15%	5%	98%	5%	5%

Figure 7. Rate of aphids mortality on variants with organic insecticides (after 24 hours and 48 hours respectively) in the orchard

Treatments with the five chemical insecticides produced a similar rate of mortality after 24 and 48 hours, respectively, reaching to almost 100% mortality after two days (Figure 8).



	Mospilan	Actara	Movento	Calypso	Karate Zeon
48 h	99%	99%	98%	98%	99%
24 h	90%	95%	90%	95%	90%

Figure 8. Rate of aphids mortality on variants with chemical products (after 24 hours and 48 hours respectively) in the orchard

This experiment will be extended along few consecutive years and will take into account a larger period after spraying to determine the pest mortality since some products gave unexpectedly results. For example Laser SC 240 gave favorable results in other experiments, such as against whitefly (*Trialeurodes vaporariorum*) in tomato (Prijović et al., 2012) and for *large pine weevil* (*Hylobius abieti)* control in spruce seedling (Brudea and Ciucla, 2007). Important to mentioned that these results were obtained at 14 days and 21 days (treatments against *Hylobius abieti),* and 23 days (treatments against *Hylobius abieti)* after spaying.

CONCLUSIONS

Preliminary results from the two experiments undertaken both, under laboratory conditions and in the orchard, revealed that Ovipron Top is an effective organic insecticide that could be used against aphids in plum crops. However, the treatments applied along vegetative period in a few consecutive years could provide further and more well documented results. Prev-Am is also a potentially valuable promising organic insecticide that required attention in the future. Treatments with the other seven organic insecticides were unsatisfactory under the study conditions since the rate of mortality was between 5-15% after 48 hours from spraying.

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SCREENING OF APRICOT ELITES REACTION TO *PSEUDOMONAS* SPP.

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Abstract

Apricot decline remains an important threat to the apricot industry. Pathogens like: fungi, bacteria, viruses and mycoplasma are able to destroy the trees after several years of good growth. Diseases caused by Pseudomonas spp. in apricot has widely spread in fruit-producing areas worldwide. During the 2018 growing period, isolations were taken from apricot trees showing "blossom blast" symptom, grown in an apricot breeding orchard at the Fruit Growing Institute of Plovdiv, Bulgaria. It was found that in this area the species causing the observed symptom belong to Pseudomonas spp. In terms of integrated pest management genetical resistance of the cultivars is the most reliable way for reducing infection. In 2019, thirty-one apricot elites, selected from the hybrid families 'Harlayne' x 'Harcot', 'Lito' x 'Silistrenska ranna' and 'Harcot' x 'SEO' and all parental cultivars, were tested for their response to Pseudomonas spp. infection after artificial inoculation. The aim of this experiment was to study the reaction of all elites to the pathogen and to select the perspective ones for future breeding purposes. The laboratory tests were conducted in 2019 by artificial inoculation of one-year-old shoots in 'full flowering' phenophase with the obtained in 2018 isolate of the phytopathogen. In 48% of the studied elites, first symptoms were observed on the second day after the inoculation. Twenty-nine percent of the elites showed the first symptoms on the 5th day after inoculation. The parental cultivar Silistrenska ranna was the only genotype that showed symptoms on the 6th day. The lowest disease severity index was recorded for elite LS 14-30 – 3,45%, followed by HH 13-45 13,68%, HH 9-2- 15%, HH 13-20 - 15,43% and HH 9-1 - 17,50%. which makes them valuable for future breeding purposes.

Key words*: apricot, artificial inoculation, blossom blast, breeding.*

INTRODUCTION

Diseases of fruit trees caused by *Pseudomonas* spp. are of major concern in fruit-producing areas worldwide, because they need precise control, and result in significant economic losses. Disease symptoms include blossom blast and spur dieback, leaf and fruit lesions, cankers with associated gummosis of woody tissue, and decreased fruit yields (Kennelly et al., 2007). *Pseudomonas syringae* pv. *syringae* van Hall 1902, is causing a bacterial blast of apricot and cherry (Young, 1987). During periods of cool, wet weather or after frost events, blossom blast symptoms can occur. Early in the growing season, pathogen populations develop on apparently healthy blossoms. Blossom blast symptoms on stone fruits are similar to those of blossom blast on pear, except the damage to pear is not accompanied by further wood infection. Frost damage is recognized as an important predisposing injury for bacterial canker infection, and cankers initiated from blossom infections are a common symptom. The bacteria overwinter in cankers, in buds and within the tree (Kennelly et al., 2007). During wet patches in spring, the bacteria multiply, ooze from the branch cankers and are spread by rain. Spring is the time of year when infections of the bacterial blast can be most conspicuous. The most susceptible growth stages of the trees are during flowering and leaf buds opening. The spreading of the pathogen is also facilitated by cold winters and humid climates (Zhebentyayeva, 2012).

The apricot flowering stage in Bulgaria takes place in early spring when the weather is often cool and wet, accompanied by spring frosts. These environmental conditions are the most conducive for disease development (Marshall, 2015). On apricot trees, grown in commercial orchards and home gardens in the Silistra region are observed untimely tree decline antecedent by canker symptoms. Initial characteristic symptoms observed are mainly on trunk and scaffold branches as small or larger bark cankers with sap flow and dark-amber gumming at bud unions, crotches, pruning wounds or at the base of affected spurs.

The subsequent development of the disease is expressed as blossom blast, dried leaves attached to trees and twig dieback (Ivanova, 2007).

According to Kennelly et al. (2007) the management of diseases caused by *Pseudomonas* spp. is almost unattainable, due to the lack of effective chemical or biological control measures, lack of host resistance, and the endophytic nature of the pathogen during some phases of the disease cycle. Thus, the use of apricot genotypes resistant to *Pseudomonas* spp. is economically and technically the most reliable method for effective management (Bassi, 1999). The objective of this study was to evaluate the reaction of apricot elites after artificial inoculation with *Pseudomonas* spp. and to select the least sensitive ones for future breeding schemes.

MATERIALS AND METHODS

This study was conducted in 2018 and 2019 at the Fruit Growing Institute in Plovdiv, Bulgaria. In the spring of 2018 during the flowering phenophase of the apricots, the weather was rainy and cool. Thus, blossom blast symptom was observed on a big number of apricot hybrids in a breeding orchard. The observed disease symptoms included necrosis flower buds. Bark below the dead buds on severely affected trees showed black streaking. After bark shaving, dark xylem tissue was visible along the branch length, in contrast to the color of the healthy tissue. A fluorescent, gram-negative bacteria were consistently isolated onto King's B medium from twigs showing symptoms. *Pseudomonas* spp. was isolated onto a standard nutrient medium by generally accepted phytopathological methods (Kiraly et al., 1974). The pathogenicity of the bacterial isolates was tested on tobacco plants (*Nicotiana tabacum*) according to Klement's method (1963).

In the spring of 2019 in the laboratory of phytopathology at the Fruit Growing Institute, an artificial inoculation was done. For this purpose 1-year-old shoots, from trees showing no symptoms, were cut in BBCH 57 phenophase and placed in water containers. The reaction of 30 apricot elites obtained from tree hybrid families 'Harlayne' x 'Harcot', 'Harcot' x 'SEO', 'Lito' x 'Silistrenska ranna' and all 5 parental cultivars was evaluated. The artificial inoculation was done by spraying the 1-year-old apricot twigs in full flowering phenophase (BBCH 63-65) with 3 x 10^8 CFU mL bacterial suspensions in sterile water. Sprayed shoots were covered with moist plastic bags and maintained in favourable conditions - t = 23°C and 80-90% air humidity for 6 days. Symptom development was monitored daily. On the basis of first symptoms observed the incubation period was recorded. After 5 days the degree of attack was determined for each flower using a 5-grade scale (Figure 1): 0 - no symptoms; 1 - symptoms of necrosis on petals; 2 - symptoms of necrosis on pistil and receptacle; 3 - symptoms of necrosis observed on sepals; 4 - symptoms of necrosis observed on all flower parts. An average degree of attack was calculated.

Figure 1. Scale for determining the degree of attack

Five days after the artificial inoculation the disease severity index was calculated on the basis of the damaged flowers.

The twigs were left in water containers for further development and twenty days after the artificial inoculation the percentage of infected leaf buds was recorded.

For statistical data processing were used Duncan's test (Steele and Torrie, 1980) and hierarchical cluster analyses using between-groups linkage method of the IBM SPSS Statistics 19 statistical software.

To prove that the observed damages were caused by *Pseudomonas* spp. reisolations of the bacteria were done using two selective media – King's B and Aesculin hydrolysis (Kaluzna et al., 2012).

RESULTS AND DISCUSSIONS

Knowledge of the incubation period of infectious diseases (time between host infection and expression of disease symptoms) is crucial to our epidemiological understanding and the design of appropriate prevention and control policies. Plant diseases cause substantial damage to agricultural systems, but there is still very little information about how the incubation period varies within host populations (Leclerc et al., 2014). The duration of the incubation period is strongly influenced by environmental factors, especially the temperature, and the host sensitivity (Karov, 2006).

In our case, the hosts were artificially inoculated in a sensitive phenophase and maintained in suitable for the pathogen development conditions. Thus, the first symptoms occurred very fast (Table 1). The cultivars 'Harcot' and 'Lito' showed necrosis on the petals 2 days after the inoculation. Symptoms on 'SEO' and 'Harlayne' occurred on the 3rd and 4th day resp. After 5 days, symptoms with different severity were observed on all of the genotypes tested except cv. Silistrenska ranna. The incubation period for that cultivar was the longest and first symptoms were observed on the 6th day after the inoculation.

The degree of attack for the elites obtained by the 'Harlayne' x 'Harcot' cross and both parental cultivars ranged from 0.56 to 2.13 (Table 2). Statistically, non-significant difference was observed between both cultivars. The least affected by the pathogen were three of the elites - HH 13-45, HH 9-2 and HH 13-20. The difference between them and the parental cultivars was statistically proven. Although HH 9-2 showed first symptoms on the 2nd day after the spray inoculation, five days later the degree of attack was still low. These three genotypes are valuable because the degree of attack is low and they are better than both parental cultivars and 65% of the tested elites obtained by this parental combination.

The elites originating from 'Harcot' x 'SEO' combination were compared to their parental cultivars. The average degree of attack for these genotypes ranged from 1.37 to 2.85. The most severe damages were observed on the twigs of 'SEO' cv. A statistically significant difference was observed between 'SEO' and the other genotypes (Table 3). All elites tested had lower values of degree of attack than both parental cultivars but the difference with 'Harcot' was non-significant.

Five days after the artificial inoculation on the 'Silistrenska ranna' cut shoots were not observed any symptoms and its calculated average degree of attack was 0 (Table 4). For one of the tested elites were observed mild symptoms - LS 14-30. Although the first symptoms occurred on the second day, the pathogen did not spread in the next 4 days and for this elite, the calculated avarage degree of attack was 0.1.

Table 1. Duration of the incubation period

Incubation period (days)						
Day	**1**	**2**	**3**	**4**	**5**	**6**
Cultivar / Elite №		'Harcot'	'SEO'	'Harlayne'	HH 13-83	Silistrenska ranna
		'Lito'	HH 13-45	HH 12-19	HH 12-38	
		HH 9-1	HH 12-33	HH 13-20	HH 12-42	
		HH 12-60	HH 13-51	HH 12-22	HH 12-36	
		HH 9-2	LS 14-30	HS 12-20	HH 12-58	
		HH 13-1	HS 12-12		HH 12-47	
		HH 12-50			HS 12-16	
		HH 12-53			HS 12-19	
		HH 13-67				
		HH 13-24				
		HH 12-66				
		HH 12-30				
		HH 12-70				
		LS 14-18				
		HS 12-8				

Table 2. Degree of attack recorded for 'Harlayne' x 'Harcot' hybrid family

Cultivar/Elite №	Number of flowers inspected	Degree of attack (average)
HH 13-45	52	0.56 l
HH 9-2	20	0.60 l
HH 13-20	47	0.62 l
HH 9-1	20	0.70 kl
HH 12-22	28	0.82 jkl
HH 13-83	35	0.89 ijkl
HH 13-67	26	0.92 hijkl
HH 12-38	17	1.06 ghijkl
HH 12-58	28	1.21 fghijk
HH 13-3	31	1.29 efghij
HH 12-42	35	1.40 defghi
'Harlayne'	18	1.44 cdefgh
HH 13-51	26	1.54 bcdefg
HH 12-60	31	1.55 bcdefg
HH 12-50	18	1.56 bcdefg
HH 12-30	31	1.58 abcdefg
HH 12-66	34	1.68 abcdef
HH 12-36	44	1.75 abcdef
HH 12-70	21	1.81 abcde
HH 12-19	13	1.85 abcde
'Harcot'	29	1.93 abcd
HH 13-24	16	1.94 abcd
HH 12-33	17	2.00 abc
HH 12-53	55	2.07 ab
HH 12-47	31	2.13 a

Table 3. Degree of attack recorded for 'Harcot' x 'SEO' hybrid family

Elite №	Number of flowers inspected	Degree of attack (avarage)
HS 12-12	30	1.37 b
HS 12-16	17	1.65 b
HS 12-8	25	1.68 b
HS 12-19	19	1.84 b
'Harcot'	29	1.93 b
'SEO'	20	2.85 a

Table 4. Degree of attack recorded for 'Lito' x 'Silistrenska ranna' hybrid family

Elite №	Number of flowers inspected	Degree of attack (average)
'Silistrenska ranna'	30	0.00 b
LS 14-30	29	0.10 b
LS 14-18	29	2.34 a
LS 12-20	14	2.43 a
'Lito'	17	2.47 a

The highest disease sevirity index was recorded for 'SEO' cv. - 71.25% and the lowest for 'Silistrenska ranna' - 0%. Low severity index was recorded also for the LS 14-30 elite (3.45%), obtained by a controlled hybridization with the presence of 'Silistrenska ranna' as a parent (Figure 1).

As the least susceptible, on the basis of flower infection, could be defined 'Silistrenska ranna' cv. and the elites - LS 14-30, HH 9-2, HH 13-20, HH 13-45, HH 9-1, HH 13-83, HH 13-47, HH 12-22 and HH 12-38. The disease severity index for these genotypes ranged from 3.45 to 26.47%.

After the pathogen has infected the flower parts of the host it continues its development in the leaf buds. Some of the tested elites had low disease severity index but after 20 days a high percentage of the leaf buds were damaged - some were necrotic and did not develop at all for others necrosis on the young leaves was observed. For example - HH 13-45 and HH 13-20. Elite LS 12-30 and 'Silistrenska ranna' cv. had low disease severity index and a low percentage of leaf buds showing symptoms. Some of the elites obtained by the parental combination 'Harlayne' and 'Harcot' had a medium value of the disease severity index but the disease did not damage a big percentage of their leaf buds. For example HH 12-19, HH 12-30, HH 12-50. A very interesting genotype from this hybrid family was HH 12-22. Its disease severity index was 20.54 and the percentage leaf buds with symptoms for this elite was the lowest recorded - 12.12%.

For better comparison of all genotypes, hierarchical cluster analyses using between-groups linkage method was used. It divided all tested cultivars and elites according to the disease severity index and the percentage of damaged leaf buds together (Figure 2). On the dendrogram could be seen that the genotypes were grouped in 3 main clusters. The 'SEO' cultivar could be evaluated as the most susceptible to *Pseudomonas* spp. with disease severity index = 71.25% and 100% damaged buds. This cultivar was not grouped with any other genotypes which shows us that none of the tested elites and cultivars had such an intensive reaction to the pathogen. Our field observations also confirm the susceptibility of 'SEO'. All of the elites obtained by the parental combination 'Harcot' x 'SEO' show a disease severity index above 35% and less damaged leaf buds than 'SEO'.

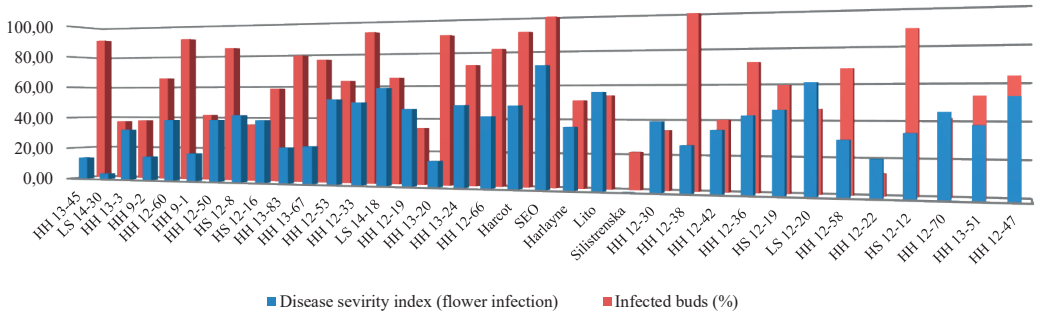

Figure 1. Disease severity index of flower infection and percentage of infected leaf buds 20 days after artificial inoculation

After the hierarchical cluster analyses as the least susceptible to the pathogen could be defined the grouped in one cluster genotypes - LS 14-30, HH 9-1, 'Silistrenska ranna' and HH 12-22.

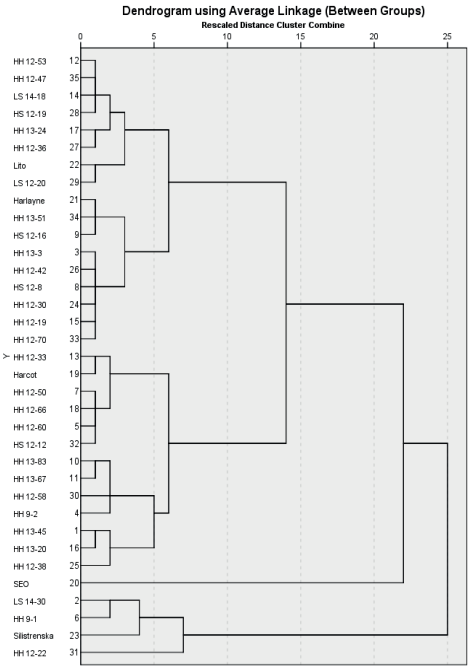

Figure 2. Dendrogram of hierarchical cluster analyses

In other previous our studies the elites were genotyped for *Plum Pox Virus* resistance by MAS (Milusheva et al., 2016). This gives us the opportunity, on the basis of this and our previous studies, to selected elites that combine low susceptibility to flower infection caused by *Pseudomonas* spp. and resistance to PPV for future breeding purposes.

Table 5. Promising elites

Elite №	Severity index to *Pseudomonas* spp. (%)	Percentage of infected leaf buds (%)	PPV resistance factor
LS 14-30	3.45	37.50	Resistant allele
HH 12-22	20.54	12.12	Resistant allele
HH 9-1	17.50	42	No data

The reisolated bacteria grown for 24-48 h on Aesculin hydrolysis gave a brown color of the medium. Grown onto King's B was observed a fluorescence reaction (Figure 3).

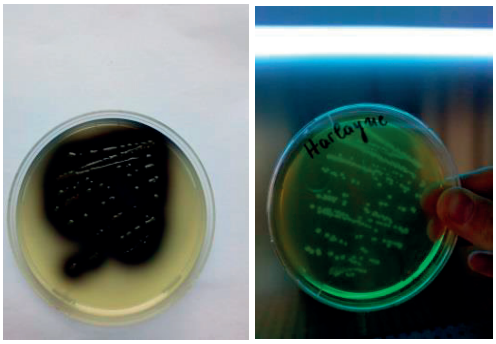

Figure 3. Reaction of the reisolated bacteria on to selective media

CONCLUSIONS

This study gives us the opportunity for conducting a very fast laboratory screening of breeding materials.

The obtained results allow us to evaluate the reliability of the tested parental combinations when used in breeding schemes aiming to low susceptibility to *Pseudomonas* spp.

The cultivar 'SEO' is proven and often used as a donor of *Plum Pox Virus* resistance but unreliable for obtaining genotypes with resistance to *Pseudomonas* spp. This indicates that when it is used in controlled crosses should be combined with cultivars showing low susceptibility to the bacterial disease.

'Silistrenska ranna' is promising cultivar for this purpose. Because it has some serious disadvantages, concerning the fruit quality, F2 and F3 generations could be obtained for combining a complex of valuable traits.

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EVALUATION OF THE BIOCHEMICAL QUALITY OF *ARONIA MELANOCARPA* FRUITS IN THE CONDITIONS OF SOUTHERN ROMANIA, UNDER THE INFLUENCE OF FERTILIZATION

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Abstract

The aim of the present study is to evaluate the chemical composition of chokeberry fruits (Aronia melanocarpa (Michx.) Elliott, variety 'Nero'), specifically total anthocyanins, total polyphenols, vitamin C and malic acid under the influence of fertilization applied to soil, foliar, and combined (soil + foliar). In the 'Nero' variety, soil fertilization and foliar fertilization were applied, respectively, with the graduations: a1 - mineral fertilization in the soil with the quantities recommended by the SMART program! Fertilizer Management software, a2 - application of Biozyme foliar fertilizer in a concentration of 0.1% and a3- soil mineral fertilizers in combination with foliar fertilizer (graduations a1 + a2). Between 2017 and 2019, in the experimental plot, the following doses and forms of mineral fertilizers were applied to the soil, adapted to the species requirements: 74.10 kg/ha MAP (ammonium monophosphate), 125.7 kg/ha Magnisal (magnesium nitrate), 122.7 kg/ha ammonium nitrate and 196.5 kg/ha Multi K (potassium nitrate). Chemical analyzes show a high content of antioxidants in chokeberry fruits. The high anthocyanin content (3535 mg kg fresh fruit, as well as total polyphenols (83128 mg/kg fresh fruit), vitamin C (over 97 mg/100 g fresh fruit), and malic acid (0.72 g/100 g fruit) emphasizes the pharmaceutical properties of the chokeberry species.

***Key words**: polyphenols, anthocyanins, antioxidant, vitamin C, organic acids.*

INTRODUCTION

Aronia, known as chokeberry, is native to North America, from where it has spread throughout the world. The genus includes the species *Aronia arbutifolia* (L.) Pers (red chocheberry), *Aronia melanocarpa* (Michx.) Elliott (black chokeberry) and *Aronia prunifolia* (Marshall) Rehder (purple chokeberry). In Europe it is cultivated on large areas as an important industrial crop (Hardin, 1973; Seidemann, 1993; Strigl et al., 1995). In recent years, the potential of chokeberry fruits has been recognized as a source of natural food and valuable phytonutrients (Slimestad et al., 2005; Nicola et al., 2012). In the future it is possible that the natural dyes extracted from *Aronia* will replace synthetic coloring, azo-types that are presently used (Snebergrova, 2014). *A. melanocarpa* is among the richest sources of polyphenols in the plant kingdom (Denev et al., 2012; Denev et al., 2013). Numerous scientific studies show the chemical composition of chokeberry fruits (Kulling & Rawel, 2008; Denev et al., 2012), their clinical efficacy and their use for various diseases (Park et al., 2013; Daskalova et al., 2015; Borowska, 2016). According to Kulling & Rawel (2008), the chemical composition of chokeberry fruits is characterized by high nutritional and biological values and depends on several factors: genotype, climate, date of harvest and use of fertilizer (Jeppsson, 2000; Skupien et al., 2007). Substances with antioxidant effect (polyphenols, organic acids, vitamins, anthocyanins, carbohydrates and proteins, etc.) are involved in inhibiting the free radical propagation reactions produced *in vivo* by reactive oxygen species, nitrogen species, and lipid peroxidation in

food (Cevallos-Casals & Cisneros-Zevallos, 2004).

Research in the field of phenols extracted from plants (Schaffer et al., 2005) shows that phenols depend quantitatively and qualitatively on genetic information (species, variety), environment and geographical conditions. Climate, season, light, temperature, maturation period strongly influence the synthesis of phenols in plants (Aherne & O'Brian, 2002).

Because oxidative damage is involved in the development of various diseases, vitamin C could have a preventive or even therapeutic effect. Vitamin C thus acts only together with other biochemically active compounds from fruits and vegetables (Dimitrović, 2006).

The hydroxyl radical, one of the strongest free radicals known, can initiate lipid peroxidation, can break DNA strands, and oxidize virtually any organic molecule (Burkitt & Duncan, 2000; McCord, 2000).

A possible mechanism for neutralizing free radicals by the ascorbic ion (Dimitrović, 2006; Halliwell & Gutteridge, 1990) is presented below:

$$AscH^- + O_2 \rightarrow AscH^{\bullet -} + O_2^{\bullet -}$$
$$AscH^- + O_2^{\bullet -} \rightarrow AscH^{\bullet -} + H_2O_2$$

or hydroxyl radical $OH^{\cdot}$) (Halliwell & Gutteridge, 1990):

$$Fe(III) + AscH^- \rightarrow Fe(II) + Asc^{\cdot -}$$
$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^{\cdot} + OH^-$$

In the literature the observations on the technology of chokeberry cultivation are contradictory. Mineral fertilizer, among other agronomic practices, can influence the nutritional value and content of biochemically active compounds in fruits. Cultivation methods can be used to improve phenol content and fruit pigmentation, although information on this topic is very rare and often contradictory (Tomás-Barberán & Espin, 2001). Carbonaro et al. (2002), observed an improvement of the plant's antioxidant defense system (peach and pear) as a possible consequence of agronomic cultivation practices. On the other hand, Häkkinen & Törrönen (2000), found a similar content of polyphenols in three conventionally grown and organically grown strawberry crops.

In this study we estimate whether mineral soil fertilizer, foliar fertilizer and combined fertilizer (soil and foliar) influence the content of basic nutrients and improve sensory attributes by (increasing sugar content) and therefore the nutritional value of chokeberry fruits.

MATERIALS AND METHODS

The present study was carried out at the Research Institute for Fruit Growing Pitesti, Mărăcineni, in the experimental field of shrubs (44° 51′ 30" N, 24° 52" E), in an *Aronia melanocarpa* plantation, year 5, Romania. The experiment was organized on a soil from the class of wet phreatic aluviosol protisols, formed on fluvial deposits, with a loam-sandy granulometric composition. The field was located in a meadow terrace of the Argeș River. The soil, is characterized by a strongly moderate acid reaction, a low humus content and a low assimilable phosphorus content in the arable layer. The biological material chosen was the chokeberry variety, 'Nero', (planting distance being 3.0 m between rows and 1.5 m between plants per row). In the 'Nero' variety, soil fertilizer and foliar fertilizer were applied, respectively, with the graduations: a_1 - mineral fertilizer in the soil, with the quantities recommended by the SMART program! Fertilizer Management software (smart-fertilizer.com), a_2 – application of Biozyme foliar fertilizer in a concentration of 0.1% and a_3- application of mineral fertilizers to the soil in combination with foliar fertilizer ($a_1 + a_2$ graduations). During the vegetation period, from 2017-2019 the following doses and forms of mineral fertilizers were applied to the soil, in the experimental plot, adapted to the species requirements for an expected harvest of 10 t/ha: 74.10 kg/ha MAP (ammonium monophosphate), 125.7 kg/ ha, Magnisal

(magnesium nitrate) 122.7 kg/ha, ammonium nitrate and 196.5 kg/ha, Multi K (potassium nitrate). The fruit samples were harvested between 2017-2019, in three replications, at the technical maturity of harvesting.

Laboratory chemical determinations consisted of the determination of total sugar content (%), using the Fehling-Soxlet volumetric method (Singleton & Rosi, 1965), titratable acidity expressed as total malic acid %, using the volumetric method with 0,1N sodium hydroxide, vitamin C (mg/100 g fresh fruit), which was dosed using the iodometric method by solvent extraction using ethyl alcohol-hydrochloric acid, and total polyphenols expressed as mg galic acid/kg fresh fruit using Folin - Ciocalteu method (Singleton & Rosi, 1965). All analytical determinations were performed on three replications, and the data were subjected to analysis of variance (ANOVA). The influence of experimental factors was analyzed by the Duncan test, with a significance level of $P \leq 0.05$. Correlations were also made between the biochemical quality indicators of the fruits. Statistical data analysis was performed using SPSS 14.0 for Windows software.

RESULTS AND DISCUSSIONS

Anthocyanins

On average, over the three years of experimentation, soil fertilizer combined with foliar fertilizer led to a significant increase in anthocyanin content up to 3252 mg/kg fresh fruit, compared to foliar fertilizer (2690 mg/kg fresh fruit) and root fertilizer (2280 mg/kg fresh fruit) (Figure 1). Both in 2017 and 2019 the anthocyanin content was higher in the case of fertilization with both soil and foliar fertilizer, compared with the application of only one type of fertilizer. However, in 2018, the difference between the three types of fertilizer applications was insignificant. It is obvious that agronomic practices influence the biosynthesis (metabolism) of anthocyanins. Some hormones (yasminic acid, abscisic acid, etc.) can increase the content of anthocyanin pigments and the biochemical quality of fruits (McClure, 1975; Lee et al., 1996). In the version of combined soil and foliar fertilizer , the product used Biozyme 1% contains cytokinins and auxins which accelerate the metabolism of the plant. Dixon & Paiva (1995) show that these nutrients actually affect the activity of the enzyme phenylalanine ammonialyase responsible for the increase in the substrate of anthocyanin biosynthesis (Figure 1).

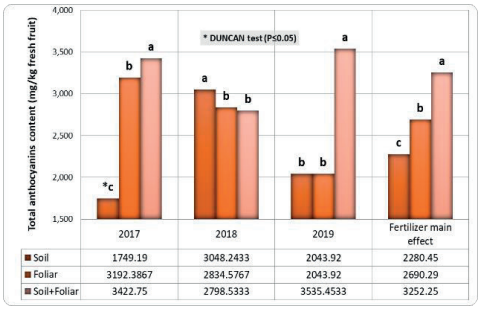

Figure 1. Influence of fertilizer on the anthocyanin content of fruits depending to the study year, in the 'Nero' variety

However, there were deviations from this trend, a1 graduation (soil fertilizer) in 2018 registering the highest content in anthocyanins; in the a2 graduation in 2018 was registered the lowest anthocyanin content (Figure 2).

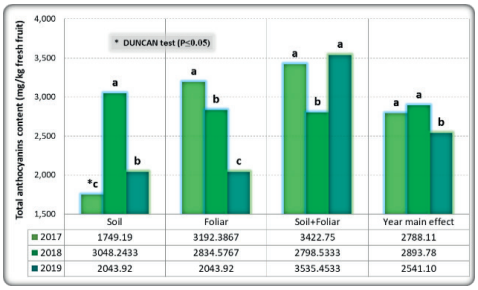

Figure 2. Influence of the study year on the anthocyanin content of fruits depending on the fertilizer, in the 'Nero' variety

Polyphenols

Overall, the polyphenol content decreased significantly in the third year of fertilizer application (Figure 3).

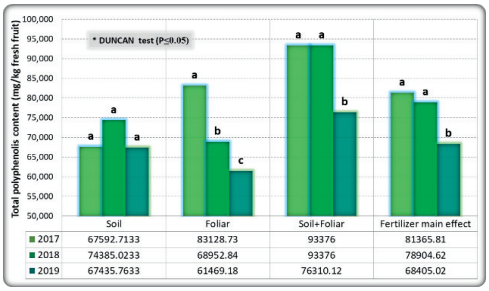

Figure 3. Influence of the study year on the content of polyphenols in fruits depending on the fertilizer, in the 'Nero' variety

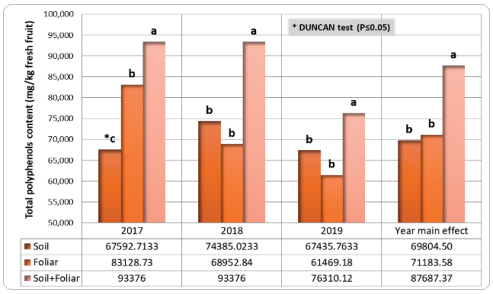

Figure 4. Influence of fertilizer on fruit polyphenol content depending to the study year, in the 'Nero' variety

On average, over the three years of the study, the polyphenol content was significantly higher in the a3 graduation (soil + foliar fertilizer) (87,687.37 mg/kg fresh fruit), compared to the other two graduations, the effect being maintained with small oscillations, in all years of experimentation (Figure 4).

Agronomic practices (McClure, 1975, McGarry et al., 1996) such as fertilization (Misra et al., 1991; McNabnay et al., 1999) influence the biochemical quality of fruits in the sense of increasing the content of polyphenols in response to stress (fertilization with insufficient doses of potassium or high doses of potassium, high doses of phosphorus, etc.).

Sanchez et al. (2000) confirm the control of the enzyme phenylalanine ammonialyase on the biosynthesis of anthocyanins and polyphenols, during the stress period of plants by inducing nitrogen toxicity to green beans.

In this case the content of polyphenols decreases due to the inhibition of the mentioned enzyme (Figure 4).

Vitamin C

On average, depending on the graduation of fertilizer applied, the vitamin C content was significantly higher in 2018 (94.01 mg/fresh fruit), compared to 2017 (64.26 mg/fresh fruit) and compared to 2019 (58.28 mg/fresh fruit). The trend was maintained on each fertilizer variant (Figure 5).

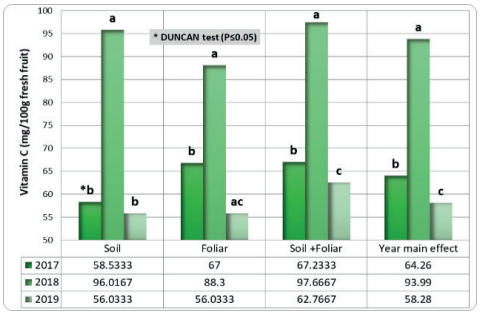

Figure 5. Influence of the study year on the vitamin C content of fruits depending on the fertilizer, in the 'Nero' variety

Over the three years of the study, the application of the third graduation, a3 (soil combined with foliar fertilizer), stimulated the increase of vitamin C content (75.89 mg/ 100 g fruit pulp), compared to the a1 (soil fertilizer only) (70.19 mg/100 g fruit pulp) or a2 (foliar fertilizer only) (70.44 mg/100 g fruit pulp). The average trend was generally maintained throughout the years of experimentation (Figure 6).

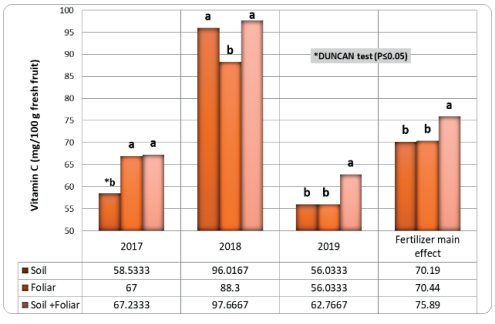

Figure 6. The influence of the fertilizer on the vitamin C content of the fruits depending to the study year, in the 'Nero' variety

Malic acid

The malic acid average content in 2018 was higher (0.64%), compared to other years. Unlike the average trend, in the a1 graduation (soil fertilizer only), the malic acid content decreased, whereas in the a3 graduation (combined soil and foliar fertilizer), the malic acid content increased over time (Figure 7).

Looking at the 3 years of study, the third graduastion, a3 (combined soil and foliar fertilizers) determined an increase in the percentage of malic acid in fruits (0.66%), compared to the other two graduations. Only in 2017, the first year of fertilization, the soil only fertilization (a1) exceeded the other two graduations in terms of the percentages of malic acid found in the fruit (Figure 8).

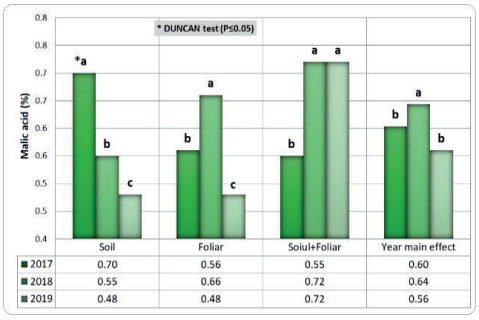

Figure 7. Influence of the study year on the malic acid content of fruits depending on the fertilizer, in the 'Nero' variety

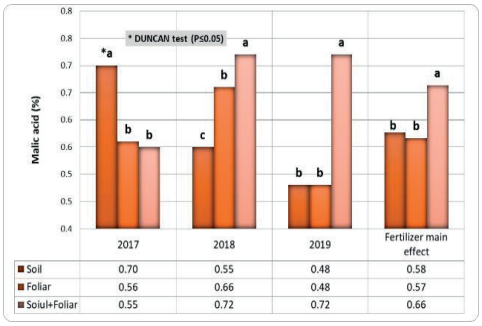

Figure 8. Influence of fertilizer on fruit malic acid depending on the study year, on the 'Nero' variety

Table 1. Matrix of correlation (Pearson "r" correlation coefficients "r") of the main physical and biochemical indicators studied in 'Nero' variety).

Indicators		Total sugar content (%)	Malic acid (%)	Total anthocyanins content (mg/kg fresh fruit)	Total polyphenol content (mg/kg fresh fruit)	Vitamin C (mg/kg fresh pulp)
Total sugar content (%)	Pearson Correlation	1.000	0.019	0.104	-0.183	0.081
	Sig. (2-tailed)		0.921	0.591	0.342	0.675
Malic acid (%)	Pearson Correlation	0.019	1.000	0.300	-0.664(**)	0.276
	Sig. (2-tailed)	0.921		0.114	0.000	0.147
Total anthocyanins content (mg/kg fresh fruit)	Pearson Correlation	0.104	0.300	1.000	-0.288	0.306
	Sig. (2-tailed)	0.591	0,114		0.130	0.106
Total polyphenol content (mg/kg fresh fruit)	Pearson Correlation	-0.183	-0.664(**)	-0.288	1.000	-0.428(*)
	Sig. (2-tailed)	0.342	0.000	0.130		0.021
Vitamin C (mg/kg fresh pulp)	Pearson Correlation	0.081	0.276	0.306	-0.428(*)	1.000
	Sig. (2-tailed)	0.675	0.147	0.106	0.021	

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

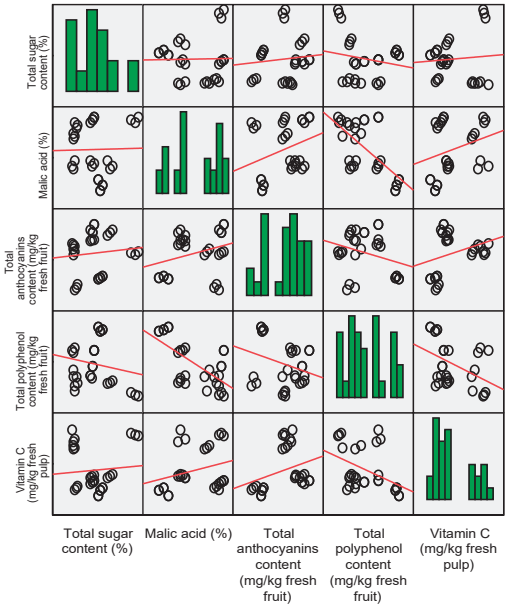

Figure 9. Chart of correlations between biometric and biochemical indicators studied in 'Nero' variety

Analyzing the values obtained (Table 1, Figure 9) a distinctly significant negative correlation is observed between the malic acid content and the polyphenol content. The phenomenon is explicable, if we consider the ripening period of the fruits. Polyphenols accumulate in fruits when day/night temperature differences are large, in response to plant stress. Also, a distinctly significant negative correlation can be observed between the content of vitamin C and the content of total polyphenols in chokeberry fruits.

CONCLUSIONS

In the end of the present study, the conclusions are that in the chokeberry culture from Research Institute for Fruit Growing Pitești Mărăcineni, under the influence of soil mineral fertilization, foliar fertilization and combined fertilization, the content of organic acids, expressed as total malic acid, accumulates in the fruit to the detriment of polyphenols biosynthesis.

Similarly, under the influence of the aforementioned fertilization, the content of vitamin C decreased as well in the fruit during the polyphenols biosyntesis.

Although still preliminary, these results have provided evidence that mineral fertilization may influence the chemical composition of chockeberry fruits. Other studies show that fertilizer application with increased doses of NPK have as result a higher yield whereas pigment content and total acidity decrease (Jeppsson, 2000).

The increase of the contents of biochemically active compounds was obtained using combined (soil and foliar) fertilization. Therefore, we recommend for the chokeberry culture as optimal the third graduation (a3), combined soil mineral fertilization and foliar fertilization with 0,1% Biozyme .

In the future, we intend to study an experimental model of fertilization with optimal doses of mineral fertilizers with NPK and microelements administered in the soil, combined with foliar administration of growth biostimulators.

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USING NORMAL AND REDUCED RATE OF PHEROMONE DISPENSERS ISOMATE A/OFM FOR CONTROL OF PESTS ON PEACH AND PLUM IN BULGARIA

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Abstract

Combined pheromone dispensers Isomate A/OFM are used for control of most important pests on peach and plum - Oriental Fruit Moth (OFM), Grapholita molesta (Busck.), Plum Fruit Moth (PFM), Grapholita funebrana (Tr.) and Peach Twig Borer (PTB) Anarsia lineatella (Zell.) by means of mating disruption (MD), at a rate of 800-1000 dispensers per hectare. The aim of the study was to evaluate possibilities for MD of these three important pests using dispensers Isomate A/OFM at a normal and a reduced rate.
Experiments have shown that pheromone dispensers Isomate A/OFM at a rate of 1000 pieces per ha provide excellent control for all three pests. At a reduced rate of 300 pieces per ha Isomate A/OFM shows a high degree of control of the OFM and PTB - no damage from both pests was found, but this rate was not sufficient for PFM and damaged plum fruits at harvest exceeded 40%. At the rate of 600 pieces per ha the results were better - damaged fruits from PFM, at harvest time, did not exceed 14%.

Key words*: mating disruption, oriental fruit moth, Grapholita molesta, plum fruit moth, Grapholita funebrana, peach twig borer, Anarsia lineatella.*

INTRODUCTION

Peaches and plums are traditional fruit crops in Bulgaria grown on large areas - more than 16 thousand hectares (Agrostatistics, 2018). Their fruits are used both for consumption and for production of juices, nectars, and purees for children and other foodstuffs. Farmers annually apply a significant number of treatments with agrochemicals to protect the orchards from pests. Both fruit crops are greatly affected by fruit moths, sawflies, aphids, scale insects, etc (Stancheva et al., 2008; Lecheva et al., 2006; Andreev and Kutinkova, 2004; Lecheva et al., 2003; Arnaudov and Andreev, 2002). On the one hand this practice is necessary to protect fruit production, but on the other hand the use of chemical pesticides poses a risk of food contamination with toxic substances and this is directly related to the health of consumers. Another important theme is the harmful effect of pesticides on beneficial insects in agrocenoses and in general on the environment. Reducing this harmful effect as well as obtaining healthy foods is a leading trend in modern plant protection. Therefore, more and more attention is paid to methods of pest control that do not use harmful chemical pesticides. Such a method is the mating disruption applied to some of the economically important pests of the two fruit crops, using synthetically derived sex pheromones. Some successful results of trials on mating disruption of different moths were reported by Kovanci (2019); Kutinkova et al. (2019, 2011); Andreev and Kutinkova (2010); Riolo et al. (2010); Brouwer et al. (2008); Toffolutti et al. (2008); Falta et al. (2007); Veronelli and Iodice (2004).
Combined pheromone dispensers Isomate A/OFM are not registered in Bulgaria. They are used in other EC countries for control of important pests on peach and plum - oriental fruit moth *Grapholita molesta* (Busck.) and peach twig borer *Anarsia lineatella* (Zell.) by means of mating disruption (MD), at a rate of 800-1000 dispensers per hectare (Biogard, 2019). The chemical composition is similar to the pheromone for the plum fruit moth *Grapholita funebrana* (Tr.).
The aim of the study was to evaluate possibilities for MD of these three important

pests using dispensers Isomate A/OFM at a normal and a reduced rate.

MATERIALS AND METHODS

The experiments were carried out in the period 2017-2019 in isolated peach and plum orchards without chemical treatments. Combined pheromone dispensers Isomate A/OFM produced from ShinEtsu Chemical LTD, Japan were used for MD of oriental fruit moth, peach twig borer and plum fruit moth. The dispenser contains (Z)-8-Dodecenyl acetate, (E)-8-Dodecenyl acetate, (Z)-8-Dodecen-1-ol, (E)-5-Decenyl acetate. (E)-5-Decen-1-ol (a.i. LOAD guaranteed 274.4 mg).

In 2017 the trial plots for oriental fruit moth and peach twig borer were located in the region of Plovdiv, Sliven and Karnobat - Central South and South-East Bulgaria.

Experiments in the Plovdiv area were conducted on a 5 ha, in an 8-year-old orchard with peaches and nectarines of different varieties. The orchard is located in the Experimental Field of the Agricultural University (AU), Plovdiv - Brestnik village, 5 km south of Plovdiv. Two conventional private orchards with a total area of 3 ha, in a distance of 1.5 km were used as control plots. Six organophosphate and pyrethroid treatments were conducted there.

Isomate-A/OFM was used at a rate of 1000 dispensers per hectare. Dispensers are hung on trees in early April when peaches and plums are in full bloom.

A similar experiment was conducted 15 km south of Sliven, in the village of Glufishevo. The trial plot was 4.5 ha with peaches and nectarines of different varieties. Located a kilometre away from it, a 7-hectare plot with conventional plant protection was selected to serve as a control orchard. It is treated with pyrethroids and neonicotinoids for 12-14 days.

In the town of Karnobat trial and control plots are two orchards with peaches and nectarines, 10 ha each and at a distance of 0.5 km. The control orchard is treated for 14-20 days with neonicotinoids and organophosphorus insecticides.

The trial plots for plum fruit moth was 0.5 ha plums, 'Stanley' and 'Chachanska Lipotica' varieties. The orchard is located at the Agro-Ecological Center at the AU, Plovdiv, which is certified for the producing of organic fruit production. The control plot was a similar one, but conventional orchard, at a distance of 0.5 km, where seven chemical treatments have been conducted with neonicotinoids, pyrethroids and organophosphates.

In 2018 and 2019, only the orchards in the area of Plovdiv were monitored. In 2018, the pheromone dispensers were set in the trial plots at a rate of 300 dispensers/ha and in 2019 - in the plum orchard only, the rate was increased to 600 dispensers/ha. Only three insecticidal treatments were conducted in the conventional orchard due to poor fertility.

The flight of oriental fruit moth, peach twig borer and plum fruit moth flights was monitored using sex pheromone trapping in the years of the study. PHEROCON® VI Delta sticky traps were installed in the trial orchards and in reference orchards, located in the vicinity, treated with conventional pesticides.

The damages to shoots from oriental fruit moth and peach twig borer, as well as the damages to fruits from the three pests, were evaluated on 20 trees, randomly selected for each block.

RESULTS AND DISCUSSIONS

In the period 2017-2019, the oriental fruit moth was developing three or four generations per year depending on weather conditions in the three regions where the experiments were conducted.

In the Plovdiv's region, adults (moths) were flying from the first decade of April to the last decade of September. Caterpillars were damaging shoots from May to August.

The last two generations were damaging fruits from the beginning of August till harvest in September (Figures 1, 2, 3).

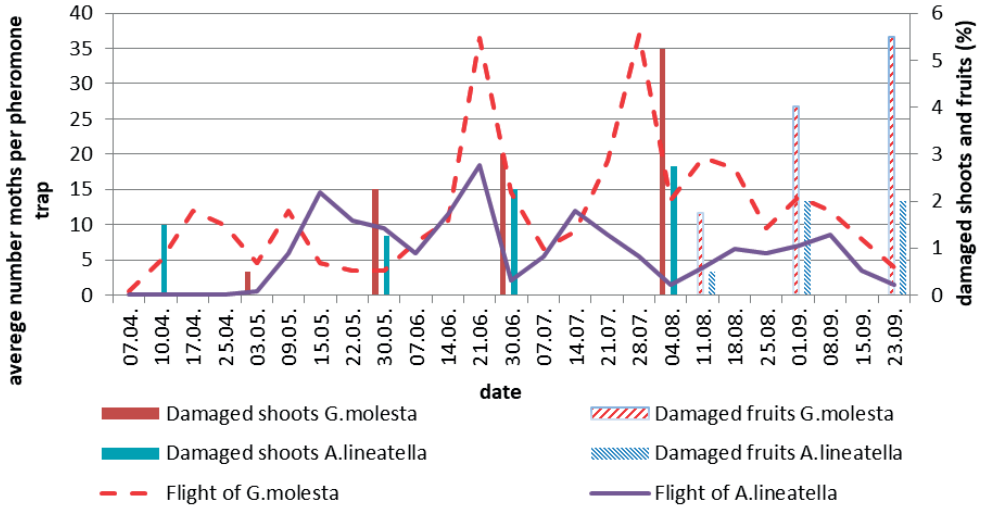

Figure 1. Flight dynamics and damages from *G. molesta* and *A. lineatella* in the control conventional orchard in the region of Plovdiv in 2017

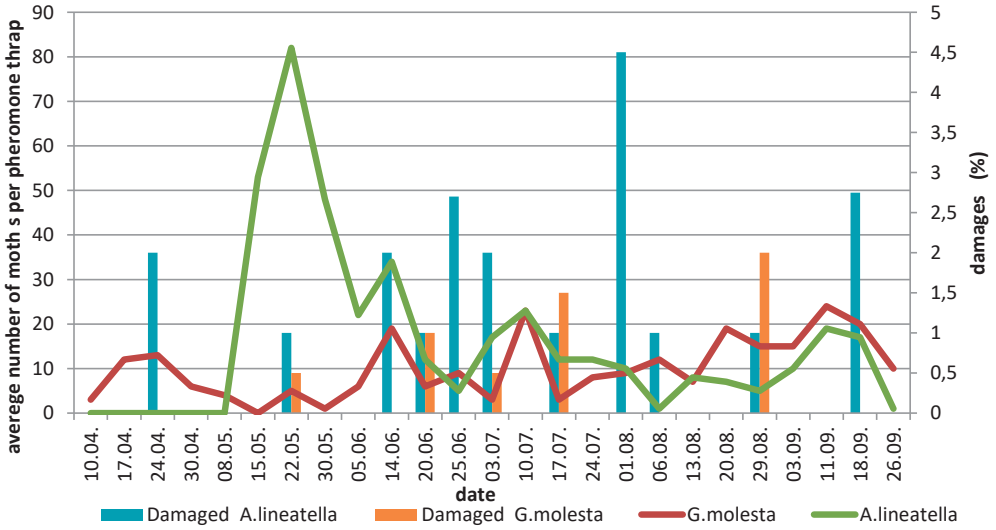

Figure 2. Flight dynamics and damages from *G. molesta* and *A. lineatella* in the control conventional orchard in the region of Plovdiv in 2018

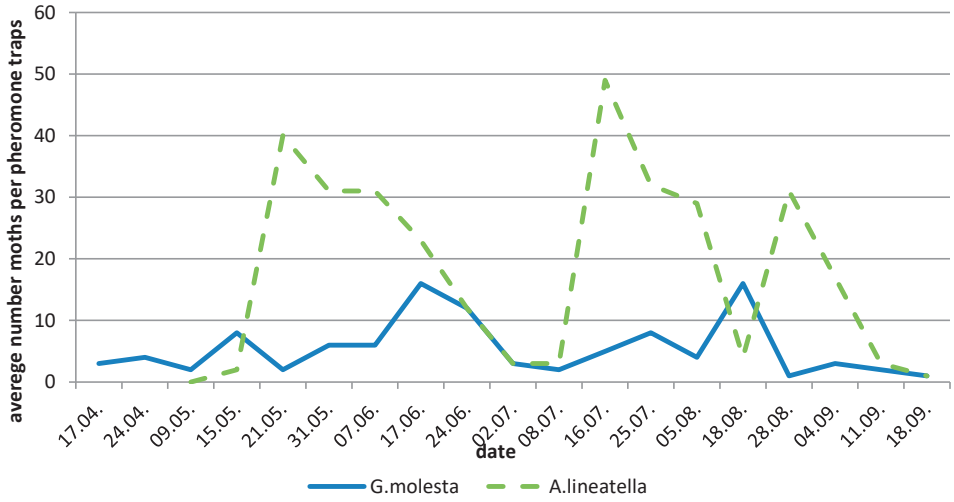

Figure 3. Flight dynamics and damages from *G. molesta* and *A. lineatella* in the control conventional orchard in the region of Plovdiv in 2019

In the other two regions the moths appeared in spring two weeks later, but also were flying to the end of September. The population density varies and depends on weather conditions but also on control measures. For control of the pest in conventional orchards, insecticide treatments were carried out from May to September every 12-14 days. With such a plant protection system, damage to shoots and fruits remained within 2-4.5% (Figures 4, 5), however, in unprotected orchards even relatively low population densities of the pest can cause a severe economic damage (Rothschild and Vickers, 1991).

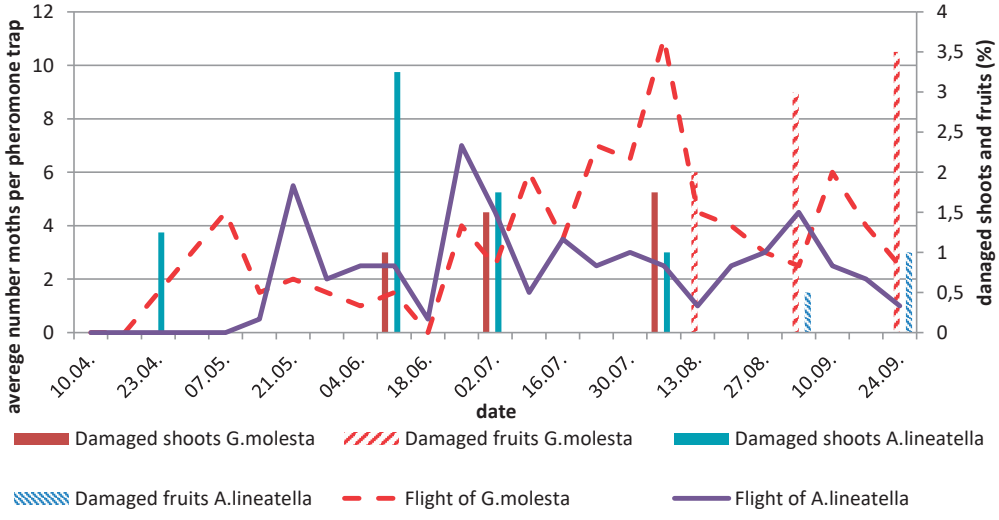

Figure 4. Flight dynamics and damages from *G. molesta* and *A. lineatella* in the control conventional orchard in the region of Glufishevo (near Sliven) in 2017

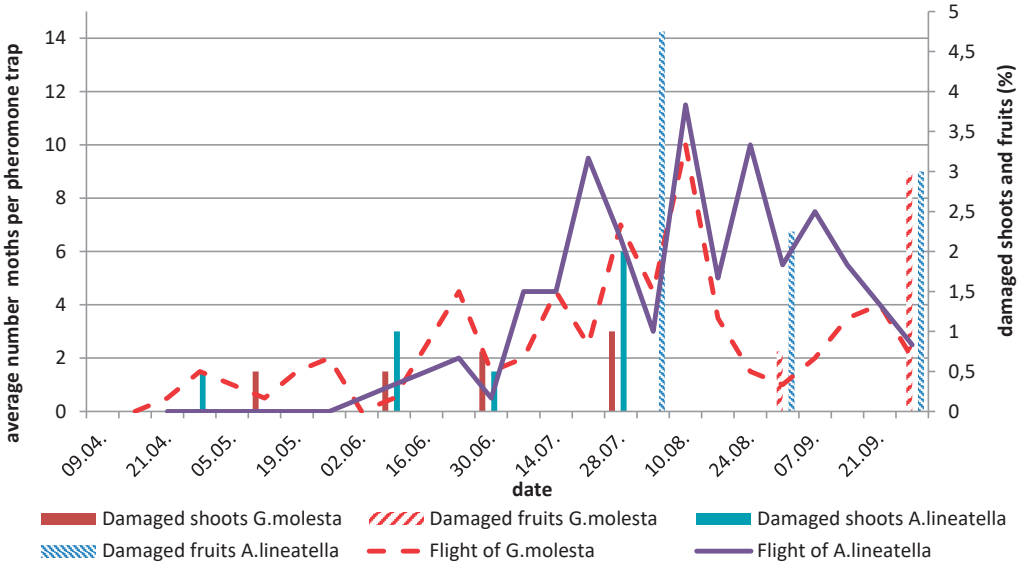

Figure 5. Flight dynamics and damages from *G. molesta* and *A. lineatella* in the control conventional orchard in the region of Karnobat in 2017

The peach twig borer also damages shoots and fruit. The pest overwinters as a caterpillar that has not completed its development and in April it began to cause damage on shoots. The moths were flying from May to September, and the species developed 3-4 generations too (Figures 1, 2, 3, 4, 5). Control was combined with that of the oriental fruit moth.

The plum fruit moth appeared a week later than the oriental fruit moth and developed two or three generations till September (Figures 6, 7, 8).

The caterpillars of the pest attacked only fruits and without control the damages exceeded 70% (Figure 8).

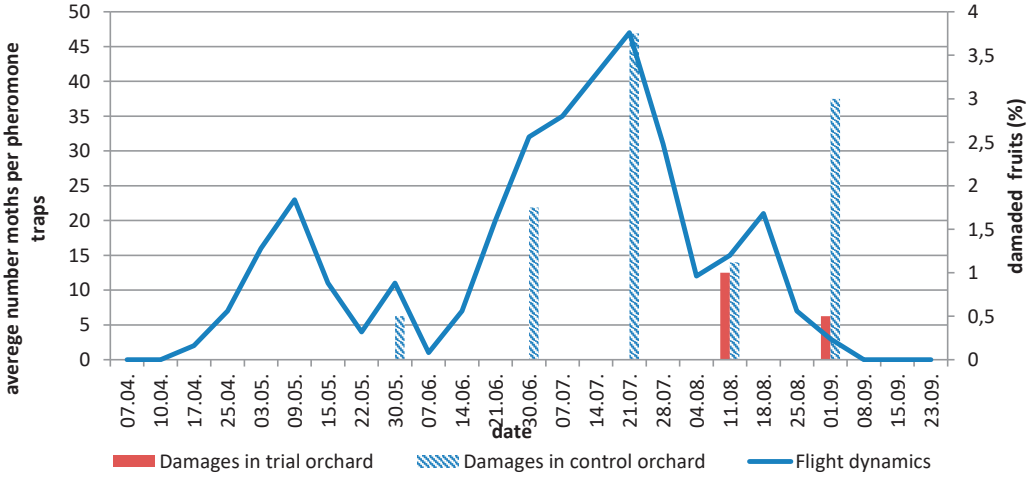

Figure 6. Flight dynamics and damages from *G. funebrana* in the control conventional and the trial organic orchards in the region of Plovdiv in 2017

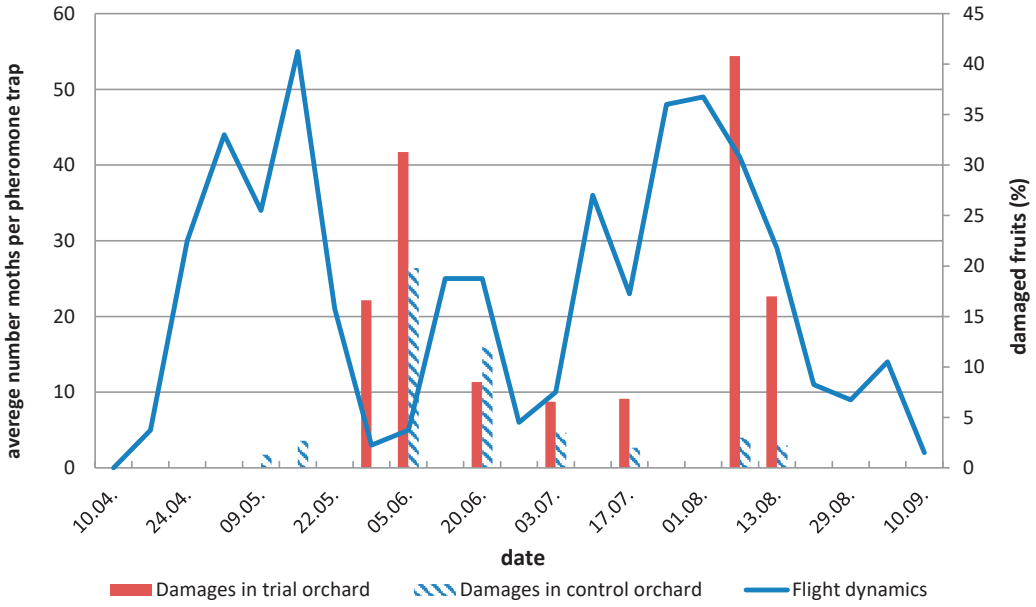

Figure 7. Flight dynamics and damages from *G. funebgana* in the control conventional and the trial organic orchards in the region of Plovdiv in 2018

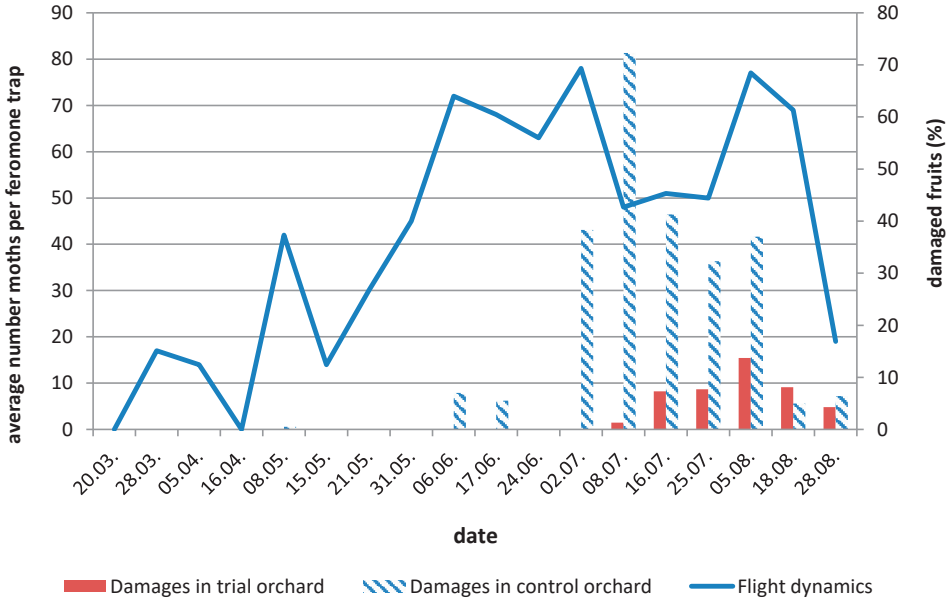

Figure 8. Flight dynamics and damages from *G. funebgana* in the control conventional and the trial organic orchards in the region of Plovdiv in 2019

The experiments in 2017 demonstrated that the pheromone dispensers Isomate A/OFM at a rate of 1000 pieces per ha provided excellent control for these three pests - no damage by *G. molesta* and *A. lineatella* was detected in any of the trial plots with peaches. Only in the trial plots with plums a small number of fruits damaged by *G. funebrana* were found. The

damages were minor (1% and less) and in fact could be ignored. In the control conventional orchards, despite the conducted insecticidal treatments, a certain percentage of damaged shoots and fruits were found (Figures 1, 2, 3, 4, 5, 6, 7, 8).

In the following year (2018) only the orchards in the Plovdiv's region were monitored. The rate of the dispensers was reduced to 300 pieces per ha. The experiments showed that Isomate A/OFM had a high degree of control of the oriental fruit moth and peach twig borer - no damage from both pests was found in the experimental orchards, but this rate was not sufficient for the plum fruit moth and the damaged plum fruits in May exceeded 30%, and in August - 40% (Figure 7).

Due to the unsatisfactory result in 2018 in the plum orchard, the rate of the pheromone dispensers in 2019 was raised to 600 pieces per ha. The result was significantly better and the damaged fruits from caterpillars of the plum fruit moth at harvest time did not exceed 14% while in the abandoned control orchard the fruit damage exceeded 72% in July and the subsequent reduction was due to the drop of damaged fruits (Figure 8).

CONCLUSIONS

The pheromone dispensers Isomate A/OFM at a rate of 1000 pieces per ha provide excellent control for the oriental fruit moth *G. molesta*, the plum fruit moth *G. funebrana* and peach twig borer *A. lineatella*.

At a reduced rate of 300 pieces per ha Isomate A/OFM showed a high degree of control of the oriental fruit moth and of the peach twig borer, but this rate was not sufficient for the plum fruit moth and the damaged plum fruits at harvest exceeded 40%.

At the rate of 600 pieces per ha, the number of fruits damaged by the plum fruit moth at harvest time did not exceed 14% but this is unsatisfactory. In order to control this pest, the recommended rate of 1000 pieces per ha must be applied.

ACKNOWLEDGEMENTS

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ASSESSMENT OF THE BACTERIUM *ERWINIA AMYLOVORA* ATTACK ON SEVERAL PEAR VARIETIES (*PYRUS COMMUNIS* L.) AND THE INFLUENCE ON FRUITS SUGAR CONTENT

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Abstract

Proven to be the first pathogenic bacterium of plants, Erwinia amylovora causes Fire Blight which is nowadays one of the most devastating disease of apples and pears in many parts of the world, especially in temperate ones. The current paper assessed in natural conditions of infection in 2016 year, the impact of fire blight on four pear genotypes (cv. Napoca, Red Bartlett, Beurre Bosc, Curè) within a plantation located in the proximity of Craiova city, using Area under the Disease Progress Curve (AUDPC) and the impact of pathogen attack on fruits yield and sugars content. The response of tested pear genotypes to the fire blight attack ranged on a large scale of variability depending on the genotype resistance or sensitivity to disease and environmental conditions. The most susceptible pear variety to fire blight was Curè, which also proved the lowest yielding capacity and sugar content in fruits under fire blight impact. AUDPC values ranged from 164 to 376 with unfavorable impact on fruits yield and sugar content (R^2 = 0.9799; R^2 = 0.9557).

Key words*: Erwinia amylovora, AUDPC, Fire Blight, pear, sugar content.*

INTRODUCTION

Belonging to Rosaceae family, European pear (*Pyrus communis* L.) has been enjoyed since centuries worldwide for its desirable taste and highly nutritious value, being a rich source of important vitamins and minerals, flavonoid antioxidants, soluble and insoluble fibre, including prebiotics which promote digestive and heart health (Gayer et al., 2019; Navaei et al., 2019). Nowadays is one of the most economically important fruit species grown in Europe, North America and temperate regions of both two hemispheres of the Earth on different soils and environmental conditions (Cichi et al., 2008).

In 2018 world pear production has been reported as 23.733.772 tons (FAO 2018). Despite their economically importance, health benefits and costumers preference for fresh fruits, the most important factor that limits pear cultivation worldwide is the bacterium *Erwinia amylovora*, which develops the disease known as Fire Blight (FB). The pathogen is considered quarantine pest on the list of European and Mediterranean Plant Protection Organization (EPPO, http://www.eppo.org/QUARANTINE/quarante.htm). Currently phytosanitary control and early eradication of any Fire Blight are the best measures to delay disease spread and avoid losses.

Since first report about Fire Blight in the USA in the late 1700s, the disease has spread in more than 46 countries from America, Australia, Europe, Middle East, Africa and other regions where pear tree is gown, despite the control measures adopted (Denning, 1794; Jock et al., 2000; Bonn and Van der Zwet, 2000; Sestras et al., 2008; Peil et al., 2009; Braun-Kiewnick et al., 2011; Jock et al., 2013; Gaaliche et al., 2018). In Romania, Fire Blight symptoms were first observed in 1992 in the south region of the country (Severin et al., 1999).

Affecting pear, apple, quince and other rosaceous plants, Fire Blight causes serious fruits losses and even whole tree dieback,

especially in young orchards (Yom Din et al., 2007; Johnson et al., 2016; Gaaliche et al., 2018; Gaganidze et al., 2018). This epiphytic bacteria attacks parts or whole tree affecting blossoms, leaves, shoots, branches, fruits, and roots, being able to devastate pear trees within one season, especially on sensitive genotypes (Kuflik et al., 2008; Braun-Kiewnick et al., 2011; Gaganidze et al., 2018). The injuries have long-term impact because sometimes it is necessary to be removed large portions of the tree increasing the dieback risk of the whole tree. It can be spread easily by vectors (wind, rain, insects, birds), but also by contaminated pruning tools and infected plant material.

Many genetically studies have been done on different crop species about the impact of management measures and climate change on different plants traits and their ability to face the stress produced by different biotic and abiotic constrainers (Duncan and Howard, 2000; Loarie et al., 2009; Wittenberg et al., 2009; Burger at al., 2012; Johnson et al., 2012; Bonciu, 2018; Bonciu et al., 2018; Bonciu, 2019). However, despite biological, chemical and cultural methods, the use of resistant genotypes remains the most efficient way to control the disease (Aysan et al., 1999; Durel et al., 2003; Bell et al., 2005; Dondini et al., 2005; Stockwell et al., 2011; Montanari et al., 2016; Calis et al., 2017; Hashman et al., 2017; Kellerhals et al., 2017; Mertoğlu and Evrenosoglu, 2017).

The aim of the present study was to determine the response of four pear varieties to the attack of the bacteria *Erwinia amylovora* under natural infection in terms of the relationship between weather conditions, varieties susceptibility to Fire Blight and pathogen impact on pear fruits yield and sugar content. However, little research has been done on the impact of Fire Blight on the affected fruits quality.

MATERIALS AND METHODS

The experiment was conducted during 2016 year to individual trees in a randomized compete block design in four replicate blocks (10 pear trees/block) within a private pear orchard established in 2006 year (3.5 m between rows x 3.5 m between trees on row) in the proximity of Craiova city, Dolj county, Romania. A total of forty pear trees including four pear genotypes (cv. Napoca, Red Bartlett, Beurre Bosc, Curè) were assessed in natural conditions of infection for their response to the attack of the bacteria *Erwinia amylovora*. There was calculated the cumulative number of Fire Blight infections per each assessed pear tree. The quantitative determination of sugars content in fruits was done using digital refractometer (WYT-J 0-32% Chong Qing, China) and reported as degrees Brix, which is equivalent in percentage (Ball, 2006; Wei and Wang, 2013; Dongare et al., 2014). Total soluble solids (TSS) values obtained from the digital refractometer have been adjusted using the factor 0.85 which means that sugars are 85% of TSS. For the pathogen isolation and identification have been taken samples of diseased young shoots, flower clusters, leaves and fruits with visible symptoms of Fire Blight (necrosis, wilting, bacterial ooze), taken after symptoms were visible for each assessed pear tree from all genotypes.

Isolation of the pathogen was made from fresh samples (symptomatic shoots, flowers, leaves, fruits) according to the EPPO protocol (EPPO, 2013). Detection of the bacterium was done using PCR assays and MALDI-TOF mass spectroscopy protocols (Sauer et al., 2008; Wensing et al., 2012). For all assessed pear trees were determined Frequency (F%) and Intensity (I%) of Fire Blight attack. These parameters were used to calculate Attack Degree (AD%) using the formula: AD% = (F% x I%)/100 (Cociu and Oprea,1989). To estimate the response of pear genotypes to Fire Blight attack was used the scale 1 (no attack) to 9 (tree dead), corresponding to AD% classes. Also, for assessing the Fire Blight evolution and disease quantity on each pear tree included in the trail was used the Area under Disease Progress Curve (AUDPC), following the formula (Campbell and Madden, 1990):

$$\text{AUDPC} = \sum_{i=1}^{a}\left[\left\{\frac{Yi + Y(i+1)}{2}\right\} x(t(i+1) - ti)\right]$$

where, Yi = disease severity at each measurement; ti = time in days of each measurement; n = number of Fire Blight (FB) assessments. AD% was used to assess disease severity at each measurement.

The fruits yield for each assessed pear tree was calculated using the formula: number of fruits/tree x average weight of the fruit.

RESULTS AND DISCUSSIONS

Since the first report regarding the occurrence of Fire Blight in Romania in 1992 (Severin et al., 1999), the disease has been spread in all regions of the country (mostly in the south and south east) with variable intensity. In the climatically conditions of 2016 year inspections in the pear orchard have been performed periodically during the growing season in order to identify typical symptoms of fire blight, assuming an infection occurred. Scouting of the disease has started for each pear genotype during blooming and continue in other three moments on leaves, shoots and fruits, because the meteorological conditions were favourable to Fire Blight development. Necrotic symptoms of Fire Blight have been observed on all pear genotypes assessed (Figure 1).

Figure 1. Fire Blight attack symptoms on pear (dry and necrotic leaves, blight, affected fruits found on diseased branches) (original photo)

For scouting optimization and to predict the disease development, rainfalls and temperatures were taken into account. Thus, climatic conditions of 2016 year favoured the infection with *Erwinia amylovora* and further Fire Blight development. Humidity was determined by the amount of rain of 825.8 mm, comparatively with multiannual average rainfall of 585.4 mm, while the average temperature was 12.4ºC comparatively with multiannual average temperature of 10.8ºC (Figure 2).

During periods of high humidity and warm temperature affected tissues of leaves, shoots and fruits became water soaked and dull, covered with small droplets of bacterial ooze rich in polysaccharide, which creates a matrix that protects the pathogen on plant surfaces and attracts insects that disseminate the pathogen.

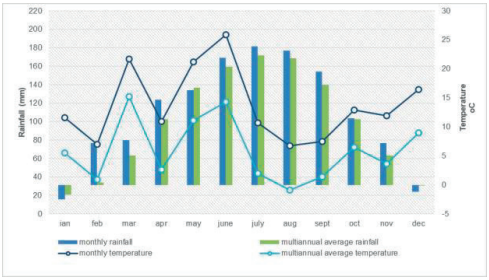

Figure 2. Weather conditions during the study period (2016 year)

The severity of the disease was noticed by Attack Degree (%) which was calculated for each scouting and introduced in the formula of AUDPC. All pear genotypes correspond to different classes for their response to Fire Blight attack. The response of tested pear genotypes to the fire blight attack ranged on a large scale of variability depending on the genotype resistance or sensitivity to disease and environmental conditions. Thus, appreciation scale indicates class 3 (low attack) for Beurre Bosc, class 5 (supra medium attack) for Napoca, class 6 (strong attack) for Red Bartlett and class 7 (very strong attack) for Curè (Table 1).

Table 1. Attack appreciation scale to Fire Blight (*Erwinia amylovora*) (Sestras et al., 2008)

Class	Attack appreciation	Attack degree (AD%)
1	No attack	0
2	Very low attack	0.1-5.0
3	Low attack	5.1-10.0
4	Medium attack	10.1-20.0
5	Supra medium attack	20.1-40.0
6	Strong attack	40.1-60.0
7	Very strong attack	60.1-80.0
8	Extreme strong attack	80.1-99.9
9	Complete scorching (trees dead)	100

The results confirms that all four pear genotypes included into the study are susceptible to *Erwinia amylovora* attack, which confirms the previous research (Zwet and Beer, 1995; Sestras, 2004; Montanari et al., 2016; Calis et al., 2017; Hashman et al., 2017; Kellerhals et al., 2017; Mertoğlu and Evrenosoglu, 2017). The most susceptible to Fire Blight (FB) was Curè, which also proved the lowest yielding capacity and sugar content

in fruits under Fire Blight impact (Table 2). The genotype Beurre Bosc has recorded lowest AUDPC value and the highest yield and fruits sugars content.

Table 2. The response of pear genotypes to Fire Blight (FB) attack and the impact on fruits yield (t/ha) and sugars content (%)

Pear Genotype	2016		
	AUDPC	Yield (t/ha)	Sugars (%)*
Napoca	210	9.75	17.8
Red Bartlett	312	6.4	16.7
Beurre Bosc	167	10.2	18.1
Curè	376	5.15	15.3

*sugars (sucrose, glucose and fructose)

For all pear genotypes assessed for their behaviour to Fire Blight attack it was noticed a very significant negative correlation between AUDPC values and fruits yield (R^2 = 0.9799) (Figure 3).

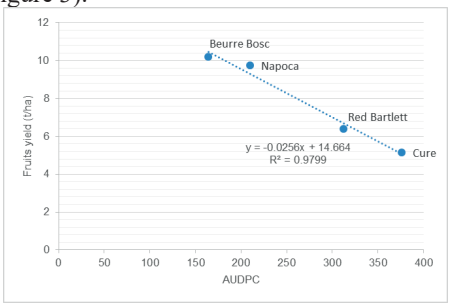

Figure 3. Relationship between Fire Blight AUDPC and pear fruits yield in 2016 year

Also it was found a very significant negative correlation between AUDPC values and fruits sugars content (R^2 = 0.9557) (Figure 4).

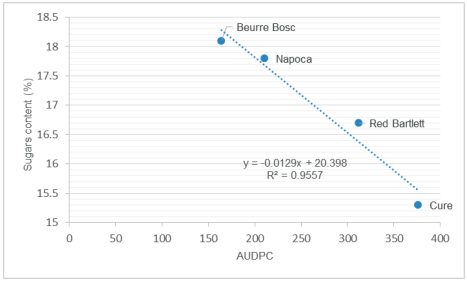

Figure 4. Relationship between Fire Blight AUDPC and pear fruits sugars content in 2016 year

However, effective management of Fire Blight is complex and largely preventative. It requires a combination of sanitation, cultural practices and chemical or biological control to keep the disease in check.

CONCLUSIONS

The present study was carried out to assess the response of four different pear genotypes to the attack of Fire Blight (*Erwinia amylovora*) in natural infections and to evaluate the impact of the pathogen attack on pear fruits and sugar contents. The response of tested pear genotypes to Fire Blight attack ranged on a large scale of variability depending on the genotype resistance or sensitivity to disease and environmental conditions. The most susceptible pear variety to Fire Blight was Curè, which also proved the lowest yielding capacity and sugar content in fruits under fire blight impact. AUDPC values ranged from 164 to 376 with unfavourable impact on fruits yield and sugar content (R^2 = 0.9799; R^2 = 0.9557).

Beside breeding programs focused on identifying sources with durable resistance to Fire Blight, severe quarantine measures attempt to reduce the disease in pear orchards and especially in private gardens. Also, monitoring of Fire Blight on ornamental plants such as *Crataegus* sp., *Sorbus* sp., *Amelanchter*, wild *Malus* and *Pyrus* sp., during the growing season when the symptoms can be visible, is effective to avoid a subsequent spread of the disease.

The obvious conclusion is that the most effective methods to control Fire Blight are section for resistance and optimization of scouting in pear orchards during growing season in order to reduce yield losses and impairment of fruit quality.

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REASEARCH CONCERNING THE APPEARANCE OF ALTERNATION OF FRUCTIFICATION IN SUPERINTENSIVE APPLE ORCHARDS

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Abstract

The researches were carried out in the experimental field of the Faculty of Horticulture Bucharest in a super intensive orchard of apple, established in 2010 for study the alternation of fructification at new varieties of apple. Three romanian varieties 'Redix', 'Generos' and 'Iris' were studied during 4 years starting with 2016. The trees were grafted on the M9 rootstock and planted at 4 x 1 m, with a density of 2500 trees/ha. The research included three varieties represented by the variety and each repetition included three trees. Determinations were made regarding the morphological characteristics of the trees, percentage of fecundations flowers, the number of fruits on the tree, and productivity. The three apple varieties have high fructification potential, 'Redix' 22 kg/tree, 'Generos' 20 kg/tree and 'Iris' 18 kg/tree. In 2017 the production on the tree decreased with 75% in the 'Redix' variety, with 64% in the 'Generos' variety and with 58% in the Iris variety. In the years with high production, the average weight of the fruits was 180 g at variety 'Redix', 168 g at 'Generos' variety and 160 g at Iris variety and in the years with low production, the weight of the fruits increased, being 230 g at 'Redix' variety, 186 g at 'Generos' variety and 185 g at Iris. Fruit production fluctuates from year to year in the same agrotechnical conditions, and the size of the fruits was influenced by the number of fruits per tree. The alternation of fructification was very intense in the varieties cultivated in superintensive system and affects the level of production.

Key words*: apple, rootstock, alternation of fructification.*

INTRODUCTION

In Romania until the establishment of the first intensive orchards, the classical system was the only tree growing system (Cepoiu N., 1996).
On the Dâmbovița Valley, until 1950, the trees were grafted onto the generative rootstocks, planted at densities of 150-250 trees / ha and were conducted with the high trunk and globular crown (Petre, 1983). In these orchards, fruit production was carried out at relatively late economic parameters, starting with 8-10 years after planting. Some main works, such as the cutting and harvesting of fruits, required a very high consumption of work force due to the high size of the trees (6-8 m) and the use of large scales (Cepoiu, 1988, 1989, 2001). The mechanized execution of the phytosanitary treatments, and the works of the soil was hampered by the dimensions and the globular form of the crown. In view of all these shortcomings recorded in the classical plantations, it was gradually replaced by intensive and superintensive orchards. The scientific results obtained in other advanced fruit-growing countries such as Italy, France, Germany, Belgium, Holland, etc., have played a special role in the introduction of new tree cultivation systems in our country. The intensification of the culture in our country was achieved by the introduction of new tree culture systems, adapted to the social-economic conditions and the technical-material base that is constantly developing. All these concerns were aimed at the application in the orchards of modern technologies focused on fertilization , mechanization, cutting and different forms of crown, planting distances and rootstocks and not least on the extension of species and varieties of high productivity and with fruits of one choice quality (Cepoiu, 1987, 1992). According to the way the land is used worldwide, 103.4 million hectares are used for growing fruit trees and shrubs, which represent 0.79% of the agricultural land in the world (Gonda, 2003). From this surface occupied with fruit plants, in the temperate zone of the globe, about 7 million hectares are cultivated with apple (Ghena, 2003). In Europe, the largest apple producing countries are France,

Italy, Germany, Spain, countries that have a high degree of intensification and high productivity of plantations. In the orchards of these countries modern culture systems with high densities, with a valuable assortment of varieties predominate, where the fertilization, mechanization and irrigation ensure apple productions of over 40-50 tons per hectar (Balan, 2001). Most varieties are grafted on rootstocks of reduced vigor (M9, M27) and fruit even from the first years after planting (Cepoiu et al., 2005). The trees used for the establishment of these orchards differentiate fruit buds in the nursery, and at high densities (10-12 thousand trees per hectare), they produce economically, even from the first year of planting 15-16 tons per hectare Amzăr et al., 2000). The realization of these orchards is done with great financial efforts, but the investments recover very quickly. In these orchards are produced of high quality fruit that can be selled at a high price. At present, the assortment at the apple is in a continuous change, determined by the demands of the consumers, productivity and quality, as well as by the resistance of the varieties to diseases and pests. A great emphasis is placed on obtaining biological crops, without traces of pollution produced by the chemicals used to treat crops (Petre, 1989). It should also be noted that the large number of very good quality apple varieties, created in recent years, tend to gradually replace the assortment of old varieties (Păun, 2017). From these varieties of perspective we mention ′Elstar′, ′Red Elstar′, ′Fiesta′, ′Elshof′, ′Gala′, ′Royal Gala′, ′Imperial Gala′, ′Gala Must′, ′Sweet Carolina′ etc. (Petre, 2005). Also varieties genetically resistant to diseases, ′Prima′, ′Priam′, ′Priscila′, ′Florina′, ′Wanda′ etc. They make their place in the assortment due to the demand for the least polluted fruits (Branişte et al., 1999). The main aim of this study was to test these varieties in orchard and obtain the unpolluted fruits at a small price.

MATERIALS AND METHODS

The researches were carried out in the experimental field of the Faculty of Horticulture Bucharest in a superintensive orchard of apple, established in 2010. Three romanian varieties 'Redix', 'Generos' and 'Iris' were studied during 4 years starting with 2016. The trees were grafted on the M9 rootstock and planted at 4 x 1m, with a density of 2500 trees/ha. The research included three varieties represented by the variety and each repetition included three trees. Determinations were made regarding the morphological characteristics of the trees, percentage of fertilized flowers, the number of fruits on the tree, the average weight of the fruits and the chemical characteristics of the fruits.

The biological material studied was represented by 3 varieties of apple obtained in Romania, respectively, 'Redix', 'Generos' and 'Iris'. During the research period, observations were made regarding the triggering of the phenophases of the generative and vegetative organs, noting the moment of the triggering and the moment of the end of these phenophases. The circumference of the trunk was measured at a height of 20 cm from the ground, using the roulette. The number of fruit branches and semi-schaffold branches was determined by careful evaluation and registration in the synthetic tables. The number of related fruits was determined by counting the flowers in the bud stage and after forming the fruits when they were 10 mm in diameter. Fruit production was determined by harvesting the fruits from the 3 repetitions when the fruits reached maturity. The determination of the evolution of the fruit mass in dynamics was carried out in the field and laboratory by weighing the fruits and measuring the height and diameter of the fruit.

RESULTS AND DISCUSSIONS

For a correct interpretation of the research results, repeated measurements were made during the study period. The vigor of the 3 varieties conducted in the form called vertical axis was expressed by the trunk circumference measured 20 cm from the ground surface (Table 1).

Table 1. The determinant vigour features

Variety	H. tree (m)	Trunck circumferince (cm)		Difference (cm)
		2016	2019	
Redix	**2.0**	**21.3**	**25.6**	**4.3**
Generos	**1.9**	**19.3**	**25.3**	**6**
Iris	**2.0**	**20.6**	**25.3**	**4.6**

Immediately after the petals were shaken, observations were made regarding the fruit binding process. Because the flowers are grouped into inflorescences, research was carried out to see if the number of flowers in the inflorescence directly influences the binding process (Table 2).

Table 2. The capacity to fecundate of flowers from inlorescences

Variety	Percent of flowers fecundated per inflorescence				
	`16	`17	`18	`19	Ave rage
Redix	70.3	69.7	76.4	69.2	**71.5**
Generos	97.1	57.3	74.2	63.4	**73**
Iris	57.5	64.5	78.2	62.6	**65.7**

The observations regarding the percentage of fecundated flower from inflorescences revealed interesting data from one variety to another. Thus, the percentage of fecundated flowers was determined according to their number in inflorescence, registering different results each time. The results obtained from the research showed that the percentage of fecundated flowers in some varieties is higher as the number of flowers in inflorescence increases. The 3 varieties studied had very high percentages of flower fecundated, which explains the high production of these varieties.
'Iris' variety has lower percentages of flower fecundated compared to the other varieties studied, but nevertheless the production of this variety is high, the explanation being of a different nature (genetic origin of variety, being obtained by irradiation of seeds). Between the variety 'Iris' and the variety 'Redix' there is a difference in the percentage of flowering inflorescence.
The 3 varieties studied behaved very well in the process of pollination and fecundation and during the period studied there were no losses of fruits due to the non-flowering. And for these morphological and physiological aspects, (good flowering, good pollination and good fecundation of the flowers) we recommend the varieties studied for culture in our country. During the research period, observation were made on the fruits. In the year 2017 and 2019, the number of fruits obtained was lower than in previous years, as a result of the appearance of the alternating fructification, which affected the level of production (Table 3, Figures 1 and 2).

Table 3. The productive capacity

Variety	Repeti tion	Vertical axis			
		Number of fruits/tree			
		2016	2017	2818	2019
Redix		**117.6**	**29.4**	**115**	**28.7**
Generos		**110**	**39.6**	**129.6**	**46.7**
Iris		**112**	**53.1**	**145**	**60.9**

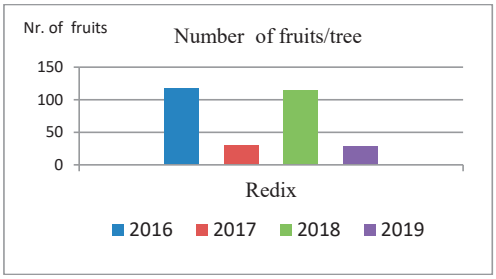

Figure 1. The fructification capacity

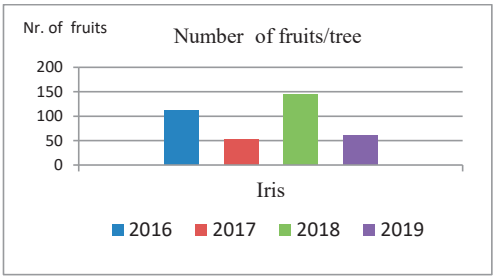

Figure 2. The fructification capacity

The fruit production obtained in 2016 was about 22 kg / tree for the 'Redix' variety, 20 kg for the 'Generos' variety and 18 kg for the 'Iris' variety (Table 4).

Table 4. The fructification capacity

Variety	Repe-tition	Vertical axis			
		Yield (kg/tree)			
		2016	2017	2018	2019
Redix		**22.0**	**6.7**	**26.4**	**6.59**
Generos		**20.0**	**4.8**	**27.8**	**8.68**
Iris		**18.0**	**9.81**	**27.5**	**11.2**

In 2016 the studied varieties were a high fructification potential, 'Redix' 22 kg/tree, 'Generous' 20 kg/tree and 'Iris' 18 kg/tree. In 2017 the production on the tree decreased with 75% in the 'Redix' variety, with 64% in the 'Generos' variety and with 58% in the 'Iris' variety.

At the end of 2019, the obtained production was small, compared to the previous year. The decrease in production can be explained by the manifestation of the alternating fructification, which is frequently encountered in this species (Cepoiu, 1996). The results regarding the production capacity of the studied varieties showed that the average multiannual production was between 37.9 t/ha ('Redix') and 41.5 t/ha in the 'Iris' variety (Table 5, Figure 3).

Table 5. The fluctuation of the yield and the annual average yield

Variety	Annual yield (t/ha)				The annual average yield (t/ha)
	16	17	18	19	2016-2019
Redix	52.7	16.7	66	16.8	37.9
Generos	49.2	12	69.5	21.7	38.1
Iris	44.7	24.5	68.7	28	41.5

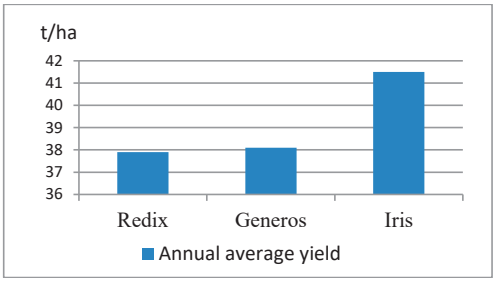

Figure 3. The annual average yield

It is noteworthy that these productions are obtained early, immediately after the establishment of the orchard. The level of production is different from year to year. The alternation of the fructification comprises a different number of trees from one year to the next. Even under the conditions of applying correct fruiting cuttings, the alternation of fruit appears and influences the production per hectare. Fruit production fluctuated from year to year as a result of the alternation of fructification (the inconstant level of production at the same tree from year to year), table 5.

In the case of the 'Redix' variety, fruit yield decreased in 2017 by 68.3% compared to 2016 yield. In 2018, the yield increased by 295.2% compared to 2017 production. In 2019, the yield decreased by 74.5 % compared to the yield of the year 2018. The causes of this fluctuations are the genetic factors and the technological measures and were observed and by other researchers.

Table 6. Average weight of the fruits (g)

Variety	Year with big yield	Year with small yield	Difference (g)
Redix	180	230	50
Generos	168	186	18
Iris	160	185	25

In the years with high production, the average weight of the fruits was 180 g at variety 'Redix', 168 g at 'Generos' variety and 160 g at 'Iris' variety and in the years with low production, the weight of the fruits increased, being 230 g at 'Redix' variety, 186 g at 'Generos' variety and 185 g at 'Iris' (Table 6). Fruit production fluctuates from year to year in the same agrotechnical conditions, and the size of the fruits is influenced by the number of fruits per tree.

The fruit production was not constant and so the alternation of fructification was manifested in the studied varieties (alternation of fructification) (Figure 4).

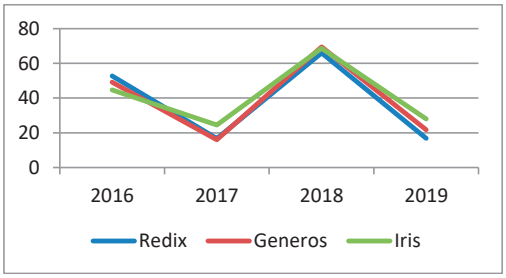

Figure 4. The fluctuation of the yield

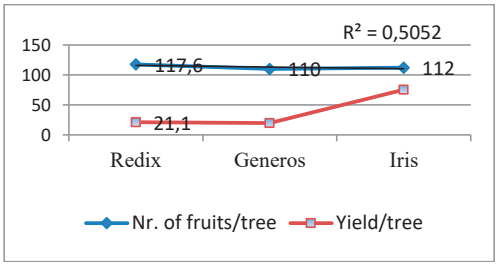

Figure 5. The correlation between nr. of fruits per tree and yield per tree in high production conditions (2016)

In the years with high production there was no strong correlation between the number of fruits per tree and the level of the production per

hectar (a great number of fruits per tree no means a great production, important is the number of the trees non affected by alternation of fructification - per hectar) (Figure 5).
In the years with smaller productions, the correlation between the production level of the tree and the number of fruits per tree is strong (small number of fruits per tree, big weight of the fruits and good production), the correlation coefficient being 0.993(Figure 6.

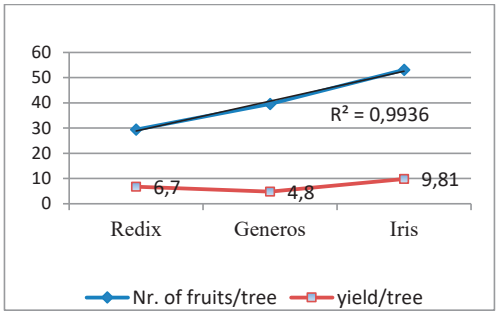

Figure 6. The correlation between nr. of fruits per tree and yield per tree in low production conditions (2017)

CONCLUSIONS

In superintensive orchards the higher the yields obtained, the greater the alternation of fructification.
The average annual yields are different depending on the variety. In the case of the studied varieties the average annual production was between 37.9 t/ha and41.4t/ha. This level of production is not a high level, if we take into account the investments that are made for the establishment and operation of the plantation and the life span of the trees.
The alternation of fructification has affected all the studied varieties.
Fruit production fell from 74.5% year-on-year in the case of the 'Redix' variety.
After a year with low production, the production increased in 2018 to the Redix variety by 295%.
The alternation of fructification was very intense in the varieties cultivated in superintensive system and affects the level of production.

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BEHAVIOUR OF SOME NEW APPLE SCAB RESISTANT CULTIVARS CULTIVATED IN BUCHAREST AREA

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Abstract

The aim of this study is to present the behaviour of new apple scab resistant varieties cultivated in Bucharest area. Apple scab (Venturia inaequalis) is an important and dangerous pathogen, specific treatment being required and, in many cases, the products exceed the new pesticides limits. Every year, new resistant varieties are released by breeders and before their spread into commercial orchards, they need to be tested in specific growing conditions. In the Didactic Experimental Orchard of the Faculty of Horticulture within USAMV of Bucharest more than 25 apple cultivars are planted and monitored. Most of the trees are grafted of M9 rootstock, planted at 3.5 x 1.0 m and led as Vertical Axe. The soil is maintained covered with a mixture of perennial grasses on the interrow and clean with herbicide on the row. Drip irrigation is applied. The paper presents the comparative results focused on the biometrical data like: average tree height, type, number and average length of fruiting shoots, trunk cross sectional area. For each variety fruit yield was calculated. Basic fruits analyses at the harvest moment are presented.

Key words: *canopy, fruit morphological parameters, Malus domestica, rootstock, Vertical Axe.*

INTRODUCTION

Apple is one of the ancient fruit that human mankind continuously appreciated and cultivated for its special nutraceutical properties (Mihăescu, 1977; Hoza, 2000; Tănăsescu, 2005; Chira, 2005; Grădinariu, 2002; Grădinariu and Istrate, 2003; Ghena et al., 2004). It can be found all over the world, in 2018 being reported 86,142,197 tons from which 643,856 tons in Romania (the second fruit crop after plums) (Faostat, 2020; INSSE, 2020).

In time, apple cumulated more pest and diseases, some of them very difficult to manage (Bowen et al., 2011). Apple scab (*Venturia inaequalis*) is an important and dangerous pathogen, specific treatment being required and, in many cases, the products exceed the new pesticides limits (Eppo Global Database, 2020).

Originally from Europe, it was first reported by Fries in 1819 in Sweden; in the USA appeared in 1834, in England in 1945 and in Austria in 1862.

In Romania it is spread in all the apple orchards and in the years with spring and rainy summers, the losses can reach 30-98%. Some of the traditional cultivars presented resistance (Ionescu et al., 2019). The fungicides have proved to be quite inefficient requiring a large number of unwanted sprays, both from the consumer's point of view and from the costs involved, due to the necessity of a very good synchronization of the fungicides application with the plant growth stages and also to the difficulty of its eradication.

The best way to control it seems to depend on the development of scab resistant cultivars (Stănică and Branişte, 2011; Petre and Petre, 2014; Chira et al., 2015; Dudu et al., 2015; Petre et al., 2015; Petre el al., 2019). Every year, new resistant varieties are released by breeders and before their spread into commercial orchards, they need to be tested in specific growing conditions.

The paper presents the comparative results for more than 25 apple scab resistant cultivars focused on the biometrical data like: average tree height, type, number and average length of fruiting shoots, trunk cross sectional area. For each cultivar, fruit production was calculated. Basic fruits analyses at the harvest moment are presented. The aim of this study is to present the behaviour of new apple scab resistant varieties cultivated in Bucharest area.

MATERIALS AND METHODS

In the Didactic Experimental Orchard of the Faculty of Horticulture within USAMV of Bucharest more than 25 apple varieties are cultivated and monitored. Most of the trees are grafted of M9 rootstock, planted in 2009, at 3.5 x 1.0 m and led as Vertical Axe. The soil is maintained covered with a mixture of perennial grasses on the inter-row and clean with herbicide on the row. Drip irrigation is applied. Apples were harvested in August 28th. Biometric and basic biochemical analyzes were made at the harvest time. Biological material consisted in more scab resistant cultivars: Ariwa, Aura, Bistrițean, Ciprian, Dalinbel, Dalinette, Dalinred, Evereste, Florina, Iris, Jonaprim, Luna, Mars, Mela, Opal, Orion, Pionier, Rajka, Real, Rebra, Red Devil, Red Topaz, Remar, Romus 3, Rosana, Sirius, Starkprim, Svatava (Figure 1).

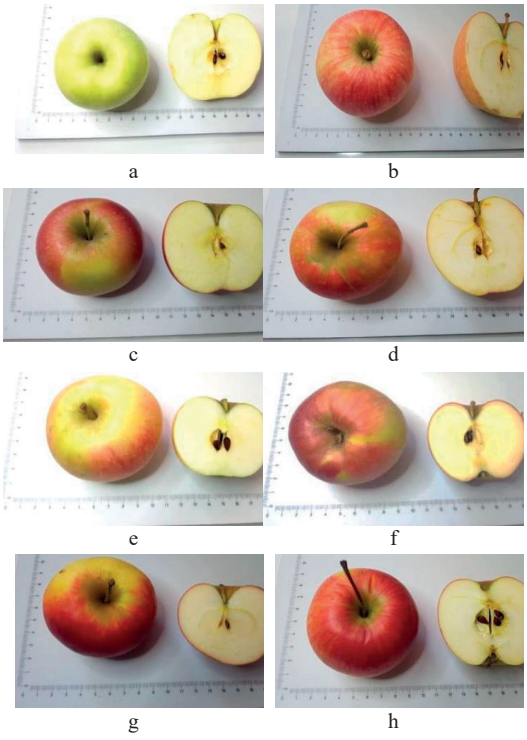

Figure 1. Apple scab resistant cultivars: Aura (a), Bistrițean (b), Dalinette (c), Iris (d), Rebra (e), Jonaprim (f), Remar (g), Starkprim (h)

Tree vigour, blooming period and rate, yield and fertility were analyzed to all above cultivars. Fruiting branches characteristics were determined at Orion, Luna, Red Topaz, Opal, Stark prim, Dalinbel, Jonaprim, Florina, Aura, Remar, Evereste and Real cultivars.

Physical and biochemical parameters were determined for several cultivars comparing with sensitive apple scab cultivars. The results were analyzed comparing with the cultivars descriptions and other researches (Asănică and Hoza, 2013; Petre et al., 2014; Ștefan et al., 2018; Cimpoieș, 2018).

The physio-chemical analyses were performed in the Researcher Centre for Study of Food Quality and Agricultural Products, USAMV Bucharest.

RESULTS AND DISCUSSIONS

Tree vigour

Tree height

Although all the trees on which measurements were made have M9 rootstocks, significant differences could be observed on their height. The highest tree cultivar was Florina, with an average height of 4.25 m in 2017 and 4.30 m in 2018, while at the opposite was the cultivar Iris, with the average height of 2.62 m in 2017 and 2.65 m in 2018 (Figure 2). Red Devil and Romus 3 registered the highest increasing rate of height, followed by Rosana, Dalinbel, Mars, Jonaprim, Red Topaz and Ariwa.

Trunk height

At the Svatava cultivar, the highest values were recorded on the trunk height, with 90 cm height in both years of monitoring. The shortest trunk variety had Evereste with 50.60 cm in 2017 and 51.60 cm in 2018, followed by Rebra and Bistrițean with 54.50 cm in 2017, respectively 56.00 cm and 55.50 cm in 2018. Although, the applied technology determined the initial trunk height, the evolution of this parameter was important for the cultivar description.

Trunk cross section area (TCSA)

The cultivar with the highest TCSA was Dalinbel, measuring 81.24 cm² in 2017 and 85.41 cm² in 2018, while Iris cultivar had the lowest values with 11.83 cm² in 2017 and 12.26 cm² in 2018 (Figure 3). Florina registered the highest TCSA increasing rate in the analyzed period, with 38.29%, highlighting the cultivar feature, followed by Mars cultivar with 5.75% and Dalinbel cultivar with 5.14%.

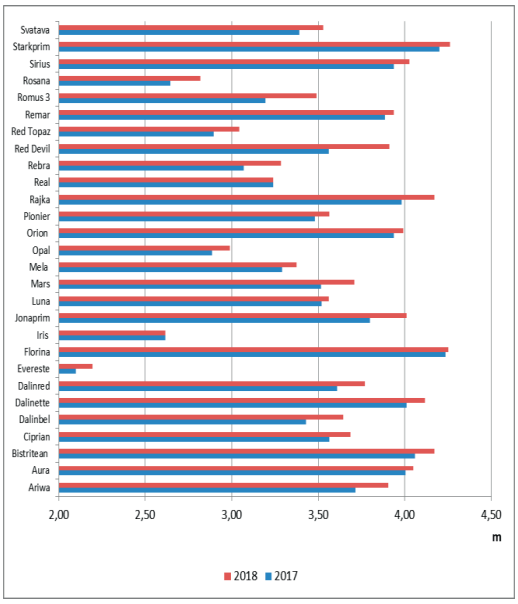

Figure 2. Tree height between 2017-2018 period

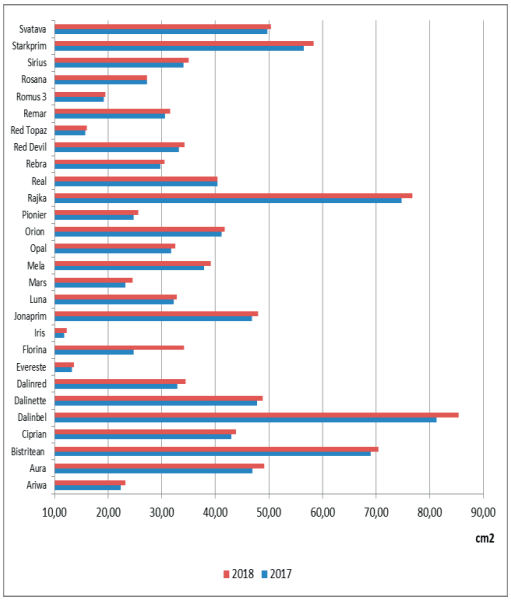

Figure 3. Trunk cross section area between 2017-2018 period

Evolution of the number and length of the fruiting branches

For fruiting branches analyses, for some of the cultivars considered in the study, spurs, water shoots, dards, brindles and offshoots were counted and measured. A detailed analyze with the evolution of their number, average length, rate of each type of fruiting branch and total vegetative growth per tree was presented. The applied agro-technics and the type of fruiting for each apple cultivar determined the obtained results.

The number of fruit branches significantly differed between cultivars. The number of spurs increased in 2018 compared to 2017 for all the analyzed cultivars. In 2017, Luna cultivar presented the lowest number of spurs (12.8) and Jonaprim cultivar (25.3) the highest number. In 2018, the Opal (18.0) and Dalinbel (25.4) were at the extreme.

The number of water shoots evolved during the two years of study different according to cultivars, in some cases increasing (Orion, Luna, Red Topaz, Dalinbel, Florina and Remar) and in others decreasing (Opal, Starkprim, Evereste and Real). At Jonaprim and Aura remained constant. Dards number increased for all cultivars in 2018 compared to 2017 with the exception of the Jonaprim cultivar.

The smallest number had the Orion cultivar (9.5 in 2017 and 12.5 in 2018) and the highest number of dards was presented by the Evereste cultivar. The smallest number of brindles was found at the Opal cultivar (3.5 in 2017 and 7.0 in 2018) and the largest number at Dalinbel cultivar (17.2 in 2017 respectively 15.6 in 2018). The smallest number of offshoots had the Starkprim cultivar (6.2 in 2017 and 9.6 in 2018) and the highest number was found at the Evereste cultivar (18.0 in 2017) and respectively Florina cultivar (18.6 in 2018) (Table 1).

The average length of the fruit branches were on average constant during the two years at spurs (2.1 cm) in the studied cultivars. The average length of the water shoots ranged from 49.9 cm in 2017 to 47.7 cm in 2018. The average length varied for dards from an average of 2.9 cm in 2017 to 2.7 cm in 2018, for brindles from an average of 23.9 cm in 2017 to 25.8 cm in 2018 and for the offshoots varied from an average of 33.4 cm in 2017 to 34.3 cm in 2018 (Table 2).

The total growth of the fruit branches varied between 2017 and 2018, several cultivars having an increasing evolution and others decreasing, influenced by the climatic factors and the applied culture technology. Thus, in 2017 the smallest growth was observed in the

Evereste cultivar with an average of 198 cm and the highest in the Jonaprim cultivar with an average of 2,791 cm. In 2018, the smallest growth was in the Opal cultivar with 991 cm and the highest in the Florina cultivar with 2,066 cm (Table 3 and Figure 4).

Table 1. Evolution of fruiting branches number between 2017-2018 period

Cultivar	Fruiting branches number									
	Spurs		Water shoots		Dards		Brindles		Offshoots	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Orion	14.5	22.3	14.0	15.5	9.5	12.5	5.5	7.3	7.0	11.3
Luna	12.8	23.6	4.6	13.4	14.6	18.0	5.2	7.0	9.8	12.8
Red Topaz	19.4	24.8	14.6	22.6	12.4	16.8	9.0	10.8	13.8	16.2
Opal	15.5	18.0	13.0	7.0	13.5	17.0	3.5	7.0	8.5	11.5
Stark prim	23.0	25.0	24.4	21.6	17.6	19.4	11.6	10.2	6.2	9.6
Dalinbel	23.4	25.4	16.2	17.2	22.4	23.6	17.2	15.6	16.4	12.8
Jonaprim	25.3	24.0	13.3	13.3	23.0	22.3	16.7	11.0	14.7	16.3
Florina	15.8	19.6	12.0	22.8	18.2	22.8	8.6	9.8	11.8	18.6
Aura	16.8	23.8	18.8	18.6	18.0	22.4	5.8	9.6	15.2	12.6
Remar	20.2	22.8	12.3	17.0	19.5	22.8	9.3	9.7	13.7	11.7
Evereste	20.0	22.0	21.0	19.0	24.0	25.0	17.0	13.0	18.0	13.0
Real	22.0	22.4	20.2	16.0	11.2	19.2	6.8	11.0	13.8	13.2
Average	19.1	22.8	15.4	17.0	17.0	20.2	9.7	10.2	12.4	13.3

Table 2. Evolution of fruiting branches length between 2017-2018 period

Cultivar	Average length of fruiting branches (cm)									
	Spurs		Water shoots		Dards		Brindles		Offshoots	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Orion	2.4	2.5	32.5	33.6	3.3	3.1	13.4	18.9	37.2	30.0
Luna	2.2	2.1	38.1	38.3	3.0	2.9	18.0	24.3	19.7	26.2
Red Topaz	2.1	2.1	42.2	45.4	3.0	2.4	20.0	25.0	35.8	36.9
Opal	1.8	2.2	54.3	55.6	2.8	2.6	19.0	25.1	25.2	29.3
Stark prim	1.8	1.9	60.6	64.0	2.6	2.3	29.0	27.3	33.9	32.5
Dalinbel	1.9	2.1	61.4	57.4	2.9	2.6	24.6	23.8	34.9	33.0
Jonaprim	1.8	1.9	67.2	50.4	2.6	2.6	27.3	24.8	37.6	34.8
Florina	2.1	2.1	56.5	45.3	3.1	3.0	29.7	28.4	40.4	36.2
Aura	2.1	2.0	50.2	47.5	2.9	3.1	25.4	31.8	39.9	40.6
Remar	2.1	2.1	54.5	55.9	2.9	3.0	29.0	25.9	26.2	34.4
Evereste	2.0	2.1	39.0	37.6	2.7	2.7	25.4	26.2	28.4	36.4
Real	2.2	2.4	42.1	41.7	2.9	2.8	25.7	28.0	41.7	41.3
Average	2.1	2.1	49.9	47.7	2.9	2.7	23.9	25.8	33.4	34.3

Table 3. Evolution of total vegetative growth between 2017-2018 period

Cultivar	Total vegetative growth (cm)											
	Spurs		Water shoots		Dards		Brindles		Offshoots		Total	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Orion	36	54	911	519	28	39	83	181	471	342	1,529	1,135
Luna	29	49	220	497	44	52	160	203	365	405	818	1,206
Red Topaz	42	52	1,019	1,018	42	40	236	264	849	602	2,188	1,976
Opal	29	39	1,413	390	76	46	133	182	429	335	2,080	992
Stark prim	41	49	1,471	1,354	45	45	452	291	359	314	2,368	2,053
Dalinbel	44	53	1,225	1,007	64	62	425	367	570	530	2,328	2,019
Jonaprim	46	45	1,371	681	60	57	467	275	848	572	2,792	1,630
Florina	32	40	1,081	1,008	55	67	251	271	603	680	2,022	2,066
Aura	37	48	898	1,126	51	68	247	308	767	497	2,000	2,047
Remar	43	48	768	922	58	68	319	303	454	397	1,642	1,738
Evereste	22	47	60	714	27	67	42	340	46	473	197	1,641
Real	49	53	860	830	53	53	266	299	955	543	2,183	1,778
Average	37.5	48.1	941.4	838.8	50.3	55.3	256.8	273.7	559.7	474.2	1845.6	1690.1

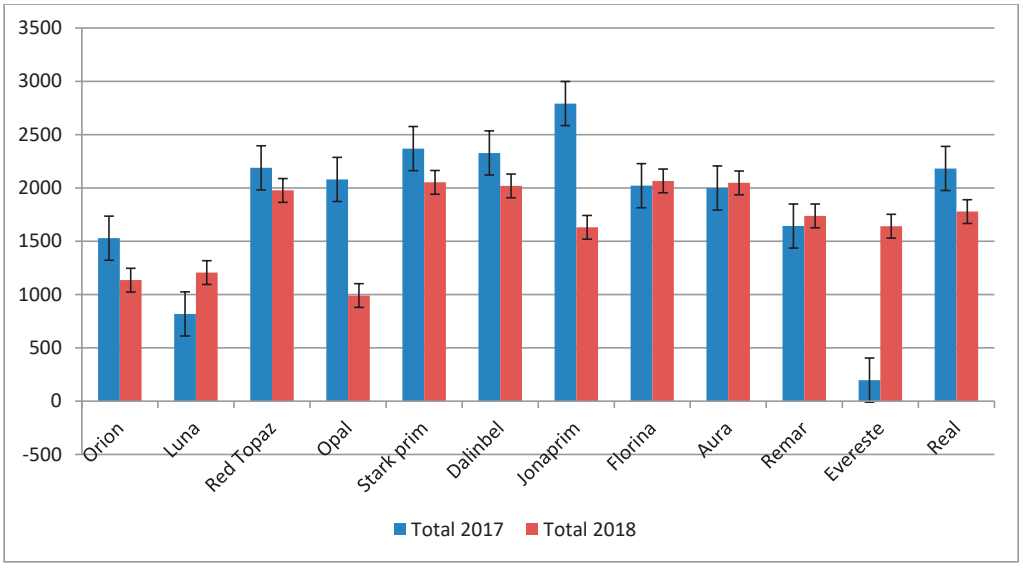

Figure 4. Evolution of total vegetative growth per tree between 2018 - 2019

The rate of the types of fruit branches was different in the analyzed cultivars. If the rate of the spurs and dards was relatively close, the rate of the water shoots in total was maximum in the cultivars Orion (60% in 2017), Opal (68% in 2017), Starkprim (62% in 2017) decreasing in 2018 (maximum 66% in Starkprim cultivar). The rate of the brindles ranged from 5% in Orion to 21% in Evereste in 2017 and between 13% in Red Topaz and 21% in Evereste in 2018. The rate of the offshoots varied between 15% in Starkprim and 39% in Red Topaz in 2017 and between 15% at Starkprim and 35% at Jonaprim in 2018 (Table 4).

Table 4. Fruiting type for each studied cultivar (2018-2019)

Cultivar	Fruiting branches percentage (%)									
	Spurs		Water shoots		Dards		Brindles		Offshoots	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Orion	2	5	60	46	2	3	5	16	31	30
Luna	4	4	27	41	5	4	20	17	44	34
Red Topaz	2	3	46	52	2	2	11	13	39	30
Opal	1	4	68	39	4	5	6	18	21	34
Stark prim	2	3	62	66	2	2	19	14	15	15
Dalinbel	2	3	53	50	3	3	18	18	24	26
Jonaprim	2	3	49	42	2	3	17	17	30	35
Florina	2	2	53	49	3	3	12	13	30	33
Aura	2	2	45	55	3	4	12	15	38	24
Remar	3	3	47	53	4	4	19	17	27	23
Evereste	11	3	30	44	14	4	21	21	24	28
Real	2	3	40	47	2	3	12	17	44	30
Average	2.92	3.17	48.33	48.67	3.83	3.33	14.33	16.33	30.58	28.50

Tree phenologic study

In order to study the specificity of phenology on the studied cultivars, the date of flowering and the percentage of average flowering/tree in the two years taken into the study were considered (Figure 5). The flowering started in April, in 2017 between April 16th-21th and in 2018 between April 11th and 14th. The flowering rate ranged from 5 (Florina, Opal, Pionier and Rajka) to 90 (Luna, Rebra, Red Topaz and Sirius) in 2017 and between 30 (Pioneer and Rosana) and 88 (Evereste) respectively 80 (Moon, Red Devil and Red Topaz) in 2018 (Figure 6).

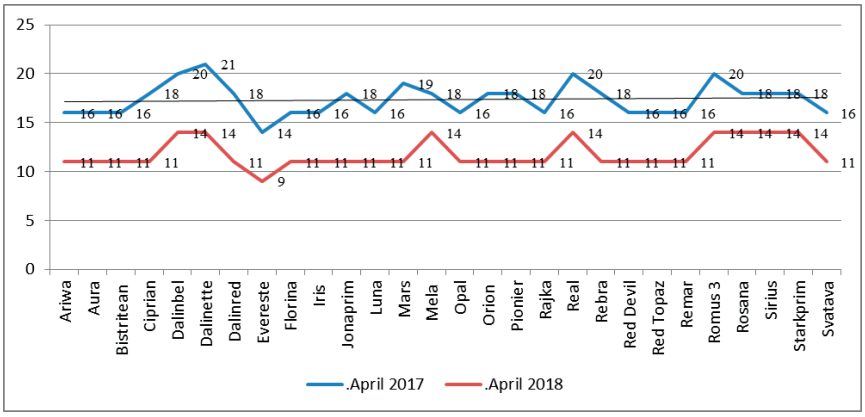

Figure 5. Blooming period of the apple cultivars

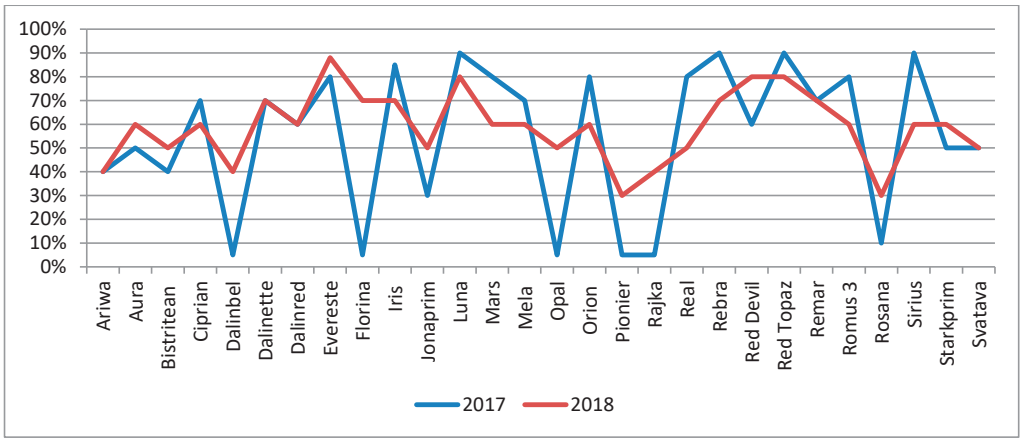

Figure 6. Blooming rate between 2018-2019 period

Tree productivity

One of the most productive cultivars was Mars, with a production of 120.5 fruits/ tree in 2018, followed closely by the Romus 3 cultivar, with a production of 109.5 fruits/ tree. At the opposite were the cultivars: Opal, with a production of 3.5 fruits/tree, and Rosana, with a production of 6 fruits/ tree (Figure 7). The analyzed cultivars presented lower yield values than the cultivars potential in 2018 year. Fertility index (kg/cm^2 TCSA) for several cultivars was presented in Figure 8.

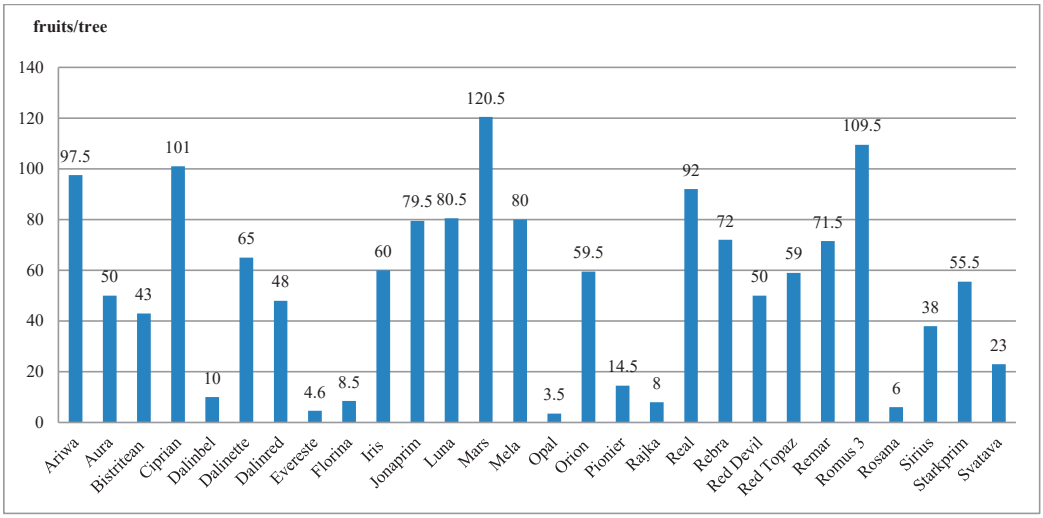

Figure 7. Fruit production registered in 2018

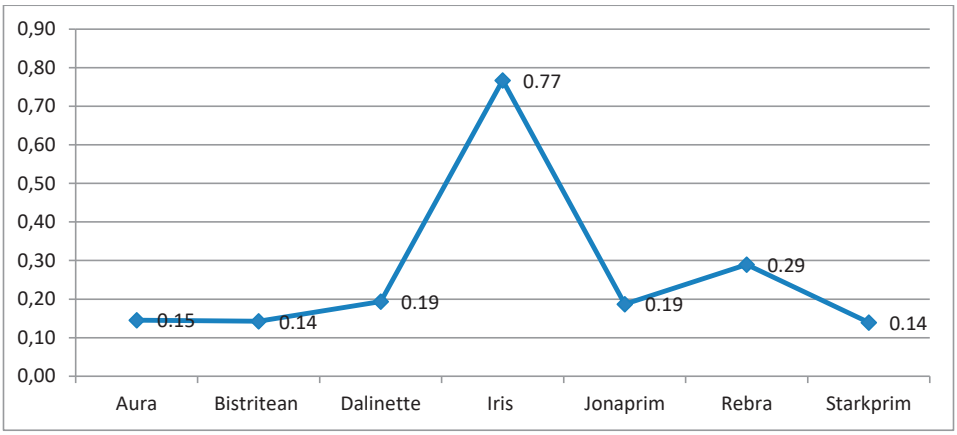

Figure 8. Fertility index in 2018 for several analyzed apple cultivars

Fruits physical and biochemical characteristics at the harvest moment

The cultivar with the highest weight/fruit was Bistrițean, registering an average weight of 233.4 g/fruit, significant higher than standard (Asănică and Hoza, 2013).

Iris with an average weight of 156.8 g/fruit was similar with the cultivar characteristics (150 g/fruit).

Aura with 143 g/fruit, Jonaprim with 112.8 g/fruit, Redix with 104 g/fruit and Starkprim with 146.6 g/fruit presented lower values than the cultivars potential (Asănică and Hoza, 2013) (Figure 9).

The fruits with the largest diameter belong to the Bistrițean cultivar (8.88 cm) and the smallest to the Rene cultivar (6.98 cm) (Figure 10).

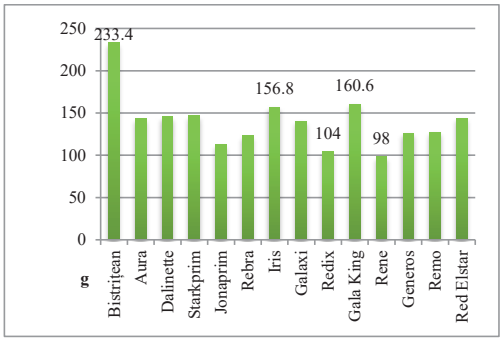

Figura 9. Average weight/fruits (2018)

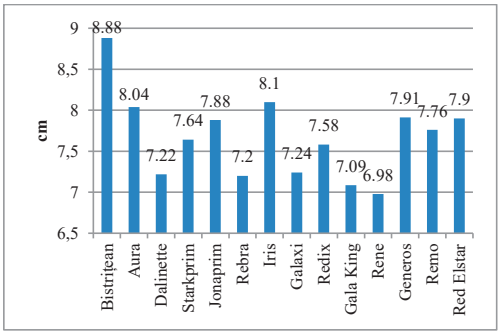

Figure 10. Average fruit diameter (2018)

Firmness, at the harvest moment, ranged between 4.57 kg/cm^2 (Starkprim), 4.88 kg/cm^2 (Red Elstar) to 7.89 kg/cm^2 (Dalinette). Soluble dry matter varied between 9.73 Brix (Bistrițean) to 13.51 Brix (Jonaprim) (Figures 11 and 12).

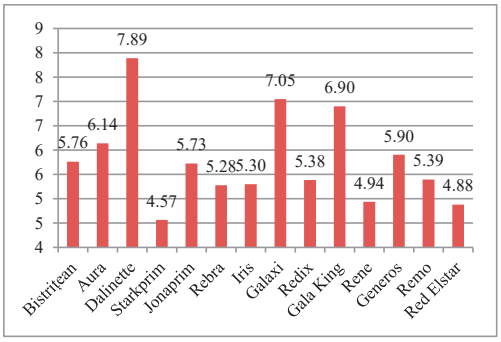

Figure 11. Flesh firmness depending on cultivars at the harvest moment (2018)

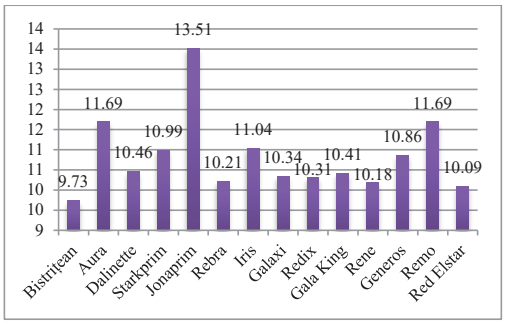

Figure 12. Soluble dry matter depending on cultivars at the harvest moment (2018)

CONCLUSIONS

This research presented several important characteristics of more than 25 apple scab resistant cultivars cultivated in Bucharest area. The applied agro-technics and cultivars specificity were highlighted through tree vigour (total height, trunk height, total cross section area), blooming characteristics, production and fertility index for several cultivars for 2017-2018 period.

Flowering rate was specific to cultivars and especially to climatic factors, blooming period being in April 16th-21th (2017) respectively 11th -14th (2018).

Apple scab resistant cultivars presented valuable characteristics and most of them can be spread in production in Romanian orchards.

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PRELIMINARY RESULTS ON THE CHANGES IN THE FLIGHT DYNAMIC OF *CYDIA POMONELLA* (L.) IN NORTH-EASTERN TRANSYLVANIA, UNDER THE INFLUENCE OF CLIMATE CHANGE

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Abstract

The climate change in the last decades have brought increased average annual temperatures all over the world. The north-eastern region of Transylvania is no exception to this trend. Under the influence of this phenomenon and of the way in which average daily temperatures are distributed across the year, we observed certain changes in the flight dynamic of the adult males of the codling moth Cydia pomonella (L.). The preliminary data collected by the use of pheromone traps (Atrapom), along the vegetative period of 2019, regarding the flight of the moths, showed an extended flight span with two to five weeks than the flight recorded in the same orchards between 2004 and 2006. There is also a significant increase of the number of moths caught in the pheromone traps in 2019 when compared to the number of moths caught between 2004 and 2006. In the next years the monitoring of the flight dynamic of Cydia pomonella (L.) will continue in order to confirm that the rising average temperatures lead to an environment that favour certain pests.

Key words: *climate change, codling moth, flight dynamic.*

INTRODUCTION

The codling moth is one of the most dangerous pests for the Romanian apple orchards. It is a pest wide-spread across all over the temperate climate zones of fruit growing, except for Japan and Korea (Figure 1).

Figure 1. Distribution of the codling moth *Cydia pomonella* (L.) February 2020 (CABI)

The codling moth larvae annually produce significant damage in Bistrița region orchards, their presence being favored by the existence of a lot of untreated apple orchards. In other orchards the sprays applied at inappropriate timing or insufficient in number not only does it not help maintaining the damage under the economic threshold, but contrary contribute to destroy the useful fauna. On this context and taking into consideration the climate changes of the last years, it is necessary to study the evolution of this pest, in order to be able to secure efficient control programs that also comply with the tendencies of low insecticide quantities applied. In Bistrița region the climate change consist of rise of annual medium temperature and increasing frequency of extreme weather conditions. There have been previous studies of the codling moth in Bistrița region between 1983-1987 (Minoiu & Boaru, 1989) that showed a low number of captures on the pheromone traps. Later on, another study conducted between 2004-2006 (Kutinkova et al., 2009), in the same region, showed a bigger number of adult males caught and a modified flight dynamic in that period of time. As the average annual temperature is constantly increasing, it is expected to modify the flight curve and the population dimensions in the region. However, there are recent studies performed in other regions of the world that suggest that the rise of the annual mean value of the temperature does not impact the flight span of some *Lepidopterae* (tortricid) species (Pak et al., 2019). Other multiannual studies

came to the conclusion that the rise of the temperature mean value may extend or shorten the flight period in moths depending on the species (Maurer et al., 2018). The aim of this study is to determine if the flight dynamics of the codling moth could shift under the climatic change in the north-eastern Transylvania and will be continued in the next years.

MATERIALS AND METHODS

The pheromone traps were placed in an apple orchard at Fruit Research & Development Station (F.R.D.S.) Bistrița (47°10' latitude North and 358 m above sea level).
The flight dynamic was established based on the number of adult males captured on specific pheromone traps ATRAPOM produced by the Chemistry Institute "Raluca Rîpan" Cluj-Napoca (Figure 2).

Figure 2. Adult male moths on pheromone trap ATRAPOM

The pheromone dispenser was replaced every five weeks and also the sticky part of the trap on which the moths were caught. There were two recordings of the captures per week starting from 25.04.2019 until 19.09.2019. Data collected were compared with the existing data from previous years, 2004-2006. A flight curve of the codling moth males was made using the weekly recordings of the four years. Data recorded along the four years (2004, 2005, 2006 and 2019) were grouped on seven days intervals, so it can be compared using graphic representation. The orchard where the trap was placed has been sprayed with insecticides five times in the period of *Cydia pomonella* (L.) flight. The dates and substances used to control the codling moth in 2019 are presented in Table 1.

Table 1. The insecticide sprays in the 2019 season

Tr. No.	Data 2019	Active ingredient
1	17 June	clorpirifos metil 225 g/l
2	01 July	clorpirifos metil 225 g/l
3	18 July	Dimetoat 400 g/l
4	08 August	Lambda-cihalotrin 50 g/l
5	22 August	Tiacloprid 480 g/l

Although there are no complete records over an extended period of time of the exact treatments made with insecticides in the plot where the traps were placed, still there are some data. For example, in the year 1997 there were three sprays applied on: 19 of June, 17 of July and 13 of August, and there were 40 adult males caught on traps. Recordings of data regarding insecticides sprays from 2004-2019 indicated a number of five or six treatments per season during the flight span of the codling moth each year.
To check if there is a correlation between the flight span and the number of male adult moths caught the recordings were statistically analysed. We used the 'Pearson' function in Excel to calculate the value of the correlation factor "r". Then was compared with the critical value for Pearson's "r" factor (for two degrees of freedom, $\alpha = 0.05$).

RESULTS AND DISCUSSIONS

The data collected showed both an extended flight span in 2019 and a much bigger number of moths caught (Figure 3).

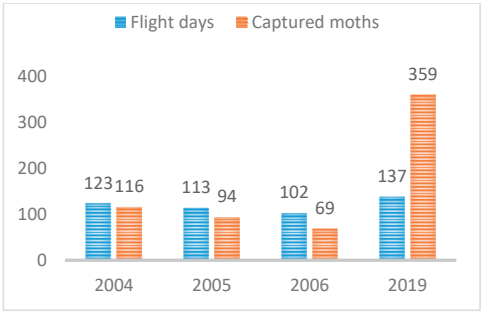

Figure 3. The number of flight days and the number of caught male moths in four years (2004-2006 and 2019)

Previous studies conducted in Bistrita area (Kutinkova et al., 2009) during 2004-2006 placed the begining of the flight of *Cydia pomonella* L. at the end of April (30.04.2004) or at the begining of May (2005, 2006), while the last captures were recorded at the end of August in all of the years of study. In 2019, the flight began in the last decade of April and ended in the second decade of September (Figure 4). The flight intensity was bigger from the last decade of June until the end of August and it was highly variable. An intensification of the flight can be observed on the second part of the summer, the same period when the medium temperatures are usually higher than the first part of the summer. The peacks of the flight curve are much higher in 2019 when compared to those in the years 2004, 2005 and 2006. Also the distribution of the peaks suggests a much bigger activity of the pest in the second part of the season of 2019 while in all the other years the first part of the season the captures were higher.

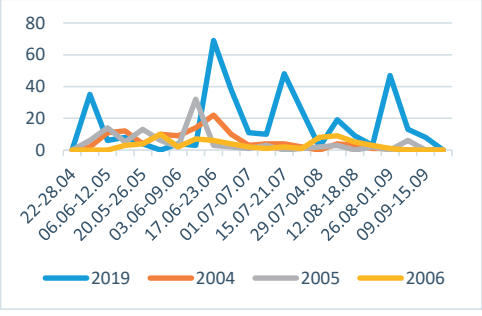

Figure 4. The flight of the codling moth in 2004-2006 and 2019

The number of captures, in 2019 season, decrease after each treatment with insecticides. This indicates that the products used were able to control the population for a period of time but the peaks that followed suggest a high reserve of moths in the area.

Although there seems to be a positive correlation between the number of days of the flight and the number of adult male moths caught, the statistics analysis of the correlation show that the correlation is not statistically significant. The calculated Pearson's "r" factor was 0.891274, smaller than the critical value for Pearson's "r" factor - 0.95 (for two degrees of freedom, $\alpha = 0.05$), that lead to this conclusion. The drastic increase in number of the moths was much likely to be the result of the growth of the pest reserve in the area over the years. There are certain questions that emerge about the efficacy of insecticides sprays in the period between 2004 and 2019. We found some data indicating that two or more consecutive treatments have been executed with the same substance on repeated occasions and also mixtures of substances that are not recommended by the producer were applied. Such technological mistakes may have had an important effect on the increasing of the pest population by lack of efficacy of the control or even by inducing resistance towards certain insecticides.

CONCLUSIONS

The preliminary results showed an extended length of the flight of the codling moth in the year 2019 compared to the previous period 2004-2006 and a big increase in number of individuals caught, but it remains to clarify the exact factors that led to this situation.

The pattern of the flight was changed, the second part of the season being much more important in the economy of the flight and in consequence the number of treatments should increase in that period of the summer.

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NUTRITIONAL STATUS OF SOIL AFTER EIGHT YEARS OF FERTILIZING WITH ORGANIC PRODUCTS

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Abstract

The study was conducted in a fruit-bearing peach plantation on the site of the Fruit-Growing Institute – Plovdiv Bulgaria. In 2019 the influence of bio-products Biohumus, Agriful and Humustim on the basic soil characteristics pH, Ec, N-NO_3^-, N-NH_4^+, K_2O, P_2O_5 has been studied. Eight years of fertilization was carried out. At the dose of 120 kg/da Biohumus, is achieved a very high degree that of available phosphorus and potassium, respectively: 80.93 mg P_2O_5/100 g and 43.13 mg K_2O/100 g soil. Fertilization with Biohumus and Agriful results in an increase of ammonium and nitrate nitrogen in the top soil layer to a depth of 30 cm. For Agriful, ammonium nitrogen values increased from 32.15 mg/100 g (0.5 L/da) to 46.93 mg/100 g (1.0 L/da). The nitrate nitrogen increase is from 39.49 to 66.34 mg/100 g soil. Continuous application of Biohumus and Agriful creates a favorable supply of nutrients in the soil (P_2O_5, K_2O, N-NO_3^-, N-NH_4^+), optimal for nutrition of peach plants.

Key words*: fertilization, biofertilizers, agrochemical parameters of soil (pH, K_2O, P_2O_5, NO_3^-, NH_4^+), peach.*

INTRODUCTION

The soil is fertile only when it contains all the mineral nutrients of vital importance for the plants in sufficient quantities and in the best ratio.

The chemical analysis of soil is used as a diagnostic tool for assessing soil nutritional status (Stoilov, 1977; Marx et al., 1996; Roy et al., 2006). Soil analysis can be a useful indicator of the relative nutritional status of fruit orchards, especially if changes are followed for a longer period of time.

Soil fertility is maintained mainly by the use of natural products, such as green fertilization, fertilization with organic phosphates and glauconites, and other natural products that provide nutrients to soil or improve its agrochemical properties (Gorbanov, 2006).

The ecological approach in organic fertilization comprises predominantly the use manure extract, a soil nutritional substance derived from the activity of California worms, natural resources rich in biologically active substances, compost derived from wood waste, soil amendment extracted from paper waste and the use of certified fertilizers for organic production (Yadav et al., 2000; Fliebach et al., 2007). The application of organic waste can increase nitrogen and phosphorus content in soil (Jakobsen, 1995), improve soil structure and water retention capacity (Johansson et al., 1999). Other authors (Dutta et al., 2003; Kaur et al., 2005) compare the use of organic and chemical fertilizers. The application of organic fertilizers has a higher positive effect on microbial biomass and therefore on soil status and the total NPK content in soil. Compost is a good source of nutrients for fruit trees, but the time of mineralization and release is difficult to predict. A many-year study in Italy evaluated the impact of organic fertilization on soil fertility, the level of nutrient supply for the nectarine fruit trees and the export of nutrients from the trees (Toselli et al., 2013). The effect of manure applied at the time of planting at a rate of 10 t per ha and of compost at 5 and 10 t per ha was studied. It was found that nitrate nitrogen content in soil increased with the application of a higher rate of compost. Biofertilizers are microbial preparations containing living cells of various microorganisms that have the ability to mobilize nutrients in soil, promote the restoration of micro flora and improve soil fertility (Rozpara et al., 2014). Soil properties are constantly changing in time and space (Rogerio et al., 2006).

The aim of the present study was to evaluate the effects of some bioproducts (Biohumus,

Agriful and Humustim) on the major soil characteristics of pH, NO_3^-, NH_4^+, P_2O_5, K_2O after eight years of fertilization in a fruiting peach plantation of 'Glohaven' cultivar.

MATERIALS AND METHODS

The trial was carried out in a fruiting peach orchard on the territory of the Fruit-Growing Institute in Plovdiv. 'Glohaven' cultivar grafted on the vegetative rootstock GF677 was used in the experiment. The soil is alluvial-meadow. Over a period of eight years, from 2011 to 2019, the bioproducts Humustim, Agriful and Biohumus obtained from California worms were applied to soil.

Biohumus was introduced into soil around the stems of the experimental trees at three rates of 40, 80 and 120 kg/da.

Agriful was applied as water solution. Two rates of 0.5 and 1.0 L/da were studied. Humustim was applied as a leaf fertilizer at three rates of 100, 120 and 150 ml/da. Treatment at the studied rates was performed five times during vegetation, every 15-20 days. The control was untreated. Another control variant was also included in the study for the period 2016 to 2019, applying fertilization with ammonium nitrate at a rate of 22 kg/da.

Soil samples for the analysis were collected in 2011 and in 2019 from the studied variants in three replications. The content of mineral nitrogen ($N{-}NH_4^+$ and $N{-}NO_3^-$) in the soil samples was determined by the distillation method after extraction with 1% KCl.

Mobile phosphorus (P_2O_5) was determined in a lactate extract (DL method) colorimetrically, with a hydrazine sulfate reducer and that of potassium (K_2O) - with a flame photometer. Soil reaction (pH) and electrical conductivity (Ec) were determined potentiometrically in water extract (1:2.5). The results were statistically processed with the Duncan test.

RESULTS AND DISCUSSIONS

The agrochemical soil properties at the beginning of the experiment are presented in Table 1.

Table 1. Soil supply in the peach plantation with the major nutrients in 2011

Depth, cm	pH (H_2O)	P_2O_5	K_2O	$N{-}NH_4^+$	$N{-}NO_3^-$
		mg/100 g	mg/100 g	mg/kg	mg/kg
Control					
0-30 cm	7.16	19.80	23.00	21.73	15.55
30-60 cm	7.13	30.00	20.00	25.40	15.03
Biohumus					
0-30 cm	7.10	26.00	32.60	13.24	19.23
30-60 cm	7.30	31.00	23.00	11.14	18.14
Agriful					
0-30 cm	7.05	22.00	30.00	12.96	16.07
30-60 cm	7.43	155.00	20.00	13.22	15.81
Humustim					
0-30 cm	7.24	66.00	27.40	16.33	20.99
30-60 cm	7.28	160.00	21.00	11.92	19.18

The soil in the experimental plot is alluvial-meadow, with a soil reaction from neutral to slightly alkaline (Table 1). There are significant differences in the supply of soil with phosphorus. According to the margin levels of supply (20-30 mg P_2O_5/100 g), all the samples fall under the category of highly supplied. Very high supply was reported for the rows fertilized with Agriful and Humustim at a depth of 30-60 cm. The amount of mineral nitrogen varied by variants of fertilization. The potassium content in the experimental plot varied from 20 to 32.6 mg/100 g of soil. The control falls under the category of moderately supplied. The results of the analyses show that the soil in the plantation is well supplied with the basic macronutrients.

The results obtained for the concentrations of nitric nitrogen, ammonium nitrogen, phosphorus, potassium, conductivity values (Ec) and soil reaction (pH) in water extract and in KCl, after eight years of fertilization with the bioproducts Biohumus, Ariful and Humustim, are presented in Tables 2 and 3.

Table 2. Phosphorus and potassium content and soil reaction in water extract and in 1n KCl after eight years of fertilization with bioproducts

Fertilization rate	**pH (H_2O)**		**pH (KCl)**		**P_2O_5** mg/100 g		**K_2O** mg/100 g	
Biohumus	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
40 kg/da	7.19 ab	7.83 a	6.40 ab	7.15 ab	29.33 ab	51.00 a	25.13 bc	16.93 bcd
80 kg/da	6.70 b	7.19 bc	6.04 b	6.13 b	41.00 ab	69.13 a	26.00 bc	15.80 cd
120 kg/da	7.40 a	7.94 a	6.79 a	7.13 ab	75.80 a	80.93 a	43.13 a	17.93abcd
Agriful	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
0.5 L/da	7.07 ab	7.37 ab	6.29 ab	6.22 b	44.6 ab	62.07 a	31.30 abc	18.47abcd
1.0 L/da	6.73 ab	7.60 ab	6.06 ab	6.74 ab	16.67 b	54.00 a	22.47 c	18.00abcd
Humustim	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
100 ml/da	6.65 b	7.67 ab	5.97 b	7.04 ab	8.93 b	58.13 a	23.47 c	13.00 d
120 ml/da	6.64 b	7.41 ab	5.78 b	6.50 ab	14.0 b	91.00 a	27.67 bc	18.50 abc
150 ml/da	6.86 ab	7.74 ab	6.24 ab	7.24 ab	15.13 b	52.33 a	40.67 a	21.67 ab
Ammonium nitrate	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
22 kg/da	4.89 c	6.72 c	4.30 c	6.33 ab	7.27 b	27.97 a	27.33 bc	19.00 abc
Control	6.72 b	7.71 ab	6.06 b	7.46 a	19.23 b	88.00 a	36.27 ab	22.83 a

Soil reaction was neutral to moderately alkaline. A significant decrease in pH values was observed as a result of fertilization with ammonium nitrate. The pH values in H_2O were 4.89 for the layer 0-30 cm and pH 6.72 at 30-60 cm depth. In KCl salt extract those values were 4.30 (0-30 cm) and 6.33 (30-60 cm). The imported ammonium nitrate led to an acidification of the soil reaction in the surface soil layer. The results obtained correspond to the studies of other authors (Sas et al., 2003; Sas Paszt & Żurawicz, 2005; Wójcik, 2000). On the other hand, the acidification also affected the content of ammonium and nitrate nitrogen (Table 3). Ammonium nitrogen in the 0-30 cm layer reached values of 181.93 mg/kg and nitrate nitrogen up to 187 mg/kg of soil, those values exceeding the untreated control many times. The differences are statistically significant.

In all the other treated variants, the soil reaction in water extract ranged from 6.64 to 7.7, which is the optimal soil acidity for the peach crop. A slight increase in pH (H_2O) was observed after the application of Biohumus - 7.19 (40 kg/da) to 7.4 (120 kg/da), as the organically bound nitrogen is mineralized and then transformed into ammonia, leading to a slightly alkaline soil reaction. The electrical conductivity ranged from 244 µS to 811 µS for the 0-30 cm layer and from 124 µS to 250 µS for the 30-60 cm layer in the different variants of fertilization. Soil electrical conductivity was relatively high, although below the threshold for damage to the peach trees (Table 3).

Table 3. Ammonium nitrogen and nitrate nitrogen content and soil electrical conductivity after eight years of fertilization with bioproducts

Fertilization rate	Ec, µS		$N–NH_4^+$ mg/кg		$N–NO_3^-$ mg/кg	
Biohumus	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
40 kg/da	305.33 ab	163.33 b	6.40 c	26.84 b	37.85 c	7.91 b
80 kg/da	811.33 a	211.33 b	133.45 ab	6.04 ab	146.00 ab	28.97 ab
120 kg/da	244.33 b	181.00 b	19.87 c	6.79 ab	16.90 c	5.79 b
Agriful	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
0.5 L/da	292.67 ab	124.67 b	32.15 bc	13.90 ab	39.49 bc	9.27 b
1.0 L/da	402.00 ab	207.33 b	46.93 bc	18.83 ab	66.34 bc	16.02 b
Humustim	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
100 ml/da	297.67 ab	184.33 b	36.69 bc	14.09 ab	44.80 bc	7.72
120 ml/da	177.33 b	135.00 b	22.79 c	15.93 ab	16.99 c	11.68 b
150 ml/da	224,67 b	200.00 b	19.79 c	14.87 ab	15.06 c	13.79 b
Ammonium nitrate	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
22 kg/da	655.67 ab	539.00 b	181.93 a	33.22 a	187.34 a	56.10 a
Control	262.33 b	250.33 a	29.26 c	20.28 ab	40,56 bc	11.78 b

Eight years of fertilization of the alluvial-meadow soil shows that with increasing the rate of Biohumus the phosphorus uptake by the plants also increases. At the highest rate of 120 kg/da the soil has sufficient reserves of absorbable phosphorus - 80.93 mg P_2O_5/100 g of soil. There is a linear relationship between the introduced rate of Biohumus and the amount of mobile phosphorus in soil, which can be expressed by the linear equation of the type y = b*x (Figure 1).

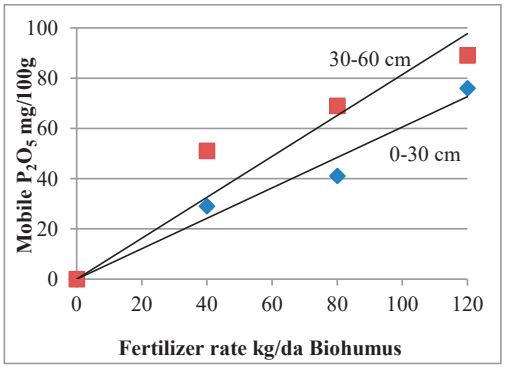

Figure 1. Relationship between the rate of fertilization with Biohumus and the mobile phosphorus content

The relationship between the amount of introduced fertilizer and the content of mobile phosphorus achieved in the 0-30 cm layer is expressed by the linear equation y = 6054*x and for the layer 30-60 cm -i by the equation y = 8143*x, where y is the mobile phosphorus in soil in mg/100 g and x is the fertilizer rate, kg/da of Biohumus.

Potassium uptake by plants gradually increases with increasing the rates of Biohumus, but the increase is not so clearly expressed as with phosphorus. At a fertilization rate of 120 kg/da of Biohumus, the potassium content in soil reached 43.13 mg K_2O/100 g of soil, showing very high supply with the nutrient. The mineral nitrogen content varied in the studied variants. Monitoring the nitrogen dynamics in soil to a depth of 60 cm with increasing fertilization rates showed a significant difference in the content and distribution of ammonium nitrogen and nitrate nitrogen in depth. Fertilization with Biohumus led to an increase in ammonium and nitrate ions only in the surface soil layer. The highest values of the ammonium (133 mg/kg) and nitrate (146 mg/kg) forms of nitrogen were observed at the rate of 80 kg/da at a depth of 0-30 cm. The nitrate form of nitrogen increased at the expense of the ammonium due to mineralization. Further to that, the application of 120 kg/da had a negative effect on the nitrogen content in soil: 19.87 mg/kg $N–NO_4^+$

and 16.90 mg/kg $N{-}NO_3^-$, the values obtained being commensurable with those in the control (Table 3).

After treatment with Agriful, there was a tendency to higher values for the phosphorus and potassium contents in the surface soil layer 0-30 cm, when applying the rate of 0.5 L/da. The increased rate of 1.0 L/da had a negative effect on the values of those nutrients. However, this is not the case with nitrogen. The introduction of the higher rate of Agriful – 1.0 L/da resulted in higher values of $N{-}NH_4^+$ and $N{-}NO_3^-$, the tendency being reported only for the 0-30 cm layer. Ammonium nitrogen values increased from 32.15 mg/kg (at the rate of 0.5 L/da of Agriful) to 46.93 mg/kg (at 1.0 L/da of Agriful). For nitrate nitrogen, the increase was from 39.49 to 66.34 mg/kg, the differences to the untreated control being statistically significant (Table 3).

It should be noted that soil application of Agriful and Biohumus products resulted in better soil parameters for the 0-30 cm layer. The most active part of the roots of the peach trees grown on a vegetative rootstock, are located at that depth.

The values obtained for the studied soil characteristics after the application of Humustim were close to those of the control variants. The bioproduct did not affect the basic soil characteristics, since its application is foliar and it does not significantly affect the studied soil parameters.

CONCLUSIONS

Fertilization of alluvial-meadow soils with high rates of Biohumus led to an increase in the mobile forms of phosphorus in the soil, with a close relation between the rate of fertilization and the amount of the mobile forms of phosphorus, expressed by a linear equation.

Despite the high rates of Biohumus and Agriful fertilizers applied, deep penetration into the soil was not observed.

The long-term application of the bioproducts contributed favorably to the soil characteristics of pH, Ec, NO_3^-, NH_4^+, P_2O_5, K_2O in the peach plantation and contributed to a favorable supply of nutrients (potassium, phosphorus and nitrogen), optimal for the nutrition of peach trees.

Continuous application of ammonium nitrate led to acidification of soil, which is unfavorable to the peach crop.

The application of bioproducts to soil confirmed the positive impact on its nutrient supply and hence, increases its productivity.

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EVALUATION OF GERMAN PLUM CULTIVARS IN THE REGION OF TROYAN

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Abstract

Due to the need to expand its plum assortment in the mountainous regions of the country, the German plum cultivars from the selection program of Prof. Jacob's collection at the Geisenheim Research Station in Germany, 'Topgigant plus', 'Topking', 'Topper', 'Top 2000', 'Top', 'Topend plus', 'p33-6-94', were introduced in the RIMSA Troyan in 2008. For the period 2016-2019, the climatic factors of the Troyan region were analyzed, the phenology of the cultivars (flowering and ripening time), vegetative and reproductive characteristics were supplemented, which were complemented by laboratory analyzes of the fruits. The 'Top 2000' cv. was first blossomed (at the end of March, the first days of April, depending on climatic conditions. The lowest growth was in the 'Topper' cv., which was adapted to intensify the planting scheme. The largest are the fruits of 'Topgiant plus' (77.34 g) and have the smallest share of stone (2.75%) for 2019, ripening relatively earlier than the others. The highest content of total sugars (9.4%) and high glucoacidimetric ratio (12.37) are of the 'Topper' cv.. 'Topper' (0.18 kg / cm^2) and 'p33-6-94' (0.15 kg / cm^2) are distinguished by high productivity values. The German plum varieties of the Top group are suitable for cultivation in the mountainous conditions of the Troyan region, using technologies that meet the specificities of each cultivar.

Key words*: plum, phenology, growth, fruit qualities.*

INTRODUCTION

Plum is the main fruit species in our country, where climatic factors are suitable for its development. The varietal assortment is constantly changing, and it is always good to be enriched with new, more productive and attractive plum cultivars.

German cultivars have been widely distributed in recent years, so it is especially important to investigate their economic suitability for a particular area. The breeding program at the Geisenheim Research Station in Germany has produced large-fruited cultivars such as 'Tophit' and high and regular bearing cultivars such as 'Topper' (Jacob, 1998).

The parameters for which the genotypes were sought in Prof. Jacob's breeding program are different maturing dates (early, mid-term, late-ripening), taste and aroma, disease resistance, especially measles, shelf life and storage, tolerance to climatic conditions for their widespread distribution (Jacob, 2007a). Most of them were created with the participation of the 'Cacanska najbolia', which the author considers to be a unique donor for the resistance to PPV and good taste (Jacob, 2007b).

Late-ripening varieties can be sold at a better price and most of them have a higher dry matter and sugars content than the earlier ones (Milatovic et al., 2019).

The aim is to study the technological and economic characteristics of the German plum cultivars of the Top series and the consumer qualities of their fruits, their suitability for cultivation under (in accordance with) the agro-ecological conditions of the Troyan region, to recommend them to other regions with similar ecological conditions.

MATERIALS AND METHODS

In RIMSA Troyan, in order to enrich the assortment list of pre-mountain plum production with new cultivars, the German plum cultivars of Top series, selected at the Geisenheim Research Station, were introduced 10 years ago.

This study covers the period 2016-2019.

The trees are grown in a plum garden in the Troyan region under the agro-ecological conditions of the Pre-Balkans. The climatic conditions for the study period (01.01.2016-30.09.2019) are compared with the conditions of a basic 30-year period (1965-2005).

The trees were planted in 2008 under a 5 x 4 m scheme, in planting pits loaded with 30-40 kg of fertilized manure, on slightly sloping terrain. The altitude is 420 m, the soils are light gray acidic forest, poorly stocked with nutrients.

The cultivation technology includes the formation of a free-growing crown, the maintenance of mowed rows that are mowed (Bozhanska et al., 2017) and once-a-year treatments in a row, standard plant protection, non-irrigation conditions, not further nourished during the years of experience.

The rootstocks is 'Myrobalan' (*Prunus cerasifera* Ehrh.). Each cultivar is an option represented by 5 threes.

The following metrics were reported:

- Phenological data;
- Vegetative parameters:

Trunk cross-section area (cm^2); Crown volume (m^3); Projection of crown (m^2);

- Reproductive parameters:

Yield per tree (kg); Fruit weight (g);

- Chemical composition of fresh plum fruits: Soluble solids (refractometrically) (%); Sugars according to Schoorl (%); Acids, as malic, by titration with 0.1 n KCl (%); Tannins – according to Levental-Neubauer (%); Antocianins - Filsky and Fransis (mg%).

The studies were conducted according to Methods for Studying Plant Resources (Nedev et al., 1979).

The experimental data were subjected to statistical analysis by Fisher's single-factors ANOVA. The significance of differences between the mean values of the factors and the interaction means was determined by LSD test at significance levels of $P \leq 0.05$.

RESULTS AND DISCUSSIONS

The climatic conditions for the Troyan region were analyzed as a comparison of data on average monthly temperatures (°C) and total precipitation (mm) for the study period (01.01.2016-30.09.2019), with the same indicators for a 30 year base period (1965-2005) (Figure 1). There is a tendency for an increase in the average monthly summer temperatures for the months of June, July, August and September, compared to the baseline for 30 years period, an increase in the total rainfall for June and July and a sharp decrease in rainfall, compared to the average 30 years period for the months of August and September, when the period of ripening of the fruit.

This makes us consider the suitability of these cultivars for the conditions of the Troyan region as suitable for extending the harvest period, but providing some (partial) irrigation at the end of the growing season, during ripening.

For the study period, 'Top 2000' cv. started **flowering** earlier (28-30.03.2016; 27.03.2017), followed by 'Top' and 'Topping' cvs. for 2016 and 'Top' and 'Topper' cvs. for 2017 (30.03.2017) (Figure 2).

Flowering of 'Top', 'Top 2000' and 'Topper' cultivars lasted and longest - 15-16 days, which is a favorable factor for the pollination period.

For 2018, the flowering period is later and extremely short for all cultivars (5-15.04). Low March temperatures (5.1°C), similar to the baseline 30-year period (5.7°C), but lower than other years of experience (Figure 1), had an impact. Precipitation for March is higher for the survey years and about 30 mm more than the baseline. The average flowering time for all cultivars is 7-9 days, as in April the temperatures rise sharply, by about 3-4°C, compared to the base period, which leads to the rapid flow of phenophase. The end of flowering of the Top series for 2018 is April 14-16, which is very similar to the data of Milatovic et al. (2019) for a period of 5 years in the Belgrade area.

In 2019, cvs. 'Top' and 'Topper' start flowering as early as March 30.03. (due to higher temperatures and lack of precipitation), with a flowering phase of 15-17 days to 17-18.04. The remaining cultivars of the group bloomed from 1-2 to 15-17 April, at the latest 3-4.04. Has started its flowering 'Topgiant Plus' cv. and has completed about 17.04. All this indicates a long flowering period for 2019, with the cultivars with the longest flowering being represented with higher yields of 8-10-11 kg ('Top 2000' and 'Topper', 'Top' cvs. respectively).

Ripening time: - 'Top' (30.8-2.09); 'Topgiant plus' (12-15.08); 'Topper' (10-13.09).

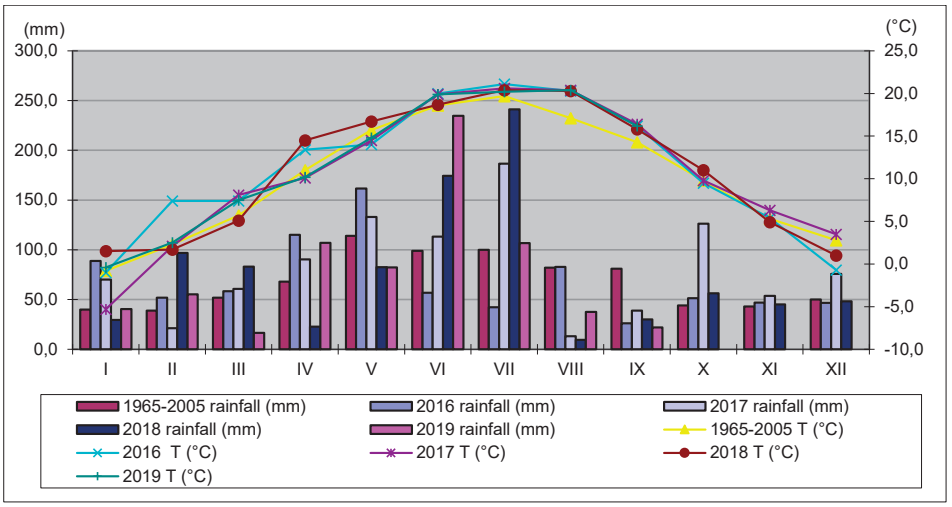

Figure 1. Evolution of climatic conditions for the Troyan region (2016-2019); a 30 year base period (1965-2005)

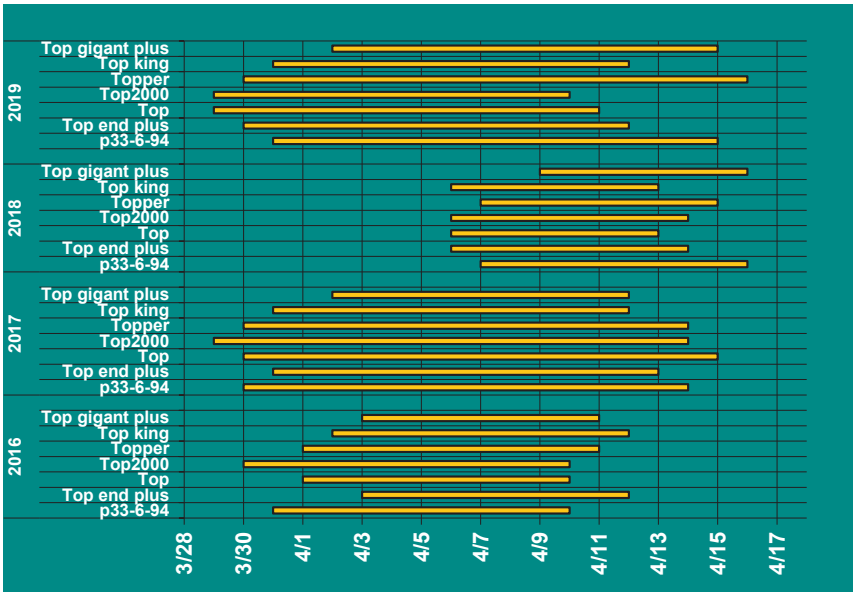

Figure 2. Phenology of plum cultivars of Top series, under the conditions of the Troyan region (2016-2019)

The growth characteristics of Top series cultivars (Table 1), showing that for 2016 the largest trunk cross section was calculated for the 'Topking' and 'Top 2000' cultivars (74.0 cm^2; 57.5 cm^2). The trunk cross-sections have minimum values for the cv. 'p33-6-94' (26.0 cm^2) and 'Topper' (11.7 cm^2), which defines it as the weakest so far.

For 2017, there is a greater increase in trunk cross-section in all varieties, with differences from 4 to 19 cm^2, with the largest increase at 'p33-6-94' and 'Topper', and the smallest increase for the year at 'Top' and 'Topgiant plus' cvs. (4 cm^2). The largest sections form the 'Topking' and 'Top 2000' cvs. (80.49 cm^2; 68.84 cm^2). For the sake of Blajek et al. (2012) consider that under their conditions the 'Top 2000' variety is weak.

The volume of crowns at the beginning of the study (2016) is quite large in the cvs, 'Topend plus', 'Top 2000', 'Top'. The smallest crowns

were measured at 'Topgigant plus' (1.06 m^3) (Table 1).

For 2017, the smallest by volume are the crowns of 'Topper' (1.13 m^3) and 'Topgigant plus' (1.89 m^3), but their growth compared to 2016 is more moderate, with the smallest expansion of 'Top 2000' and 'Topend plus' (with 0.17-0.21 m^3). Growth differences, assuming that they will continue to exist, suggest the possible choice of different schemes specific to each cultivar. In the studies conducted by Čmelik et al. (2007) observed lower growth in 'Toptaste' and 'Top 2000' cvs., stronger in 'Topfirst' and 'Topfive' varieties, and stronger in 'Tophit' cv.

By the end of the study period, 2019, the growth of the stems is extremely uneven in variants. The differences are from 1-2 cm^2 for 'Top' and 'Topper' cvs., to -13 cm^2 for 'Topgiant plus' cv. The largest crown had Topking (7.4 m^3) and Top 2000 (5.29 m^3).

The smallest and most harvested crowns, like the previous year, have 'Topper' and 'p33-6-94' trees. Their volume is 0.80-1.13 m^3.

Reproductive qualities

The productivity factor (yield per tree, per unit cross-section of the trunk kg/cm^2) for 2019 is extremely small in the case of the taller trees 'Topking', 'Top', etc. (0.04 kg/cm^2), but with high values are the 'Topper' (0.18 kg/cm^2), 'p33-6-94' (0.15 kg/cm^2), 'Topend plus' (0.14 kg/cm^2), 'Top 2000' cvs. with 0.10 kg/cm^2 which are more cost effective (Table 1).

Average for the study period, with the highest fruit mass being the early ripening cultivar 'Topgigant plus' (77 g) followed by 'p33-6-94' (37 g), with their stones are large, but the relative share of 'Topgigant' is 2.57%. For the other cultivars, the average weight of the fruit is 28-30 g and the smallest are the 'Top 2000' (20 g) (Table 2).

Fruit sizes - with greater height and almost equal width and thickness, with a confidence of results at p = 0.05, determine the elongated shape of the fruit in all cultivars.

Radiković (2014) shows remarkable differences between the studied cultivars in terms of pomological and physicochemical properties. The highest percentage of fruit meat and the smallest stone was found in the 'Topgigant plus' cv. (93.7% fruit flesh, stone share 3.97%, with us 2.57%), followed by the 'Top 2000' cv. (93.5% fruit flesh, stone share 5.24%, 3.44% for us), so they are the sorts desired for consumption as fresh fruits.

Chemical composition

Chemical analysis of fresh fruits was performed on 7 cultivars for 2019 at the analytical laboratory in RIMSA Troyan.

The highest dry matter content (22.5%) has the 'Topking' cv. and the lowest 'Top' (15.5%) (Table 3). Content of total sugars also is the lowest (5.7%) in the 'Top' cv. The highest amount of total sugars was determined in the 'Topper' cv. (9.4%), most of them in the form of invert sugar (7.2%).

Table 1. Vegetative characteristics of Top series cultivars (2016-2019)

	2016		2017		2018		2019		2019
	TCSA (cm^2)	Crown volume (m^3)	TCSA (cm^2)	Crown volume (m^3)	TCSA (cm^2)	Crown volume (m^3)	TCSA (cm^2)	Crown volume (m^3)	Yield efficiency (kg/cm^2)
To gigant plus	53.9	1.06	58.25	1.89	65.66	1.30	79.96	2.69	0.05
Topking	74.24	6.12	80.49	6.43	83.17	4.82	93.68	7.41	0.04
Topper	11.78	0.67	28.22	1.13	30.31	0.58	31.24	1.13	**0.18**
Top 2000	57.52	8.45	68.84	8.61	70.51	3.43	76.88	5.29	0.10
Top	49.84	7.04	54.19	3.66	67.65	4.64	69.06	3.49	0.07
Topend plus	49.76	1.70	65.92	1.91	66.28	1.97	71.47	5.05	**0.14**
p33-6-94	25.88	0.99	43.99	3.09	48.14	0.96	54.18	0.83	**0.15**

Table 2. Reproductive characteristics (average 2016-2019)

				Fruit dimensions (mm)			Stone dimensions (mm)			
	Fruit weight (g)	Stone weight (g)	Stone share (%)	length	width	thickness	length	width	thickness	Yield per tree (kg)
Topgigant plus	77.34	1.99	2.57	59.1	51.4	47.1	29.3	17.3	10.3	7.5
Topking	28.20	1.25	4.43	40.0	35.0	33.8	22.6	15.1	8.9	3.0
Topper	28.49	1.31	4.60	41.9	34.8	35.5	24.6	14.1	9.1	8.0
Top 2000	20.03	0.69	3.44	36.9	30.7	31.1	19.8	12.3	7.8	10.0
Top	30.57	1.15	3.76	43.2	36.3	35.1	23.3	15.2	9.6	11.0
Topend plus	36,39	1.35	3.71	43.3	37.6	37.5	24.1	16.0	8.9	2.5
p 33-6-94	36,81	1.38	3.75	44.1	38.4	38.3	24.9	16.7	9.6	3.5
LSD=0,05	*5,03*	*0.15*		*2.59*	*3.93*	*1.83*	*1.49*	*0.83*	*0.57*	

Table 3. Chemical composition (2019)

	Soluble solids (%)	Total sugars (%)	Invert sugar (%)	Sucrose (%)	Total acids (%)	Sugar/acid ratio	Tannins (%)	Anthocyanins (mg%)
Topgigant plus	15.5	7.35	5.85	1.43	0.88	12.32	0.163	7.26
Topking	22.5	8.90	6.65	2.14	0.76	11.71	0.208	8.55
Topper	17.5	9.40	7.20	2.09	0.76	12.37	0.229	11.61
Top 2000	18.0	6.15	5.00	1.09	0.50	12.30	0.125	11.61
Top	15.5	5.70	4.70	0.95	0.63	9.05	0.312	9.68
Topend plus	17.5	9.20	2.00	1.10	0.70	13.14	0.230	10.11
p33-6-94	16.5	7.20	4.85	2.23	0.63	11.43	0.229	12.58

It also has the lowest value of tannins and a relatively high value of anthocyanins (11.61%). 'Topper' is also the highest Glycoacidimetric factor (12.37).

The 'Top 2000' is represented by 18% dry matter (approximately as standard Stanley) and very low acid content (0.5%), so its sugar/acid ratio is high. This defines a balanced taste. The other cultivars contain acids from 0.63 to 0.76%.

By conducting serological analysis for the presence of Plum pox virus, the cultivars 'Topend plus' it was positive.

CONCLUSIONS

The soil-climatic and agro-ecological conditions of the region of Troyan are favorable for the normal development and regular bearing of plums from the introduced plums of German cultivars 'Topgigant plus', 'Topking', 'Topper', 'Top 2000', 'Top', 'Topend plus', 'p33-6-94'.

The flowering of the 'Top 2000'' cv. begins at the earliest. For all cultivars, the duration of the phenophase is 12-14 days, and in 2018 it is later and shorter (7-8 days).

The smallest (with crowns removed) are the 'Topper' trees (0.58 m^3), 'p33-6-94' (0.96 m^3), 'Topgigant plus' (1.89 m^3), which makes them suitable for thickening the planting scheme. The largest fruits have the early-ripening 'Topgigant plus' cv. (77 g), followed by 'Topend plus' cv. (39 g).

With high values of yield efficiency are distinguished 'Topper' (0.18 kg/cm^2), 'p33-6-94' (0.15 kg/cm^2), 'Topend plus' (0.14 kg/cm^2), 'Top 2000' cvs. with 0.10 kg/cm^2 which are more cost effective.

Based on the results obtained, for cultivation in the Troyan region, it is possible to recommend the cultivars of the Top group, as cultivars with

combined traits (suitable for both fresh consumption and processing). In addition, the early cultivar 'Topgigant plus' can be grown mainly as a table (for fresh consumption).

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TOP GRAFTING RESPONSE OF SOME PAWPAW (*ASIMINA TRILOBA* DUNAL) GENOTYPES

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Abstract

Pawpaw or Northern banana (Asimina triloba Dunal) is a recently introduced and nearly unknown fruit with high nutraceutical potential. Originated from the Eastern part of USA, is the only representative of the Annonaceae family cultivated in temperate areas. In Romania was introduced in 1926 by Ioan Suciu family in Pianu Nou, Alba County, who brought some seed from Ohio State. In the last 25 years, extended researches on the plant and new varieties were conducted at the Faculty of Horticulture within USAMV Bucharest. The pawpaw usual applied propagation method is by seeds while the new selected genotypes require grafting to be extended in production. The aim of this study is to present the top grafting response of Simina, a new Romanian variety under registration and several genotypes from Lehman collection, Illinois, USA. The new genotypes were over grafted on mature trees, planted at 4.0 x 2.0 m. The grafting shoots were placed at different height levels in the tree to compare the success rate. Number of shoots, lengths, buds and flowering were monitored with the success grafting rate.

Key words*: Lehman collection pawpaw, Northern banana, Simina.*

INTRODUCTION

Pawpaw or Northern banana (*Asimina triloba* L. Dunal) is a recently introduced and nearly unknown fruit with high nutraceutical potential (Nam et al., 2018). Originated from the Eastern part of United States of America, is the only representative of the *Annonaceae* family cultivated in temperate areas (Layne, 1996). It has the largest edible fruit native in this country (Pomper et al., 2003a). In the last years it is well known for the natural compounds (annonaceous, acetogenins) from leaf, bark, and twig tissue with insecticidal and anticancer properties (Johnson et al., 1996; Ratnayake et al., 1993; McLaughlin et al., 1986; Huang et al., 2003; Avula et al., 2018).

In Romania was introduced in 1926 by Ioan Suciu family in Pianu Nou, Alba County, who brought some seed from Ohio State (Stănică, 2012). In the last 25 years, extended researches on the plant and new varieties were conducted at the Faculty of Horticulture within USAMV Bucharest (Cepoiu et al., 2004). The pawpaw usual applied propagation method was by seeds while the new selected genotypes require other methods like top grafting for production (Stănică et al., 2002). The aim of this study is to present the top grafting response of Simina, a new Romanian variety under registration and of several genotypes from Lehman collection, Illinois, USA.

MATERIALS AND METHODS

Description of the study site and experimental design

The experience was placed in the Experimental Field of the Faculty of Horticulture, USAMV Bucharest. New genotypes were over grafted on mature trees, planted at 4.0 x 2.0 m. Six mature trees were grafted with eleven genotypes: Simina, L-NS, 250-30, G4, G6, SN-15, MJ, 275-17, VE-21, 166-66, 275-56. In the same time, several plants in containers were grafted with Simina variety using the same top grafting method.

The grafting shoots were placed at different height levels in the tree to compare the success rate. Biometric parameters like height and diameter of the grafting point, shoots length, number of the vegetative and flower buds were determined. Success grafting rate was compared.

Biological material

From the grafted genotypes, ten of them are new hybrids from Lehman collection. Simina (sin. Vitroplant 2) (Figure 1) is a new cultivar obtained by selection from hybrids achieved by pollination of Sunflower x Overleese cultivars.

The main characteristics are medium-size vigor tree with spherical crown, auto sterile, recommended as pollinating cultivars being Sunflower, Prima or Overleese.

Figure 1. Flowers of the cultivar Simina

The fruit (Figure 2) is very large (over 200 g), with irregular shape, yellowish green, covered with fine prubine, whitish. White, creamy pulp is sweet, with accentuated taste and a very fine, specific flavor.

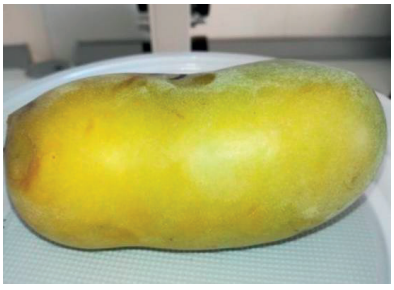

Figure 2. Fruit of Simina at maturity

Contains 23% dry matter, low acidity 0.12% (malic acid), ascorbic acid 35.2 mg/100 g, mineral substances 0.81% of which: calcium 29.79 mg/100 g, magnesium 16.91 mg/100 g and potassium 301.63 mg/100 g.

Seeds are dark brown, mugged, elliptical, large, one fruit having usually 10-12 seeds (Ştefan et al., 2018).

Statistical analysis

Data statistical analysis were performed with Excel (MS Office) and Quattro programs. Anova one-way together with T tests were used.

For correlation between two data sets Excel statistical functions with a significance level $p < 0.05$ were used (Pomohaci and Vâșcă-Zamfir, 2017).

RESULTS AND DISCUSSIONS

The MJ genotype and Simina cultivar had the best results comparing success top grafting rate after the two years taken into study (Figure 3). Genotypes SN-15, 275-17, VE-21, 166-66, 275-56 haven't results after grafting.

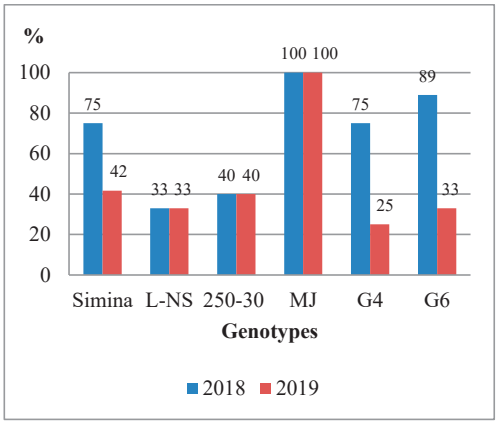

Figure 3. Success grafting rate

Simina cultivar grafted on container plants at different heights had a success grafting rate in average of 67% (Figure 4).

The results for the first year are comparable with those registered by Pomper et al. (2006), where in a rootstock trial field mortality was greatest (58%) for Susquehanna budded onto Susquehanna seedling rootstock, whereas mortality was about 25% with other scion/rootstock combinations.

In another experiment, Pomper et al. (2003b), had similar results on chip budding grafting method, where 95% of trees survived in Frankfort plot. The cultivars Wilson and Taylor had the poorest survival rate (75%). All other cultivars and advanced selections had survival rates bigger than 88%.

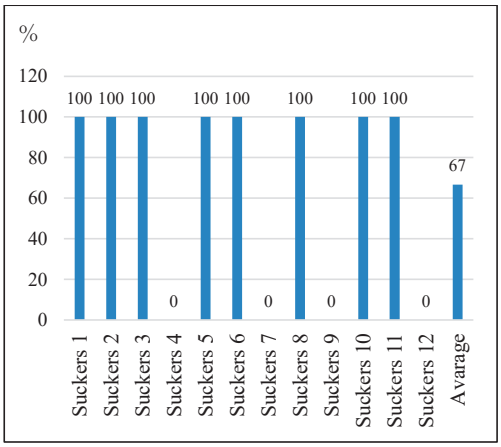

Figure 4. Simina variety success grafting rate

Grafting success rate didn't present influences from the height of grafting point at Simina cultivar (Figure 5).

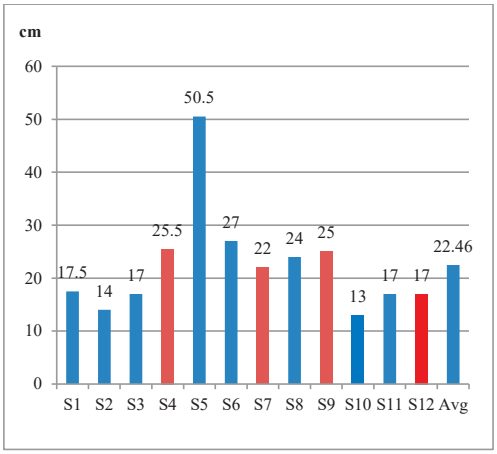

Figure 5. Influence of grafting point heights on success rate

Shoots number

For the shoots number formed on the grafted branches, the best results were obtained by the Simina cultivar, genotypes 250-30 and MJ, the last one multiplied the number of shoots four times in the second year (Figure 6).

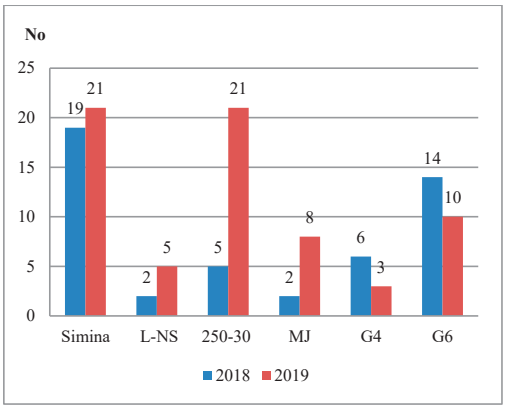

Figure 6. Shoots number formed on grafting branches

Influence of grafting point diameter

On the field trees, at Simina cultivar, viable shoots were registered for diameters of grafting point over 2.05 cm. In the pot plants, this diameter varied from 0.89 to 1.50 cm (Figure 7), without specific influence in this interval.

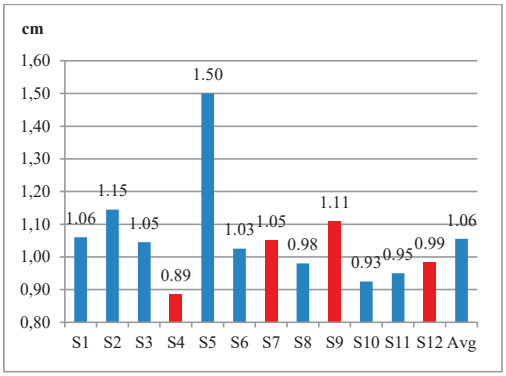

Figure 7. Influence of grafting point diameter on success rate

On L-NS genotype, the diameter varied from 1.55 to 1.65 cm without specific influence. On 250-30 genotype, the diameter varied from 1.7 to 3.67 cm, successful grafting registered on the lower values. On G4 genotype, diameter varied from 1.5 to 2.1 cm, the lowest being successful. On G6 genotype, diameter varied from 1.4 to 3.8 cm, viable shoots being on the 2.35, 3.1 and 3.8 cm.

The average grafting points diameter for these genotypes was significant positive correlated with the shoots number (in 2018: $R^2 = 0.93$, y = 7.39 x – 8.49; in 2019: $R^2 = 0.48$, y = 5.94 x – 3.88 where y = shoots number and x = average genotype diameter).

Total vegetative growth, vegetative and flowering buds

Simina cultivar with genotypes MJ and 250-30 had the most vigorous growth after two years. Comparing the second year genotypes, MJ and 250-30 had nine or eight times higher vegetative growth increase compared to the first year (Figure 8). The average grafting points diameter was significant positive correlated with the shoots length (in 2018: $R^2 = 0.78$, y = 106.79 x – 133.1; in 2019: $R^2 = 0.49$, y = 337.45 x – 399.89 where y = shoots length and x = average genotype diameter).

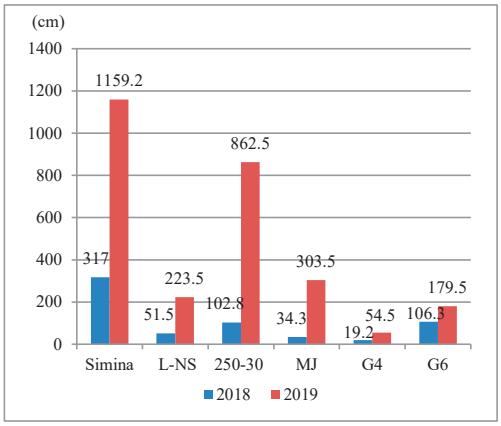

Figure 8. Total graft shoots length (cm)

On the Simina cultivar grafted on pot rootstocks, the most vigorous growth in grafted shoots were recorded on the sucker 1, grafted at 17.5 cm height with a point of grafting diameter of 1.06 cm (Figure 9).

Average shoots length at Simina grafted on field trees was 14.39 cm (2018) compared with 11.90 cm in pot plants (2018).

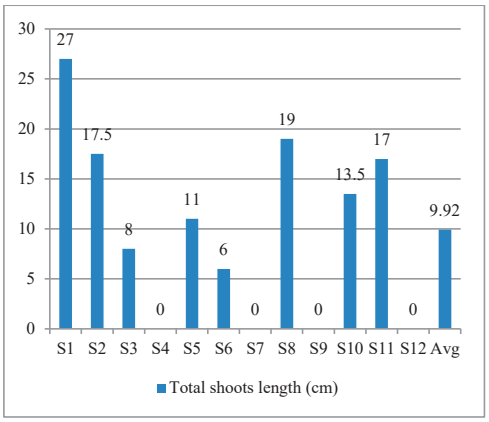

Figure 9. Total graft shoots length at Simina cultivar grafted on pot plants

Simina cultivar recorded the highest number of vegetative buds every year but MJ and 250-30 genotypes in the second year had five respectively four times higher increase rate of vegetative buds number (Figure 10). Similar significant positive correlation were registered between grafting point diameter and vegetative buds number (in 2018: $R^2 = 0.87$, y = 55.5 x – 60.83; in 2019: $R^2 = 0.53$, y = 91.73 x – 97.77 where y = vegetative buds number and x = average genotype diameter).

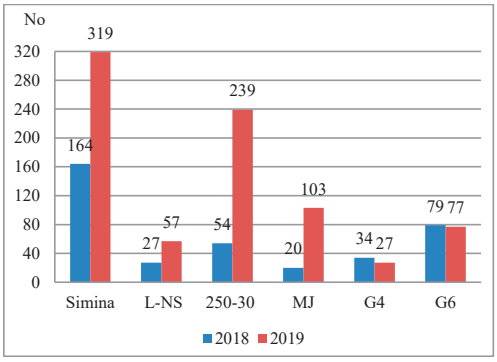

Figure 10. Number of vegetative buds on grafted shoots

On Simina grafted on pot, sucker 10 recorded the highest number of vegetative buds having the lowest height of the grafting point (13 cm) and one of the smallest diameters of the grafting point (0.93 cm) (Figures 11 and 12).

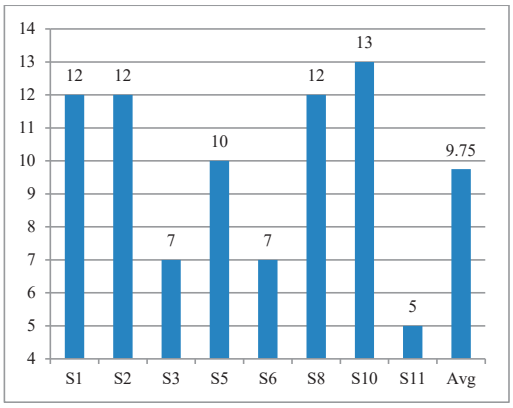

Figure 11. Number of vegetative buds on Simina grafted on pot

Figure 12. New shoots on the grafting branch

In the process of differentiation of the flower buds, the best results were recorded by the MJ genotype (28) (Figures 13 and 14) and Simina cultivar (34). No flower buds were on the pot grafted plants.

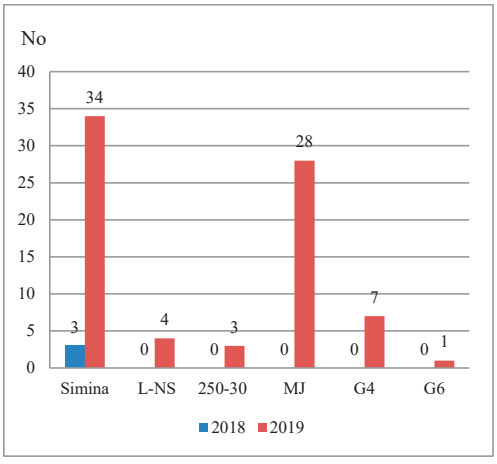

Figure 13. Number of flower buds on grafted shoots

Figure 14. Flower bud of the MJ genotype

For the percentage of flowering, the best results were recorded by the MJ genotype and the Simina cultivar (Figures 15 and 16), in the second year with a number of 17 respectively 20 flowers.

Simina was the only genotype with flowers from the first year. The genotype influenced the flower number similar with Pomper et al (2006) where Sunflower (3.46) had more flower buds per tree than Susquehanna (0.43).

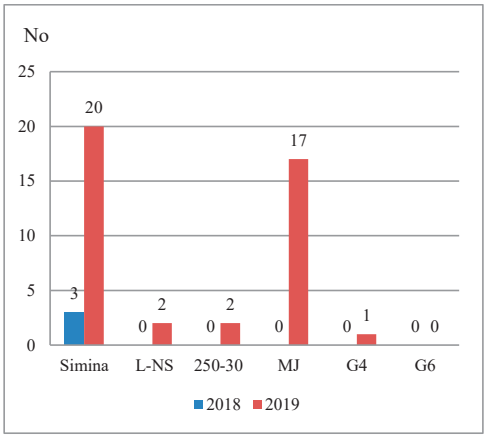

Figure 15. Number of flowers on grafted branches

Figure 16. Flower of the Simina cultivar

CONCLUSIONS

From the initial 11 genotypes, six were successful with the top grafting method. The genotype 250-30 had good results in formation of shoots, growth vigour and formation of buds.

The genotype MJ had good results in formation of shoots, growth vigour and formation of buds, differentiation of flower buds, flowers bound and grafting rate.

Simina had good results in differentiation of flower buds, flowers bound and grafting rate. Simina grafted on the mature plants in orchards had a bigger success grafting rate than the container grafted plants.

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FURTHER EVIDENCE OF DURABLE RESISTANCE IN HONEYSWEET TRANSGENIC PLUM UNDER NATURAL INFECTION WITH D AND REC STRAINS OF PLUM POX VIRUS

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Abstract

'HoneySweet' is a transgenic plum protected against Plum pox virus (PPV). Its behaviour to natural PPV infection was the subject of different field trials undertaken in several endemic European countries. The first experiment in Romania was performed between 1996-2006 and no 'HoneySweet' tree was found infected under high natural PPV infection pressure. To further asses the durability of resistance to PPV of 'HoneySweet', a new field trial was established in 2013. As inoculum sources of PPV were used plants, previously artificially inoculated with D or Rec strains, grown in pots and placed inside of the blocks. Limited treatments with insecticides were made within the plot in order to stimulate the virus spread by aphids. The monitoring of PPV spreading was made by serological and molecular assays. Strains discrimination was made by RT-PCR. The temporal spread of PPV revealed a continual evolution of infection in conventional plums. No trees of transgenic plum 'HoneySweet' expressed PPV symptoms or were found infected, by serological and molecular assays, confirming its durable resistance to natural PPV infection with D or Rec strains.

Key words*: plum pox virus, D and Rec strains, resistance, transgenic plum.*

INTRODUCTION

In Europe, the plum is highly affected by infections with *Plum pox* virus (PPV) for more than a century, being considered the most dangerous viral pathogen affecting *Prunus* spp. It produces severe symptoms on fruits, and also premature dropping, causing serious yield losses (Cambra et al., 2006).

Plum pox virus, the causal agent of Sharka disease, was described for the first time in Bulgaria, at the beginning of the 20th century (Atanasoff, 1932). Since then, PPV has progressively spread around the Mediterranean basin and Middle East. It has also been found in North and South America, North Africa and Asia (Barba et al., 2011). So far, PPV has not been found in Australia, New Zeeland and South Africa (García and Cambra 2007; EPPO, 2019). The virus is naturally spread by aphids in a non-persistent manner (Labonne et al., 1995).

Ten strains of PPV have been reported so far (Kamenova and Borisova, 2019): Dideron (D) and Marcus (M) - (Kerlan and Dunez, 1976), Recombinant (Rec) - (Glasa et al., 2002), El Amar (EA) - (Wetzel et al., 1991), Cherry (C) - (Kalashyan et al., 1994; Crescenzi et al., 1995; Maxim et al., 2002), Winona (W) - (James and Varga, 2004), Turkey (T) - (Serçe et al., 2009), Ancestor Marcus (An) - (Palmisano et al, 2012), Cherry Russian (CR) (Glasa et al., 2013) and Cherry Volga (CV) - (Chircov et al., 2018). The most common PPV strains are D, M and Rec, which are largely distributed in Europe (Šubr and Glasa, 2013). Although PPV is widespread in all plum growing areas from Romania and causes economic losses, a large-scale study revealed that only two strains (PPV-D and PPV-Rec) are present in this endemic country (Zagrai et al., 2010). PPV-C was also reported in a few sweet cherry trees, in an orchard from Bistrița area (Maxim et al., 2002), which was promptly rooted out, and a survey done ten years later did not found PPV-C in Romania (Zagrai et al., 2012).

Sharka disease strongly reduces the profitability of stone fruits crops in endemic areas, and can compromise the most part of yield of susceptible plum varieties (Minoiu, 1997; Zagrai et al., 2001). An eradication program of *Plum pox* virus in endemic areas is difficult to establish due to a fast spread of the virus via aphids and also, by the presence of

many potential host. Thus, using of resistant cultivars represents the most efficient solution to control PPV infection. In the context of over 80 years of conventional breeding which did not led to the expected results regarding the resistance to PPV, genetic engineering was used as a complementary approach to develop resistant plums by introducing a virus gene fragment into the DNA of *Prunus* host plants. Thus, a transgenic European plum (*Prunus domestica* L.) containing the coat-protein (CP) gene of PPV has been developed inside a cooperation between U.S. and France (Scorza et al., 1994). 'HoneySweet' (Figure 1) is a transgenic plum protected against *Plum pox* virus based on RNA interferance.

Figure 1. Fruits of 'HoneySweet' transgenic plum (original)

It was found highly resistant to PPV both under greenhouse conditions (Ravelonandro et al., 1997; Scorza et al., 2001) and in the field to natural PPV infection in several endemic European countries. Field trials carried out in Poland and Spain (Malinowski et al., 2006), Czech Republic (Polak et al., 2008) and Romania (Zagrai et al., 2011) demonstrated that 'HoneySweet' transgenic plum shows a highly effective and durable resistance to natural PPV infection.

The first experiment in Romania was performed between 1996-2006, and no 'HoneySweet' tree was found infected under high natural PPV infection pressure (Zagrai et al., 2011).

To further asses the durability and stability of resistance to *Plum pox* virus of 'HoneySweet' transgenic plum, a new field trial was established in 2013, and a new cycle of investigation was undertaken until 2019.

MATERIALS AND METHODS

Plant material and experimental field plot. The field trial, established in 2013, was statistically designed in twelve blocks of four trees (two trees of 'Honey Sweet' transgenic plum and two trees of conventional tolerant plum cultivars, used as control, 'Stanley' and 'Reine Claude d'Althan'). Thus, twenty-four trees of transgenic plum and twenty-four trees of conventional, all of these having virus-free status, were in the subject of this study.

The plot was surrounded by a large apple orchard in order to secure a buffer zone of minimum 500 m.

Plants of conventional plum, previously artificially inoculated with D (Čačanska rana 15/16) or Rec (Oneida 10/12) strains of PPV, were grown in pots and used as inoculum sources of PPV. The infected plants were then placed inside of the blocks, one infector per block, alternately each one of the two strains, to ensure a high inoculum pressure of the virus inside the experimental field plot (Figure 2). Limited treatments with insecticides were made within the plot in order to stimulate the virus spreads by aphids.

Virus monitoring. The monitoring of PPV spreading was made three times on each vegetative period by visual observations for potential symptoms of sharka disease, and annually by serological and molecular assays. In the case of trees which expressed typical symptoms of PPV, samples were collected from symptomatic leaves. In all the other cases, samples consisted in asymptomatic leaves taken from different parts of the canopy. All forty-eight trees belonging to transgenic and conventional plums were tested for the presence of PPV by DAS-ELISA (Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay) - (Clark and Adams, 1977) using polyclonal antibody (Bioreba, Switzerland) according to the manufacturer's instructions.

Molecular detection was done by IC-RT-PCR (Immunocapture - Reverse Transcription - Polymerase Chain Reaction) using pairs of primers (P1/P2) that allows the production of the 243 bp fragment located at the C-terminus of PPV-CP gene (Wetzel et al., 1991). PPV

immunocapture was trapped with PPV polyclonal antibodies (Bioreba, Switzerland). Qiagen one-step kit (Qiagen, Germany) was used for RT-PCR. The thermal cycling scheme used was the following: RT - 30 min at 50ºC, denaturation/RT inactivation - 2 min at 94ºC followed by 35 cycles: template denaturation - 30 s at 94ºC, primer annealing - 45 s at 61ºC and DNA elongation - 60 s at 72ºC. Following to the last cycle, amplified DNA was elongated for 10 min at 72ºC. An aliquot of the amplified products (10µl) was fractionated onto 1.5% agarose gel electrophoresis in 1 x TAE buffer. Bands were visualized by ethidium-bromide staining under UV light.

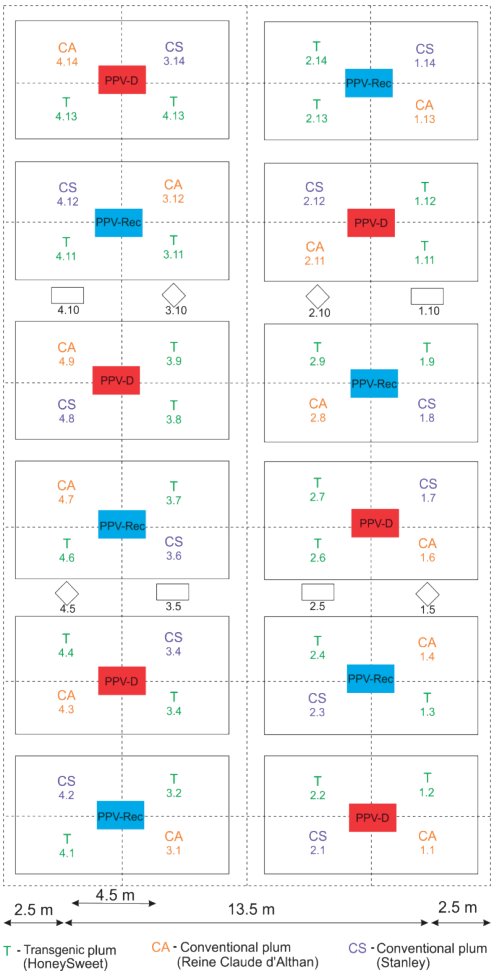

Figure 2. The design of field trial with transgenic and conventional plums (2013-2019)

Molecular discrimination of PPV strains. Infected trees were then analyzed for the presence of PPV-D and PPV-Rec strains. PPV strain discrimination was performed by RT-PCR targeting the C-terminus of CP genomic region using P1/PD and P1/PM primer sets (Olmos et al., 1997) and 6K1-CI genomic region of PPV using CIP-M/CIP-MR and CIP-D/CIP-DR primer pairs (Kamenova et al., 2011). Total RNA was extracted by RNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's instructions.

RESULTS AND DISCUSSIONS

PPV incidence in experimental plot. All artificially infected plants with D or Rec strains of PPV, used as inoculum sources, showed PPV typical symptoms since 2013, ensuring thus a high natural infection pressure of the virus for transgenic and conventional plums grown in the same experimental plot. After three years of study, typical symptoms of sharka has been observed on a tree of conventional plum, 'Stanley' cultivar. The presence of *Plum pox* virus was confirmed by laboratory tests (Figure 3). The first tree of 'Reine Claude d'Althan' cv. became infected with PPV after four years (2017). In the fifth year (2018), two trees belonging to 'Reine Claude d'Althan' and another one of 'Stanley' cvs. confirmed the presence of PPV by serological and molecular tests. In the last year of the study, additional three trees of 'Reine Claude d'Althan' and five of 'Stanley' cvs. proved to be infected by PPV.

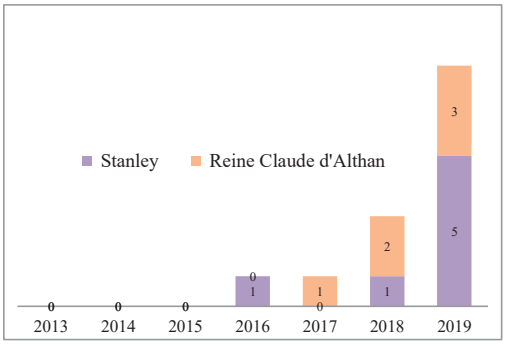

Figure 3. Newly conventional plum trees infected by *Plum pox* virus

In conventional plum, the number of infected trees with PPV has increased year by year approximately similar on both cultivars, 'Reine Claude d'Althan' and 'Stanley'.
Typical symptoms of PPV were expressed on leaves of both conventional plum cultivars and on the fruits of 'Reine Claude d'Althan' (Figure 4).

Figure 4. PPV symptoms on fruits ('Reine Claude d'Althan' plum cv.) - original

During six years of field testing, thirteen out of twenty-four conventional plums, seven trees of 'Stanley' and six of 'Reine Claude d'Althan' cultivars, became infected with PPV (Figure 5).

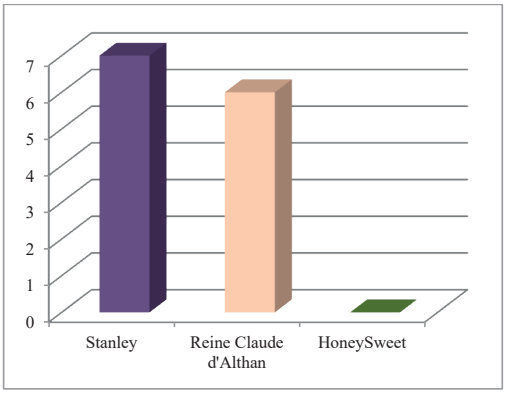

Figure 5. No. of trees infected by PPV (2013-2019)

The results revealed an annually increase of PPV infection inside the plot between 4% to 33% on conventional plum (Table 1), being similar with results obtained by Blazek et. al (2003) that state an over 4% newly PPV infected trees per year on young (three to five years old) plum trees.

Table 1. Newly PPV infected trees per year

Year/ Specification	Conventional plum		Transgenic plum	
	Infected/ total trees	%	Infected/ total trees	%
2013	0/24	0	0/24	0
2014	0/24	0	0/24	0
2015	0/24	0	0/24	0
2016	1/24	4.2	0/24	0
2017	1/24	4.2	0/24	0
2018	3/24	12.5	0/24	0
2019	8/24	33.3	0/24	0
Total	13/24	54.2	0/24	0

Therefore, the temporal spread of *Plum pox* virus revealed a continual evolution of infection in both conventional plum cultivars, from about 4% in 2016 to over 50% in 2019 (Figure 6).

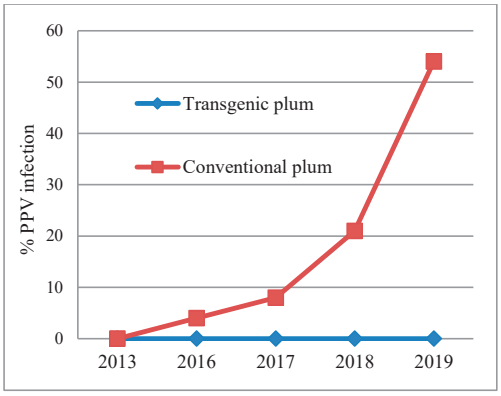

Figure 6. Temporal spread of PPV in experimental plot (2013-2019)

In the same conditions, no PPV symptoms appeared on 'HoneySweet' leaves or fruits, and no infection was found in transgenic plum trees by DAS-ELISA and IC-RT-PCR tests.

PPV strain discrimination. All PPV infected trees, belonging to conventional plum 'Reine Claude d'Althan' and 'Stanley' cultivars, were subjected to PPV discrimination. The results revelead that six out of thirteen trees proved to be infected by PPV-D strain, and the other seven, by PPV-Rec strain (Table 2).

Table 2. Strains discrimination of conventional plums infected with *Plum pox* virus

Plum cultivars/Code	PPV-D	PPV-Rec.
Stanley/1.7	+	-
Stanley/1.8	-	+
Stanley/2.1	+	-
Stanley/3.4	+	-
Stanley/3.6	-	+
Stanley/3.14	-	+
Stanley/4.12	-	+
Reine Claude d'Althan/1.1	+	-
Reine Claude d'Althan/2.8	-	+
Reine Claude d'Althan/2.11	+	-
Reine Claude d'Althan/3.12	-	+
Reine Claude d'Althan/4.3	+	-
Reine Claude d'Althan/4.7	-	+

Three trees belonging to Stanley cultivars were found infected by PPV-D, and four by PPV-Rec strains. Three of each PPV-D or PPV-Rec strain were detected in the six 'Reine Claude d'Althan' infected trees. Thus, the occurrence of the two strains in newly infected trees, along of six vegetative periods of study, revealed a quite similarity of D and Rec strains of PPV rate occurring in the two conventional plum cultivars. No mixed infection (D+Rec) of PPV was found by molecular tests in conventional plums.

Durability of resistance to PPV of transgenic plum. The virus infection in conventional plums suggest a high inoculum pressure of *Plum pox* virus for natural transmission by aphids inside the field plot. In situation in which incidence of PPV in conventional plums increased over 50%, 'HoneySweet' transgenic plum did not express PPV symptoms along six years of field trial. Moreover, no tree of transgenic plum 'HoneySweet' was found infected by PPV in both serological and molecular assays. These results coroborated with those obtained between 1996-2006 in Romania (Zagrai et al., 2011), Poland and Spain (Malinowski et al., 2006), and Czech Republic (Polak et al., 2008) demonstrated the durability and stability of 'HoneySweet' transgenic plum under high inoculum pressure of PPV over time.

CONCLUSIONS

Plum pox virus was rapidly spread by natural transmission in conventional plum cultivars 'Reine Claude d'Althan' and 'Stanley' (over 50% along 6 years).

A similar rate of D and Rec strains of PPV was occurred in the conventional plum cultivars.

In the same conditions, 'HoneySweet' transgenic plum remained uninfected under high *Plum pox* virus inoculum pressure confirming its durable resistance to natural *Plum pox* virus infection with D or Rec strains.

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INFLUENCE OF SOME ROOTSTOCKS ON THE GROWTH, YIELD AND FRUITS QUALITY AT THE 'JOJO' PLUM CULTIVAR

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Abstract

Mirobolan seedling (Prunus cerasifera) is the most popular rootstock for plums. Recently researches were begun about the vegetative and generative rootstocks suitability to conditions of Romania. In 2017-2019 periods the influence of six rootstocks on growth, yield and fruits quality at 'Jojo' cultivar was carried out at Genetics and Breeding Department, in Research Institute for Fruit Growing Pitesti, Romania. The trees were planted in the spring of 2015 at 4 x 3 m and comprised 3 trees/3 replications. As results of the investigations we found that: 'Mirobolan dawrf' and 'Mirodad 2' rootstocks induced a very low vigor; the smallest increase of trunk diameter was on the rootstocks 'Adaptabil' and 'Mirodad 2'; the 'Jojo' cv. yielded significantly better on 'Mirodad 1' and 'Mirodad 2' rootstocks; the largest fruits were obtained when the variety was grafted on 'Mirobolan dwarf', and the best taste was obtained in the case of the 'Mirodad 2' rootstock. The 'Jojo' cv. grafted on the 'Mirodad 2' rootstock was noted for its low vigor, high yielding capacity and fruits with high soluble solids content. It can also be observed that in the case of the 'Mirodad 2' rootstock the fruits weight was slightly lower due to the very high production.

Key words*: plum cultivar, rootstocks, growth, yield, fruits quality.*

INTRODUCTION

In Romania, plums are the main types of fruit crops, occupying an area of 65,910 hectares and producing 842,132 tons of fruit (Data FAO, 2020).

Like in the most European countries, in Romania plums were grown until the end of the last century in classical orchards (maximum 400 trees/ha) from which economically yields began in the 6th year after planting or later (Blazek and Pistekova, 2009; Butac et al., 2014, 2015; Kaufmane et al., 2007).

The most popular rootstock in these plum orchards was 'Myrobolan' seedling which is very vigorous, incompatible with some cultivars, causes late bearing and intensive suckering.

In the last 20 years have been established new plum orchards in an intensive system (1,250 trees/ha) with trees training form spindle bush, under fertirigation (Blazek and Pistekova, 2009, 2012; Botu et al., 2002; Butac et al., 2015, 2016; Hartman et al., 2007; Sosna, 2002; Zamfirescu et al., 2019).

Modern fruit growing, besides valuable cultivars, also require rootstocks suitable for a high density plum orchard (Sosna, 2002).

The objective of this paper is to study the influence of some rootstocks (obtained at RIFG Pitesti from the rootstocks breeding program) on the 'Jojo' cultivar. The 'Jojo' cv. was chosen for this study, because due to its resistance to Plum Pox Virus, it was extended in orchards from different European countries.

MATERIALS AND METHODS

The experimental field was established in 2015 at RIFG Pitesti - Maracineni, Genetic and Breeding Department. 'Jojo' cultivar grafted on six rootstocks were planted in a spacing of 4 m between the rows and 3 m between trees, according to the following experimental scheme: Factor A - cultivar, with one graduation (a1-'Jojo'); Factor B - rootstock, with six graduations (b1 -'Adaptabil'; b2 - 'BN4Kr'; b3 - 'Mirodad 1'; b4 - 'Mirodad 2'; b5 - 'Mirobolan dwarf'; b6 - 'Mirobolan'). The experiment was carried out in a randomized

block design, in 3 replications with 3 trees per plot.

In 2017-2019 periods, the following measurements were carried out: tree vigor expressed as trunk diameter at 30 cm above the soil in mm; fruit yield in kg/tree; mean fruit weight in g, soluble solids content with a digital refractometer in % Brix and titratable acidity in % or g/100 g fresh matter with the device Minititrator Hanna Instrument 84532.

The results of the experiment were analyzed statistically using Duncan's multiple range test at a 0.05% significance level.

RESULTS AND DISCUSSIONS

Rootstock effect on tree vigor, yield efficiency and fruit quality is well known (Webster, 2001; Botu et al., 2002, 2004; Hrotko et al., 2002).

Regarding ***tree vigor***, there are not significantly differences between combinations studied. However, the lowest tree vigor, expressed by the average trunk diameter was recorded when 'Jojo' cv. was grafted on 'Mirobolan dwarf' rootstock (58.39 mm - the average on three years) and the largest vigor was recorded on 'Mirobolan' rootstock (65.98 mm) (Table 1). Among the new registered rootstocks, 'Mirodad 2' also induced low vigor of the 'Jojo' cv. From previous studies it is known that the 'Adaptabil' rootstock induces high growth of the varieties grafted on it (Butac et al., 2016). It can be seen, from table 1, that the young plum trees on 'Adaptabil' grew more vigorously, but in the bearing age growth slightly decreased, and finally the increased growth was lower than in the other rootstocks (Table 1).

Table 1. Influence of the rootstocks on the vigor of the 'Jojo' cultivar

No.	Rootstock	Trunk diameter (mm)				Increased growth
		2017	2018	2019	Average	
1	Adaptabil	32.74 a	67.99 a	89.83 a	**63.52 a**	**57.09**
2	BN4Kr	31.65 a	64.98 a	94.43 a	**63.69 a**	**62.78**
3	Mirodad 1	33.01 a	66.96 a	96.40 a	**65.46 a**	**63.39**
4	Mirodad 2	32.93 a	65.81 a	92.33 a	**63.69 a**	**59.40**
5	Mirobolan dwarf	26.78 b	60.84 a	87.54 a	**58.39 a**	**60.76**
6	Mirobolan	32.33 a	66.99 a	98.62 a	**65.98 a**	**66.29**
	Average	**31.57**	**65.60**	**93.19**	**63.46**	

Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P>0.05).

Regarding the ***fruits yield***, it can see that there are significant differences between combinations studied (Table 2).

Fruits production was recorded only in 2018 and 2019. In 2017, due to late spring frosts recorded in the young fruit stage, production was totally compromised. The average yield per tree of 'Jojo' was the highest on 'Mirodad 2' and 'Mirodad 1' rootstocks (23.21 kg/tree and 18.78 kg/tree, respectively). The average yield per tree on 'BN4kr' and 'Adaptabil' was lower than on other rootstocks (6.92 kg/tree, respectively, 10.00 kg/tree).

Table 2. Influence of the rootstocks on the yield of the 'Jojo' cultivar

No.	Rootstock	Yield (kg/tree)		
		2018	2019	Average
1	Adaptabil	8.27 cd	11.72 d	**10.00 de**
2	BN4Kr	5.47 d	8.36 e	**6.92 e**
3	Mirodad 1	20.93 a	16.62 b	**18.78 b**
4	Mirodad 2	25.08 a	21.34 a	**23.21 a**
5	Mirobolan dwarf	14.26 b	14.22 c	**14.24 c**
6	Mirobolan	11.52 bc	11.39 d	**11.46 cd**
	Average	**14.26**	**13,95**	**14,11**

Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P>0.05).

It can also be seen that in 2018 fruits yield was higher than in 2019 (Table 2). The differences of yield among years can be explained not only by weather conditions, but also by a tendency

to biennially (Rubauskis et al., 2003), caused by too abundant cropping for 'Jojo' cv. (Kaufmane et al., 2007). 'Jojo' cv. had the regular yields on 'Mirodad 2', 'Mirobolan dwarf' and 'Mirobolan' rootstocks. However, the plums had lower biennially (Skrivele et al., 2000).

Fruit weight. Usually plum rootstocks have not significant effect on fruit weight (Hrotko et al., 2002; Sosna, 2002; Lanauskas, 2006). Statistical analysis of data on fruit weight, show that, between cultivar-rootstocks combinations were not significant differences. The largest fruits were obtained on 'Mirobolan dwarf' (55.07 g) and 'BN4Kr' (53.07 g) rootstocks (Table 3). Regarding 'Jojo'/'BN4Kr' combination the size of the fruits can be explained by the fact that the fruits yield was small in both years of study.

Table 3. Influence of the rootstocks on the fruits weight of the 'Jojo' cultivar

No.	Rootstock	Fruit weight (g)		
		2018	2019	Average
1	Adaptabil	50.30 b	51.27 b	**50.79 b**
2	BN4Kr	54.37 a	51.77 b	**53.07 ab**
3	Mirodad 1	51.80 ab	50.23 b	**51.02 b**
4	Mirodad 2	51.03 ab	50.13 b	**50.58 b**
5	Mirobolan dwarf	54.17 a	55.97 a	**55.07 a**
6	Mirobolan	49.17 b	57.00 a	**53.09 ab**
	Average	**51.81**	**52.73**	**52.27**

Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P>0.05).

Fruit soluble solids, acid content and soluble solids/acid content ratio (SS/AC).

The *soluble solids content* (SSC) gives the information about value of the fruits. In the present study the SSC varied from 17.63% in 'Jojo'/'Mirobolan' combination to 15.05% in 'Jojo'/'Mirodad 1' combination. After statistical analysis of fruit soluble solids content data, no significant differences between cultivar-rootstock combinations were found. The highest soluble solids content was recorded on 'Mirobolan' and 'Mirodad 2' rootstocks (17.63 % Brix, respectively 16.57 % Brix) and the lowest on 'Mirodad 1' (15.05 % Brix) (Table 4). The same results were obtained in the same experience in previous years (Zamfirescu et al., 2019). In conclusion, soluble solids content were not affected by rootstock. The same results were reported by Sitarek and co-workers (2007) and also by Milosevic and Milosevic (2012).

Organic acids of fruits have a good effect on stomach and the intestine tract and they also determine the taste qualities of fruits (Bozhkova, 2014). As a whole, the 'Jojo' cv. has a low acids content varying from 0.39% on 'Mirobolan' rootstock to 0.65% on 'Mirodad 1' rootstock. The differences between cultivar-rootstock combinations regarding acids content were statically insignificant, which is in agreement with a previous study of plum rootstock (Sitarek et al., 2007). In our study it can be seen that the 'Jojo'/'Adaptabil' combination which has higher soluble solids content had a higher percentage of titratable acids. The 'Jojo'/'Mirobolan' combination which has higher soluble solids content had a lower percentage of titratable acids. On the other hand, it can also be seen that the 'Jojo' cv. on the 'Mirodad 1' rootstock has low soluble solids content, but a high percentage of acids (Table 4).

For plum, higher *soluble solids/acid content* ratio (SS/AC) is correlated with higher eating quality (Crisosto et al., 2007). Effects of interaction on the SS/AC ratio were not significant. There was a significantly higher SS/AC ratio on 'Mirobolan' rootstock and lower SS/AC ratio on 'Mirodad 1' rootstock (Table 4). A high SS/AC ratio means high soluble solids by low acidity (Milosevic and Milosevic, 2012).

Table 4. Influence of the rootstocks on the fruits soluble solids content and titratable acidity of the 'Jojo' cultivar

No.	Rootstock	Fruits soluble solids content (% Brix)			Titratable acidity (%)			Soluble solids: Titratable acidity
		2018	2019	Average	2018	2019	Average	
1	Adaptabil	16.53 a	15.20 c	**15.86 a**	0.38 b	0.87 a	**0.63 ab**	**25.17**
2	BN4Kr	15.47 ab	15.50 c	**15.48 a**	0.30 d	0.61 b	**0.45 ab**	**34.40**
3	Mirodad 1	13.70 bc	16.40 bc	**15.05 a**	0.44 a	0.86 a	**0.65 a**	**23.15**
4	Mirodad 2	14.73 ab	18.40 ab	**16.57 a**	0.35 c	0.61 b	**0.48 ab**	**34.52**
5	Mirobolan dwarf	12.80 c	18.13 ab	**15.47 a**	0.28 e	0.60 b	**0.44 ab**	**35.16**
6	Mirobolan	15.33 ab	19.93 a	**17.63 a**	0.40 b	0.38 c	**0.39 b**	**45.20**
	Average	**14.76**	**17.26**	**16.01**	**0.36**	**0.66**	**0.51**	**32.93**

Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P>0.05).

CONCLUSIONS

As results of the investigations we found that:
- 'Mirobolan dwarf' and 'Mirodad 2' rootstocks induced a very low vigour; the smallest increase of trunk diameter was on the rootstocks 'Adaptabil' and 'Mirodad 2';
- the 'Jojo' cv. yielded significantly better on 'Mirodad 1' and 'Mirodad 2' rootstocks;
- the largest fruits were obtained when the variety was grafted on 'Mirobolan dwarf', and the best taste was obtained in the case of the 'Mirodad 2' rootstock.
- The 'Jojo' cv. grafted on the 'Mirodad 2' rootstock was noted for its low vigor, high yielding capacity and fruits with high soluble solids content. It can also be observed that in the case of the 'Mirodad 2' rootstock the fruits weight was slightly lower due to the very high production.

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INFLUENCE OF MARC USED AS AN ORGANIC FERTILIZER ON BIOCHEMICAL AND BIOMETRIC CHARACTERISTICS OF THE GRAPES

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Abstract

Organic fertilizer obtained from marc is a green technology that converts organic wastes into rich nutrient organic fertilizer available for plant. This work shows how to optimize the fertilization technologies of grapes and their influence on the evolution of some biometric characteristics (number of fruit/plant, the average mass of the fruit, mean weight for 100 grape beans, production) and biochemical grape indicators (total acidity, total sugar content, anthocyanins and polyphenols content). The experimental factors underlying it the organization scheme are: A Factor - grapes varieties: 'Feteasca Neagra', 'Pinot Noir', 'Merlot'; B factor - quantity of marc used as an organic fertilizer, with three graduations: 3, 4, respectively 5 kg marc/plant. In all three varieties studied, the dose of 5 kg marc/plant induced a higher production and ensured statistically compared to the doses of 4 and 3 kg/plant, respectively. On the average of the studied cultivars, the dose of 5 kg marc/plant induced a higher production, which was with 0.53 kg/plant higher compared to the treatment by 3 kg marc/plant. That meant a harvest increase of over 2.4 tons per hectare.

Key words: *marc, organic fertilizer, weight fruit, production, fruit quality.*

INTRODUCTION

"Organic Agriculture" is an sustainable alternative to conventional system as it aids in environmental protection, improved food quality and human health (S. Suthar, 2009; R. Pratap, 2011). It restricts use of agro-chemicals and genetically modified organisms; rather focuses on other agricultural practices like organic manure (compost, vermicompost, green manures, animal manures), crop rotations and biological control of pests to maintain productivity. Increasing awareness on consumers has uplifted the demand of organic products in global scenario. However, the organic supply has not been competent to meet the demand. Therefore, farmers are encouraged to move into organic farming.

Organic marc, a by-product of the wine industry, is another category of less used organic fertilizer, despite the low price and abundance in wine farms. It is a heterogeneous product, whose composition and texture vary according to the treatments it undergoes: recovery of alcohol, anthocyanins, etc. On average, organic marc contains between 14-25% rachis; 48-69% skins and 14-27% seeds (Bejan C., 2008).

Product quality determined by its indicators, such as: soluble dry matter and acidity, is influenced by the intake of water and fertilizers (Sumedrea, 2017).

Bio-fertilizers are essential components of organic farming the preparations contain live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic micro-organisms. They are used for application objective to seed, soil or composting areas, with the increasing of the number of such micro-organisms and accelerate those microbial processes which augment the availability of nutrients that can be easily assimilated by plants (Mishra, 2013). Organic agriculture controls and intensifies the natural processes in order to balance the yield, with respect of ecosystems, including human health. It promotes the quality, not the quantity.

Development of durable agriculture takes in account the multifunctional role that it must have, in respect of the following principles: to use production methods capable to provide qualitative products by protecting the environment; to preserve and renew the natural resources; to promote those technologies friendly with the environment.

Researches all over the world proved that the ecological products obtained by non-polluting technologies have a better quality comparing with those obtained by conventional technologies, which imply a series of chemical products risky for human health.
The present experiment was, therefore, conducted to evaluate influence of the organic fertilizer obtained marc grape of biometric characteristics (number of fruit/plant, the average mass of the fruit, mean weight for 100 grape beans, production) and biochemical grape indicators (total titrable acidity, total sugar content, anthocyanins and polyphenols content).

MATERIALS AND METHODS

The experimental factors studied were: Factor A - grapes cultivars: 'Feteasca Neagra', 'Pinot Noir', 'Merlot', grafted on the SO 4-4 and factor B - dose of marc used as an organic fertilizer, with three graduations: 3 kg/plant, 4 kg/plant, 5 kg/plant. The experience presents average data for two years from the application of marc (2018-2019). The experience took place in a plantation which was established in 1997 at the National Research and Development Institute for Biotechnology in Horticulture Stefanesti, with the planting distance of 2.20 x 1 m. The process of separating the marc is very important, because each component has a specific composition and post-processing technology. Thus, the marc resulting as a by-product of pressing grapes, which consisted of bunches, skins, seeds and residues of must or wine not extracted by pressing, was dried before application, using a microwave equipment -MW-6kW.
The set of biometrical and chemical analyses consisted in the following indicators: number of fruit/plant, total titrable acidity (TTA), total sugar content (TSC), anthocyanins and polyphenols. The total acidity was determined by the titrimetric determination method. The total sugar content was determined by the Fehling-Soxlet titrimetric method. Total polyphenol content was measured by colorimetric Folin-Ciocalteu method. Total anthocyanins content was measured by spectrophotometric absorbance at wave length λ = 540 nm (adapted method after Bărăscu et al., 2016). The extracts were filtered under vacuum and completed up to 50 ml volume. The results were calculated using the formula: Total anthocyanins = DO540 x F, where DO540 is absorbance at wavelength λ = 540 nm and factor F = 11.16. The total anthocyanins content was expressed in mg/100 g in fresh weight.
For the statistical interpretation of the results, the data were included in an Excel database and then statistically analyzed with the SPSS 14.0 program, which uses the Duncan test (multiple t test) for a 5% level of significance.

RESULTS AND DISCUTIONS

Both the number of grapes per plant and their weight are basic indicators used in viticulture to express the productive potential of the varieties. According to the data from Table 1, there are positive correlations between the biometric indicators: number of grapes per plant, average mass of grapes and production per plant.

Table 1. Correlation matrix of the biometric indicators

		Number of grapes/plant	Fruit weight (g)	Production
Number of grapes/plant	Pearson Correlation	1	-,733(**)	,458(**)
	Sig. (2-tailed)		,000	,000
	N	81	81	81
Fruit weight (g)	Pearson Correlation	-,733(**)	1	,220(*)
	Sig. (2-tailed)	,000		,049
	N	81	81	81
Production	Pearson Correlation	,458(**)	,220(*)	1
	Sig. (2-tailed)	,000	,049	
	N	81	81	81

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

As a result, the following coefficients in correlation with the production of grapes on the plant were recorded: r = 0.458* for the number of grapes per plant, r = 0.220* for the average mass of grapes between fruit production. Further, the influence between the two experimental factors (variety and dose of marc) on the biometric and biochemical quality indicators of the grapes was analyzed. The quantity of the harvest depends on the size of the grapes and the number of inflorescences, so that cultivars with the same
percentage of fertile leaves or with the same fertility coefficient give different grape harvests in terms of production level (Bădulescu, 2013).
Analyzing the interactions between the two experimental factors, it is noted that, in terms of the number of grapes/plant, fertilization

doses induced significant differences only at the 'Merlot' cultivar. It registered the highest number of grapes per plant at the fertilization dose with 5 kg marc/plant (33.11 grapes/plant), compared to 27.67, respectively 27.55, in the case of 4 kg marc/plant (Figure 1). On the average of the cultivars, the best results on the number of grapes per plant were obtained at fertilization with 5 kg of marc/plant, the average number of grapes per plant reaching 21.85 grapes/plant, exceeding the sample treated with 3 kg of organic fertilizer with 3.14 grapes/plant (18.71 grapes/plant at dose of 3 kg of marc/plant).

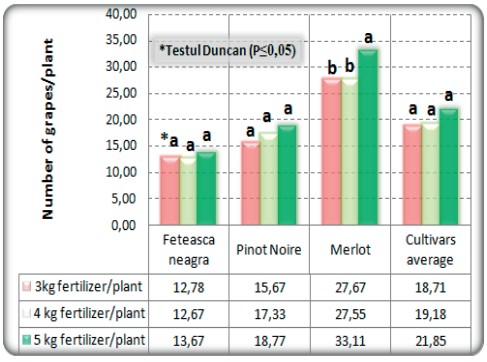

Figure 1. Influence of the marc dose on the number of grapes depending on the cultivars

In the 'Feteasca Neagra' and 'Pinot Noire' cultivars, the organic fertilization dose led to significant differences in the average fruit mass, thus, the highest mass in the case of the two mentioned cultivars was registered at the organic fertilization dose with 5 kg marc/ plant. In the case of the 'Merlot' cultivar, the organic fertilization did not induce significant changes in the average mass of the grapes (Figure 2).

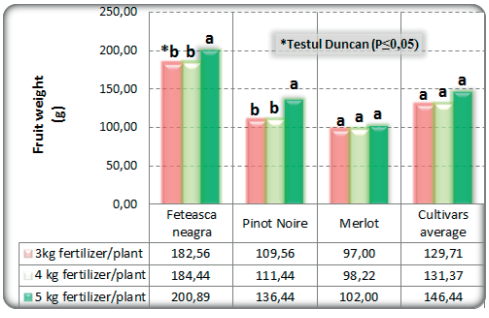

Figure 2. Influence of the marc dose on the fruit weight of grapes depending on the cultivar

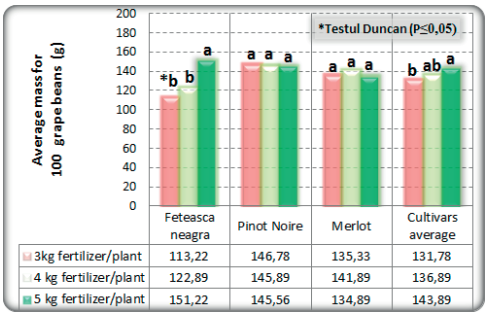

Figure 3. Influence of the marc dose on the mass of 100 grape beans depending on the cultivar

On the average of the cultivars (Figure 3), the highest average mass of 100 grape beans were obtained at fertilization with 5 kg of marc per plant, which reaching 143.89 g, value that exceeding the sample treated with 3 kg of organic fertilizer with 12.11 g (131.78 g).

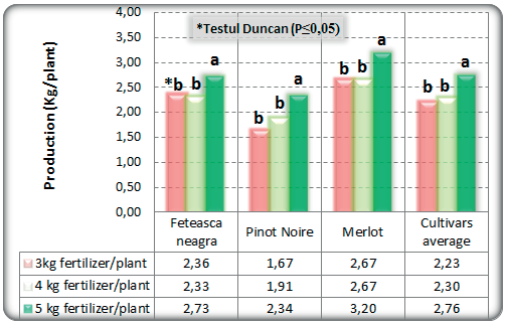

Figure 4. Influence of the fertilizer dose on the production depending on the cultivar

Grape production is the basic indicator in establishing economy and profitability in the application of a measure or a whole complex of agrotechnical measures (Badulescu, 2013).
Organic fertilization dose induced a significant increase in grape/plant production.
Thus, on the average of the cultivars, the highest production was registered in the case of organic fertilization dose with 5 kg marc/ plant (2.76 kg/plant), compared to the treatment with the lower doses of organic fertilizer, which recorded 2.30, respectively 2.23 kg/plant) (Figure 4). It was observed that, for all three varieties studied, on the average of the two years of study, dose of 5 kg marc/plant induced a higher production and these are ensured statistically compared to the doses of 4 and 3 kg/plant, respectively. The highest production

was recorded for the 'Merlot' cultivar, at all dose of organic fertilization, and the smallest to the 'Pinot Noire' cultivar.

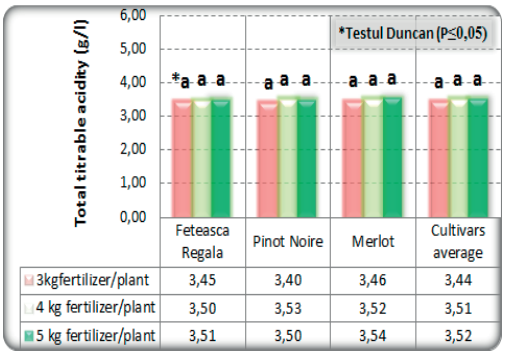

Figure 5. Influence of the marc dose on the total acidity of grapes depending on the cultivar

If we analyze the influence on the total acidity of the grapes depending on the fertilizer dose, the effect on the TTA wasn't statistically different for any variety. As compared to the other varieties, 'Merlot' displayed the highest value of TTA (between 3.46 of the doze with 3 kg/plant and 3.54 g/l in the case of the fertilization dose with 5 kg, but this was entirely due to the genetic characteristics, not to the fertilization (Figure 5).

Fruits accumulate starch in the early stages of maturation, which are subsequently hydrolyzed in sugars at consumption maturity (Magein and Leurquin, 2000; Sumedrea, 2017).

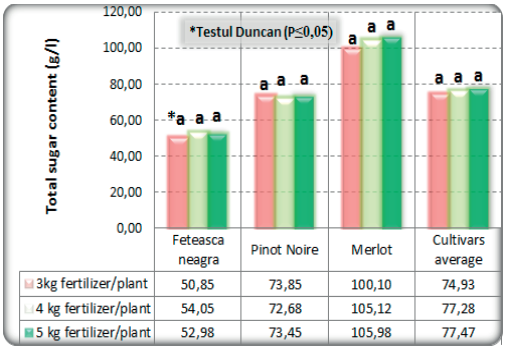

Figure 6. Influence of the fertilizer dose on the total sugar content (g/l) of grapes depending on the cultivar

At all the 3 applied doses of fertilizer, the level of sugar accumulation in the grapes, didn't recorded significantly different for any of the three cultivars studied (Figure 6). The results also show that among the three cultivars studied, 'Merlot' accumulated the highest sugar content in the grapes, at all three doses of fertilization, but this is a trait of the cultivar and isn't dependent on fertilization.

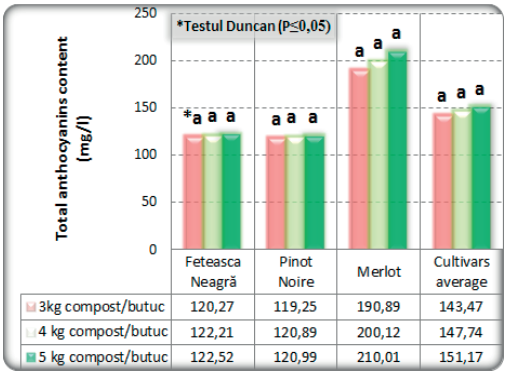

Figure 7. Influence of the marc dose on the total anthocyanins content (mg/l) of grapes depending on the cultivar

At all the three applied doses of marc, the anthocyanin content accumulation in the skins, was not significantly different for any of the three cultivars studied (Figure 7). 'Merlot' cultivar recorded the highest anthocyanins concentration in the skins, at the all three doses of marc, but this is a trait of the cultivar and is not dependent on fertilization.

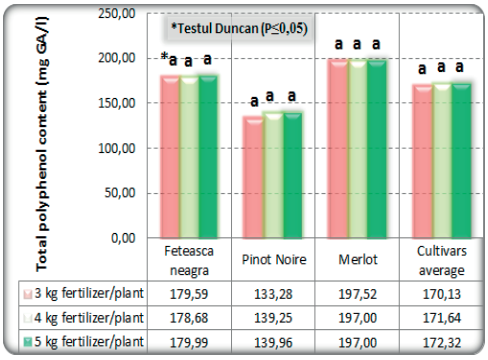

Figure 8. Influence of the marc dose on the total polyphenol content (mg/l) grapes depending on the cultivar

'Merlot' and 'Feteasca Neagra' cultivars registered the higher levels of polyphenol content than the 'Pinot Noire' variety, but this is a characteristic of the cultivar and does not depend on the dose of marc applied. Research

in the field of phenols extracted from plants (Schaffer et al., 2005) shows that phenols depend quantitatively and qualitatively on genetic information (species, variety), environment and geographical conditions. Climate, season, light, temperature, maturation period strongly influence the synthesis of phenols in plants (Aherne and O'Brian, 2002). On the average of the cultivars, the marc dose didn't determines significant changes of the grape content in polyphenols (Figure 8).

CONCLUSIONS

The different doses of fertilization led to significant differences between the basic indicators used in viticulture for the characterization of fruiting processes and finally on grape production.

In all three varieties studied, the dose of 5 kg marc/plant induced a higher production and ensured statistically compared to the doses of 4 and 3 kg/plant, respectively. Thus, on the average of the studied cultivars, the dose of 5 kg marc/plant induced a higher production, which was with 0.53 kg/plant higher compared to the treatment by 3 kg marc/plant. That meant a harvest increase of over 2.4 tons per hectare.

The growing up of the yield with the increase of the quantity of marc applied was possible due to the increase of the average number of grapes/plant and respectively of the average weight of the grapes. Thus, on the average of the studied varieties, the average number of grapes per plant was higher with 3.14 and the average weight of 100 grape beans increased with 12.11 g.

There is therefore a positive correlation between the simple correlation coefficients between fruit production and quantitative indicators, these being: r = 0.458** for the number of grapes per plant, r = 0.220* for the weight of a grape).

Merlot cultivar recorded the highest content in total sugar, anthocyanins and polyphenols, but these were not influenced by organic fertilization with marc, being rather a characteristic of the variety.

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THE INFLUENCE OF THE CLIMATIC CONDITIONS ON THE WINTER BUDS VIABILITY AND FERTILITY OF THE VINE VARIETIES FROM ISTRIA VITICOL CENTER

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Abstract

The viability and fertility of the vine varieties from the plantations of Istria viticol Center were studied, according to the climatic conditions in the period of years 2017-2018. In the year 2017, the winter began gentle, with high temperatures for this time of year, in November was between +1 up to +12.3°C, and in December between minus 2.3°C up to +12.0°C. In the first half of January 2018, the temperature was positive (0-11°C), and in the second half, this decreased to a minimum of minus 20°C on January 28. In February 2018, the temperature was extremely low, between minus 22°C on February 2-nd and minus 20.5°C on February 10. Before the frost, from January 20, in the laboratory of Horticultural faculty from Ovidius University, were analyzed the fruit shoots and found normal values of viability and fertility of winter buds, values characteristic of each analyzed vine variety, but shortly after freezing, were reanalyzed the shoots from the same varieties and we have seen that these have suffered lost of winter buds, the percentage being between 27-56.25 %. For this reason in spring, when the vine is cut, the lost buds must be properly compensated, for the production to be ensured.

Key words*: fertility, fruit shoot, viability, winter bud.*

INTRODUCTION

Any grape variety, manifests its economic usefulness through the features of fertility and productivity. Fertility, is the vine ability to form fruiting organs, as the first step in the formation of grape production (Oslobeanu et al., 1980; Olteanu, 2000). The fertility is determined in each spring, for practical purposes, before vine cutting, to establish the fruit load of the buds, the length of the fruit elements and allow to forecast the future grape harvest (Dobrei, 2003; Ion, 2009). The knowledge of the vines fruiting capacity, by buds categories and their positions on the annual shoot, allow us to take technological measures, which will increase the potential of fruiting of the vines. Climate changes have profound implications on the winter buds viability and fertility, on the grapes productions, high quality of wines and implicitly on the maintenance technologies of the vine plantations.

Generally, in the vineyard Istria-Babadag, the climatic factors levels develop according to the temperate-continental climate which presenting certain particularities related to the geographical position (located in the central-eastern area of Dobrogea, bordering of the Black Sea and the lake Razelm and near the Danube Delta) of the territory. Before the vine's fruit cutting the vinegrower checks the resitence of the annual vine shoots over the winter and the viability and fertility of the winter buds, to determine the vine cuting system. The viability of the winter buds and especially of the main bud, can be affected by temperatures of minus 20ºC - minus 25ºC, which exceeds the variety's resistance limits.

The buds viability can also be affected by temperatures of minus 8ºC - minus 10ºC, that appear after days with positive temperatures.

The vinegrower is interested of the winter buds, because they will generate fertile shoots usually (Oslobeanu et al., 1980; Dejeu, 2004; Bucur, 2011).

The study was carried out in the viticol period of 2017-2018 years in Istria viticol centre, from Istria-Babadag vineyard.

MATERIALS AND METHODS

The air temperature description (average, maximum, minimum) from November 2017 to Octomber 2018, used the data recorded with the own Wather Master 2000 weather station.
The viability and fertility evolution of the winter buds were studied to the following vine varieties existing in Istria viticol centre (from Istria-Babadag wineyard): 'Chardonnay', 'Muscat Ottonel', 'Sauvignon Blanc', 'Riesling Italian', 'Cabernet Sauvignon', 'Merlot' and 'Feteasca Neagra'.
Also in the plantation, it was verified how the annual shoots passed over the winter. The health of the annual shoots were checked by longitudinal cutting of the internode and node to see the state of diaphragm and leading tissues.
The control of the winter buds viability was carried out by the method of longitudinal cutting as follows: - in plantation by cutting them directly on the annual shoot; - in laboratory by reading sections under microscope, establishing in the same time the buds fertility.
Before the frost from January 20, three annual shoots in length of 12-15 winter buds from each vine variety were collected.
After the shoots have been cut, they are labeled (date, variety, vine plot, slope position) and brought in laboratory and kept for 2-3 days in water (Figure 1) for the winter buds enlarge their size (Irimia et al, 2007).

Figure 1. Vine annual shoots in water

To avoid confusion refering the position of the winter buds on the annual shoot, this was divided into three intervals: 1-4, 5-8 and 9-12 winter buds, in order, from the base of the shoot and there were extracted one by one and put on the table. Each winter bud was fixed in the Sambucus marrow so as not to be crushed (Figure 2). Using a new blade they were longitudinally sectioned in very thin sections, collected in a Petri dish with distilled water to prevent sections drying and than these were analyzed under the microscop to determine their viability and fertility.

Figure 2. Winter bud in the Sambucus marrow

The data, obtained from this study were analysed, interpreted and included in the tables and figures from the next chapter.

RESULTS AND DISCUSSIONS

The results regarding the climatic conditions are specific to each viticol year (Table 1). Analysing the data presented in table 1, which highlights the termal regim, can be appreciated that the period November 2017-October 2018, compared to the multiannual average, was a capricious one. In the year 2017, the winter began gentle, with high temperatures for this time of the year, in November was between +1 up to +12.3°C, and in December between minus 2.3°C up to +12.0°C. In the first half of January 2018, the temperature was positive (0-11°C), and in the second half, this decreased to a minimum of minus 20°C on January 28. In February 2018, the temperature was extremely low, between minus 22°C on February 2-nd and minus 20.5°C on February 10.
The evolution of negative minimum temperatures recorded in January continued to decrease and remained at negative values almost all February 2018.

Table 1. The thermal regim in the viticol year 2017-2018

Year	Month	Average monthly temperature		Absolute max. temp	Absolute min. temp	Σ of temperature degrees					
						Global		Active		Effective	
		Normal	2017-2018			Normal	2017-2018	Normal	2017-2018	Normal	2017-2018
2017	XI	7.2	5.5	16.0	-2.0	228.1	159.8	134.9	46.1	34.9	6.1
2017	XII	2.3	5.0	17.0	-4.2	255.1	152.7	24.2	12.0	3.4	2.0
2018	I	0.5	0.1	11.0	-20.0	4.1	3.2	2.6	0.0	0.6	0.0
2018	II	1.3	-2.2	9.0	-22.0	62.6	-66.3	14.7	0.0	2.7	0.0
2018	III	4.2	7.2	23.0	-3.0	125.6	218.3	41.3	128.2	9.3	28.2
2018	IV	10.5	14.6	28.6	-2.0	369.7	436.2	219.8	402.5	53.8	152.5
2018	V	16.2	19.7	31.0	8.4	513.7	612.7	513.7	612.7	203.7	302.7
2018	VI	20.4	21.3	37.2	12.6	620.1	754.7	620.1	754.7	328.1	454.7
2018	VII	22.6	28.1	38.2	13.1	726.3	868.6	726.3	868.6	416.3	558.6
2018	VIII	22.6	26.2	39.7	10.7	671.0	808.9	671.0	808.9	361.0	498.9
2018	IX	17.6	20.6	36.0	7.8	521.2	619.7	552.7	619.7	252.7	319.7
2018	X	12.0	16.7	30.0	0.9	373.1	506.9	311.4	484.0	81.4	204,0
Σ/year		126.9	162.8			4,470.6	5,075.6	3,832.7	4,737.4	1,747.9	2,527.4
Average/month		10.57	13.56								

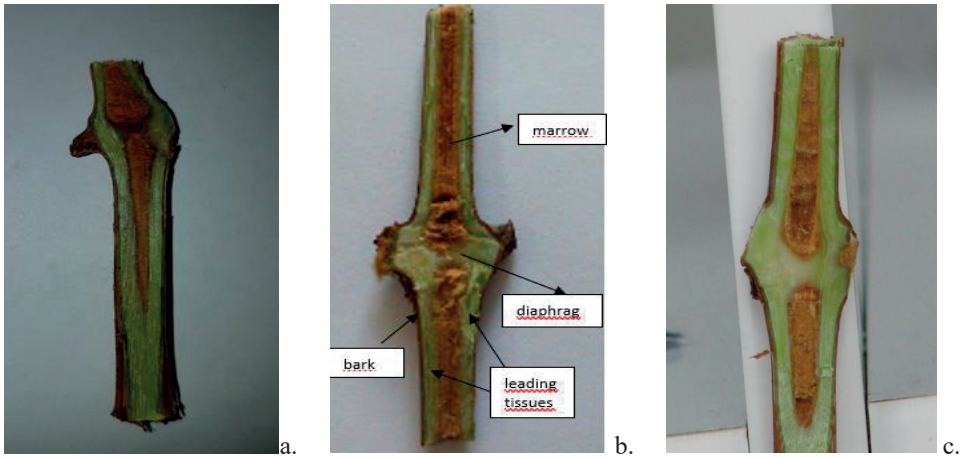
The beginning of March 2018, is a normal one for this period of the year, with positive daily average temperatures wich were not dangerous to the vines development.

Starting with March 15, temperatures between 10ºC up to 14.6ºC were recorded. Compared to the normal, amount of temperature degrees in March is with 92.7ºC higher, which favored the entry into the vegetation of the vine to earlier.

The sum of the temperature degrees for the period November 2017 - October 2018 was 5,075.6ºC compared to 4,470. 6ºC, with 605ºC more than normal. The maximum temperature recorded during this period was 39.7ºC (in August), and the minimum temperature recorded during the same period was minus 22ºC (in February).

The active temperature was 904.7ºC higher than the normal temperature, totaling 4,737.4ºC, compared to the normal temperature of 3,832.7ºC. And the effective temperature of these months amounted to 2,527.4ºC, compared to 1,747.9ºC to normal, with 779.5 more than this.

- Within the Istria viticol centre it was possible to analyze the annual shoots before freezing on January 20, 2018 and it was found that these were healthy, not frozen (Figure 3). The marrow brown colored and the bright green color of the diaphragm and leading tissues show us that the annual shoots did not freeze and were healthy.



a. b. c.

Figure 3. The annual shoots healthy- longitudinal section:
a - 'Merlot'; b -'Sauvignon Blanc'; c -'Riesling Italian' (original)

- The control of the winter buds viability in plantation

First, the winter buds viability was tested directly on the annual shoots in plantation and the results were that all of them were alive (Figure 4). Remember that in autumn, after the leaves fall, the axillary buds complex becomes winter bud, which is formed from: a main bud, 2 secondary buds (or replacement buds) and 2-4 tertiary buds, all protected from fluffy and scaly formations (Figure 4). All the above mentioned buds are fixed in a common tissue called meristematic zone or common generating area, which exist at the level of each node.

- The control of the winter buds viability and fertility in laboratory

This consisted in reading under microscope the sections made from the winter buds and the result was that the winter buds viability and fertility were characteristic for each analized variety (Figures 5, 6, 7, 8 and Table 2).

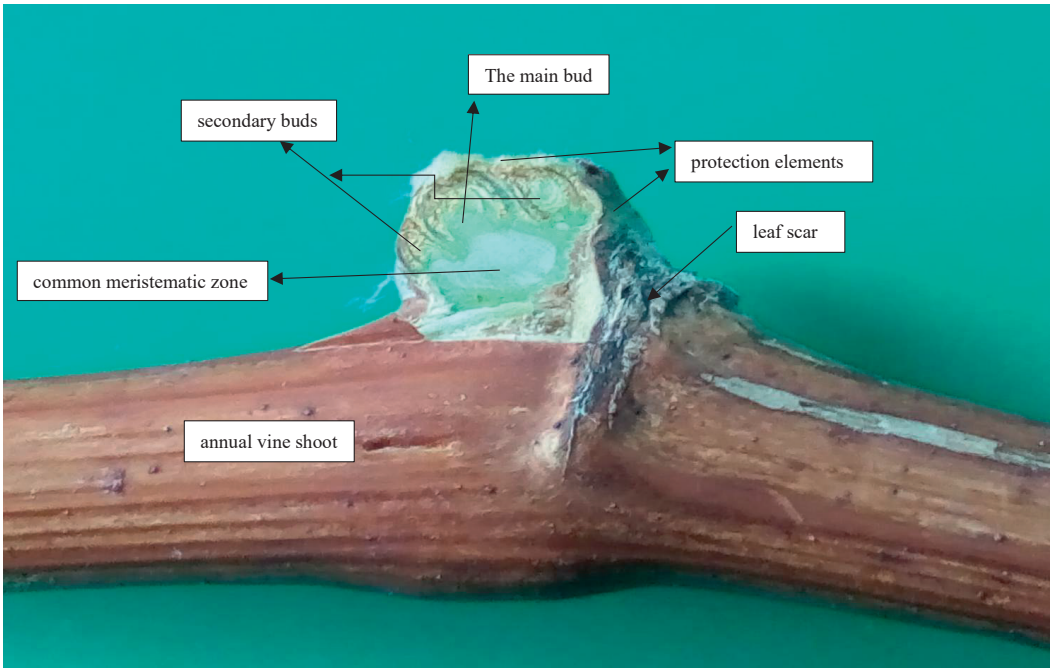


Figure 4. Viable winter buds on the annual shoot of 'Riesling Italian' variety (original)

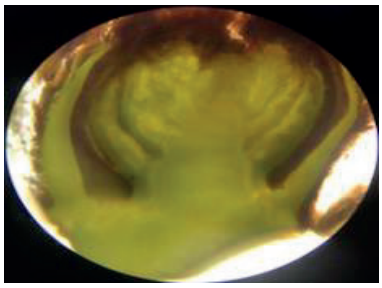


Figure 5. Main bud of 'Chardonnay' variety (original)

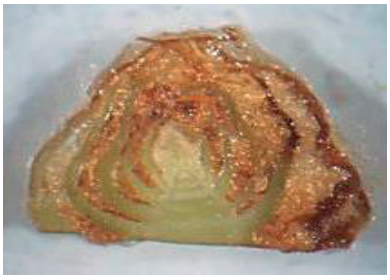


Figure 6. Main bud of 'Riesling Italian' variety (original)

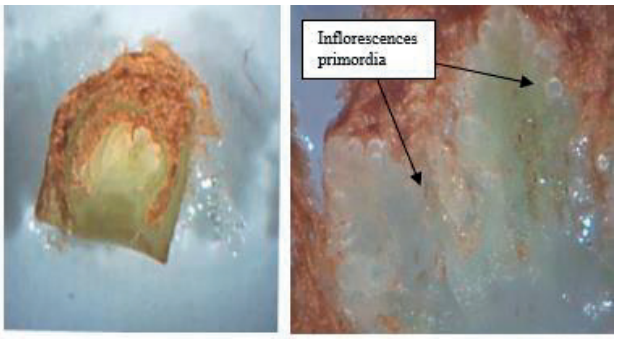


Figure 7. Main bud and inflorescences primordia of 'Merlot' variety (original)

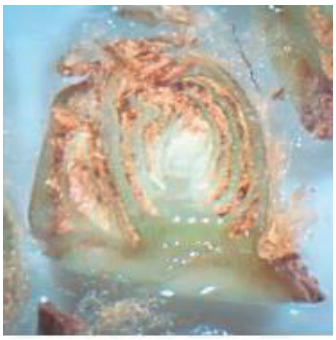


Figure 8. Main and secondary buds of 'Fetească Neagră' variety (original)

In the case of very severe winters, with very low temperatures, all buds (main, secondary and tertiary) of the winter bud are examined, in order to determine the possibilities of recovering the productive and vegetative potential of the vine variety. The losts of buds are expressed in percentages (% dead buds), taking into account the distribution of losses along the annual shoot (base, middle, tip), so that at cutting to reserve part of the shoot with the maximum percentage of viable winter buds (Dejeu, 2004) .

Generally, the best viability is recorded at the base of the annual shoot, where the buds are less developed, but more resistant to frost (Oslobeanu et al., 1980).

Table 2. Winter buds viability and fertility values in 2018 before freezing

No crt	Vine variety	Viability (%)/Fertility (%) in the interval winter buds:			X Average viability/ fertility	Normal fertility values %
		1-4	5-8	9-12		
Samples collected in 20.01.2018						
1	'Chardonnay'	41/32	94/68	56/57	64/52	50-60
2	'Muscat Ottonel'	74/68	100/82	100/83	91/78	75-83
3	'Riesling Italian'	75/65	80/74	45/68	79/69	80
4	'Sauvignon Blanc'	51/68	92/100	74/66	72/78	60-70
5	'Cabernet Sauvignon'	77/59	82/75	74/82	78/72	40-60
6	'Merlot'	84/93	83/92	82/49	83/78	60-80
7	'Fetească Neagră'	84/68	100/67	91/82	92/72	70-80

According to the data from the Table 2, the lowest viability/fertility was recorded at ‚Chardonnay' variety, on the interval 1-4 from the annual shoot base. The average fertility of the analyzed varieties was close to the normal value of the variety, sometimes exceeding it (eg. 'Sauvignon Blanc' variety).

After recording the absolute minimum temperatures harmful to the vines, the winter buds were reanalyzed, and a decrease of viability and fertility could be observed.

All the vine varieties in Istria viticol centre have suffered winter buds losts (Table 3).

In section the dead bud, analyzed under microscope, has a brownish-black color. Sometimes only the main bud of the winter buds is dead and the secondary are viable (Figure 9).

Other times, the main and secondary buds are dead and only the tertiary ones remain viable, other times the whole winter buds complex is dead (Figure 10).

Due to the small size and poor development of the winter buds, the tertiary buds are difficult to put in evidence in the sections.

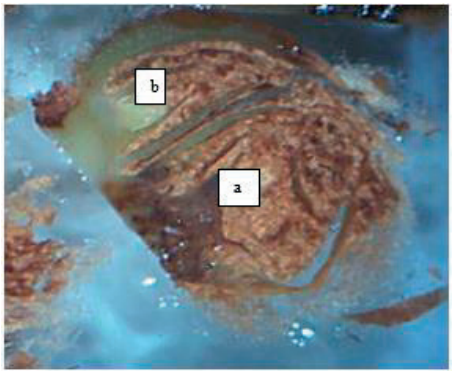


Figure 9. 'Fetească Neagră', main bud dead (a) and the secondary viable (b) (original)

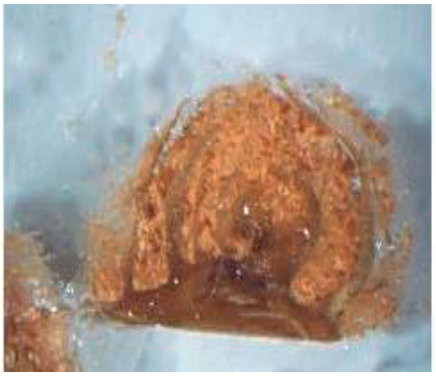


Figure 10. 'Cabernet Sauvignon'-All the buds are dead (original)

Table 3. Winter buds viability and fertility values in 2018 after freezing

No crt	Vine variety	Viability (%)/Fertility (%) in the interval winter buds:			X Average viability/ fertility	Normal fertility values %
		1-4	5-8	9-12		
Samples collected in 07.02.2018						
1	'Chardonnay'	7/0	43/52	57/31	36/28	50-60
2	'Muscat Ottonel'	9/7	41/44	24/24	25/25	75-83
3	'Riesling Italian'	58/52	68/46	36/41	54/46	80
4	'Sauvignon'	51/59	24/24	34/33	36/39	60-70
5	'Cabernet Sauvignon'	16/7	34/34	24/15	25/19	40-60
6	'Merlot'	15/17	34/16	66/24	38/19	60-80
7	'Fetească Neagră'	51/51	34/15	66/66	50/44	70-80

For all the analyzed varieties the viability and fertility losts of the winter buds were calculated and it was found that: viability losts were between 27.47-68.35% and fertility losts were between 24.35-66.66% (Table 4). For this reason in spring, when the vine is cut, the lost buds must be properly compensated, to ensure the production.

Table 4. The viability and fertility losses of the winter buds in Istria viticol centre in viticol year 2018-2019

No crt	Vine varieties	Average viability/ fertility before freezing	Average viability/ fertility after freezing	% losses	
				Viability	Fertility
1	'Chardonnay'	64/52	36/28	56.25	53.84
2	'Muscat Ottonel'	91/78	25/25	27.47	32.05
3	'Riesling Italian'	79/69	54/46	68,35	66,66
4	'Sauvignon'	72/78	36/39	50.0	50.0
5	'Cabernet Sauvignon'	78/72	25/19	32.05	26.38
6	'Merlot'	83/78	38/19	46.98	24.35
7	'Fetească Neagră'	92/72	50/44	54.43	61.11

CONCLUSIONS

As a biological material for the study of viability and fertility, during the viticol year 2018-2019, the vine varieties were used: 'Chardonnay', 'Sauvignon Blanc', 'Muscat Ottonel', 'Riesling Italian', 'Cabernet Sauvignon', 'Merlot', and 'Fetească Neagră', existing in the plantations from Istria viticol centre, situated in Istria-Babadag vineyard. The variable viability and fertility of the winter buds are influenced by environmental conditions.

The most important aspect for the vine fruit cuttings is to determine and reserve on each plant/m^2/ ha the correct number of winter buds

corresponding to the age and vigor, the environmental conditions and the qualities of the cultivated variety, to ensure the relationship between quantity and quality of the harvest.

This work is carried out every year in the Istria viticol centre, from December to March,

First, the control of the buds viability was done directly in plantation and than in laboratory by the longitudinal sectioning method, and the fertility was evaluated in the laboratory only.

Of the investigated varieties, all corresponded as fertility to the normal values established for them. All the data obtained by us provided information regarding the evolution of winter buds, the viability and fertility of the varieties and the position of buds along the annual shoots.

The data are important for determining the total number of winter buds retained on the vine plant after the fruit cuts.

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PHYSIOLOGICAL PROFILE OF SOME PATHOGENIC BACTERIA ASSOCIATED WITH GRAPEVINE CROWN GALL

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Abstract

The most devastating bacterial infection of grapevine is crown gall disease, especially for young vineyards and orchards. Considering these, a better understanding of the pathogen physiology will improve the diagnostic of the causal agent of tumours induction. Therefore, small tumours collected from young grapevine plants of Italian Riesling and Fetească neagră cultivars were analyzed in order to identify and characterize the causal pathogenic bacteria. Several bacteria were isolated from grapevine tumours of Miniş-Măderat vineyard. Only four isolates were selected (vv1, vv2, vv3, vv4) for their identical or similar colony morphology to a phytopatogenic reference strain of Agrobacterium tumefaciens. The isolates vv1, vv2 and vv3 were positive for esculinase and urease, but negative for 3-ketolactose. In the tumour inducing tests, the isolates vv1, vv3, vv4 and the reference At12 were found to induce hyperplasia on carrot slices. The isolates vv1, vv2 and vv3 were identified as Rhizobium vitis bv 3 (formerly known as Agrobacterium biovar 3 or Agrobacterium vitis, comb. nov. Allorhizobium vitis). The fourth isolate (vv4) was identified as Pantoea agglomerans. In vitro assay revealed that cooper sulphate inhibits bacterial growth at less than 5% concentration, and completely suppresses their growth at 5% or higher concentration.

Key words*: A. vitis, Pantoea agglomerans, grapevine crown gall.*

INTRODUCTION

Romania is currently ranked as the 4th in Europe, considering the area planted with grapevine (www.fao.org). The Food and Agriculture Organization of the United Nations - FAOSTAT has mentioned that, in 2016, Romania was having 175 057 ha of grapevine. This placed Romania as the tenth state worldwide in growing grapevine, after Spain (920 108 ha), France (757 234 ha), China (843 407 ha), Italy (668 087 ha), Turkey (435 227 ha), United States of America (409 947 ha), Argentine (223 944 ha), Israel (207 329 ha), and Chile (203 127 ha).

Crown gall is a serious grapevine disease worldwide causing vine decline and mortality in vineyards with important economic losses. In cold climate regions, grapevine is more susceptible to infection, expressing a particularly severe attack (Vizitiu & Dejeu, 2011). The causal agent is the phytopathogenic bacteria *Agrobacterium vitis*, syn. *Agrobacterium* biovar 3, named *Rhizobium vitis* by Young et al. (2001), and currently proposed to be renamed *Allorhizobium vitis* (Mousavi et al., 2015). However, in some cases, there was also found *Agrobacterium tumefaciens* (Szegedi et al., 2005; Bazzi et al., 2008), currently named *Rhizobium radiobacter* (Young et al., 2001, 2003). These pathogens are causing hyperplasia and hypertrophy in the infected plant tissue, generating tumours or galls (Burr et al., 1998, 1999; Kawaguchi et al., 2017). The infection process can occur naturally through the wounds caused by frost. Thus, colder and humid climate increase plant vulnerability to infection, especially in less winter-hardy cultivars. Severe winter weather, as well as the current climatic changes with extreme temperature fluctuations, late spring frosts or early winter frosts, damage the grapevine trunks increasing infection risks. Tumorigenic infection and pathogen spread is higher during grafting, pruning and other mechanical activities in the vineyard. Therefore, careful monitoring of plant phytosanitary status is important in order to minimize the pathogenic infection and disease

spread. Preventive measurements and treatments are of great importance since the infections are established before the symptoms occur.

Taking into consideration the impact of crown gall infection in young grapevine, we analysed the presence of the pathogen in some small tumours, collected from young plants of Miniş-Măderat vineyard in order to characterize the causal agent of the tumour induction.

MATERIALS AND METHODS

Grapevine canes and cordons with small or abundant tumours (Figure 1) were collected from young plants of Miniş-Măderat vineyard in order to characterize the causal agent of the crown gall. The samples were taken from young plants of *Vitis vinifera* L., Italian Riesling and Fetească neagră cultivars, grafted on SO4 rootstocks. The samples were collected in 2016, when the vineyard was seven to eight year-old.

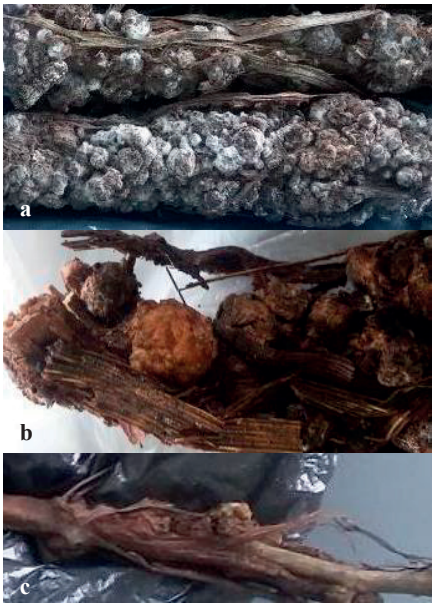

Figure 1. Tumours on canes and cordons from Italian Riesling (a, c) and Fetească neagră (b) cultivars

In order to detect the causal agent of the tumours, the samples were subjected to the analysis. Bacterial isolation was carried on MG-Te medium (Ophel & Kerr, 1990). This is an Agrobacterium semi-selective medium based on mannitol and glutamic acid, supplemented with potassium tellurite. To purify the bacterial cultures we used the streak plate method on MG-Te and Yeast extract Mannitol Agar (YMA) supplemented with Congo red. These two semi-selective media were also used to grow the reference strains *Agrobacterium tumefaciens* At12 and *Agrobacterium rhizogenes* Ar8196, which allowed a comparative analysis between the bacterial growths. The newly isolated strains were selected based on their morphological characteristics and growth similarities with the reference strains.

The isolated bacteria were subjected to some biochemical tests for agrobacteria (Campillo et al., 2012), like urease, β-glucosidase (esculinase), and 3-ketolactose. For the urease test fresh bacterial biomass was suspended in specific medium containing 2% urea, 0.3% L-tryptophan, 0.1% monopotassium phosphate, 0.1% dipotassium phosphate, 0.5% sodium chloride, 0.95% of pure ethanol, and 0.0025% phenol red in distilled water. The esculinase test was performed on broth medium containing 1% peptone, 0.1% esculine, and 2% ferric ammonium citrate in distilled water. These two specific solutions were filter sterilized, through 0.22µm membrane. For 3-ketolactose test, bacteria were grown on Lactose Agar medium for two days and cultures were flooded with Benedict's reagent. The yellow colour around microbial growth reveal lactose conversion to 3-ketolactose. This positive reaction is specific to *A. tumefaciens* (Shams et al., 2012).

The purified strains were biochemical characterized and identified based on their physiological profile using the Biolog GEN III microbial identification system and the standard IFA protocol, according to the manufacturer guidelines.

The pathogenicity test of the isolated strains was carried out *in vitro* on carrot slices inoculated with fresh bacterial suspension (10^7cfu/ml), using o similar protocol as described by Milijašević et al. (2007). Carrots were disinfected with Dakin's solution (0.5% sodium hypochlorite) and aseptically cut in slices with a sterile scalpel. The carrot disks were placed in sterile humid chambers of moistened filter paper in Petri dishes. For this

test, a negative control of sterile distilled water was used. As positive control, the reference strain *A. tumefaciens* At12 was also used. Inoculated carrot slices were incubated in moistened chambers for three weeks at room temperature. The pathogenicity test was repeated two times, each performed in four replicates.

Bacterial sensitivity to copper based treatments was also studied. In this study a modified version of disc diffusion method was used. For these test, 100 µl of fresh bacterial suspension was plated on Yeast Mannitol Agar (YMA). Several paper disc impregnated with 10 µl of fungicide solution, in different test concentrations, were placed on top of the agar plate. The fungicide solution was based on different concentrations of copper sulphate, neutralized with slaked lime, $Ca(OH)_2$. The Bordeaux mixture was used as reference. Thus, ten different concentrations of copper sulphate solution were tested: 0.75%, 1%, 1.5%, 2%, 3%, 4%, 5%, 6%, 6.5%, and 7% $CuSO_4$. Plates were incubated at 28ºC for 5 days, before analysing the growth inhibition zone.

RESULTS AND DISCUSSIONS

Four bacterial isolates were purified from the grapevine crown galls: vv1, vv2, vv3 and vv4 (Table 1). During isolation, bacterial growth obtained on MG-Te was compared with the reference strains *A. tumefaciens* At12 and *A. rhizogenes* Ar8196. Bacterial colonies having identical or similar colony morphology with the reference strains were harvested and bacterial cultures were purified on MG-Te and YMA with Congo red.

Table 1. Bacterial strains isolated from grapevine tumours

Bacterial isolates	Vineyard	ID code	Harvest area (ha)	Cultivar	Rootstock	Grapevine age	Disease symptoms
vv1	Miniș-Măderat vineyard	BRA-M	1.2	Italian Riesling	SO4 (*V. riparia* × *V. berlandieri*)	7 years	Crown galls on cane
vv2		ELI-M	3.5	Fetească neagră		8 years	Tumours on cane and cordons
vv3		BRA-M	1.2	Italian Riesling		7 years	Tumours on cane
vv4			1.2				Corky galls and cracking of bark on grapevine cordon

On MG-Te media, the bacterial isolates vv1, vv2 and vv3 exhibited black coloured, smooth, circular, convex, isolated colonies, identical with the reference agrobacteria (Figure 2a). Bacterial isolate vv4 presented similar morphology; however it had a much abundant growth, of dark grey colour and semi-translucent appearance (Figure 2b).

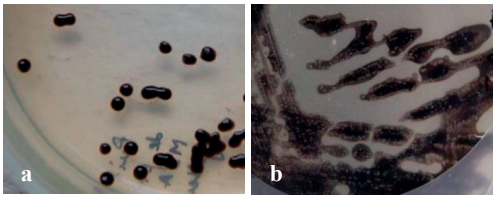

Figure 2. Bacterial growth on MG-Te semi-selective media: a) typical agrobacteria colonies of the vv1 isolate; b) vv4 bacterial growth

On YMA with Congo red, *A. tumefaciens* At12 reference developed bright red colonies. *A. rhizogenes* Ar8196 produced smooth, circular, convex colonies, whitish-pink in colour, with a central reddish spot after prolonged incubation on YMA with Congo red. Bacterial strains vv1, vv2 and vv3 developed pink colonies, with red pigmentation in the centre. Bacterial strain vv4 developed pink, punctiform colonies (Figure 3).

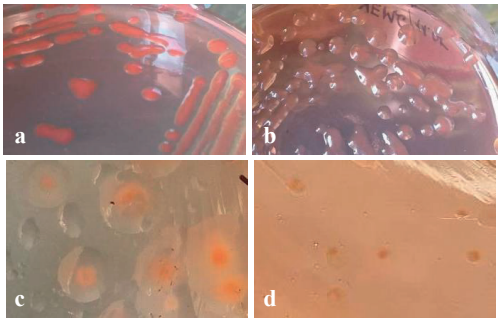

Figure 3. Bacterial growth morphology on YMA with Congo red: a) At12; b) Ar8196; c) vv1 strain; d) vv4 strain

Bacterial maintenance was made on YMA growth medium, on which luxuriant growth was obtained. Bacterial strains vv1, vv2, vv3 presented translucent, gummy, glistening, elevated colonies with entire margins, and vv4 strain had a fluid, translucent growth (Figure 4).

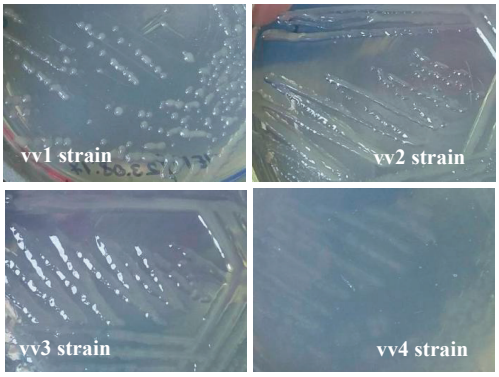

Figure 4. Bacterial strains on YMA growth media

The urease, esculinase and 3-ketolactose tests were performed for all isolates, and compared to the reference strain At12. Urease production was detected in At12 reference, vv1 and vv3 isolates. Regarding vv2, urease reaction was weak-positive after overnight incubation. The reaction was clear negative in vv4 (Figure 5). All bacterial strains, including the reference, were esculinase positive. Regarding these two test, a positive reaction should be obtained for agrobacteria detection (Campillo et al., 2012). Production of 3-ketolactose was detected only by the reference strain At12, the isolates tested being negative. According to several authors, 3-ketolactose is produced only by *A. tumefaciens*, *A. vitis* showing a negative response (Ophel & Kerr, 1990; Argun et al., 2002). Corroborating the results obtained for urease, esculinase and 3-ketolactose it is suggested that vv1, vv2 and vv3 could be affiliated to *A. vitis*.

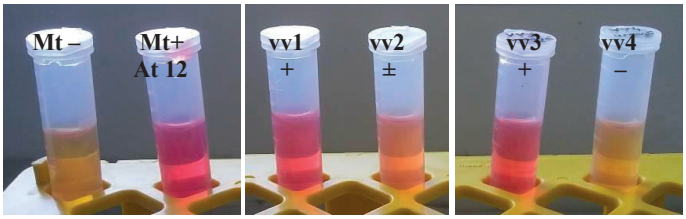

Figure 5. Urease production by different bacterial strains isolated from grapevine tumours

Species identification was made using the Biolog Gen III phenotypic microarray, based on 96 tests revealed by a redox reaction. Among these tests, 71 are biochemical tests and 23 are chemical tolerance tests. This technique is able to detect bacteria, at species and biovar level. The vv1, vv2 and vv3 isolates were assigned to *Rhizobium vitis* bv.3, also known as *Agrobacterium vitis* or *Agrobacterium* biovar 3, and currently reclassified as *Allorhizobium vitis* (Mousavi et al., 2015), which is the main pathogen involved in grapevine crown gall. This identification confirmed the results revealed by the previous tests. Unlike the first three isolates, vv4 strain was assigned to *Pantoea agglomerans*.

Studying the metabolic profile of vv1, vv2 and vv3 strains compared to *Rhizobium vitis* from the system database, it was shown that these newly isolated strains can metabolize dextrine faster than the references. Compared to vv1, vv3 and the database references, vv2 strain metabolised slower the fucose. Another difference among strains is that vv1 used L-arginine slower than vv2, vv3 and the database references. The vv1 and vv2 strains used L-histidine substrate slower compared to the references; vv1 and vv3 revealed a less intense redox reaction on p-hydroxyphenylacetic acid. All three strains (vv1, vv2 and vv3) presented rifamycin SV, lincomycin and potassium tellurite resistance, and vv1 was tolerant to fusidic acid.

Regarding the identified *Pantoea agglomerans* vv4 strain, it presented more rapid redox reactions on fucose, L-aspartic acid and citric acid compared to references from the system database. Although we isolated vv4 strain from grapevine tumours, *P. agglomerans* is however mentioned as a saprophytic specie with a cosmopolitan distribution, being an epiphyte colonizer of the vegetal material or an

endophyte symbiont (Nadarasah & Stavrinides, 2014; Walterson & Stavrinides, 2015). Although *P. agglomerans* is known to be a good competitor against *Erwinia amylovora* plant pathogen (Wright et al., 2001, Pusey et al., 2011), some strains are associated to plant, insects and human infections (Dutkiewicz et al., 2016), such as seed-born decay of maize (Silva-Rojas et al., 2016), or onion bulb rot (Edens et al., 2006).

Virulence or tumour induction capacity was evaluated for each newly isolated strain, in comparison with the reference bacterial strain At12. Therefore, artificially infected carrot slices were analysed after 3 weeks of incubation in humid chamber, at room temperature. Tumorigenic agrobacteria are able to induce tumour-like callus form the meristematic tissue. The carrot disks were checked for tumours in the pericycle area. Bacterial virulence was visually evaluated, as follows: grade 0 - for isolates that are not capable to induce tumours, grade 1 - for induction of solitary tumours in the pericycle area, grade 2 - for induction of several differentiated tumours, grade 3 - for confluent tumours in arc circle shape, grade 4 - for abundant tumours on the pericycle ring. The At12 positive control induced tumours classified as grade 3 and 4. In the negative control, were carrot slices were treated with sterile distilled water, no tumour was generated (Figure 6).

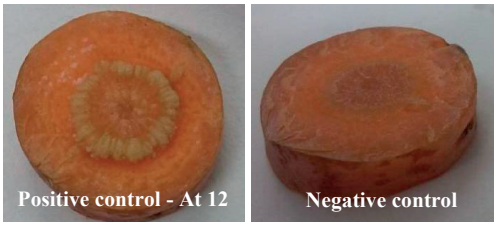

Figure 6. Young galls around the central vascular system induced by virulent agrobacteria

The vv1 and vv3 strains generated tumours of 3 and 4 grade, suggesting an increased virulence. The vv2 strain presented a lower virulence, as it generated small isolated tumours, of grade 1, in not more than two slices of each replicate (Figure 7).

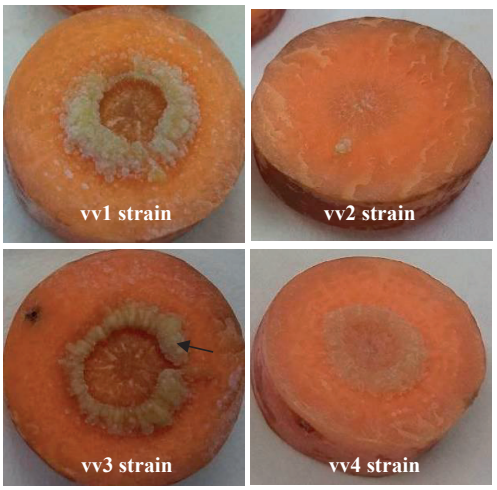

Figure 7. Hyperplasia and hypertrophies of the meristematic tissue from carrot pericycle, symptoms were induced by *R. vitis* vv1, vv2 and vv3 strains, and by *P. agglomerans* vv4

Although vv4 strain is not an agrobacteria, it produced foal, laced hypertrophies on the carrot slices (Figure 7). Since the result was not to be expected from a *P. agglomerans* strain, we hypothesized that meristematic tissue was stimulated to grow due to a high amount of auxine phytohormones, probably produced by vv4 strain. In *P. agglomerans* bacteria, formerly known as *Enterobacter agglomerans*, auxins production is a commonly found feature (Ludwig-Muller, 2014). These plant hormones regulate cell division and increases cells size (Perrot-Rechenmann, 2010).

Although copper bactericides could kill pathogenic agrobacteria by contact, they do not penetrate systemically in the vine (Burr, 2004). Studies performed by Vizitiu (2016) revealed that combination of copper sulphite with garlic tincture decreased gall apparitions caused by agrobacteria in grapevine and stopped the proliferation of pathogenic *A. tumefaciens*. Previous studies of Vizitiu & Rădulescu (2013) showed that the application of copper sulphate in combination with garlic tincture can reduce *A. tumefaciens* density in soil. Moreover, increased copper concentrations enhance some plant enzymes activity, like Phenylalanine ammonia lyase (PAL), cinnamyl alcohol dehydrogenase (CAD), and caffeic acid peroxidase (CA-POD), triggering lignin synthesis (Liu et al., 2018). Therefore copper

applications can help plants in the healing process of wounds caused by frost, or mechanical injuries. Thus reducing the incidence of bacterial infections.

Considering the tumorigenic potential of the isolated strains, bacterial sensitivity to copper based products, including Bordeaux mixture, was studied. Therefore, ten different concentrations of copper sulphate solution (0.75% to 7% $CuSO_4$) were used. All tested copper sulphate solutions were neutralized with calcium hydroxide, in equal concentration. Results suggested that in concentrations lower than 5% bacterial growth was inhibited. The inhibition zone being proportional with the tested concentration. At 0.75% CuSO4, reduced bacterial inhibition zones, of 0.1 to 0.3cm, were obtained (Figure 8b).

Treatment solutions based on copper sulphate in 5% or higher concentrations generated clear inhibition zones, were bacterial growth was completely blocked (Figure 8c), However, at less than 5% concentration bacterial inhibition zones were detected, but bacterial cells remained viable, and started to develop a weak/pale growth. Higher concentrated copper solutions (≥ 5%) induced clear zones, with no bacterial growth in the first 2-3 cm, surrounded by a pale growth, due to copper diffusion through agar.

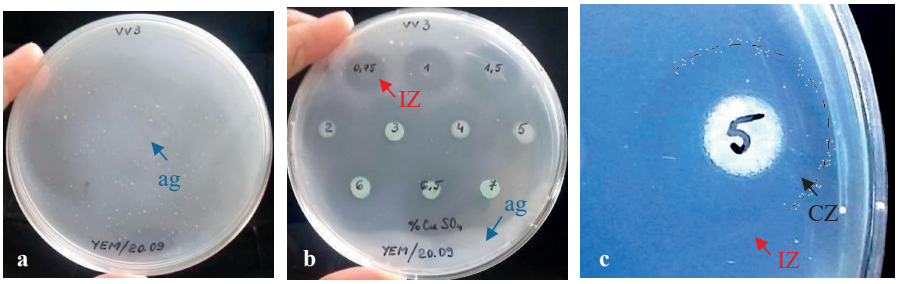

Figure 8. The inhibitory activity of copper based treatments against bacterial growth:
a) Bacterial growth in untreated culture; b) Inhibited bacterial growth, due to the copper sulphate solutions, tested in different concentrations; c) Clear zone with blocked bacterial growth neat the copper treated disks revealed at ≥ 5% $CuSO_4$ concentrations
Legend: ag = abundant bacterial growth; CZ = clear zone with blocked bacterial growth;
IZ = inhibition zone, were bacterial growth was diminished but not completely blocked;
- - - - = marking line between CZ and IZ.

Similar results were obtained among all bacterial strains (vv1, vv2, vv3 and vv4), including the reference strains At12 and Ar8196. This confirming that copper based products have a wide antibacterial spectrum.

These results support farmers' effort to reduce agrobacteria infections during winter and spring, when high concentrations copper treatments are limiting the spread of bacterial infection, favouring plants to heal their natural or mechanical wounds without contacting an infection.

CONCLUSIONS

A. vitis was found to be the causal agent of the grapevine crown gall of Italian Riesling and Fetească neagră cultivars, from Miniş-Măderat vineyard. Although *P. agglomerans* is generally considered not to be a threat to plants health, our tests suggested that *P. agglomerans* vv4 strains could induce proliferation in meristematic tissues most probably due to an increased phytohormone production. The isolated bacteria were identified through classical microbiological assays and Biolog Gen III microarray. Regarding farmer's need to treat and prevent pathogenic bacterial infection we recommend a spring treatment of 5% Copper sulphate solution before budding. These would reduce plant phyto-toxicity, but will eradicate the pathogen, preventing the spread of crown gall disease.

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RESEARCHES ON THE FROST RESISTANCE OF GRAPEVINE WITH SPECIAL REGARD TO THE ROMANIAN VITICULTURE. A REVIEW

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Abstract

Under the temperate continental climate of Romania, very low temperatures during the winter (below -20ºC) can cause severe damage in several wine regions. The relatively high frequency of the low temperatures in winter allowed the classification of the autochthonous varieties of grapevine by their resistance/tolerance to frost. Thus, the varieties Aromat de Iaşi, Alidor, Ozana, Victoria, Transilvania, Băbească neagră, Cadarcă and Frâncuşă are frost sensitive, while Silvania, Crâmpoşie selecţionată, Şarba, Coarnă neagră, Fetească albă are resistant to winter frosts. The relatively high frequency of minimum temperatures harmful to grapevine during the dormant period, makes it necessary to assess the wintering behavior of vineyards. If during the reference period (1961-1990) the frequency of minimum temperatures below -20ºC in the Southern part of Romania (Bucharest) was 16.7%, in the period 1991-2018 it increased significantly to 39.3%. The higher frequency of minimum temperatures harmful to grapevine recorded in the main wine regions of the country (Hills of Moldova, Hills of Walachia and Oltenia, Transylvanian plateau) resulted in a significantly lower total wine production in the years 2005, 2010, 2012, 2015 and 2016. In a long-term experiment carried out on Fetească regală variety, a reduction of the average grape yield by 27-34% was found under the conditions of minimum temperatures of -20 ... -22ºC, in the dormant period. The paper reviews research on the physiological and biochemical aspects, frequency and intensity of winter frosts, the resistance/tolerance of the different varieties as well as factors that influence the resistance to frost of grapevine.

Key words*: grapevine varieties, winter frost resistance .*

INTRODUCTION

Among the abiotic factors with negative influences on the vine are also low temperatures, which, depending on the time of registration, by the minimum level they reach, cause less or greater damage, up to the total destruction of the plants.

Under the conditions of the continental temperate climate in Romania, especially the low (negative) temperatures recorded during the winter are conditioning the area of cultivation of certain varieties, grapevine training systems, as well as other viticulture practices (Oprean, 1975; Ţârdea and Dejeu, 1995; Olteanu, 2000; Dobrei et al., 2011; Hill, 2011; Ivanov et al., 2016).

In the traditional viticulture in Romania, up to mid -20th century, in many wine regions the protected culture of vines was practiced, by covering the plants with soil, especially in the plantations located on the valleys, on the plains and at the base of slopes (Martin, 1978; Oşlobeanu et al., 1980; 1991).

In the second half of the 20th century, as a result of several studies on the resistance to frost of the varieties and the delimitation of areas of unprotected culture, the vineyard plantations were modernized using the semi-high and high training systems (Laszlo et al., 1974; Metaxa and Kovacs, 1976; Macici et al., 1983; Mustea, 2004).

Under the influence of the low temperatures exceeding the resistance level of the different organs of the vines, a necrosis of the buds can occur, reduction of fertility and productivity, partial or total loss of the yield, necrosis of the free annual canes, cracking of the wood, installation on sap leakages of saprophytic fungi and bacteria that can penetrate into tissues, installation of crown gall, anthracnose, etc., becoming dangerous for the survival of the vine (Mănescu et al., 1990; Burzo et al., 2005;

Podosu et al., 2009; Călugăr et al., 2009; Blaich, 2010).
The present review presents a synthesis of the experimental results in a large number of winegrowing centers and years and on an important number of varieties, from which the main morpho-anatomical and physiological-biochemical aspects involved in the frost resistance of grapevine, winter behavior of varieties and factors of influence. All these aspects are useful for recovering the losses from the affected plantations and a better zoning of the varieties (Dobrei et al., 2017; Cragin et al., 2017).

MORPHO-ANATOMICAL AND PHYSIO-LOGICAL-BIOCHEMICAL ASPECTS

In our country there have been numerous researches on the resistance to frost of the grapevines in correlation with the morpho-anatomical particularities of the plants during the dormancy period (Radu et al., 1968; Chirilei et al., 1970; Bădiţescu et al., 1978; Burzo, 2015; Dami et al., 2016; Kaya and Kose, 2018).
The freezing temperatures vary depending on how the shoots maturation process was carried out. The acclimation of the grapevine is a gradual process. It starts with the formation of the periderm and thus browning of the shoots, the entry of the winter buds into dormancy, the redistribution of the nutrients from the leaves into the permanent organs of the grapevine (trunk, canes, roots) and the fall of the leaves.
The beginning of maturation is marked by the change in the color of the bark, as a result of the occurrence of the subero-felodermal zone and the formation of the suber. The depth of formation of the suber determines the degree of frost resistance. In the varieties with better resistance, the suber appears at a greater depth and with a greater number of layers.
Prolonged growth of shoots, excess rainfall in autumn causes insufficient maturation of the canes and therefore, a frost-sensitization of the grapevines (Dejeu, 2010).
The acclimatization of the vines to the action of freezing temperatures is achieved by accumulating reserve substances and by increasing the concentration of the solution in the cells. The resistance to frost is genetically controlled and only manifests after the plant has undergone a period of acclimatization, during which the decrease of the free water content in the tissues and the increase of osmotic pressure take place. This protects the cells from intracellular ice formation (Burzo et al., 2005).
In the first phase of the acclimation process, which overlaps with the ripening of the canes, an intense accumulation of starch takes place in the annual and multi-annual wood, the accumulation of total and protein nitrogen, and the decrease of the non-protein nitrogen quantity.
In the second phase, enzymatic hydrolysis of the starch takes place, during the short days of the autumn, in simple soluble carbohydrates, which increase the osmotic force of the vacuolar content and the concentration of cytoplasmic juice, especially in the bark tissues. Rapid hydrolysis of starch and biosynthesis of glucose, fructose, sucrose, and raffinose occur in frost resistant varieties (Popa and Popa, 1976; Tacu and Beznea, 1994; Eibach and Töpfer, 2015; Keller, 2015).
The frost resistance of the tissues of the grapevines is also determined by the increase of the water retention force in the cells, which can be achieved by modifying the predominant water form and especially by decreasing the amount of free water and increasing the bound water. The content in free and bound water is closely linked to the intensity of dehydration of the canes in various moments during the autumn-spring interval.
In the process of preparing the plant for wintering, reactions of enzymatic hydrolysis of the proteins and transformation into free amino acids, which are the most resistant forms to frost of the nitrogenous organic compounds take place. During the winter, a reduction in the intensity of respiration and transpiration of the annual canes and buds was ascertained, with a minimum in January-February; the decrease was more pronounced in the varieties with better resistance to frost.

FREQUENCY AND INTENSITY OF WINTER FROSTS

The frequency of low winter temperatures affecting the aerial part of the grapevine is

relatively high. Between 1888 and 1985, the critical frosty winters for the unprotected grapevine were at intervals of 2-20 years, returning once on average every 10 years (Popa et al., 1966; Georgescu et al., 1986). The very cold winters were in the years: 1888, 1893, 1907, 1909, 1929, 1942, 1954, 1963, 1969, 1980, 1985. A particularly difficult winter for grapevines was that of 1929, when on sandy soils the root system (more sensitive than other organs) froze and entire plantations of grapevines grown on their own roots were lost (Teodorescu, 1929).

In the last three decades, as a result of climate change, it has been observed that, besides global warming, a higher frequency and intensity of absolute low temperatures were damaging to the grapevine (Bucur and Babeş, 2016; Bucur and Dejeu, 2016a; Bucur et al., 2019). Bucur and Babeş (2016) kept track of the frequency of low temperatures in the period 1991-2016, compared to the reference period 1961-1990 (Table 1).

Table 1. Minimum temperatures below -20°C recorded in Bucharest-Baneasa in the period 1991-2015 compared to the reference period 1961-1990 (Bucur and Babeş, 2016)

1961-1990	1991-2015
1963: -23.7°C (18.01.1963)	1997: -20.0°C (18.12.1997)
1969: -21.7°C (05.02.1969)	1998: -20.3°C (25.12.1998)
1980: -24.5°C (15.01.1980)	2002: -25.7°C (26.12.2002)
1985: -24.6°C (14.02.1985)	2003: -20.0°C (14.02.2003)
1987: -21.7 °C (31.01.1987)	2004: -20.8°C (13.02.2004)
	2005: -23.7°C (08.02.2005)
	2010: -24.6°C (26.01.2010)
	2012: -24.3°C (29.01.2012)
	2015: -20.8°C (08.01.2015)

Between 1961 and 1990, the minimum temperatures were recorded at intervals of 2-11 years, returning on average every 2 years out of 10. As a result of the climatic changes of the last three decades (1991-2015), the frequency of the low temperatures harmful to the grapevine (under -20ºC) has increased significantly, and low temperatures were registered at intervals of 1-5 years, returning on average every 4 years out of 10.

Absolute minimum temperatures are recorded mostly in January. Another negative influence on the grapevines are low temperatures (-10 to -16ºC) which occur during November and lead to losses of up to 50-70% of the bud viability, depending on the variety, the degree of hardening, the depth of dormancy (Oprea and Oprea, 1976).

Following the evolution of the absolute minimum temperatures in time for Bucharest-Băneasa and Iaşi (Bucur and Babeş, 2016), a decrease of temperature can be observed (Figure 1).

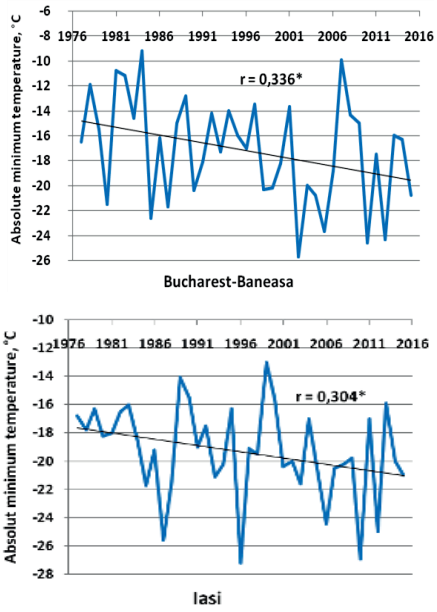

Figure 1. Evolution of absolute minimum temperatures between 1977 and 2015, Bucharest and Iaşi

In the southwest of the country, within the Banu Mărăcine winegrowing center, Cichi (2005); Cichi et al. (2006a and 2006b) a frequency greater than 26% of the absolute minimum temperatures was ascertained, with values between -18.1ºC and -22ºC, with a frequency of over 14% of minimum temperatures falling below -22ºC.

In the Târnave vineyard (Blaj) the largest winegrowing area in Transylvania, the low temperatures harmful to grapevines, between 2000 and 2018 (19 years) were recorded in the years: 2004 (-22.6ºC), 2005 (-22.1°C), 2006 (-20.2°C), 2010 (-21.4°C), 2012 (-21.6°C), 2014 (-21.7°C), 2015 (-23.3°C), 2017 (-24.1°C). In this wine-growing area, one of the coldest in Romania, the frequency of minimum

temperatures below -20ºC for the studied period was 42.1% (Iliescu et al., 2019).

Extreme climatic phenomena have been frequently recorded in recent years and in the northeastern part of the country (frosts, prolonged drought, etc.) having more or less unfavorable impact on production and quality (Rotaru, 2008; Rotaru et al., 2008, 2010a, 2010b; Rotaru and Colibaba, 2011; Zaldea et al., 2013).

Numerous researches have considered the behavior of the grapevine varieties in the winegrowing centers Iasi and Cotnari, regarding the influence of the low harmful temperatures under the conditions of the winters 2010/2011 and 2011/2012 (Jităreanu et al., 2011; Irimia et al., 2012; Rotaru et al., 2013; Zaldea et al., 2014; Planchon et al., 2015).

During a long-term study (Bucharest, 1998-2018) on the Fetească regală variety, grafted on the Kober 5BB rootstock (Bucur et al., 2019), as a result of the high frequency of minimum temperatures below -20ºC large variations in production were ascertained, with a significant tendency of its decrease (Figure 2).

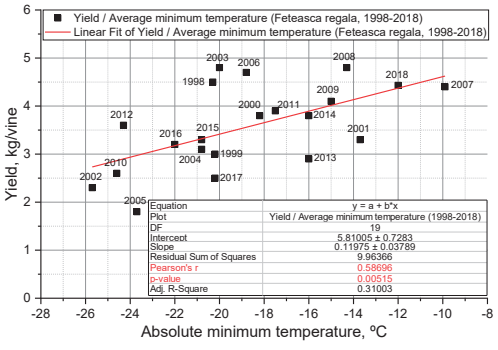

Figure 2. The negative effect of winter temperatures on grape yield (Fetească regală, Bucharest 1998-2018)

At minimum temperatures below -22ºC, a significant reduction in the average grape production (kg/vine) was recorded, compared to the normal winter years (from 4.75 kg/grapevine to 3.2 kg/grapevine), representing a reduction by 33%. For the entire country, the minimum harmful temperatures recorded in many winegrowing regions of the country (mainly in Moldova, Walachia, Transylvania and Banat) lead to decreases in the total wine production (Figure 3) in 2005, 2010, 2012, 2015, 2016 by up to 50% (2005).

An important indicator of the conditions of grapevine wintering is the average of the minimum temperatures of the coldest month of the year (January). Following the average of the minimum temperatures in January in different regions of the country over a period of 38 years, Bucur and Dejeu (2016a) found large differences, from -3.57°C (Constanţa) to -6.0°C (Iaşi) and -6.26°C Cluj-Napoca; the last two regions have the most frequent temperatures harmful to grapevines.

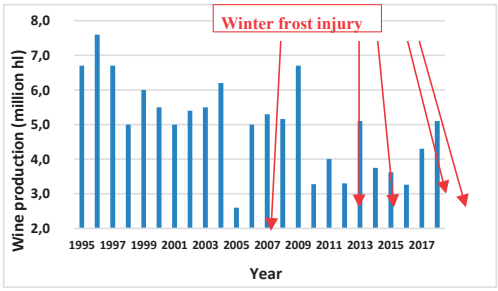

Figure 3. The evolution of the Romanian wine production and the influence of winter frosts between 1995-2018

BEHAVIOR OF VINE VARIETIES UNDER WINTERING CONDITIONS

The research carried out by Popa et al., 1966, in 7 wine centers, with reference to the influence of the low temperatures between -20ºC (Pietroasele) and -34ºC (Blaj) from winter 1962-1963, has shown that the varieties with best resistance to frost are: Coarnă neagră, Cabernet Sauvignon, Pinot noir, Riesling italian, Riesling de Rhin, Fetească albă, Aligoté and Perla de Csaba.

In the winter of 1984-1985, minimum temperatures critical for grapevine occurred in most of the winegrowing regions of the country. In Bucharest, -22.6ºC was registered on January 13, with a 44 cm snow layer, contributing to the protection of the base of the trunk, and intensifying the negative effect on the cordons, located near this layer (Georgescu et al., 1986).

The newly created varieties for table grapes (Timpuriu de Cluj, Cetăţuia, Victoria, Silvania, Roz românesc, Coarnă neagră selecţionată, Greaca, Xenia, Tamina), compared to the

variety of Chasselas had a very low resistance to frost. Very low frost resistance varieties were considered Cetăţuia, Victoria şi Xenia. Newly created wine grape varieties (Aromat de Iaşi, Ozana, Codană) proved to be sensitive to low temperatures, except for Roz de Miniş variety.

A higher resistance, shown in the percentage of main and secondary viable buds, had Rkatiteli and resistant varieties Villard noir and Seyval. It is appreciated that a territorial distribution of varieties is required, according to their resistance to frost and the application of differentiated pruning based on the influence on the bud viability.

After the low winter temperature recorded in Bucharest-Baneasa weather station (-23.7ºC in February 9, 2005), Dejeu et al. (2005) have tested the frost resistance of 45 *Vitis vinifera* varieties. All the varieties were affected on different levels and they were grouped into four classes of resistance, according to the viability of the primary buds:

- high resistance to frost, with viability of the primary buds between 80 and 100% (Burgund mare, Columna, Furmint, Traminer roz, Riesling italian, Oporto, Muscat Ottonel, Cabernet Sauvignon);
- moderate resistance to frost, with viability of the primary buds between 50 and 80% (Rkaţiteli, Steinschiller, Şarba, Tamina, Muscat Hamburg, Frâncuşă, Pinot noir, Grasă de Cotnari, Augusta, Merlot, Fetească regală, Chardonnay, Fetească neagră, Aligoté, Coarnă neagră selecţionată, Sauvignon, Plăvaie, Timpuriu de Cluj, Creaţă, Chasselas doré);
- low resistance to frost, with viability of the primary buds between 30 and 50% (Novac, Azur, Fetească albă, Galbenă de Odobeşti, Kişmiş alb, Tămâioasă românească, Afuz Ali);
- very low resistance to frost, with viability of the primary buds between 0 and 30% (Crâmpoşie, Cardinal, Sangiovese, Victoria, Băbească neagră, Cadarcă, Timpuriu de Pietroasa, Italia, Muscat timpuriu de Bucureşti).

During the winter of 2009-2010, starting with January 24, three consecutive days with minimum temperatures below the limit of resistance of grapevine to frost were recorded, in most of the winegrowing areas of the country (Şerdinescu and Ion, 2010). In the vineyards in the northeast of the country, where temperatures up to -27ºC were recorded, the viability of the eyes of the representative varieties was between 0% (Feteasca albă) and 50% (Sauvignon). The largest losses of buds were recorded in the plots with northern, north-eastern exposure and at the base of the slopes, these being between 93% (Fetească regală) and 100% (Fetească albă).

In Odobeşti vineyard, after recording an absolute minimum temperature of -23.8ºC, the viability was between 33% (Şarba) and 81% (Fetească regală).

In Murfatlar vineyard (-20.0ºC absolute minimum temperature), the viability was lower for table grape varieties (Afuz Ali 93Mf - 3%, Perlette - 5%, Victoria - 10%, Muscat de Hamburg - 30%) and for wine grape varieties located at the base of the slope and in the lower third of it (Cabernet Sauvignon - 22%, Cristina - 30%, Mamaia - 35%, Fetească neagră - 40%). The viability was better for wine grape varieties located in the upper third of the slope (Columna - 62%, Pinot gris - 54%, Muscat Ottonel - 50%, Chardonnay - 50%).

In the Blaj winegrowing center (-21.4ºC absolute minimum temperature), the variety Fetească regală showed viability between 32% in the plantations located at the base of the slope, 43% in the plantations located in the first third of the slope and 83% in the plantations located in the highest third of the slope. In the variety Italian Riesling, the viability, under the same conditions, was between 42 and 91%.

Studying the behavior at low temperatures of -24.2ºC under the conditions in Bucharest (February 9, 2012), Stroe and Bucur (2012) found higher sensitivity in new table grape varieties to be in close correlation with that of the parents. Thus, the Azur variety (Coarnă neagră x Cardinal) had lower eye losses (56%) compared to Greaca (Bicane x Afuz Ali), where the losses were 96%.

Following the effect of the minimum temperatures of -20.8ºC (January 8, 2015) on a number of 45 new varieties (Bucur and Dejeu, 2016b), they found a better resistance to frost of the varieties Napoca (56% viability), Milcov (43%), Greaca and Xenia (42%), among table grape varieties. Of the varieties for wine studied, better behavior had Şarba and Crâmpoşie selecţionată (56%), Columna

(50%), Novac (50%) and Roz de Miniş (40%). The varieties Călina (6% viability), Transilvania (3%), Triumf (5%), Victoria (9%), Selena (3%) and Mamaia (5%) were the most sensitive to the action of low temperatures in winter.

Following the minimum temperatures of -22ºC (January 23, 2016), a better behavior in wintering had the varieties Silvania (48% viability), Câmpoşie selecţionată (72%), Şarba (59%), Haiduc (48%), Arcaş (43%), Negru de Drăgăşani (40%). The most sensitive ones were Azur, Călina, Muscat timpuriu de Bucureşti, Someşan, Selena, Blasius and Aromat de Iaşi (between 2 and 9%).

In the winter of 2016-2017, the minimum temperature harmful for the grapevine, of -20.2ºC was recorded again (January 10, 2017). Under these conditions, the viability of the buds was higher compared to previous years. The most resistant were the varieties Greaca (93%), Otilia (81%), Tamina (81%), Azur (78%), Timpuriu de Pietroasa (76%), Silvania (68%), Şarba (94%), Columna and Crâmpoşie selecţionata (79%), Pandur and Novac (93%), Negru de Drăgăşani (85%), Cristina (83%). The following varieties were the most sensitive: Muscat timpuriu de Bucureşti (21% viability), Transilvania (25%), Istriţa (28%), Milcov (30%), Băbească gri (12%), Selena (21%), Blasius (24%), Furmint de Miniş (25%), Codană (24%), Mamaia (28%).

Following a minimum temperature of -20°C recorded in the southwest of the country (Pleniţa - January 1, 2015) Cichi et al. (2016) classified the varieties studied in two categories:

- varieties with medium resistance to frost (25-50% killed buds): Fetească neagră, Cabernet Sauvignon and Riesling italian;
- varieties with low resistance to frost (50-75% killed buds): Syrah, Tămâioasă românească, Merlot and Sauvignon.

FACTORS DETERMINING THE FROST RESISTANCE OF PLANTS

There are a variety of factors determining the frost resistance of grapevines: the species, the variety, the organ of the vine, the duration and level of low temperatures, how low temperatures occur and pass, the alternation of low and high temperatures, variation of frost resistance in winter, topographic features, the level of previous year production, the rootstock, culture techniques, etc. (Gagea et al., 1991; Cotea et al., 2009; Haras and Rotaru, 2012; Costescu et al., 2012; 2013; Hajdu, 2013).

The *V. amurensis* species is very resilient; the American species used as rootstocks have a medium resistance and the varieties of the *V. vinifera* species are less resistant. Some of them (Cabernet Sauvignon, Coarnă neagră, Riesling de Rhin, etc.) have a slightly higher resistance than others (Cardinal, Perlette, Afuz Ali, Victoria, etc.). Martin, 1978, found that the freezing temperature of the winter buds is between -15 and -22ºC, the annual canes of the *Vitis vinifera* freeze at -22 ... -24ºC and the rootstocks at -28 ... -30ºC.

Buds losses due to low winter temperatures result in crop losses, variable based on temperature level and duration.

The longer the duration of the lower temperatures, the higher the degree of injury, not only the organs with lower resistance (winter eyes) are affected, but also those with better resistance (annual canes, multiannual canes of grapevine). For the same organ, the effects of low temperatures increase with their duration of action (Oşlobeanu et al., 1980; Olteanu, 2000; Cichi and Capruciu, 2018).

If at a temperature -18ºC no special losses are noticed, when the temperature drops to -22ºC, the production significantly starts decreasing, especially in the varieties with a lower resistance to frost. Thus, Fetească neagră variety showed a production loss of 45%, Cabernet Sauvignon 62%, Muscat Ottonel 86%, and Merlot, the less resistant, 91%. The decrease in temperature to -26ºC totally compromised the production. The yield losses, in the mentioned varieties, increased with the increase of the duration of low temperatures; compared to a duration of 3 hours, losses increased by 52% for a duration of 12 hours (Beznea D., 1986).

Generally, in the vineyards of Romania, a tolerance threshold at low temperatures up to -18 ... -20ºC for most varieties of table grapes and -20 ... -22ºC for wine varieties is considered (Oşlobeanu et al., 1980; Olteanu, 2000; Irimia, 2012).

If the low temperatures occur slowly, gradually, the grapevines can endure more easily the bud losses, which are lower than if the temperatures occur suddenly. With slow passage, there are lower injuries, even when they occur suddenly, compared to the sudden passage (Severin, 1972).

The alternation of low and high temperatures increases the effect of the low temperatures. The resistance to low temperatures is not the same throughout the winter. Under the conditions in Bucharest area, Oprea and Oprea (1976) found that in the first part of the vegetative dormancy (November, 27), the temperature of -16.2ºC affected 21% of the buds of the Fetească neagră variety and 58% of the buds of the Afuz Ali variety.

The highest resistance of grapevine to frost was found in January, in the middle of the vegetative dormancy (Severin, 1970; Milică and Severin, 1978). The topographic conditions influence the variations of the minimum temperature, which affect the behavior to frost differently. Irimia et al. (2012) found differences in the minimum temperature in Cotnari vineyard of up to -7.1ºC, depending on the location of the plots on different forms of relief.

Şerdinescu and Ion (2010) found important differences in the behavior under wintering conditions of the varieties depending on their location on the plot. Also, for the vineyards located in the north and northeast of the country (Iaşi), the plantations with northern and northeast exposure were the most affected by the frosts of winter 2009-2010.

The large grape yields obtained in 2004 with the variety Fetească regală, as a result of large bud loads to the pruning (20 buds/m^2), led to appreciable losses of buds in the winter 2004-2005, compared to the loads of 10 buds/m^2 (Dejeu et al., 2005).

The influence of the rootstock on the frost resistance of variety graft is indisputable. The varieties not grafted compared to the grafted ones, under the same environmental conditions, have a slightly higher sensitivity to low temperatures. The use of rootstocks with a longer vegetation period, of high vigor, leads to decreased resistance to frost, compared to rootstocks with a shorter vegetation period and normal vigor. Thus, the Cardinal variety grafted on the rootstock Riparia gloire, showed 75% lost buds, compared to 85.3% when grafted on Berlandieri x Riparia Kober 5 BB, after a frost of -19.5ºC (December 13, 1977). When grafting on Riparia gloire rootstock, the canes showed a content richer in starch, monosaccharides, bound water (Mănescu et al., 1990).

The high grapevine training systems and the attribution of moderate bud loads when pruning stimulate the growth vigor of the grapevines, improve the maturation of the annual canes and provide conditions for a better wintering of the grapevines (Burzo et al., 2005). Abundant fertilization and late irrigation delay the maturation process, cause accumulation of larger amounts of water in the cells and thus predispose the plants to frost.

Fertilization with nitrogen in moderate doses (100 kg/ha) on a background with phosphorus and potassium contributes to the increase of certain resistance properties of vines to low temperatures in winter (Popa and Motoi, 1980).

CONCLUSIONS

The frost resistance of grapevine is affected by genotype, organ, the duration and level of the low minimum temperatures, how low temperatures occur and pass, the variation of frost resistance during the winter, topographic conditions.

In the last decades, scientific research in the field of frost resistance of grapevines has significantly increased, due to the increased frequency and intensity of this abiotic factor, characteristic to climatic changes.

This knowledge can be used for a better zoning of the grapevine varieties, to establish measures that diminish the effects of this negative factor under sustainability conditions of the wine production.

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INFLUENCE OF CORBOHYDRATE CONTENT ON GRAFTING IN WINE GRAPE VARIETIES 'AROMAT DE IAȘI' AND 'GOLIA'

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Abstract

In the present work determinations were made regarding the behavior of wine grape varieties 'Aromat de Iași' and 'Golia', respectively Chasselas doré variety, that was taken as control, grafted on three rootstocks Riparia Gloire, Berlandieri x Riparia Selection Oppenheim 4-clone 4 Blaj and Berlandieri x Riparia Selection Crăciunel 2. It was found that the highest total content of carbohydrates had the rootstock Riparia gloire (13.49%), followed by Crăciunel 2 (12.78%) and SO_{4-4} (12.02%). In the Vinifera varieties the same indicator was higher for 'Golia' grape variety (15.64%) and for 'Aromat de Iași' variety (14.97%), compared to the control variety Chasselas doré (13.83%). As a result of the grafting and forcing, the following aspects resulted: the percentage of vines suitable for planting was the highest in the 'Golia' variety grafted on Riparia Gloire, respectively 98%, and in the 'Aromat de Iași' variety grafted on Crăciunel 2, of 96%. The vines for which the grafting point was complete, where the buds entered vegetation and the root primordia was in maximum percentage were found at the 'Golia'/SO_{4-4} variant, at 74%.

Key words*: callus, carbohydrates, primordial roots.*

INTRODUCTION

In grafted vines, the root development and healing of the graft union are particulary affected by the water content and by carbohydrates stored in scions and rootstock. The accumulation, transformation and translocation of carbohydrates in individual parts of the grapevine have been described in the literature by various authors (Koblet, 1969, 1975; Schaefer, 1978, 1986a, 1986b; Koblet and Perret, 1990; Koblet et al., 1993; Candolfi-Vascocnelos et al., 1994, 1995; Warmund et al., 1986; Schaefer and Schropp, 1987; Schumann and Schaefer, 1988; Vršič, 1996) examined various effects of individual procedures for the cultivation of grafted vines on the level of substances stored in the grafted vines.

Rootstock plays a role in the partitioning of biomass between root, shoot, trunk and fruit. Not only are carbohydrates stored in vine canes evidence of the health and vigor of the previous season's growth (Balasubrahmanyam et al., 1978), in many plant species, root carbohydrates are responsible for shoot development, growth in stem and root diameters as well as new root length, flower bud initiation and growth, and fruit set (Loescheret et al., 1990).

In grafted vines, the root development and healing of the graft union are particulary affected by the water content and by carbohydrates stored in scions and rootstock. The accumulation, transformation and translocation of carbohydrates in individual parts of the grapevine have been described in the literature by various authors and examined various effects of individual procedures for the cultivation of grafted vines on the level of substances stored in the grafted vines (Vršič et al., 2009).

High carbohydrates and levels of specific plant hormones are required for successful callus formation (Hunter et al., 2004), but little work describing relationships between the two has been conducted. Starch is directly involved in callus formation and vegetative growth of rootstocks during callusing (Hunter et al., 2004). Rootstock cultivars affect starch levels

in scions to differing degrees and also vary with respect to starch depletion during callusing, which impact time required for callus development (Phillips et al., 2015).

MATERIALS AND METHODS

Research was carried out at the Research and Development Station for Viticulture and Winemaking (SCDVV) in Iasi in 2019. Two varieties of wine grapes, 'Aromat de Iași' and 'Golia', obtained at SCDVV Iasi, respectively Chasselas doré variety taken as control (Table 1), were grafted on three rootstocks (Riparia Gloire, Berlandieri x Riparia Selection Oppenheim 4-clone 4 and Berlandieri x Riparia Selection Crăciunel 2).

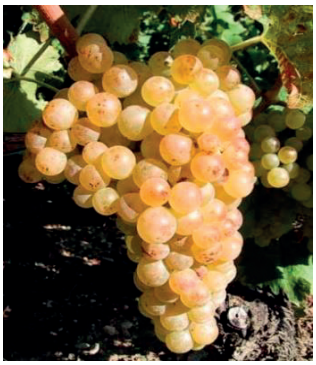
Using the two varieties taken into consideration, respectively the three rootstocks on which the varieties were grafted, two series of determinations were made:

Determinations regarding the total carbohydrate content of the rootstock and scion canes (soluble sugars and starch) by the chemical method with the anthrone reagent and a representation of the starch in the shoots by the colorimetric method based on the staining reaction of the starch with Lugol reagent.

Determinations on several growth parameters after the end of the process of forcing of the grafted cuttings: the percentage of vines fit for planting, the percentage of successful grafting, the percentage of vines with eyes not provided in the vegetation, the callus formation on diameter of the grafted calves, the degree of callus formation of the grafted vines and the location of the roots on the grafted cuttings.

Table 1. Studied biological material

Grape variety	Genitors	Author	Year of homologation
Aromat de Iași 	Free fecundation of Tămâioasă românească seeds	Dănulescu Dumitru	1980
Golia 	Intraspecific hybridation of Sauvignon x Șarbă	Dănulescu Dumitru	1999

Chasselas doré (control) 	Ancient grape variety with uncertain origin. It is supposed to be Swiss.	Unknown	Cultivated since the 11th century

Carbohydrates were determined by the chemical method with the anthrone reagent. The sugars were extracted with ethyl alcohol, respectively starch with perchloric acid, under certain conditions, and treated with anthrone reagent.

When the sugars were extracted, chopped chunks in pieces under 0.5 cm were dried in the oven at 65°C to a practically constant mass. The material thus dried was finely milled to the destruction of the cell structure and then passed through the mill once more. An amount of 0.5 g of the prepared material was weighed to the nearest 0.01 g, mixed with about 0.5 g of sand and 5 ml of ethyl alcohol and stirred by mixing until a homogeneous mass was obtained. To the obtained mixture were added 40 ml of water and then introduced into a polyethylene centrifuge tube. It was centrifuged for 20 minutes at 8000 rpm. The liquid consisting of water and alcohol was poured into a 200 ml graduated flask, and over the remaining sediment in the centrifuge tube 5 ml of warm ethyl alcohol was poured and mixed with a glass wand, until homogenized. Then 20 ml of warm ethyl alcohol were added and centrifuged for 20 minutes. The liquid containing the alcohol was poured over that obtained at the first centrifugation, and the operations were repeated two more times. The flask with the solutions obtained after all the centrifugations is filled to the mark with water. From the obtained basic solution 100 ml were pipetted into a 200 ml volumetric flask and 1 ml of lead acetate solution was added. After 5 minutes it was filled up to the mark with water. After another 10 minutes the solution was filtered and 100 ml of the filtrate was pipetted into another 200 ml volumetric flask. The excess of lead acetate was neutralized by the addition of 1 ml of sodium acid phosphate solution. After 5 minutes, the mixture was made with water and then stirred vigorously. 40 ml of the solution were taken and centrifuged, and the clear and transparent liquid constitutes the sugar extract.

The mixture remaining in the centrifuge tube after the extraction of sugars was mixed with 5 ml of water until a homogeneous mass was obtained and 6.5 ml of perchloric acid solution were added dropwise. The mixture was stirred continuously for 15 minutes then diluted with water and centrifuged again for 20 minutes. The liquid was decanted into a 500 ml graduated flask and the treatment was repeated with perchloric acid and water as well as centrifugation twice more. The liquid was decanted and introduced into the same balloon which was brought to the mark with water. The extract thus obtained represents the starch from the rootstock and from the scion canes respectively.

The calculation, expression and interpretation of the results of the total carbohydrate content (soluble sugars and starch) in the extract are expressed as glucose and calculated with the formulas:

% Soluble sugars (glucose): $\frac{Ec-Ea}{Eb} x\ 50 \left[\frac{\mu g}{ml}\right]$

% Starch (glucose): $\frac{Ed-Ea}{Eb} \times 50 \left[\frac{\mu g}{ml}\right]$

Ea, Eb, Ec, Ed = extinctions of solutions a, b, c, d (average of the 3 determinations);

50 = concentration of standard glucose solution, in µg/ml.

The content of soluble sugars, respectively of starch of the planting material expressed as glucose and related in percentage to the dry planting material at 65°C, is calculated with the formula:

% sugars (glucose) = Z $\frac{200\times4}{m\times10^6}$ x 100 [%]

% starch (glucose) = A $\frac{500}{m\times10^6}$ x 100 [%]

Z = the sugar content of the extract calculated in µg/ml;

A = starch content of extract calculated in µg / ml;

4 = factor for the dilutions performed during the determination;

500 = volume of starch extract in ml;

200 = volume of filtrate from which sugars are determined in ml;

m = mass of dry planting material at 65°C, taken for determination.

RESULTS AND DISCUSSIONS

For a representation of the starch in ropes by the colorimetric method that is based on the staining reaction of the starch with Lugol reagent, the analyzed parts are represented by annual elements of the freshly harvested kernel (fruit ropes). The starch that accumulates in the woody tissues of the annual cords, gives them resistance to the low temperatures in winter and constitutes the nutritional reserve for starting the buds/winter eyes in the spring of next year.

At the end of the resting period, canes were harvested for both rootstock varieties and scion varieties through which cross sections were made using the microtome. These sections were treated with Lugol reagent and allowed for a period of time to dry thus obtaining preparations that were analyzed under a microscope (Figure 1).

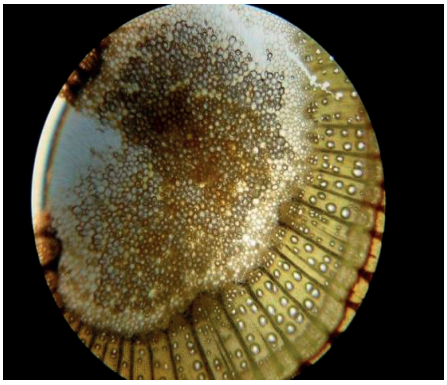


Figure 1. Aspects of the starch content accumulated in the ropes of rootstock and grape varieties

Table 2. Carbohydrates from the ropes of rootstock varieties

Rootstock	Total carbohydrate (%)	Soluble sugars (%)	Starch (%)	Rope moisture (%)
Riparia Gloire	13.49	10.29	3.20	52.71
Selection Oppenheim 4-clone 4 (SO_{4-4})	12.02	9.58	2.44	52.33
Berlandieri x Riparia Selection Crăciunel 2	12.78	9.79	2.99	52.17

Regarding the results obtained regarding the total carbohydrate content in the string rootstocks (Table 2), the best result was registered at the rootstock Riparia Gloire (13.49%) of which the percentage of soluble sugars was 10.29%, and the starch percentage was 3.20%.

The other two rootstock varieties had results close to each other, Berlandieri x Riparia Selection Crăciunel 2 having a percentage of 12.78% total carbohydrates, of which soluble sugars 9.79% and starch 2.99%, and Selection Oppenheim 4-clone 4 (SO_{4-4}), 12.02% carbohydrate of which 9.58% represents the percentage of soluble sugars and 2.44% the percentage of starch. Both varieties had inferior results to the rootstock Riparia Gloire.

Analyzing these results it can be seen that all three varieties of rootstocks are suitable for grafting because the material with a total carbohydrate content (sugars, starch) below 12% is rejected from propagation, the varieties taken into operation having values above that which would have led to the removal from graft.

Table 3. Carbohydrates from the ropes of grape varieties

Scion	Total carbohydrate (%)	Soluble sugars (%)	Starch (%)	Rope moisture (%)
Aromat de Iași	14.97	9.56	5.41	53.74
Golia	15.64	11.38	4.26	56.50
Chasselas doré (control)	13.83	8.63	5.20	57.36

After analyzing the carbohydrates from the rootstock varieties, the content of the carbohydrates from the scion varieties was also analyzed (Table 3). Of the three varieties taken into consideration, the best results were obtained by 'Golia' grape variety, which recorded a total carbohydrate content of 15.64% (11.38%, representing the percentage of soluble sugars , respectively 4.26% starch), followed by the other variety, 'Aromat de Iași', having a total carbohydrate content of 14.97% of which 9.56% represent soluble sugars and 5.41% starch. Both varieties recorded higher values compared to the control variety Chasselas doré (13.83%, of which 8.63% soluble sugars and 5.20% starch).

After it was found that the material used fulfills the necessary conditions for being grafted, it was prepared for grafting. They were grafted using a pedal operated device, by uniting the two partners through a joining tip, in the form of the letter omega, they were covered in paraffin, they were layered and forced into vegetation. The process lasted 21 days in which the temperature was raised in the first days (30°C) and then gradually reduced (25°C), the humidity was between 68% and 91%, in the absence of light on the entire duration of the cycle, due to the fact that the crates were covered with a canvas of geotextile material, over which lay a 5 cm layer of sawdust. After the forcing process was completed, the grafted vines were acclimatized for 5 days. After the completion of the forcing, the grafted vines were analyzed following a series of parameters whose results are presented in table 4.

As for the percentage of vines suitable for planting, 'Golia'/Riparia Gloire variant recorded the best result (98%), while in the 'Aromat de Iași' variety, the best association was at the grafting on the Crăciunel 2 rootstock (96%), both varieties having better results compared to the best variant obtained in the control variety, Chasselas doré/Riparia Gloire (94%).

From the vines that are suitable for planting, the percentage of a good grafting was also analyzed. The best results were obtained by the 'Golia'/SO_{4-4} variant (74%), respectively 'Aromat de Iași'/Riparia Gloire (72%), while in the control variety, the best result was Chasselas doré/Riparia Gloire (68%).

The percentage of vines with the bud that entered vegetation was good, with values of 74% ('Golia'/Riparia Gloire), respectively 72% ('Aromat de Iași'/Crăciunel 2), compared to 68% (Chasselas doré/Riparia Gloire). For both varieties taken into consideration, the percentage of vines where the bud did not enter vegetation was analyzed. The variants 'Golia'/Crăciunel 2 (21%) and 'Aromat de Iași'/Riparia Gloire (23%) had lower values compared to Chasselas doré/Riparia Gloire (26%), the control variety.

The degree of callus formation was another parameter analyzed, here the best results were obtained by the variant 'Golia'/Riparia Gloire (98%) and 'Aromat de Iași'/Crăciunel 2 (96%), these having fully formed callus. The control variety, Chasselas doré/Riparia Gloire (94%) recorded the best results, having a lower value compared to the results obtained in the two varieties taken into consideration. Callus formation on different diameters was also tracked here. The grafted vines were calibrated on the three diameters found in the specialized literature (7-8.5 mm; 8.6-10 mm; 10.1-12 mm), the results obtained being very varied for each variant, both in the varieties taken into operation and in the control variety.

The last parameter analyzed was the one regarding the location of the roots on the grafted vines, namely the place where the grafted vines formed root primordia. All the variants had a high percentage of root primordia occurring at the base of grafted vines, compared to their formation at the second node, where the highest percentage was obtained in 'Aromat de Iași'/SO_{4-4} (7%) and 'Golia'/SO_{4-4} (5%) while the control variety Chasselas doré/Riparia Gloire, had lower values, namely 2%.

Table 4. Results obtained after the completion of the forcing process

Scion	Rootstock	*Vines suitable for planting (%)	**Percentage of grafting (%)	Vines where the buds did not enter vegetation (%)	Diameter of callus (%)			Degree of callus (%)		Root placement (%)	
					7-8,5 mm	8,6-10 mm	10,1-12 mm				
Aromat de Iași	Riparia Gloire	95	72	23	20	26	49	95	5	91	4
	Selection Oppenheim 4-clona 4 (SO_{4-4})	95	70	25	34	31	30	95	5	88	7
	Berlandieri x Riparia Selection Crăciunel 2	96	71	25	30	32	34	96	4	92	4
Golia	Riparia Gloire	98	70	28	30	35	33	98	2	95	3
	Selection Oppenheim 4-clona 4 (SO_{4-4})	96	74	22	38	32	26	96	4	91	5
	Berlandieri x Riparia Selection Crăciunel 2	93	72	21	32	38	23	93	7	89	4
Chasselas doré (control)	Riparia Gloire	94	68	26	29	42	23	94	6	92	2
	Selection Oppenheim 4-clona 4 (SO_{4-4})	92	67	25	37	30	25	92	8	91	1
	Berlandieri x Riparia Selection Crăciunel 2	91	66	25	29	36	26	91	9	90	1

*Percentage of vines suitable for planting (%) - vines with complete callus formed at the point of grafting, with buds started in vegetation and with root primordia + vines with complete callus formed at the point of grafting, primordia of root formed, without buds started in vegetation.
**Percentage of grafting (%) - calves with complete callus formed at the grafting point, with buds started in vegetation and with root primordia.

CONCLUSIONS

The total content in carbohydrates was maximal in the case of the rootstock Riparia Gloire (13.49%), which was also reflected on the high percentage of grafted vines that developed a root system (98%).

The physiological humidity of the analysed canes was within normal limits, both in the scion and in the rootstocks (52-57%), which showed that the graft material was kept under proper conditions.

The callus formation for different diameters shows that the best ratio was at Riparia gloire and Selection Crăciunel 2 with the diameters of 8.6-10 mm, respectively 10.1-12 mm, and at the rootstock SO_{4-4} at the diameters of 7-8.5 mm, respectively 8.6-10 mm.

The percentage of vines with the most numerous basal roots was registered at the Riparia gloire rootstock (95-96%).

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NURSERY VEGETATIVE GROWTH OF 'GELU' AND 'PAULA' GRAPE VARIETIES, BY ANALYSIS OF FOLIARY PHOTOSYNTHETIC PIGMENTS

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Abstract

The physiological role of the assimilating pigments is complex, being involved in the processes of oxidation-reduction, in the photosynthesis and protection processes against ultraviolet radiation. The study aims to track vegetative growth during the vegetation period by spectrophotometrically determining the content of chlorophylls (a and b) and carotenoids from the leaves of vine varieties (Vitis vinifera L.) for table grapes 'Gelu' and 'Paula', new varieties created within the Research and Development Station for Viticulture and Winemaking Iasi. The two varieties were grafted on three rootstocks (Riparia Gloire, Berlandieri x Riparia Selection Oppenheim 4-clone 4 and Berlandieri x Riparia Selection Crăciunel 2), thus creating six working variants. Following the completion of the observations, 'Gelu'/Riparia gloire variant recorded the best results in terms of vegetative growth in the vegetation period measuring 45.39 cm (before wood maturation) followed by 'Paula'/SO4-4, which recorded 37.32 cm at the last measurement. Regarding the determination of the content of photosynthetic pigments (chlorophyll a and b, respectively carotenoids), the best results were obtained in the 'Gelu' variety on all rootstocks. 'Paula' grape variety registered smaller values.

Key words*: carotenoids, chlorophyll a and b, photosynthetic pigments, vegetative growths.*

INTRODUCTION

First isolated by Caventou and Pelletier in 1817, chlorophyll (gr. *chloros* - green; *phyllon* - leaf) is a biomolecule critical in photosynthesis (gr. *photos* - light; *synthesis* - building a whole), which allow plants to absorb energy from light (Davies, 2004).

Chlorophyll *a* and chlorophyll *b* are the major types of chlorophylls found inplants (Wilows, 2004). They have a characteristic green colour due to strong absorbance of blue and red light. The increased proportion of chlorophyll *b* is due to its absorption in the 450-480 nm range, capturing effectively light at low intensity (Lichtenthaler and Wellburn, 1983).

Photosynthetic pigments are represented by green chlorophyll pigments (chlorophylls a and b) and yellow carotenoid pigments (carotenes and xanthophylls), being essential compounds in light energy conversion (Toma and Jităreanu, 2007). Carotenoids are a large group of deeply red or yellow fat-soluble pigments (Pfander, 1992). Carotenoids are found in all photosynthetic organisms, being involved in photosystem assembly and contributing to light harvesting by absorbing light energy in a region of the visible spectrum where chlorophyll absorption is lower and by transferring the energy to chlorophyll. Also, carotenoids provide protection from excess light, free radical detoxification and limiting damage to membranes (Cuttriss and Pogson, 2004).

Rootstock influences vegetative growth thereby increasing the photosynthesis of vine (Somkuwar et al., 2015; Köse and Çelik, 2017). The differences in vegetative growth patterns affect gas exchange by altering source/sink relations (Ezzahouani, 1995). The effect of rootstocks on photosynthetic activity is scion specific (During, 1994).

The study aims to track vegetative growth during the vegetation period depending on rootstock, measuring the content of chlorophylls (a and b) and carotenoids from the leaves of new vine varieties (*Vitis vinifera* L.) for table grapes 'Gelu' and 'Paula', new

created within the Research and Development Station for Viticulture and Winemaking Iaşi.

MATERIALS AND METHODS

In order to carry out this study, research was conducted within the Research and Development Station for Viticulture and Winemaking in Iaşi, in 2019. Two varieties of table grapes, 'Gelu' and 'Paula', obtained at SCDVV Iaşi (Table 1), were grafted on three rootstocks (Riparia Gloire, Berlandieri x Riparia Selection Oppenheim 4-clone 4 and Berlandieri x Riparia Selection Crăciunel 2), thus creating six working variants.

Using the two varieties taken into consideration, respectively the three rootstocks on which the varieties were grafted, two series of determinations were made:

Determinations regarding the content in photosynthetic pigments, chlorophyll (a and b) and leaf carotenoids for each created variant and determinations on vegetative growth during the vegetation period.

Table 1. Studied biological material

Grape variety	Genitors	Author	Year of homologation
 Gelu	Free fecundation of local grape variety Coarnă neagră and irradiation with X rays of its seeds	Calistru Gheorghe Damian Doina	1999
 Paula	Intraspecific sexual hybridation of Bicane x Aromat de Iaşi	Calistru Gheorghe Damian Doina	1997

The determination of the content in photosynthetic pigments from leaves was performed by the extraction of chlorophyll (a and b) and carotenoids (xanthophylls and carotenoids). The harvested leaves were crushed and 0.5 g was weighed for each variant, after which this amount was infused with 10 ml acetone 99.98%. 0.5 mg of $MgCO_3$ was added during extraction to neutralize the acids responsible for the formation of pheophytin a in chlorophyll a. The samples thus obtained were stored overnight in a cold environment. The fractions obtained were subsequently centrifuged using a Nahita 2816 cooling centrifuge for 15 minutes, at 3000 rotations per minute, at a temperature of 10 °C. The analytical determinations were performed using a Shimadzu 1700 Pharmaspec UV-vis spectrophotometer at wavelengths 662, 645 and 710 nm for chlorophyll a and b, respectively 470 nm for carotenoids. The pigment content was calculated in mg/g fresh substance, using the equations proposed by Lichtenthaler and Buschmann and completed by the Carnegie Institute of Science by Spectranomis Protocol.

Chl *a* ($\mu g\ mL^{-1}$) = 11.24 × (A662 – A710) – 2.04 ×(A645 – A710);

Chl *b* ($\mu g\ mL^{-1}$) = 20.13 × (A645 – A710) – 4.19 ×(A662 – A710);

Carotenoids (μg mL^{-1}) = (1000 × (A470 – A710) – 1.90× Chl *a* – 63.14 × Chl *b*)/214.

Simultaneously with the determination of the content in photosynthetic pigments from the leaves, the humidity of the harvested leaves was determined, by drying for four hours in the oven, at a temperature of 105°C.

The determinations of the vegetative growths during the vegetation period were made by measuring the shoots every 15 days starting with 1st June 2019, the last measurement being on 15th August 2019.

RESULTS AND DISCUSSIONS

In order to determine the content of chlorophyll pigments, the leaves moisture content was also determined (Table 2).

From the table above it can be observed that the leaf moisture for all six variants taken into account registered higher values in June, gradually decreasing in July and August respectively. In June, the highest values were recorded for the variants 'Paula'/Crăciunel 2 (78.12%) and 'Gelu'/Crăciunel 2 (75.59%),

Table 2. Leaves moisture (%)

Scion	Rootstock	Humidity (%)		
		June	July	August
Gelu	Riparia Gloire	74.19	73.85	71.28
	Selection Oppenheim 4-clone 4 (SO_{4-4})	75.13	74.12	70.00
	Berlandieri x Riparia Selection Crăciunel 2	75.59	72.68	70.42
Paula	Riparia Gloire	74.84	73.81	72.87
	Selection Oppenheim 4-clone 4 (SO_{4-4})	76.56	75.16	71.67
	Berlandieri x Riparia Selection Crăciunel 2	78.12	76.79	73.91

after which they decreased slightly and the best results were in July registered in the 'Paula'/Crăciunel 2 (76.79%) and 'Gelu'/SO_{4-4} (74.12%) variants.

In August the results were even lower compared to the previous months, the best values being obtained at 'Paula'/Crăciunel 2 (73.91%) and 'Gelu'/Riparia gloire (71.28%).

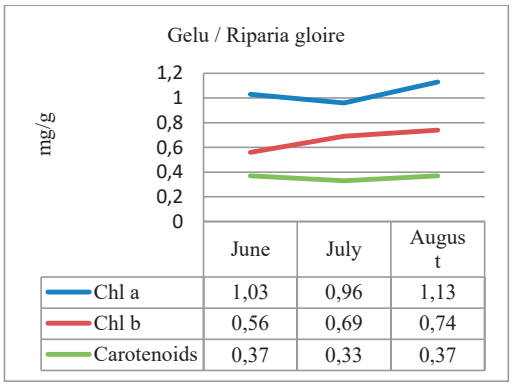
After determining the humidity of the harvested leaves, the analysis of the foliar photosynthetic pigments was studied (Figure 1).

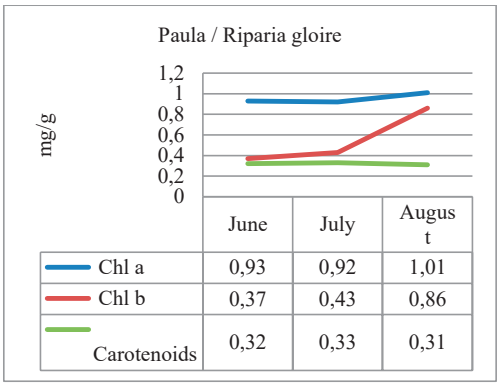
Regarding the determination of the content of photosynthetic pigments (chlorophyll a and b, respectively carotenoids), the best results were obtained in the 'Gelu' variety for all the variants on which it was grafted, compared to the 'Paula' variety which had weaker results.

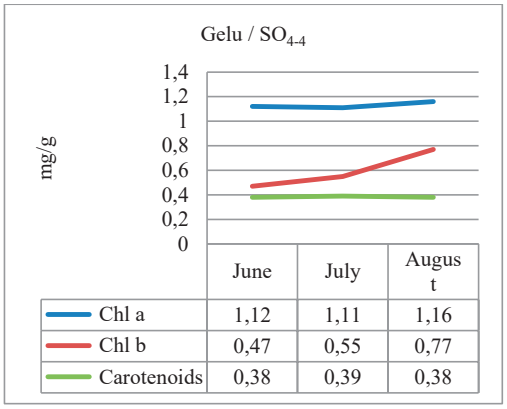
Chlorophyll a, in June, in the variant Gelu/SO_{4-4} had the highest values of 1.12 mg/g fresh substance, and in the Paula variety the best result was recorded at the grafting with Riparia gloire having 0, 93 mg/g fresh substance. For chlorophyll b, the best results were recorded for the variants 'Gelu'/Riparia gloire (0.56 mg/g) and 'Paula'/SO_{4-4} (0.39 mg/g), and for the carotenoids the best results were recorded at 'Gelu'/SO_{4-4} (0.38 mg/g) and 'Paula'/Riparia gloire (0.32 mg/g).

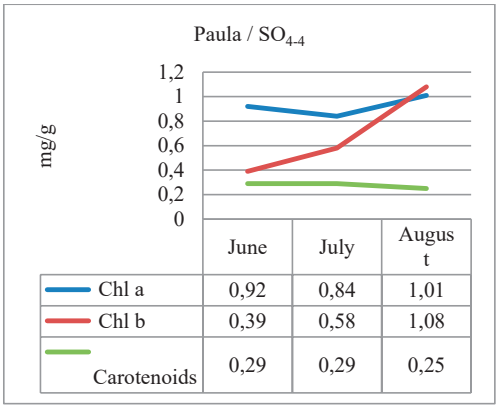
The determination of the content of photosynthetic pigments from the leaves was carried out in July, where for the chlorophyll the best results were obtained by the variants 'Gelu'/SO_{4-4} (1.11 mg/g), respectively 'Paula'/Riparia gloire (0.92 mg/g). Chlorophyll b, recorded higher values compared to the previous month for all variants taken into consideration, and the best results were obtained at 'Gelu'/Riparia gloire (0.69 mg/g) and 'Paula'/SO_{4-4} (0.58 mg/g). For the last pigment analyzed, the carotenoids, the variants that were highlighted were 'Gelu'/SO_{4-4} (0.39 mg/g) and 'Paula'/Riparia gloire (0.33 mg/g).

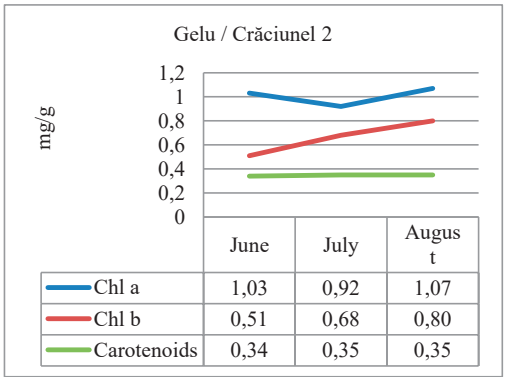
The last determination of the content of the photosynthetic pigments from the leaves was made in August, before the process of maturation of the shoots and of the appearance of the phellogen, the results obtained being higher in comparison with the other two months in which they were analyzed. For chlorophyll a, the best results were recorded for the variants 'Gelu'/SO_{4-4} (1.16 mg/g) and 'Paula/Riparia gloire respectively 'Paula'/SO_{4-4} at which the same values were obtained (1.01 mg/g). Chlorophyll b was found in the leaves of the Paula/SO_{4-4} variant (1.08 mg/g) and Gelu/Crăciunel 2 (0.80 mg/g), and the last pigment analyzed, the carotenoids, had the highest results in the 'Gelu' variant./SO_{4-4} (0.38 mg/g) and 'Paula'/Riparia glory (0.31 mg/g).











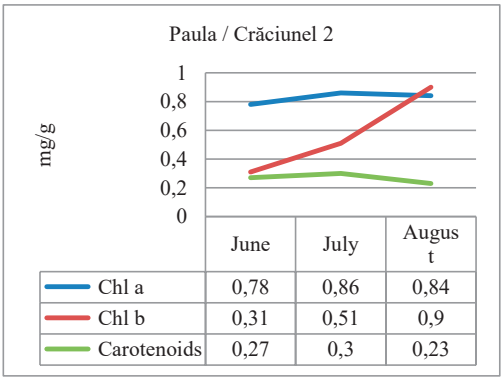


Figure 1. Dynamics of the content of photosynthetic pigments

The vine is a plant adapted to the conditions of insolation or semi-shade (Warren, 2013). Mittal et al. (2011), states that the ratio of chlorophyll a and b varies between 2.0 and 3.2 for plants adapted to shade conditions and 3.5 to 4.9 for plants adapted to insulation conditions.

According to Toma and Jitareanu (2007), the ratio of chlorophyll a / b, to the species *Vitis vinifera* L. is maximum at the beginning of the vegetation period, reaching up to a ratio of 3/1 and decreases during the maturation period of the grapes, while the ratio chlorophyll / carotenoids can record ratiosof 4/1.

After determining the content of photosynthetic pigments, two reports were made between the analyzed pigments, the first being between chlorophyll a and b, and the second one was between the sum of the two chlorophyll pigments and carotenoids (Table 3).

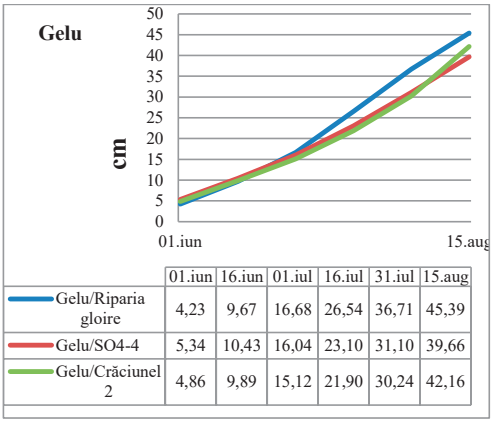
Table 3. Photosynthetic pigment reports

Scion	Rootstock	Chlorophyll a/b ratio			Chlorophyll ratio (a + b) / carotenoids		
		June	July	August	June	July	August
Gelu	Riparia Gloire	1.83	1.39	1.52	4.29	5.00	5.05
	Selection Oppenheim 4-clone 4 (SO_{4-4})	2.38	2.01	1.50	4.18	4.25	5.07
	Berlandieri x Riparia Selection Crăciunel 2	2.01	1.35	1.33	4.52	4.57	5.34
Paula	Riparia Gloire	2.51	2.13	1.17	4.06	4.09	6.03
	Selection Oppenheim 4-clone 4 (SO_{4-4})	2.30	1.44	0.93	4.51	4.89	8.36
	Berlandieri x Riparia Selection Crăciunel 2	2.51	1.68	0.93	4.03	4.56	7.56

The chlorophyll a/b ratio for the Gelu variety in June registered the highest value (2.38) at grafting on the SO_{4-4} rootstock while the same ratio for the Paula variety, obtained the best result (2.51), both at the grafting on the rootstock Riparia Gloire and on the rootstock Crăciunel 2. In July both varieties had the best values of the ratio of chlorophyll a / b to the variants Gelu/SO_{4-4} (2.01) and Paula/Riparia gloire (2.13), and in August the variants with the highest values were Gelu/Riparia gloire (1.52) and Paula / Riparia gloire (1.17).

For the second report, between chlorophyll (a + b) / carotenoids, the variant that was highlighted in June, Gelu / Craciunel 2 (4.52), was closely followed by Paula/SO_{4-4} (4.51). In July the variants grafted on the SO_{4-4} rootstock recorded the highest values, Paula (4.89) and Gelu (4.25). In the last analysis in August the variant Paula/SO_{4-4} (8.36), had the best result, while in the other variety taken into account the variant Gelu/Crăciunel 2 (5.34) was highlighted.

In parallel with determining the content of foliar photosynthetic pigments, measurements were also made on the vegetative growths of the vines nursery. Six measurements were made, the first being on 01/06/2019. The measurements were made at an interval of 15 days from each other, the last being on 15/08/2019 (Figure 2).



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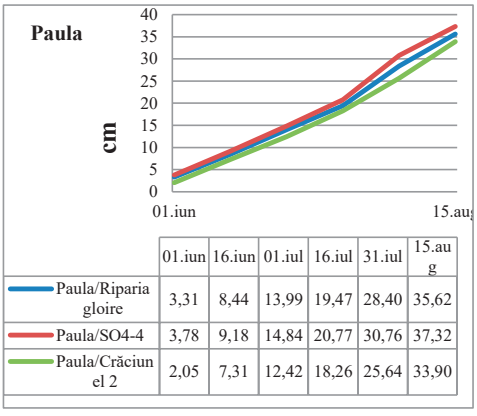
Gelu/Riparia gloire	4,23	9,67	16,68	26,54	36,71	45,39
Gelu/SO4-4	5,34	10,43	16,04	23,10	31,10	39,66
Gelu/Crăciunel 2	4,86	9,89	15,12	21,90	30,24	42,16



	01.iun	16.iun	01.iul	16.iul	31.iul	15.aug
Paula/Riparia gloire	3,31	8,44	13,99	19,47	28,40	35,62
Paula/SO4-4	3,78	9,18	14,84	20,77	30,76	37,32
Paula/Crăciunel 2	2,05	7,31	12,42	18,26	25,64	33,90

Figure 2. Dynamics of vegetative growth

From the above figure it can be observed that in the 'Gelu' variety, the first two measurements the largest vegetative growths were recorded at the graft on the SO_{4-4} rootstock, after which the 'Gelu'/Riparia gloire variant had the most significant increases. On the other hand, in the 'Paula' variety, the best results were recorded by the 'Paula'/SO_{4-4} variant for all the measurements made.

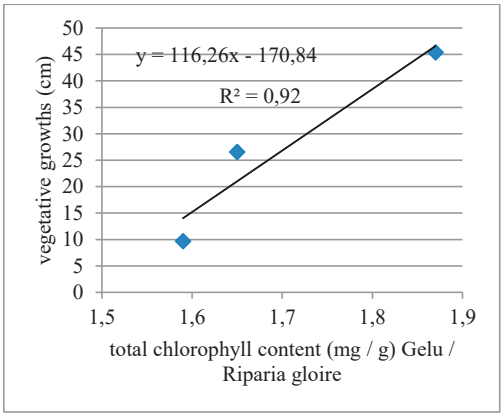
At the first measurement performed on 01/06 the best results were recorded by the 'Gelu'/SO_{4-4} variant which was 5.34 cm, and

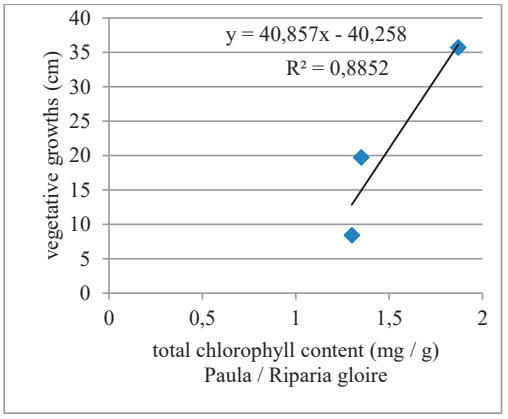
'Paula'/SO_{4-4} was 3.78 cm. The second measurement was made after 15 days, more precisely on 16/06, and the variants that had the largest increases were 'Gelu'/SO_{4-4} (10.43 cm) and 'Paula'/SO_{4-4} (9.18 cm).

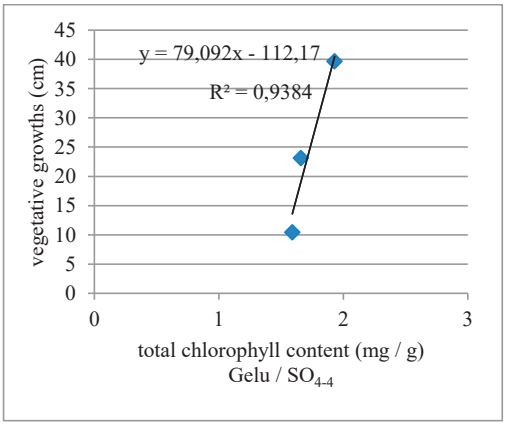
After another 15 days, the third measurement was made on 01/07, where the highest results were recorded in the 'Gelu'/Riparia gloire variant (16.68 cm), respectively 'Paula/SO_{4-4} (14.84 cm). The fourth measurement on 16/07 highlighted the variants Gelu/Riparia gloire (26.54 cm) and Paula / SO_{4-4} (14.84).

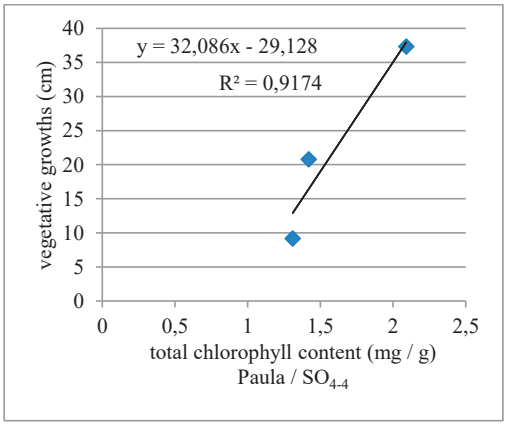
The fifth measurement on 31/07, highlighted the variants 'Gelu'/Riparia gloire (36.71 cm) and 'Paula'/SO_{4-4} (30.76 cm), and on the last measurement on 15/08, the highest vegetative growths were in the 'Gelu'/Riparia gloire (45.39 cm) and 'Paula'/SO_{4-4} (37.32 cm) variants.

Following the correlation between the vegetative growths and the amount of chlorophyll (a + b), a direct and linear relation is found, in the sense that the higher the content in the chlorophyll the higher the vegetative growths. This behavior was observed also in other situations, probably because of the mineral uptake stimulation by rootstocks (Fekete et al., 2013). From the analysis of figure 3, it can be seen that the values of the correlation coefficient (R2) for the Gelu variety were 0.9200 for the grafted variant on the Riparia gloire rootstock, 0.9384 on the SO_{4-4} rootstock and 0.9583 on the Crăciunel rootstock 2. For the other variety taken into consideration, Paula, the values of the correlation coefficient had values of 0.8852 in the variant grafted on the rootstock Riparia gloire, 0.9174 on the rootstock SO_{4-4} and 0.9995 on the rootstock Crăciunel 2. In both cases the value of the coefficient correlation was over 75%, indicating that there is a direct linear correlation between the two factors analyzed.









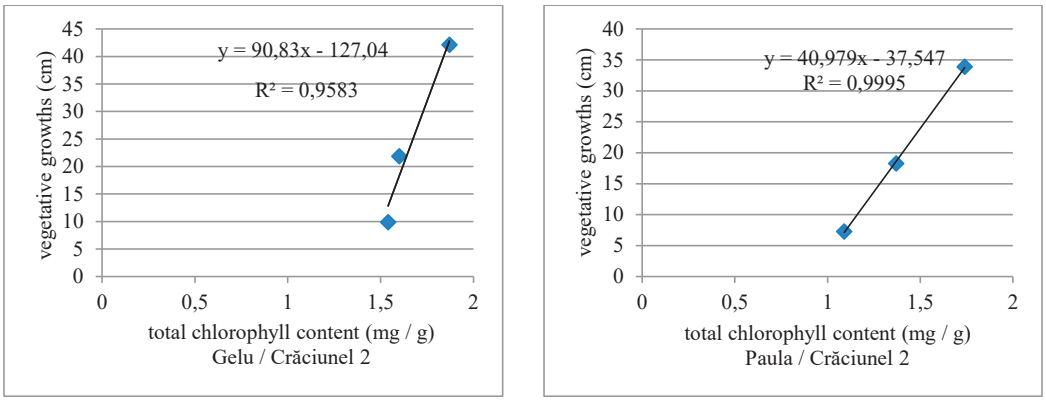


Figure 3. Correlation between total chlorophyll content and vegetative growth of vines

CONCLUSIONS

From the analysis of the moisture of studied vine leaves, it is ascertained that it decreases as they go towards the senescence phase; however, the values are kept within the physiological limits of the plant. The highest humidity was recorded in June on the 'Paula'/Crăciunel 2 version, and the lowest on the 'Gelu/'SO_{4-4} version, in August.

Regarding the ratio of chlorophyll a/chlorophyll b, it is found that it is specific to a semi-shade plant, being the highest in June, between 1.83-2.38 in the variety 'Gelu' and 2.30-2.51 in the Paula variety. As the plant grows older, it has lower values, due to the fact that here we are witnessing the development of a vine and an adult plant. The ratio of chlorophyll (a + b)/carotenoids has been 4/1 higher since June, increasing as the age progresses, to 8.36 in the Paula/SO_{4-4} variant. The correlation coefficient R^2 was calculated in order to establish the direct correlation between the vegetative growths and the total chlorophyll content. It had values indicating the existence of a direct and linear correlation between increasing the content of chlorophyll pigments and the vegetative growths of the vines, having values of over 0.75.

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ASSESSMENT OF THE OAK CHIPS INFLUENCE ON THE COLOUR AND SENSORY PARAMETERS OF FETEASCA NEAGRA BY RAPID MATURATION SIMULATION

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Abstract

Feteasca Neagra is a Romanian black grape variety, which is vinified in various styles, but seems to evolve better when maturated in oak barrels. To rapidly assess the evolution of this variety in the presence of wood, an experiment was designed to account for the contact with wood, as well as the presence of lower or medium oxygen levels. Oak chips of different origins (American and French) were used, each with a medium and high degree of toasting, respectively. Freshly obtained wines were kept in contact with 3 g/l of these oak chip samples for 2 months, either protected from air or in conditions simulating the oxygenation level obtained during barrel maturation. The colour of the samples was assessed spectrophotometrically after 2, 4, 6 and 8 weeks of wood contact. Sensory analysis of wines was also performed after 2 months of wood contact. After this short experimental period it was observed that oxygen is correlated with a reduced clarity and is inducing a more yellow hue in the wines, irrespective of the time of assessment. Furthermore, chips are changing the initial colour of wines bringing some yellow hues of their own, especially in the case of medium-toasted oak chips, but in time this effect fades away. The intensity of colour is, however, increased by the highly-toasted chips, the effect being also reduced by oxidative maturation. The sensory analysis showed that the overall quality of the control sample is the most affected by the presence of oxygen, while the aftertaste also scored lower than in the case of wines produced with chips contact. During this short time of experimentation the toasting degree of the chips did not significantly influence the colour parameters and sensory scores, but the origin of the chips is starting to show a sensory influence when wines maturate, proven by a slight increase in the sensory scores for samples treated with American chips in the presence of moderate oxygenation level. Considering these findings it seems that Feteasca Neagra would indeed benefit from wood contact, especially with oak of American origin. The abstract is too long (maximum 180 words or 10 rows)

Key words*: French oak; American oak; chips toasting level; CIELab parameters; sensory analysis*.

INTRODUCTION

Wine maturation is a well-known winemaking practice for improving wine quality and organoleptic characteristics (Breton *et al.*, 2018). The maturation of red wines in oak barrels is a technique commonly used in many wineries (del Álamo-Sanza *et al.*, 2004; OIV, 2019a resolution 8/2001), but alternative methods are also available (Oberholster *et al.*, 2015; OIV, 2019a resolution 9/2001; OIV, 2019b 3/2005, 430/2010, 406/2011). The most common oak species used for barrel making are the French and Eastern European oak *Quercus petraea* Liebl. and *Q. robur* L., respectively or the American oak, *Q. alba* L. (Jordão *et al.*, 2005; Cadahía *et al.*, 2009; Alañón *et al.*, 2011). Besides the positive effects of oak wood on wine flavour, traditional wine maturation in oak barrel allows wine compounds to interact with constant low doses of oxygen, which is an important factor for producing quality red wines (Bautista-Ortín *et al.*, 2008) with stable colour and sensory characteristics. Many studies revealed that barrel walls are permeable to oxygen and permit the transfer of the oxygen from the air to wine at moderate rates, between 15 and 45 mg/l/year, on the condition that the barrels are new, hermetically sealed and with wet staves (Ribereau-Gayon, 1933; Vivas and Glories, 1997; del Alamo-Sanza and Nevares, 2018). Due to the porosity of oak wood a dose of ≈0.135 mg O_2 are transferred by any gram of wood into the wine at the moment of first contact (García-Estévez *et al.*, 2017). A decreasing permeability for oxygen is observed in old barrels with a rate of about 10 mg/l/year for five-year-old barrels (Vivas and Glories,

1997; del Alamo-Sanza and Nevares, 2018). However, in order to reduce the time and costs, maturation of red wines can be accomplished directly in the inox tanks, by using oak-based alternative products (powder, chips, cubes or staves) in combination with micro-oxygenation (MOX) technique. Introducing oak wood products directly in wines proved to be a suitable practice, economically beneficial for early release of young red wines on the market (Oberholster *et al.*, 2015; Kyraleou *et al.*, 2016). Anyway, for applying these techniques with positive results, winemakers also need a micro-oxygenation unit fitted to the stainless tanks, as well as experience and knowledge regarding oxygen management. Time of contact, oak type and toasting, dosage levels in wine are also important factors. Many oak wood studies evaluated the volatile compounds released into the wines (Jordão *et al.*, 2005; Jordão *et al.*, 2006a; Chira and Teissedre, 2013), mostly phenols (Jordão *et al.*, 2007; Jordão *et al.*, 2012; Chira and Teissedre, 2013; 2015; Izquierdo-Cañas *et al.*, 2016), but also other compounds with impact on wine chemical matrix and sensory properties (de Coninck *et al.*, 2006; Gonçalves and Jordão, 2009; Oberholster *et al.*, 2015).

MATERIALS AND METHODS

This study used a young red wine produced from Feteasca Neagra in 2018 through classic red winemaking technology at Pietroasa Viticulture and Enology Research and Development Station. The grapes were harvested on 25th September 2018, sulphited with 30 mg/kg SO_2, then crushed and destemmed. Crushed grapes were treated with an extraction enzyme Enovin Color 2 g/q and, after homogenization, with a mixture of condensed and hydrolysable tannins Tanicolor Super 15 g/hl and then inoculated with 20 g/hl active dry yeast Viniferm TTA (Agrovin). The maceration-fermentation was conducted at 25-28°C for 4 days in a roto-fermentor. After 4 days of skin contact during maceration, the fermented grapes were pressed and the resulted wine allowed to continue the alcoholic and malolactic fermentations in 100 hl tanks. Once the malolactic fermentation finished, the wine was racked off lees, acidified with 1 g/l tartaric acid and sulphited with 50 mg/l SO_2. The main wine parameters determined based on the standardized methods (OIV, 2018) were: 15.6% vol. alc.; 6.04 g/l total acidity expressed as tartaric acid; 0.78 g/l volatile acidity expressed as acetic acid; 25.9 g/l total dry extract; 24.1 g/l non-reducing extract; 1.8 g/l reducing sugars. For the maturation experiment, the base young wine was introduced in bottles of 1.0 litre capacity with a dose of 3 g/l oak chips and left for 2 months at 16°C. The chips used were of different types (two different levels of toasting, medium or high, and two botanical origin, French or American) and maturation was performed with two different levels of oxygen. Wine sample variants were prepared in triplicate and are detailed in Table 1.

Table 1. Types of oak chips and level of oxygen used for the variants of fast maturation of red wine

Codification of samples	Oak botanical origin	Toasting level	Oxygen level low*	Oxygen level moderate (barrel-like)**
FR_M	French oak	Medium	x	
FR_M_OX	*Quercus petraea*			x
FR_H		High	x	
FR_H_OX				x
AM_M	American oak	Medium	x	
AM_M_OX	*Quercus alba*			x
AM_H		High	x	
AM_H_OX				x

*Low oxygenation obtained by fully occupying the space in the bottles; **Moderate oxygenation obtained by leaving a headspace of 7 ml of air in 1-litter bottles, resulting in rough <4 mg O_2/l/month.

The level of oxygenation was implemented by taking into account the reference value of 50 mg/l/year of oxygen, considered valid for new oak barrels, which means that approximately 0.14 mg O_2/l/day are required to simulate an oak barrel oxygenation medium, with a very good oxygen transmission rate (OTR). To accomplish this OTR, the experiment was

designed to have the bottles opened every two weeks to permit the oxygen to enter into the headspace in a controlled manner. To be able to do this, the headspace was readjusted every time the bottle was opened in order to introduce about 1.92 mg O_2/l/2 weeks. To put this scenario into practice, based on the desired oxygen level per bottle, we have calculated the required headspace and the filling level, taking in to account the ambient temperature of 16°C. Our estimations used the ideal gas law, the transformation of mg into ml of oxygen, the calculated density of oxygen at our ambient temperature, the molar mass of oxygen which is 31.99 g/mol, temperature (16°C) and universal gas constant, 0.08206 $l \cdot atm \cdot K^{-1} \cdot mol^{-1}$. The resulted density of oxygen ($\rho^{16°C}$ O_2=1.348216 kg/m^3) allowed us to estimate the volume of oxygen in ml (≈1.42 ml O_2) for our experimental conditions. However, knowing that the content of oxygen in the atmosphere is 20.95% by volume (Williams, 2019), the headspace would be about 6.78 ml of air.

Because every 2 weeks when the bottles were opened wine was also taken for analyses, in order to simplify the methodology and keep the headspace in the bottles constant, the removed wine for analyses was replaced with the same amount of control wine, keeping in each bottle a headspace of 7 ml.

A UV-VIS spectrophotometer Analytik Jena AG Specord 250 equipped with WinAspect software version 2.2.7 was used to determine CIELab parameters. The CIELab parameters (OIV, 2018: method OIV-MA-AS2-11) were calculated automatically for each wine from the transmittance spectrum measured every 5 nm in the range of 380-780 nm, using glass cuvettes of 2 mm optical path length.

The sensorial analyses were performed after the maturation period, by a team of five evaluators, using a simplified U.C. Davis 20-point system (Table 2) developed for rating of large number of experimental samples (Amerine *et al.*, 1959; Bamforth and Cook, 2019; AWS, 2020). Accordingly, the evaluators analysed the following sensory traits awarding scores for each as follows: visual aspect (clarity and colour), maximum 3 points; olfactory characteristics (intensity and quality), maximum 6 points; olfacto-gustatory and mouthfeel traits (intensity and quality), maximum 6 points; aftertaste, maximum 3 points and overall impression (harmony), maximum 2 points. Wine rating according to their scores and the perceived quality can be: Extraordinary (18 to 20 points); Excellent (15-17 points); Good (12-14 points); Acceptable (9 to 11 points); Deficient (6 to 8 points); Poor and Objectionable (0 to 5 points).

Table 2. Implementing scores according to the perceived quality characteristics of the wine (AWS, 2020)

Assigned score	Visual (0-3 points)	Olfactory (0-6 points)	Olfacto-gustatory and mouthfeel (0-6 points)	Aftertaste (0-3 points)	Overall impression (0-2 points)
6	-	Extraordinary	Extraordinary	-	-
5	-	Excellent	Excellent	-	-
4	-	Good	Good	-	-
3	Excellent	Acceptable	Acceptable	Excellent	-
2	Good	Deficient	Deficient	Good	Excellent
1	Poor	Poor	Poor	Poor	Good
0	Objectionable	Objectionable	Objectionable	Objectionable	Poor

RESULTS AND DISCUSSIONS

The influence of the oak origin, regardless of oxygenation level

The evolution of clarity (parameter *L*, limpidity or lightness, Figure 1, left) followed a specific pattern in all samples during the experimental period, uncorrelated with the oxygenation level, chips presence or degree of chips toasting. An increase in clarity during the first 48 days of maturation was observed in all samples, followed by a decrease during the next 16 days. On the last 16 days of the maturation, control samples, without oak chips contact, recorded a higher drop in clarity than the samples treated with oak chips. However, the French oak seems to provide the best final clarity (*L*), while the American oak showed a behaviour closer to that of the control samples. An explanation can be the different level of tannin in the oak of different origins (able to precipitate some of the proteins and modify the colour intensity). Other

explanation for the reversal of the wine lightness after the first 48 days could be the beginning of precipitation of unstable colour matter in young red wines, a temporary phase in their path towards stabilisation, as well as an increase in colour intensity due to wine oxidative evolution.

On the other hand, the yellow component of the colour, the parameter +*b* (Figure 1, right) followed a decreasing trend during the first 48 days, while after another 16 days of maturation the positive evolution was reversed, the parameter b showing a sharp increase. The other CIELab parameter, +*a*, which correlates with the red colour, followed an increasing trend in the first 48 days, but remained constant afterwards (Figure 1, right). On the 64th day, the reddest were the repetitions of the control wine (Figure 1 right, parameter +*a*), followed by those treated with French oak and then by those obtained in the presence of the American oak. In the same time (Figure 1, right), the less yellow samples (parameter +*b*) samples were those treated with French oak, thus better preserving the hue of the red young wines, in which the yellow shades are not appreciated, being considered a sign of premature evolution.

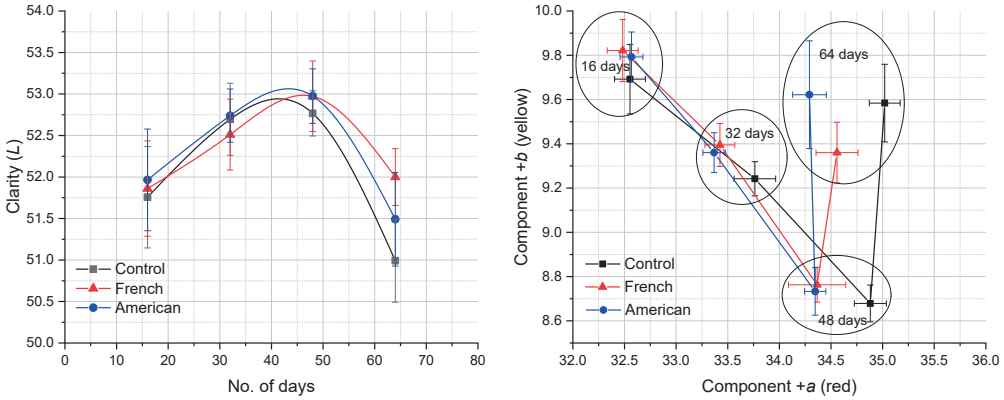

Figure 1. The influence of the oak origin, regardless of oxygenation level, on the CIELab parameters: left - clarity (*L*) evolution; right - red and yellow colour evolution in the *ab* colour space

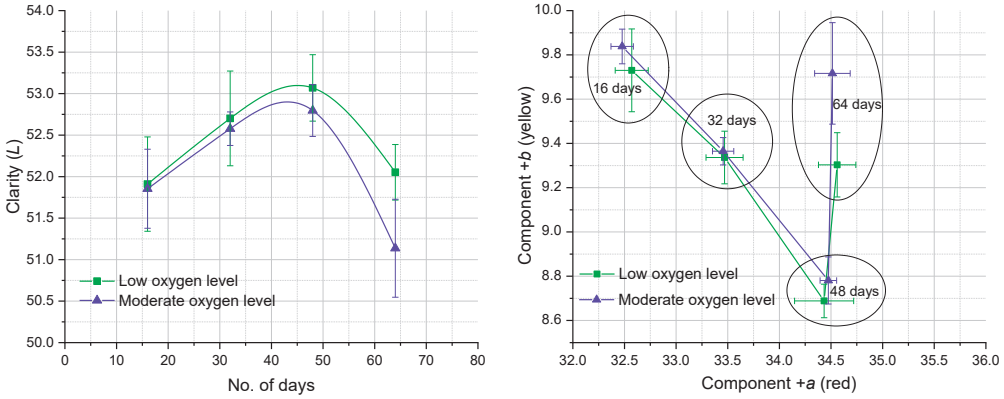

Figure 2. The influence of the oxygenation level, regardless of the oak chips type, on the CIELab parameters: left - clarity (*L*) evolution; right - red and yellow colour evolution in the *ab* colour space

The influence of the oxygenation level, regardless of the oak chips type

Both moderate and low oxygen levels (Figure 2, left) increase the clarity (*L*) similarly during the first 48 days, while on the last 16 days, when the wines start to stabilize, the samples exposed to low oxygen levels, have the tendency to keep a better clarity than those exposed to more oxygen (moderate levels).

Thus, introducing a moderate level of oxygen in the wine leads to a lower lightness, which can also correlate to a higher colour intensity. On the other hand, the results from Figure 2, right, show an increase of redness (parameter +*a*) and a decrease of yellowness (parameter +*b*) during the first 48 days in a similar way for both groups, low- and moderate-oxygen level. On the last 16 days, the redness (parameter +*a*) stagnates, while yellowness (parameter +*b*) increases significantly in all cases, but more pronounced for samples exposed to moderate oxygen level, a sign of higher oxidation. These results suggest an accelerated maturation process in samples exposed to a moderate level of oxygen, as compared to the cases of only low oxygen levels.

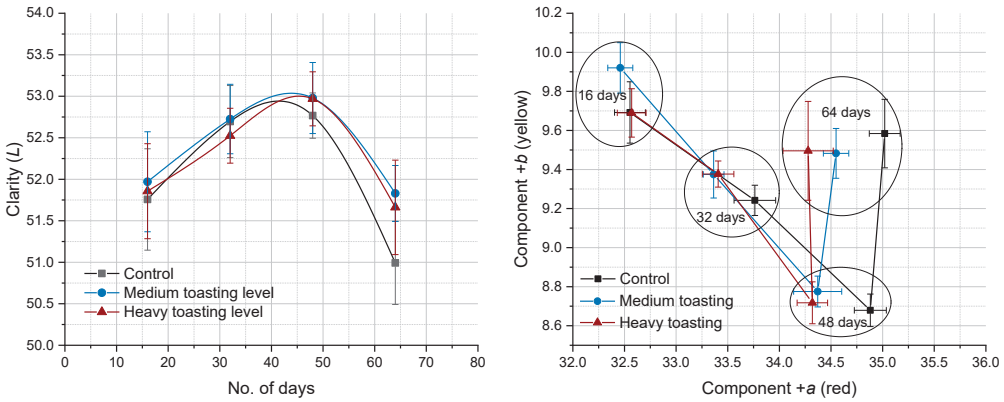

Figure 3. The influence of the oak chips toasting level, regardless of oak chips origin and oxygenation level, on the CIELab parameters: left - clarity (*L*) evolution; right - red and yellow colour evolution in the *ab* colour space

The influence of the oak chips toasting level, regardless of oak chips origin and oxygenation level

The level of toasting did not affect directly the clarity (*L*), the effect of an increased clarity coming from the contact with the oak wood of any type (Figure 3, left). The evolution of this parameter was similar to the one seen and explained already in Figure 1, left, the samples with oak showing a better clarity (*L*) than the control, in the 64th day of evolution. However, as it results from Figure 3, right, the control samples were the reddest (parameter +*a*) and the yellowest (parameter +*b*) after 64 days of maturation, while the oak wood contact decreased both parameters a little, irrespective of their toasting level. Nevertheless, medium toasting seems to give slightly better results regarding the red parameter (+*a*) after 64 days of maturation.

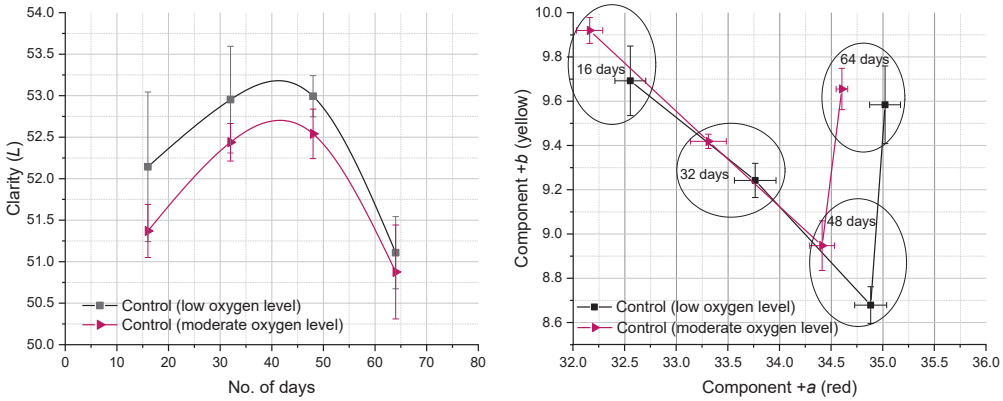

Figure 4. The influence of the oxygenation level on the control samples on the CIELab parameters: left - clarity (*L*) evolution; right - red and yellow colour evolution in the *ab* colour space

The influence of the oxygenation level on the control samples (without any wood contact)

The overall influence of oxygen can be observed in Figure 4, left, where moderate levels of oxygen induce lower colour lightness over the entire period of 64 days, which means that the colour is more intense in those samples. In the Figure 4, right, the parameter +*a* (red) increase is more pronounced in the low oxygen samples during all the 64 days. The parameter +*b* showed decreasing values during the first 48 days, increasing then sharply by the 64th day. The samples exposed to low oxygen level systematically maintained lower yellow hues as compared to the samples exposed to higher doses of oxygen (moderate level), confirming that the yellow increases with the oxidation of wine.

In all cases presented, the constant increase in the red parameter values over the entire period of 64 days can be explained by the condensation reactions known to take place between tannins with anthocyanin. On average, oxygenated samples have a more intense colour, while, in low oxygenated samples, the clarity (*L*) and red (+*a*) values are higher.

The control samples without oxygen treatment showed the lowest values for parameter +*b*. The yellowness is influenced on one hand by slow oxidation and on the other hand by extraction of yellow-brown compounds from oak.

Table 3. Detailed sensory evaluation scores on the main categories and total for experimental wines

Samples	Visual (≤3 pct.)	Olfactory (≤6 pct.)	Olfacto-gustatory and mouthfeel (≤6 pct.)	Aftertaste (≤3 pct.)	Overall impression (≤2 pct.)	Total score (≤20 pct.)
CT	1.60±0.22^{a}	2.60±0.22^{cd}	2.80±0.27^{bc}	1.60±0.22^{c}	1.10±0.22^{a}	9.70±0.67^{cd}
FR_M	1.80±0.27^{a}	***3.80±0.57^{ab}***	***3.40±0.42^{ab}***	**1.90±0.22^{abc}**	1.10±0.22^{a}	12.00±1.41^{ab}
FR_H	1.80±0.27^{a}	***4.40±0.65^{a}***	***4.24±0.34^{a}***	**2.24±0.25^{ab}**	1.10±0.22^{a}	13.78±1.02^{a}
AM_M	1.90±0.42^{a}	***4.30±0.57^{ab}***	***3.80±0.57^{a}***	**2.40±0.22^{a}**	1.10±0.22^{a}	13.50±0.94^{a}
AM_H	1.80±0.27^{a}	***4.20±0.57^{ab}***	***4.24±0.49^{a}***	**2.30±0.27^{ab}**	1.10±0.22^{a}	13.54±0.75^{a}
CT_OX	1.60±0.22^{a}	2.30±0.45^{d}	2.10±0.22^{c}	1.50±0.35^{c}	1.00±0.00^{a}	8.50±0.61^{d}
FR_M_OX	1.80±0.27^{a}	3.30±0.57^{bcd}	3.40±0.89^{ab}	1.70±0.27^{bc}	1.10±0.22^{a}	11.30±1.44^{bc}
FR_H_OX	1.80±0.27^{a}	3.50±0.61^{abc}	3.30±0.45^{ab}	1.70±0.45^{bc}	1.00±0.00^{a}	11.30±1.20^{bc}
AM_M_OX	1.80±0.27^{a}	**3.60±0.42^{abc}**	**4.20±0.27^{a}**	1.90±0.22^{abc}	1.10±0.22^{a}	12.70±0.67^{ab}
AM_H_OX	1.90±0.22^{a}	**3.70±0.27^{ab}**	**3.60±0.42^{ab}**	1.90±0.42^{abc}	1.10±0.22^{a}	12.20±0.45^{ab}

^{abcd}ANOVA - Tukey Test ($p < 0.05$)

The sensory evaluation results are summarized in Table 3. Sensory scores of experimental wines showed that the oak chips considerably induce sensorial changes on the following levels: Olfactory, Olfacto-gustatory and mouthfeel and on Aftertaste.

In accordance to the sensory evaluation scores, the most appreciated wines were, as follow: 1) the group less exposed to oxygen and treated with either French oak or American oak (medium and heavy toast alike) - scores between 12.00-13.78, not significantly different; 2) the group exposed to moderate oxygen levels and treated with American oak (medium and heavy toast alike) - scores between 12.20-12.70, not significantly different, 3) the group exposed to moderate oxygen levels and treated with French oak (medium and heavy toast alike) - scores of 11.30, not significantly different and 4) the control wines, with scores of 9.7 (exposed to low oxygen levels) and 8.5 (exposed to moderate oxygen levels), not significantly different.

The statistical differences among total scores showed that oak chips, regardless of botanical origin, under the condition of low oxygenation are the most suitable for maturation of wines produced from Feteasca Neagra.

CONCLUSIONS

The effect of oxygen during maturation. The wine samples exposed to more oxygen levels tend to have a decreased clarity (*L*) and a more intense colour, as well as an increased yellowness (parameter +*b*). The redness (parameter +*a*) is slightly decreased over 64 days of maturation in samples exposed to moderate oxygen level as compared to low level. The wines produced from Feteasca Neagra treated with a low dose of oxygen

showed a higher redness (parameter +*a*) and a lower yellowness (parameter +*b*), suggesting a better wine development under these conditions.

The effect of oak chips during maturation. Oak chips slightly increase the clarity (*L*) during 2 months of maturation of wine samples as compared to control wines, while an increased stability regarding colour matter precipitations is expected after the treatment. On the other hand, oak chips increased yellowness (parameter +*b*) by extraction of yellow-brown compounds into wines, further influenced by oxidation. The yellowest samples were those treated with American oak, followed by control samples and then the samples treated with French oak.

The effect of chips toasting level. The different level of toasting did not significantly influence the clarity (*L*), redness (parameter +*a*) or yellowness (parameter +*b*), during 64 days of maturation. The changes compared with control samples are induced by the treatment with oak rather than the toasting level of oak.

The effect of treatments on sensory properties. The best rated wines were those treated with oak wood (irrespective of the oak origin or toasting level) matured under low oxygen levels. Under the conditions of moderate oxygen exposure, the American oak chips proved more suitable than the French oak for Feteasca Neagra, but the scores at the sensory evaluation are still lower than for any wine treated with oak and exposed to low oxygen level. All the oak treated wines obtained higher scores than the samples not in contact with oak. However, because when exposed to more oxygen the American oaked wines behaved better than the French oaked wines, it is expected that the wines in contact with American oak will evolve better in time. Thus, we can safely recommend for the wines of Feteasca Neagra to be maturated in contact with American oak, under low levels of oxygen.

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EFFICACY ASSESSMENT OF SEVERAL CRYSTALLIZATION INHIBITORS USED FOR TARTARIC ACID STABILIZATION IN RED WINES

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Abstract

Red wine tartaric acid stabilization is a challenging process, due to the presence of tannins which interfere with the tartrate crystallisation. For this reason, of the known methods for tartaric acid stabilization, the crystallization inhibition seems more appropriate for the red wines. This paper presents several treatments with new crystallization inhibitors (mannoproteins and potassium polyaspartate) applied for an unstable red wine, in the presence of colour stabilization agents (arabic gum, tannin, egg albumin). For the tested wine, known to be difficult to stabilize due to its rich extract, none of the two mannoproteins tested resulted in stability, while the potassium polyaspartate proved to be highly efficient. Kept at cold, the mannoprotein treated samples lead to an important deposit of flocculated proteins, in lower quantity in the case of additional treatment with tannin, while the wines containing potassium polyaspartate did not form deposit, irrespective of the additional colour stabilization treatment. The sensory analysis revealed some better characteristics of the wines treated with polyaspartate, such as reduced perception of bitterness and a slight increase in mouthfeel perception. The influence on colour stabilization of combined treatments is also discussed.

Key words*: colour stabilization, mannoproteins; polyaspartate, sensory characteristics.*

INTRODUCTION

Wine stabilization is a very important stage in wine production, its effectiveness being crucial for the overall quality and marketing strategies of the wine. Young red wines are released on the market soon after their production, if not properly stabilized, have high potential to precipitate both the colour matter and potassium bitartrate. In order to avoid these incidents, several types of treatments can be applied, in compliance with the recommendations of the International Organisation of Vine and Wine (OIV).

The classical stabilization method widely used against colour matter and potassium bitartrate precipitation is the cold treatment (OIV, 2019b 5/88 and 2/04). During cold stabilization, wines are kept for a period of time at very low temperatures, close to the wine freezing point, to remove in this way the unstable colour matter and to induce the crystallization and precipitation of potassium bitartrate. However, the method is time-consuming and expensive, thus alternative solutions are also available or under research.

Two modern methods for stabilization of wines against potassium bitartrate precipitation involve removing their salt-forming excess ions. First method applies cation exchangers to remove the potassium cations (OIV, 2019a, 43/2000; OIV, 2019b 1/93 and 447/2011), while the second uses electrodialysis to extract of potassium cations and tartrate anions in super-saturation (OIV, 2019a 29/2000; OIV, 2019b 1/93). These methods are very efficient, but still have the disadvantage of high costs for both equipment acquisition and operation.

The most economical methods for potassium bitartrate stabilization are the use of crystallization inhibitors, but these are not always efficient for a long period of time, due to their hydrolysis, and in certain cases they may interact with colour matter, which in red wines is causing precipitation, limiting the stabilizing effect benefits. Generally, these inhibitors are added in clarified wines just before bottling.

One of the most common and oldest inhibitors for potassium bitartrate stabilization is the metatartaric acid, used in a dose of maximum 10 g/hl (OIV, 2019a 31/2000; OIV, 2019b 16/70), this being able to stabilize wines for a short time (several months) after bottling. The treatment with metatartaric acid induces stability only for a limited time due to its hydrolysis into tartaric acid, the rate being temperature-dependent (with higher rates at higher temperatures).

Aside of the metatartaric acid, which is the classical inhibitor, other new substances are approved. Using specific yeast mannoproteins for potassium bitartrate stabilization is a possible option for winemakers (OIV, 2019a 26/2004; OIV, 2019b 4/01 and 15/05). The specific mannoproteins are obtained by enzymatic hydrolysis of yeast cell walls and purification by ultrafiltration of those mannoprotein fractions with a molecular weight of about 40 kDa (Feuillat *et al.*, 1998, Gerbaud *et al.* 1996; Moine and Dobordeau, 1996; Moine-Ledoux *et al.*, 1997). These specific fractions have a stabilizing effect when added in a dose between of 15-25 g/hl, while exceeding these doses results in a reduced stabilizing (Moine-Ledoux *et al.*, 1997). Other fractions of mannoproteins with different molecular weights attracted the attention of researchers as well, due to other important effects in winemaking technology, such as improvement of wine protein stability, which in turn leads to a decrease in bentonite doses required for protein stabilization of white wines (Dupin *et al.*, 2000; Gonzalez-Ramos *et al.*, 2008; Gonzalez-Ramos 2006 and Gonzalez 2006; Gonzalez-Ramos *et al.*, 2009; Moine and Dobordeau, 1996; Waters *et al.*, 1994). Other mannoproteins may be used for the stabilization of foam in sparkling wines (Nunez *at al.*, 2005). Sensory effects are also produced by some mannoprotein fractions with edulcorant properties, thus increasing the sweetness and balancing sourness and bitterness (Moine, 2004). Having a proteic component, these complex macromolecules can interact with polyphenols acting as protective colloids, improving the stability of colour matter and reducing the perception of astringency (Fornairon *et al.*, 2002; Trione and Martínez, 2001; Vidal *et al.*, 2004; Escot *et al.*, 2001).

Other inhibitors against potassium bitartrate precipitation are cellulose gums or sodium carboxymethylcellulose (OIV, 2019a 366/2009; OIV, 2019b 2/08). Due to the interaction with colour compounds, these treatments are allowed only for white and sparkling wines, where they can ensure a long term potassium bitartrate stability. The doses must be under 10 g/hl.

Potassium polyaspartate is the most recently approved additive for potassium bitartrate stabilization (OIV, 2019a 572/2017; OIV, 2019b 543/2016). The potassium polyaspartate can be used in all wines. Red wines must be stabilized against high colloidal instability before this treatment. The dose must not exceed 10 g/hl, not only because at higher doses the stability is not improved but also because in certain cases an increase in turbidity may occur.

For commercialisation of young red wines, aside of tartaric stabilization, colour matter stabilization is another important aspect to take into account. The classical cold stabilization may contribute also to colour stabilization, by removing certain unstable colloids and pigments. But, when cold stabilization is not used, along with alternative methods for tartaric acid stabilization, alternative methods for colour stabilization are needed.

Tannins addition (OIV, 2019a 6/2008, 352/2009, 554/2015 and 574-2017; OIV, 2019b 16/70) is mainly used to facilitate clarification of young wines by removing the excess of proteins through flocculation and sedimentation, with or without other fining processes. However, the tannins can bring other benefits as well. They are commonly added in low tannin red wines made from varieties with thin skins or with short maceration, to improve sensory properties and to stabilize the colour through interaction with free anthocyanins, which leads to many anthocyanin-derived pigments (García-Estévez *et al.*, 2017; Cheynier *et al.*, 2006).

Arabic gum (OIV, 2019a 27/2000; OIV, 2019b 12/72) is an additive which can be used to prevent the precipitation of colloidal colour matter in red wines or to avoid copper haze and protect wines against light iron haze. Treatment with arabic gum is done at bottling time, the maximum dose being 0.3 g/l.

Using combined stabilization treatments for both potassium bitartrate and colour matter stabilization is an appealing solution for a winemaker, as the time and work-load are reduced. However, in order to establish the combinations and dosages laboratory trials are needed to evaluate their efficiency. Also, sensory changes of final product should be evaluated as well. In this paper, some combined stabilization protocols are assessed, including newly approved products and some recommendations are formulated based on the findings.

MATERIALS AND METHODS

The red wine used for the trials was produced in 2018 from the Romanian grape variety Negru de Dragasani. The wine was made through classic technology at industrial scale at Negrini wine cellar, located in Dragasani, a traditional wine growing region of South Romania. The main wine parameters are (OIV, 2018): 13.8% vol. alc.; 5.9 g/l total acidity as tartaric acid; pH = 3.65; 0.18 g/l volatile acidity as acetic acid; 28.1 g/l non-reducing extract; free SO_2 = 1.6 mg/l; total SO_2 = 9.2 mg/l; 0.26 g/l malic acid; 1.39 g/l lactic acid; T_{SAT} = 20.2 (very unstable wine in relation to potassium bitartrate). The theoretical saturation temperature (T_{SAT}) was determined by measuring the difference in electrical conductivity of wine at 20°C before (C_1) and after an excess of potassium bitartrate was added to wine (C_2). Knowing the average value of electrical conductivity (33 µS/cm) necessary for a decrease of 1°C in theoretical saturation temperature, then the relation describing T_{SAT} is (Ribéreau-Gayon *et al.*, 2006; Würdig *et al.*, 1982):

$$T_{SAT} = T - \frac{C_2 - C_1}{33}$$

The values lower than 15 for red wines mean that the wine is stable; between 15 and 20, the wine is unstable; over 20, the wine is very unstable.

Once the malolactic fermentation finished, the wine was racked off sediments and sulphited with 60 mg/l SO_2 using 10% m/v $K_2S_2O_5$ solution. The young wine was treated first for colour stabilization by 3 different methods and after 7 days the samples were racked and treated by other 3 different methods, this time for potassium bitartrate stabilization. After 20 days the samples were racked again, sulphited by an additional 10 mg/l SO_2 and bottled. By combining the treatments for colour and potassium bitartrate stabilization, 9 experimental variants resulted. The control sample was only treated by the classical cold stabilization method. All variants (Table 1) were prepared in two sets and in triplicate. The bottled samples were stored for two months, one set of wines at cellar temperature of 12-16°C and the second one at 4°C.

Table 1. Experimental variants and the detailed treatments used for colour and potassium bitartrate stabilization all the table in the same page

Codification	Technological variants for colour stabilization	Technological variants for potassium bitartrate stabilization
Control	cold stabilization treatment, 14 days at 0°C	
AG_MP1	30 g/hl arabic gum, ®Araban Spray Dry,	20 g/hl mannoprotein, ®Mannostab, Laffort
AG_MP2	Vason extracted from *Acacia verek*	20 g/hl mannoprotein, ®MPA, Vason
AG_KPA		10 g/hl potassium polyaspartate, ®Zenith Uno, Enartis
TAN_MP1	10 g/hl mixture of ellagic, gallic,	20 g/hl mannoprotein, ®Mannostab, Laffort
TAN_MP2	catechinic and procyanidic *tannins*,	20 g/hl mannoprotein, ®MPA, Vason
TAN_KPA	®Premium Color SG, Vason	10 g/hl potassium polyaspartate, ®Zenith Uno, Enartis
ALBU_MP1	20 g/hl micro-granulated egg albumin,	20 g/hl mannoprotein, ®Mannostab, Laffort
ALBU_MP2	®Albuclar SG, Vason	20 g/hl mannoprotein, ®MPA, Vason
ALBU_KPA		10 g/hl potassium polyaspartate, ®Zenith Uno, Enartis

A sensory assessment was performed on the first set of samples, by a team of five tasters who analysed taste and mouthfeel characteristics (sweetness, sourness, bitterness, astringency and body of wine) on intensity scales from 0 to 5. The second set of wines, cold stored, was inspected for sediments, and if they were present, they were gravimetrically evaluated by using a drying balance (KERN MLB 50-3N), after being separated by centrifugation (Hettich Mikro 220R centrifuge, with 50 ml tubes, set for 10 minutes at 6000

rpm). The drying balance ran at 120°C and reading was done after the automatic shutoff, occurring for a change of weighing value < 1 mg within 60 seconds. The results were reported as g/l dry weight sediment.
The CIELab colour parameters and total polyphenols index were determined with WinAspect software version 2.2.7 and a computer-controlled double beam spectrophotometer Specord 250 from Analytik Jena AG.

RESULTS AND DISCUSSIONS

The CIELab parameters and total polyphenols for all performed stabilization treatments are presented in Table 2. The main results show some differences induced by certain treatments, such as an increase of lightness (parameter L) and a decrease of total polyphenol index in samples treated with egg albumin as a fining agent or that the arabic gum treatment induces an increase of yellowness in wine samples (*b* parameter). In order to establish the individual effect of the performed treatments, the influence of colour stabilization treatments (Table 3) or the influence of the potassium bitartrate stabilization treatments (Table 4) were statistically analyzed.
The effect of colour stabilization treatments (Table 3), without taking into account the effect of potassium bitartrate stabilization treatments, showed that tannin and arabic gum treatments seem to have the highest efficiency on colour stabilization. Even though the cold treatment along with egg albumin fining removed the pigment supposed to be unstable, the resulted wines remained unstable. The most efficient mechanism for colour stabilization is proven to be the interaction of tannins with anthocyanins, which prevents the loss of free and polymerized anthocyanins, which is here evidenced by an increase in redness (a parameter) and polyphenol index (TPI), along with a reduced yellowness (b parameter) comparing to arabic gum. The use of arabic gum as protective colloid works well with the mention that induces a slightly increase in yellowness (b parameter).
Comparing the wine samples stored at cellar temperature with those stored in the refrigerator, we can observe an increase in lightness (L parameter), which indicates a more advanced loss in pigments and other solid matter. However, in potassium bitartrate highly unstable wines, the phenomenon of tartaric acid co-precipitation with tannins, pigments and other molecules can also occur (Manns *et al.*, 2005; Vernhet *et al.*, 1999).
The mechanism of co-precipitation is not well understood but it is likely to appear because of hydrophobicity or surface charges on the crystal faces and could be dependent on the pH of the wine (Manns *et al.*, 2005; Vernhet *et al.*, 1999).
Considering the effect of colour stabilization treatments in our experimental samples, a highly unstable wine regarding potassium bitartrate leads to co-precipitation of crystals along with pigments (Figure 2), even though the wine is stable regarding colour matter (Table 3).

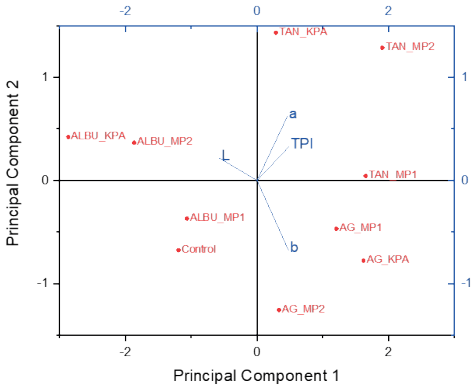

Figure 1. Principal Component Analysis bi-plot of CIELab parameters and Total Phenolic Index

The results indicated in Table 4, revealed that potassium polyaspartate is the only treatment efficient to prevent crystal formation in our red wine samples and because of its efficiency, the possibility of colour matter loss is avoided (Figure 2). Thus, the influence of potassium bitartrate stabilization technique is equally important for the colour matter stabilization, stabilizing the tartrate preventing also some pigment loss in red wines.
To highlight the effect of stabilization treatments on the CIELab parameters and total polyphenol index (TPI), the results from the Cellar stored samples are plotted in a Principle Component Analysis (PCA) diagram (Figure 1). As we can observe in the PCA bi-plot, the

parameter L is increased by subtractive techniques (egg albumin fining and cold treatment which remove part of the compounds from wine), while it is relatively constant in additive techniques. The positive value of parameter a indicates the shift of colour towards red, indicating a better colour appearance and stability, which can be obtained by tannin treatment followed by a period to form associations with anthocyanins. However, the tannin treatment increases the total polyphenols, which provide a better structure in red wines. The treatment with arabic gum is associated with similar effects as tannin, but has the disadvantage of increasing the parameter b, shifting the colour slightly towards yellow. A similar behaviour for CIELab and total polyphenol index was not present for mannoprotein or potassium polyaspartate treatments. The combined effect of stabilization (colour matter and potassium bitartrate) can be observed in Figure 2, where the dry weight of sediment was measured and analysed statistically.

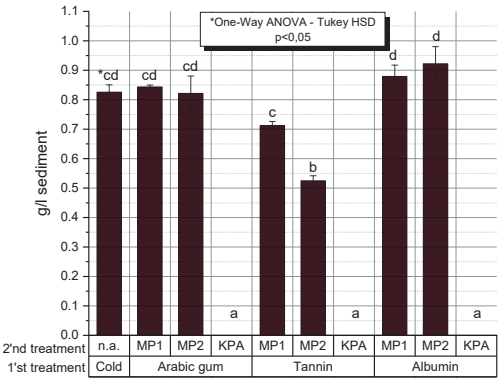

Figure 2. Measured dry weight of sediment (potassium bitartrate and colour matter)

The efficacy of potassium polyaspartate on our red wine samples is undeniable, because no sediment was detected in those samples, proving the attainment of stability regardless of the treatment for colour stabilization. However, in the case of tannin treatment for colour stabilization, both mannoproteins showed a reduced co-precipitation while combined with egg albumin or arabic gum the co-precipitation effect is increased in a similar way as in control samples.

The results highlight a combined beneficial effect of both stabilization treatments on the final product stability (lower colour matter loss and more efficient potassium bitartrate stabilization). If one of these compounds is not well stabilized in the final product, a co-precipitation may occur and a visual sediment of crystals with pigments or tannin will appear at the bottom of bottles. The parameter L from CIELab method seems to be a good indicator for the assessment of colour stability in red wines, as an increase in its value after storage at 4°C indicates a loss in pigments with potassium bitartrate crystals.

One Way ANOVA applied to the dry weight of sediment (Figure 2) indicates that there are certain significant differences ($p < 0.05$) among experimental variants. Although both colour and tartaric acid stabilization treatments were combined in each variant the statistical analyses were run only for the treatment combination, thus independent treatment effects were not evaluated in this way. Even so, the results are strongly suggesting that, as compared to other treatments, tannin combined with MP's may lead to an increased stability, at least for a short period of time comparing with the other combinations.

However, the most efficient treatment for stabilization of colour matter and potassium bitartrate remains potassium polyaspartate (KPA) regardless of its combination with a treatment for color stabilization. Potassium polyaspartate (KPA) provided very good stability in our samples with no detected sediment in bottles (Figure 2).

The classical cold treatment for 14 days at 0°C did not provide the desired physico-chemical stability in our samples. A lower temperature of about -4°C probably would have been a better way to stabilize the potassium bitartrate and colour for this type of wine with a very unstable character.

Table 2. CIELab parameters and total polyphenols in samples stored in cellar against samples stored in refrigerator

Samples	*L_1±sd	*a_1±sd	*b_1±sd	*TPI_1±sd	*L_2±sd	*a_2±sd	*b_2±sd	*TPI_2±sd
Control	51.86±0.96	47.21±0.63	1.56±0.17	57.21±3.71	53.87±0.22	45.97±0.17	1.68±0.17	61.07±0.24
AG_MP1	51.47±0.23	47.28±0.34	1.97±0.08	63.13±2.39	52.84±0.19	46.22±0.13	1.83±0.08	60.99±1.74
AG_MP2	51.61±0.83	46.95±0.80	1.99±0.21	61.43±0.43	51.47±1.04	46.63±0.72	1.92±0.15	61.68±2.52
AG_KPA	50.94±0.24	47.55±0.45	2.10±0.18	61.24±0.9	51.52±0.44	46.71±0.4	2.19±0.24	59.21±1.80
TAN_MP1	51.27±0.61	47.73±0.39	1.95±0.17	62.35±0.49	51.38±1.66	46.26±0.85	1.87±0.17	63.78±1.28
TAN_MP2	51.21±0.61	48.16±0.28	1.67±0.21	62.81±0.79	51.48±0.77	47.24±0.42	1.71±0.19	61.73±2.45
TAN_KPA	51.94±0.35	47.70±0.23	1.31±0.04	62.52±2.22	50.7±0.74	46.68±1.05	1.54±0.03	60.69±1.81
ALBU_MP1	52.54±0.84	47.08±0.72	1.64±0.21	59.95±1.41	53.55±0.07	46.58±0.13	1.43±0.04	59.57±1.56
ALBU_MP2	52.82±0.13	47.12±0.44	1.27±0.18	59.37±0.79	53.65±0.46	46.29±0.46	1.39±0.06	58.63±3.03
ALBU_KPA	53.21±0.71	46.82±0.31	1.04±0.35	59.16±1.38	53.13±0.22	46.09±0.05	1.39±0.03	59.18±0.55

*L_1, a_1, b_1, TPI_1 - samples stored in cellar (12-16°C); L_2, a_2, b_2, TPI_2 - samples stored in refrigerator (4°C).

Table 3. Comparison of samples stored in cellar (12-16°C) against samples stored in refrigerator (4°C) as regards CIELab parameters and TPI of wines treated for colour matter stabilization (Test Tukey $p < 0.05$)

Treatment	*L_1	*a_1	*b_1	*TPI_1	*L_2	*a_2	*b_2	*TPI_2
Control	51.86^{ab}	47.21^{ab}	1.56^{ab}	57.21^{c}	53.87^{b}	45.97^{a}	1.68^{b}	61.07^{a}
Arabic gum	51.34^{a}	47.26^{ab}	2.02^{c}	61.93^{ab}	51.94^{a}	46.52^{a}	1.98^{c}	60.63^{a}
Tannins	51.47^{a}	47.86^{a}	1.66^{b}	62.56^{a}	51.19^{a}	46.73^{a}	1.71^{b}	62.07^{a}
Albumin	52.85^{b}	47.01^{b}	1.32^{a}	59.49^{cb}	53.45^{b}	46.32^{a}	1.40^{a}	59.13^{a}

*L_1, a_1, b_1, TPI_1 – samples stored in cellar (12-16°C); L_2, a_2, b_2, TPI_2 – samples stored in refrigerator (4°C).

Table 4. Comparison of samples stored in cellar (12-16°C) against samples stored in refrigerator (4°C) as regards CIELab parameters and TPI of wines treated for potassium bitartrate stabilization (Test Tukey $p < 0.05$)

Treatment	*L_1	*a_1	*b_1	*TPI_1	*L_2	*a_2	*b_2	*TPI_2
Control	51.86^{a}	47.21^{a}	1.56^{ab}	57.21^{b}	53.87^{b}	45.97^{a}	1.68^{a}	61.07^{a}
MP1	51.76^{a}	47.36^{a}	1.85^{b}	61.81^{a}	52.59^{a}	46.35^{a}	1.71^{a}	61.45^{a}
MP2	51.88^{a}	47.41^{a}	1.64^{ab}	61.20^{a}	52.20^{a}	46.72^{a}	1.68^{a}	60.68^{a}
KPA	52.03^{a}	47.36^{a}	1.48^{a}	60.97^{a}	51.79^{a}	46.49^{a}	1.71^{a}	59.69^{a}

*L_1, a_1, b_1, TPI_1 – samples stored in cellar (12-16°C); L_2, a_2, b_2, TPI_2 – samples stored in refrigerator (4°C).

The sensorial results (Figure 3, left) showed that arabic gum in combination with potassium bitartrate stabilizers brings substantial differences on bitterness, astringency and wine body. The body of wine samples was increased to a greater extent when arabic gum was combined with potassium polyaspartate, showing a lower effect and different behaviour regarding the MP1 and MP2 mannoproteins.

The perceived bitterness was very different regarding the two mannoproteins, MP1 being perceived with a lower bitterness than control samples, while MP2 increased the bitterness in the wine samples. The lowest bitterness perception was found in wines treated with potassium polyaspartate.

The astringency perception is reduced by potassium polyaspartate and MP1 comparing to control, while MP2 increased this perception. The sweetness appears to be less affected by the treatments involved, with a slight increase induced by all stabilizers for potassium bitartrate.

The sour taste is not affected by mannoproteins, while it is slightly masked by potassium polyasparatate treatments.

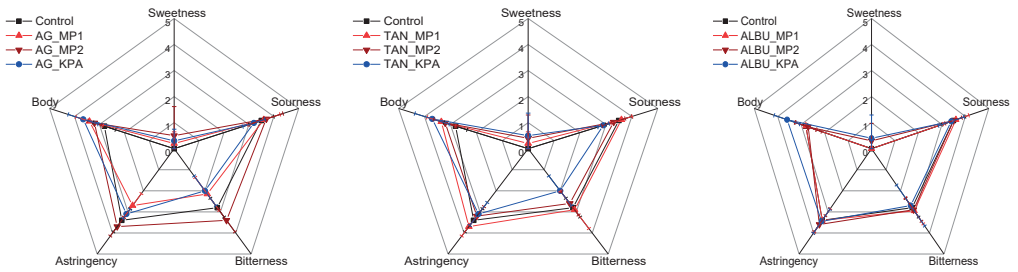

Figure 3. Sensory changes induced by the stabilization treatments: **left** - *arabic gum* in combination with mannoproteins (MPs) or polyaspartate (KPA); **middle** - *tannin* in combination with MPs or KPA; **right** - *egg albumin* with MPs or KPA

The results from the combination of tannin with potassium bitartrate stabilizers (Figure 3, middle) stand out for a similar behaviour on bitterness, astringency and wine body. The most notable effect was given by potassium polyaspartate which appears to mask the bitterness and sourness and lower astringency, while increasing the wine body.

The sensory results regarding egg albumin fining combined with potassium bitartrate stabilizers (Figure 3, right), appear to show reduced wine complexity because of various phenolic compounds removal form wine, causing the samples to be close together for most of sensorial parameters. An exception can be observed regarding potassium polyaspartate which has a clear influence on increasing wine body.

CONCLUSIONS

- ***Arabic gum*** ensures colour matter stability by its efficacy as a protective colloid, but this mechanism may not be enough if the wine is not stable regarding potassium bitartrate (KHT). Co-precipitation of potassium bitartrate with pigments and tannins may occur when no or an inadequate treatment to prevent crystallization was applied. Other induced effect is a slight increase of yellowness (parameter b) in the treated wines.
- ***Tannin*** provides the most interesting effect on colour matter, increasing the redness (parameter a) and total polyphenolic index (TPI), while keeping the yellowness (parameter b) similar to control samples. The structure of wines treated with tannin were improved, but this may lead to a longer maturation period required to attenuate the astringency. The co-precipitation phenomenon can also occur if the potassium bitartrate remains unstable.
- ***Egg albumin*** treatment is often used, but we find it not recommendable for quality wines, because it reduces the colour and structure, enhancing the acidic and vegetal sensations, also reducing the overall complexity. This treatment may be beneficial in certain oxidized wines of lower quality, where the reducing of yellowness (parameter b) is needed, but with the compromise of reducing some desired phenolic compounds as well. Even if the unstable phenolic compounds are reduced by this treatment, the unstable potassium bitartrate co-precipitates with the rest, bringing an additional loss of pigments and tannins.
- ***Mannoproteins*** MP1 and MP2 may increase the body of wine, but they are not able to mask the sour taste. Astringency and bitterness perceptions depended on the type of mannoprotein and on other treatments combined with them. A certain degree of stability for short term can be observed when MP1 and MP2 mannoproteins were combined with tannin treatment, but in the end they can co-precipitate with pigments in the case of wines with highly unstable potassium bitartrate. The treatment with mannoproteins may be a solution for wines with a longer stabilization period as in the case of barrel matured wines, but may not be a good choice for young red wines.
- ***Potassium polyaspartate*** is providing positive features in young red wines with highly unstable potassium bitartrate. The structure of wine is improved and a more complex mouthfeel is perceived, lowering the sourness and astringency perceptions. It gives the best results in combination with tannins, but also in combination with arabic gum. Potassium polyaspartate was able to stabilize potassium bitartrate in highly unstable young red wine and to prevent co-precipitation, thus being recommended for use for these purposes.

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INFLUENCE OF COPPER SPRAYING ON PHOTOSYNTHETIC PERFORMANCE, GRAPE RIPENING, WOOD MATURATION AND FROST RESISTANCE IN GRAPEVINE

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Abstract

The research was carried out in a family vineyard from Arad County, Buteni village, during 2018-2019, on Cardinal, Victoria, and Merlot, varieties. In the experimental versions, 1 or 2 copper treatments were added in the normal scheme of phytosanitary treatments for diseases and pest control. Copper is essential in grapevine growing, for grapevine downy mildew (Plasmopara viticola) control. The research plots were superior to the control concerning the percentage of annual wood maturation, the canes content in carbohydrates and the buds viability. In the V_7 variant were obtained the best results, sprayed with 2.42 kg/ha copper hydroxide ($Cu(OH)_2$) applied three times: before flowering, berry development, respectively after veraison. The second was V_5 variant in which was applied 1.61 kg/ha of copper hydroxide ($Cu(OH)_2$) two times: at berry development, and after veraison. In the last place as concern the percentage of wood maturation, the content of the cane in carbohydrates and the buds viability, was the control variant V_1 on which was applied a single treatment with copper, respectively 0.807 kg/ha of copper hydroxide ($Cu(OH)_2$) after veraison.

Key words*: copper, carbohydrates, wood maturation, sensitive varieties, frost resistance.*

INTRODUCTION

Copper is very important for plants, with unique fungistatic and bactericidal activity due to the copper ions (Cu ++) released in the water (Yruela, 2005). Copper ions are passively absorbed by fungi and bacteria during growth and accumulate until the concentration becomes lethal to the cells (Sommers, 2008). Copper is component of enzymes and enzyme activator and acts as a catalyst (Festa and Thiele, 2011). Copper is vital to all plants and is involved in the chlorophyll biosynthesis, seed germination, in increasing drought resistance and in water supply (Yamasaki et al., 2008). Copper is involved in respiration and protein synthesis, in the nutrients and water assimilation, in the lignin synthesis, in the stiffening of the cell wall and in increasing of plant resistance to pathogens (Sundin et al., 2016).

Copper-based fungicides used in agriculture took place for the first time in the seventeenth century, when farmers treated the wheat seeds for sowing with copper sulphate (blue stone) against corn cockle (*Agrostemma githago*) (Morton and Staub, 2008). In 1882, the French scientist Millardet discovered the properties of copper as a fungicide, using copper sulphate as "Bordeaux Mixture" (*Bouille Bordelaise*) to control the grapevine downy mildew (*Plasmopara viticola*), and in 1956 the first copper-based pesticide was approved.

Nowadays copper has become essential for the grapevine growing, due both to the grapevine downy mildew (*Plasmopara viticola*) control as well as for several side effects (Andras-Sauca et al., 2018a; Borca et al., 2018).

When is applied in the late growing season in vineyards, the copper decreased the powdery mildew (*Erysiphe necator*) infection due to effect on cleistothecia and mycelium, decrease the noble rot (*Botrytis cinerea*) infection by thickening the berries skin, delays the leaves falling, which helps to ripen the shoots tissues and increase the resistance to winter low temperatures (Gruau et al., 2016; Blanco-Ulate et al., 2015).

Copper-based treatments are currently included in all grapevine integrated disease and pest

control management, which finally increase the production costs (Gadoury et al., 2012).

High amount of nitrogen and phosphorus can lead to copper deficiency (Brunetto et al., 2015). For example, measureless amounts of nitrogen increase the abundant development of canopy, the sequestration of total copper and the decrease of the grapevine photosynthesis rate (Hendrickson et al., 2004). Copper deficiency, correlated with climate variability, decreases the vines frost resistance, with negative financial impact on vineyards management (Dobrei et al., 2010).

However, the copper use must be moderate, because the copper excess can cause chlorosis and burns on the leaves, it can negatively affect the quality of grapes, must and wine (Lamichhane et al., 2018). Achievement of high quality wine by-products is essential to withstand an increasingly demanding market with more and more efficient competitors (Andraş-Sauca et al., 2018b).

MATERIALS AND METHODS

The research was carried out during 2018-2019 in a private vineyard from Buteni village, Arad County, during the full maturity growing stage. Two table grapes varieties (Victoria and Cardinal) and Merlot wine grapes variety were investigated, known as being more sensitive to the lower temperatures in winter, therefore with issues concerning the maturation of the one year old canes and the buds viability (Dobrei et al., 2018; Nistor et al., 2018).

In the experimental plots were add one or two copper treatments, in the current scheme of phytosanitary treatments for diseases and pests control (usually with only one copper treatment applied after veraison). Copper-base treatment applied was Kocide which has as active substance copper hydroxide ($Cu(OH)_2$) 53.8% concentration; the treatment dose was 1.5 kg/ ha commercial product.

The experimental plots were: V_1 - treatment with copper (0.807 kg/ha $Cu(OH)_2$) applied after early veraison stage, considered as control; V_2 - treatment with copper (0.807 kg/ ha $Cu(OH)_2$) applied before flowering stage; V_3 - treatment with copper (0.807 kg/ha $Cu(OH)_2$) applied in lag phase; V_4 - two treatments with copper (0.807 kg/ha $Cu(OH)_2$) applied before flowering stage + (0.807 kg/ha $Cu(OH)_2$) applied at lag phase; V_5 - two treatments with copper (0.807 kg/ha $Cu(OH)_2$) applied in lag phase + (0.807 kg/ha $Cu(OH)_2$) applied after early veraison stage; V_6 - two treatments with copper (0.807 kg/ha $Cu(OH)_2$) applied before flowering stage + (0.807 kg/ha $Cu(OH)_2$) applied after starting veraison stage; V_7 - three treatments with copper (0.807 kg/ha $Cu(OH)_2$) applied before flowering stage + (0.807 kg/ha $Cu(OH)_2$) applied to lag phase + (0.807 kg/ha $Cu(OH)_2$) applied after veraison stage starting.

During the research, minimum temperatures of -17ºC were recorded in three nights, respectively -18ºC in one night.

Observations and determinations were made regarding several indicators: sugar concentration in grape must (g/l), was determined with Bellingham + Stanley OPTi digital hand held refractometer; the amount of sugar per hectare was calculated, respectively the canopy area necessary for the accumulation of one kg of sugar (or photosynthetic efficiency); the total annual growth (expressed in m/vine and calculated in m/ha were determined); following the first frosts the measurements were redone, after the wood the fragments affected by the frost were removed, resulting the one year old matured wood (measures were in m/vine, m/ha and as a percentage of total annual wood growth; the amount of carbohydrates in canes was determined in two different stages: before early autumn frosts (15th November), respectively after the late frosts in spring (1th March). Measurements were done by the antrona reagent method by which the soluble sugar and starch were determined, and their amount was evaluated as the total carbohydrates; the rate of viable eyes was determined after the late frosts in spring and before the bleeding start (1th March). The research aimed to study the influence of copper treatments on the grape yield and quality, photosynthesis efficiency, the well-matured canes wood, the carbohydrate amount in canes and the buds viability.

Statistics were done by GraphPad InStat version 5.04 version for Windows (GraphPad Software, Inc. 5755 Oberlin Drive #110 San Diego, USA) and data analysed using statistical ANOVA and Tukey's test ($p \leq 0.05$) for quantitative variables.

RESULTS AND DISCUSSIONS

The quality of grapes or wine is essential for successful products on the market. Quality cannot be achieved without a performant growing technology, which provides high quality grapes for winemaking with economic efficiency (Nan et al., 2018).

Table 1. Influence of copper applied during phytosanitary treatments on the grapes quality and photosynthetic efficiency

Treatment variants	Variety	Sugar g/l	Sugar kg/ha	m^2 canopy/kg sugar	Difference to control (kg sugar/ha)	Significance
V_1 -control	Victoria	148	1538	12.7	-	-
	Cardinal	127	1201	20.43	-	-
	Merlot	194	1675	9.09	-	-
V_2	Victoria	144	1461	12.75	-77	0
	Cardinal	124	1170	20.2	-40	0
	Merlot	190	1640	8.87	-35	0
V_3	Victoria	151	1532	12.76	-6	-
	Cardinal	131	1239	19.81	38	*
	Merlot	196	1693	8.99	18	-
V_4	Victoria	153	1591	11.71	53	*
	Cardinal	135	1275	18.54	74	**
	Merlot	198	1709	8.51	34	-
V_5	Victoria	161	1676	11.66	171	***
	Cardinal	144	1364	17.99	163	***
	Merlot	208	1798	8.51	123	***
V_6	Victoria	157	1632	11.42	94	**
	Cardinal	138	1304	18.12	103	**
	Merlot	202	1745	8.33	70	*
V_7	Victoria	159	1654	11.26	167	***
	Cardinal	141	1333	17.73	132	***
	Merlot	204	1763	8.25	88	**
DL - Victoria			5% - 37.1		1% - 67.4	0.1% - 117.9
DL - Cardinal			5% - 34.9		1% - 62.5	0.1% - 109.9
DL - Merlot			5% - 42.3		1% - 71.1	0.1% - 122.6

In Table 1 is presented the data regarding the influence of copper applied in the mixture of phytosanitary treatments on the sugar concentration and the photosynthetic efficiency. Regardless of whether were grapes for wine or fresh consumption, varieties from experimental plots reaction was positive to the copper doses applied. In all three varieties the sugar concentration was influenced both by the amount of copper applied and by the growing stage of treatment application.

The highest concentrations of sugar resulted in V_5 plot: 161 g/l for the Victoria, 144 g/l for the Cardinal and respectively 208 g/l for the Merlot variety. In V_5 plot was applied 1.61 kg/ha copper twice: in lag phase and in the first stage of veraison.

In the second place was ranked the V_7 plot on which was applied 2.42 kg/ha copper in three stages: before flowering, lag phase and veraison. The V_2 was the only experimental plot that recorded values lower than the control, with 144 g/l sugars in the Victoria variety, 124 g/l sugars in the Cardinal variety, respectively 190 g/l sugars in the Merlot variety. Compared to the control, in V_2 was applied the same copper dose (0.807 kg/ha), but much earlier - before flowering.

The largest concentration of sugars was reported in the V_5 experimental plot: 1676 kg sugars/ha in the Victoria variety, 1364 kg/ha in the Cardinal variety, respectively 1798 kg sugars/ha in the Merlot variety.

Statistically the V_5 experimental plot was the highest statistical significant variant; the differences between V_5 variant and the control variant, were for all three varieties very significant.

The photosynthetic efficiency recorded higher values in V_7 due to the lower canopy area per kilogram of sugar amount, in which was applied 2.42 kg/ha copper.

In varieties more sensitive to low winter temperatures, a proper maturation of the one year old canes is essential for an adequate resistance in winter time. The maturation of the shoots tissues begins in the summer and can be positively influenced by appropriate technology, so that the possible early frosts which are quite frequent during the month of October to affect less the canes maturation. Therefore, the introduction of copper into the phytosanitary treatments is beneficial from all points of view. Copper is efficient in downy mildew (*Plasmopara viticola*), does not charge the cost of treatments due to the reasonable prices of copper fungicides, it is also accepted in organic viticulture and in addition it is very efficient in wood maturation.

Research should establish precisely both the optimum amount of copper per vine and for each vineyard, as well as the most appropriate growing stage for application.

The results regarding the influence of the copper treatments on the total annual growths and one year old wood matured are presented in the Table 2.

As concern the total annual cane wood increases, it is noted that the high doses of copper (V_7 variant) and especially the early application of copper - before flowering (variant V_2), has led in all three varieties to a slight slowing of growth. In all three varieties, V_2 recorded the lowest values of the total growth of shoots. Therefore, the variant with the best results regarding the total one year old cane wood increases was the V_5 variant, in which were applied two treatments with copper: one applied in lag phase and the second in the first stage of veraison.

The one year old cane wood matured is very important indicator, especially in varieties with lower resistance to winter frosts (Dobrei et al., 2015). Therefore copper influence is higher on one year old canes wood than on the well-matured canes wood.

Table 2. The influence of the copper treatments on the one year old canes and well-matured canes

Treatment variants	Variety	One year old canes		Well-matured canes			Difference from control (% from total)	Significance
		m/vine	m/ha	m/vine	m/ha	% from total		
V_1 - control	Victoria	18.8	85465	13.3	60680	71	-	
	Cardinal	24.1	109534	13.9	63189	58	-	
	Merlot	13.6	61812	9.8	44451	72	-	
V_2	Victoria	16.7	75901	12.2	55461	73	2	-
	Cardinal	20.9	94990	12.3	55916	59	1	-
	Merlot	12.4	56358	9.3	42278	75	3	-
V_3	Victoria	17.4	79083	13.4	60916	77	6	*
	Cardinal	22.8	103626	13.7	62280	60	2	-
	Merlot	13.2	59994	10.3	46824	78	6	*
V_4	Victoria	16.9	76810	13.7	62280	81	10	**
	Cardinal	21.6	98172	14	63644	65	7	**
	Merlot	13.1	59539	10.7	48642	82	10	**
V_5	Victoria	18.9	85900	15.7	71372	83	12	**
	Cardinal	24.5	111352	16.6	75464	68	10	**
	Merlot	13.8	62721	11.7	53188	85	13	***
V_6	Victoria	16.8	76356	13.1	59553	78	7	*
	Cardinal	21.5	97739	13.5	61371	63	5	*
	Merlot	12.7	57734	10	45460	79	11	**
V_7	Victoria	16.7	75918	14.4	65462	86	15	***
	Cardinal	21.3	96830	15.3	69554	72	14	***
	Merlot	12.5	56825	11	50006	88	16	***
DL - Victoria		5% - 4.21		1% - 7.11			0.1% - 12.38	
DL - Cardinal		5% - 3.87		1% - 6.45			0.1% - 11.32	
DL - Merlot		5% - 4.72		1% - 7.78			0.1% - 12.92	

Regarding the well matured canes ratio from the total one year old wood, the best results were recorded in V_7 variant, in which was applied the highest amount of copper. In V_7, the well-matured canes represent 88% of the total one year old canes wood in the Merlot variety, 86% in the Victoria variety, respectively 72% in the Cardinal variety. The differences recorded between the V_7 and the control, were 15% in the Victoria variety, 14% in the Cardinal variety, respectively 16% in the Merlot variety, and were very highly significant.

The experimental variant that recorded the highest values of well-matured canes wood/ha was V_5, on which were applied two copper treatments (the first in lag phase and the second in the first decade of veraison stage).

In the varieties sensitive to the low temperatures during the winter, it is very important the sugars and starch concentration in one year old canes, which influence the time for wood maturation and the buds viability. In Victoria variety the carbohydrate amount in canes was influenced both by the number of copper treatments applied and by the growing stage of treatment application.

At the beginning of winter, the highest concentration of carbohydrates in canes was recorded in the V_7 variant, the only one in which were applied three copper treatments. On the second and third rank the variants V_5 and V_4, in which were applied two copper treatments. The same ranking of variants was noted for the carbohydrates in canes in the early spring; all the experimental variants recorded higher significant limits compared with the control.

In the Cardinal variety, the canes concentration in carbohydrate was lower compared to the Victoria variety, but the experimental variants ranking was similar. In Cardinal variety the higher differences between the variants and the control showed the higher influence of the copper treatments on the cane carbohydrates concentration (Table 4).

In Merlot variety was recorded the highest carbohydrates concentration in canes compared with other two varieties, both at the beginning of winter and spring. The differences between the control and the experimental variants were also smaller. In Merlot variety the best results were recorded in the variant with three copper treatments (Table 5).

Table 3. Carbohydrates concentration in Victoria variety canes

Variant	Carbohydrates concentration (g %)		Carbohydrates concentration (%)		Difference compared to control (%)		Significance	
	15 XI	1 III	15 XI	1 III	15 XI	1 III	15 XI	1 III
V_1 (Control)	10.2	7.7	100	100	-	-	-	-
V_2	10.6	8.3	103.9	107.79	3.9	7.79	-	*
V_3	10.9	8.5	106.9	110.38	6.9	10.38	*	**
V_4	12.7	10.6	124.5	137.66	24.5	37.66	***	***
V_5	13	11.1	127.4	144.15	27.4	44.15	***	***
V_6	12.1	9.9	118.6	127.27	18.6	27.27	**	***
V_7	13.3	11.5	130.4	149.35	30.4	49.35	***	***

DL - 15XI 5% - 5.92 1% - 10.71 0.1% - 19.48
DL - 1 III 5% - 5.21 1% - 9.33 0.1% - 17.28

Table 4. Carbohydrates concentration in Cardinal variety canes

Variant	Carbohydrates concentration (g%)		Carbohydrates concentration (%)		Difference compared to control (%)		Significance	
	15 XI	1 III	15 XI	1 III	15 XI	1 III	15 XI	1 III
V_1 (Control)	8.7	5.7	100	100	-	-	-	-
V_2	9.2	6.1	105.74	107.01	5.74	7.01	-	*
V_3	9.4	6.4	108.04	112.28	8.04	12.28	*	**
V_4	10.9	8	125.28	140.35	25.28	40.35	***	***
V_5	11.2	8.4	128.73	147.36	28.73	47.36	***	***
V_6	10.4	7.5	119.54	131.57	19.54	31.57	**	***
V_7	11.9	9	136.78	157.89	36.78	57.89	***	***

DL - 15XI 5% - 6.53 1% - 11.98 0.1% - 21.42
DL - 1 III 5% - 6.21 1% - 11.16 0.1% - 20.82

Zufferey et al. (2012) found in Chasselas variety the lowest level of carbohydrates concentration in the two-year-old cane wood around flowering growing stage.
In Chardonnay variety, Vaillant-Gaveau et al. (2014), found that grapevine is able to correlate the inflorescences with the available carbohydrate amount from perennial wood.

The buds are some of the most sensitive organs of vines at frost. The level of frost damage on grapevine buds is directly related to the grape yield in the current year and of the next year.
The varieties chosen for the research are well-known as low buds viability, especially Cardinal and Victoria. Merlot variety is ranking among red wine varieties frequently influenced by climate change and variability (Table 6).

Table 5. Carbohydrates concentration in Merlot variety canes

Variant	Carbohydrates concentration (g %)		Carbohydrates concentration (%)		Difference compared to control (%)		Significance	
	15 XI	1 III	15 XI	1 III	15 XI	1 III	15 XI	1 III
V_1 (MT)	12.7	10.1	100	100	-	-	-	-
V_2	13.2	10.4	103.9	102.97	3.9	2.97	-	-
V_3	13.6	10.6	107.08	104.95	7.08	4.95	*	**
V_4	14.6	12	114.9	118.81	14.9	18.81	**	***
V_5	14.9	12.1	117.32	119.8	17.32	19.8	***	***
V_6	13.9	11.8	109.44	116.83	9.44	16.83	**	***
V_7	15.1	12.3	118.89	121.78	18.89	21.78	***	***

DL - 15XI 5% - 5.31 1% - 9.12 0.1% - 16.21
DL - 1 III 5% - 4.91 1% - 7.98 0.1% - 13.78

Table 6. Buds viability

Variant	Victoria			Cardinal			Merlot		
	Buds viability			Buds viability			Buds viability		
	%	Difference to control	Significance	%	Difference to control	Significance	%	Difference to control	Significance
V_1 (MT)	63	-	-	41	-	-	83	-	-
V_2	66	3	-	46	5	*	85	2	-
V_3	67	4	*	48	7	*	86	3	*
V_4	72	9	**	58	17	***	90	7	**
V_5	76	13	***	61	20	***	92	9	**
V_6	70	7	**	52	11	**	88	5	**
V_7	78	15	***	62	21	***	94	11	***

DL - Victoria 5% - 3.85 1% - 6.12 0.1%- 12.03
DL - Cardinal 5% - 4.37 1% - 7.33 0.1%-14.12
DL - Merlot 5% - 2.72 1% - 4.98 0.1%-9.75

Although the temperatures during research years were high, in the Cardinal variety, the buds viability ratio was relatively low, with limits between 41 and 62% depending on the variant.
In the Victoria variety, the buds viability ratio was higher than in the Cardinal variety, ranging between 63 and 78%, while in the Merlot variety was recorded the highest buds viability ratio, ranging from 83 to 94%. In all three varieties, the highest buds viability was recorded in the V_7 variant, in which was applied the highest amount of copper, followed by the V_5 and V_4 variants, in which was applied 1.61 kg of copper/ha. In the variants V_1, V_2 and V_3, in which were applied the smallest amounts of copper, was recorded the lowest ratio of buds viability.
In Figures 1, 2 and 3 are presented the correlation between cane carbohydrates concentration and buds variability in all three varieties. The highest correlation is recorded in Victoria variety while in Cardinal and Merlot varieties cane carbohydrates concentration influence on buds viability is moderate. Buds viability in experimental variants V_4 and V_6 are the most influenced by the cane carbohydrates concentration recorded in the first decade of

March during the research. On contrary, in V_1 (control), V_2 and V_3 was recorded the less influence of cane carbohydrates concentration on buds viability. Calugar et al. (2010) reported significant correlations between canes carbohydrates concentration and buds viability in grape varieties from Blaj vineyards.

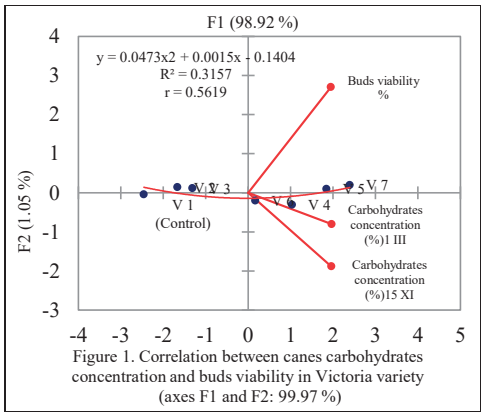

Figure 1. Correlation between canes carbohydrates concentration and buds viability in Victoria variety (axes F1 and F2: 99.97 %)

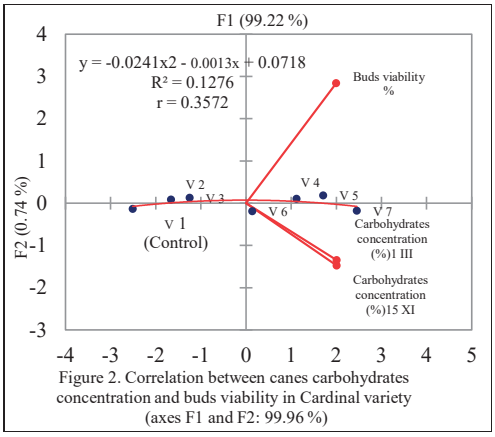

Figure 2. Correlation between canes carbohydrates concentration and buds viability in Cardinal variety (axes F1 and F2: 99.96 %)

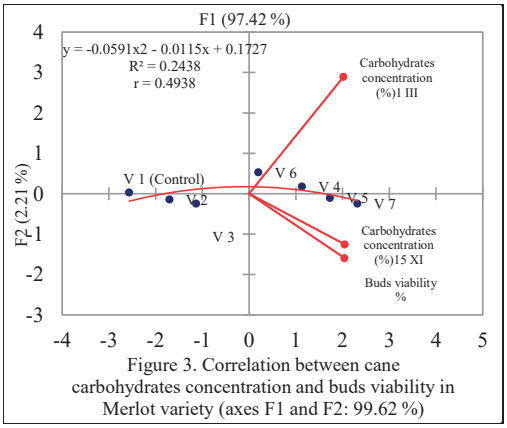

Figure 3. Correlation between cane carbohydrates concentration and buds viability in Merlot variety (axes F1 and F2: 99.62 %)

CONCLUSIONS

The must concentration in sugars was influenced both by the direct amount of copper applied during the phytosanitary treatments, as well as by the growing stage of the treatment application.

Too early copper application in V_2 variant (before flowering), led to the poorest results because the copper applied during the intense growth of the stems, decrease for short time the rate of photosynthesis.

The application of higher doses of copper maintains the canopy area in appropriate phytosanitary status in all three varieties and therefore the photosynthetic efficiency increased proportionally with the amount of copper applied. V_7, in which was applied 2.42 kg copper/per hectare, recorded the lowest canopy area necessary to synthesize one kilogram of sugars.

Copper treatments were favourable for canes carbohydrate concentration in all three grape varieties. The concentration carbohydrates in canes increased proportionally with the amount of copper applied; the highest values were recorded in V_7 variant, for which was applied 2.42 kg copper/ha.

The carbohydrate concentration in canes was influenced not only by the copper doses but also by the growing stage when copper treatment was applied. The control variant to which copper was applied only ones - in late ripening growing stage, recorded for all three varieties, the lowest concentrations of carbohydrates in canes. In the V_2 and V_3 variants were recorded higher concentrations of carbohydrates in canes compared to the control, for copper applied in a single treatment, but much earlier, before flowering and in lag phase respectively.

Copper treatments also had a favourable influence on the buds viability; number of viable buds increased gradually with the amount of copper applied. Besides the copper amount of copper applied, the buds viability was also influenced by the growing stage when treatments was applied; the lowest buds viability was registered when copper treatments were applied later in the ripening stage.

The late copper application does not have the expected effect on the one year old wood

maturation and buds viability due to the short time available until the first hoar frost. Since the maturation of the cane wood starts in the summer, it is very important for the vines to benefit from copper in moderate doses at least from the lag phase stage.

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IDENTIFICATION BY PCR ITS-RFLP TECHNIQUE OF NEW YEAST ISOLATES FROM PIETROASA VINEYARD

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Abstract

The biotechnological potential of local yeast is far to be completely investigated. Our work was focused on isolating and identifying by molecular tools different yeast isolated during the winemaking process (2017 and 2018), from the different varieties of grape, grape pomace and residual biomass (Burgund Mare, Busuioaca de Bohotin, Feteasca Neagra, Italian Riesling, Tamaioasa Romaneasca) originated in Pietroasa vineyard. A total of 14 different colonies were isolated and cultivated under specific conditions. In the next step, these isolates were subject to molecular identification by PCR-ITS-RFLP technique. The 5.8S-ITS region was amplified using the universal primers ITS1 and ITS4, followed by digestion with HhaI, HinfI, HaeIII. As expected, most frequent species was found to be Saccharomyces cerevisiae. Other non-Saccharomyces strains were identified as Dekkera anomala or Metschnikowia pulcherrima. The new isolated Saccharomyces and non-Saccharomyces strains will be subject of further test for the biomass production of different industrial application.

Key words*: local yeast, PCR-ITS-RFLP, grape pomace, biomass, Pietroasa vineyard.*

INTRODUCTION

Numerous studies have been reported about yeast frequency and diversity (classified in *Saccharomyces* and the non-*Saccharomyces*) associated with grape berries (vineyards) or wine production (must, during the fermentation process and wine, wineries) (Pretorius, 2000; Fleet et al., 2002; Prakitchaiwattana et al., 2004; Burcea & Radoi, 2005; Doaré-Lebrun, 2005; Renouf et al., 2005, 2007; Matei et al., 2011, 2014; Nemcová et al., 2015; Grangeteau et al., 2017; Abdo et al., 2020).

Yeast frequency is low, ranging from 10^2-10^3 CFU.g^{-1} on immature grapes and increase to 10^3-10^5 CFU.g^{-1} on grapes during harvest, with an increase in diversity of genera and species encountered (Prakitchaiwattana et al., 2004; Renouf et al., 2005, 2007; Barata et al., 2008; Grangeteau et al., 2017). Yeast populations present on grapes damaged or affected by rot are higher, ranging between 10^5-10^7 CFU.g^{-1} (Nisiotou and Nychas, 2007; Barata et al., 2008; Guzzon et al., 2014). Yeast diversity associated with grape berries has been reported in different vineyards from several countries: Argentina (Combina et al., 2005); Australia (Prakitchaiwattana et al., 2004), Brazil (Baffi et al., 2011); China (Li et al., 2010); France (Doaré-Lebrun, 2005; Renouf et al., 2005, 2007; Grangeteau et al., 2017); Germany (Brysch-Herzberg and Seidel, 2015), Greece (Nisiotou and Nychas, 2007); Slovakia (Nemcová et al., 2015); Spain (Sabate et al., 2002; Clavijo et al., 2010); Portugal (Barata et al., 2008).

According to the International Organisation of Vine and Wine (OIV), Romania is situated in top grape-producing countries in the world, alongside traditional wine-making countries (France, Italy, Spain, etc.) or more recent producers as United States, Chile Argentina or Australia (OIV, 2019). Several studies have been conducted on the isolation and characterization of indigenous yeasts from different Romanian vineyards and it was proven an important potential for industrial application of the autochthonous isolates (Burcea & Radoi, 2005; Viziteu et al., 2008;

Antoce et al., 2011; Matei and Găgeanu, 2011; Matei Radoi et al., 2011; Matei et al., 2014).

In the winemaking process, the wide use of commercial starter cultures of active dry yeast has become a routine practice in order to lead the controlled alcoholic fermentation, to produce of desirable compounds, as well to reduce the risk of spoilage and unpredictable changes of wine flavour (Lu et al., 2016; Belda et al., 2017; Berbegal et al., 2018). On the other hand, trends of wine market have been focused toward the production of wines by indigenous yeasts (*Saccharomyces* and non-*Saccharomyces*) isolated from grape berries (Grangeteau et al., 2017) or winery (Abdo et al., 2020), which can contribute to the specific aromatic fingerprints of wines.

The yeast identification based on cultural, morphological, and biochemical traits are laborious and time-consuming. On the other hand, various molecular methods based on DNA analysis have been developed enabling rapid and reliable identification of a large number of yeast at specie level in a much shorter period: Restriction Fragment Length Polymorphism (RFLP) based on the ITS-5.8S region (Esteve-Zarzoso et al., 1999; Sabate et al., 2002; Baffi et al., 2011; Csutak et al., 2016); Real-time quantitative PCR (qPCR) (Hierro et al., 2006; Zott et al., 2010) PCR-denaturing gel gradient electrophoresis (DGGE) (Prakitchaiwattana et al., 2004; Renouf et al., 2007), pyrosequencing (Grangeteau et al., 2017).

The present work is part of a larger national project focused on the yeast biodiversity of grape pomace after the winemaking process, as well on the selection of valuable indigenous yeast for added valued products.

MATERIALS AND METHODS

Grape samples and yeast isolation

Grape samples (grape or grape pomace or residual biomass) were picked up from vineyards owned by USAMV of Bucharest at Pietroasa Station, during the harvests 2017 and 2018. The samples originate from different grape varieties: (Burgund Mare, Busuioaca de Bohotin, Feteasca Neagra, Italian Riesling, Tamaioasa Romaneasca). A grape sample of 1 g was suspended in 9 ml distilled water for decimal dilution; 0.1 ml suspended solution was spread in the Petri dish with Malt Extract Agar (MEA) (VWR, USA) supplemented with colaramphenicol (to inhibit bacterial growth). The plates were incubated at 25°C, for 72 hours. Different colonies of the representative yeasts were purified until obtaining a pure culture and cryopreserved at -20°C in culture medium containing Difco™ Malt Extract Broth (Vwr, USA) with 25% (v/v) glycerol until further identification (Begea et al., 2012). All isolated yeasts were deposited in the culture collection of the UASVM Bucharest.

Morphological characterization

Isolated yeasts were grown on MEA medium at 25°C for 72 h and grouped based on their colony aspect and microscopically observations according to Lodder (1974), Pitt and Hocking (2009).

Extraction of the genomic DNA

Each isolate was cultivated in MEA medium for 72 hours. Yeasts cells were washed with sterile distilled water, transferred to a 1.5 ml Eppendorf tube and centrifuged at 5,000 *g* for 10 minutes 4°C to sediment the yeast cells. DNA was extracted according to procedures described by Ausubel et al. (2002) with some modifications. Cell pellets were resuspended in 200 µL lyse buffer (1% SDS, 2% Triton X-100, 100 mM NaCl, 10 mM Tris pH 8.0, 1 mM EDTA, pH 8.0), 200 µL phenol:chloroform:isoamyl alcohol (25:24:1) solution, and homogenized with 0.3 g of glass beads (0.5 mm in diameter) in a Vortex agitator. After, TE buffer (200 µl) (10 mM Tris, 1 mM EDTA, pH 8), was added and centrifuged at 12,000 *g* for 5 min at 4°C. The supernatant was transferred to a new tube and treated with 2 µl RNase (10 mg/mL) (Thermo Scientific, USA) for 30 minutes at 37°C. DNA was precipitated with sodium acetate (3M) and cold absolute ethanol and kept in -20°C for 30 minutes. A new centrifugation was performed at 12,000 *g* for 5 min at 4°C, washed with 70% ethanol and centrifuged again at 12,000 *g* for 5 min at 4°C. DNA pellets were air dried at room temperature and solubilized in 50 µL ultrapure water. The concentration and purity of DNA were measured spectrophotometrically (SpectraMax® QuickDrop™ (Molecular Devices, USA). Finally, the DNA was stored at -20°C until use.

PCR-ITS

Molecular identification of the ITS1-5.8S-ITS2 region of each yeast isolate was performed using the ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers. The mixture of PCR (50 µL) consisted of 10X DreamTaq Green Buffer (contains 20 mM $MgCl_2$), 0.2 mM dNTPs, 0.5 mM of each primer, 0.025 U of DreamTaq DNA Polymerase (Thermo Scientific, USA) and 10 ng of yeast DNA. PCR was carried out in MultiGene thermal cycler (Labnet International, Inc., Cambridge, United Kingdom) with an initial denaturation cycle for 2 min at 94°C, followed by 34 cycles of amplification (with 1 min at 94°C, 1 min at 55°C, 2 min at 72°C) and a final extension cycle for 7 min at 72°C.

Restriction digestion

Digestion of ITS products was performed using *Hha*I, *Hinf*I, *Hae*III (Thermo Scientific, USA), according to ITS-RFLP method developed by Esteve-Zarzoso et al. (1999). The digestion reaction of each enzyme was performed in a volume of 20 µl containing 2 µL 10X digestion buffer, 1 U enzyme, 10 µL PCR product and ultrapure water to complete the volume. The digestions were performed at 37°C for 90 min.

PCR-RFLP analyses

PCR products and restriction fragments were checked by electrophoresis on 2% (w/v) agarose gel respectively, added with 0.05 µg/L of ethidium bromide in 1× TBE buffer (Tris-Borate-EDTA). The migration had lead at 90V for 1h. After electrophoresis, gels were visualized under UV light using GelDoc-It Imaging System (Analytik Jena, USA). All fragment sizes were approximated using standard molecular weight markers (GeneRuler 100bp Plus DNA Ladder, Thermo Scientific, USA). RFLP profile obtained with each enzyme was analysed and compared with the data of Esteve-Zarzoso et al. (1999), in order to identify at specie level.

RESULTS AND DISCUSSIONS

Fourteen strains have been isolated during the winemaking process (2017 and 2018), from the different varieties of grape (5 yeast isolates), grape pomace (8 yeasts isolates) and residual biomass (1 yeast isolate) originated in Pietroasa vineyard (Table 1).

Table 1. Classical identification based on macroscopic and microscopic characteristics of yeasts isolates

Yeast code	Origine	Colony aspect (shape, colour, aspect of the margin and texture)	Microscopic observations
PS1	grape pomace - Tămăioasă Românească	Round, white to cream, smooth, butyrous	globose to ovoidal
PM1	grape - Fetească Neagră	Round, white to cream, smooth, butyrous	globose to ovoidal
PC1	grape pomace- Busuioacă de Bohotin	Round, white to cream, smooth, butyrous	globose to ovoidal
PM2	grape pomace - Burgund Mare	Round, white to cream, smooth, butyrous	globose to ovoidal
PM3	grape pomace - Burgund Mare	Cream with red-brown from bottom, convex; smooth, opaque.	ovoid to ellipsoidal
PS2	residual biomass- Tămăioasă Românească	Round, white to cream, smooth, butyrous	globose to ovoidal
PM4	grape pomace - Fetească Neagră	Round, white to cream, smooth, butyrous	globose to ovoidal
PC2	grape pomace- Busuioacă de Bohotin	Cream with red-brown from bottom, convex; smooth, opaque.	ovoid to ellipsoidal
PC3	grape pomace- Busuioacă de Bohotin	Round, white to cream, smooth, butyrous	globose to ovoidal
PC4	grape pomace - Riesling Italian	Cream with red-brown from bottom, convex; smooth, opaque.	ovoid to ellipsoidal
PC5	grape - Busuioacă de Bohotin	Irregular, white to cream, smooth, butyrous to membranous	spheroidal to ellipsoidal
PM5	grape	Irregular, white to cream, smooth, butyrous	spheroidal to ellipsoidal
PC6	grape - Riesling Italian	Round, white to cream, smooth, butyrous	globose to ovoidal
PC7	grape	Round, white to cream, smooth, butyrous	globose to ovoidal

Initially, the strain identification of the yeast isolates was carried out on the basis of their microscopic and macroscopic observations (Table 1). 9 yeasts isolates showed round, white to cream, smooth, butyrous colonies and globose to ovoidal cells. Three yeasts isolates

were grouped by cream with red-brown colour on the bottom, convex; smooth, opaque aspects with ovoid to ellipsoidal cell morphology. Spheroidal to ellipsoidal cells were observed in two other yeasts isolates with irregular, white to cream, smooth, butyrous to membranous colonial aspects.

The identification of yeast isolates on specie level was performed by molecular methods. The internal transcribed spacer (ITS) region consisting of the 5.8S rRNA gene and two variable flanking is considered genetic markers (White et al., 1990). Polymerase chain reaction (PCR) methods based on this targeted region are developed to identify and differentiate between fungi species (Esteve-Zarzoso et al., 1999; Sabate et al., 2002; Prakitchaiwattana et al., 2004; Baffi et al., 2011; Zott et al., 2010). In our study, based on the amplification of the 5.8S-ITS region using primers ITS1 and ITS4, the amplicons were estimated to be approximately 400 bp (for 3 yeasts isolates), 800 bp (for 2 yeasts isolates) and 880 bp (for 9 yeasts isolates) (Table 2). Esteve-Zarzoso et al (1999) developed a PCR-RFLP method of the 5.8S-ITS region to discriminate 132 yeast species belonging to 25 genera using a combination of three endonucleease such as *Cfo*l, *Hae*lll and *Hinf*l. The PCR products were digested separately with three different restriction endonucleases, *Hha*I, *Hae*III and *Hinf*I and the restriction fragments obtained are presented in the Figure 1. Digestion with each restriction enzyme yielded three distinct restriction profiles (Table 2). The isolates were grouped according to distinct restriction patterns: nine yeasts isolates generated a restriction pattern corresponding to *Saccharomyces cerevisiae*, three yeasts isolates corresponding to *Metschnikowia pulcherrima* and two yeasts isolates corresponding to *Dekkera anomala* (Tabel 2).

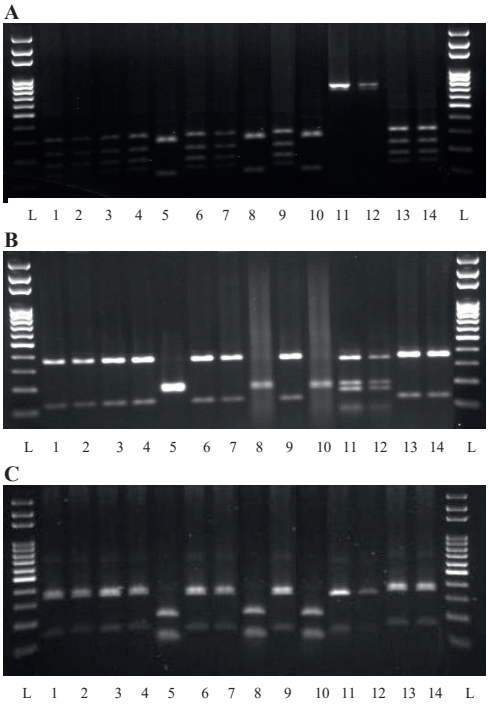

Figure 1. The restriction fragments of the PCR-ITS products of yeast isolates with *Hae*III (**A**); *Hinf*I (**B**); *Hha*I (**C**)
1- PS1; **2**- PM1; **3**- PC1; **4**- PM2; **5**- PM3; **6**- PS2; **7**- PM4; **8**- PC2; **9**- PC3; **10**- PC4; **11**- PC5; **12**- PM5; **13**- PC6; **14**- PC7

Table 2. Identification of yeasts isolates by RFLP analysis of 5.8S-ITS region

Yeast species	**Yeast isolate**	**PCR-ITS (pb)**	**Restriction fragments (bp)**		
			***Hae*III**	***Hinf*I**	***Hha*I**
Metschnikowia pulcherrima	PC6; PC2; PC4	400	280 + 120	200	210 + 110
Dekkera anomala	PC4; PM5	800	800	350+200+170+80	340+130
Saccharomyces cerevisiae	PS1; PM1; PC1; PM3; PS2; PM4; PC3; PC6; PC7	880	320+230+180+150	370+ 120	380+ 360+140

Saccharomyces cerevisiae, the main yeast involved in alcoholic fermentation, was detected on grapes and grape pomace. For some authors it is even undetectable on grapes (Combina et al., 2005) or present in a very low proportion of about 1-10 CFU.mL^{-1} (Prakitchaiwattana et al., 2004; Renouf et al. 2007) or its presence increased on spoilage grapes.

Metschnikowia is considered as a component of non- *Saccharomyces* yeasts in mature grape berries and during the first stages of grape

musts (Pretorius, 2000; Fleet et al., 2002; Prakitchaiwattana et al., 2004; Combina et al., 2005). *Metschnikowia* strains have been investigated as biocontrol agent, against filamentous fungi, yeasts, bacteria and for the high enzymatic activities to release aromatic precursors from grapes which enhance aromatic profiles of wines (Sipiczki, 2006; Csutak et al., 2013; Oro et al., 2014; Morata et al., 2019).

Among non-*Saccharomyces* yeast, two isolates have been identified as *Dekkera anomala*. Yeasts species belonging to the genus *Dekkera* (anamorph *Brettanomyces*) are associated with the off-flavours, due to the production of ethyl phenols, which lead to spoilage of wine (Pitt and Hocking, 2009; Oelofse et al., 2008; Berbegal et al., 2018). *Dekkera anomala* was detected as yeast spoilage of spoil beer, cider and soft drinks (Gray et al., 2011), but is not linkage to wine spoilage (Loureiro and Malfeito-Ferreira, 2003). For this reason, future investigations will be performed on these yeast isolates focussed on their ability to wine spoilage.

CONCLUSIONS

In the present study, 14 yeasts colonies were isolated, purified and grouped on the basis of appearance of colony and cell morphology. RFLP analysis of the ITS-5.8S region was completed the identification of yeast isolates at the species level: *Saccharomyces cerevisiae* (9 yeasts of all total isolates), followed by *Metschnikowia pulcherrima* (three of the total isolates) and *Dekkera anomala* (two of the total isolates). Future researches will lie on the use of indigenous *Saccharomyces cerevisiae* isolates mixed with *Metschnikowia pulcherrima* isolates and others non-*Saccharmomyces* as starter cultures to obtain and promote new wines with lower alcoholic degree and strong indigenous aromatic profiles. Also, the use of the *Saccharomyces* and non-*Saccharomyces* strains as biomass for other biotechnological application will be investigated (e.g. as potential feed source).

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MECHANICAL COMPOSITION AND FERTILITY ELEMENTS OF CLONES 48, 1089, and 1091 cv. RIESLING

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Abstract

Agroecological conditions and production technology have the greatest impact on the composition of wine, sensory characteristics, and consumer attitudes. The analysis of the connection between these factors is the basis for the recommendation of a certain variety (clone) in a production region. In this study, three French clones of cv. Riesling: 48, 1089, and 1091 were analyzed for the first time in Ukrina vine-growing region (the northwestern part of the Republic of Srpska - Bosnia and Herzegovina). The research was conducted during the 2016-2017 period in the vineyard of a private winery, by determining its production potential and technological characteristics. The basic elements of fertility and mechanical characteristics of grapes and berries were analyzed. In years with very variable climatic conditions, the examined clones had a satisfactory mass of grapes (85.7-131.8 g), a high share of berries in the structure of the cluster (94.70-96.00%) and a slightly lower yield (5.4-7.5 t·ha^{-1}). Tested clones deserve attention for further examination of their characteristics (wine quality) with regard to product characteristics and the possibility of their cultivation in the examined area

Key words*: riesling, clone, productivity, quality.*

INTRODUCTION

The choice of the appropriate grape variety is given great importance in modern viticulture (Kerridge and Antiliff, 2004). Variety as a quality factor, with its specificity has a crucial role in wine production especially when it comes to the production of high quality wines (Jovanović-Cvetković et al., 2008). Today, there are many different clones of certain varieties, with different quality characteristics. Clone selection is a way to correct old or significant varieties, i.e. to single out individuals who have not experienced negative mutations in production traits (Manninia, 2000; Cindrić, 2003). As it has been in production for many years cv. Riesling has shown a great deal of heterogeneity so its clonal selection has been done in Europe. Clonal selection can increase the yield of cultivar Riesling by up to 36% (Jacsons and Lombard, 1993). New clonal selections provide an excellent opportunity for growers to improve the production of this variety in different environmental conditions and to achieve satisfactory wine quality. Examination of the characteristics of the cultivar Riesling indicated the need to determine the influence of macro and micro climates, soil type and technology of grape and wine production on the composition of wine, sensory characteristics and attitude of consumers. Without a better knowledge of the correlation between these elements in production, classification of wines based on geographical origin may be inappropriate (Fischer, 2011). Examination of the characteristics of the cultivar Riesling (Cindrić et al., 2000; Todić et al., 2000; Friedel et al., 2016) and its clonal selections (Pejović and Maraš, 1994; Ćirković and Garić, 2006; Jovanović-Cvetković et al., 2011; Stroe and Ioana, 2015) is of great importance for the recommendation of further expansion in production. The research aims to determine for

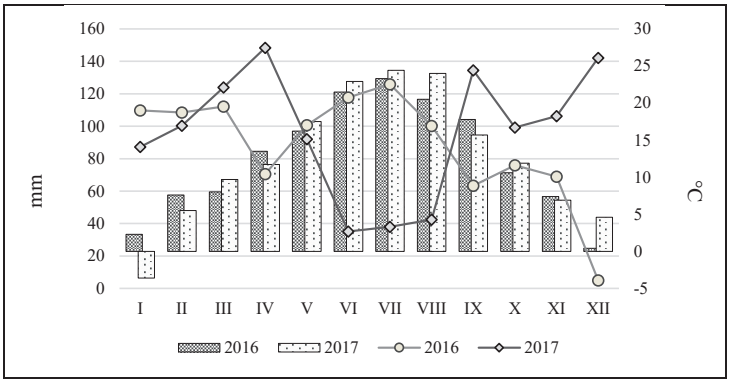
the first time in the area of Ukrina vine-growing region (the northwestern part of the Republic of Srpska - Bosnia and Herzegovina) the production potential and technological characteristics of Riesling clones and indicate the justification of their cultivation in this region.

MATERIALS AND METHODS

The field experiment was performed in 2016 and 2017 in the vineyard of the "Fazan" winery, which belongs to the Ukrina vine-growing region. The characteristics of French clones 49, 1089 and 1091 of the Riesling were examined. So far, vineyard was established in 2008 with a planting distance of 3.0 x 1.0 m. The training system of the vine is Gijo simple. Mixed pruning was performed on 10 randomly selected vines - one long spur with 10 buds and one renewal spur with 3 buds (with total load of 13 buds per vine). The basic elements of fertility (fertility coefficient, grape mass and grape yield per grapevine) and uvological characteristics of grapes and berries were analyzed. The fertility coefficient was obtained from the ratio of the number of inflorescences per vine and the number of fruiting shoots per vine. Mechanical analysis was performed on bunches at the time of optimal maturity on 10 bunches and 100 berries. Elements of the mechanical composition of the grapes and berries were made according to the method of Prostoserdov (1946). The statistical analysis was performed using Statgraphics Centurion. Obtained results were subjected to analysis of variance (ANOVA) according to a factorial design, where the sources of variation were year and clone, and their interaction. Comparison of means was performed by the Tukey test ($\alpha = 0.05$). The results are presented as the mean value ± standard error of mean (SEM), and coefficient of variation (CV).

RESULTS AND DISCUSSIONS

Variable climatic conditions prevailed in the research period (Graphic 1). Slightly higher air temperatures and smaller precipitation amounts were recorded in 2017 which was therefore somewhat more favorable for the wine production and protection against pathogens. Clones 1089 and 1091, respectively, had a higher cluster mass and a mass of berries in the cluster in 2016 compared to 2017 (Table 1) which like other parameters was likely influenced by climatic conditions. Clone 1089 had the highest mass of grapes in 2016 (131.8 g) but at the same time the lowest in 2017 (85.7 g). The difference in cluster mass of clone 1089, was statistically significant between the years, but not in comparison with the other two clones in the survey years. Variations in the mass of grapes in the cultivar Riesling ranged from 90.71 g to 150.00 g (Todić et al., 2000; Cindrić et al., 2000; Stroe and Ioana, 2015). Similar characteristics are shown by clones 198, Gm 239 (Pejović and Maraš, 1994) and clone B 21 (Jovanović-Cvetković et al., 2011).



Graphic 1. The average monthly temperatures (°C) and total precipitation (mm) during 2016-2017 period

Accordingly, the mass of berries in the cluster at clone 1089 was statistically significantly higher in 2016 (124.7 g) compared to 2017 (81.5 g). Interaction Year × Clone is significant in bunch weight and mass of berries. Berries were heavier in 2016 (81 g/bunch) than in 2017 (69), in average clone 49 had heavier berries (82 g per bunch) than clone 1091 and 1089 (72 and 71 g per bunch, respectively). The number of berries in the cluster during the research period ranged from 58.9 to 87.0, which can also be considered as a cultivar characteristic (Žunić and Garić, 2017). The suitability of grapes for processing depends also on the percentage ratio of individual components, as determined by mechanical analysis of the grapes and berries. These elements, with the characteristics of the grape of variety grown under certain conditions, provide enough elements to draw conclusions about the quality of grapes for processing into wine. The percentage of shellfish and berries (Figure 1) is uniform in the clones examined. Clone 1091 had the highest percentage of berries during the research (96.00 %). Riesling clones Gm 239 (95.97 %) and B21 (96.50 %) have a similar percentage of berries in the cluster structure (Jovanović-Cvetković et al., 2011). In both years of research, the largest percentage of meat with juice (Figure 2) had clone 49 (86.45%), which is significantly higher compared to clones Gm 239 and B 21 as well as the standard cultivar Riesling (Jovanović-Cvetković et al., 2011).

Table 1. Structural bunch indicators of tested Riesling clones

Year/clones	Average bunch weight (g)	Number of berries per bunch	Mass of berries per bunch
	$\overline{X}$ ± SEM		
2016/49	99.9^{ab} ± 8.81	87.0^{a} ± 4.97	94.9^{ab} ± 8.38
2016/1089	131.8^{b} ± 10.04	82.7^{a} ± 4.98	124.7^{a} ± 9.37
2016/1091	106.3^{ab} ± 9.84	73.2^{a} ± 4.79	102.1^{ab} ± 9.56
2017/49	104.7^{ab} ± 9.80	77.2^{a} ± 5.91	99.9^{ab} ± 9.34
2017/1089	85.7^{a} ± 6.63	58.9^{a} ± 4.53	81.5^{b} ± 6.12
2017/1091	94.4^{ab} ± 9.32	70.3^{a} ± 5.72	90.0^{ab} ± 8.88

$^{a, b}$means (interaction Year × Clone) followed by different letter(s) are significantly different (Tukey, $\alpha = 0.05$)

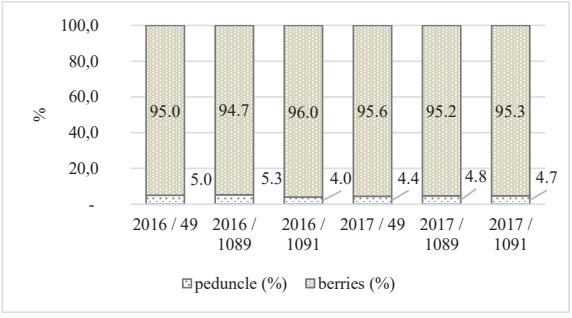


Figure 1. Mechanical composition of the bunch

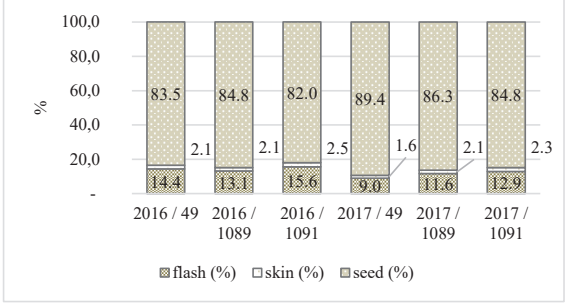


Figure 2. Mechanical composition of berry

The clones tested had a larger mass of 10 berries in 2017 compared to 2016 (Table 2). Interaction Year × Clone is significant in the mass of ten berries.

Table 2. Mass and number of seeds in berries

Year/clones	Mass of 10 berries (g)	Number of seeds in 10 berries
	$\bar{X}$ ± SEM	
2016/49	$13.7^{b} \pm 0.36$	$15.8^{a} \pm 1.19$
2016/1089	$15.8^{a} \pm 0.58$	$19.1^{a} \pm 0.98$
2016/1091	$15.4^{ab} \pm 0.35$	$16.4^{a} \pm 0.76$
2017/49	$17.1^{a} \pm 0.59$	$23.2^{a} \pm 1.14$
2017/1089	$16.3^{a} \pm 0.36$	$22.3^{a} \pm 1.06$
2017/1091	$15.3^{ab} \pm 0.52$	$21.0^{a} \pm 0.77$

[a, b]means (interaction Year × Clone) followed by different letter(s) are significantly different (Tukey, $\alpha = 0.05$)

Clone 49 in 2016 had a statistically significantly lower mass of 10 berries than in 2017. This clone also had a statistically smaller mass of 10 berries than clone 1089 in both survey years.

The results obtained are in accordance with the statements of other authors who found that the average weight of the cultivar Riesling berry averages 1.38 g (Friedel et al., 2016a) and 1.32 g (Stroe and Ioana, 2015).

The size of the berry of the Riesling is influenced by the position of the berry in the cluster (Friedel et al., 2016a) but also by the measures applied in the production process (Friedel et al., 2016b). Berries had a significantly higher number of eeds in 2017 than in 2016 (22 and 17 in ten berries, respectively).

The teritility coefficient was fairly uniform with the clones tested in the years of research (Table 3) and significantly higher than the standard variety (Todić et al., 2000). Clones 49 and 1091, had a slightly higher fertility rate than clone 1089, indicating that they belong to the group with high fertility rate (Žunić and Garić, 2017), which, depending on the load in this variety, ranges from 1.82 to 1.93.

Table 3. Fertility elements - fertility coefficient

	2016		2017	
	$\bar{X}$ ± SEM	CV (%)	$\bar{X}$ ± SEM	CV (%)
49	2.2 ± 0.48	22.1	2.1 ± 0.18	8.8
1089	1.9 ± 0.17	9.0	1.8 ± 0.27	15.3
1091	2.0 ± 0.31	15.6	2.0 ± 0.30	15.3

Clones 1089 and 1091 had higher yields ($t \cdot ha^{-1}$) in 2016 compared to 2017 (Table 4). Interaction Year × Clone is significant in the grape yield. Clone 1089 had a statistically significantly higher yield in 2016 (7.5 $t \cdot ha^{-1}$) compared to 2017 (5.4 $t \cdot ha^{-1}$).

Table 4. Fertility elements - grape yield ($t \cdot ha^{-1}$)

	2016		2017	
	$\bar{X}$ ± SEM	CV (%)	$\bar{X}$ ± SEM	CV (%)
49	$6.2^{ab} \pm 1.24$	20.1	$6.6^{ab} \pm 1.32$	20.0
1089	$7.5^{a} \pm 1.62$	21.7	$5.4^{b} \pm 1.22$	22.7
1091	$7.0^{ab} \pm 1.63$	23.3	$5.7^{ab} \pm 1.73$	30.6

[a, b]means (interaction Year × Clone) followed by different letter(s) are significantly different (Tukey, $\alpha = 0.05$)

The difference from the other two clones in the survey years was not statistically significant. The different fertility of the tested clones in France (1089 - 2.3 kg per vine; 1091 - 3.1 kg per vine; 49 - 3.8 kg per vine) indicates different predispositions of these clones, as well as the influence of the growing region (www-pepinieres-jenny-com).

The yield of the Riesling variety depends on the number of the buds (Stroe and Ioana, 2015) which certainly has an impact on the quality of the grape.

CONCLUSIONS

During the two-year study, the tested clones showed variety characteristics specific to the Riesling and his clones. The mass of the cluster was uniform, except for clone 1089, which in 2016 had a significantly higher mass than other clones. The largest variations were observed in this clone in the other analyzed parameters as well, which was not found in the other two clones. Mechanical analysis of grapes and berries indicates good predispositions of these clones in terms of flesh ration in wine production. The tested clones had a lower yield compared to data from other regions, but also compared to the original cultivar and some other clones. The issue of yield is also closely related to the desired quality, so the level and intensity of vine management can greatly affect the realization of the final yield.

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CLIMATE VARIABILITY AND CANOPY MANAGEMENT INFLUENCE ON GRAPE BERRIES QUALITY IN MERLOT AND PINOT NOIR VARIETIES

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Abstract

The aim of the study was to investigate the climate and canopy management effects on Merlot and Pinot Noir berries quality, from Minis vineyards, Romania. During four growing seasons (2016-2019), crop load and cluster thinning were investigated on double Guyot training system. For grape berries composition analysis samples were individual harvested on each vine. Each season was applied for both varieties the same winter pruning level, for 10, 20, 30 and 40 crop load. For vine balance, bunches were removed in three stages of the growing season: fruit set, lag phase and 20% veraison. Crop load associated with climate from growing season influenced the grape berries composition concerning the sugar, titratable acidity and pH, more than cluster thinning treatments. Weather within growing season override the canopy management practices: cold and wet weather in the spring decrease the grape production while warm weather mainly in the ripening time had little effect on Merlot and Pinot Noir berries composition. Therefore, cluster thinning is expensive with high production losses and small increase in grape berries qualities to can balance the profit of winemakers.

Key words: *climate, crop load, growing season, Merlot, Pinot Noir.*

INTRODUCTION

Grapevine is cultivated more than 4,000 years B.C. and has about 60 wild species across Europe, North America and Asia (Zohary and Hopf, 2000). The story of vine spreading around the world is related to the Europeans colonies; Columbus and other explorers, brought *Vitis vinifera* to Mexico and then throughout Latin America, on California, Australia, or to Far East (Buxó, 2008). About 1,600 years ago, some North American hybrids were created and selected specifically to withstand *Phylloxera* (Arnold et al., 2005). Planted in Europe, these varieties have led to a spectacular increase in grape yield and, consequently, in wine production. The story of wine is, in fact, just as old as grape growing (Grassi et al., 2003). Egyptian inscriptions mention the production and storage of wine, the Etruscans and the Greeks were delighted with Bachus's drink, and the Romans created powerful vine-growing areas throughout almost the entire empire, but especially in some areas of nowadays France, Germany and Austria (Guasch-Jané et al., 2013). Around 4,000 different varieties of table grapes and wine grapes are grown today in the world and 1368 are included in commercial production (Robinson et al., 2012).

Relationship of climate variability and viticulture

Connection between grape yield and berries composition has been studied extensively but climate variability from last decades require new research in grape growing regions to found the best management practices in vineyards (Kliewer and Dokkozlian, 2005; Sipiora, 2009). During the grapevine growing seasons there are many possibilities for interventions in vine and canopy development through different management practices. Two common practices like winter pruning and cluster removal involve variation in berries development and further berries and wine composition (King et al., 2015). Vine balance and yield are strongly influenced by weather. Global warming is

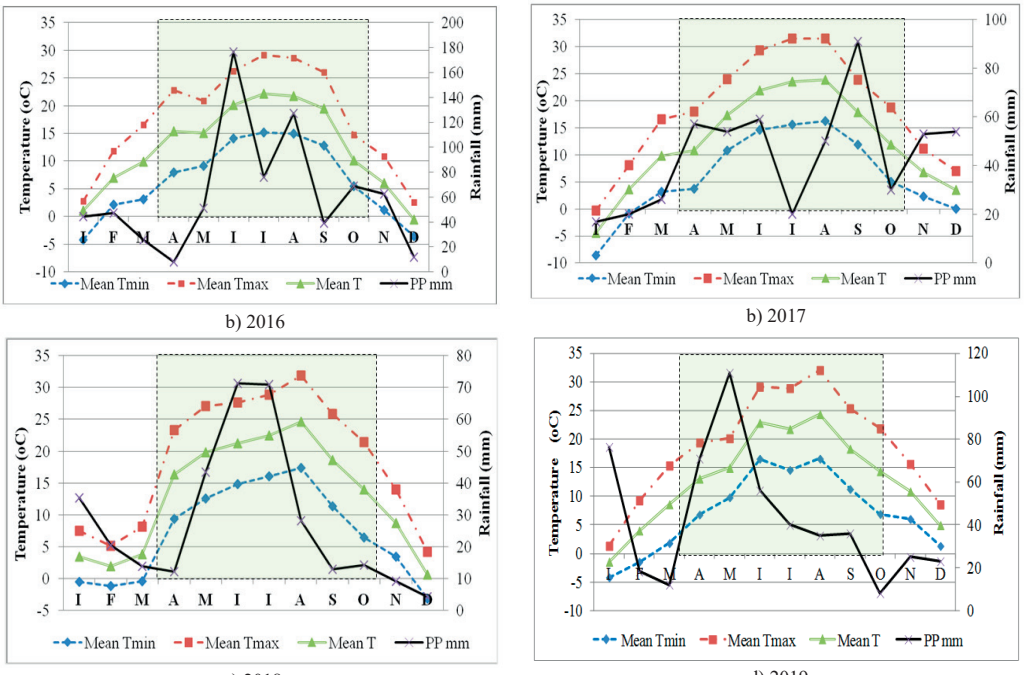
increasingly evident in recent decades but climate variability during growing stages has major influence on vines and grapes growth and development (Nistor et al., 2018). Grapevine growing regions are "moving up" for some cultivars towards potential northern areas, while many traditional wine regions will not be suitable anymore for wine grapes (Mozell and Thach, 2014).

MATERIALS AND METHODS

Minis vineyard is located in the west of Romania on the Siria-Lipova alignment, bordered by the Zarand Mountains in the east, which drop from 800 m to the surrounding hills and plains. Soils from vineyards area are cambisols characterized by accumulated layer of humus, clay, iron oxides, or soluble salts. First soil layers are sandy loam form on medium parent material texture, and are different from brown clay to regosols.

Vineyard is southern exposure; climate in the area has a Mediterranean influence that brings long, warm and dry autumns. During the ripening period, the average monthly temperature ranged between 22.5-23.6°C (July-August) and 18.56°C in September. The average temperature during the growing season is 17.2°C (Figure 1). Most rainfall is recorded in May, June and July, a humidity favourable for fungal infections. The vine and row spacing was 1.5 x 2.5 m. In pruning trial at the Minis Vineyard with Merlot and Pinot Noir varieties, four types of crop load were imposed: 10, 20, 30 and 40 buds per vine, and bunches were removed in three stages of the growing season: fruit set, lag phase and 20% veraison. Each treatment was replicated three times with six vines per replicate plot design.

Vines were pruned to double Guyot system. The ratio of clusters removed ranged between 10 and 30% over four growing seasons in time of fruit set, lag phase and 20% veraison. Clusters from lateral shoots were removed. Temperature and rainfall data were collected from the Minis Research weather station during 2016-2019.



b) 2016 b) 2017

c) 2018 d) 2019

Figure 1. Temperature and rainfall in Minis area, during 2016-2019

Measurements for berries quality were conducted immediately after samples collecting. The chemical parameters of berries (sugars, titratable acidity, and pH) were

subsequently made in the laboratory. For sugars, the digital refractometer HI96811 was used and expressed in Brix scale. For the determination of acidity, the acids in the must were neutralized with NaOH 0.1 n solution in the presence of 1% phenolphthalein, with a semi-automatic titrator and the required volume was recorded. The acidity was expressed in g/L. The pH value in the must was measured before titration.

Data were analyzed using SAS ver. 9.13 (SAS Institute Inc., Cary, NC 27513, USA). A randomized design and statistical significance establish at 5% level. Significant differences were assessed by two ways - ANOVA.

The objectives of the paper were to examine several canes pruning level with variable number of crop load per vine and cluster removal effect on grape berries composition in Merlot and Pinot Noir varieties, during 2016-2019 growing seasons.

RESULTS AND DISCUSSIONS

High canopy vigor and shading can have negative impact on inflorescence development, poor fruiting set, ripening and fruit quality (Feng et al., 2015). Imbalanced canopies can be managed by trellising, training systems, pruning, or thinning practices (Archer & Van Schalkwyk, 2007). Merlot variety has high yield potential but can often reach excess vigor produced by lateral shoots; to avoid overshadow/over cropping pruning and thinning operations can balance the grape yield (Spayd et al., 2002). On the contrary, Pinot Noir is less vigorous variety, with smaller grapes and yield, but cluster tinning is believed to increase the berries quality and ripening (Nistor et al., 2019).

The average yield after cluster thinning at fruit set stage in Merlot and Pinot Noir was lower in all four growing seasons compared to the control (Figure 2).

Grape yield was higher in 2016, a season with better climate for grapevine than other years during fruit set, favourable for higher clusters, and larger berries.

Crop removal influence on grape yield and each growing stage was higher in 2017 when winter cold and later frost from the spring damaged buds and young shoots respectively.

Grape yield/vine decrease in all thinning treatments compared with the control and was very significantly lower in Minis vineyard than yield/vine recorded by King et al. (2025) in Merlot variety (3.80-6.68 kg/vine) for the same thinning treatments.

In other researcher's opinion (Kliewer et al. 1983; Palliotti et al., 2000; Bubola et al., 2011), yield decrease in lower rate after cluster thinning due to the increase of cluster and berries weight.

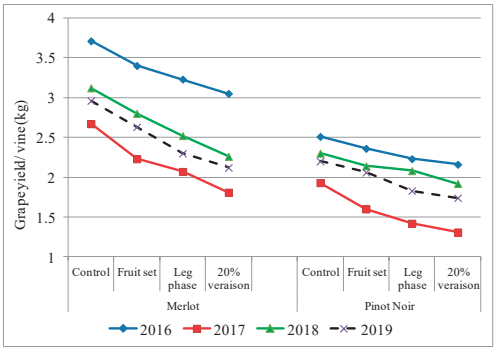


Figure 2. Effect of crop removal (CR) on Merlot and Pinot Noir grape yield/vine (kg), in 2016-2019 growing seasons

Obviously, cluster thinning reduce the number of clusters and the yield per vine relative to the control in all thinning treatments, with differences induce by temperature and rainfall during growing season from each year, but without significant difference between cluster removal treatments, which means that the treatment in each variety was evenly applied (Figure 3).

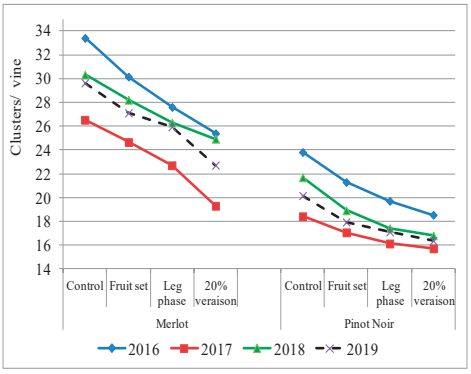


Figure 3. Effect of crop removal (CR) on Merlot and Pinot Noir clusters/vine, in 2016-2019 growing seasons

Cluster number/vine was in close relation with the results of King et al. (2015) which reported after cluster thinning during fruit set cluster number/vine between 23.1 and 29.9; in 20% veraison treatment, cluster number also ranged between close values (22.6 -26.0) compared with Merlot from Minis vineyards.
Cluster weight was higher by 30-40% in 2016 compared with the unthinned control in other research years (excepting 2018 when the cluster weight increased around 48% in Merlot) when clusters were removed during fruit set stage.

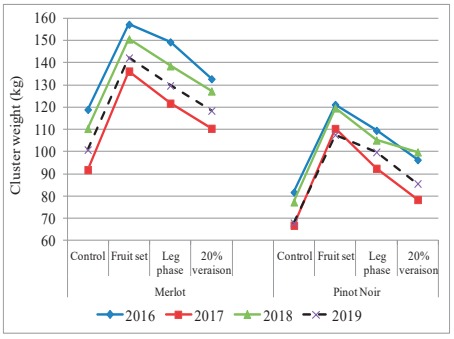


Figure 4. Effect of crop removal (CR) on Merlot and Pinot Noir clusters/vine, in 2016-2019 growing seasons

In Pinot Noir, cluster thinning in fruit set stage show higher cluster weight than control. In the balanced growing season 2016 compared with the result from the other research years, indicate that climate from growing season had greater influence than treatments on cluster weight. The growing season 2017 was characterized as the worst year in viticulture since 1961, cold and wet in the spring (poor fruit set), very hot and dry in July and very hot and wet in harvest time.
Results concerning the influence of thinning treatments and growing season on cluster weight are similar in Pinot Noir with those reported by Mawdsley et al. (2018) and Dobrei et al., (2013). For the same topic, research results are very different: Nuzzo and Matthews (2006) found no differences between cluster thinning and un-thinning treatments, whereas Reynolds et al. (1994) reported an improved cluster weight.
Must composition in Merlot and Pinot Noir at harvest for all four seasons is presented in figures 4, 5, 6. Grape berries composition was influenced both by thinning time and year of growing season. In the unbalanced year 2017 there was no effect of cluster thinning on sugars, pH or titratable acidity (TA).
In 2019 even the grape yield was lower than 2018, because of cold and wet April and May with problems in floral and berries development, was the best year for berries quality.
Due to the very high temperatures during the day (28-30ºC) the harvest was made more at night at 18-20°C, the quality of the berries was higher, considered as the best of the last 38 years. In all four growing seasons the effect of cluster removal treatments on must composition had lower significance compared with climate, but °Brix and pH were higher and titratable acidity was lower compared with the unthinned control.
Cluster thinning at 20% veraison in both Merlot and Pinot Noir variety increased sugar concentration in berries (Figure 5).

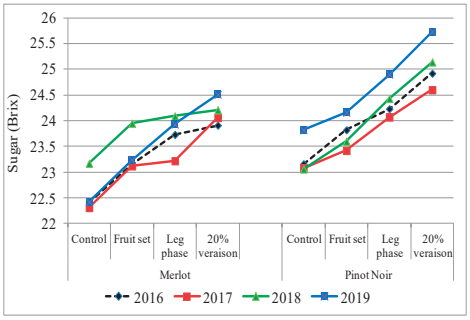


Figure 5. Cluster thinning influence on must sugar concentration in Merlot and Pinot Noir at harvest, during 2016-2019 growing seasons

The higher sugar (°Brix) associated with lower titratable acidity without significant influence of thinning treatment, was confirmed in 2019. The same association was reported in Merlot variety by King et al. (2015). Cluster thinning in veraison period favours the faster maturation and improves the berries composition because vegetative growth is reduced and sugar concentration increased (Reynolds et al., 2007). Mota et al., (2010) recommended after research in cluster thinning in Merlot variety grown in Brazil, to practice only shoot trimming for increase sugars in berries without cluster removal.

The pH is one of the grape quality parameter with impact on taste, flavour or colour stability of wines (Boulton, 1980).
Results (Figure 6) show that pH was influenced mostly by growing season 2017 with heavy rainfall in ripening stage and less by cluster thinning treatments, with the lowest values in both Merlot and Pinot Noir varieties.

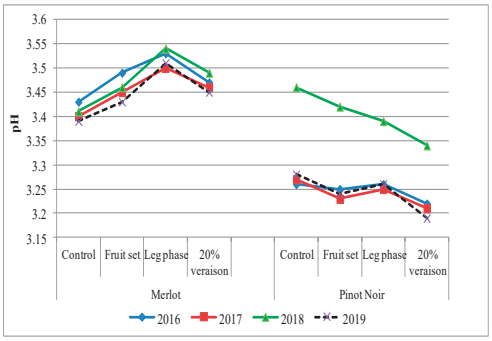


Figure 6. Cluster thinning influence on must pH in Merlot and Pinot Noir at harvest, during 2016-2019 growing seasons

The pH value in Pinot Noir variety was influenced by dry spring and autumn associated with cluster thinning in 2018, when were found the highest pH levels in grape must.
The juice pH affects the taste, sugar / acid balance and stability of the wines; in red wine also affects colour (Dami et al., 2006). It is determined by the balance between the main anions (malate and tartrate) and the presence or absence of major cations (mainly K).
Thus, it is important to achieve the proper balance of potassium in grapes. In France, for example, the levels of potassium accepted in the wine of French wines are between 1-2.5 g/L, resulting in the final wine 0.7-1.6 g/L (Anderson et al., 2008). High pH values can also change the colour of anthocyanin solutions - responsible for the colour of red wine - by changing the structure of the anthocyanin molecule, which becomes bluer and therefore less desirable (Peterlunger et al., 2002).
A high pH in must result in wines with a flat taste and red wines with a brown colour. A pH higher than 3.6 is undesirable because has negative effect on a number of wine quality characteristics (Prajitna et al., 2007).

Warm growing seasons (2016, 2018, and 2019) influence the titratable acidity (TA) concentration (Figure 7).
Wet and cold weather from the late spring associate with cluster thinning in fruit set stage from 2019 growing season, influenced the most titratable acidity which had the lowest level in the research period in both Merlot and Pinot varieties. Highest level of titratable acidity was registered in 2017 growing season
The level of acidity is special trait for the taste of any wine. The acidity gives the wine a fresh and clean taste. Together with the sugar, the acidity of the grape juice represents an important guide for the quality of the wine. Acids - malic and tartaric - account for over 90% of total TA (organic acids) acidity in grapes (Boulton et al., 1999).

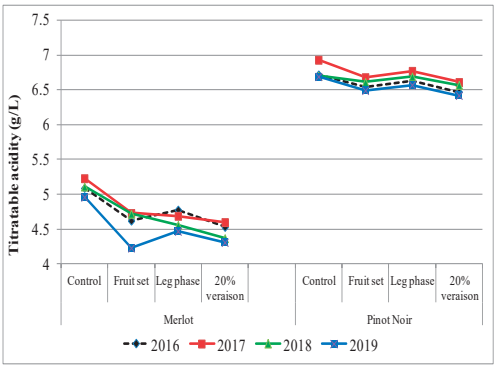


Figure 7. Cluster thinning influence on must TA in Merlot and Pinot Noir at harvest, during 2016-2019 growing seasons

In the hot climate, in grapes exposed to direct sunlight for longer periods, the malic acid content decline during maturity is the highest (Bergqvist et al., 2001). On the contrary, grapes grown in low temperature areas have higher concentration of malic acid in wine (Cirami, 1973).
Wine with higher acidity (> 10 g/L equivalent TA) is sour taste and requires de-acidification (Bardi et al., 1997). In hot environments, titratable acid become too low (< 6-7 g/L TA), thus producing a soft wine (Sadras et al., 2013).
The aromatic components, which are part of titratable acids, are important in the sensory or organoleptic quality of the vintage wine. In any type of wine there are usually over 500 of these

substances, all of which are found in minimal quantities (Sadras et al., 2013).

Canopy microclimate is strongly influenced by pruning and crop load; short summer pruning improved cluster exposure to light and ventilation while the *Botrytis cinerea* incidence is reduced (Kliewer and Dokkozlian, 2005; Dobrei et al., 2014). The highest sugar (°Brix) was recorded in Pinot Noir from 2019 growing season and at 30 buds/vine crop load (Figure 8). The most balance growing season for sugar concentration in both varieties was 2018.

In Pinot Noir sugar concentration was more balanced over the years regardless the crop load. On opposite, sugar concentration in Merlot was influenced by crop load mostly by short (10 buds/vine) and long (40 buds/vine) pruning treatments.

During 2014-2015 growing seasons, Dobrei et al. (2016), found in Pinot Noir for 30 buds/vine crop load lower sugar limits between 197 and 237 g/L, while in Merlot sugar concentration in must was higher for 40 buds/vine crop load and ranged from 190 to 224 g/L; in both years titratable acidity (g/L) steadily increased with crop load in Pinot Noir and decrease in Merlot.

In Merlot variety, with more vigorous canopy than Pinot Noir, pH values were higher for the same crop load. Temperature variability from day/night in growing seasons from last years, associated with high crop load changed the titratable acidity level in grape berries.

The lower pH the cleaner fermentation will be and the wine less spoiled (Dobrei et al., 2016). None of the pH values exceed 3.6 values; therefore the wine from both varieties was high quality without astringency. A study of Kliewer (1973) stated that the rise of pH is associated with warm nights during growing season.

The lower titratable acidity was balanced with yeasts during fermentation about 11%.

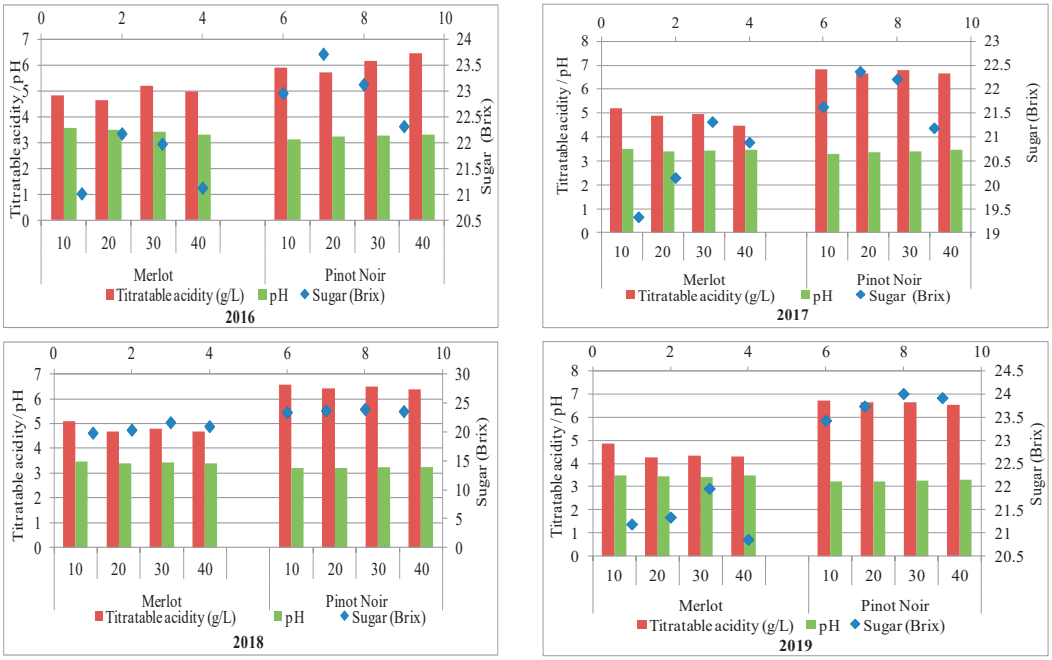


Figure 8. Summary for crop load influence on must components in Merlot and Pinot Noir varieties during 2016-2019 growing seasons

CONCLUSIONS

Both crop load and cluster thinning are frequently used in grapevine growing management, but are expensive and with high yield losses when cluster thinning is applied. Crop loss was higher in 2017 when late spring frost affects buds and then rainy weather the flowering stage. In the same growing season rainy days from ripening stage decrease the

berry composition and was the worst year in viticulture in the last 60 years.

On the contrary, in the growing season 2019 - even the yield was lower by 10-12 % then 2018 - result the higher quality berries composition from last 40 years. Cluster thinning increase ripening and sugar concentration whereas TA is decreased, but not influence major the pH. In response to crop load, cluster thinning and climate variability influence, the response of two varieties was different as regards the yield and berry composition. In the conclusion, cluster thinning is expensive with high production losses and small increase in grape berries qualities to can balance the profit of winemakers; cluster removal in vineyards from areas with more and more hot growing seasons is not efficient.

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STUDY OF THE CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF SOME VARIETIES OF YOUNG RED WINES FROM DIFFERENT VINEYARDS IN SOUTHERN ROMANIA

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Abstract

Red wine samples were taken from the Stefanesti-Arges, Babadag and Dragasani vineyard. The study was done on young red wine varieties from 2019 vintage. The chemical parameters were determined from the following varieties of wine: 'Feteasca Neagra', 'Merlot' and 'Pinot Noir'. For the wines studied was observed a higher concentration of total polyphenols and anthocyanins from Dragasani vineyard as compared to the wines from the other two vineyards. The concentration of alcohol in Dobrogea vineyard wines is higher than in the other vineyards studied for 'Merlot' varieties (15.55%) and 'Feteasca Neagra' (15.45%). For the 'Pinot Noir' variety (15.5%) the alcoholic concentration is higher in the Stefanesti vineyard centre. The total acidity at the 'Merlot' (5.93 g/l) and 'Feteasca Neagra' (5.67g/l) varieties from Dragasani vineyard are the highest compared to the other wine varieties, and their pH is the lowest (3.22) compared to the pH of 'Pinot Noir' (3.72) from Stefanesti. Antioxidant activity has been studied by the DPPH radical scavenging assay method and the highest values for are found in Dobrogea, 'Feteasca Neagra' ((EC50)7.19 mg GAE/l) and 'Merlot' ((EC50) 6.56 mg GAE/l).

Key words*: red wine, varieties, antioxidant activity, chemical composition.*

INTRODUCTION

"Wine is the healthiest and most hygienic drink" (Louis Pasteur). It is considered as one of the major sources of energy in human nutrition, it can be counted as a source rich in potassium (K = 0.5-1.2 g/l), or as a vitamin. (Mihalca Al., 2010). Vitamins don't come as a source of energy. They are generally activated in the traces status, their synthesis is not carried out in the body and thus must be consumed with food. Wine contains in variable doses all soluble vitamins (Ouranac A., 1970). Well-developed wine contains vitamin C 10 mg/l from grape pulp. Vitamin B1 in wine is 7-10 mg/l. Vitamin B2 (riboflavin), which plays an important role in protein metabolism and energy production, is found up to 0.5 mg/l. Other vitamins in lower doses are vitamins B3, B5, B6, B12, H and vitamin factors P(C2). (Mihalca Al., 2010).

Since ancient times, wine was considered a food, but also a good medicine. But simultaneously, the wine has its detractors, known to excessive consumption. So if Hippocrates recommends wine to his patients, Pythagoras condemns him. (Mihalca Al., 2010). Numerous studies done by Masquelier and Bourzeix in 1989, devoted to the effects of moderate wine consumption on health, have highlighted a multitude of properties: vascular protection, anticancer factors, antihistaminic factors, antiviral agents, protection against ionizing radiation, sun protection, free radical captors, dietetic supplement etc. (Masquelier J., 1982, 1987).

Studies conducted at Davis University in California have established that phenolic compounds in wine, considered as antioxidants, or other constituents in wine can significantly reduce cancer. (Health Qviz,1996; Watson R., 2018). Camargo's study highlighted the protective effect of consuming 1-2 glasses of wine per day for ischemic brain injuries. (Dejeu L., 2000). Prof. Orgogozo considers that the protective action of wine in relation to strokes

accidents is explained by: - inhibition of the aggregation of cells responsible for the formation of atheroma plaque; - decrease dining of blood content in low-density lipoprotein (LDL); - increase in high-density lipoprotein (HDL) blood content (Orgogozo J.M., 1995 and 1997).

The authenticity and the typicality of the wine are basic attributes that guarantee the quality of a wine. First of all, the wine must meet the requirements of authenticity and typicality, because wines with false identity cannot be offered to consumers. (Giosanu D., 2011). The notions are closely linked, without being identical. The authenticity and the typicality of the wines are highly appreciated by the consumers and rigorously controlled, given that the consumption of wine has decreased in all countries, and the consumers increasingly prefer "the authentic wines of quality". The most important parameters used in the typicality of wines are the anthocyanin spectrum in red wines, the content in shikimic acid in white wines, the imprint of amino acids in wine, the isotopic composition of alcohol and water in wine. The quality of the wine is given by the physico-chemical composition of the wine and its organoleptic, taste-olfactory properties. In the case of wine that has a very complex composition, the quality remains a random feature, difficult to define and to establish (Geana E.M., 2016). However, the quality is very severely controlled in all wine producing and importing countries.

Red wine is a tannic drink, so it contains polyphenols, which is not the case with other alcoholic beverages. Polyphenols are, in fact, very important constituents and characteristics of wine. A greater interest is in studies is the antioxidant action of some chemical compounds in wine, respectively polyphenols. Polyphenols taste (tart) and colour (anthocyanins) of wine. The colour of the wine can be determined by the shade of the wine and by the colour intensity of the wine. Wine polyphenols form a diverse family, including phenolic acids, monoacids, anthocyanins, flavonili, leucoanthocyans and tannins, which are made up of phenolic compounds. The most sustained beneficial action of polyphenols in wine is the antioxidant one. It is achieved by the capture by polyphenols of free radicals circulated through the blood (Woraratphoka J., 2007; Valkom J., 2007; Yang J., 2009; Visnja K., 2010; Bunea C.I., 2012). Consequently, a vigorous research activity was initiated regarding the analysis of these antioxidants in different grape products and the preparation of extracts and wines with a high content of phenolic antioxidants (Heinonen I.M., 1998).

MATERIALS AND METHODS

For this experiment, 3 wine-growing areas in Romania were studied with 3 varieties of young red wine from 2019 of the grape varieties: 'Feteasca Neagra', 'Merlot' and 'Pinot Noir'. Climate data recording and physico-chemical determinations were made for each vineyard.

For climate records, weather stations were used for each area studied: the one in Stefanesti is an automatic station in the research pilot station with the coordinates 44°51' N and 24°57' E, 300m; Babadag (Dobrogea) weather station has the coordinates 44°53' N and 28°58' E 80 m; respectively Dragasani weather stations has the coordinates 44°317' N and 23°678' E, 195 m.

Our studies were made on many sorts of wines, from different areas, but in this paper we present just only three of them from 2019: 'Merlot', 'Pinot Noir' and 'Feteasca Neagra' - Stefanesti area, important vine area for red wine production, Dobrogea vineyard and Dragasani. We started the wine analysis with an important stage: preliminary examination of samples. This includes colour analysis and microbiological stability. Usually, shade and intensity of wine colour are calculated by optical methods (Niskanen I., 2009).

For each wine variety we determined: minimum alcohol content (alcoholic concentration), relative density, total acidity, contents of SO_2 and anthocyanins. The alcoholic concentration, one of the most important parameters of wine quality, was determined by indirect method, (pycnometer method), after prior separation of alcohol from wine by distillation. Acidity provides physico-chemical stability of wine, gives colour, brightness and freshness of taste. To characterize acidity of wine the following types of acidity are taken into consideration: total acidity, volatile acidity, fixed acidity and ionic/ real acidity of wine. The determination of total

acidity was made by titration with bromothymol blue. The method consists of wine sample titration (acid neutralization) with a solution of sodium hydroxide in the presence of bromothymol blue, after prior removal of carbon dioxide (Giosanu D., 2011).
The total remaining unfermented sugars in wine were evaluated by refractometric method, by measuring the percentage of soluble solids or refractive index, after prior removal of alcohol and volatile compounds from wine (which changes the refractive index value). Sulphur dioxide is the only antiseptic allowed in wine conservation. The determination of free SO_2, combined SO_2 and total SO_2 is rapidly made by using iodometric oxidation. The anthocyanins are visible phenolic compounds (pigments), which are getting accumulated in grapes and give red colour to wine. They represent 38% of the total phenolic compounds present in wine. The quantitative determination of anthocyanins is made by visible spectrophotometry and is based on the change of anthocyanins colour depending on the pH. We measured the absorbance variation of anthocyanins colour at two pH values, 0.6, 3.5, and compared with distilled water. The measurements were made at 520 nm, the absorbance of the samples being proportional to the anthocyanins content (Giosanu D., 2011).
The total content of polyphenolic compounds (CTCF) was determined by the Folin - Ciocalteu method and by the enzyme method with the BS200 analyser. Tannins are chains of flavonols (promainidines) more or less polymerized in leaves and strings. The method of leucoantocyanids for the determination of tannins is based on the property of tannins to turn hot and into the strong acidic environment (Concentrated HCl) into cyanidine, which is red in colour.
The samples were analyzed according to the technique reported by Brand-Williams and others (1995), with some modifications. Briefly, 1 volume of sample [5 μL for red wine (1: 1, v/v with methanol)] was added to 1 volume of 2,2-diphenyl-1picrylhydrazyl (DPPH) (Sigma) 0.094 mM in methanol up to completing 1 mL. The free radical scavenging activity using the free radical DPPH• reaction was evaluated by measuring the absorbance at 515 nm after 60 min of reaction at 20°C in a spectrophotometer (Specord 205).
Antioxidant activity represented by the amount of antioxidant needed to decrease the initial DPPH radical concentration by 50% (Efficient concentration = EC50 mg GAE/l). (Woraratphoka J., 2007; Rockenbach I. I., 2011)
For the statistical interpretation of the results, the data were included in an Excel database and then statistically interpreted with the SPSS 14.0 program, which uses the Duncan test (multiple t test) for a 5% statistical assurance.

RESULTS AND DISCUSSIONS

The values recorded in Stefanesti are influenced by the climatic data of the 2019 wine year and presented a number of peculiarities whose effects were found both in the course of the vegetative cycle and especially in the level and quality of the grape harvest.
Among these specific features we mention the most significant: low temperatures in the period of April - May leading to a delay of the flowering of the vine by one week (3ºC/April 1st, 5.9ºC/May 1st, 6.1ºC/May 7th), June does not exceed the temperature of 35ºC until 2 days (37ºC June 25 and 35.6ºC June 27), the same in July, there were no reports of very high temperatures (maximum 37.8ºC July 21 and July 26), but in the two months the temperatures during the night were quite low (14ºC/June 03 - June 10, 8.2ºC/July 11) led to the extension of the ripening period of the grapes. (Table 1)
The climatic data in Dragasani do not showed any peculiarities in terms of temperatures, they are kept within the normal limits of the Dragasani wine area (Table 1).

Table 1. Temperature dynamics in 2019 in the ripening stage of the three vineyards studied

Month	Air temperature			Rainfall (mm)	Huglin Index	No days with precipitation > 10 mm	Σ °t global (°C)	Σ °t active (°C)	Σ °t useful (°C)
	T med (°C)	Average T min (°C)	Average T max (°C)						
Dragasani									
April	11.92	6.53	17.83	39.9	146.25	1	357.5	314.5	64.5
May	14.08	9.42	19.06	14.9	203.67	-	436.5	436.5	166.6
June	22.81	17	29.23	139.2	480.6	7	684.5	684.5	384.5
July	22.82	15.84	30.16	63.8	511.19	3	707.5	707.5	397.5
August	25.13	17.39	32.87	1	589	-	779	779	469
Babadag									
April	13.3	8.43	17.97	810.2	163.05	8	401	354.5	124.5
May	17.5	13.1	22.29	518	241.65	4	542.5	542.5	232.5
June	20.5	16.2	24.73	707.1	378.45	7	614.5	614.5	314.5
July	27.3	21.45	32.42	602.7	615.66	6	845	614.7	304.7
August	27.35	20.58	31.94	199.2	609	3	848	848	538
Stefanesti									
April	11.2	5.4	19.1	42.2	171	1	338.1	244.4	53.4
May	16.3	9.8	24.6	93.6	323.95	2	504.1	494.5	194.5
June	22.1	15.5	32.0	193.6	511.5	6	664.6	664.6	364.6
July	22.2	14.5	32.2	70.6	531.95	1	687.1	687.1	377.1
August	24.6	15.7	35.8	6.6	626.2	0	762.1	762.1	452.1

Huglin Index =$[(T_{med} - 10)+(T_{max} - 10)]/2$ x no.days of the month.
Σ°t global = sum of average positive daily temperatures.
Σ°t activate = sum of average daily temperatures > 10 °C.
Σ°t useful = sum of differences between average daily temperature > 10°C and the biological threshold for starting in the vegetation of the vine (10°C).

In March having small peculiarities represented by the minimum of -2°C in March 15 and the maximum of 25°C March 18 and poor rainfall, only in March 18 were recorded of 18.7 mm. April with minimum temperatures of 2°C/April 3 and 21 and maximum of 25°C/April 27, with poor precipitation, with rain in April 18 of 15.4 mm. In May, climate data remained normal (22-28°C) of this rain-free area, only on May 31 were recorded the maximum temperature of 20°C. In the grapes ripening time of June - August there were deficient months in terms of precipitation, there were no particular manifestations of temperatures, only in August there were higher temperatures (36°C/August 10 and 11).

Climate data from the Dobrogea vineyard showed peculiarities such as low temperature in March (T max 5°C/March 25) and high rainfall (99.5mm/March 16 and 17, 99.8 mm/March 21 and 22). The April month was evidenced by low maximum temperatures (7-18°C), the highest was 25°C/April 29 and 30 and heavy rainfall (99.8 mm/April 25, 26 and 29). May is not a particular manifestation of temperatures, only heavy rainfall (99.5 mm/May 4, 10, 24 and 31). June recorded high temperatures above 30°C and heavy rainfall due to storms (99.3 mm/June 4, 8, 13, 20 and 28). The months of July and August had no specific features in terms of temperatures, instead there were storms with high precipitation (99.5 mm/July 2, 13 and 24; 99.0 mm/August 6 and 26) (Table 1).

From a chemical point of view, wine is a polydisperse hydroalcoholic solution, in which several organic and mineral substances are dissolved or present in colloidal state.

The chemical analysis of the wine aims to establish the composition parameters that define the quality of the wine.

Table 2 show the correlations between the indicators studied, of which we highlight the following: It is noted that pH correlates positively with alcoholic concentration, density, volatile acidity, and antioxidant activity (r = 0.237, r = 0.128, r = 0.185, respectively r = 0.064) and significantly positive with sugar (r = 0.432*), which explains that there is a balanced ratio between these indicators.

Table 2. Matrix of correlations between biometric and biomedical indicators studied in wine

		Alcohol concentration % vol	Dry extract, g/l	Dens at 20 degreesC	Total acidity, g/l AT	Volatile acidity, g/l AA	Sugar g/l	pH	Total anthocyanins content (mg/l)	Total polyphenol content (mg GA/g)	Tannins (mg/l)	Antioxidant activity, mg GAE/l
Alcohol concentration % vol	Pearson Correlation	1	,628(**)	-,284	-,298	-,088	,088	,237	,171	,106	,249	,260
Dry extract, g/l	Pearson Correlation	,628(**)	1	,067	,059	-,532(**)	-,048	-,217	,538(**)	,253	,251	-,089
	Sig. (2-tailed)	,000		,711	,744	,001	,792	,226	,001	,156	,158	,621
Dens at 20 degreesC	Pearson Correlation	-,284	,067	1	-,054	,148	,814(**)	,128	,116	-,439(*)	-,416(*)	-,072
	Sig. (2-tailed)	,110	,711		,765	,411	,000	,479	,519	,011	,016	,691
Total acidity, g/l AT	Pearson Correlation	-,298	,059	-,054	1	-,145	-,281	-,667(**)	,338	,051	,179	-,232
	Sig. (2-tailed)	,093	,744	,765		,422	,114	,000	,054	,778	,318	,193
Volatile acidity, g/l AA	Pearson Correlation	-,088	-,532(**)	,148	-,145	1	,477(**)	,185	-,196	-,084	,083	,643(**)
	Sig. (2-tailed)	,627	,001	,411	,422		,005	,304	,274	,640	,644	,000
Sugar g/l	Pearson Correlation	,088	-,048	,814(**)	-,281	,477(**)	1	,423(*)	-,034	-,557(**)	-,368(*)	,250
	Sig. (2-tailed)	,626	,792	,000	,114	,005		,014	,850	,001	,035	,161
pH	Pearson Correlation	,237	-,217	,128	-,667(**)	,185	,423(*)	1	-,348(*)	-,431(*)	-,352(*)	,064
	Sig. (2-tailed)	,184	,226	,479	,000	,304	,014		,047	,012	,045	,722
Total anthocyanins content (mg/l)	Pearson Correlation	,171	,538(**)	,116	,338	-,196	-,034	-,348(*)	1	,474(**)	,606(**)	,116
	Sig. (2-tailed)	,341	,001	,519	,054	,274	,850	,047		,005	,000	,521
Total polyphenol content (mg GA/g)	Pearson Correlation	,106	,253	-,439(*)	,051	-,084	-,557(**)	-,431(*)	,474(**)	1	,659(**)	,385(*)
	Sig. (2-tailed)	,558	,156	,011	,778	,640	,001	,012	,005		,000	,027
Tannins (mg/l)	Pearson Correlation	,249	,251	-,416(*)	,179	,083	-,368(*)	-,352(*)	,606(**)	,659(**)	1	,169
	Sig. (2-tailed)	,163	,158	,016	,318	,644	,035	,045	,000	,000		,348
Antioxidant activity , mg GAE/l	Pearson Correlation	,260	-,089	-,072	-,232	,643(**)	,250	,064	,116	,385(*)	,169	1
	Sig. (2-tailed)	,145	,621	,691	,193	,000	,161	,722	,521	,027	,348	

The anthocyanin content was correlated positively with dry extract, total polyphenols and tannins, (r = 0.538**, r = 0.474**, r = 0.606**) having in all three cases distinctly significant and negative correlations with, sugar and volatile acidity, (r = -0.034 and r = 0.196 respectively), which explains that anthocyanins are formed in the presence of sugar and acids in grapes (Table 2). Their presence in grapes leads to an increase content in anthocyanins. Some acids (tartaric acid) may increase the content of anthocyanin pigments and the biochemical quality of grape (Lee, 1996).

Figure 2 showed the differences between the Dobrogea vineyards and the other two (Drăgăşani vineyard and Ştefăneşti vineyard centre) regarding alcoholic concentrations, both as concern 'Feteasca Neagră' from Dobrogea vineyard, which is around 15.45% vol., and the one from Ştefăneşti it reaches max. 13.2% vol., respectively from Drăgăşani vineyard to 14.77% vol.

By considering the influence of the area on the alcohol strength on the average of the varieties, it can be observed that the highest values were recorded in the vineyards of Dragasani and Dobrogea, and the lowest in Stefanesti vineyard centre (Figure 1). As regards the alcoholic strength of the three varieties analysed in the three geographical areas, the highest alcohol content was recorded at the 'Pinot Noir' wine in Stefanesti vineyard centre.

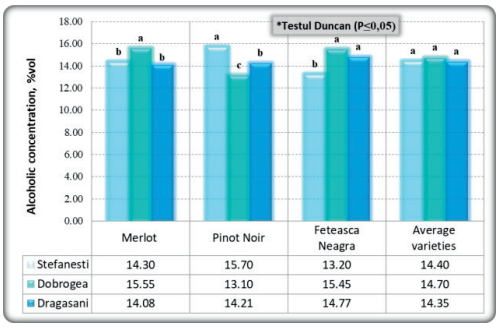

Figure 1. Influence of the geographical area on the alcoholic concentration of wine, depending on the variety

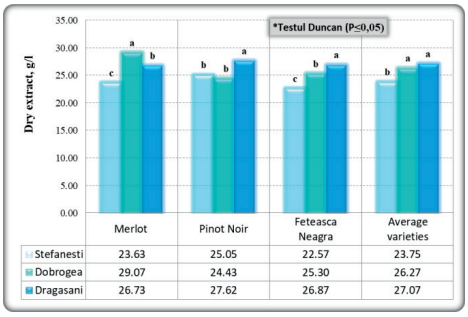

Figure 2. The influence of the geographical area on the dry extract of the wine, depending on the variety

Figure 2 shows the influence of the area on the dry extract of wines, on the average of the varieties, it can be observed that the highest value of extracts were recorded at the Dragasani and Dobrogea vineyards, and the lowest at Stefanesti vineyard centre, and the differences between the two classes of homogeneity are statistically assured.

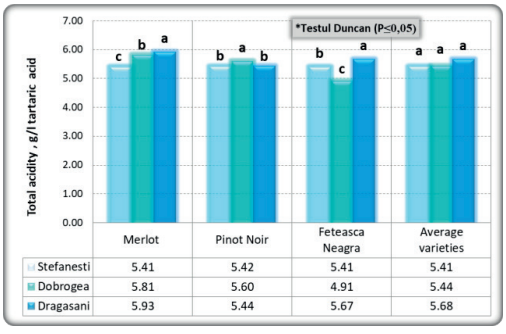

Figure 3. Influence of the geographical area on total acidity in wine, depending on the variety

total acidity (expressed in g/l tartaric acid) recorded the high value at different variety depending on the area. Thus, the highest value of total acidity was registered to the Merlot and 'Feteasca Neagra' variety at Dragasani vineyards, respectively at Dobrogea in the case of 'Pinot Noir' variety (Figure 3).

The differences of total acidity depend on the pedological composition of the soil in each vineyard.

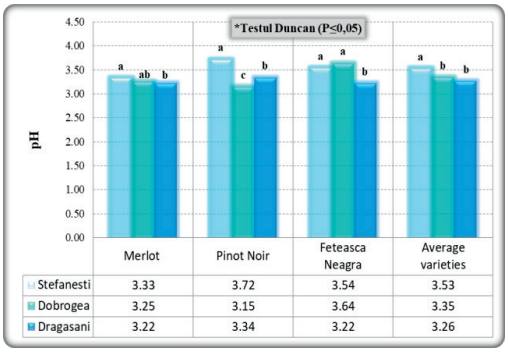

Figure 4. The influence of the geographical area on the pH of the wine, depending on the variety

The pH depends on the variety of wine and the geographical area, for example at 'Pinot Noir' from Stefanesti is 3.72, and for the variety from Dobrogea it is 3.15 (Figure 4).

On average of the varieties, the highest pH was recorded to the Stefanesti vineyard centre.

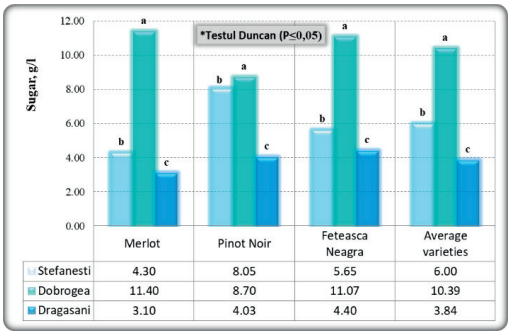

Figure 5. Influence of geographical area on wine sugar, by variety

In all three cultivars studied, due to the climate conditions the highest sugar content was induced at Dobrogea vineyard and the values are ensured statistically compared to Stefanesti and Dragasani areas, respectively (Figure 5).

On average of the varieties studied, sugar level recorded at Dobrogea was 2.7 times more than Dragasani area.

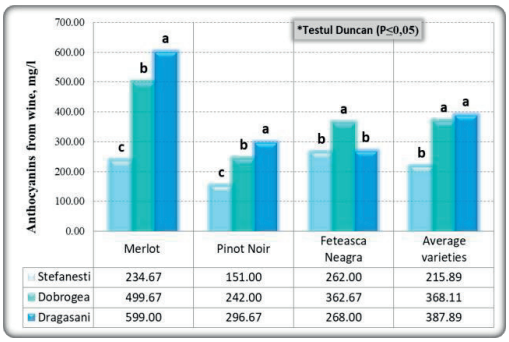

Figure 6. Influence of the geographical area on the anthocyanins from wine, depending on the variety

The highest anthocyanin content is at 'Merlot' wine (599 mg/l) from Dobrogea vineyard and the lowest is at 'Pinot Noir' (151 mg/l) from Stefanesti (Figure 6). On average of the varieties studied, at Dragasani and Dobrogea areas, anthocyanin levels recorded high value than Stefanesti area. The content of anthocyanin is given primarily by the winemaking technology (the time of contact of the must with marc) and to a lesser extent by the soil pH.

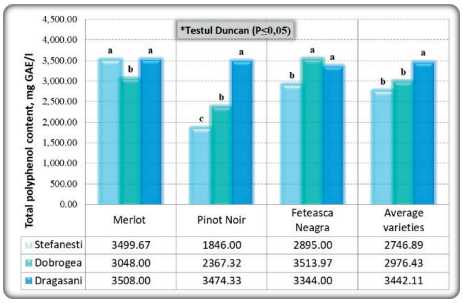

Figure 7. Influence of the geographical area on the content of total polyphenols in wine, depending on the variety

In Figure 7 is showed influence of the geographical area on the content of total polyphenols in wine, depending on the variety and the highest content of total polyphenols was recorded at 'Merlot' (3508 mg GAE/l) from Dragasani vineyard, while 'Pinot Noir' from the Stefanesti vineyard center had the lowest total polyphenols content (1846 mg GAE/l). Polyphenols have a fundamental property they are antioxidant compounds.

Antioxidant activity was influenced by the climate and pedological characteristics. For all studied varieties, the highest values of this chemical parameters content were recorded at Dobrogea vineyard (Figure 8).

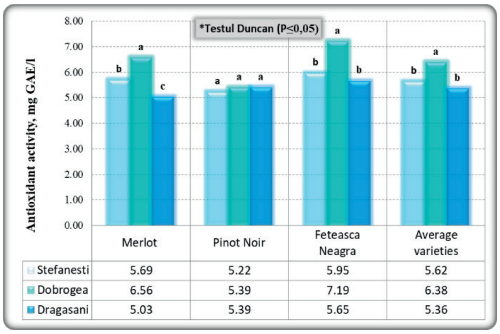

Figure 8. Influence of the geographical area on antioxidant activity, depending on the variety

Antioxidant activity is also influenced by the variety a higher antioxidant activity is observed at Dobrogea vineyard at 'Feteasca Neagra' variety (EC(50) = 7.19 mg GAE/L) and the lowest value at 'Merlot' variety (EC(50) = 5.03 mg GAE/L) from Dragasani vineyard.

CONCLUSIONS

There are differences of concentration of alcohol in the same variety of wine in example for 'Feteasca Neagra' variety from all three vineyards, due to the differences of soil, climatic conditions and winemaking technology.

Among the correlations between the studied indicators we highlight the following: It is noted that pH correlates positively with alcoholic concentration, density, volatile acidity, and antioxidant activity, (r = 0.237, r = 0.128, r = 0.185, respectively r = 0.064) and significantly positive with sugar (r = 0.432*) which explains that there is a balanced ratio between these indicators.

The anthocyanins content was correlated positively with dry extract, total polyphenols and tannins, (r = 0.538**, r = 0.474**, r = 0.606**), having in all three cases distinctly significant. Also, the anthocyanins content had a negative correlations with sugar, volatile acidity and pH (r = -0.034, r = -0.196 and r = -0.348*, respectively). That explains that anthocyanins are formed in the presence of sugar and acids from grapes, their presence in grapes leads to an increase of the content in anthocyanins.

Total acidity recorded the high value at different variety depending on the area. The total acidity at the 'Merlot' (5.93 g/l) and 'Feteasca Neagra' (5.67 g/l) varieties from Dragasani vineyard are the highest compared to the other wine varieties from Dobrogea and Stefanesti area.

In general, the pH of the wines depends mainly on the area. The highest pH value was recorded in Stefanesti for most of studied varieties (3.33-3.72 depending by the variety).

In all three cultivars studied, due to the climate conditions, the highest sugar content was induced at Dobrogea vineyard and the values are ensured statistically compared to Stefanesti and Dragasani areas, respectively. On average of the studied varieties, sugar level recorded at Dobrogea was 2.7 times more than Dragasani area.

On average of the varieties studied, at Dragasani, Dobrogea and Stefanesti areas, anthocyanin levels recorded high value at Stefanesti area. The content of anthocyanin is given primarily by the winemaking technology (the time of contact of the must with marc) and to a lesser extent by the soil pH.

Antioxidant activity was influenced by the variety, geographical area and winemaking technology, for all varieties studied, the highest values of this parameter was recoded at Dobrogea vineyard.

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STUDY OF THE EXTRACTION OF BIOACTIVE COMPOUNDS FROM THE WINEMAKING WASTE OF THE CABERNET SAUVIGNON VARIETY

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Abstract

The by-products of winemaking have a high impact on the environment, due to their biochemical behaviour. This study aims to extract bioactive compounds from the marc obtained from the winemaking of Cabernet Sauvignon variety from 2019 vintage. Extraction of bioactive compounds was performed using a solvent under various experimental conditions: the traditional method of mechanical stirring, ultrasonic assisted extraction (UAE) and microwave assisted extraction (MAE). In this study, total polyphenols and anthocyanins in the marc result of winemaking of the Cabernet Sauvignon variety were determined by Folin - Ciocalteu method and by ITV method. Resveratrol (cis- and trans-resveratrol) was also identified and quantified by the HPTLC-UV densitometry and confirmed by the ESI-MS method. Trans- and cis-resveratrol were identified in the two extractions and only cis-resveratrol could be quantified at a concentration of 9.75 µg/ml from UAE extraction and 5.94 µg/ml by MAE.

Key words*: bioactive compounds, resveratrol, polyphenols, UAE, MAE.*

INTRODUCTION

Grapes are rich in phenolic compounds, being important for human health having antioxidant, anti-inflammatory, antimicrobial activity (de Lange et al., 2003; Gaziano et al., 1993; Kuulasmaa et al., 2000; Renaud & de Lorgeril, 1992; Stoclet et al., 2004; Tjonneland, Gronbaeck, Stripp, & Overvad, 1999). There are also studies with the beneficial effects of these compounds in the heart and other chronic diseases. At 2019, the vineyards worldwide reached a total area (including areas not yet in production) of 7,400,000 ha, global grape production of 78,000,000 tons and global wine production (excluding juice and musts). 292.000.000 hl. (OIV 2019). Wine production generates significant quantities of waste. These wine wastes have a gross residual biomass (that is residues from winemaking marc and yeast, branches or leaves) (Neamţu, 1983; Neamţu et al., 1983 and 1986; Ruberto et al., 2007; Brist et al., 2014).

From fresh, unfermented pomegranate resulting from the pressing of red grapes, intensely colored, dye substances can be extracted by diffusion, and the solution obtained is concentrated and then used as food coloring (Haslam et al.,1996; Pulido et al., 2000; Moure et al., 2001; Makris et al., 2007; Campos et al., 2008).

Taking into account the percentage of by-products (marc, yeast and tartrate) obtained in the wine making process, which, in some cases, is evaluated at 18 ÷ 20%, and estimates that the rate of grape seeds in marc is 18 ÷ 25% (the rest it has liquid remnants and 55 ÷ 65% skins), this process is cost-effective, only if significant quantities of marc are collected from wine producers. In the world, the processing of grape brands is done entirely, mainly grape seeds - to obtain oil and skins for the food industry and natural dyes (Moţa et al., 2017). From a biochemical point of view, resveratrol is a stilbene (the main stilbene in grapes), belonging to the class of non-flavonoid polyphenols (for example, curcumin or lignans belongs too)

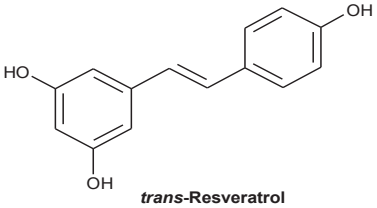

Figure 1. Structure of *trans*-resveratrol

It has been found that some plants synthesize it in response to different stressful circumstances (injury, exposure to high doses of ultraviolet radiation, fungal parasites, etc.). It is found in both stereo-isomeric forms (*cis* - in very small quantities and *trans*-resveratrol most present) and is liposoluble (Geana et al., 2011).

This study aims to extract and bioactive compounds from the mark obtained from the winemaking of the Cabernet Sauvignon variety from the 2019 vintage.

In this study it will be establish which method of resveratrol extraction is more suitable for Cabernet Sauvignon by-products, resulted from wine production (marc). For that, it will be compared two extraction methods, MAE (Microwave Assisted Extraction) and UAE (Ultrasound Assisted Extraction), using the optimized parameters of each method, established elsewhere (Cho, 2006; Ghafoor, 2009; Wang, 2012; Soural, 2015; Garcia, 2016; Pezzini, 2018).

It will be analysed the efficiency of these methodologies concerning total phenolics (TP) and resveratrol content, followed by antioxidant activity (AA) evaluation.

MATERIALS AND METHODS

The marc was harvested in Sept 24th 2019, brought to the laboratory immediately and frozen at -20° C to avoid oxidation. Samples were oven dried, to a constant moisture of about 4-5%. The dried marc was then separated manually into its components, seeds, skins and stalks, by means of sieves. The skins, that would have been finally extracted, were milled by a mortar to obtain a fine powder with a medium particle size of 0.8 mm (Casazza, 2010).

The extraction of polyphenols and anthocyanins from marc was performed by the traditional method ITV: maceration for one hour of 50 g of marc in 85 ml of 1% HCl solution and 15% ethyl alcohol, after which it was centrifuged at 3000 RPM from the clear solution (Ţârdea, 2007). The determination of the anthocyanin content from Cabernet Sauvignon marc was used by the ITV spectrophotometric method with its reading at 520 nm (Rockenbach, 2011; Rivas-Gonzalo, 1992), and the total content of polyphenolic compounds (CTCF) was determined by the Folin-Ciocalteu method and by the enzyme method with the BS200 analyser. (Fogarasi *et al.*, 2018; Ratola *et al.*, 2004; Singh *et al.*, 2015; Vincenzi *et al.*, 2013; Weiskirchen & Weiskirchen, 2016).

In this study, catechins, monomeric flavonolic units were determined by the reaction method of catechins with vanillin and are based on the reaction of the floroglucinol cycle with vanillin, producing red color, stable in concentrated H_2SO_4 and HCl solutions. Tannins are more or less polymerized flavonol (procyanidine) chains in leaves and strings. The leuco-anthocyanidins method for determining tannins is based on the property of tannins to be transformed into hot and strongly acidic medium (concentrated HCl) in cyanidine, which has a red color. The determination of catechins in wine was done by the method in which vanillin reacts with floroglucinol and produces a red color and is read at 500 nm wavelength. (Ţârdea, 2007; O.I.V, 1990; Ough, 1988; Ribereau-Gayon, 1965)

Antioxidant activity was determined at marc using DPPH-1,1-diphenyl-2-picryl hydrazyl (Sigma) 25 mg/1000 ml methanol (solution A), 1:10 of solution A = solution B, 100 µl of solution B together with 2 ml of marc extract in methanol solution, it was incubated for 30 minutes at t = 25°C. The free radical scavenging activity using the free radical DPPH• reaction was evaluated by measuring the absorbance at 515 nm after 60 min of reaction at 20°C in a spectrophotometer (Specord 205). Antioxidant activity represented by the amount of antioxidant needed to decrease the initial DPPH radical concentration by 50%. This value is called the "effective concentration" or EC50 value (Tarola et al., 2019). It was determined at a spectrophotometer at wavelength 515 nm the initial absorbance and the one after 30 minutes.

$$\% \text{ DPPHrem} = [A_{t0min} - A_{t30min}/A_{t0min}] \times 100$$

A_{t0min} - Absorbance before incubation
A_{t30min} - Absorbance after incubation

Extraction of bioactive compounds - *trans*-resveratrol and *cis*-resveratrol from marc was done by two methods: ultrasound extraction (UAE - Ultrasound - Assisted Extraction

with HIELSCHER UIP1000h DT) and microwave - assisted extraction (MAE - Microwave - Assisted Extraction with MILESTONE NEOS-GR). HPLC and HPTLC were used to determine resveratrol. Sample processing was performed by liquid-liquid extraction.

To a 250 ml Erlenmeyer beaker was added a volume of sample to be analysed (Vprob) and a volume of mixture of organic solvents [cyclohexane-n-pentanol 3: 7 (v/v) - Vsolv] (Table 1). The solution was stirred (200 RPM) at room temperature for two hours. The two phases were then decanted from the separating funnels after 24 hours to ensure efficient separation. The organic solvent mixture was removed by vacuum evaporation in a Heidolph rotory evaporator (bath temperature 80°C) to obtain a dry extract. Preliminary studies have shown that pure trans-resveratrol does not degrade at 80°C. The dried organic phases were then taken up in 2 ml of 95% ethanol (Rabesiaka et al., 2011).

Table 1. Sample volume and volume of mixture of organic solvents for liquid-liquid extraction processing

Sample processed	V_{sample} [ml]	V_{solv} [ml]
Cabernet Sauvignon_U_UAE_10:1	8	21
Cabernet Sauvignon_M_MAE_10:1	7.2	14

Vsample - the volume of sample processed; Vsolv - volume of a mixture of organic solvents [cyclohexane-n-pentanol 3: 7 (v/v)].
Cabernet Sauvignon_U_UAE_10:1 - sample of marc extracted by the ultrasonic method, 10:1- ratio between solvent and dry matter.
Cabernet Sauvignon_M_MAE_10:1 - sample of marc extracted by the microwave method, 10:1- ratio between solvent and dry matter.

Resveratrol was separated, identified and quantified by thin layer chromatography high performance (HPTLC) *High-Performance Thin Layer Chromatography* coupled with UV densitometry, taking into account the following experimental conditions (Agatonovic-Kustrin et al., 2015; Babu et al., 2005; Király-Véghely et al., 2013; Lopez et al., 2007; Lotz & Spangenberg, 2016):

- stationary phase: HPTLC silica gel G 60 F254, preformed glass plates 20 × 10 cm (Merck, Darmstadt, Germany);
- stationary phase pre-washing: chloroform – methanol mixture (1: 1, v/v);
- activation of the stationary phase: drying in the oven (110ºC, 30 minutes);
- mobile phase: mixture of toluene-ethyl acetate-formic acid (7: 3: 1, v/v/v), 20 ml in chromatographic graph, with supersaturation for 20 minutes, at 25ºC;
- standard (reference): methanolic solution 200 µg/ml of *trans*-resveratrol;
- the samples to be analysed: two extracts from the Cabernet Sauvignon marc, previously processed by liquid-liquid extraction;
- the migration distance: 62 mm (the sample application line set to 8 mm, and the solvent front set to 70 mm);
- the volumes applied to the starting line: 2, 3, 4, 5 and 6 µl for the standard solution (calibration curve), respectively 2, 4 and 6 µl for the two samples to be analysed;
- the samples to be analyzed were applied by spray, in the form of strips with a length of 8 mm, using the semi-automatic system CAMAG Linomat 5 (CAMAG, Muttenz, Switzerland): gas-air spray, syringe volume - 100 µl, syringe solvent - methanol/ethanol, application rate - 150 nl/s (methanol) and 100 nl/s (ethanol), pre - dose volume - 0.2 µl;
- drying of the chromatographic plate, after development: 5 minutes, at 25°C (with the help of a cold air blower);
- examination (detection): in UV (for photographing the chromatographic plate - λ 254 nm/366 nm (and at λ 320 nm (Figure 2), for obtaining densitocharts), without derivatization (respectively chemical treatment), using the photodensitometer CAMAG TLC Scanner 3: scan speed of chromatographic plate - 20 mm/s, resolution - 100 µm, lamp - deuterium and tungsten, measurement mode - absorption.

For analysis by electrospray ionization (ESI - Electrospray Ionization) coupled with mass spectrometry (MS - Mass Spectrometry), the samples were eluted directly from the chromatographic plate, using the TLC – MS 2 CAMAG interface coupled with the Waters 1525 binary pump. The elution was pure methanol. For the molecular ion detection m/z, the Waters AcquityQDa mass detector in negative mode (ESI–) was used. The source temperature was 120ºC and the capillary was 250ºC. The cone energy was 10 V for *cis*-resveratrol and 15 V for *trans*-resveratrol, and the capillary energy was 0.8 kV. A range of 100–500 M / Z was used (Careri et al., 2004; Chen et al., 2009; Flieger et al., 2017; Mark et al., 2005).

The solvents used for HPTLC (LiChrosolv® purity) analysis and the *trans*-resveratrol standard were sourced from Merck - Millipore (Darmstadt, Germany).

RESULTS AND DISCUSSIONS

The phenolic compounds of the grapes are responsible for the color and flavor of the red wines. In 2019, the parameters studied in red grapes were improved greatly due to the climatic conditions, without rains during the ripening period, the grapes besides sugars also accumulated total polyphenols and anthocyanins (Tănase, 2019).
Table 1 presents the polyphenolic potential of grapes at harvest, detecting the total polyphenols, anthocyanins, tannins, catechins content of the resulting marc and the total anthocyanin potential.

Table 1. Phenolic composition of marc obtained from Cabernet Sauvignon grapes at the time of harvest

Harvest date	Variety	The polyphenolic potential of grapes at harvest					
		PFT	PAT	T	C	A	V/La
Sept 24th 2019	Cabernet Sauvignon	191	1291	3.99	0.817	143	0.694

PFT - total polyphenols content mg GAE/l
PAT - total polyphenolic potential mg/kg
C - catechins, mg/l T - tannins, mg/l
A - anthocyanins, mg/l
V/La - degree of tannin polymerization

The results showed that Cabernet Sauvignon marc had an polyphenolic content of 191 mg GAE/l, which represents almost 10% of the total polyphenol content of the wine. Anthocyanins content 143 mg/l also represents almost 1/3 of the anthocyanin concentration of wine (Geana, 2014). The concentration of tannins retained in marc was by 3.99 mg/l.
The total polyphenolic potential registred by the Cabernet Sauvignon variety was 1291 mg/kg marc. The antioxidant activity of Cabernet Sauvignon marc was 8.93%, having a high capacity to eliminate the free hydroxyl radical (Ginjom, 2010).
Data obtained from HPTLC - UV densitometry analysis, with ESI - MS confirmation, about the resveratrol content of the two extracts obtained by ultrasound extraction (UAE – Ultrasound - Assisted Extraction) and microwave assisted extraction (MAE - Microwave - Assisted Extraction).

Resveratrol was separated, identified and quantified by thin layer chromatography high performance (HPTLC) (Figure 1). The examination (detection) was: in UV (for photographing the chromatographic plate - λ 254 nm/366 nm (Figure 1) and at λ 320 nm (Figure 2), for obtaining densitocharts, without derivatization (respectively chemical treatment), using the photodensitometer CAMAG TLC Scanner.

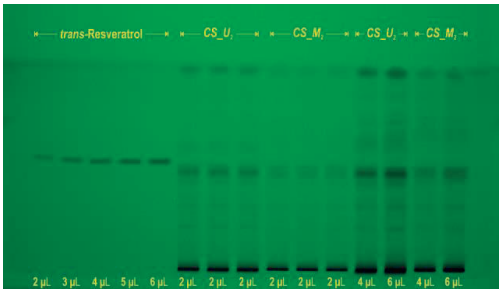

Figure 1. HPTLC chromatochart obtained by analyzing the resveratrol content of the two Cabernet Sauvignon marc extracts. UV photography, λ 254 nm, without derivatization. CS_U: Cabernet Sauvignon Extract_U_2_UAE_10: 1; CS_M: Cabernet Sauvignon Extract_M_2_MAE_10: 1

In Figures 1 and 2 it was observed that *trans*-resveratrol after extraction by both methods (ultrasound and microwave) is detected only qualitatively and not quantitatively.

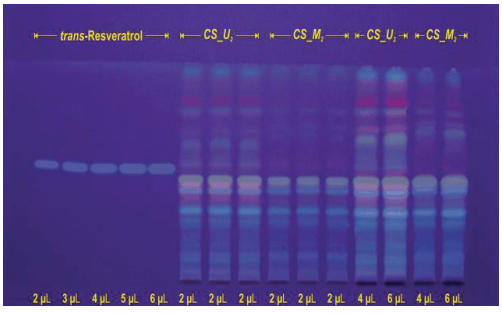

Figure 2. HPTLC chromatogram obtained by analyzing the resveratrol content of two extracts from the Cabernet Sauvignon marc. UV photography, λ 320 nm, without derivatization. CS_U: Cabernet Sauvignon Extract_U_UAE_10: 1; CS_M: Cabernet Sauvignon Extract_M_MAE_10: 1

Figure 3 shows the HPTLC obtained densitochart of the *trans*-resveratrol content of the Cabernet Sauvignon marc extract at 320 nm wavelength without derivatization.

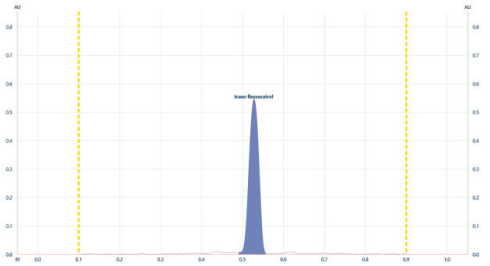

Figure 3. Densitochart obtained by HPTLC analysis of the trans-resveratrol standard (UV λ 320 nm, without derivatization)

Figure 4 shows the densitochart obtained by HPTLC of the cis-resveratrol content of the Cabernet Sauvignon extract of marc obtained by the ultrasonic extraction method, at 320 nm wavelength without derivatization.

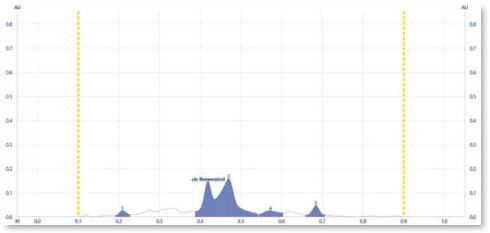

Figure 4. Densitochart obtained by HPTLC analysis of the cis-resveratrol content of Cabernet Sauvignon_U_UAE_10: 1 extract (UV λ 320 nm, without derivatization)

Figure 5 shows the densitochart obtained by HPTLC of the cis-resveratrol content of the Cabernet Sauvignon extract of marc obtained by the microwave extraction method, at 320 nm wavelength without derivatization.

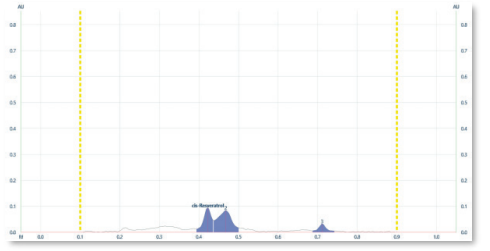

Figure 5. Densitochart obtained by HPTLC analysis of the cis-resveratrol content of Cabernet Sauvignon_M_MAE_10: 1 extract (UV λ 320 nm, without derivatization)

Using the electrospray ionization analysis method (ESI - Electrospray Ionization) coupled with mass spectrometry (MS - Mass Spectrometry) a chromatogram was performed for *trans*-resveratrol in Figure 6. For the electrospray ionization analysis (ESI - Electrospray Ionization) method with mass spectrometry (MS) a chromatogram was performed for *trans*- resveratrol in Figure 6.

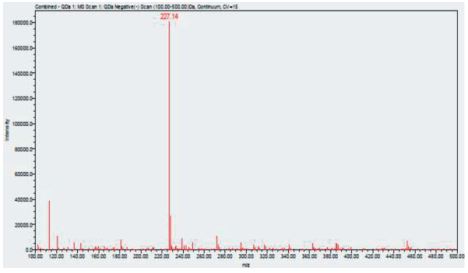

Figure 6. ESI - MS spectrum for the *trans*-resveratrol standard identified in the two extracts from the Cabernet Sauvignon marc (main ion m / z 227)

Figure 7 also shows by electrospray ionization (ESI - Electrospray Ionization) coupled with mass spectrometry (MS - Mass Spectrometry) a chromatogram for *cis* - resveratrol.

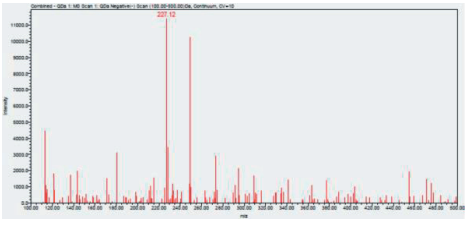

Figure 7. ESI - MS spectrum for *cis*-resveratrol identified in the two extracts from Cabernet Sauvignon marc (main ion m/z 227)

Figure 8 shows the calibration curve for the trans-resveratrol standard at 320 nm wavelength, without derivatization.

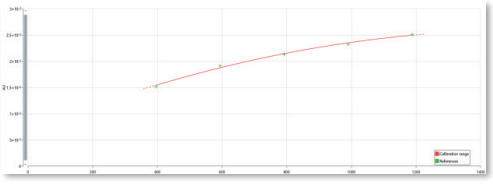

Figure 8. Calibration curve for the *trans*-resveratrol standard (UV λ 320 nm, without derivatization). Polynomial regression, equation: y = -7.998 × 10-15x2 + 2.472 × 10-8x + 6.9 × 10-3, coefficient of variation (CV): 1.21%, correlation coefficient (R): 0.997286

In the two extracts of Cabernet Sauvignon_U_UAE_10:1 and Cabernet Sauvignon_M_MAE_10:1, respectively - it was identified only *cis*-resveratrol from *trans*-resveratrol, by total isomerization, during ultrasound-assisted extraction procedures (UAE - Ultrasound - Assisted Extraction) and, respectively, microwave - assisted extraction (MAE - Microwave - Assisted Extraction).

Table 2. HPTLC - UV densitometry analysis results, confirmed by ESI - MS, for the content of resveratrol in the extracts of Cabernet Sauvignon marc (according to densitochart)

Sample analyzed	Peak No.	ESI–MS $[M–H]^-$ *m/z*	R_f	Concentration [µg / band] (on HPTLC board)	Concentration of *cis*-resveratrol [µg / ml] from the analyzed samples
trans-Resveratrol (standard)	1.	227	0.517	0.396	
	2.			0.594	
	3.			0.792	
	4.			0.99	
	5.			1.188	
*Cabernet Sauvignon*_U_UAE_10:1	2.	227	0.424 *cis*-Resveratrol	0.078	9.75
*Cabernet Sauvignon*_M_MAE_10:1	1.	227	0.424 *cis*-Resveratrol	0.05	5.94

In Table 2 it can be observed that the *trans*-resveratrol by the two methods is detected qualitatively, but not quantitatively. Instead, *cis*-resvatrol is detected in both methods and the highest amount (9.75 µg/ml) is in the method of extracts by ultrasonic processes compared to the microwave extraction method (5.94 µg/ml). From the experiment, it is observed that by extraction, both with ultrasound and microwave, *trans*-resveratrol isomerizes into *cis*-resveratrol.

CONCLUSIONS

Regarding the content of total polyphenols in marc, it is observed that the Cabernet Sauvignon variety has 191 mg GAE/l, almost 10% of the total polyphenols content in the wine and anthocyanins content was 143 mg/l, which represent 1/3 of the anthocyanin content in the wine. It showed, that the Cabernet Sauvignon variety a sufficiently large amount of total polyphenols and anthocyanins remain in marc.

As a result, the antioxidant activity of the marc from the Cabernet Sauvignon variety was high, 8.93%, having a high capacity to eliminate the free hydroxyl radical.

Cis-resveratrol in the composition of the marc can be identified and quantified by HPTLC - UV densitometry, with confirmation by ESI - MS method. The highest concentration of *cis*-resveratrol (9.75 µg/ml) was determined in the method of extracts by ultrasound procedure.

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QUALITATIVE AND QUANTITATIVE PERFORMANCES OF THE 'FETEASCĂ NEAGRĂ' VARIETY - A TRUE AMBASSADOR OF VITICULTURAL ROMANIA

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Abstract

The development of this study began from the consideration that the Severin vineyard in the viticultural Area of the Muntenia and Oltenia Hills offers an extremely varied ecological environment and viticultural landscape, capable of meeting the requirements of the various grape vine varieties, even of those considered sensitive. Throughout this entire oenological offer, which reflects, within a limited territory, the whole country's winemaking ability, Oltenia may be considered, and rightly so, a small viticultural Romania. It is not by accident that we chose the 'Feteasc㠠neagră' variety, as it is considered a close descendant of the wild grape vine (which may be admired in the forests of Oltenia); it is considered the most representative local variety for high-quality red wines, a variety the origin of which has been lost over time. In essence, the nature of the climate here remains favorable to the 'Feteasc㠠neagră' variety, which seems well adjusted to the vine training system with fruiting woody shoot of 12 buds and the grape yield obtained and all of the wine's quality parameters meet the necessary for obtaining high-quality wines.

Key words*: climate, grape, temperature, terroir, yield.*

INTRODUCTION

Romania's international recognition in the winemaking field is based upon the various medals obtained by the Romanian wines in the national and international contests in which our country has participated ever since the 19th century. An area's vocation and terroir elements, its recognition, supported by its mark on the qualitative value of the products obtained, are hard to achieve. They require time, terroir studies, the variety's structure, the plant's and soil's agricultural engineering, a specific vinification technology, a permanent interest in promoting the wine (Stroe and Barcanu-Tudor, 2011; Popa et al., 2015; O.I.V., 2010).

Basically, the environment conditions offered by the Romanian viticultural territory ensure the cultivation of a diverse range of varieties, both from worldwide and local sorts, intended for raw consumption, for white and fortified wines, as well as for red wines, due to the generosity of the heliothermic resources. The wines obtained may thus be included in different quality-related categories, from those with a protected designation of origin and degrees of quality, to those with a geographical indication or varietal wines, dry, semidry, semisweet or sweet, and each type of consumer may find, in the current viticulture and vinification, a partner that meets each and everyone's requirements.

The variety structure existing in Romanian's viticulture, one may notice that it is oriented towards the culture of white wine varieties, in a percentage of 67.3% (Oșlobeanu et al., 1980).

The percentage of black grape varieties is of 21.4%, while aromatic varieties hold 6.6%; other varieties, 4.7% (Oslobeanu et al., 1991). The registered data has a setting point of reference in this sense, due to the fastidiousness with which the study has been developed.

At the time, 11 varieties held a percentage of 87.4% of the surface, and the remaining ones, including the 'Feteasc㠠neagră' local variety (0.8 % - 650 ha), represented a total of 12.6 %. In 2017 (www.agrinet.ro) the dominant share

was still held by the white wine varieties and‘Fetească regală’ represent 18% of the total surface on which white wine grape varieties were cultivated. The surface on which red wine grape varieties were cultivated is of 27,135 ha (representing 15.9%). An analysis by comparison reveals an increase of the share held by the white grape varieties from 67.3% to 84.1% and a decrease of the position held by the black grape varieties from 21.4% to 15.9%. With respect to the share held by the ‘Fetească neagră’ variety, according to the data of the National Office of Vine and Wine Products, it is cultivated on 2,961.99 ha, and within the regions, the surfaces are distributed as follows (www.capital.ro): the Transilvania Plateau Viticultural Area: 34.01 ha; the Moldavian Hills Viticultural Area: 966.9 ha; the Muntenia and Oltenia Hills Viticultural Area: 1,036.16 ha; the Banat Hills Viticultural Area: 110.95 ha; the Crişana and Maramureş Viticultural Area: 154.92 ha; the Dobrogea Mounds Viticultural Area: 581.85 ha; the Danube Terraces Viticultural Area and other favorable lands in the south of the country: 77.20 ha. The development of this study began from the consideration that the Muntenia and Oltenia Hills Viticultural Area offers an extremely varied ecological background and viticultural landscape, capable of satisfying the requirements of the various grapevine varieties, so that, in this historical region of Romania, one may obtain a diverse range of wines: from white to red, from dry to semisweet or sweet, from still to sparkling, from varietal to high quality, with protected designation of origin or even white wines having black grapes as raw material for vinification (Cabernet Sauvignon - the Oprișor viticultural center). Throughout this oenological offer, which reflects, within a limited territory, the winemaking abilities of the entire country, Oltenia may be considered, and rightly so, a small viticultural Romania. It is not by accident that we have chosen the ‘Fetească neagră’ variety. Considered a close descendant of the wild grape vine (which may be admitted in the forests of Oltenia), it is the most representative local variety for high-quality red wines, a variety the origin of which has been lost over time; many authors consider it to have Dacian origins (Constantinescu et al., (84.1%). From among them, ‘Fetească albă’ 1959). It should be iconic for our viticulture and it should become a true ambassador of viticultural Romania. One cannot ignore its significance in the activities of viticultural tourism when, offered for tasting, along other local varieties, it is capable of promoting the quality and tradition of Romanian viticulture. Despite being cultivated over almost 3,000 ha, the biggest share is held by the vineyards and the viticultural centers in the South and South Eastern region of Romania, namely the region of Moldova, Dobrogea and the Hills of Muntenia and Oltenia. If we refer to the Oltenia viticultural territory, it may be found at Breasta, Banu Mărăcine, Stârmina, Oprișor, Vânju Mare and others. In the Corcova viticultural area, located in the north of the Mehedinți County, this variety is cultivated on over 6 ha; this is also the place where this study has been developed. The Mehedinți County benefits from a long viticultural tradition and a fame built especially on the quality of the red wines obtained here.

MATERIALS AND METHODS

Plant material and growth conditions

The chosen variety was ‘Fetească neagră’ (Figure 1) an ancient local variety, considered Dacian in origin, which seems to be a selection of *Vitis silvestris*. It is part of *Proles orientalis - subproles caspica.* It has 32 synonyms, the most well known of which are: ‘Poama fetei neagră’ (Black Maiden Fruit), ‘Păsărească neagră’ (Black Bird), ‘Coada rândunicii’ (Swallow's Tail) (Rotaru, 2009; Stroe, 2014; www.vivc.de, www.eu-vitis.de/index.php). The pruning technique used was Guyot, in the Single Guyot version (1 short element 3 buds + 1 woody shoot of 12 buds - V1), Double Guyot (2 short element of 3 buds + 2 woody shoot of 10 buds - V2) and multiple Guyot (3 short element of 2 buds + 3 woody shoot of 8 buds - V3).

This way, the fruit load was gradual 15, 26 and 30 buds/vine, finalized after the pruning performed in early spring. Spacing between vines are reduced to 1.8 meters between the rows and 0.9 meters between the vines (6,172 vines/ha), meaning a high density planting,

because the growers main objective is to obtain the experimental variants: V1: 15 buds/vine, and 9.2 buds/m^2; variant V2: 26 buds/vine and 16 buds/m^2; variant V3: 30 buds /vine and 18.5 buds/m^2. During the growing period, observations were made during the entire phenological spectrum, calculating the fertility coefficients (absolute, relative), the yield indices (absolute and relative - g/vine shoot), the length of the vegetative shoots and, at the time of harvesting, on an average sample of 10 vine for each experimental variant, the following determinations were made: the number of grapes per vine the average weight of a grape, the yield kg/vine, the yied/ha, sugars (g/l) by the refractometric method, acidity (g/l tartaric acid) by the titrimetric method, the alcohol potential was achieved by the ebuliometric method and the non-sugar dry extract was determined as being the difference between the total dry extract (calculated by the direct method) and the total sugars.

The climatic conditions registered in the area

The evolution of the oenoclimatic background was based upon the calculation of certain classical synthetic indicators (the annual amount of rainfall, the amount of rainfall during the growing season, the average annual temperature, the average temperature in the month of July, the Martonne aridity index), over two viticultural years (2015-2016 and 2016-2017) and their interpretation in accordance with the information contained in the specialized literature (Oşlobeanu et al., 1980; Teodorescu et al., 1987; Paltineanu et al., 2007; Mărăcineanu, 2010). The meteorological data comes from the specialized literature, if we take into account the older entries to which our research period relates (Teodorescu et al., 1987) and from the current ones, available at the address www.wunderground.com. Thus, the average annual temperature provides information on the nature of the climate; the average temperature in the month of July is a synthetic indicator that leads the viticultural center towards a certain yield direction, the annual amount of rainfall and the amount of rainfall in the growing period indicate the area's favorability for viticulture; the Martonne aridity index indicates the nature of the climate, taking quality wines. This way were obtained into account the temperature - humidity interaction and is calculated in accordance with the formula:

$$IAM = \frac{\text{Annual rainfall amount}}{\text{Average annual temp.}+10^{\circ}C}.$$

From a lithological point of view, the area has a diverse lithological structure and is rich in minerals, specific to the Motru Piedmont, which is part of the largest piedmont unit in the country, the Getic Piedmont.

RESULTS AND DISCUSSIONS

The oenoclimatic data that characterizes the Corcova viticultural center is presented in Table 1, by comparison to the multiannual average in the respective area and its analysis reveals that there are significant differences with respect to the defining elements of the climate. These are generally pointed out by an increase in the value of certain temperature indicators and the decrease of those corresponding to the humidity, as follows: the annual temperature increased by 1.75ºC; the average temperature in the month of July increased by 1.63ºC; the annual amount of rainfall decreased by 383.76 mm; the amount of rainfall in the growing period decreased by 191.13 mm.

Figure 1. 'Fetească neagră' grape, Corcova

From the point of view of the aridity index, which integrates in a single formula the value of the annual average temperature and the annual rainfall, in view of characterizing the climate, it follows that, between 1961 and 1970, Corcova had a rather humid climate. Over the years, because of the decrease in rainfall and increase in temperature, the climate may be characterized as semi-arid Mediterranean in nature.

The collected data have, of course, a indicative value, but in the same time they confirm the current climatic changes, but for a precise characterizations are needed the values for the last 30 years. In ths context, for the studied period, it was appreciate that the climate nature was favorable for quality viticultural practices. Climatic challenges may arise due to the lack of precipitation, their uneven distribution or excessive temperatures, but the presence of surroundings forest ecosystems may improve the existing microclimate. During the research, the terroir of wine-growing center Corcova was studied and also the behavior of phenological spectrum of 'Feteasc㠠neagră' variety. It was observed that the triggering of all the phenophases and their length were influenced, in a small extent, by the number of bunch load, this in turn, being influenced by ecoclimatic conditions recorded in the area, but also according to the wine year.

According to the research it appears that a differentiated load of bud/vine has influenced the values of fertility coefficients (Table 2). Fertility is a measurable parameter of each variety (genetic imprint), but it can be influenced, to the same extent, by environmental conditions and by agro-technology applied to the plant and soil.

It was observed that the increase of the fruit load correlates with the decrease of the absolute fertility coefficient, so it is correlated with the decrease of the fertility buds.

There is a correlation between fertility and number of elements production elements, (fruiting elements); as their length decreases, the fertility of buds is reduce. As a results, under the given conditions, the 'Feteasc㠠neagră' variety has a better agrobiological behavior when the pruning is made with 12 buds/shoot (V1).

The relative fertility coefficient value remains constant in the first two variants and on the third variant has a slight increase, recorded at the highest load of fruit (30 buds/vine). However, the increase of the number of inflorescences reported to the number of shoots is small, and does not compensate for the disadvantages of exaggerated vegetative growths.

Regarding the yield, measured by the two indices (api, rpi), it can be noted that as fruit load increases, the average weight of a grape decrease (Table 2*)*.

The same trend is observed in analysis data and highlights that the length of the shoots is reducing with the increase of the buds on vine. On average, it ranges from 140 cm to 156 cm, but in the same way it also evolves the matured length of the woody shoots - the small fruit load ensures an obvious maturation of the woody shoots.

In practice, this aspect is very important considering that the extreme minimum temperatures during winter have a tendency to accentuate, at least in recent years, in most of the wine-growing areas of Romania.

The grape yield increase in proportion with the fruit load, the maximum registered was V2, with a load of 26 buds/vine (V2).

Table 1. Synthesis regarding the oenoclimatic data characterizing the Corcova viticultural center

Northern latitude	**Altitude (m)**	**Rainfall (mm)**		**Average temperature (°C)**		**Martonne aridity index**
		Annual	**Growing period**	**Annual**	**July**	
44°35'	150	**1961-1970 Period** (Teodorescu et al., 1987)				
		741.00	374.00	10.70	22.70	35.79
		2015-2017 Period				
		357.24	182.87	12.45	24.33	15.92
		-383.76	**-191.13**	**+1.75**	**+1.63**	**-19.87**

This can be explained by the fact that the quantitative yield is influenced both by the average weight of a grape and by the number of grapes on the vine, ultimately by the relative productivity index. From a qualitative point of view, too high yields are not desirable if they are not supported from a qualitative point of view, especially in the case of varieties with high growth rate (Stroe et al., 2013).

Table 2. Quantity and quality yield attributes

Experimental varieties	Fertility coefficient		The average weight of grapes (g)	Productivity index (g/shoot)		Length of shoots (cm)			Yield Kg//vine	Yield ha (kg)	Sugars (g/l)	Total acidity (g/l H_2SO_4)
	absolute	**relative**		**absolute (g/shoot)**	**relative (g/shoot)**	**total**	**matured**	**%**				
V1 15 buds/vine 1 short element of 3 buds + 1 woody shoot of 12 buds	1.3	0.38	210	273.0	79.8	156	142	91	1.25	5,932	235	4.47
V2 26 buds/vine 2 short element of 3 buds + 2 woody shoot of 10 buds	1.1	0.38	194	213.4	73.7	145	125	86	1.70	8,495	223	5.10
V3 30 buds/vine 3 short element of 2 buds + 3 woody shoot of 8 buds	1.0	0.40	175	175.0	70.0	140	115	82	1.60	7,944	203	5.30

By comparison, if for the table grapes the visual perspectives are important, for wine grapes the major importance are the elements that ensure the composition of the wine and its balance such as: sugar content, acidity, color compounds and so on (Table 3).

As shown in table 3, the content in sugars decrease as the fruit load increases. The total acidity marks a slight inverse variation, so that higher sugar content is associated with a lower acidity and vice versa. From the maturity evolution point of view, there is sufficient accumulation of compounds responsible for ensuring the quality of the wine production.

For example, the sugar content is sufficient to provide a potential alcoholic degree of wine of approximate 13-14% volume of alcohol, under conditions of sufficient acidity.

It can be noted, that the harvest fulfills the required conditions for obtaining quality wines, both with protected designation of origin and with geographical indication (Figure 2).

Table 3. Quality indices of grape harvest and obtained wine (average 2016-2017)

Quality indices of grape harvest	
Total acidity (g/l H_2SO_4)	5.3
Sugars (g/l)	220
Yield (t/ha)	5.3
Wine quality indices	
Total acidity (g/l acid tartric)	4.2
Alcohol (vol %)	13.0
Non-reducing dry extract (g/l)	28.9

It can be seen that all the qualitative parameters, found in Table 3, provides quality elements that support the classification of 'Fetească neagră' variety in protected designation of origin category (www.onvpv.ro).

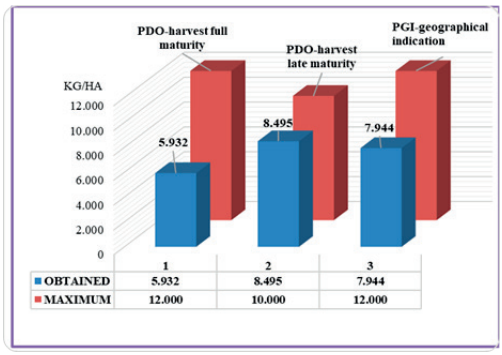

Figure 2. Achieved yield in relation with the maximum accepted for quality wines

CONCLUSIONS

Due to global warming, we observe obvious differences in the climate, highlighted by the increase values of some temperature indicators and the decrease of those corresponding to humidity.

The climate of the Corcova-Mehedinti wine-growing center, can be characterized as a Mediterranean semiarid, while almost 50 years ago it was rather a humid climate.

The climate remains favorable for the cultivation of the 'Fetească neagră' variety, and the attribution of a different fruit load, this variety seems well suited for pruning in 12 buds (V1), 15 buds/vine.

The assessment of the oenological potential revealed that the values of the compounds responsible for ensuring the quality of the wine production are sufficient to ensure an alcoholic potential of approx. 13-14 vol % alcohol, under conditions of balanced and sufficient acidity.

The grape harvest and all the quality parameters of the wine meet the necessary conditions existing in the specifications that aim to obtain quality wines with protected designation of origin.

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AGRO-MORPHOLOGICAL CHARACTERIZATION OF HABANERO PEPPERS FROM THE GERMPLASM COLLECTION OF VEGETABLE RESEARCH DEVELOPMENT STATION BUZĂU

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Abstract

The aim of this study was to assess the agro-morphological traits of different accessions of Habanero peppers from the germplasm collection of V.R.D.S. Buzau. Ten accesions of Habanero peppers were taken into study and had been characterized from agro-morphological point of view, analysing 15 quantitative and 10 qualitative traits. The quantitative parameters were statistically analysed and significant differences were observed in terms of plant height, therefore the maximum value was recorded by accession A110 (131.4 cm), followed by A12A (128.35 cm), A12B (124.85 cm) while the lowest plant height was recorded by genotype A500 (78.4 cm). The length of the fruits varied between 6.70 cm (A128) to 3.50 cm (A13A). Regarding the weight of the one fruit, the highest values was reported by A128 (30.10 g) and the lowest was also registered by A 500 (7.15 g) Following the agro-morphological characterization, it can be concluded that there was a great variability within studied genotypes of chilli peppers. The results will be used in the breeding program to obtain new genotypes adapted to the pedo-climatic conditions of Romania.

Key words: *ANOVA, biodiversity, Capsicum chinense, Romania.*

INTRODUCTION

Hot pepper (*Capsicum* sp.*)* belongs to the Solanaceae family and many authors attribute to the genus thirty-five species, but this is only an estimate number, the list remaining open until the discovery of new species. Of these, only five species are widely cultivated. *Capsicum annuum* L.*, Capsicum baccatum* L.*, Capsicum chinense* Jacq., *Capsicum frutescens* L.*, Capsicuum pubescens* Ruiz & Pav. (Bebeli and Mazzucato, 2008; Padilha and Barbieri, 2016; Xiao-min et al., 2016). The *Capsicum sp*. represent one of the most economically important vegetable crops in the word due to their innovative and versatile uses, both in the food and pharmaceutical industry (González-Pérez et al., 2014) as well as ornamental plants (Rêgo et al., 2009, Lagunovschi et al., 2016).

The centre of origin for hot peppers is considered to be in South America, either in Brazil, along the Amazon or in central Bolivia along the Rio Grande (Kang and Kole, 2013). The first countries in Europe where peppers crops have expanded were Spain and Portugal in 1494, followed by Germany (1542), England (1548) and Hungary (1560) (Ciofu et al., 2004), (Lagunovchi et al., 2016).

In Romania the pepper began to be grown in the 18th century (Andronicescu and Angelescu, 1968).

Habanero peppers (*Capsicum chinense)* are a unique group of plants with a distinct variability in different traits of interest (Montalvo-Peniche et al., 2007). In Mexico, Habanero pepper is traditionally grown as a culinary product for export due to the specific taste and aroma, as well as the content of oleoresin, characteristics that have generated a significant growth in the international markets (Zakia et al., 2013). Furthermore, this species is part of the hottest peppers in the world, their fruits are used in the industrial extraction of capsaicin a valuable component in various pharmaceutical and food products (Butcker et

al., 2012; Canton-Flick et al., 2008; Montalvo-Peniche et al., 2007; Yordanova et al., 2015).
Butcker et al. (2012) states that few Habanero pepper breeding programs exist worldwide, limiting the potential exploitation of this diverse germplasm. For this reason, Vegetable Research and Development Station Buzau (V.R.D.S.) has been studying this species intensively since 1996, but concerns for breeding and large scale crop production of the hot pepper have existed since its foundation in 1957. V.R.D.S. was the first location in Romania where this crop was grown under greenhouse conditions. Over time, Research Station has patented and registered in Official Catalogue of Romanian Crop Plants, four varieties with distinct phenotypic expressivity, respectively, *Decebal, Jovial, Roial* and *Vladimir*.
V.R.D.S. Buzau owns a valuable collection of *Capsicum sp.,* it consists of two hundred genotypes, of these, ten genotypes of Habanero peppers were selected for this study and characterized from an agro-morphological point of view.

MATERIALS AND METHODS

The researches were carried out at the Genetic, Breeding and Biodiversity Laboratory from Vegetable Research and Development Station Buzau. The study aimed the evaluation of ten accessions of Habanero peppers noted A10B, A10C, A12A, A12B, A13A, A13B, A76, A110, A128, A500. The breeding method used was repeated individual selection, consisting in homozygosity of the main characters, followed by retention of typical elite plants. Afterwards, the elite plants were isolated under greenhouse conditions in order to keep biological purification of the accessions. The crop technology applied was the one specific for chilli peppers. The seed were sown at the beginning of March in alveolar pallets with 70 cubes with a volume of 50 cm^3 in a mixture a peat and sand, the planting was made in first decade of May, and the planting scheme used was 70 x 35cm. The plants were grown in fence system, during the vegetation period a special care was made using mechanical and manual hoeing and pest disease management. Throughout the vegetation period a sets of 10 qualitative and 15 quantitative descriptors showing continuous variation were selected from the available literature on the crop, IPGRI and UPOV Guidelines. Similar studies were made by Ortiza et al. (2010), they used the qualitative and quantitative traits in the five domesticated species of *Capsicum* for grouping them after assessing inter- and intra-specific variation. Also, Rahman et al. (2017) have been using 22 qualitative and 5 quantitative traits to characterize sixty chilli germplasm collected from different parts of Bangladesh.
The qualitative traits targeted in the study are presented in Table 1.
Statistical analysis was done using the analysis of variance (ANOVA) and statistics indices use for each character were: the means, standard deviation (SD) and coefficient of variation (CV%).

Table 1. Qualitative traits

Descriptors	Polymorphism
Fruit: color before maturity (FCBM)	1. white 2. yellow 3. green 4. Orange 5. purple 6. dark purple 7. other
Fruit: color at maturity (FCAM)	1. white 2. lemon yellow 3. pale orange-yellow 4. yellow orange 5. pale orange 6. orange 7. light red 8. red 9. dark red 10. purple 11. brown 12. black 13. other
Fruit: shape at pedicel attachment (FSP)	1. sharp 2. obtuse 3. truncated 4. cordate 5. lobed
Fruit: neck at base (FNB)	0. absent 1. present
Fruit: shape of blossom end (FSB)	1. sharp 2. bont 3. deep 4. deep and sharp 5. other
Fruit: blossom end appendage (FA) (FA)	0. absent 1. present
Fruit: shape in cross section (FSCS)	3. eliptic 5. angular 7. circular
Fruit: number of locules (FNL)	
Fruit: texture of surface (FS)	1.smooth or very slightly wrinkled 2.slightly wrinkled 3.strongly wrinkled
Placenta: length (PL)	1. <1/4 fruit 2. ¼-1/2 fruit 3. >1/2 fruit

The 15 *quantitative* traits used for agro-morphological characterization were: plant length (PL), plant diameter (PDM), leaf length (LL), leaf width (LW), petiole length (PL),

number of fruits per plants (NF), yield of fruits per plant (YF), fruit length (FL), fruit width (FW), fruit weight (W), weight of fruit pulp (WP), weight of fruit receptacle (WR), pedicel length (PDL), pedicel diameter (PD), pericarp thickness (PT).

RESULTS AND DISCUSSIONS

Throughout the study, a wide variability was note in most of the characters included as descriptors in this research.

Qualitative traits

A total of 10 qualitative characters were recorded and evaluated in order to establish the variability among the studied accessions. During the assessment, six characters showed distinct variation among the studied accessions (FCBM, FCAM, FSB, FSA, FSCS, FNL) and a number of four characters (FNB, FA, FS, PL) showed slightly variation among the accessions. The result of qualitative traits used in this study can be found in Table 2.

Table 2. Qualitative characters of the studied genotype

Accession	FCBM	FCAM	FSP	FNB	FSB	FA	FSCS	FNL	FS	PL
A10B	3	2	4	0	4	0	5	5	3	3
A10C	3	9	3	0	4	0	5	2	3	3
A12A	2	4	4	0	4	0	3	3	3	3
A12B	3	4	4	0	4	0	3	3	3	3
A13A	3	8	4	0	4	0	7	4	3	3
A13B	3	8	4	0	4	0	7	4	3	3
A76	3	4	3	0	1	0	3	3	3	3
A110	3	8	4	0	4	0	5	3	3	3
A128	3	8	5	0	4	0	7	4	3	3
A500	3	2	5	0	1	0	7	3	3	3

The number of fruit locules and fruit color at physiologically maturity has shown a great variability within studied traits. None of accessions have blossom end appendage or neck at the base of fruit. The length of placenta occupied more than half of fruit on all studied accessions and, also, all genotypes have a strongly surface wrinkled. Regarding the fruit color before maturity stage, accession A12A presented yellow fruit, and the rest of accessions have green fruit. The fruit shape of blossom end was deep and sharp for 80% for genotypes, while 20% for genotype presents sharp characters. Majority of the studied accessions have cordate shape at pedicel attachment (60%), and the rest have truncated (20%) or lobed (20%) shape. In cross section, A13A, A13B, A125, A500 showed circular fruits, A10B, A10C, A110 presented angular fruits and A12A, A12B, A76 have elliptic fruits. Number of locules of fruits varied from 2 for A10C, to 5 for A10B. At physiologically maturity, fruit colour in Habanero accessions was lemon yellow (A10B, A500), yellow orange (A12A, A12B, A76), red (A13A, A13B, A110, A128) or dark red (A10C) (Figure 1).

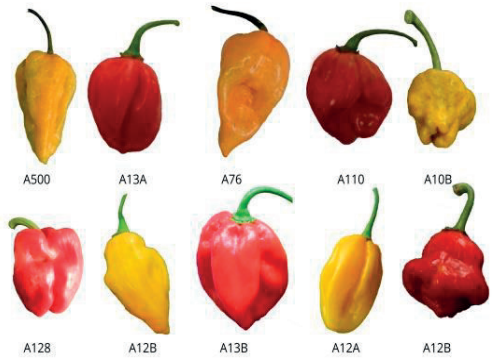

Figure 1. Different types of fruits

Quantitative traits

The quantitative parameters were statistically analyzed and significant differences were observed in terms of plant height, therefore, the maximum value was recorded by accession A110 (131.40 cm), followed by A12A (128.35 cm), A12B (124.85 cm) while the lowest plant height was recorded by genotype A500 (78.40 cm). As regards plant diameter, the maximum value was 127.55 cm (A12B), and the minimum value was 47.25 cm (A500). Crop detail of accession A12B is presented in Figure 2. The intermediate values are found in table 3. The length of the fruits varied between 3.50 cm (A13A) to 6.70 cm (A128**)** and the fruits width varied between 2.77 cm (A76) to 6.43 cm (A128).

Table 3. Means of studied accession

Code	A10B	A10C	A12A	A12B	A13A	A13B	A76	A110	A128	A500	Mean	SD	CV%	p value
PL	93.45	107.0	128.35	124.85	117.06	81.50	84.45	131.40	99.30	78.40	104.58	20.09	19.21	< 0.0001
PDM	72.05	82.35	103.25	127.55	79.60	90.50	51.30	100.85	64.15	47.25	81.89	24.78	30.26	< 0.0001
LL	18.20	16.30	15.25	15.85	13.50	18.05	16.15	16.80	19.85	12.89	16.28	2.12	13.00	ns
LW	5.60	4.85	6.05	5.50	7.10	9.60	6.77	8.35	10.70	4.85	6.94	2.02	29.09	ns
PL	5.60	5.00	2.80	2.65	4.10	4.75	2.90	3.15	3.40	2.77	3.71	1.07	28.94	ns
NF	29.50	38.00	35.00	7.85	35.00	23.50	22.50	39.50	13.50	36.00	28.04	10.87	38.77	< 0.0001
FL	3.87	4.57	3.90	5.28	3.50	4.83	6.45	4.17	6.70	5.71	4.90	1.11	22.69	ns
FW	4.33	4.33	3.55	3.00	3.62	5.08	2.77	3.95	6.43	2.77	3.98	1.14	28.60	ns
W	9.06	13.07	11.88	11.40	9.09	24.39	15.79	12.36	30.10	7.15	14.43	7.29	50.55	< 0.0001
WP	5.40	10.12	9.86	9.24	8.21	21.47	12.21	10.94	22.61	6.50	11.65	5.83	50.04	<0.0001
WR	3.66	2.95	2.02	2.17	0.88	3.51	4.28	1.37	7.34	1.04	2.92	1.94	66.35	< 0.0001
PDL	5.45	5.75	3.55	3.69	3.32	4.13	2.48	3.42	4.29	3.15	3.92	1.02	26.00	ns
PD	0.37	0.38	0.36	0.32	0.21	0.35	0.74	0.25	0.54	0.16	0.37	0.17	45.72	< 0.0001
PT	0.72	1.67	1.61	1.95	1.69	2.23	4.15	2.35	2.56	1.47	2.04	0.91	44.43	< 0.0001

SD-standard deviation, CV-coefficient of variation, NS-insignificant PL-plant length, PDM-plant diameter, LL-leaf length, LW-leaf width, PL-petiole length NF-number of fruits per plants,), FL-fruit length, FW-fruit width, W-fruit weight, WP-weight of fruit pulp, WR-weight of fruit receptacle, PDL-pedicel length, PD-pedicel diameter, PT-pericarp tickness

Figure 2. Crop detail A12B

Regarding the weight of the fruits, the highest values was reported by A128 (30.10 g) and the lowest was also registered by A500 (7.15 g). The weight of fruit pulp had the highest value for A13B with 21.47 g, followed by A76 with 12.21 g and the smallest value was 5.40 g had by A10B. The highest value for fruit receptacle weight was registered by A128 with a value of 7.34 g and the smallest value was 0.88 g (A13A). A detailed crop imagine for A13B can be found in Figure 3 and also a crop detail for A13A is presented in Figure 4. Regarding the fruit yield per plant highly significant differences were observed. The highest yield per plant was found in germplasm A13B (826.44 g) and the lowest yield per plant was found in A12B (102.03 g).

The fruits of A76 showed the highest pericarp thickness (4.15 mm), followed by A128 (2.56 mm), and lowest value was recorded by A10B (0.72 mm). The length of pedicel varied from 2.48 cm to 5.75 cm; the highest value was found in A10C and lowest in A76. The diameter of the pedicel varied from 0.16 cm (A500) cm to 0.74 cm (A76).

Figure 3. Crop detail A13B

In Table 3 are presented the mean values of studied accessions followed by standard deviation, coefficient of variation and p value. The results of statistical analysis showed a great variability within studied genotypes.

Figure 4. Crop detail A13A

CONCLUSIONS

Following the agro-morphological characterization, it can be concluded that there was a great variability within studied genotypes of chilli peppers. Significant variation was observed in terms of quantitative parameters and the highest variation was observed in weight of fruit receptacle. At the same time, most of the qualitative characters showed distinct variation among the germplasm studied accessions. The number of fruit locules and fruit color at physiologically maturity has shown a great variability within studied traits.
The results will be used in the breeding program to obtain new genotypes adapted to the pedo-climatic conditions of Romania.

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ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICALS COMPOUNDS ON LEAF CABBAGE (*BRASSICA OLERACEA* L. VAR. *ACEPHALA*) AND CHINESE CABBAGE (*BRASSICA RAPA* L. VAR. *CHINENSIS*)

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Abstract

The aim of the study was the comparative analysis of some varieties of leaf cabbage in terms of their content in total phenolic compounds, flavonoids, foliar pigments, antioxidant activity and antioxidant enzyme activity (superoxide dismutase, catalase and soluble peroxidase). The biological material was represented by two cultivars of kale: Dwarf Green Curled and Nero Di Toscana and by the cultivar Pak Choi White, a variety of Chinese cabbage, cropped under the same conditions. The total phenolics, flavonoids, chlorophyll and carotene content were determined by colorimetric methods. The antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and enzymatic antioxidant activities were determined colorimetric using appropriate substrate. The results show that Nero Di Toscana cultivar has the highest content of phenolic compounds (275.1 mg GAE/100 g fm), total soluble peroxidase (50.33 ΔA/min/g f.m) and low Superoxide dismutase activity (30.06 IC 50% mg) and at chinese cabbage, Pak Choi White cultivar recorded a high catalase activity (44.96 mM H_2O_2/g fm/min) and the highest antioxidant activity (1294 TE/100 g fm). The study recommends the introduction into the diet of the varieties studied due to the high content of phytochemicals with health-promoting benefits.

Key words*: leaf cabbage, total phenolics, flavonoids, chlorophyll.*

INTRODUCTION

Green leafy vegetables are sources of important nutritional value for human diet and health. Recent studies suggest that foods rich in carbohydrates and dietary fiber have the potential to reduce risks of diseases, including obesity, diabetes, cancer and heart disease.

Brassicaceae vegetables are an abundant source of health-promoting substances and which reduce the risk of diseases (Leja et al., 2010; Podsedek, 2007).

Brassica species are vegetables rich in phytochemicals that include polyphenols, phenolic acids, flavonoids, carotenoids (zeaxanthin, lutein, β-carotene), alkaloids, tannins, saponins, anthocyanins, chlorophyll phytosterols, glucosinolates, phytosteroids, E vitamin. (Nawaz et al., 2018). Flavonoid compounds produce biological activity with beneficial effects on human health: suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses (Agati et al., 2012).

The phenolic compounds are an important group of biologically active substances found in plants. They are secondary metabolites that are able to neutralize the free radicals and numerous studies indicate that phenols may play a significant role in protecting biological systems from the effects of oxidative stress.

This is especially important because under oxidative stress, excessive formation of reactive oxygen species (ROS) can damage biomolecules, such as DNA, proteins, lipids and carbohydrates and can lead to many diseases. Superoxide dismutase enzyme (SOD) catalyzes the elimination (O^{-2}) of H_2O_2, whereas peroxidase (POX) and catalase (CAT) are involved in the reduction of H_2O_2 in cells (Babeanu et al., 2017).

Kale (*Brassica oleracea* var. *Acephala*) is one of the oldest forms of the cabbage family. It has origins in the eastern Mediterranean (Acikgoz,

2011). Kale is commonly cultivated in Central and Northern Europe and North America (Korus, 2011).
Pak Choy plants (*Brassica rapa* var. *Chinensis*) belong to a group of plant crops derived from the Far East (Kalisz, 2011). Chinese cabbage is a very popular vegetable in China that is also widely known in the USA and in European countries there are attempts to introduce it into the culture (Kalisz, 2011). These species of *Brassica* are of major importance due to their nutritional but also medicinal value, being rich in proteins, minerals, cellulose and antioxidants.
Due to its antioxidant, anti-inflammatory and antibacterial properties, cabbage is widely used in traditional medicine to relieve symptoms associated with gastrointestinal disorders. As food plants can be used raw in the form of salads or juices, or other preparations much appreciated by consumers.
Kale (*Brassica oleracea* L. *var. Acephala*) and Chinese cabbage (*Brassica rapa var. Chinensis*) are little known in Romania as sources of vitamins and mineral salts. The regions most favorable for cabbage culture are represented by the river beds in the area of Transylvania, Moldova, but also in the Plain of the South and West of the country (Soare et al., 2016a; Balcău et al., 2013).
The crop of these species can have multiple advantages through the high income of the producers and the introduction in the diet of vegetables with high nutritional value. The aim of the study was the comparative analysis of the content of total phenols, flavonoids, antioxidant activity and enzymatic activity in varieties of leaf cabbage and Chinese cabbage, in order to promote them for cropping in southwest Romania.

MATERIALS AND METHODS

The biological material was represented by two cultivars of leaf cabbage (*Brassica oleracea* var. *Acephala*): Dwarf Green Curled and Nero Di Toscana and a variety of Chinese cabbage (*Brassica rapa* var. *Chinensis*), cultivar Pak choi White.
The crop was established at the Didactic Station of the University of Craiova, Romania (44°19'N and 23°48'E), in 2018-2019, a favorable area for the cropping of the species of *Brassicaceae*, by seedlings. The sowing was done at the beginning of April, and the planting of seedlings in the field was carried out in the last decade of May. At planting, the seedlings were 50 days old. Planting distances were 80 cm between rows and 60 cm between plants in a row for leaf cabbage, and in Chinese cabbage 40 cm between rows and 25 cm between plants in a row. Within the experimental trials, the specific technological sequences were applied.

Characterization of genotypes
Dwarf Green Curled Kale genotype is characterized by large, densely curled, fleshy, petiolated, dark green leaves. It forms a vigorous plant, with rich foliage, spirally arranged. Also, the leaves are obliquely upward oriented and outward rolled.
Nero Di Toscana genotype has the appearance of a palm tree, with oblong, embossed blue-green leaves, covered with a thick layer of rhyme, vertically oriented. These cultivars have a dual purpose both for food and ornamental, being winter resistant.
Pak Choi White genotype forms a rosette of green, smooth, leaves, with well-developed petiole, whose color is white. The leaves are sessile, they have a very well developed central string, which is white and a lush foliar limb, soft and with a fine texture (Soare et al., 2016a).
To carry out the study, fresh leaves were harvested from the cultivated varieties and the content of phenolic compounds, flavonoids, foliar pigments, antioxidant activity and antioxidant enzyme activity (superoxide dismutase, catalase and soluble peroxidase) were analyzed.

Chemical analysis methods
For the spectrophotometric determination of chlorophyll a, chlorophyll b and carotenoids, 1 g leaf samples were extracted in 100 mL methanol and absorbance of the extracts was measured at 470, 653 and 666 nm.
The results were calculated using the formulas of Lichtentaler and Wellburn (1983). Chlorophyll a (mg/mL) = 15.65*A666 – 7.340*A653; Chlorophyll b (mg/mL) = 27.05*A653 – 11.21*A666; Total carotenes (mg/mL) = (1000*A470 – 2.860*Chlorophyll a – 129.2*Chlorophyll b)/245

Taking into account the extraction rate (1 g: 100 mL), the amount of pigments in leaves was expressed as mg/g fm (fresh matter).

For antioxidant enzymes extraction, fresh tissue was homogenised with 0.1 M phosphate buffer (pH 7.5) containing 0.1 mM EDTA. The homogenates were centrifuged for 20 min at 6000 rpm and the supernatants were used for enzyme assays.

The Superoxide dismutase (EC 1.15.1.1) activity (SOD) was assayed by measuring ability of sample extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Soare et al., 2017). SOD activity was expressed as the amount of sample (mg) which caused 50% inhibition of photochemical reduction of NBT.

Total soluble peroxidase (guaiacol-type E.C.1.11.1.7) activity (POX) was assayed by measuring the increase in A470 due to guaiacol oxidation to tetraguaiacol on addition of H_2O_2 and their activity was expressed as ΔA/min/g fm (Matei et al., 2014).

Catalase activity (E.C.1.11.1.6) CAT was assayed through the spectrophotometric method at 240 nm (Soare et al., 2017) and expressed as as mM H_2O_2/min/g fm at 25°C.

The extracts for the determination of total phenolic content, total flavonoids content and antioxidant activity were prepared into 80% aqueous methanol (1: 10 w/v) at 24°C for 16 h. The resulting slurries were centrifuged at 4000g for 5 min and the supernatants were collected.

Total phenolic content was determined colorimetrically with Folin - Ciocalteu reagent (Dinu et al., 2018a). To 1mL methanolic extract 5 mL reactive Folin - Ciocalteu (diluted 1: 10 with ultrapure water) were added. After two minutes, 4 mL 7.5% sodium carbonate was added and the samples were kept in the incubator at the room temperature for 90 minutes. The absorbance was measured at 765 nm and the total phenolic content was calculated using a standard curve with gallic acid and expressed as mg GAE/100 g fm.

The total flavonoids content was determined by colorimetric method with 10% $Al(NO_3)_3$ and 5% sodium nitrite ($NaNO_2$) in alkaline medium (Soare et al., 2015a). The absorbance was read at 500 nm and the results were calculated from quercetin calibration curve and expressed as mg QE/100 g fm.

Antioxidant activity, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay: The capacity of extracts to scavenge the DPPH radical has been evaluated colorimetrically at 517 nm (Dinu et al., 2018b). The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The results were expressed as μM Trolox equivalents (TE)/100 g fm.

All the spectrophotometric measurements were carried out with Evolution 600 UV-Vis spectrophotometer, Thermo Scientific, England, with VISION PRO software.

Statistical method

All analyzes were performed in three repetitions, and mean values were processed by analysis of variance (ANOVA). The significance of the differences was estimated with the Duncan test at the $p \leq 0.05$ level The correlation coefficients between the analyzed characters were also calculated, their significance being determined by Pearson values.

RESULTS AND DISCUSSIONS

Brassicaceous species biosynthesize high levels of chlorophylls and carotenoids (Hanson et al., 2009). The pigment content confers certain sensory characteristics on the edible organs and is a feature with a role in the evaluation and acceptability of consumers of these species.

Carotenoids (α and β carotene, xanthophylls and oxygenated carotenoids) are compounds with antioxidant properties that play an important role in scavenging free radicals and maintaining the immune system. Of these, beta-carotene (provitamin A), lutein and zeaxanthin, are involved in the process of vision. Carotene content has shown great interest recently because of its importance in human nutrition (Dinu and Soare, 2017).

Other components that contribute to the beneficial effects of consuming Brassicaceae are chlorophylls. Recent studies have investigated a wide range of medicinal and pharmacological properties of chlorophylls a and b, including antimutagenic, anti-inflammatory and antioxidant properties and their use in cancer therapy (Gopi et al., 2014). Regarding the content of chlorophyll and

carotene in the present study, the values were higher in the varieties of leaf cabbage compared to the Chinese cabbage. The cultivar Nero Di Toscana recorded the highest values in chlorophyll a of 1.83 mg/g f.m., in chlorophyll b of 0.833 mg/g f.m. and in carotene, of 0.753 mg/g f.m. Our results are similar to those recorded by Jurkow, (2019) for two kale cultivars (blue green and red purple leaves) harvested before frost, a content of chlorophyll a by 1.24 mg/g f.m., chlorophyll b 0.632 mg/g fm and 0.142 mg/g fm carotenoids in 2015/2016 season and chlorophyll a by 1.531 mg/g f.m. chlorophyll b 0.49 mg/g f.m. and 0.324 mg/g fm carotenoids in 2016/2017 season, and Samec (2018), raported at Kale, chlorophyll a by 2.27 mg/g dw, Ch b de 1.19 mg/g dw and on caroten by 0.53 mg/g dw, and at Chinese cabbage, chlorophyll a by 1.88 mg/g dw, chlorophyll b 0.85mg/g dw, caroten 0.38 mg/g dw.

Also, Korus and Kmiecik (2007) report for 3 kale crop investigated in 2 successive years an average content of total chlorophylls of 118-145 mg/100g f.w.; carotenoids 23.1-26.0 mg/g fw and beta-carotene 3.8-4.53 mg/g fw. It can be stated that this variability in chlorophyll and carotene content can be greatly influenced by the species, cultivar, date harvest and leaf maturity.

Table 1. Content in chlorophyll and carotene in *Brassica oleracea* var. *Acephala* and *Brassica rapa* var. *Chinensis*

Indices / Cultivar	chlorophyll a (mg/g fm)	chlorophyll b (mg/g fm)	Carotene content (mg/g fm)
Nero Di Toscana	1.83a	0.833a	0.753a
FC Dwarf Green Curled	1.40b	0.749b	0.533b
Pak choi White	0.32c	0.114c	0.21c
LSD $p \leq 0.05$	0.11	0.13	0.13

Values represent the mean in the same column followed by different superscript letters are significantly different at $p \leq 0.05$

In a study by Kapusta-Duch and Leszczyńska (2013), the difference in carotene content was influenced according to the variation of climatic and agrotechnical conditions (cultivated organic farms compared to vegetables grown in steel or retail available).

The content of total phenolic compounds varies between 217.9 mg GAE/100 g fm (Pak choi White) and 275.1 mg GAE/100 g fm (Nero Di Toscana) (Table 2). The investigated species have a high content in phenolic compounds, justifying the beneficial effects of the diet rich in *Brassicaceae*. The data obtained in this study are similar to those presented by Korus (2011) in kale, variety Winterbor, a content in phenolic compounds ranging from 273 to 381 mg chlorogenic acid/100 g fw., and Polish variety, medium high Green Curly between 256 and 395 mg chlorogenic acid/100 g fw at different stages of maturity, and Samec et al. (2018), report for kale 15.13 mg GAE/g dm (dry weight 11.53%) and for Chinese cabbage 13.2 mg GAE/g dm (dry weight 12.03%).

In a previous study showing the content of bioactive compounds in some varieties of *Brassicaceae*, our group (Soare et al., 2015b), reported the highest content of phenolic compounds in kale (206 mg GAE/100 g fw) followed by broccoli (101.66 mg GAE/100 g fw), red cabbage (100 mg GAE/100 g fw), white cabbage (73.6 mg GAE/100 g fw) and cauliflower (33 mg GAE/100 g fw) and Samek et al. (2018) found for total phenolic content a variation in the order of white cabbage > kale. > broccoli > Chinese cabbage. There is a significant variation in the content of phenolic compounds in the two varieties of *Brassica* influenced primarily by the species, from the moment of harvest and the crop conditions.

The results obtained for total flavonoids content are shown in the Table 2.

The content of total flavonoid compounds was higher in Chinese cabbage, 124.2 mg QE/100 g fm cultivar Pak choi White, and leaf cabbage 109.86 mg QE/100 g fm Dwarf Green Curled cultivar 117.12 mg QE/100 g fm cultivar Nero Di Toscana.

The literature shows that in the composition of the studied varieties, a wide range of flavonoids has been identified and quantified, the majority of which are: quercetin, kaempferol and rutin (Cartea et al., 2011).

Table 2. Content in total phenols, total flavonoids and antioxidant activity in *Brassica oleracea var. Acephala* and *Brassica rapa* var. *Chinensis*

Indices / Cultivar	Total Phenols (mg GAE/100g) fm	Total Flavonoids (mg QE/100 g)	Antioxidant activity (µmol TE/100g fm DPPH)
Nero Di Toscana	275.10[a]	117.12[a]	982.04[b]
Dwarf Green Curled	267.43[a]	109.86[b]	830.92[c]
Pak choi White'	217.09[b]	124.30[a]	1294[a]
LSD 0.05	25.24	8.74	67.48

Values represent the mean in the same column followed by different superscript letters are significantly different at $p \leq 0.05$

Some authors have evaluated the content of flavonoid compounds in different species of *Brassicaceae.* Samec et al. (2018) analyzing comparative phytochemicals content in five *Brassicaceae* species reports a TFC of 3.67 mg CE/g dw for kale and 2.57 mg CE/g dw for Chinese cabbage, Hagen et al. (2009) finds in kale variety Reflex, total flavonol content 661 mg/100g dm, Agarwal et al. (2017) determine for kale 13.98 mgQ/g dw (dw = 9.08%) while Bahorun et al. (2004) show for Chinese cabbage TF of 944 µg QE/g fw. It can be stated that there is a great variability of the flavonoid content influenced by the species, variety, genetic potential, the extra time for the plants remaining in the field after the coming of the frost and different harvesting times.

Numerous studies indicated that the beneficial effects of eating fruits and vegetables are partly explained by the content in compounds with antioxidant activity that scavenge free radicals and contribute to the defense against oxidative stress (Agati et al., 2012; Gopi et al., 2014; Podsedek, 2007).

The antioxidant activity of the studied *Brassica* vegetables was determined by the ability of extracts to reduce the DPPH radical. The results obtained are shown in table 2.

The antioxidant activity determined by DPPH recorded 1294 µM TE/100 g fm in Chinese cabbage, Pak choi and Kale cultivars, from 830.92 µM TE/100g fm (Dwarf Green Curled) to 982.04 µM TE/100 g fm (Nero Di Toscana). In some reports, DPPH values determined for radical scavenging activity ranged from 56.3 µmol TE/100 g fw (cauliflora) and 965.76 µmol TE/100 g fw (kale), high values were also recorded for red cabbage of 566.08 µmol TE/100g fw and also of 388.56 µmol TE/100 g fw for broccoli (Soare et al., 2015b). The variation of the antioxidant potential depends on the species, the maturity stage of the plant or the variety.

Korus (2011) reports for kale, the antioxidant activity measured by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method values ranging from 14.7 µM Trolox/g to 19.6 µM Trolox/g at different stages of maturity. (Samec et al., 2018; Korus, 2011) show for kale and Chinese cabbage 34 µM Trolox/g dw (dw = 11.53%, respectively 12.03%). Within the *Brassica* species, there is a great deal of variability, and depending on the cultivated genotype (Soare et al., 2016b; Korus and Kmiecik, 2007).

To evaluate the enzymatic antioxidant capacity of the investigated *Brassica* species, the activity of the enzymes was determined: superoxide dismutase-SOD, catalase - CAT and soluble peroxidase - POX.

SOD is an enzyme that catalyzes the dismutation of the superoxide anion into oxygen and hydrogen peroxide, peroxidase (POX) catalyzes oxidation of various electron donor substrates concomitant with the decomposition of H_2O_2. Peroxidase is an enzyme involved in the control of various essential physiological processes of plant growth and development, lignification, biotic and abiotic stress responses and the catabolism of growth regulators (Caverzan et al., 2012). Catalase catalyses the reduction of H_2O_2 to H_2O and molecular oxygen.

The obtained results show that the activity of antioxidant enzymes varies with the investigated variety (Table 3). The enzymatic activity of CAT depending on the species and genotype, varies in the order: Dwarf Green Curled (26.8 mM H_2O_2/g fm/min) <Nero Di Toscana (36.18 mM H_2O_2/g fm/min) <Pak choi White (44.96 mM H_2O_2/g fm/min). In previous research, our group determined for the enzymatic activity of CAT at kale, 33.6 mM H_2O_2/min/g fm, and this enzymatic activity determined for some varieties of Brassicaceae, varied in the order: broccoli> kale> red cabbage> white cabbage> cauliflower (Soare et al., 2017). The results obtained by us are very close to those reported

by Samec et al., (2018) for kale and Chinese cabbage.

Table 3. Enzyme activity in *Brassica oleracea* var. *Acephala* and *Brassica rapa* var. *Chinensis*

Indices / Cultivar	CAT (mM H_2O_2/g fm/min)	POX (ΔA/min/g fm)	SOD (IC 50% (mg)
Nero Di Toscana	38.6[b]	50.33[a]	30.06[a]
Dwarf Green Curled	26.8[c]	19.6[c]	12[c]
Pak choi White	44.96[a]	34.85[b]	21.6[b]
LSD 0.05	3.86	6.95	4.17

Values represent the mean in the same column followed by different superscript letters are significantly different at $p \leq 0.05$

In the present study, the enzymatic activity of POX ranges from 19.6 ΔA/min/g fm (Dwarf Green Curled) to 50.33 ΔA/min/g fm (Nero Di Toscana). Of the two kale cultivars, Nero Di Toscana has the highest enzyme activity of CAT and POX, 35%, respectively 156.78% higher than the value determined in the Dwarf Green Curled cultivar. In a previous study investigating antioxidant enzyme activity in five species of *Brassica*, our group showed a 54.6 ΔA/min/g fm enzyme activity of POX, the highest value for peroxidase activity was determined at red cabbage, followed by broccoli, white cabbage, kale and cauliflower (Soare et al., 2017). In other studies, for 2 varieties of kale, values between 3.89 and 16.06 ΔA/min/g fm are reported, depending on the degree of maturity (Korus, 2011). Peroxidase activity among different vegetables varies significantly. In a comparative study evaluating the enzymatic activity of peroxidase in selected vegetables the authors report the greatest activity in the case of cabbage while lowest peroxidase activity was observed in the case of green chilli and spinach (Pradeep et al., 2017).

The enzymatic activity of SOD varies with genotype and species. A low IC 50% expressed as the amount of sample (mg) which caused 50% inhibition of photochemical reduction of nitrobluetetrazolium corresponds with high superoxide dismutase enzymatic activity. The results obtained for superoxide dismutase activity, expressed as IC 50% (mg), varied between 12.00 mg (Dwarf Green Curled) and 30.06 mg (Kale Nero Di Toscana) (Table 3).

Of the cultivars investigated, Dwarf Green Curled with the highest enzyme activity of SOD 2.5 times higher than the value determined at Nero Di Toscana and 1.8 times higher than the value determined at Pak choi White. Our previous results indicate values for the kale species of 32.4 IC 50% mg (Soare et al., 2017). Although a high level of enzyme activity investigated increases the value of antioxidant capacity, bioavailability and food quality, high enzymatic activity can cause unwanted changes in foods that occur as a result of oxidation of different substrates leading to loss of quality attributes such as aroma and appearance.

The enzymatic activities and the antioxidant activity vary depending on the analyzed species and cultivar (Korus, 2011; Babeanu et al., 2017; Soare et al., 2017). In a study of different species of *Brassica*, superoxide dismutase activity recorded high levels in kale, peroxidase recorded high values in red cabbage, highest value for catalase activity was determined in broccoli, and highest value for antioxidant activity in kale (Soare et al., 2017).

Regarding the correlation coefficient (r) between the analyzed phytochemicals, both positive and negative correlations were registered (Table 4).

Significant positive correlations were recorded between chlorophyll a and chlorophyll b (r = 0.974), between carotene and chlorophyll a and b, (r = 0.940; r = 0.890); between total phenols and chlorophyll b and between CAT and total flavonoids (0.862), between CAT and antioxidant activity (r = 0.913), as well as between SOD and POX (r = 0.934).

Table 4. Correlations between the analyzed compounds in the investigated *Brassica* species

Chemical compound	Chlorophyll a	Chlorophyll b	Carotenes	Total phenols	Total flavonoids	Antioxidant activity	POX	CAT
Chlorophyll b	0.974**							
Carotenes	0.940**	0.890**						
Total phenols	0.935**	0.965**	0.84**					
Total flavonoids	-0.514	-0.580	-0.607	-0.540				
Antioxidant activity	-0.802**	-0.896**	-0.717	-0.870**	0.784*			
POX	0.279	0.098	0.348	0.065	0.434	0.326		
CAT	-0.509	-0.655	-0.476	-0.642	0.862**	0.913**	0.634	
SOD	0.244	0.063	0.363	0.092	0.359	0.352	0.934**	0.615

p 5% = 0.67; p 1% = 80

CONCLUSIONS

The results of this study show that the species studied of cabbage for leaves, less known and marketed in Romania, are a rich source of compounds with antioxidant activity. Among the cultivars studied, Nero Di Toscana kale cultivar has the highest content of phenolic compounds, flavonoides and the highest enzyme activity of CAT and POX, while chinese cabbage, Pak choi White cultivar recorded the highest catalase activity and antioxidant activity.

Regarding the analysis of the compounds investigated, a positive correlation is observed between chlorophyll a and chlorophyll b, between carotene and chlorophyll a and b, between total phenols and chlorophyll b and between determined antioxidant activity and content in flavonoids and in the enzymatic activity of catalase. The results of the study indicate that Chinese cabbage and kale are sources of antioxidants. The emergence of new species of *Brassica* on the market, such as kale and Chinese cabbage, can have multiple benefits for humans, such as sources of antioxidants in food, opportunities to diversify the vegetable assortment and as ornamental plants.

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INVESTIGATION OF THE INFLUENCE OF THE HUMIC ACIDS ON THE CULTIVARS OF LONG-DAY ONION (*ALLIUM CEPA* L.), DRIP IRRIGATED IN THE CONDITION OF SOUTH BULGARIA

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Abstract

- During 2014-2015 the varieties of long-day onion (Allium cepa L.), sown in spring were examined at the experimental field of Department of Horticulture in Agricultural University, Plovdiv, Bulgaria. It has been estimated that the foliar treatment with liquid Humustim (23.4% humic acids) during the phase of luxuriant growth of vegetative organs of the onion plants contribute to overcome the negative factors of an environment. The treatments with Humustim gave the higher onion yield of 4% (by varieties Citation and Tamara) to 15.4% (Pueblo and Aspen).

Key words*: long-day onion (Allium cepa L.), humic acids, drip irrigation.*

INTRODUCTION

In the last decades it has been observed global use of increasing in the crop production, including a great number of vegetable crops. The tendency is more clearly expressed by the vegetables, because of the shorter growth period - annual, biennial or triennial. This feature of the intensive vegetable - growing development imposes annually application of the mineral fertilizers in order to achieve maximum biological potential and higher yield. Many times biological value and ecological purity of the crops by reaching the aims are in the background. The application of the chemical fertilizers has increased 10 times compared to 50-ies of the last century, and 17 times in the last ten years. In these cases the increase of the yields was only 3 times. In the most of the cases the excessive application of the mineral fertilizers like base dressing, foliar spray or fertigation leads to disturbance of the plant immunity. This is the cause for decreasing of the crop production and lowering of the vegetables quality. The high requirements very often have posed the questions about quality of the vegetable production, related with valuable nutrient constituents, possibilities for post-harvest handling and storage, good commercial appearance as well as good chemical - technological features of the crop production, intending for processing. The great consumption of the mineral fertilizers has initiated the problems with an application of different bio regulators in the crop production, especially in vegetable production under irrigation in the studies of (Daly and Stewart, 1999; Feibert Erik et al., 2002; Gaur and Adholeya, 2000).

The investigations of the plant bioprocesses activation have particularly attained a great actuality with participation of the soluble humic substances. The humic and fulvous acids from natural origin are the most active biochemical substances to which the plants respond best. Mechanism of the humic acids as bio regulators in the plants have not been enough studied although there are available literature about investigation carried out. This is still incomplete knowledge about the topics on the stimulating effect of humic substances on growth and production.

The investigation of new biological active substances requires detailed research on all the aspects of their action including on their effect on biological habits of plants and soil microflora (Gaur and Adholeya, 2000; Schloter et al., 2003; Hashimoto et al., 1999). Chen and Aviad (1990), Feibert Erik et al. (2002) and David et al. (1994) have reported promoted growth and nutrient uptake of plant due to the addition of humic substances. The plants take more mineral elements due to the better-

developed root systems. In addition, the stimulation of ion uptake in applications with humic materials led many investigators to propose that these materials affect membrane permeability (Zientara, 1983). It is related to the surface activity of humic substances resulting from the presence of both hydrophilic and hydrophobic sites (Chen and Schnitzer, 1978). Therefore, the humic substances may interact with the phospholipids structures of cell membranes and react as carriers of nutrients through them. On the other hand, the application of the very high dose of humic acid is less effective (Lee and Bartlett, 1976). According to several researches, results were changing due to the levels of treatment, growing media and origin of humic substances (Chen and Aviad, 1990; Hashimoto et al., 1999). Feibert Erik et al., 2002, had evaluated products which are nonconventional fertilizers, containing humic acids on the onion yield and quality. Agri-Plus supplied the highest total amount of humic acids (31.4 kg/ha), but this wasn't enough to get higher yield. The granular fertilizer, containing humic acid would be incapable of significantly increasing soil organic matter and consequently improve crop production. The obtained results, Kandil et al. (2013), showed that foliar spraying with humic acid resulted in highest growth characters, total and marketable yields, total culls and bulb weight as well as TSS %, dry matter and total weight loss percentage at storage period compared with the control treatment.

The aim of the present study was to do investigation on the effectiveness of humic acids (trade name Humustim) application on the annual long-day onion, drip irrigated, and estimate the influence on the biological behavior of the plants.

MATERIALS AND METHODS

During 2014-2015 the trials were conducted at the Experiment Station of the Department of Horticulture, Agricultural University - Plovdiv, Bulgaria to test the varieties of long-day onion (*Allium cepa* L.), sown in spring. The soil was light alluvial - meadow solonetz, silt loam with field capacity FC of 30%, and bulk density of 1.2 g/cm^3. A soil sample taken from the top foot showed a pH of 7.88, 1.56% organic matter, 43.38 mg N per 100 g, and 96.33 mg K per 100 g. The experimental plots were replicated four times and onion plants were irrigated by drip installation, maintaining optimal moisture of 70% to 100% of field capacity. The beds were 1m wide with experimental block area of 5 m^2 with two drip laterals. The soil samples were taken at the rooting depth. The object of investigation were the following cultivars of long-day onions and treatments: 1. Aspen (Enza Zaden); 2. Aspen + Humustim; 3. Tamara (Enza Zaden); 4.Tamara + Humustim; 5. Citation (Enza Zaden); 6. Citation + Humustim; 7. Twist (Enza Zaden); 8.Twist + Humustim; 9. Pueblo (Enza Zaden); 10. Pueblo + Humustim; 11. BGS 217 (Enza Zaden); 12. BGS 217 + Humustim; 13. BGS 196 (Enza Zaden); 14. BGS 196 + Humustim.

The sowing was done during February, 15th and March, 20th with precise sowing machine in the scheme of 70+30+30+30/3 cm at sowing rate of 1 g/m^2. Drip irrigation system with emitters spaced 10 cm for vegetative irrigation applications was used. The single treatment with liquid humic acids Humustim (23.4% humic acids) during the luxuriant growth of vegetative organs of the onion was done with a solution of 0.25% in a dose of 4 l/ha, according the producer. The controls were untreated variants with Humustim. For the investigation of vegetative behavior and onion production were estimated following indices:

1. Dynamics of the plant growth - height (cm), fresh matter (g); 2. Beginning of the bulb formation; 3. Crop production - total yield (kg/ha); 4. Dry matter and sugar content (%). The results were calculated using Fisher's method for the probability of differences.

RESULTS AND DISCUSSIONS

The most important meteorological components for the tested plants in Plovdiv are graphically present in Figures 1 and 2 for experimental period 2014-2015. The favorable climate condition in the spring of 2014 permitted to be the sowing in the second part of February, 20th. The optimal positive air temperatures (from +12^{0}C to +24^{0}C) continued to the end of May, and together with uniform rainfall distribution in April and May allowed the fast development of the investigated cultivars (Figure1). The soil moisture of the top

layer flucktuated continously in accordance to rainfall and irrigation application during 2014. Significant differences among the onion cultivars, sowed at the same date by treatment with Humustim were observed.

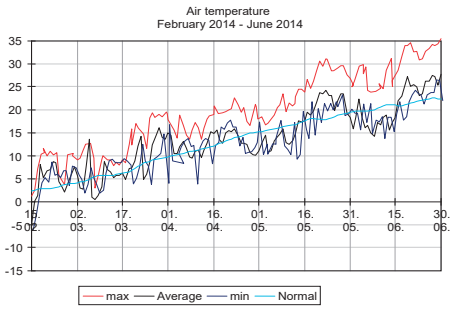

Figure 1. Air temperature in the period of February-June, 2014

The winter of 2015 (Figure2) prolonged to the end of March, when the sowing was on 19th. The rate of development of the onion plants retarded in spite of the a sudden change in the air temperature above +30°C in May and heavy rainfall in the end of April.

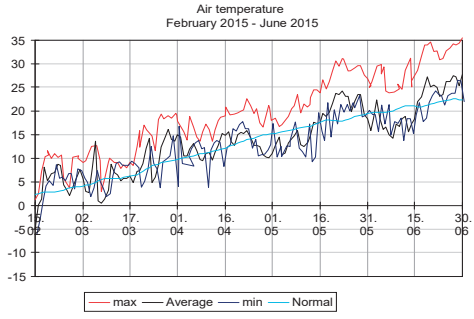

Figure 2. Air temperature in the period of February-June, 2015

In Table 1 it can be seen the dynamics in fresh matter increase for different varieties onions per plant in g during 2014.

The faster growing rate was of the cv. Citation. The onion plants of cv. Citation accumulated the greatest fresh matter of 239 g, while the treatment with Humustim increased the accumulated fresh matter to 250 g. Comparatively high fresh matter was accumulated by cv. Tamara and cv. BGS-196 (Table 1).

Table1. Dynamics in the fresh matter increase per onion plant in g at sowing date February, 2014

Variety	15.04	30.04	15.05	15.06	30.06	15.07
Aspen	10	22	35	90	122	131
Aspen +H	10	22	37	102	140	155
Tamara	11	27	70	184	226	228
Tamara+H	11	27	87	212	232	238
Citation	11	30	85	206	233	239
Citation+H	11	30	108	229	246	250
Twist	11	24	38	144	174	181
Twist+H	11	24	52	161	194	202
Pueblo	11	24	37	106	138	148
Pueblo+H	11	24	44	129	162	175
BGS217	12	25	68	170	203	204
BGS217+H	12	25	68	181	213	216
BGS196	10	27	59	176	216	216
BGS196+H	10	27	76	193	224	228

The treatment with Humustim increased the fresh matter of these cultivars with 4.2% and 5.3%, respectively. Cv. Twist showed slow vegetative growth, compared with other investigated cultivars in 2014. Aspen and Pueblo had the smallest yield of fresh matter. By harvesting in the end of July, 2014 these cultivars had the lowest yield of bulbs in comparison with others. The treatment with Humustim gave the highest yield of bulbs for Citation and Tamara (Table 2).

Table 2. Yield kg/ha from different varieties of long-day onion at sowing date February, 2014

Variety	Mean bulb weight g	Bulb height cm	Bulb diam. cm	Number of dry layers	Dry matter %	Sugars %	Total yield kg/ha
Aspen	107	4.6	6.6	3-4	12.5	15.8	18 270
Aspen +Humustim	114	4.7	6.6	3-4	12.5	15.8	21 000
Tamara	198	6.9	7.8	3-4	6.6	5.82	47 910
Tamara +Humustim	204	6.9	7.8	3-4	6.6	5.82	50 430
Citation	232	7.7	8.8	3	6.3	5.41	56 180
Citation +Humustim	238	7.7	8.8	3	6.3	5.41	59 140
Twist	143	6.1	7.2	3-4	8.4	7.41	26 710
Twist +Humustim	145	6.1	7.2	3-4	8.4	7.41	29 680
Pueblo	135	5.7	7.1	3-4	11.8	15.4	19 780
Pueblo +Humustim	138	6.0	7.1	3-4	11.8	15.4	23 000
BGS217	160	6.5	7.3	3-4	7.2	6.22	34 410
BGS217 +Humustim	165	6.5	7.3	3-4	7.2	6.22	37 000
BGS196	189	8.0	7.5	3-4	7.4	6.46	40 250
BGS196 +Humustim	194	8.0	7.5	3-4	7.4	6.46	42 820

In Table 2 were shown the yield of cultivars in 2014, as following: 59 140 kg/ha for Citation + Humustim, 50 430 kg/ha for Tamara + Humustim, and 42 820 kg/ha for BGS 196. The lower content of dry matter and sugar – 6.3% and 6.6% of the onion bulbs had Citation and Tamara, respectively. Cultivars Pueblo and Aspen are with white colored onion bulbs, suitable for processing, but they could not accumulate high content of dry matter and sugar during the first year, characterized with heavy rainfall. The yields of Pueblo and Aspen were 23 000 kg/ha and 21 000 kg/ha, respectively. The treatment with Humustim for these variants increased the yield of 14.9% and 15.4%, than with untreated.

Table 3. Dynamics in the fresh matter increase per onion plant in g at sowing date March, 2015

Variety	15.04	30.04	15.05	15.06	30.06	15.07
Aspen	6	14	35	90	122	127
Aspen+H	6	14	37	107	140	145
Tamara	9	15	68	176	208	210
Tamara+H	9	15	68	183	211	217
Citation	9	15	76	190	218	221
Citation+H	9	15	86	197	223	227
Twist	9	15	38	124	153	162
Twist+H	9	15	47	145	175	182
Pueblo	7	15	37	97	129	139
Pueblo+H	7	15	41	125	155	163
BGS217	9	15	54	148	181	189
BGS217+H	9	15	54	158	189	196
BGS196	9	15	59	162	194	200
BGS196+H	9	15	59	171	200	206

During the next 2015 the beginning of the intensive grow of the over ground parts started in the middle of May, when was the treatment with Humustim. The extreme high air temperature > + 33°C in the end of May and lower relative air humidity in June (Figure 2) impeded the growth of the investigated onion cultivars during the second year.

The bigger fresh matter had cv. Citation, treated with Humustim, shown in Table 3 (227 g per plant), than the untreated of 221 g. Treated cv. Tamara and BGS 196 had influence on yield too.

In Table 4 are shown the yield of all investigated cultivars of long-day onion during 2015. The best cultivar was Citation of 49 470 kg/ha. The treatment with Humustim increased the yield of 3% to 51 000 kg/ha. The next good cultivars of high yield were Tamara - 42 270 kg/ha and BGS 196 - 32 000 kg/ha. Aspen turned out more sensitive to the factors of the environment.

Table 4. Yield kg/ha from different varieties of long-day onion at sowing date March, 2015

Variety	Mean Bulb weight g	Bulb height cm	Bulb diameter cm	Number of dry layers	Dry matter %	Sugars %	Yield kg/ha
Aspen	96	6.3	6.7	3-4	18.5	23.6	14 790
Aspen +Humustim	97	6.3	6.7	3-4	18.5	23.6	17 000
Tamara	158	6.9	7.3	3-4	7.4	6.46	40 580
Tamara +Humustim	160	6.9	7.3	3-4	7.4	6.46	42 270
Citation	192	7.7	7.5	3	7.2	6.22	49 470
Citation +Humustim	198	7.7	7.5	3	7.2	6.22	51 000
Twist	122	6.1	6.6	3-4	8.4	7.41	20 160
Twist +Humustim	125	6.1	6.6	3-4	8.4	7.41	22 400
Pueblo	107	5.2	5.0	3-4	17.8	16.4	16 340
Pueblo +Humustim	110	5.2	5.0	3-4	17.8	16.4	19 000
BGS217	140	6.5	7.1	3-4	8.4	7.41	29 100
BGS217 +Humustim	144	6.5	7.1	3-4	8.4	7.41	30 000
BGS196	150	8.0	7.2	3-4	8.4	7.41	31 040
BGS196 +Humustim	152	8.0	7.2	3-4	8.4	7.41	32 000

CONCLUSIONS

The treatment with humic acids - Humustim on varieties of long-day onion (*Allium cepa* L.) in the phase of development of the vegetative parts contributed to overcome more quickly the negative factors of the environment (extremely high air temperatures and low relative humidity of air, typically for this region). The onion yield of treated variants with Humustim was bigger of 4% (cv. Citation and cv. Tamara) to 15.4% (cv. Pueblo and cv. Aspen). Citation and Tamara, treated with Humustim had yield of 59 140 kg/ha and 50 430 kg/ha during 2014, which was the highest of all investigated long-day onions. These cultivars are with lowest contents of dry matter. That is confirmation of the studies of Kandil et al. (2013), who estimated relationship between increasing the yield with 1tonne and decreasing of onion dry matter to 1% using humic acid.

The treatment with humic acid by foliar application on varieties of long-day onion (*Allium cepa* L.) didn't influence on the content of sugar, but increase the yield under condition of South Bulgaria.

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THE QUALITATIVE AND QUANTITATIVE CHARACTERISTICS OF SOME ROMANIAN TOMATO VARIETIES IN GREENHOUSE CONDITIONS

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Abstract

Tomato (Solanum lycopersicum L.), known to belong to the Solanaceae family, is considered one of the most important vegetable in the world since the fruits are widely consumed either fresh or processed. The ripe fruits are a valuable source of vitamin C, carotenoids and minerals such as iron and phosphorous that is daily required for a healthy diet. Fruit growth and ripening are the result of multiple physiological and metabolic processes that occur during the plant development. Knowledge of the physiological characteristics of tomato plants is necessary to improve the cultivation technology under greenhouse conditions. This work highlights the evolution of the quality study of tomato's fruits varieties as regard the fruit indicators (plant height, number of inflorescences, diameter, fruit length, biometric indicators (weight, diameter, weigh fruit) and biochemical (dry mater content % Brix, acidity).The following varieties obtained at NRDIBH Stefanesti were studied: Argeş 11, Argeş 20, Argeş 16, Argeş 123, compared to the control variety Notorius.

Key words*: tomato, fruit quality, weight, dry substance, acidity.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), known to belong to the *Solanaceae* family, is considered one of the most important vegetable in the world since the fruits are widely consumed either fresh or processed.

In the world, tomato is one of the most consumed vegetables and one of the most produced agricultural products. According to FAO, in 2018, Romania produce 742.899 tons of tomatoes.

The interest in consuming high quality fresh or processed tomatoes continues to increase. In Romania, the annual average of tomatoes consumption per capita was recorded in 2015 as 38.6 kg/inhabitant (Soare et al., 2017), which is a relevant indicator for the vegetable market.

Tomato is considered an important antioxidant source in human nutrition. Compounds with essential antioxidant properties in tomato fruit include phenolics, carotenoids and pigments (Coyago-Cruz et al., 2019). Beside the high nutritional value, the ripe tomato fruits are a valuable source of vitamin C, carotenoids and minerals such as iron and phosphorous that are daily required for a healthy diet (Mubarok et al., 2019; Nour et al., 2013).

Fruit growth and ripening are the result of multiple physiological and metabolic processes that occur during the plant development (Bertin & Génard, 2018; Li et al., 2019). Leaves are considered to be the main providers of carbon for fruit growth (Hetherington et al., 1998).

MATERIALS AND METHODS

Four tomato hybrids patented by the National Research and Development Institute of Biotechnology in Horticulture Ştefăneşti (INCDBH) were investigated: 'Arges 11', 'Arges 20', 'Arges 16', 'Arges 123', compared to the control 'Notorius' cultivar. 'Arges 11' hybrid is a tomato with determined growth, big fruit (average weight 180 g), and ideal for consumption in fresh and preserved condition (Badulescu & Uleanu, 2017). 'Arges 20' hybrid is characterized by determined growth, very big fruits (average weight 220 g), suitable for consumption in fresh or preserved state. 'Arges 16' are tomato hybrids with undetermined growth, which produce big elongated fruits

(180-200 g) and 'Arges 123' which produce big fruits (average weight 270 g) (Bădulescu & Uleanu, 2017). 'Notorius' variety was chosen as a Control cultivar. The selected tomato hybrids were cultivated in protected systems (greenhouse) that provided controlled conditions for plant growth.

The following bioindicators regarding the growth and fruiting processes were determined: the number of inflorescence per plant, the number of fruits in inflorescence, the average length of a fruit, the average diameter of the fruit, the production (Badulescu & Tita, 2014) and the biochemical indicators: acidity and soluble solids content.

The total soluble solids (TSS) was determined with KRUSS GMbH mobile optronic refractometer model DR 101-60, in Brix % of fruit juice. The total acidity was determined by the titrimetric determination method (Tudor-Radu et al., 2016).

For the statistical interpretation of the results, the data were included in an Excel database and then statistically interpreted with the SPSS 14.0 program, which uses the Duncan test (multiple t test) for a 5% statistical assurance.

RESULTS AND DISCUSSIONS

The statistical analysis of the fruit took into account the following biometrics: inflorescence number, number of fruit blossom, fruit average height, the diameter of a fruit, production, total acidity and dry matter.

Average height of plants. In general a normal distribution is symmetric when the asymmetry value of the coefficient is equal to zero.

The sample average was 76.4933 the values being between the minimum value of 59.00 and the maximum value of 86.00.

The histogram of all the plant height values is asymmetrical to the left, (the values are higher than the average), being different from the normal distribution, a sign that there are significant influences between the varieties studied about of plant height (Figure 1).

Analyzing indicators of dispersion or genetic and experimental diversity, in terms of inflorescence number, the mean sample was 3.8267, the values being between 2 and 6 (Figure 2). The histogram of the number of fruits in the inflorescence is bimodal, a sign that the sample is no longer homogeneous due to the influence of the different varieties studied, regarding the number of fruits in the inflorescence (Figure 3).

In the case of the number of fruits from the average inflorescence it was 8.4467, with a standard deviation of 2.03, the values being between the minimum value 4 and the maximum value 12.0 (Figure 3).

The diameter of the fruit, expressed in mm, of an average of 8.4467 mm with a standard deviation of 2.03505 (Figure 4). If fruit weight, average was 197.9427 kilograms with a standard deviation of 37.18824 (Figure 6).

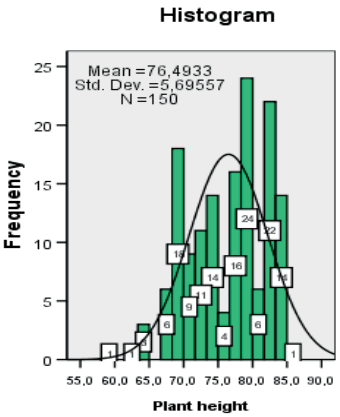

Figure 1. Histogram of the distribution by absolute frequency classes of plant height, in the studied varieties (mm)

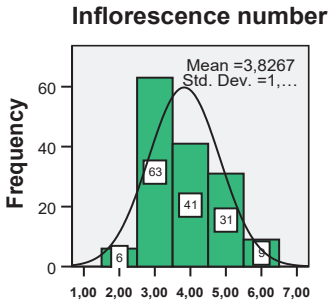

Figure 2. Histogram of the distribution by absolute frequency classes of the number of inflorescences per plant, in the studied varieties

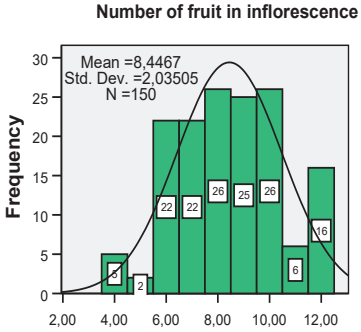

Figure 3. Histogram of the distribution by classes of absolute frequency of the number of fruits in the inflorescence, at the studied varieties

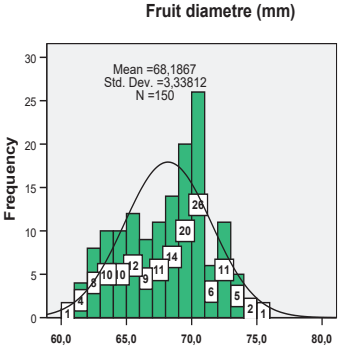

Figure 4. Histogram of the distribution by absolute frequency classes of the diameter of the fruits, for the studied varieties

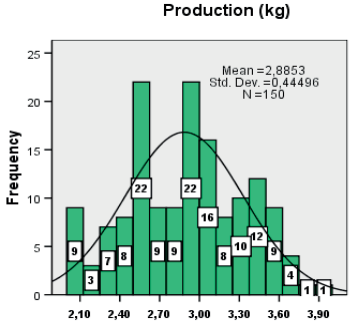

Figure 5. Histogram of the distribution by classes of absolute frequency of fruit production, for the studied varieties

The histogram of the average mass of the fruits deviates significantly from the normal distribution, being asymmetrical to the right (predominating values lower than the average) sign that there are significant influences between the studied varieties (Figure 6).

The values measurements at the production, at the 5 varieties of tomatoes studied on a sample of 150 samples are between the minimum value of 2.0 and the maximum value of 3.90, with a maximum oscillation of 1.90 (Figure 5).

For acidity, the sample average was 3.3073, with a standard deviation with 0.30, values ranging from a minimum of 2.50 to a maximum of 4.20 (Figure 7). It is observed that the histogram deviates from the normal distribution, having asymmetry to the right (the values lower than the average predominate), sign that there are significant differences between the values of the acidity of the fruits recorded by the 5 varieties.

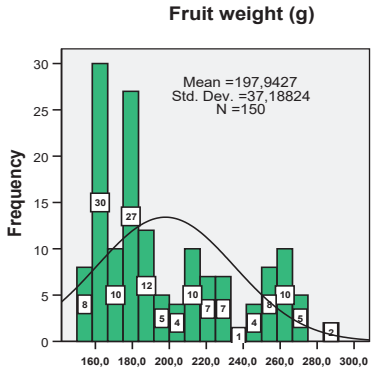

Figure 6. Distribution histogram by frequency classes absolute of the average fruit mass, in the studied varieties

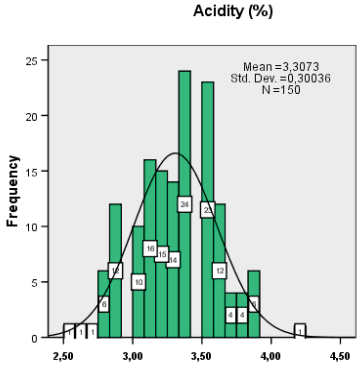

Figure 7. Histogram with the distribution of fruit acidity values, in the studied varieties

As total soluble solids, the sample average was 3.8487 with values between 2.80 and 5.00 with a standard deviation of 2.20 (Figure 8).

The histogram of all the values analyzed regarding the dry substance in the 5 varieties

studied is asymmetric to the left, the coefficient of asymmetry being -0.633, which means that the values above the average predominate (Figure 8), and the sample is no longer homogeneous due to the influence of the variety regarding the dry matter (% Brix), (Figure 8).

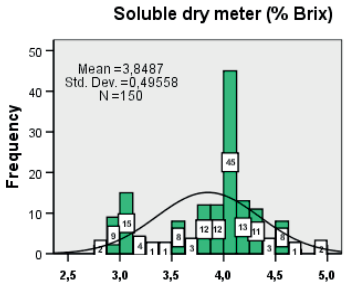

Figure 8. Histogram of the distribution by frequency classes of the total soluble solids in fruit (% Brix), in the studied varieties

Table 2 and graph below (Figure 9) shows the correlations between indicators studied, we highlight the following:

- Between the average fruit weight and production there is a positive correlation (r = 0.250**) significant. The weight of the fruit implies a high production.
- Is a significant negative correlation between the fruit weight and number of inflorescence (r=-0.711**), which explains the fact that, as the number of fruit per plant is higher, the lower the average weight of a fruit;
- Number of fruits in inflorescence correlate significant negative with fruit weight, production and total soluble solids (r=-0.742**; r=-0.247**; r=-0.127**; r=-0.220**), negative acidity significant (r = -0.129 **), and then we have a decrease in the percentage of the tomato juice.
- It is known that, as total soluble solids (% Brix) has higher values as the total acidity (%) will have lower values (Gurteg Singh, 2017). Soluble dry matter correlate positive and distinct significant negative with acidity, indicating that there is a relation between these parameters balanced.

Table 1. Indicators sample central tendency (mean, median and mode) and indicators value dispersion around the average (maximum amplitude, limits, standard deviation and asymmetric coefficient)

Statistics		Plant height	Inflorescence number	Number of fruits in inflorescente	Fruit diameter (mm)	Fruit lenght (mm)	Fruit weight (g)	Production (kg)	Total acidity (%)	TSS (% Brix)
N	Valid	150	150	150	150	150	150	150	150	150
Mean		76.4933	3.8267	8.4467	68.1867	70.4060	197.9427	2.8853	3.3073	3.8487
Median		78.0000	4.0000	8.0000	68.9000	74.9000	183.0000	2.9000	3.3000	4.0000
Mode		79.00	3.00	8.00(a)	67.90	75.90	179.00(a)	2.90	3.40	4.10
Std. Deviation		5.69557	1.00165	2.03505	3.33812	10.50128	37.18824	.44496	.30036	.49558
Skewness		-.414	.477	.005	-.272	3.783	.764	.000	.004	-.633
Std. Error of Skewness		.198	.198	.198	.198	.198	.198	.198	.198	.198
Kurtosis		-.577	-.592	-.584	-.813	32.107	-.698	-.813	-.067	-.399
Std. Error of Kurtosis		.394	.394	.394	.394	.394	.394	.394	.394	.394
Range		27.00	4.00	8.00	14.50	101.70	136.00	1.90	1.70	2.20
Minimum		59.00	2.00	4.00	60.90	56.90	154.00	2.00	2.50	2.80
Maximum		86.00	6.00	12.00	75.40	158.60	290.00	3.90	4.20	5.00

(a) Multiple modes exist. The smallest value is shown.

Table 2. Matrix of correlation (Pearson "r" correlation coefficients "r") of the main physical and biochemical indicators (average for the five tomato cultivars studied).

		Plant height	Inflores-cence number	Number of fruits in inflo-rescence	Fruit diameter (mm)	Fruit weight (g)	Producti-on (kg)	Total acidity (%)	TSS (% Brix)
Plant height	Pearson Correlation	1	.367(**)	.289(**)	.303(**)	-.382(**)	.081	.021	-.244(**)
Inflorescence number	Pearson Correlation	.367(**)	1	.433(**)	.579(**)	-.614(**)	-.220(**)	-.212(**)	-.433(**)
Number of fruits in inflorescence	Pearson Correlation	.289(**)	.433(**)	1	.603(**)	-.742(**)	-.247(**)	-.129	-.220(**)
Fruit diameter (mm)	Pearson Correlation	.303(**)	.579(**)	.603(**)	1	-.711(**)	-.208(*)	.005	-.216(**)
Fruit weight (g)	Pearson Correlation	-.382(**)	-.614(**)	-.742(**)	-.711(**)	1	.431(**)	.250(**)	.384(**)
Production (kg)	Pearson Correlation	.081	-.220(**)	-.247(**)	-.208(*)	.431(**)	1	.452(**)	.441(**)
Total acidity (%)	Pearson Correlation	.021	-.212(**)	-.129	.005	.250(**)	.452(**)	1	.530(**)
TSS (% Brix)	Pearson Correlation	-.244(**)	-.433(**)	-.220(**)	-.216(**)	.384(**)	.441(**)	.530(**)	1

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

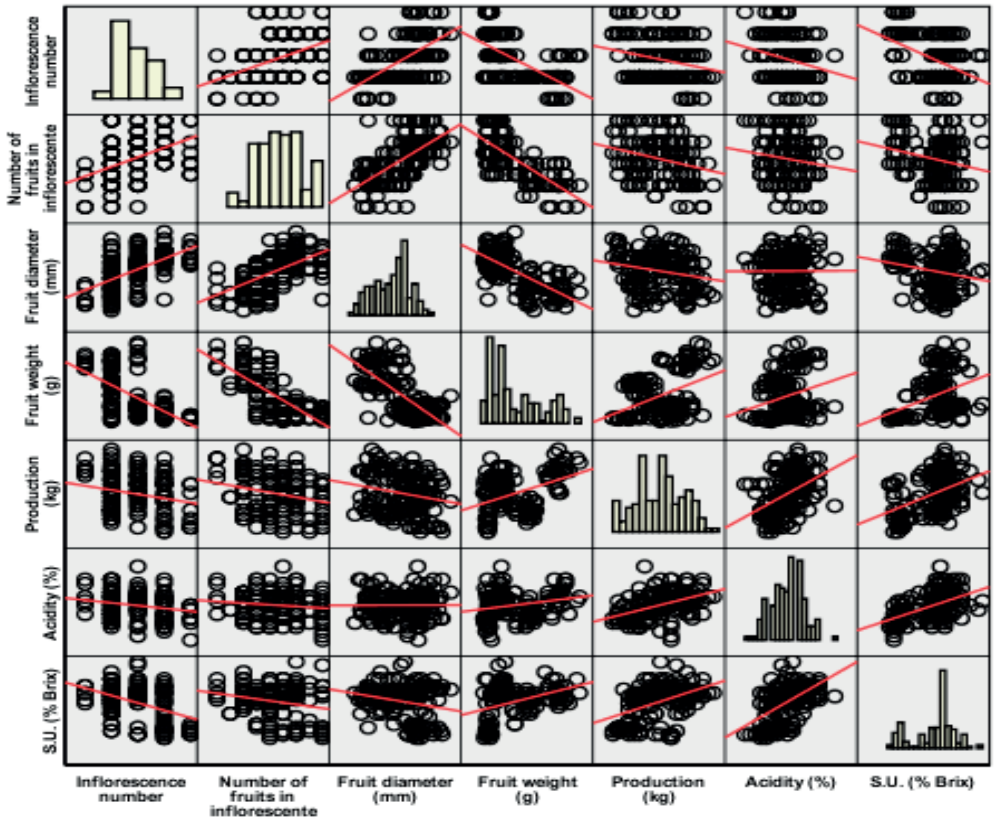

Figure 9. Matrix of correlations between biometric and biochemical indicators, for studied tomato cultivars

Table 3. Morphometry of tomato varieties

Varieties	Plant height (cm)	Inflorescence number	Number of fruit in inflorescence	Fruit diameter (mm)
Arges 11	75.1b	4.03b	10.00a	70.84a
Arges 20	70.23c	3.03c	7.00c	65.31c
Arges 16	80.10a	4.03b	9.07b	69.63b
Arges 123	75.83b	3.03c	6.13d	64.65c
Control (Mt)	81.23a	5.00a	10.00a	70.51ab

Duncan. Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 30,000

The plant height shown that all studied varieties are shorter than Control ('Notorius' variety), which could be an advantage in the field. The number of inflorescence varied between 3.03% on 'Argeș 20' and 'Arges 123' and 5.00% on 'Notorius' variety (Table 3, Figure 10).

Table 4. Physical and biochemical properties of studied tomato varieties

Varieties	Fruit weight (g)	Produc-tion (kg)	Total acidity (%)	TSS (% Brix)
Arges 11	179.03c	2.9c	3.5a	4.3a
Arges 20	215.20b	2.5d	3.10b	3.81c
Arges 16	175.10c	3.13b	3.42a	4.0b
Arges 123	260.30a	3.40a	3.5a	4.11b
Mt	160.08d	2.49d	3.02b	3.1d

Duncan
Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 30,000.

The fruit weight varied between 175.10 g on 'Argeș 16' and 260.30 g ('Arges 123'), being cultivar characteristic that influence significantly tomato production (Table 4).

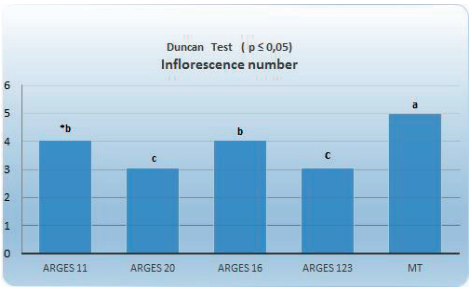

Figure 10. Inflorescence number of the fruit on the tomato varieties

The fruit diameter was higher in varieties 'Arges 11' and 'Notorius' (control), with significant differences compared to all the other varieties to 5% statistical assurance. The lowest values of this indicator were registered variety 'Arges 123' (Figure 11).

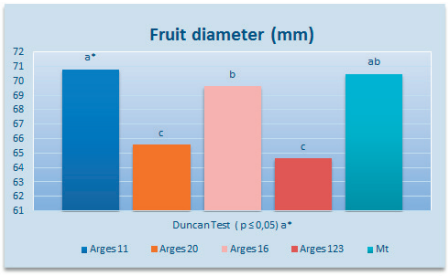

Figure 11. Fruit diameter of the fruit on the tomato varieties

The average weight is a characteristic that expresses the size of the fruit, the index of commercial importance, especially for fresh fruits market. Analyzing the average weight of the fruit from 5 varieties of tomato is found that all varieties belongs to tomato large fruits group, like St. Pierre or Mirsini F1 (Mandru et al., 2019). The variety 'Arges 123' has the heaviest fruit (260.30 g), its average weight being significantly different from all other varieties, for statistical assurance 5% (Figure 12). The smallest fruit varieties were recorded at 'Arges 16' and 'Notorius' (Control), that influence strongly the production.

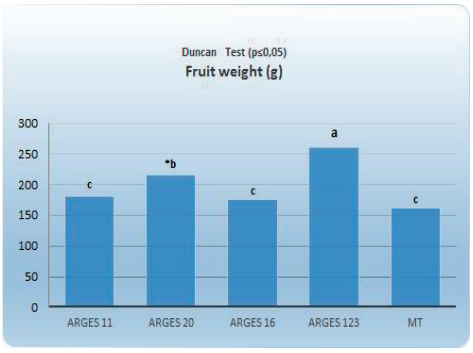

Figure 12. Fruit weight of the fruits on the tomato varieties

Analysing the production were significant differences between genotypes studied (Figure 13). The variety 'Arges 123' recorded the highest yield (3.4 kg/plant), with significant differences compared to the other varieties. The small amount of tomato fruit varieties were

recorded to 'Notorius' and 'Arges 20' (control), the differences between the two varieties were statistically insignificant for a statistical assurance 5%, (Figure 13).

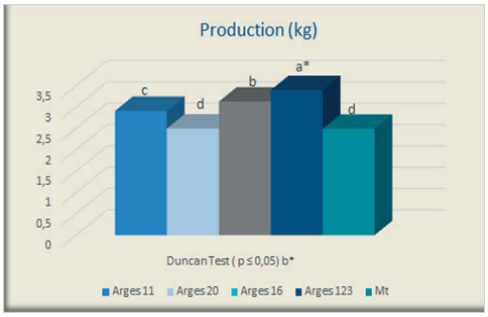

Figure 13. Production of the fruits on the tomato varieties

The content of organic acids (total acidity %) in fruits and vegetables depends on several factors, including differences in genotypic, climatic conditions pre-harvest and post-harvest handling procedures (Lee & Kader, 2000). It is known that during periods of heavy rainfall or cooler areas, total acidity values become larger (Gherghi, 1972).

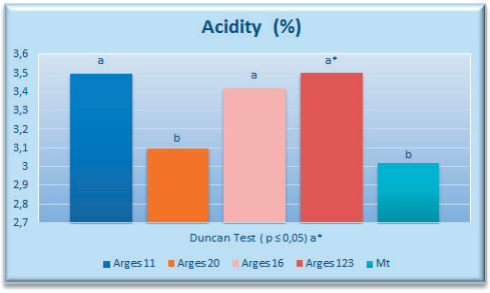

Figure 14. Acidity of the fruits on the tomato varieties

The fruits acidity between 3.02% on 'Notorius' and 3.5 % on 'Arges 11' variety The highest values of this indicator showed varieties 'Arges 11', 'Arges Arges 123' and 'Arges 16', and the smaller varieties 'Notorius', 'Arges 20' (control), the differences between the two classes is significant (Figure 14).

The dry matter content depends on the cultivar, the growing technology, and the environmental factors during the growing season (Helyes, 2007). János Ágoston, following the studies done on tomatoes intended for both fresh consumption and industrialization, states that the varieties intended for fresh consumption should fall between 3.5-4.5% Brix values, while destined for industrialization must exceed the value of 5% Brix (J. Agoston, 2017).

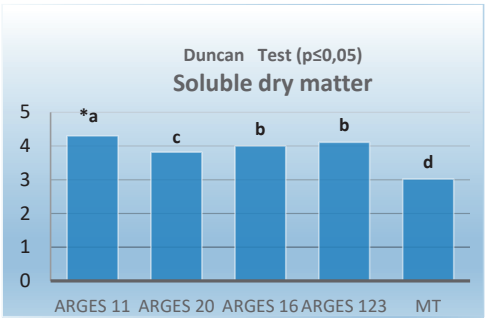

Figure 15. Soluble dry matter of the fruits on the tomato varieties

From the point of view of the content of tomato fruit dry matter (% Brix) was studied varieties within the normal limits of variation of this index ranging from 4.11 to 'Arges 123' and 3.1 from variety 'Notorius' (control).

The results on the soluble dry matter (% Brix) showed significant statistically differences between genotypes, and the mean there of was classified into four classes of statistical significance homogeneous. The highest value of dry matter (Figure 15) was recorded in the variety 'Arges 11' (4.3%), which differs significantly from the varieties Arges 20, 'Arges 16', 'Arges 123' and 'Notorius' - Mt (3.81%, 4.0%, 4.11%, 3.1%).

CONCLUSIONS

The following conclusions were drawn from the study:

All the studied varieties had large fruits with a mean weight over 175.10 g and a diameter of more than 64 mm;

Average fruit weight was significantly correlated and distinct positive with production (r = 0.250 **). Fruit weight entails a high production

Soluble dry matter was correlates significantly with acidity (r = 0.530**), indicating that there is a relation between these parameters balanced.

'Arges 123' variety presented the best results for the production of fruit soluble dry matter and acidity.

The low solids content (% Brix) and acid recorded in the control variety ('Notorius').

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AGRONOMIC ASSESSMENT OF *PHYSALIS* SP. ACCESSIONS FROM VEGETABLE RESEARCH DEVELOPMENT STATION BUZĂU, ROMANIA

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Abstract

Physalis sp. is widely cultivated in South America, but little known and cultivated plant in Romania, although varieties belonging to the species are found in wild flora. The aim of this paper was to assess agronomic traits with emphasis on the phenotypic expressiveness of the accessions in terms of yield, quality and earliness production. The research consisted in agronomic evaluation of seven genotypes of Physalis (P. peruviana, P. ixocarpa, P. philadelphica and P. alkekengi) and the selection of valuable genotypes suitable for cultivation under the pedoclimatic conditions of the southern part of Romania. The study targeted 30 qualitative descriptors and 16 quantitative descriptors subjected to ANOVA analysis. A percentage of 67% of the studied genotypes contains fruits with a diameter exceeding 2 cm, belonging to the genus P. ixocarpa and P. philadelphica, 16.5% fall in the typical form of the Colombian ecotype of 1.85 cm, and 16.5% are small fruits, with a diameter below 1.5 cm. Accession A7 obtained a yield of 3.9 kg/plant and accessions A4 and A3 have small fruits, but with a higher content in soluble substances.

Key words*: Tomatillo, cape gooseberry, diversity, germplasm variability, husk tomato.*

INTRODUCTION

Physalis is the fifth largest genus of Solanaceae family and embrace over 90 native species in America, and the greatest richness is concentrated in Mexico, which hosts 65 species (Martinez et al., 2017; Saavedra et al., 2019; Lagunovschi et al., 2016). Some of the Physalis species such as *P. divaricata*, *P. alkekengi* L. occur jointly in America and Asia (Mozaffarian, 2013; Sayadi and Mehrabian, 2016). *Physalis alkegengi* was distributed in wild flora of southern Europe (Filipovic et al., 2019). *Physalis alkekengi* is a perennial plant with preferences for limestone areas and can be found in the forests of Romania, from the plains to the hillsides.

The plant is completely toxic, except for fruits. The fruits contain citric acid, mineral substances and have a pleasant bitter-sour taste (Pârvu C., 2006). In traditional medicine references, Physalis is known as effective in treatment of several disorders such as: jaundice, asthma and shortness of breath, tissue necrosis, liver, kidney and bladder dysfunctions, wounds, haemorrhoids and helminths diseases (Mirzaee, 2019). *Physalis peruviana* L., *P. ixocarpa* L. and *P. philadelphica* L. are very little cultivated plants in Romania, but once with the promotion of fruit and vegetable consumption for a healthier diet, Physalis sp. it began to be required in the market.

At the moment, in the Official Catalogue of Romanian Crop Plants does not have a Physalis variety, which is the reason why the Vegetable Research and Development Station Buzau undertook a breeding program for this species. The aim of this study was to evaluate the germplasm collection, from which seven accessions are the subject of this work and to select valuable genotypes suitable for cultivation in the pedoclimatic conditions of southern part of Romania.

MATERIALS AND METHODS

The Breeding and Biodiversity Laboratory from Vegetable Research Development Station Buzau, Romania, holds a valuable germplasm collection, structured on several stages of breeding, of which seven stable genotypes were retained. The studied genotypes were: two genotypes belonging to *P. philadelphica* (tomatillo), two genotypes of *P. peruviana (cape gooseberry)*, two genotypes of *P.*

ixocarpa (husk tomato) and one genotype of *Physalis alkekengi*.

There are some studies referring that *P. philadelphica* and *Physalis ixocarpa* are synonyms, but despite the morphological similarities, these are different species. Fernandes (1974) and Hanelt and IPK (2001) - states that the *P. ixocarpa* has relatively smaller flower and fruit and also a capitate stigma.

Geographic location and conditions of the experiment

The evaluation process was carried out in the field. The temperature has registered a maximum of 37.3°C (middle of August) and a minimum of 6.4°C (late September), and relative humidity had recorded a maximum of 95.0% and a minimum of 23.0%.

The soil was clay-sandy, with a pH of 6.8 and with a content of organic matter of 6%, a phosphorus value of 106 mg kg^{-1} and exchangeable potassium, 320 mg kg^{-1}.

Agronomic management

The seedlings were obtained by sowing in alveolar pallets with 70 cubes with a volume of 50 mL/cubes. The planting was made after 56 days after germination, using the planting scheme: 70 cm between rows and 40-50 between plants cm. The seedlings were planted in a completely randomized distribution. The special care works were the usual one: manual and mechanical hoeing, dripping water and fertilization.

Throughout the plant season, no chemical treatments were applied for management of diseases and pest. So far, no pathogens have been identified to endanger the crop.

During the vegetation period, phenological and biometric observations were made for 30 qualitative descriptors and 16 quantitative descriptors according to UPOV Guidelines.

The agronomic variables were established according to studies of Arbelaez and Mora (1990), Lagos (2006), Bonilla et.al (2008) and Herrerra et al. (2012); Drăghici (2014); Panayotov et al. (2015); Panayotov (2018); Ozturk et al. (2017) were focused on fruit quality and yield.

The qualitative traits targeted in the study can be found in Table 1.

Table 1. Qualitative characters

Descriptors	Polymorphism
Plant habit (PH);	1.upright 3.semi-upright 5.prostrate
Stem: anthocyanin coloration of internodes (SAC)	1.absent 9.present
Stem: intensity of anthocyanin coloration of internodes (SIC)	3.weak 5.medium 7.strong
Stem: pubescence of internodes (SP)	1.absent 9.present
Leaf blade: shape (LS)	1.narrow elliptic 2.medium elliptic 3.broad elliptic
Leaf blade: dentation of margin (LM)	1.absent or weak 2.medium 3.strong
Leaf blade: colour (LC)	1.yellowish green 2.green 3.purplish green
Leaf blade: intensity of green colour (LIC)	3.weak 5.medium 7.strong
Petiole: attitude (PA)	1.semi-erect 2.intermediate 3.drooping
Flower: attitude of pedicel (FP)	1.erect 3.intermediate 5.drooping
Flower: number of anthers (FNA)	1.five 2.more than five
Fruit: shape in longitudinal section (FSLS)	1.oblate 2.circular 3.cordate 4.triangular
Fruit: shape in cross section (FSCS)	1.elliptic 2.circular 3.angular
Fruit: depth of stalk cavity (FDSC)	1.absent or very shallow 3.shallow 5.medium 7.deep
Fruit: shape of apex (FSA)	1.pointed 2.rounded 3.depressed
Fruit: main colour (at harvest maturity) (FMC)	1.white 2.green 3.yellow 4.orange 5.purple
Fruit: intensity of main colour (at harvest maturity) (FIMC)	1.light 2.intermediate 3.dark
Fruit: main colour (at physiological maturity) (FMCP)	1.white 2.green 3.yellow 4.orange 5.purple
Fruit: intensity of main colour (at physiological maturity) (FIMP)	1.light 2.intermediate 3.dark
Fruit: colour of flesh (FCF)	1.white 2.yellow 3.greenish yellow 4.green 5.purplish green 6.purple
Fruit: predominant number of locules (FPNL)	1.two 2.three 3.four 4.five 5.more than five
Fruit: adherence of calyx (FAC)	3.weak 5.medium 7.strong
Fruit: enclosure of calyx (FEC)	1.fully enclosed 2.slightly open 3.widely open
Calyx: pubescence (CP)	1.absent 9.present
Calyx: ribbing (CR)	1.absent 9.present
Calyx: anthocyanin coloration (CC)	1.absent 9.present
Calyx: intensity of anthocyanin coloration (CIAC)	1. very weak 3.weak 5.medium 7.strong 9. very strong
Fruit: firmness (FF)	3.soft 5.medium 7.firm
Fruit: number of seeds (FNS)	3.few 5.medium 7.many
Seed: colour (SC)	1.yellow 2.brown yellow

The quantitative characters targeted in the study were: plant height (cm), height at first bifurcation (cm), internode length in first order branches (cm), leaf length (cm), petiole length (cm), flower diameter (cm), fruit mass (g), fruit mass without calyx (g), equatorial diameter of fruit (cm), polar diameter of fruit (cm), fruit shape, fruit size, total soluble solids (°Brix), potential yield (g/plant) and number of fruits per plant.

Measurements of equatorial and polar diameter fruit where made with standardized electronic digital caliper. The fruit were measured with electronic scale, with accuracy +/- 0.0005 g and the total soluble solids content was measure using refractometer.

For statistical analysis, ANOVA was used, followed by the Tukey test.

RESULTS AND DISCUSSIONS

Descriptive analysis of the evaluated traits

For a better understanding of general behaviour of the accessions in relation with each trait, a basic descriptive analysis for quantitative (Table 2) and qualitative (Table 3) characteristics was carried out.

Tabel 2. Means and standard deviation of quantitative traits

Variable	A1	A2	A3	A4	A6	A7
Plant height (cm)	67.45 ±5.59d	43 ±3.9b	87.1 ±1.8e	**28.25** ±3.46a	54.1 ±1.83c	**107.05** ±5.16f
Height at first bifurcation (cm)	**18.4** ±0.28f	14.5 ±0.11e	10.25 ±0.07c	**1.6** ±0.14a	8.7 ±0.14b	13.05 ±0.35d
Internode length in first order branches (cm)	6,35 ±0.21c	**4.25** ±0.21a	**7.7** ±0.14d	6.375 ±0.04c	5.525 ±0.11b	7.465 ±0.05d
Leaf length (cm)	5.7 ±0.14b	**4.25** ±0.07a	**16.97** ±0.18f	8.70 ±0.15d	9.71 ±0.13e	6.475 ±0.03c
Leaf width (cm)	2.37 ±0.03a	**2.35** ±0.07a	**10.4** ±0.28c	4.865 ±0.05b	4.85 ±0.06b	2.79 ±0.02a
Petiole length (cm)	2.04 ±0.08bc	**1.15** ±0.07a	**6.35** ±0.35d	2.6 ±0.14c	2.65 ±0.07c	1.53 ±0.04ab
Flower diameter (cm)	2.3 ±0.14b	2.27 ±0.03b	2.15 ±0.07b	**0.775** ±0.03a	2.11 ±0.01b	**2.65** ±0.07c
Fruit mass (g)	17.15 ±0.21c	20.3 ±0.99d	3.55 ±0.07b	**1.55** ±0.07a	16.35 ±0.77c	**29.3** ±0.81e
Fruit mass without calyx (g)	16.55 ±0.49b	19.76 ±1.04c	3.11 ±0.01a	**1.05** ±0.07a	15.75 ±0.91b	**28.75** ±0.8d
Equatorial diameter of fruit (EDF) (cm)	3.82 ±0.11c	3.6 ±0.14c	1.75 ±0.07a	**1.29** ±0.01a	3.05 ±0.21b	**3.92** ±0.1c
Polar diameter of fruit (PDF) (cm)	2.75 ±0.18cd	3.04 ±0.08d	1.915 ±0.02ab	**1.375** ±0.05a	2.4 ±0.3bc	**3.22** ±0.09d
Fruit shape (EDF/PDF) (cm)	**1.39** ±0.05c	1.18 ±0.01b	**0.91** ±0.02a	**0.91** ±0.02a	1.27 ±0.06bc	1.21 ±0.08b
Fruit size (EDF+PDF)/2	3.29 ±0.14c	3.32 ±0.11c	1.83 ±0.04a	**1.33** ±0.03a	2.72 ±0.24b	**3.57** ±0.09c
Total soluble solids of fruits (TSS) °Brix	8.7 ±0.14c	7.2 ±0.14b	**13.55** ±0.07e	11.25 ±0.07d	**5.15** ±0.08a	8.75 ±0.21c
Yield (g/plant)	2177 ±143b	2989±6 9.3c	661.6 ±29.5a	**359.8** ±6.6a	2271 ±38.7b	**3957** ±239d
Number of fruits per plant	**127.2** ±9.97a	148.1 ±10.3a	187.1 ±12.3b	**232.9** ±14.5c	139.4 ±4.46a	135.1 ±4.2a

The quantitative traits were subject to analysis of variance followed by Tukey test and almost all evaluated traits have showed significant differences. Means follow by the same letter do no differ significantly according to Tukey test (Table 2).

The plant height values varied from A4 with a height of 28.25 cm to 107.05 cm at A7. The height at first bifurcation was taller at *P. philadelphica* (A1 and A7) with a mean value varying from 14.5 cm to 18.4 cm and the smaller one was recorded by *P. peruviana* (A4) with a mean value of 1.6 cm. Leaf length, leaf width and petiole length had the highest value on A3 and the smallest one was recorded by A2. Flower diameter had the smallest record on A4 with only 0.775 cm, and the highest was record by A7. Accordingly, the fruit mass, fruit mass without calyx, polar diameter of fruit, fruit size and yield followed the same pattern. A percentage of 67% of the studied genotypes contains fruits with a diameter exceeding 2 cm, belonging to the genus *P. ixocarpa* and *P. philadelphica*, 16.5% fall in the typical form of the Colombian ecotype of 1.85 cm, and 16.5% are small fruits, with a diameter below 1.5 cm. The maximum number of fruits per plant was 232.9 fruits and was registered by A4 and the minimum was registered by A1 with 127.2 fruits. Regarding the total soluble solids, the highest value was recorded by A3, followed by A4 and the lowest value was obtained by A5.

The quality characteristics can be found in Table 3.

Table 3. Quality characters and their variations in relation to studied accessions

Descriptors	A1	A2	A3	A4	A5	A6	A7
PH	1	1	3	5	1	3	3
SAC	9	9	9	9	9	9	9
SIC	7	5	5	3	3	7	5
SP	1	1	9	9	1	1	1
LS	2	2	3	2	3	2	1
LM	1	2	3	3	3	3	3
LC	1	1	2	3	2	2	2
LIC	3	3	5	5	5	5	5
PA	2	1	2	2	1	3	3
FP	3	3	3	3	1	3	5
FNA	1	1	1	1	1	1	1
FSLS	2	2	2	2	2	2	2
FSCS	2	2	2	2	2	2	2
FDCS	3	3	1	3	5	1	1
FSA	2	2	2	2	2	2	2
FMC	1	1	4	1	4	5	2
FIMC	1	1	1	1	3	2	2
FMP	3	3	4	3	4	5	5
FIMP	1	1	1	1	3	2	2
FCF	3	3	4	3	2	4	3
FPNL	3	1	1	3	3	2	1
FAC	3	3	3	3	3	3	3
FEC	2	2	1	1	1	3	3
CP	1	1	9	9	1	1	1
CR	9	9	9	9	9	9	9
CC	1	1	1	1	1	9	1
CIAC	1	1	1	1	1	5	1
FF	7	7	7	5	5	7	7
FNS	3	3	7	5	5	3	5
SC	1	1	1	1	1	1	1

Plant habit had an upright position on three genotypes, semi-upright position was on three genotypes and one genotype had prostrate habit (A4). All the studied accessions had anthocyanin coloration of internodes, but the intensity of coloration varied from weak (A4 and A5) to medium (A2, A3, A7) and strong (A1, A7). The pubescence of the internodes was present in 28.6% of accessions and absent

on 71.4%. Leaf shape varied from narrow elliptic on one accession (A7), to medium elliptic on four accessions (A1, A2, A4, A6) and broad elliptic to two accessions (A3, A5).
Dentation of margin was weak on A1, medium on A2 and strong on the rest of studied genotypes.
Leaf blade had a yellowish green colour on 28.6 genotypes, 57.1% had a green colour and 14.3% genotypes had purplish green colour. Intensity on green colour was weak on 28.6% genotypes and medium on 71.4%.
Petiole attitude was: semi-erect (A2, A5), intermediate (A1, A3, A4) and drooping (A6 and A7). Flower attitude of pedicel was semi-erect on 14.3% accessions, intermediate on 57.1% accessions and drooping in 14.3% accessions. Number of anthers was five on all genotypes. Fruit shape in longitudinal section and fruit shape in cross section was circular on all studied accessions.
Depth of stalk cavity of fruit was absent or very shallow on A3 and A7, shallow on A1, A2 and A4 and medium on A5. Shape of fruit apex was rounded on all studied genotypes.
Main colour of fruit at harvest maturity was white on 3 genotypes (A1, A2, A4), green on one genotype A7, orange on A3 and A5 and purple on A6.
Intensity of the main colour fruit at harvest maturity was light on A1, A2, A3, A4; intermediate on A6, A7 and dark on A5.
Fruit colour of flesh was yellow on genotype A5; greenish yellow on four accessions (A1, A2, A4, A7); and green on two accessions A3 and A4.
The predominant number of locules in fruit had a value of two on A2, A3, A7; three locules on A6; and four locules on A1, A4 and A5.
The fruit adherence of calyx was weak on all studied accessions. The enclosure of fruit calyx was fully enclosed on A3, A5, A5; slightly open on A1, A2; and widely open on A6 and A7. Pubescence of calyx was absent on 71.4% of genotypes and 28.6% was present.
Ribbed calyx was present on all studied genotypes. Anthocyanin coloration of calyx was present on 14.3% genotypes (A6) and absent on 85.7% genotypes. Firmness of fruit was firm on five accessions and medium on (A4, A5). The number of seeds in fruit was few (A1, A2, A6), medium (A4, A5, A7) and many on A3. The colour of seeds was yellow on all studied accessions.

Accessions summary

Physalis philadelphica - A1 (Figure 1)
Plant has indefinite growth, with erect habit and with an average internode length. The leaves have an average length of 5.8 cm and a width of 2.4 cm

Figure 1. A1 crop detail and fruits images

The flowers have a diameter of 2.4 cm. The fruits have a low adhesion of calyx and at maturity the calyx is slightly open. The fruits have a yellowish green colour and an average weight of 17.3 g, the total soluble content has a value of 8.8 º Brix.

Physalis ixocarpa - A2 (Figure 2)
Plant has an erect habit and with a height of over 50 cm, the height of the stem to the first branch and the length of internodes is 20% lower than A1. The anthocyanin coloration of internodes is medium. The leaves are shorter by 27% compared to A1. The genotype is noted for its earliness, its fruit reaching maturity 74 days after germination period.

Figure 2. A2 crop detail and fruits images

The fruits are yellow-green and average in size, with an average value of 21.2 g and a content of total soluble of 7.3º Brix.

Physalis peruviana - A3 (Figure 3)
Plant has an indefinite growth, with erect habit and a plant height of over 1 m. The height of the stem to the first branch is 10.1 cm. The length of the internodes is short at the base of the plant, only 4.5 cm, and the distance increases as the plant grows in height, reaching a length of internodes of 16.3 cm. The plant has strong pubescence and medium anthocyanin coloration of internodes. The leaves are long, with an average length of 17.2 cm and weakly purple veins.

Figure 3. A3 crop and fruits images

The fruits are orange and pubescent in colour and the calyx completely closes the fruit. The small fruits, when are immature, have anthocyanin coloration on the calyx, but at maturity is no longer present. The fruits have the highest total soluble content, in average, 13.56º Brix, the fruits are small (4g/fresh fruit), tasty and aromatic.

Physalis peruviana A4 (Figure 4)
Plant has an indefinite growth, with semi-erect habit (crawling to semierect). The height of the stem to the first branch is below 1 cm. The leaves are up to 50% narrower than those in genotype A3, and the main veins of the lower leaflets have higher anthocyanin intensity than at A3.
The fruit closure of the calyx is completely closed, and the calyx has a superficial pubescence. Compared to A3, the colour of the pulp is greenish-yellow compared to orange on A3. A4 is productive; it forms a very large number of fruits, but with a low weight (1.6 g/fruit) and with a high TSS content, 11.19º Brix.

Figure 4. A4 crop and fruits images

Physalis alkekengi - A5 (Figure 5)
The plant has a height of over 40 cm, and the stem has up to the first branch, on average, 1.9 cm.

Figure 5. A5 crop detail

The length of internodes is short, and anthocyanin coloration at internodes is weak. The flower differs from the other genotypes, being white and with an average diameter of 2.2 cm. A5 is late variety, its fruit reaching maturity in 124 days after germination period.

Physalis ixocarpa - A6 (Figure 6)
Genotype has a semi-erect habit and average plant height of 52.3 cm. The height of the stem to the first branch is, on average, 8.8 cm, and the length of internodes is 5.4-6.2 cm. The flowers have a diameter of about 2 cm, with erect attitude.

Figure 6. A6 crop and fruits images

Genotype has a calyx with anthocyanin colouring (green with purple vein) when fruits are immature, but it loses once the fruits reach maturity. The closure of the calyx on is wide open; the calyx is smooth, without pubescence. The average weight of the fruit is 16.9 g and has the lowest content in TSS, of only 5.8º Brix from all studied genotypes.

Physalis philadelphica - A7 (Figure 7)
Accession 7 recorded the shortest period from germination to flowering, but its fruits have reached maturity after 102 days.

Figure 7. A7 crop and fruits images

The plant with an erect habit and a height of over 105 cm, the length of the internodes is average, and the leaves have strong edge ripples, the flowers are large, over 2.6 cm, 13% more than the L6. The length of the internodes increases with the height of the plant. The fruits do not have anthocyanin colour on the calyx.

CONCLUSIONS

The researches were completed with a valuable germplasm collection, out of which seven accessions were the subject of this study. The accessions were evaluated in terms of phenotypic expressivity, finding that they have different phenological and phenotypic behaviour. The best results regarding yield potential was obtained by A7 with 3957 g/plant, and the highest total soluble content was obtained by A3 with a value of 13.56º Brix, the fruits were small (4 g/fresh fruit), tasty and aromatic In summary, the agronomic assessment of the accessions using descriptive analysis for quantitative and qualitative traits facilitated a better identification and documentation of the variability between accessions. Also, helped for establishment of a valuable genetic resource for *Physalis* species, which will be useful in future breeding programs.

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RESEARCH ON THE CONSUMPTION OPTIMIZATION OF CHEMICAL AND ORGANIC FERTILIZERS BY USING FOLIAR FERTILIZERS BASED ON NATURAL ORGANIC COMPOUNDS OF BORON IN THE PRODUCTION OF WATERMELONS

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Abstract

Agricultural soils in Romania contain in average around 15-68 ppm of boron, and the mobile boron quantity is between 0.1-0.8 ppm. The interest in the watermelon culture in Romania, especially in the south of the country, in Dolj County (is reflected by over 3,000 ha cultivated only in the Danube Plain). The Folibor product were applied in a quantity of 5 liters/ha, in 4 treatments, recording some productions of 58.3 t/ha the gains being of 17.8% comparing with the non-fertilized foliar variant. The fertilization with organic compounds of boron determined the increasing of average weight of fruits (1-10 %), increasing of glucose content (7.92-8.50%) and vitamin C content (from 7.08 mg/100 FWg to 13.28 mg/100 FWg).

Key words**:** *foliar fertilization, organic compounds, watermelons.*

INTRODUCTION

Watermelon belongs to the gourd family called Cucurbitaceous and the genus *Citrullus* (Edwards *et al*., 2003). In watermelon is mostly water (about 92 %) but this refreshing fruit is full with nutrients. Has significant levels of vitamins A, B6 and C, lots of lycopene and antioxidants (Inuwa *et al*., 2011). Watermelon rind and seed also have many health benefits due to the presence of important amino acids citrulline, fibres, minerals and phenolic compounds (Masudul& Abdullah, 2015, Reetu & Tomar, 2017, Zubairu *et al*., 2018). The boron content in plants varies from 5 ppm to 654 ppm according to species. Boron fertilization is an efficient technological work, especially when boron is applied on crops that are fertilized organically (Jifon & Lester, 2006; Dawson & Hilton, 2011; Medeiros *et al*., 2016). The organs of plant which contain the most quantity of boron are the reproductive ones (anthers, stamen, stigma, ovary), a fact which explains the role of boron in the fructification process (Sienkiewicz-Cholewa, 2002; Razavi & Milani, 2006; Wang *et al*., 2013; Gil *et al*., 2006; Aguyoh *et al*., 2011; Dawson *et al*., 2011; Geleta *et al*., 2011; Dimkpa & Bindraban, 2016; Bommesh *et al*., 2017). Also a higher quantity of boron is found in the young tissues than in the old ones, the leaves containing more boron than the seeds and roots (Kashi *et al*., 2003; Reid *et al*., 2004; Young *et al*., 2005; Wang *et al*., 2013; Kadu *et al*., 2018). Generally speaking, the need of boron of dicotyledonous plants is higher in comparison to those of monocotyledonous plants (Guppy *et al*., 2005; Deswal & Patil, 1984; El-Bassiony *et al*., 2012; Do Nascimento *et al*., 2016). The agricultural soils in Romania contain in average around 15–68 ppm boron, and the mobile boron quantity is between 0.1 – 0.8 ppm (Rățoi *et al*., 2010; Popescu, 2012). The correction of boron deficit into the plant nutrition is made frequently through administration of foliar fertilizers with boron (Jifon *et al*., 2006; Medeiros *et al*., 2016). The interest in the watermelon culture in Romania, especially in the south of the country, in Dolj County is reflected by over 6500 ha cultivated only in the Danube Plain. Besides mineral fertilization, the supply of organic matter to the soil is of great importance for the cultivation of watermelon and other vegetables, especially in

sandy soils of arid regions that have low contents, because the climate conditions favor the acceleration of oxidation of soil organic matter (Nicolae *et al*., 2014). Regarding the fertilization strategies, the classical fertilizers in reduced doses correlated with consumption from the expecting crops, and also the partially substitution of fertilization through supplementary foliar fertilizers was observed (Nicoli *et al*., 1999; Jifon *et al*., 2006; Santos *et al*., 2016). By applying a modern technology, the watermelons culture on sandy soils in the south of Oltenia is profitable (Rățoi *et al*., 2010).

MATERIALS AND METHODS

In the 2019 period, the Folibor product, based on organic compounds of boron, was used on different agrofunds, the variants of the experiment being as following:
V1 - non-fertilized;
V2 - chemically fertilized with N 150 P_2O_5 100 K_2O 100;
V3 - fertilized organically with 30 t/ha, dung
V4 - Non-fertilized agrofund, Folibor, 5 l/ha, 2 treatments;
V5 - N 150 P_2O_5 100 K_2O 100+ Folibor, 5 l/ha, 2 treatments
V6 - N 75 P_2O_5 50 K_2O 50+Folibor, 5 l/ha, 2 treatments;
V7 - Dung, 30 t/ha+Folibor, 5 l/ha, 2 treatments;
V8 - Dung, 15 t/ha+Folibor, 5 l/ha, 2 treatments.
The experiment was placed in randomized blocks, four times.
The surface of the experimental place: 18 m^2.
The surface of the experiment: 720 m^2;
The moments of applying the foliar fertilizations were:
- treatment I at the beginning of forming the stem, on 6th of June, 2018;
- treatment II at 10 days after the first treatment, on 16th of June, 2018.
Quantity of solution was 600 l/ha. The watermelon crop was grown on a sandy soil in the field under a dripping irrigation system. All fruits were grown in an open field in the same area (Dăbuleni). During the 2018 campaign, samples were transported to our laboratory (Research Center for monitoring of the ecological and bioeconomical indicators for some horticultural species at regional level (BCUM) from University of Craiova) within a day after harvest and processed.
The dry matter content (%) was determined by dehydration of the plant material at 105°C, up to a constant mass.
Chemical content of watermelons in the following substances: water and TDM (total dry matter) (%) by the gravimetric method; SDM (soluble dry matter) (%), by the refractometric method.
A Boehringer enzymatic kit (combination test) was used to determine the D-glucose content. Samples for the above test were prepared from fresh material taken from the heart of the fruit which was triturated to extract the juice. The results are expressed in % of sample solution. Sweetness values were calculated on the basis of those reported by Eisenberg (1955).
For the determination of titratable acidity (g malic acid/100 g f.m.), the bromothymol blue indicator reagent was prepared in a concentration of 10.4 mol/1 in 5 x 10-3 tool/1 Britton-Robinson buffer (pH 7.5) (from a stock buffer solution containing 0.1 mol/1 sodium acetate, 0.1 mol/1 boric acid and 0.1 mol/1 potassium dihydrogen phosphate and adjusting the pH to 7).
Vitamin C standard solution was prepared by dissolving 0.250 g ascorbic acid in the beaker with 100 ml distilled water. The solution was transferred into 250 ml volumetric flask and diluted to 250 ml with distilled water. Standardization of iodine solution with the vitamin C standard solution was by pipetting 25ml of vitamin C solution into a 125 ml Erlenmeyer flask. 10 drops of 1% starch solution were added and then titrated against iodine solution until blue-black colour was observed. Titrations were repeated in triplicates. The volume of each fruit sample used was measured and the concentration of ascorbic acid per 100 ml fruits was calculated using: Concentration of ascorbic acid used in mg/100ml = Concentration (mg/ml standard)/ Weight of samples in gram x 1000 (Nweze *et al*., 2015).
The NPK content of watermelon leaves: total nitrogen (%) - Kjeldahl method, total phosphorus (%) - colorimetric method, total potassium (%) - flame photometric method.

The nitrate concentration can be estimated visually or determined spectrophotometerically at λ_{max} 400 nm in the range of 4-40 mg/l N. When the color change is very small, a coupling solution is added, and the violet color that develops can be measured colorimetrically at λ_{max} 545 nm in the range of 0.5-5 mg/l N (Szekely, 1991).

RESULTS AND DISCUSSIONS

The fertilization of watermelon culture on sandy soils determines higher production gains for the studies variants, being in average for two years between 45.6 and 58.9 t/ha. The highest production was obtained when the mineral fertilization at the level of N 150 P_2O_5 100 K_2O 100 was complemented with extraradicular fertilization through two treatments with Folibor in a dose of 5 l/ha/treatment, determining a production gain of 157%, the average difference of 21.4 t/ha being very significant (Table 1). It is important to mention the fact that the treatments with Folibor in the organic fertilized variant with 15 t/ha dung, determined the production gain to be 9.6 t/ha, and in the organic fertilized variant with 30 t/ha dung, the production gain was 10.7 t/ha.

The using of Foliborfor the foliar fertilization in watermelons cultures that were organic fertilized, leads to a better usage of fertilized substances, so that the reducing by half of quantities of 30 t/ha dung has determined in the average on two years of study an insignificant loss of production, 1.1 t/ha. It can be concluded that on the sandy soils the efficiency of fertilization based on organic compounds of boron in the watermelons culture is high, especially in the case of exclusive fertilization with mineral fertilizers. The cultures that are exclusively fertilized with organic fertilizers allow the reducing by half of the fertilizer quantity. Using the fertilizers based on organic compounds of boron in combination with organic and chemical fertilizers showed the role of boron in the development and growth of plants (Table 2). The content of total dry matter (TDM), soluble dry matter (SDM), glucose and vitamin C has recorded higher values in the variants that were fertilized in comparison to the non-fertilized variant.

The best results were obtained in the variants fertilized with dung, Folibor, Folibor + N 150 P_2O_5 100 K_2O 100, Folibor + dung 15 t/ha (8.85-9.30% TDM, 8.80-9.20 SDM, 8.50-8.77 % glucose and 11.44-16.80 mg to 100 g FW vitamin C.

The acidity of fruits recorded the lowest values in the variants with dung 30 and respectively 15 t/ha in combination with Folibor (0.076-0.089 g malic acid to 100 g FW), which are representative values for watermelons.

The content of nitrates was very variable, ranging from 12.40 mg/Kg fruit in the non-fertilized variant or fertilized with dung + Foliborto 105.43 mg/kg fruit in the variant fertilized with N 150 P_2O_5 100 K_2O 100, value which exceeds the effective standard of 100 mg/kg fruit.The lowest values were recorded by variants fertilized with dung 30 t/ha, dung 30 t/ha and 15-t/ha +Folibor (24.42 mg/kg fruit, 18.60 mg and 16.40 mg/kg fruit).

The soluble dry matter and glucose presented close values.

The climatic factors, especially the intensity of light, have a special influence on the content of nitrates in plants, the activity of nitrate reductasis being caused by high temperature. Excess of darkness and humidity and low temperature create conditions which contribute to accumulation of nitrates in the plant and fruit. When reducing by half the doses of chemical and organic fertilizers and applying Folibor ferti-stimulator to vegetation, during the phenophases of growing and development of plants, the titratable acidity and the content of nitrates decreased, but the content of vitamin C increased. Scientific evidence have shown that watermelon contains vitamin C which is an essential nutrient for humans because it aids in the synthesis of collagen in addition to protecting against oxidative damage. Inuwa et al. (2011) have also performed such studies and obtained similar results (15.0 mg/100 FWg ascorbic acid).And Reetu & Tomar (2017) obtained similar results (8.1 mg//100 FWg Vitamin C) in their study.

In terms of the content of macroelements in leaves, the best results were obtained bythe variants treated with Folibor + chemical fertilizers and Folibor + dung, 4.12-4.52 % Nt, 0.20-0.30% Pt and 1.50-2.07 % Kt (Table 3).

Table 1.The influence of organic, mineral and Folibor foliar fertilization on the watermelons production

Variant of fertilization	Year 2017 t/ha	Year 2018 t/ha	Average 2018-2019			
			t/ha	%	Difference t/ha	Significance
V1 - non-fertilized	27.0	47.9	37.5	100	Mt	Mt
V2 - chemically fertilized with N 150 P_2O_5 100 K_2O 100	42.06	69.4	55.7	148	+18.2	***
V3 - fertilized with dung, 30 t/ha	37.6	43.6	45.6	122	+8.1	
V4 - Non-fertilized agrofund, Folibor, 5 l/ha, 2 treatments	34.8	47.5	46.2	123	+8.7	
V5 - N150 P_2O_5 100 K_2O 100 + Folibor, 5 l/ha, 2 treatments	44.2	73.5	58.9	157	+21.4	***
V6 - N75 P_2O_5 50 K_2O 50 + Folibor, 5 l/ha, 2 treatments	39.6	58.3	47.5	127	+10.0	*
V7 - Dung, 30 t/ha + Folibor, 5 l/ha, 2 treatments	39.5	56.8	48.2	126	+10.7	*
V8 - Dung, 15 t/ha + Folibor, 5 l/ha, 2 treatments	38.2	56.0	47.1	126	+9.6	*
DL 5% = 10.8	7.1	8.9				
DL 1 % = 15.0	9.6	12.4				
DL 0.1% = 20.7	13.0	16.9				

Table 2. The biochemical compound of watermelons fruit according to boron treatment on agrofunds of chemical and organic fertilizers (the 2017-2018 average)

Variant of fertilization	TDM[1] (%)	Water (%)	SDM[2] (%)	Titratable Acidity (g malic acid/ 100 FW^3g)	Vitamin C (mg/100 FW^3g)	Nitrate (NO_3) (mg/Kg fruit)	Glucose (%)
V1 - non-fertilized	8.20	91.8	8.0	0.128	7.08	12.40	7.92
V2 - chemically fertilized with N 150 P_2O_5 100 K_2O 100	8.60	91.4	8.5	0.110	10.56	105.43	8.22
V3 - fertilized with dung, 30 t/ha	9.00	91.0	8.95	0.110	9.68	24.42	8.73
V4 - Non-fertilized agrofund, Folibor, 5 l/ha, 2 treatments	9.30	90.7	9.2	0.102	11.44	74.42	8.50
V5 - N150 P_2O_5 100 K_2O 100 + Folibor, 5 l/ha, 2 treatments	8.85	91.1	8.8	0.110	13.28	68.04	8.50
V6 - N75 P_2O_5 50 K_2O 50 + Folibor, 5 l/ha, 2 treatments	8.50	91.5	8.3	0.108	14.16	37.21	8.15
V7 - Dung, 30 t/ha + Folibor, 5 l/ha, 2 treatments	8.60	91.4	8.5	0.076	11.52	18.60	8.30
V8 - Dung, 15 t/ha + Folibor, 5 l/ha, 2 treatments	9.00	91.0	9.0	0.089	16.80	16.40	8.77

[1]TDM = total dry matter, [2]SDM = soluble dry matter, FW^3 = fresh weight

Table 3. The influence of fertilizers based on organic compounds of boron in complex with organic and chemical fertilizers on the content of NPK in the leaves of watermelons (the 2017-2018 average)

Variant of fertilization	Nt (%)	Pt (%)	Kt (%)
V1 - non-fertilized	3.12	0.14	0.97
V2 - chemically fertilized with N 150 P_2O_5 100 K_2O 100	3.68	0.22	1.37
V3 - fertilized with dung, 30 t/ha	3.88	0.23	1.45
V4 - Non-fertilized agrofund, Folibor, 5 l/ha, 2 treatments	4.36	0.16	1.25
V5 - N150 P_2O_5 100 K_2O 100 + Folibor, 5 l/ha, 2 treatments	4.12	0.25	1.57
V6 - N75 P_2O_5 50 K_2O 50 + Folibor, 5 l/ha, 2 treatments	4.40	0.20	1.50
V7 - Dung, 30 t/ha + Folibor, 5 l/ha, 2 treatments	4.32	0.30	2.07
V8 - Dung, 15 t/ha + Folibor, 5 l/ha, 2 treatments	4.52	0.28	1.84

All the fertilized variants recorded a higher content of NPK in leaves in comparisonto the non-fertilized variant.

CONCLUSIONS

Applying foliar fertilization based on the organic compounds of boron to different agrofunds of watermelons culture on sandy soils it has influenced positively the quantitative and qualitative indexes of production.

The highest production was recorded when mineral fertilization at the level of $N150P_2O_5$ $100K_2O100$ was complemented with extraradicular fertilization through two treatments with Foliborin a dose of 5 l/ha/treatment, determining a production gain of 157%, the average difference of 21.4 t/ha being very significant.

It is worth mentioning the fact that the treatments with Folibor applied on the organic fertilized variant with 15 t/ha dung has determined a production gain of 9.6 t/ha, and on the organic fertilized variant with 30 t/ha dung, the production gain was 10.7 t/ha, the difference of 1.1 t/ha obtained by reducing by half the organic fertilizer quantity per surface unit is not significant.

Reducing by half the doses of chemical and organic fertilizers and applying the Folibor ferti-stimulator on vegetation, during the phenophases of growing and development of plants, the titratable acidity and content of nitrates decreased, the content of vitamin C increased, and the soluble dry matter and glucose presented close values.

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MAIN PHENOTYPIC EXPRESSION ON VALUABLE AMARANTHUS ACCESSIONS FROM VEGETABLE RESEARCH DEVELOPMENT STATION BUZĂU

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Abstract

Amaranthaceae family has a valuable number of over 600 species, but in Romania is not so much cultivated. The plant has multiple uses: decorative, food, medicinal and energetic, but also is a weed in the wild flora. VRDS Buzau, through Breeding and Biodiversity Laboratory, has shown more interest for this plant, realizing until now a valuable germplasm collection. In this study were selected 11 genotypes of Amaranthus from the germplasm collection. The research aimed was to study the adaptability to the pedoclimatic conditions in south of Romania and analysing the agronomic traits. The agro-morphological characters were statistically analysed and significant differences were found in the diameter of the bush, the length of the panicle, as well as the number of main branches. The genotypes had different types of the inflorescences, thus 9.1% were semi-drooping, completely drooping 36.5% and straight inflorescences 54.4%.The colour of the inflorescence also had variations from light green to dark pink. In conclusion, V.R.D.S. has a large variety of genotypes in this species and the research will continue by enriching the germplasm collection in order to obtain new cultivars.

Key words: *ANOVA, biodiversity, breeding, leafy vegetable, Romania*.

INTRODUCTION

The Amaranthaceae family comprises a number of 175 genera and over 2000 species of herbs, shrubs and small trees, mostly distribute in tropical and also in temperate regions (Rahman and Gulshana, 2014, Mrosczek, 2015). It is considered as one of the most species-rich lineage within the flowering plant in Caryophyllales order (Müller and Borsch, 2005; Simpson, 2010). The Amaranthaceae family are native to tropical and subtropical areas of Central America, Africa and Australia, whereas occur predominantly in arid to semiarid, saline, disturbed, and agricultural habitats of temperate and subtropical regions (Kedereit et al., 2003). The *Amaranthus* is a genus with multiple uses: ornamental purpose known for their multi-coloured foliage and beautiful inflorescence (*A. caudatus*, *A. tricolor*, *A. salixifolius*), or as edible plant (*A. caudatus, A. hybridus, A. spinosus, A. tricolor*), medicinal plant, also as energetic plant (Kiwicha) or as a weed in wild flora (*Amaranthus blitoides, Amaranthus retroflexus, Amaranthus albus*).

The vegetable amaranths it has been reported that vegetable amaranths have been largely ignored by the world of science (National Academy of Science, 2006). Many authors actually labelled them as „neglected crops" (Mnzava et al., 1999; Van der Walt et al., 2009, Vînătoru et al., 2019). In South Africa Amaranthus is regarded as a plant that can reduce poverty and malnutrition (Gerrano, A.S. et al., 2014).

Family Amaranthaceae has 20 times more calcium compared to spinach leaves and 7 times more iron compared to salad (Akaneme F. I. and Ani G.O., 2007).

The modern pharmacological studies showed that extracts from Amaranthaceae plants exhibited antioxidant (Nana et al., 1998; Escudero et al., 2011; Stintzing et al., 2004; Steffensen et al., 2011; Kraujalis et al., 2013), antidiabetic (Lo´pez et al., 2011; Adewale and Olorunju, 2013; Girija et al., 2011; Rahmatullah et al., 2013), tonic (Girija et al.,

2011), immunostimulatory (Sun, 2006), antitumor (Sun, 2006), antibacterial (Sun, 2006), antiinflammatory (Sun, 2006; Salvador et al., 2002; Kambouche et al., 2009), anti-osteoporotis (Sun, 2006), antiulcer (Das et al., 2012), hypolipidemic (Pushpa Latha et al., 2011), diuretic (Salvador et al., 2002; Metwally et al., 2012), larvicidal (Doligalska et al., 2011), antihypertensive (Biancardi et al., 2012), hypoglycemic (Biancardi et al., 2012; Ninfali and Angelino, 2013), and analgesic (Yoshikawa et al., 1997; Sun, 2006) activity.

In Romania, first vegetable Amaranthus (*Amarantus paniculatus*) was obtained at Vegetable Research and Development Station Buzau in 2017 and it can be find under name "Cezar" in Official Catalogue of Romanian Crop Plants. The aim of this study was to assess from an agro-morphological point of view eleven genotypes of *Amaranthus* sp. in order to start a new breeding program regarding this valuable, yet not really appreciate this species.

MATERIALS AND METHODS

Vegetable Research Development Station (VRDS) Buzau, through the Breeding and Biodiversity Laboratory has shown interest for *Amaranthus* sp., realizing until now a valuable germplasm collection. From the germplasm collection were selected a number of eleven genetically stable genotypes of Amaranthus that are the subject of this work. The research aim was to study the adaptability to the pedo-climatic conditions in Romania and analysing the quantitative and qualitative descriptors. The agro-morphological characters were analysed using the ANOVA followed by the Duncan test.

The sowing was done in the first decade of March in unheated greenhouse. In the first decade of May, the seedlings were manual planted in the research plot of VRDS Buzau. The seedling distance was 70 cm between rows and 25-30 cm within the plants. During the vegetation period, mechanical and manual hoeing was done in order to loosen the soil and for weed control. No chemical treatments were made during the whole growing season.

Throughout out the growing season, biometric and phenological measurements were made. In order to make an accurate characterization of agro-morphological characters it was use the Amaranthus descriptors from the Minimal Descriptors of Agri-Horticultural Crops from the National Bureau of Plant Genetic Resources, India (Mahajan et. al, 2000) In table 1 are found the quality traits used in this study.

The quantitative characters used were: plant height (cm), bush diameter (cm), leaf length (cm), petiole length (cm), number of primary branches, length second panicle (cm), length of primary panicle (cm).

Table 1. The quality descriptors

Plant habit (PH)	1.Erect, 2.Spreading 3. Drooping, 99. Others.
Leaf colour (LC)	1.Yellow, 2. Yellowish orange, 3. Yellowish green, 4. Orange, 5. Green, 6. Greenish orange, 7. Pink, 8. Pinkish green, 9. Reddish yellow, 10. Reddish green, 11. Red, 12. Dark red, 99. Others.
Inflorescence colour (IC)	1. Light yellow, 2. Yellow, 3. Yellowish orange, 4. Yellowish green, 5. Orange, 6. Pink, 7. Pinkish green, 8. Purple, 9. Red, 10. Reddish green, 11. Green, 99.Others.
Inflorescence compactness (IC)	3. Lax, 5. Intermediate, 7. Dense, 99. Others.
Stem colour (CS)	1. Yellow, 2.Yellowish green, 3. Orange, 4. Pink, 5. Red, 6. Reddish green, 7. Reddish orange, 99. Others.
Stem surface (SS)	1. Smooth, 2. Ridged, 99. Others.
Inflorescence shape (IS)	1. Globose, 2. Semi drooping, 3. Completely drooping, 4. Straight, 99. Others.
Inflorescence spininess (IS)	1. Smooth, 2. Glabrous, 3. Prickly, 4. Spiny, 99. Others.
Seed shattering (SSH)	3. Low (%), 5. Intermediate (10-50%), 7. High (50%), 99. Others.
Seed transparency (ST)	1. Translucent, 2. Opaque, 99. Other.
Seed colour (SC)	1. White, 2. Creamish, 3. Pale yellow, 4. Pink, 5. Red, 6. Brown, 7. Black, 8. Golden, 99. Others.

RESULTS AND DISCUSSIONS

The quality traits used in this study and their descriptor values were found in Table 2. In Figures 1-7 are presented the studied accessions.

As regards the port habit it was found that nine genotypes have erect port, one genotype has drooping (A4B) port and one genotype has diffused port (A4A). Concerning leaf colour three genotypes have yellow-green colour (A6A, A7, A12), five genotypes have green leaf (A4B, A5, A6B, A9, A13) and three genotypes have red-green leaf (A1, A4A, A11).

Table 2. The quality descriptors and their value on studied accessions

Character	A 1	A 4A	A 4B	A 5	A 6A	A 6B	A 7	A 9	A 11	A 12	A 13
PH	1	3	2	1	1	1	1	1	1	1	1
LC	10	10	5	5	3	5	3	5	10	3	5
CI	9	6	6	6	9	8	6	4	8	4	11
I	5	3	3	3	5	5	5	7	7	5	5
IT	5	2	2	2	4	2	4	2	4	2	6
CT	1	2	2	2	2	2	2	2	2	2	2
IF	4	3	2	3	4	4	3	4	4	3	4
IS	4	1	1	3	3	4	3	4	4	3	3
DS	5	7	7	5	5	5	3	5	5	5	3
TT	2	2	2	2	2	2	1	2	2	1	2
SC	7	7	6	6	7	7	2	2	2	3	7

The greatest variability was recorded by the colour of the inflorescence: two genotypes were having yellow-green inflorescence (A9, A12), four genotypes were having pink inflorescence colour (A4A, A4B, A5, A7), two genotypes were having purple inflorescence (A6B, A11), two genotypes were having red colour inflorescence (A1, A6A) and one genotype was having green inflorescence (A13).

Figure 1. Crop view A13 and A4A (from left to right)

The type of inflorescences varied was dense on percentage of 18.2%, 27.3% had lax type and 54.5% had intermediate type. A wide variability can also be found in the colour of stem: six genotypes with yellow-green stem (54.5%), three genotypes having pink stem (A6A, A7, A11), one genotype having the red stem (A1) and one genotype having the red-green stalk (A13). The surface of the stem was smooth in one genotype (A1) and ten accessions with ridged surface.

Figure 2. Crop view A12 and A5 (from left to right)

Figure 3. Crop view A11 and A9 (from left to right)

The inflorescence shape recorded a maturity time was semi drooping on 9.1%, completely drooping on 36.5% accessions and straight on 54.4% accessions. Regarding spininess of inflorescence, two genotypes had a smooth surface, without pubescence (A4A, A4B), four genotypes had spiny inflorescence (A1, A6B, A9, A11) and five genotypes (A5, A6A, A7, A12, A13) had prickly surface. Seed shattering is essential for propagation of their off springs in wild types, but is a major cause of yield loss in crop, from the studied accessions, just two genotypes (A4A, A4B), had a high degree of shattering, six genotypes had intermediate degree of shattering and two genotypes had low rate of shattering (A5, A6A). The seed transparency was translucent on 18.2% and opaque on 81.8% on studied accessions. The

colour of seed differed from pale yellow (A12), to creamish (A7, A9, 11), brown (A4B, A5) and black (A1, A4A, A6A, A6B, A13), as can be seen in Figure 8.

Figure 4. Crop view A6B and A7 (from left to right)

Figure 5. Crop view A1 (Cezar) and A6A (from left to right)

Figure 6. Crop view A4B

The quantitative traits were the subject to ANOVA followed by Duncan test and their results can be found in Table 3. Different letters means significant differences between studied accessions. Accession 9 had the highest height of 254 cm and it can be used as an efficient energy source plant, at the other end, the lowest height was recorded by A6 with 57.86 cm and the plant might to suitable for ornamental borders.

Table 3. Means and standard deviation of quantitative characters

Acc-ssion	PH (cm)	PD (cm)	LL (cm)	PL (cm)	NB	LSP (cm)	LPP (cm)
A1	*63.9+ 10.51A*	62.33+ 7.23CDE	**7.36+ 1.1 A**	*5.96+ 1.19AB*	14.3+ 1.15B	12 + 2.08 ABC	22.36+ 3.23B
A4A	137.33+ 5.21 BC	*29.66 + 4.93 A*	17.73+ 3.86 BC	6.43+ 1.2 AB	**21.7+ 6.65D**	7.83 + 3.01 ABC	39.63+ 7.05 DE
A4B	105.33+ 14.01B	53.00+ 15.39 BCDE	14.53+ 8.26 ABC	**11.33+ 6.55C**	20 + 5.29 CD	16.36+ 5.32 BCD	28.66+ 7.02 BCD
A5	113.83+ 5.61 CD	**28.66+ 3.21 A**	**19.6+ 2.47C**	7.1+ 1.21 ABC	18 + 1 BCD	17.46+ 5.25 CD	*45.16+ 4.35E*
A6A	**57.86+ 6.77A**	47 + 3.6 ABCD	11.2+ 1.73 AB	*5.53+ 1A*	7.66+ 1.52A	**35.33+ 3.21 F**	35.83+ 2.84 DE
A6B	125.96+ 18.27D	**88.33 + 7.63F**	13.56 + 1.18 ABC	**5.23 + 0.9A**	15.66+ 2.08 BC	21.73+ 7.34 DE	24.36+ 4.75 BC
A7	89.33+ 11.23B	49.33 + 8.32 ABCDE	14.16+ 2.38 ABC	*6.86+ 1.52AB*	13.3+ 4.16B	25.7+ 5.08DE	45.2+ 16.5E
A9	**254+ 20.51E**	69+ 12.12 DEF	*19.5+ 6.76C*	10.16+ 0.76 BC	4.33+ 0.57A	11.5+ 1.5ABC	27.33+ 2.51 BC
A11	*62.66+ 2.51 A*	41.66+ 13.2ABC	14.76 + 2.82 ABC	7.8 + 0.51 ABC	**2.33 + 0.57A**	6.56+ 0.92AB	**10.4+ 0.83A**
A12	90.33+ 5.68B	71.66 + 28.43EF	18.0+ 2.6BC	6.93 + 1.22 AB	15 + 1 BC	28.33+ 12.01 EF	**74.2+ 7.12F**
A13	59+ 3.6A	34.66+ 4.16 AB	12.+ 2.53 ABC	*5.5+ 2.34A*	3.33+ 0.57A	**5.43+ 0.05A**	22.1+ 0.15B

The plant diameter (PD) recorded the highest value with the accession 6B and a value of 88.33 cm, and the smallest value was recorded by A5 with 28.66 cm, followed very close by A4A with 29.66 cm. Accession A1 had the shortest leaf with a length of 7.36 cm, and the longest was recorded by A9 with a length of 19.5 cm. The petiole length varied from 11.33 cm to A4B to 5.23 to A6A.

Figure 7. Crop view A5 (detail)

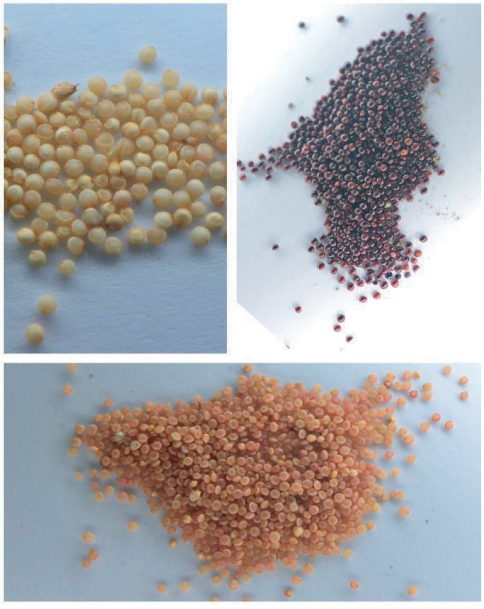

Figure 8. Seed types of Amaranthus

The number of main branches per plant varied from 21.7 registered by A4A to 2.33 branches recorded by A11. The lateral spikelet length had great variations from 5.43 cm to A13 from 35.33 cm to A6A. The length of inflorescences had also a great variation from 10.46 cm on A11 to 74.23 cm to A12.

CONCLUSIONS

Significant differences were found in the diameter of the bush, the length of the panicle, as well as the number of main branches. The genotypes had different forms of the inflorescences, thus 9.1% were semi-flowing, flowing 36.5% and straight inflorescences 54.4%. The colour of the inflorescence also had variations, from yellow to green (18.2%), to pink (36.4%), purple (18.2%), green (9.1%) and red (18.2%). In conclusion, VRDS Buzau has a large variety of genotypes in this species and the research will continue by enriching the germplasm collection and also due to the breeding program obtaining of new cultivars suitable for growing in pedoclimatic conditions of Romania.

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OPTIMIZATION OF TOMATO CULTIVATION TECHNOLOGY UNDER GREENHOUSE THROUGH THE USE OF CONTINUOUS ELECTRIC CURRENT

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Abstract

One of the most important groups of vegetables grown in our country is represented by Lycopersicon esculentum, due to the importance of tomato fruits in the food sector. The present study follows the reaction of the plant to the external stimulation of electrical and magnetic nature on the metabolism of the plants, which will be reflected in the indicators of quality and quantity of the production. The study was carried out on a population of tomatoes in the research greenhouse of the teaching Farm "V. Adamachi", from the University of Agricultural Sciences and Veterinary Medicine of Iaşi. The most favourable results in terms of morphological parameters were recorded when using a magnetic field that used a DC with an intensity of 0.15 A, and the most favourable results regarding the quality of the actual fruit production were recorded using a magnetic field generated by a DC with an intensity of 0.45 A.

Key words*: tomato, electrical current stimulation, magnetic field.*

INTRODUCTION

The need for food is one that is increasingly accentuated, an aspect that results from the statistics that envisage population growth, which leads to new innovations in the scientific field that can solve this disadvantage.

Over time, scientists have tried to use various technical methods in order to obtain higher output, using electricity as one of the boldest attempts to increase production.

Nowadays, the influence of electric, electromagnetic (EM) and static magnetic fields on cells, tissues, plants, animals, and human beings in the laboratory and in the environment is of considerable interest (Berg, 1993).

Electrical stimulation of plants is an attractive hypothesis but relatively unexplored scientifically (Black et al., 1971).

Electrostimulation of plants was studied by scientists which had studied the influences of an electric current to observe the plant movement under the effect of electrostimulation, ion transport, activation of ion channels, plant growth, plant cell damage, enhanced wound healing, enzymatic system activation, gene expression, electrical signalling (Valkov et al., 2018).

The purpose of application of low intensity electric and electromagnetic stimuli was to determine the effect they have on the morphological characteristics of the plants (Belyavskaya, 2004).

Parvaiz Ahmad also studied the application of DC in tomato plants and concluded that the DC current can cause significant differences in root development, showing a typical gradient with high developed plants (Ahmad et al., 2013).

MATERIALS AND METHODS

Plants of *Lycopersicon esculentum* Mill., Qualitet F1 hybrid with a determined growth, were used to carry out the study. The dominant feature of this hybrid is that it lends itself well to growing conditions in protected areas. The experiment was carried out in the greenhouse within the Farm "V. Adamachi" Iasi, under controlled conditions of humidity and temperature, during 2019. The experiment was organised in a split plot design with four replicates.

The seedlings were sown in alveolar tray, on 15th of March 2019, after which the seedlings were transplanted in pots with 9 cm diameter. At the 45 day after sowing, plants were

transplanted in 12 l pots, on 01st of May, at which point the experiment began, by assembling the equipment which will follow the influence that the electric current has on the metabolism of plants (Popescu et al., 2013; Voican et al., 2004).
The substrate used was peat, using ORGEVIT® as a fertilizing agent. The experience was realized on a group of 24 plants which were divided into 6 variants of 4 repetitions.
The equipment used were: 5 DC sources stabilized by the laboratory 0-30V/0-5A; electrical conductors with a length of 4.5 m, arranged in the form of a spiral with a diameter of 5 cm, these having a resistance of 1.3 Ω, the two ends of the spiral being each connected to a different electric conductor with a length of 4 m and 1.7 Ω resistance; 3 electrical resistors of 20 W and 15 Ω that were used in the case of variants 1, 2 and 3, these being connected at the output of the terminal + within the circuit.
The voltage sources were adjusted in such a way as to provide in the electrical circuit created, currents of different intensities, linking in parallel to the terminals of the laboratory source the electrical conductors for each of variants 1, 2 and 3.
A low intensity DC was used in order to prevent the plant death whereas the polarization time up to membrane breakdown is influenced by the field strength wich can cause the plant death (Angersbach et al., 2000).
Version 1 - used an electric current that crossed the circuit thus created using a current with an intensity of 0.15 A, DC.
Version 2 - used an electric current that crossed the circuit thus created using a current with an intensity of 0.30 A, DC.
Version 3 - used an electrical current that crossed the circuit thus created using a current with an intensity of 0.45 A, DC.
In the case of variant 4 (polarity) were used syringe needles that crossed the plant stem, these being inserted in number of two for each plant of the 4 repetitions, one being mounted in the apical area, and the other at the base of the stem. In this version, the 4.5 m wire was not used as a spiral, but only the 2 wires with the length of 4 m and the resistance of 1.7 Ω, the electrical conductors being connected to the two Syringe needles with the help of crocodile clips. The DC power applied was 1.5 V, DC.
For the 5th version (soil variant), the entire circuit was mounted using the same conditions as in the case of the 4th version, the difference being that the two electrical conductors were connected to 2 electrodes that were inserted in the soil.
The 6th version was represented by control, they served as a reference sample.
The experiment was conducted between 01st of May 2019 - 30th of June 2019.
During this period, all the plants were watered daily with 2 liters of water each, being applied practical measures of growing according with Stoleru et al., 2014.
To fight pests and diseases were applied four treatments, according with Munteanu, 2003. The treatments were applied foliar to all the plants, using an automatic pesticide sprayer.
The existing climatic conditions in the greenhouse compartment where the experiment was conducted are shown in Tables 1 and 2.

Table 1. Average temperature

Period	Max. temp. (ºC)	Min. temp. (ºC)	Average temp. (ºC)
01st of May 2019 - 31st of May2019	33.7	10.9	20.9
01st of June 2019 - 30th of June 2019	39.9	16.9	26.1

Table 2. Average humidity and light intensity

Period	Average humidity	Light intensity (Lx)
01st of May 2019 - 31st of May2019	73.4%	45609.7
01st of June 2019 - 30th of June 2019	70.2%	53543.3

On 30th of June 2019, the aerial part of the plant (fruits, leaves and stem) was harvested for analysis, followed by the dismantle of the crop. Immediately after dismantling the crop, measurements were made on the morphological and physiological particularities of the tomato plants: the height of the plant; number of leaves; number of fruits; area surface; the mass of the plant; fruit mass; determination of the content of chlorophyll pigments (Dannehl et al., 2012).

RESULTS AND DISCUSSIONS

On 30th of June 2019, measurements were made in order to determine the results of the experiment, in order to observe whether the application of an DC current directly or

indirectly on tomato plants by using a low intensity electromagnetic field which can induce morphological changes and/or physiological changes, similar observations were being made by De Souza using magnetic treatments on tomato seeds before sowing, magnetic treatments increasing the growth and yield of tomato crops (De Souza et al., 2006).
The results of the variance analysis are presented in Table 3, in order to determine the significance of the differences for the Duncan test.

Table 3. The analysis of the variant

Analysis of variance (ANOVA) using p≤0.05					
Plant hight					
Interaction	Sum of squares	df*	Mean square	F**	Sig.***
Between Groups	1741.00	5	348.20	0.864	0.524
Within Groups	7251.00	18	402.83		
Total	8992.00	23			
Plant weight					
Interaction	Sum of squares	df*	Mean square	F**	Sig.***
Between Groups	39088.77	5	7817.75	8.671	0.000
Within Groups	16228.58	18	901.58		
Total	55317.36	23			
Numer of fruits					
Interaction	Sum of squares	df*	Mean square	F**	Sig.***
Between Groups	31.70	5	6.34	2.272	0.091
Within Groups	50.25	18	2.79		
Total	81.95	23			
Average weight of a fruit					
Interaction	Sum of squares	df*	Mean square	F**	Sig.***
Between Groups	6968.97	5	1393.79	3.947	0.014
Within Groups	6356.16	18	353.12		
Total	13325.14	23			
Average weight of fruits per plant					
Interaction	Sum of squares	df*	Mean square	F**	Sig.***
Between Groups	243260.87	5	48652.17	3.005	0.038
Within Groups	291430.36	18	16190.57		
Total	534691.24	23			
Leaves number					
Interaction	Sum of squares	df*	Mean square	F**	Sig.***
Between Groups	29.33	5	5.86	1.072	0.408
Within Groups	98.50	18	5.47		
Total	127.83	23			
Leaf area					
Interaction	Sum of squares	df*	Mean square	F**	Sig.***
Between Groups	8310116.50	5	1662023.30	3.141	0.033
Within Groups	9524845.50	18	529158.08		
Total	17834962.00	23			
Clorophyll					
Interaction	Sum of squares	df*	Mean square	F**	Sig.***
Between Groups	109.07	5	21.81	2.023	0.124
Within Groups	194.06	18	10.78		
Total	303.13	23			

* - Degree of freedom; ** - Fischer factor; *** - Significance

Regarding the morphological parameter that refers to the height of the plant. the differences are insignificant, these being based on the genetic characteristics of the variety used, which shows that the use of DC does not significantly influence this parameter (Figure 1).

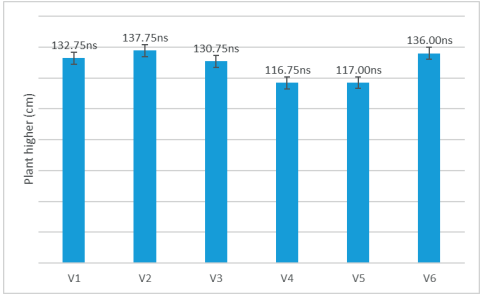

Figure 1. Plant height under continuous electric current

On the other hand, the average weight of the plants can be influenced by the electric current used, both positively as is the case of variant V1, where the highest value was recorded, slightly exceeding the weight of the control variant, while in the case of variant V4 plants were significantly negatively affected (Figure 2).

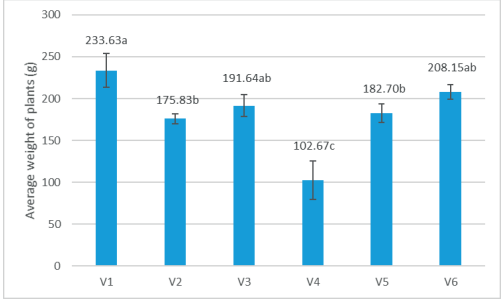

Figure 2. Influence of direct current on plant weight
*Letters are significantly different according to the Duncan test using p≤0.05, the letter a represents the highest value

Positive results regarding the use of electric currents and low intensity magnetic fields were obtained for the plants of *Helianthus annuus* and *Triticum aestivum* (Fischer et al., 2004), *Hordeum vulgare* (Lebedev et al., 1977, cited by Dannehl, 2018).

The average values of the number of fruits varied significantly, the lowest being recorded in the case of variant V4, followed by the variant V6 (reference), while the highest number of formed fruits was recorded in the case of variant V5 (Table 4).

Table 4. Influence of the direct current on the number of fruits, average weight of the fruit per plant and the average weight of a fruit

Electric treatment	Number of fruits	Average weight of a fruit (g)	Average weight of fruits per plant (g)
V1	7.75±0.48a	75.13±6.09bc	576.11±32.31ab
V2	6.00±0.91ab	60.05±12.53c	368.37±108.43b
V3	6.00±0.58ab	107.99±4.71a	642.70±47.99a
V4	5.00±1.08b	78.38±13.56bc	367.91±59.25b
V5	8.25±0.75a	60.88±7.04c	490.16±39.88ab
V6	5.75±1.03ab	93.06±8.90ab	510.67±63.86ab

*Within each column, letters are significantly different according to the Duncan test using p≤0.05, the letter a represents the highest value.

In the case of the weight of the fruits per plant there are significant differences, the best results were recorded in the case of V3, where the average mass of a fruit also recorded the highest value. Variant 3 is followed by Variant 1 as the average weight of fruits per plant, while Variant 5 shows an insignificant difference from Control.

Favourable results for fruit production efficiency (calculated as the number of fruits harvested from an area of 100 square meters, divided to the average weight of the fruit) using the electric current for 20 days at the parameters of 1500 nT, 10 Hz, were obtained by at the plants of *Glycine max* (Radhakrishnan and Kumari., 2012).

The number of leaves registered insignificant differences (Figure 3), the same results being obtained using the phenomenon of electrostimulation on the plants of *Solanum scabrum* (Gogo et al., 2016).

The parameters related to the leaf area have varied by registering the highest values in the V1 variant, being followed by the V3, V6 and V2 variants, while the V4 variant has recorded the lowest leaf area (Table 5).

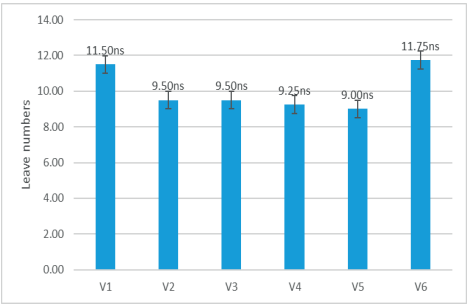

Figure 3. Leaves number under continuous electric current treatment

Table 5. Influence of direct electric current on leaves number, leaves area and chlorophyll content index

Electric treatment	Number of leaves	Leaves area (mm^2)	CCI
V1	11.50± 0.86ns	4,290.25±343.38a	21.03±1.48a
V2	9.50± 1.19ns	3,265.50±344.93ab	14.62±0.73b
V3	9.50±0.29 ns	3,695.50±203.61a	16.25±0.47ab
V4	9.25± 2.06ns	2,358.50±630.75b	18.78±2.90ab
V5	9.00± 1.08ns	3,201.75±215.82ab	17.36±1.62ab
V6	11.75± 0.75ns	3,636.50±266.37a	15.60±1.45b

CCI - chlorophyll content index

ns - insignificant; within each column, letters are significantly different according to the Duncan test using p≤0.05, the letter a represents the highest value.

Similar to the leaf area, the chlorophyll content index also presented the highest values for V1

Chlorophyll parameters were analysed using electric current on *Lepidium sativum* plants where favourable results were also obtained (Dannehl et al., 2018).

CONCLUSIONS

The plants weight variation was significant, best results being obtained for the sample that used a DC current with an intensity of 0.15 A. For the samples that used a DC current with an intensity of 0.30 A, 0.45 A, a DC of 1.5 V and the reference sample the results were very similar, while for the sample that used a DC of 1.5 V by using syringe needles in the apical area and at the bottom of the stem of tomato plants could be observed a significant lower rate of development.

The best results for the average fruits weight per plant was registered by the sample that used DC current with a intensity of 0.45 A, followed by the sample that used a DC with a intensity of 0.15 A.

The average number of leaves was relatively similar for each sample, the higher number of leaves being observed for the reference sample

and for the sample that used a DC current with 0.15 A.

The leaves area was measured using Li-3100 LI-COR. The best results were obtained again for the sample that used an DC current with an intensity of 0.15 A.

Based on the measured values it was concluded that a low intensity electric current stimulates the vegetative growths (they develop at a faster rate), but the higher intensities lead to a better absorption of the nutritional elements corroborated with a higher growth of fruit mass.

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NUTRITIONAL QUALITY CHARACTERISTICS OF TWO PUMPKINS TYPE CULTIVATED IN BULGARIA

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Abstract

Among the plant species of the diverse genus Cucurbita L., C. maxima Duchesne and C. moschata Duchesne have the greatest agro-economic importance. Both species are cultivated for food, feed and ornamentation, widely distributed in the world and known for their high biological and nutritional value. The main aim of this study was to assess two types of pumpkins (C. maxima and C. moschata) in terms of the traits of agronomic importance with the emphasis on basic fruit flesh quality parameters. The following traits of agronomic importance were analyzed: total yield (kg/da), fruit weight (kg), an thesis day and length of main stem; whereas the chemical analyses of fruit flesh included the content of dry weight (%), ash content (%), sugars (mg/g fresh weight), and carotenoids (mg/kg fresh weight) and pH.

Key words*: yield, productivity, nutrition value, carotenoids, pumpkins.*

INTRODUCTION

In recent years, consumer's interest in the health enhancement role of specific foods or physically active food components, so-called nutraceuticals or functional foods, has exploded (Hasler, 1998; Javaherashti et al., 2012).

Pumpkins are gourd squashes of the genus *Cucurbita* and the family *Cucurbitaceae*. The pumpkin species available include *C. pepo*, *C. moschata*, and *C. maxima*. These three species are cultivated worldwide and have high production yields (Kim et al., 2012). They are an increasingly popular vegetable used as a component in various dishes with dietary properties (caloric value 17 g/100 g; sugars 10%, high content of carotenoids and vitamin C).

Pumpkin is one of the vegetables that meet the requirements of healthy nutrition. They are widespread, because they can grow under different climate conditions.

Pumpkin fruit is a rich source of valuable nutrient components, such as carotenoids, potassium, vitamins C, B2, and E, characterizes low energetic content and high amount of fiber (Kunachowicz et al., 1997; USDA, 2006; Biesiada et al., 2011), which allows to produce different foods for adults and infants (as a component of purees, jellies, jams, juices). Moreover, the high percentage of pectin in the pumpkin helps to cleanse the intestine and helps the body to release from the accumulated radionuclides (Slavin, 2013).

Because the nutrient composition of pumpkins will differ depending on their origins and cultivation environments it may be important to know the nutritional profiles of the various pumpkin species (Park et al., 1997; Kim et al., 2012).

For the effective utilization of pumpkin fruit and its parts as a functional food component or medicinal herb, qualitative and quantitative information on the nutritional is essential.

The Bulgarian food composition include several types of pumpkins -mature pumpkin, zucchini squash, summer squash, mainly *C. moschata* and *C. maxima*.

Despite its importance, pumpkin has not gained adequate research attention in Bulgaria to harness its potential.

In Bulgaria, there are separate publications on the use, yield and development of pumpkins. They are dating back to the 70's and 80's of the last century. There is limited current research on the nutritional quality of the varieties grown in Bulgaria.

This study was initiated to generate information on fresh quality of the crop. The main aim of this study was to assess two types of pumpkins (*C. maxima* and *C. moschata)* in terms of the traits of agronomic importance with the emphasis on basic fruit flesh quality parameters.

MATERIALS AND METHODS

The experiment was conducted in the period 2015-2017 years. The selected varieties were representative of two main types of pumpkins: *Cucurbita maxima* - cultivar Plovdivska 48/5 and *Cucurbita moschata* - cultivar Muschatna 51/17. The plants were grown in the Experimental field at the Agricultural University - Plovdiv on technology for field production of pumpkins (Cholakov, 2009). The feeding area for each plant was 2 x 2 m. The sowing of the seeds was performed in May. Each cultivar was grown in 4 replicates x 5 wells. For the optimal development of the plants, during the growing season, the necessary agro technical measures were carried out, according to the applied technology. The harvesting of the fruits was carried out in the botanical maturity, at the end of September.

The following traits of agronomic importance were analyzed: total yield (kg/da), fruit weight (kg), number of fruits per plant, an thesis day and length of main stem; whereas the chemical analyses of fruit flesh included the content of dry weight (%), ash content (%), sugars (mg/g fresh weight), and carotenoids (mg/kg fresh weight) and pH.

The moisture (dry matter) was determined by gravimeter by BSS 7133-81 and refractometer by BSS 17257-91, respectively. pH values was determined by BSS 11688-93. Ash content was determined according to the standard AOAC procedures (AOAC, 2007). The total soluble sugars content was evaluated by the phenol-sulfuric acid method (Dubois et al., 1956). The amount of present carbohydrates was determined from a calibration curve constructed with glucose. The reducing sugars were evaluated by the PAHBAH method (Lever, 1972).

For the carotenoid extraction, 25 mL of acetone was successively added until a paste was obtained. The paste was transferred to a sintered funnel (5 μm) coupled to a 250 mL Buchner flask and filtered under vacuum. This procedure was repeated at least three times until the sample was colorless. The obtained extract was transferred to a 500 mL separatory funnel containing 40 mL of petroleum ether. The acetone was removed through the slow addition of ultrapure water (Milli - Q - Millipore) to prevent the formation of emulsion. The aqueous phase was discarded, and this procedure was repeated four times until no residual solvent remained. The extract was then transferred with a funnel to a 50 mL volumetric flask containing 15 g of anhydrous sodium sulfate. The final volume was adjusted with petroleum ether, and the samples were analyzed at 450 nm (de Carvalho et al., 2012).

RESULTS AND DISCUSSIONS

The two species differ in terms of growth and growing season. Environmental conditions, soil type, nutrient availability and their absorption of plants at different stages of relativity determine their vegetative manifestations and productivity (Table 1).

Table 1. Agronomic traits of two types of pumpkins

Type	Thesis day	Length of main stem, cm	Fruit weight, kg	Yield kg/da
C.maxima	163	542.00	5.152	2546.250
C. moschata	156	355.00	3.732	1865.938

Two species of pumpkins differ in terms of the length of main stem, thesis day, length of main stem, fruit weight and yield. *C. maxima* is distinguished by a longer growing season, larger fruits and higher yield. *C. moschata* ripens 7 days earlier than *C. maxima*. Considering the traits of agronomic importance, the variation among the pumpkin species was observed for thesis day, length of main stem, fruit weight, yield, which is common for complex traits and in accordance to the results of Sultana et al., 2015, Chaudhary et al., 2017a, and Brdar-Jokanović et al., 2019.

The variability of *C. maxima* and *C. moschata* genotypes and their specific responses to different environmental conditions and constraints are well-documented by other authors, e.g. Mladenović et al. (2014), Conti et

al. (2015), Bakhtouri et al. (2017), Mishra (2017), Martínez et al. (2018), Brdar-Jokanović et al. (2019). Our results are in line with established trends in previous studies.

The main factors that determine the culinary use of pumpkin fruits is the content and balance of sugars and the content of carotene. By defining these indicators, fruits can be standardized in nutritional and biological value, which are directly related to consumer preferences and expand the range of available fruits on the market.

The presented data showed that soluble dry matter was the highest in *Cucurbita moschata* - cv. Muschatna 51/17 (10.50%) in the comparison with *Cucurbita maxima* - cv. Plovdivska 48/5 (9.25%). The values were statistically significate ($p<0.05$) due to the different variety. The moisture content in the analyzed pumpkin was in the range of 88-89% (on the fresh basis), ash content 0.9% and pH 6.3-7.5 (Table 2).

According to the specificity of the selected variety of pumpkins, technological measurements showed that after weighing, peel and seed separation, the fruit mass for processing for both varieties was over 70%, the seeds were from 6.27% for *Cucurbita maxima* to 9.50% at butternut squash *Cucurbita moschata* (Figure 1). Unusable for culinary purposes part of the fruits of both types of pumpkins (peel) is less than 20%.

Table 2. Physicochemical characterization of pumpkin

Type	Dry matter, %	Moisture content, %	pH	Ash content, %
Cucurbita maxima	9.25	89.08±0.26	7.57	0.93±0.05
Cucurbita moschata	10.50	88.85±0.15	6.73	0.98±0.07

Variation in DM content of pumpkin fruit has also been reported by Paulauskiene et al. (2006) and Zinash et al. (2013), who studied the quality of pumpkin cultivar in relation to their electrochemical and antioxidant properties. The difference could be due to variation in starch content of the genotype of the pumpkin fruit; with high DM content there is high content of starch (Hazzard, 2006).

These results to be close to the pH values of pumpkin reported by Paulauskiene et al. (2006) and Zinash et al. (2013) varying between 5.87 to 6.99. In addition, Zinash et al. (2013) noted that there is a tremendous variation among pumpkin genotypes for pH and titratable acids.

Pumpkin fruit is composed of pulp and seeds. Pumpkin pulp contains polysaccharides, pigments, amino acids, active proteins, and minerals. Pumpkin seeds are high in lipids and proteins, and they are a good source of many elements such as potassium, phosphorus and magnesium (Zhou et al., 2007)

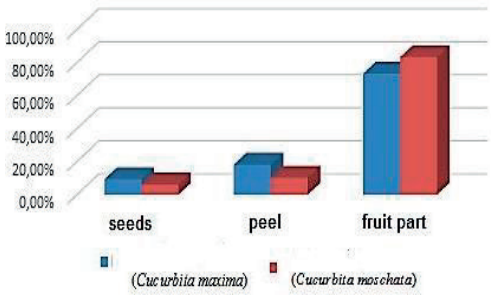

Figure 1. Technological characterization for *Cucurbita maxima* and *Cucurbita moschata*

The results for total soluble sugars in pumpkins varied from 5,8 to 4,9 % on fresh matter basis. *Cucurbita maxima* cultivar Plovdivska 48/5 as evaluated as the rich source of sugars (5.8%), especially sucrose (5.12%). The result of this study agrees with the findings of Sudhakar et al. (2003) who reported that sugar content of pumpkin fruit varied from cultivar to cultivar. Cantwell and Suslow (1998) also reported variations in total sugar contents among 36 varieties of pumpkin and indicated that sugar is the major component of total soluble solids and it determines the flavor and sensory quality of pumpkin fruit.

Terazawa et al. (2001) reported that, oligosaccharides, monosaccharides and sucrose were the principal soluble sugars accumulated by the fruit. The content of total sugars in C.maxima is higher than C. moschata by approximately 1%. The same variety is with a higher content of sucrose and fructose. The data for the carbohydrate composition in the raw pumpkin (Figures 2 and 3) showed that all these samples were source of sucrose

(Seroczynska et al. 2014). Fructooligosacchides were not detected in all investigated pumpkins, which was in accordance to Malinovska et al. (2014).

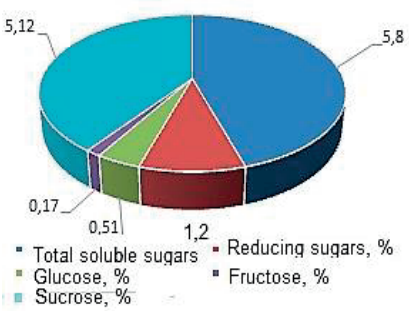

Figure 2. Sugars content in *Cucurbita maxima*

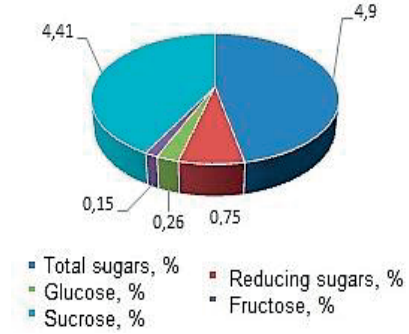

Figure 3. Sugars content in *Cucurbita moschata*

C. moschata demonstrated higher levels of carotenoids (254 µg/g fresh weight), compared with *C. maxima* - 135 µg/g fresh weight (Figure 4).

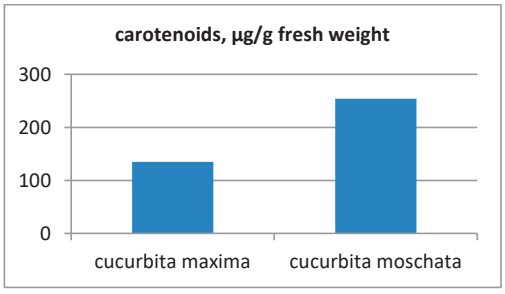

Figure 4. Carotenoids content in *C. maxima* and *C. moschata*

The high carotenoid content is one of the reasons why pumpkin is such a nutritionally valuable fruit (Dinu et al., 2016; El Khatib and Muhieddine, 2019). Carotenoids are considered a major source of vitamin A which is necessary for embryonic development, growth, and normal eyesight. Pumpkin is an excellent source of pro-vitamin A carotenoids (Zhou et al., 2007; El Khatib and Muhieddine, 2019).

Major qualitative and quantitative differences in carotenoids can be noted in relation to cultivar, environmental conditions (temperature, nutrient availability, intensity of sunlight, ripening stage, harvest time), and genetic factors. Different factors may be responsible for the different concentrations detected. It is well known that the climate has a significant influence on the content of carotenoids in vegetables. Fruits of the same cultivars produced in different regions exhibit higher or lower carotenoid concentrations in relation to warmer or more temperate climates. Bergantin et al. (2018) suggest that it is probably associated with an increase in carotenogenesis, when fruits are more exposed to sunlight, even if it may cause photodegradation. Based on the results of our study, we suppose that the difference in carotene content is a species and variety characteristic. The reason for this hypothesis is that both varieties are grown under the same climatic conditions and soils.

CONCLUSIONS

Vegetative manifestations and productivity of two species differ in terms of growth and growing season. Environmental conditions, soil type, nutrient availability and their absorption of plants at different stages of relativity determine their differences.

Cucurbita maxima is rich source of sugars, especially sucrose, compared with *Cucurbita moschata*, but contains less carotenoids.

The results of the economic productivity of the plants give us reason to believe that they may be recommended for cultivation in order to enrich the assortment of pumpkins. In addition, further research on the optimization of agro-technical procedures for the production of this type of pumpkins is required in order to maximize their potential for yield and quality.

The complex characteristics of fruits make them suitable not only for the fresh market but also for processing.

ACKNOWLEDGEMENTS

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RESEARCH REGARDING THE INFLUENCE OF SOME RHIZOGENOUS PRODUCTS AND OF THE WETTING TIME ON THE GERMINATION OF SEEDS AND GROWTH OF PEPPER SEEDLINGS

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Abstract

In Romania, the pepper culture is established exclusively by planting seedlings, which is why more attention must be paid to this technological link. For peppers, the germination of the seeds is slower, but to increase the germination capacity this experiment was carried out. As biological material, the Alexandru variety was used, 3 rhizogenous products for seed wetting, respectively, Raiza 0.2%, Keramin 0.25% and Kerafol 0.25% and 2 time cycles for seeds wetting, 6 h and 12 h, compared with non-wet seeds and seeds wet only in water 24 h. It was found that the best results regarding seed germination were recorded after wetting the seeds with 0.25% Keramin, for 12 hours and after wetting the seeds in water 24 h. Biometric features of the seedlings were more influenced by treating the seeds with 0.25% Keramin for 6 hours. Also, all the rhizogenic products led to better results compared to the non-wet seeds and to almost similar results as the seeds wet in water for 24 h.

Key words*:germination, growth, pepper, seedlings.*

INTRODUCTION

Pepper is a very important vegetable plant that is cultivated within different culture systems and is globally consumed under different forms. It is well known as one of the richest vegetable in Vitamin C, the red pepper having107-154 mg/100 g fresh product (Martinez et al., 2005).

Pepper has a high content of capsaicin, especially the hot pepper, which is why it is highly used specific cuisines such as Asian, Indian, Mexican and South American.

In addition, due to capsaicin, hot pepper has anti-septic and anti-inflammatory properties and protects the human body against rheumatoid diseases and conditions, (Krishna, 2003).

It is a species with slow growth of plants, so it is recommended to use biofertilizers for the intensification of physiological processes and the growth of the seedlings from the first stages (Vlahova and Popov, 2014).

Due to slow seed germination, to large periods of time between sowing and plant emergence and to uneven plant emergence, stimulation of pepper seeds' germination is a necessity. For this, pre-germination treatments are applied, with positive effects on the germination percentage and time, such as seed wetting through various methods and with different wetting agents (Lutts, 2016). Pre-germination treatment through osmosis (osmo-priming) of the pepper and tomato seeds reduces the time period until plant spring and ensures a better uniformity of the spring even when temperatures are below the optimum level (Giuliani et al., 1992; Rogers, 1989). Osmotic pressure, temperature and length of the treatment depend on the species (Bradford et al., 1990). Studies show that different seed wetting techniques enrich the germination for pepper seeds. This way, Kaewduangta et al., 2016, shows that seeds wet with osmotic solutions (osmo-priming) have germinated better that the ones soaked in water (hydro-

priming) and among those, the ones soaked in solution with PEG 6000 for 14 days have recorded a higher germination percentage and index (germination speed). Moreover, treating the seeds though osmo-priming leads to obtaining higher and longer seedlings, with an increased number of roots compared to using other treatments. Soaking pepper seeds with germination between 66% and 98%, in a solution with KNO_3 2%, for 4 days at 20°C led to an increased germination percentage of the seeds that had a lower germination capacity (Ermis et al., 2016), which makes it possible to use older seeds for which the germination is lower. Treating pepper seeds with GA_3 0.01 mg/l anticipates the germination process with several days compared to the untreated seeds (Hoza, 1995). Treating the seeds with gibberellic acid associated with mechanical scratching of the tegument and using the gibberellic acid led to an increase in the physiological potential of the studied pepper genotypes regarding the germination speed and percentage (Guadalupe et al., 2019). Treating the pepper seeds, California Wonder variety, with cu nanochitine, chitosa or hidropriming significantly reduced the average germination time compared to untreated seeds (Samarah, N.H., et al., 2016). Similar results were obtained for corn, for which the chitosan solution reduced significantly the average germination time, demonstrating that chitosan can be a new treatment applied to the pepper seeds (Shao et al.., 2005; Guan et al., 2009). Treating the seeds with warm water (47°C-49°C, 50°C-52°C and 53°C-55°C) for 30, 45 and 60 min in vitro, showed that for a water temperature of 50-52°C, and a 30 min hydration, seed germination is very good and the obtained seedlings are vigorous and qualitative (Singh et.al., 2019). The treatment of cereal seeds with collagen hydrolyzate determined the increase of their speed and germination rate, stimulated the increase of the seed content in gibberellic acid and the amount of biomass of the seedlings (Epure et al., 2018). Other researchers (Hanieh et al., 2013) observed that by treating the pepper seeds with salicylic acid of 0.1 and 0.5mM concentrations for 24 h the seed germination percentage and plant growth rate were improved, especially for the 0.5mM concentration. Wetting pepper seeds with different chemical products (NaCl 50 mM, $CaCl_2$ 50 mM, ABA 100 µM and others) for 24 h, at 25 ± 2°C, with moderate stir, increases the seed germination percentage and induces to the obtained plants tolerance against various types of stress compared to the seeds soaked in water at 40°C or untreated (Yadav et al., 2011).

MATERIALS AND METHODS

Research was conducted within the experimental field of USAMV Bucharest, Faculty of Horticulture, inside a greenhouse specialized for producing vegetable seedlings, during March-April 2018. The experiment was organized with 2 variable factors, one being the wetting product and the other the wetting time of the seeds.

Figure 1. USAMV Bucharest greenhouse

The purpose of the research was to establish the wetting duration for the pepper seeds and the influenced of the used product on seed germination. The biological material used was the long pepper variety Alexandru, semi-late variety, productive, with elongated-conic fruits, a red colour when matured and average weight of 80-120 g. Three products were used to stimulate seed germination, as detailed below:
Raiza, product with rhizogenous effect, contains oligopeptides, polypeptides, oligosaccharides, polysaccharides, polyamines, vitamins and seaweed extract (*Ascophyllum nodosum)*. It is totally water soluble and enriched with free amino acids (10.7%) and total nitrogen (N) 4%. It stimulates root growth and the capacity to absorb water and nutrients.
Kerafol contains a complex of hydroisolated proteins and activators that improve plant growth and increase the cellular division speed and plant growth speed, phenomenon that is

also met when the product is used for seed wetting. Kerafol contains amino acids 28%, out of which 24% are free amino acids, 5.7% total nitrogen (N) out of which 4.3% organic nitrogen, 3.1% soluble potassium oxide (K_2O) and 14% carbon organic.

Keramin has a complex composition, respectively free amino acids 14%, total nitrogen (N) 3.7% out of which organic nitrogen (N) 1.9% andammonial nitrogen (N) 1.8%, potassium oxide (K_2O) 6%, copper (Cu) 0.07%, copper chelated with EDTA 0.07%, iron (Fe) 0.10%, iron chelated with EDTA 0.10%, manganese (Mn) 0.05%, zinc (Zn) 0.07%, and zinc chelated with EDTA 0.07%. Functions like a stimulator for seed germination, by influencing the cellular growth and division and the growth of plants, roots and meristematic tissues.

The pepper seeds were soaked in different products for different durations, as detailed in Table 1.

Table 1. Experimental scheme

Variant	Wetting product	Wetting duration (h)
V1	Not soaked	0
V2	Water	24
V3	Kerafol 0.25 %	6
V4	Kerafol 0.25 %	12
V5	Keramin 0.25 %	6
V6	Keramin 0.25 %	12
V7	Raiza 0.20 %	6
V8	Raiza 0.20 %	12

The experiment was organized in subdivided parcels, with 3 replications each of 15 plants/replication. Seed soaking was made within plastic bottles of 500 ml, the quantity of solution or water being equal for all variants, respectively 300 ml. The quantity of seeds used was 8 g, one gram of seeds for each variant. The seeds were introduced into the bottles containing the solutions and stored within the warm greenhouse until the end of the wetting period, then removed and sowed immediately in plastic crates, on substrate of wet peat Kekkila. When the first real leaf emerged, the seedlings were transplanted into alveolar pallets with 45 alveoli. During seedling production, the temperature was maintained between 22°C and 26°C during the day and between 16-18°C during the night, repeated irrigation procedures were applied to maintain the substrate wet and 2 phyto-sanitary treatments were applied, one with Dithane M45 0.2% and one with Topsin 70 WDG 0.1%.

When the seedlings reached the plating phase, determinations were made regarding plant height and root length by measurements, number of leaves, weight of the aerial part, stem diameter with callipers and root system volume with the graded cylinder. The interpretation of the results was made through variant analysis.

RESULTS AND DISCUSSIONS

As a result of the measurements made, it was noted that soaking pepper seeds in rhizogeneous solutions, regardless of the product and wetting duration, determined a higher germination percentage than for the untreated seeds (Table 2). Pepper responded well to seed soaking with rhizogeneous products for 12 h compared to soaking the seeds in the same products for 6 h.

Thus, during day 6 since sowing, the best results were obtained for the seeds soaked in Keramin 0.25%, Raiza 0.2% and Kerafol 0.25%, the germination being 61 %, 59 % and 58 %. For the seeds soaked for 6 h, the germination percentage was between 50% and 56%, the best product being Keramin 0.25 %. Eight days after sowing, seed germination recorded the same tendency as for the 6-day milestone; at the end of the germination period, meaning 10 days after sowing, the highest germination percentage was obtained for the seeds treated with Keramin 0.25%, for 12 h, respectively 90%.

All products stimulated seed germination. Soaking the seeds in water for 24 h had a positive effect and similar to the effect of Raiza 0.25%, respectively 82%. The lowest percentage of plant emergence was recorded for the untreated seeds (Table 2). The results were statistically ensured.

By graphically representing the influence of the wetting products (Figure 2), it was observed that the best results were obtained when using the products Keramin and Kerafol for all 3 moments of measuring the germination percentage.

Table 2. Dynamics of plant emergence for pepper seeds depending on the used product and wetting duration (%)

Variant	Wetting product	Wetting duration (h)	Day		
			6th	8th	10th
V1 (Mt)	Not soaked	0	32 Mt	55 Mt	76 Mt
V2	Water	24	43N	76 ***	82N
V3	Kerafol 0.25 %	6	54*	67***	89*
V4	Kerafol 0.25 %	12	58*	68 ***	87*
V5	Keramin 0.25 %	6	56*	70 ***	88*
V6	Keramin 0.25 %	12	61**	80 ***	90**
V7	Raiza 0.20 %	6	50 N	62 *	85N
V8	Raiza 0.20 %	12	59 N	69 ***	84N
DL 5%			20.13	6.54	9.84
DL 1%			27.97	9.05	13.68
DL 0.1%			38.91	12.60	19.03

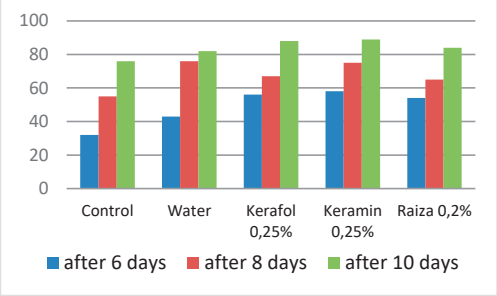

Figure 2. Influence of the wetting product on the seed germination (%)

Different wetting duration of the pepper seeds demonstrated that it influences the germination percentage, compared to the untreated seeds (Figure 3).

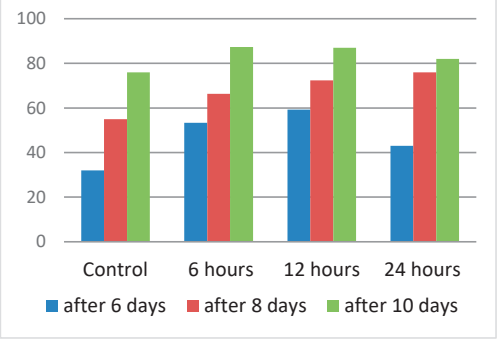

Figure 3. Influence of wetting time on the seed germination (%)

Analyzing the seedlings obtained from seeds soaked before sowing, compared to the untreated seeds, it was noted that the used products influenced also the plant growth during the first life phases, as per the description of those products (Table 3).

Soaking the seeds in Keramin 0.25% for 6 and 12 h had a greater influence on the pepper seedling growth than the other wetting products and durations. Thus, plant diameter at parcel was 3.8 mm for seeds soaked for 12 h and 3.7 mm for seeds soaked for 6 h. Similar results were obtained also for the seedlings obtained from seeds treated with Kerafol 0.25%, meaning 3.4 mm for soaking for 6 h and 3.7 mm for 12 h. Wetting the seeds with Raiza 0.2%, for 12 h, had the same results as soaking the seeds with water for 24 h, respectively 3.2 mm.

The seed wetting stimulated the growth of the roots; however, among the used products there were no significant differences, but for the plants obtained from untreated seeds the growth was weaker.

The volume of the root system varied between 2.1 $cm^3$for plants obtained from untreated seeds and 2.7 cm^3 for those obtained from seeds treated with Keramin 0.25% and Raiza 0.2%, for 6 and 12 h. Also, the weight of the roots was influenced by the treatment, especially by Keramin 0.25% and Raiza 0.2%, for 6 and 12 h.

Table 3. Seedling characteristics for pepper plants

Variant	Wetting product	Wetting duration (h)	Stem diameter at parcel (mm)	Seedling height (cm)	Number of leaves	Root length (cm)	Root volume (cm^3)	Weight of aerial part (g)
V1 (Mt)	Not soaked	0	2.4 Mt	11.7 Mt	5.3 Mt	11.4 Mt	2.1 Mt	2.9 Mt
V2	Water	24	3.2***	15.4***	6.8***	12.1***	2.7**	3.9 ***
V3	Kerafol 0.25 %	6	3.4***	15.5***	6.3***	12.2***	2.5 N	3.7***
V4	Kerafol 0.25 %	12	3.7***	15.1***	7.2***	12.3***	2.6 *	3.7***
V5	Keramin 0.25 %	6	3.5***	16.9***	7.1***	12.9***	2.7**	4.3***
V6	Keramin 0.25 %	12	3.8***	16.9***	7.5***	12.7***	2.7**	4.2***
V7	Raiza 0.20 %	6	3.4***	16.8***	7.4***	12.5***	2.7**	4.1***
V8	Raiza 0.20 %	12	3.2***	17.7***	7.4***	12. 6***	2.7**	3.8***
DL 5%			0.16	1.02	0.47	0.31	0.40	0.40
DL 1%			0.23	1.42	0.66	0.43	0.56	0.56
DL 0.1%			0.32	1.97	0.92	0.60	0.77	0.77

The results were statistically ensured through variant analysis. The effect of the wetting products was manifested also during the root growth phase.

The best results being obtained as a result of using rhizogeneous products (Figure 4). Wetting duration influenced seedling growth, especially soaking the seeds for 6 h and 12 h (Figure 5). Between the morphological parameters of the seedlings were shown the correlation. Thus, between the height of the plants and the number of leaves there was a direct correlation, with a correlation coefficient $r^2 = 0.8219$ (Figure 6).

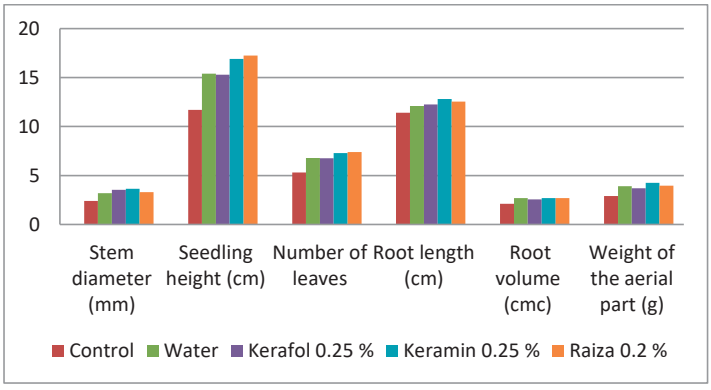

Figure 4. Influence of wetting product on seedling growth

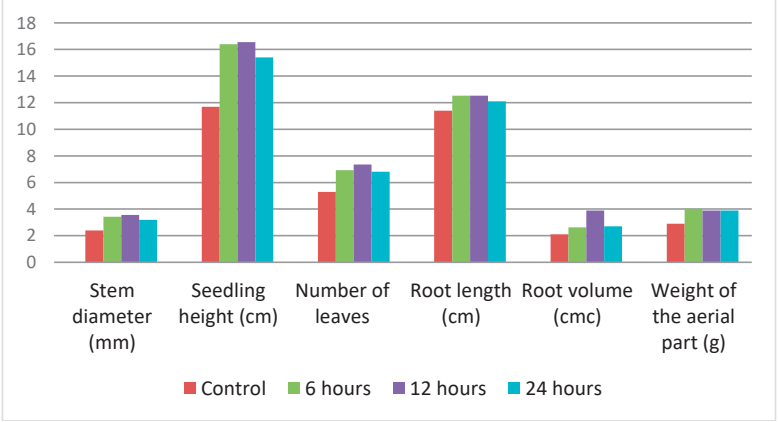

Figure 5. Influence of wetting duration on seedling growth

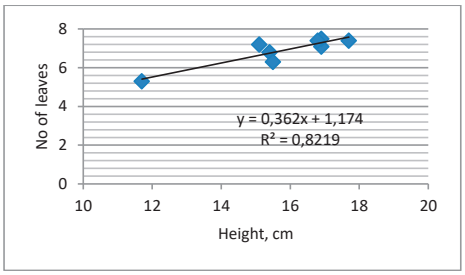

Figure 6. Correlation between height of plant and number of leaves

Between the height of the plants and the volume of the root a strong positive correlation was determined, with a high correlation coefficient, $r^2 = 0.8542$ (Figure 7).

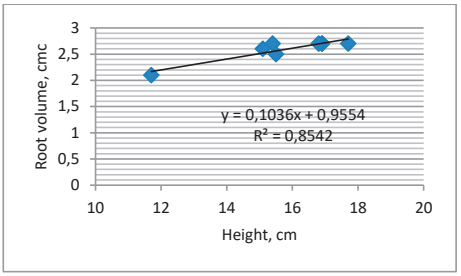

Figure 7. Correlation between height of plant and number of root volume

Between the height of the plants and the weight of the aerial part there was also a positive and strong correlation, with a high correlation coefficient, $r^2 = 0.7778$ (Figure 8).

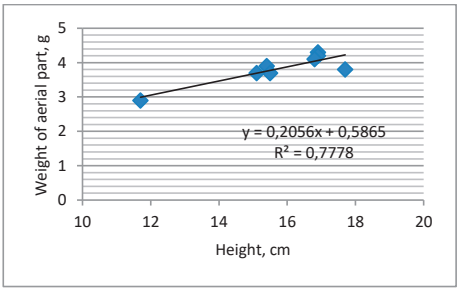

Figure 8. Correlation between height of plant and weight of aerial part

CONCLUSIONS

Research regarding wetting pepper seeds with rhizogenous products for 6 and 12 h showed that pepper reacted well and the seed germination process was improved. It was noticed that when soaking the seeds with Keramin 0.25% for 12 h. The germination was of 61% for the 6th day since sowing, compared to untreated seeds, which after the same time period germinated only at 32%. This showed a very good germination speed. The increased being of 52.45% compared to the control variant. Better results, compared to the control, were also obtained for soaking the seed with Kerafol 0.25% and Raiza 0.2%, for 6 and 12 h. Soaking the seed with water for 24 h led to an increase in germination of 7.3%, compared to the untreated seeds. The wetting products influenced the growth of the seedlings, element that was determined by the seedling characteristics. The seedlings obtained from seed soaked in rhizogenous products were more vigorous, fact determined by a higher root diameter (3.2-3.8 mm),seedling height (15.5-17.7 cm),a higher number of leaves (6.3-7.4),root volume (2.5-2.7 cm^3) and weight of aerial part (3.7-4.3 g). It was observed that by soaking the seeds in water for 24 h better results could be obtained regarding seed germination and also growth of the seedlings, compared to the untreated seeds.

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VARIATION OF BIOACTIVE COMPOUNDS IN ORGANIC *OCIMUM BASILICUM* L. DURING FREEZE-DRYING PROCESSING

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Abstract

Common basil (Ocimum basilicum L.), one of the most important aromatic perennial herbs due to its essential oil composition, belongs to Lamiaceae (Labiatae) family. Basil is an economically important herb and it is considered one of the finest aromatic herbs, being widely used as flavor in food industry. Basil samples were characterized in terms of chlorophyll content, total polyphenols, antioxidant activity, and volatile oil content. The fresh harvested leaves and the processed powder from leaves were hydro-distilled for 3 h in a Clevenger-type apparatus. The volatile oil was measured and collected for further GC-MS analysis. As drying technology, freeze-drying was used until the samples reached a loss of 85% from the fresh weigh, with the final dry matter content of 95.86%. Variations for the main constituents of volatile oil: 1,8-cineole, linalool, methyl chavicol, eugenol, α-bergamotene, and α-epi-cadinol were observed after processing.

Key words*: organic basil leaves, drying process, volatile oil, total phenolic content, DPPH antioxidant capacity.*

INTRODUCTION

Common basil (*Ocimum basilicum* L.), is an aromatic perennial herb, belonging to Lamiaceae (Labiatae) family. *Ocimum basilicum* L. can be marketed as fresh or dried products, according to their intended use and the supply chain. Consumer demand for organic processed products that keep more of their original fresh plant characteristics has increased in the last years. Fresh herbs (especially Lamiaceae) usually contain 75–80% water, and these water levels need to be lowered to less than 15% for preservation (Ghasemi Pirbalouti et al., 2013). Both the fresh and dried leaves are widely used to enhance the flavor of foods such as salads, pasta, tomato products, vegetables, pizza, meat, soups, marine foods, and other food products (Attokaran, 2017). *Ocimum basilicum* L. can also be used as a medicinal plant (Lee et al., 2005), along with other plants like *Arnica montana* L. (Nikolova et al., 2013) and *Matricaria chamomilla* L. (Baglou et al., 2017), or for microencapsulation in food products (Alexe et al., 2014).

Drying is by far the most widely used preservation method, as drying inhibits microbial growth, and is also the easiest way to preserve chemical composition. Different types of drying have been applied for herb processing, such as: shade-drying, sun-drying, hot air drying, freeze drying (Ghasemi Pirbalouti et al., 2013), CO_2 drying (Bušić et al., 2014), vibrofluidization (Lima-Corrêa et al., 2017) and convective-pre-drying and vacuum microwave finish-drying (Calín-Sánchez et al., 2012). The quality standard for dried products is freeze drying, which preserves the overall appearance of the original product (Telfser et al., 2019). However, the drying processes can affect the nutritional quality of the herbs, fruits and vegetables. Their phytochemical components like carotens, phenolic compounds and essential oils are of further interest due to their antioxidant and anti-inflammatory activities (Złotek et al., 2016).

The chemical composition of *Ocimum basilicum* L. consists of a wide and varying array of volatile compounds, depending on variations in chemotypes, leaf and flower colors, aroma and origin of the plants (Lal Saran et al., 2017; Vînătoru et al., 2019). Methyl eugenol, methyl chavicol, methyl cinnamate, eugenol, and linalool are generally

the main constituents of the basil essential oil Pistelli et al., 2020).

This study assessed the variation in bioactive compounds (chlorophyll content, total phenolic content, antioxidant activity and volatile oil content) using freeze-drying technology as the processing method for organic basil leaves.

MATERIALS AND METHODS

Chemicals

Folin - Ciocalteau reagent (2 N), DPPH (1,1-diphenyl-2- picrylhydrazyl), and anhydrous sodium carbonate were purchased from Sigma-Aldrich Company. Trolox ((±)-6-hydroxy-2,5,7,8 - tetramethyl chromane-2-carboxylic acid) was purchased from Acros Organics, Fisher Scientific (Geel, Belgium). Methanol and hexane were purchased from Honeywell (Riedel-de Haën, Seelze, Germany). Gallic acid was purchased from Carl Roth, and acetone was purchased from Chemical Company.

Organic basil materials

Organic basil leaves (*O. basilicum* var. *crispum*) were purchased from Vegetables Research and Development Station of Buzau at commercial maturity, in September 2019. Two types of leaf samples were used for analysis: a) fresh leaves and b) freeze-dried leaves.

For the freeze-drying of the samples, the batches of basil leaves (approximately 20 g each) were frozen in an ultra-low temperature freezer MDF-594-PE from Panasonic Corporation (Osaka, Japan) at −80°C for 24 h. After freezing, the samples were freeze-dried in an Alpha 2-4 LSCplus (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) freeze drier at a pressure of 0.5 mPa and with the shelf temperature of −55°C.

Moisture determination

Moisture content was determined when the sample was received and after drying. An amount of 0.5 g of fresh sample was dried until constant mass in a Sartorius thermobalance at 105°C.

Determination of chlorophylls a and b, and of total carotenoids content

Extraction of chlorophyll content was made based on Lichtenthaler & Wellburn (1983) using acetone 80% as solvent and expressed as µg/mL of extract:

$$C_a = 12.21A_{663} - 2.8A_{646} \quad (1)$$

$$C_b = 20.13A_{646} - 5.03A_{663} \quad (2)$$

$$C_{x+c} = \frac{1000\ A_{470} - 3.27C_a - 104C_b}{229} \quad (3)$$

The results were further calculated for mass and final extraction volume and the final results expressed as mg per g of dry matter (mg/g DM).

Essential oil extraction and GC-MS analysis

The fresh harvested leaves (300 g) and freeze-dried powders (60 g) were hydro-distilled for 3 h in a Clevenger-type apparatus. The obtained oil samples were diluted with hexane and analyzed by Gas Chromatography coupled with Mass Spectrometry (GC-MS). Analysis of the essential oils was performed on an Agilent 6890 GC coupled with a 5973 Network single quadruple mass spectrophotometer detector in Electron Ionization (EI) mode and 7673 injector on a HP-5MS capillary column (30 m × 0.25 mm id, 0.25 µm film thicknesses). The following operating initial conditions were employed: 50°C for 8 min, then a 4°C/min ramp to 280°C. Helium was used as carrier gas with a constant flow of 1.0 mL/min, injection volume 3 µL with a split ratio 50: 1. The temperatures for inlet, MS transfer line and ion source was 250°C, 250°C and 230°C, respectively. The GC column was coupled directly to the spectrometer in EI mode at 70 eV with the mass range of 50-550 amu at 2 scan/s.

Extraction of polyphenols

Extraction of polyphenols from basil samples was based on the method described by Stan et al. (2017). To 1 g of dried sample or 0.2 g of fresh sample, 10 mL of 70% aqueous methanol were added and the samples were incubated in the dark overnight at 4°C. After that, the extracts were shaken at 500 rpm for 1 h and then centrifuged at 5000 rpm, 4°C, for 10 min. The supernatant was recovered in a 50 mL centrifuge tube and the residue was re-extracted two more times with 10 ml of 70% aqueous methanol. All three supernatants were combined and then the volume of each extract

was adjusted to 30 mL with the extraction solvent.

Total phenolic content (TPC)

The total phenolic content of the extract solutions was determined by the Folin - Ciocalteu spectrophotometric method described by George et al. (2005). A 2.5 mL of water-diluted Folin - Ciocalteu reagent (1/10) was added to 0.5 mL methanolic extract. The mixture was incubated for 2 min at room temperature, and after incubation, 2 mL of sodium carbonate (7.5%) was added. The mixture was heated for 15 min at 50°C and finally cooled in a water-ice bath. A mixture of solvent and reagents was used as a blank.

Absorbance was measured in a Specord 210 Plus UV-Vis spectrophotometer (Analytik Jena, Jena, Germany) at 760 nm. The amount of total phenolic content was expressed as mg Gallic acid equivalents per g dry matter (mg GAE/g DM). Triplicates of independent extract solutions were analyzed.

DPPH radical scavenging activity

The DPPH test was adapted from a method described by Bujor et al. (2016) with some modifications. Briefly, 0.1 mL of the sample extract was added to 2 mL of 0.2 mM solution of DPPH in methanol (prepared daily, protected from the light and kept in ice). The solutions mixture was put under dark and shaking at 500 rpm (IKA KS 260 homogenizer) for 30 minutes. Then the absorbance was measured at 515 nm. Methanol was used as a blank reference. The results were expressed as micromoles of Trolox equivalents per gram of dry matter (μM TE/g DM).

RESULTS AND DISCUSSIONS

Fresh samples showed a moisture content loss of 86.8% after freeze-drying. The remaining powder (13.2% of the fresh sample), showed a residual moisture of 4.14%. The same range of basil leaves moisture contents were obtained by Ghasemi Pirbalouti et al. (2013) (80.72% for purple and 83.97% for green basil) and Bušić et al. (2014) (89.72% and 90.45% for fresh leaves, and 6.05% for freeze-dried leaves). As mentioned by Bušić et al. (2014), the European Spice Association (2018) recommends that the maximum moisture content of dried basil to be up to 12%. So in the case of the present study, more freeze-drying time was required in order to ensure a good quality to the product.

Content of leaves' pigments

The analysis of photosynthetic pigments (total chlorophyll, total carotenoids) showed a variation between dried and fresh leaves (Table 1). The chlorophyll a content decreased with 16.5% and chlorophyll b with 2.7% after drying, resulting in an approximately total decrease of 20% of total chlorophyll content in freeze-dried leaves. The carotenoids content decreased 43% in freeze-dried leaves compared to fresh leaves.

Table 1. Determination of foliar pigments (chlorophyll a, chlorophyll b, carotenoids)

	Fresh leaves	Freeze-dried leaves
Chlorophyll a (mg/g DM)	6.31 ± 0.63	5.27 ± 0.39
Chlorophyll b (mg/g DM)	2.12 ± 0.11	2.06 ± 0.20
Total chlorophyll (mg/g DM)	8.43 ± 0.74	7.33 ± 0.59
Total carotenoids (mg/g DM)	1.53 ± 0.15	1.07 ± 0.08

Variations in the essential oil composition

The identification of the individual compounds in leaves of organic basil for fresh and freeze-dried material was carried out using mass spectra and their identities were confirmed by comparing their mass spectra with NIST Mass Spectral Library and literature as showed in Table 2.

The essential oil (EO) extraction yield of the basil samples was 0.067% w/w for fresh leaves and 0.158% w/w for powdered leaves. EO composition is reported in Table 2, with 61 compounds identified in both fresh and freeze-dried leaves. More than 50% of the total chemical composition of fresh leaves essential oil (Figure 1A) was composed of linalool 27.59%, methyl chavicol 11.43%, α-epi-cadinol 10.52% and eugenol 7.30%. For freeze dried leaves the concentration (Figure 1B) of the main constituents varied compared to fresh leaves: linalool 18.14%, α-epi-cadinol 14.30%, eugenol 9.11%, γ-cadinene 5.28%, and methyl chavicol, 4.93%.

This chemical composition reveals that essential oil of *O. basilicum* L. processed by freeze-drying shows a decrease in monoterpenes hydrocarbons (1.11%) and oxygenated monoterpenes (20.42%). The

compound with higher molecular mass and boiling point maintained its concentration, whereas sesquiterpene hydrocarbons decreased with 14.43% and oxygenated sesquiterpenes with 5.85%. Other chemical compounds maintained similar concentrations for fresh leaves (9.61%) and freeze dried leaves (10.86%), as showed in Table 2.

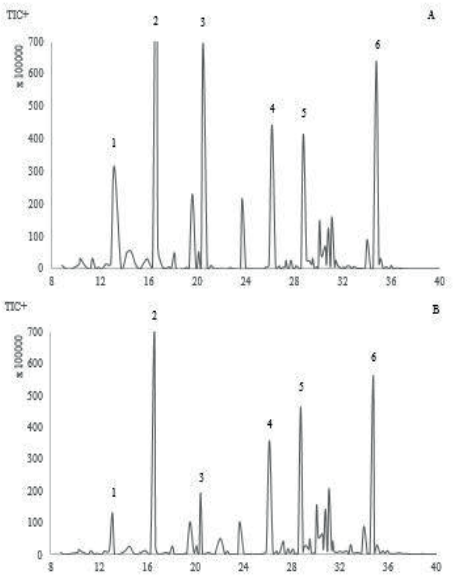

Figure 1. Essential oil chromatographic profile of fresh basil leaves (A) and freeze-dried basil leaves (B): 1) 1,8 Cineole; 2) Linalool; 3) Methyl chavicol; 4) Eugenol; 5) α-Bergamotene; 6) α-epi-Cadinol

Variation of total phenolic content and antioxidant activity

The influence of freeze-drying process on total phenolic content and antioxidant activity of basil leaves are presented in Figure 2. In this study, the highest TPC was found for freeze-dried leaves (4.76 mg GAE/g DM) compared to only 0.32 mg GAE/g DM for fresh leaves (Figure 2A).

In the case of the antioxidant activity (Figure 2B), the trends are similar to the one observed for the total phenolic content. Freeze dried leaves remains the samples which display higher antioxidant activity (295.00 µM TE/g DM) compared to fresh leaves (42.76 µM TE/g DM). These results are in agreement with those of Bušić et al. (2014) who determined that both TPC and antioxidant activity of fresh samples were lower than freeze dried basil samples.

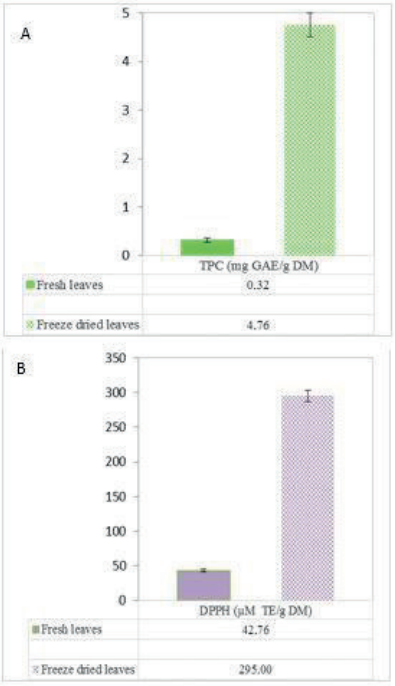

Figure 2. Total phenolic content (A) and antioxidant activity (B) of common basil leaves

Moreover, these findings are not surprising, since freeze-drying is known as the best solution to preserve foods' quality (Raponi et al., 2017). Recent work of Pistelli et al. (2020) also reported results of TPC in accordance with the results of our study (3.75 and 4.25 mg GAE/g DM for natural dried leaves of basil). Zlotek et al. (2016) founded slightly higher TPC and DPPH results compared to present study, but this could be attributed to the different type of solvents, method of extraction and sample origin.

CONCLUSIONS

The changes in volatile oil constituents during freeze-drying vary due to the different boiling points of the compounds. Although freeze drying is one of the most recommended techniques for herbs drying, significant changes can occur in the chemical composition of the essential oil of *Ocimum basilicum* L.

Table 2. Variation in chemical composition of *Ocimum basilicum* L. leaves after processing

	Compound	Chemical class	*RT	Fresh leaves (%)	Freeze-dried leaves (%)	**Ref
1	Camphene	Monoterpenes hydrocarbons	8.86	0.19	0.13	Pistelli et al., 2020; Tshilanda et al., 2016; Ghasemi Pirbalouti et al., 2013; Amaral-Baroli et al., 2016
2	Sabinene	Monoterpenes hydrocarbons	10.27	0.26	0.16	Amaral-Baroli et al., 2016; Sonmezdag et al., 2018
3	β-Pinene	Monoterpenes hydrocarbons	10.36	0.55	0.37	Koroch et al., 2017; Sonmezdag et al., 2018
4	1-Octen-3-ol	Alkenyl alcohol	10.78	0.16	0.12	Tshilanda et al., 2016; Ghasemi Pirbalouti et al., 2013
5	2,3-Dehydro-1,8-cineole	Pyrans	11.18	0.02	0.03	
6	β-Myrcene	Monoterpenes hydrocarbons	11.38	0.54	0.31	Tavallali et al., 2020
7	3-Octanol	Aliphatic alcohol	11.64	0.03	0.02	Amaral-Baroli et al., 2016
8	α-Phellandrene	Monoterpenes hydrocarbons	11.94	0.10	0.09	Tavallali et al., 2020
9	α-Terpinene	Monoterpenes hydrocarbons	12.52	0.27	0.29	Sonmezdag et al., 2018; Tavallali et al., 2020
10	p-Cymene	Alkylbenzene	12.88	0.25	0.26	Tavallali et al., 2020
11	1,8 Cineole	Oxygenated monoterpenes	13.16	5.22	3.34	Koroch et al., 2017
12	trans-β-Ocimene	Monoterpenes hydrocarbons	13.75	0.04	0.03	Sonmezdag et al., 2018; Tshilanda et al., 2016; Tavallali et al., 2020
13	β-Ocimene	Monoterpenes hydrocarbons	14.21	0.84	0.28	Tavallali et al., 2020
14	γ-Terpinene	Monoterpenes hydrocarbons	14.57	0.89	0.66	Sonmezdag et al., 2018; Tshilanda et al., 2016
15	cis-Sabinene hydrate	Oxygenated monoterpenes	14.91	0.26	0.11	Pistelli et al., 2020; Tshilanda et al., 2016
16	1-Octanol	Primary alcohol	15.35	0.04	0.04	Al-Maskri et al., 2011
17	Terpinolene	Monoterpenes hydrocarbons	16.00	0.05	0.32	Tavallali et al., 2020
18	cis-β-Terpineol	Oxygenated monoterpenes	16.30	0.12	0.05	Anand et al., 2019
19	Linalool	Oxygenated monoterpenes	16.61	27.59	18.14	Sonmezdag et al., 2018; Tavallali et al., 2020
20	Nonanal	Aldehyde	16.77	1.06	0.58	Jiang et al., 2016
21	1-Octen-3-ol acetate	Ester	17.20	0.06	0.04	Tavallali et al., 2020
22	(E)-p-2-Menthen-1-ol	Oxygenated monoterpenes	17.33	0.07	0.07	
23	4-Acetyl-1-methylcyclohexene	Oxygenated monoterpenes	17.72	0.12	0.16	
24	Camphor	Oxygenated monoterpenes	18.14	0.84	0.67	Sonmezdag et al., 2018; Tavallali et al., 2020
25	trans-2-Nonen-1-al	Primary alcohol	19.00	0.05	0.03	
26	Isoborneol	Oxygenated monoterpenes	19.10	0.07	0.05	Amaral-Baroli et al., 2016;
27	α-Terpineol	Oxygenated monoterpenes	19.20	0.09	0.08	Calín-Sánchez et al., 2012
28	Terpinen-4-ol	Oxygenated monoterpenes	19.62	3.80	2.60	Tshilanda et al., 2016
29	α-Terpinol	Oxygenated monoterpenes	20.16	0.88	0.69	Koroch et al., 2017
30	Methyl chavicol	Oxygenated monoterpenes	20.50	11.43	4.93	Koroch et al., 2017; Tshilanda et al., 2016
31	n-Octyl acetate	Ester	21.17	0.19	0.16	Pistelli et al., 2020;
32	Linalyl acetate	Oxygenated monoterpenes	22.76	0.10	0.28	Tavallali et al., 2020
33	L-α-Bornyl acetate	Oxygenated monoterpenes	23.73	3.58	2.61	Tavallali et al., 2020
34	exo-2-Hydroxycineole acetate	Oxygenated monoterpenes	25.68	0.10	0.08	Özcan et al., 2002
35	δ-Elemene	Sesquiterpene hydrocarbons	25.94	0.19	0.23	Tavallali et al., 2020
36	Eugenol	Allylbenzene	26.19	7.30	9.11	Koroch et al., 2017; Tavallali et al., 2020
37	α-Copaene	Sesquiterpene hydrocarbons	26.80	0.17	0.27	Koroch et al., 2017; Tavallali et al., 2020
38	β-Cubebene	Sesquiterpene hydrocarbons	27.28	0.14	0.24	Koroch et al., 2017; Tavallali et al., 2020

39	β-Elemene	Sesquiterpene hydrocarbons	27.35	0.46	0.81	Koroch et al., 2017; Tavallali et al., 2020
40	α-Cubebene	Sesquiterpene hydrocarbons	27.46	0.08	0.14	Calín-Sánchez et al., 2012
41	Methyleugenol	Phenylpropene	27.76	0.45	0.47	Pistelli et al., 2020; Tavallali et al., 2020
42	Caryophyllene	Sesquiterpene hydrocarbons	28.18	0.17	0.27	Ahmed et al., 2019;
43	α-Bergamotene	Sesquiterpene hydrocarbons	28.79	6.84	11.81	Pistelli et al., 2020;
44	cis-β-Farnesene	Sesquiterpene hydrocarbons	28.98	0.14	0.21	Amaral-Baroli et al., 2016
45	Epi Bicyclosesquiphellandrene	Sesquiterpene hydrocarbons	29.05	0.29	0.55	Ahmed et al., 2019; Al-Maskri et al., 2011
46	a-Humulene	Sesquiterpene hydrocarbons	29.26	0.45	0.71	Ghasemi Pirbalouti et al., 2013
47	γ-Muurolene	Sesquiterpene hydrocarbons	29.56	0.55	1.25	Amaral-Baroli et al., 2016
48	Germacrene D	Sesquiterpene hydrocarbons	30.12	2.47	3.97	Sonmezdag et al., 2018
49	(E)-β-Famesene	Sesquiterpene hydrocarbons	30.27	0.66	1.27	Pistelli et al., 2020;
50	Bicyclogermacrene	Sesquiterpene hydrocarbons	30.60	1.17	1.65	Pistelli et al., 2020; Tavallali et al., 2020
51	α-Selinene	Sesquiterpene hydrocarbons	30.85	2.08	3.62	Amaral-Baroli et al., 2016
52	γ-Cadinene	Sesquiterpene hydrocarbons	31.14	2.65	5.28	Tavallali et al, 2020
53	δ-Cadinene	Sesquiterpene hydrocarbons	31.45	0.46	1.09	Tavallali et al., 2020
54	Cubedol	Oxygenated sesquiterpenes	32.10	0.11	0.24	Maurya et al., 2019
55	4-epi-Cubedol	Oxygenated sesquiterpenes	32.36	0.15	0.17	Tshilanda et al., 2016
56	Nerolidol	Oxygenated sesquiterpenes	32.64	0.15	0.30	Tavallali et al., 2020
57	Spathulenol	Oxygenated sesquiterpenes	32.96	0.14	0.78	Tavallali et al., 2020
58	1,10-di-epi-Cubenol	Oxygenated sesquiterpenes	34.06	1.50	2.27	Milenković et al., 2019
59	α-epi-Cadinol	Oxygenated sesquiterpenes	34.82	10.52	14.30	Koroch et al., 2017; Tavallali et al., 2020
60	β-Eudesmol	Oxygenated sesquiterpenes	35.02	0.27	0.42	Koroch et al., 2017; Tshilanda et al., 2016
61	α-Cadinol	Oxygenated sesquiterpenes	35.15	0.55	0.77	Koroch et al., 2017

*RT-retention time; **references where similar compounds were found.

No significant changes in chlorophyll content was observed. Given the high content of phenolic compounds and antioxidant activity of dried leaves, the freeze-drying is a sustainable processing technique for preservation of phenolic compounds and antioxidant activity. Further studies and trials are required in order to optimize the freezing temperature for a better understanding of the freeze-drying temperature effect on the quality of organic *Ocimum basilicum* L.

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USE OF MOLECULAR MARKERS IN IMPROVING RESISTANCE TO BIOTIC STRESS IN SOLANACEAE - A REVIEW

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Abstract

Solanaceae family comprises tens of genera and thousands of species, including numerous cultivated plants such as tomato, potato, eggplant, tobacco, deadly nightshade and petunia, used for human diet, ornamental and pharmaceutical purpose as well as biological model systems. Plants must continuously defend against attacks from pest, viruses, bacteria and fungi for improving biotic stress tolerance. Molecular markers linked to specific genes responsible for resistance / tolerance to pathogens or pests can be used to accelerate the breeding process in order to create new varieties that are not only desirable for the quality of the end product but are also resistant to biotic stress. This review brings together data referring to molecular markers linked to various phenotypic traits related to plant biotic stress resistance and the benefits of resistance versus chemical protection.

Key words*: biotic stress, molecular markers, SNP, SSR, resistance, Solanaceae.*

INTRODUCTION

The Solanaceae family comprises species present on all continents except Antarctica, species with various durations of life cycle and adapted to a wide range of life environments. Members of this family are used not only for human diet or ornamental purposes, but also in the pharmaceutical field, since some of the substances they metabolize have medicinal properties, and last but not least they are useful as model plants in scientific research. As the gene content of the different species remains constant despite their different phenotypes, Solanaceae family represents an excellent model for studying plant adaptation to natural or agricultural environments (Mueller et al., 2009).

During their life cycle, plants must continuously defend against attacks from pests, viruses, bacteria and fungi. Traditional cultivated tomato lack genetic diversity. Therefore, it has been suggested to transfer the desired resistance traits from their wild type relatives (Rick & Chetelat, 1995). Plant breeders are looking for novel techniques to hasten the breeding process with the aim of creating new varieties of plants tolerant/ resistant to biotic stress. In the past three decades, with the advent of large scale DNA sequencing, new ways to improve the classic breeding techniques have been discovered. More and more genes putatively responsible for specific or broad resistance to biotic stress are being revealed every year, and changes in the DNA composition, either in the coding regions or in the regulatory regions, led to the identification of numerous molecular markers that are linked to tolerance/resistance to biotic stress. For instance, Paterson et al. (1988) used for the first time a complete Restriction Fragment Length Polymorphism (RFLP) linkage map in tomato to identify quantitative trait loci (QTLs).

QTL mapping can be used to further understand the genetic basis of various traits, including resistance to biotic stress (Barone et al., 2008). Once QTLs for a specific trait (e.g. resistance/susceptibility to a certain pathogen or pest) have been identified, there are two

avenues of follow-up research. In case the genome of the species under study is not sequenced, the identified QTL region can be sequenced. Then, the putative functions of the genes found within that region can be assessed by sequence similarity comparisons with homologue genes from other organisms. In case the genome of the species under study has been sequenced, and the genes from that particular QTL region already identified, the focus of next studies will be on the gene/genes connected to the trait of interest, and what are the differences at DNA level between cultivars resistant and susceptible to a certain biotic stress. At this point, molecular markers are extremely helpful.

Molecular markers point out variations in DNA and can appear due to DNA mutations, such as substitutions (point mutations), rearrangements (deletions or insertions) and repeated DNA sequences. Markers localized within DNA close to the genes of interest are called gene tags (Collard et al., 2005). Plant breeding that uses molecular markers - marker-assisted selection (MAS) - has numerous advantages: selection of genotypes at seedling stage (hence time saving compared to traditional methods of selection), gene pyramiding (combining multiple genes responsible for a particular trait in a single genotype), avoidance of the transfer of undesirable genes, selection of traits with low heritability, etc. (Devi et al., 2017). For example, gene pyramiding is extremely useful tool when resistance to some biotic stress factors is controlled in small measure by several genes, rather than strong resistance due to only one or two main genes, as is the case of resistance to late blight (Adhikari et al., 2017).

The present review aims to catalogue data related to the use of molecular markers in species belonging to Solanaceae family, to point out the progress made using them for developing resistance to different pathogens and pests that affect species belonging to this family, as well as the benefits of resistance versus chemical protection.

An overview on molecular markers used for Solanaceae family studies

Some of the molecular markers employed to help the breeding process of cultivated plants from Solanaceae family are Single Nucleotide Polymorphism (SNP), Random Amplification of Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Cleaved Amplified Polymorphic Sequence (CAPS), Sequence-Related Amplified Polymorphism (SRAP), Single-Nucleotide Amplified Polymorphism (SNAP), Conserved Orthologs Set (COS), Sequence Characterized Amplified Region (SCAR) (see Table 1).

Once the markers have been mapped to a specific place in the genome, they can be linked to various genes responsible to desirable/undesirable traits and can be used for selection in the breeding process.

Table 1. Molecular markers used for Solanaceae species

Marker type	Species	Reference
SNP	eggplant	Barchi et al., 2011 Acquadro et al., 2017
	potato	Hamilton et al., 2011 Draffehn et al., 2013 Mosquera et al., 2016 Berdugo-Cely et al., 2017 Enciso-Rodriguez et al., 2018 Santa et al., 2018 Tagliotti et al., 2018
	tomato	Jimenez-Gomez and Maloof, 2009 Sim et al., 2011 Hamilton et al., 2012 Sim et al., 2012 Iquebal et al., 2013 Viquez-Zamora et al., 2013 Kevei et al., 2015
	Solanum pimpinellifolium L.	Celik et al., 2017
RAPD	eggplant	Demir et al., 2010 Toppino et al., 2008
	pepper	Ilbi, 2003
	okra	Aladele et al., 2008 Nwangburuka et al., 2011 Prakash et al., 2011 Ikram-ul-Haq et al., 2013 Kaur et al, 2013
CAPS	eggplant	Toppino et al., 2008
	potato	van der Voort et al., 2000
	tomato	Yang et al, 2014
SSR	tomato	He et al., 2003; Ruiz et al., 2005; Grushetskaya et al., 2007; Sim et al., 2011; Ning et al., 2012; Todorovska et al., 2014
	eggplant	Stàgel et al., 2008; Tümbilen et al., 2011; Demir et al., 2010; Barchi et al., 2011
	potato	Feingold et al., 2005; Ghislain et al., 2001; Ghislain et al., 2009
	pepper	Kim et al, 2008; Ibarra-Torres et al, 2015
	russian box thorn	Chen et al., 2017
	cape gooseberry	Garzon-Martinez et al., 2012

AFLP	potato	van der Voort et al., 2000; Ghislain et al., 2001; Isidore et al., 2003; van Os et al., 2006
	tomato	Ning et al., 2012
	pepper	Caranta et al., 1999
	okra	Akash et al., 2013; Kyriakopoulou et al., 2014
RFLP	pepper	Kim et al, 2008
	tomato	Tanksley et al., 1992
	potato	Tanksley et al., 1992
	Solanum pimpinellifolium L.	Sharma et al., 2008
ISSR	Pepper	Ibarra-Torres et al., 2015
SRAP	tomato	Ruiz et al., 2005; Shaye et al., 2018
	eggplant	Li et al., 2010
SNAP	pepper	Kim et al., 2008
COS	potato	Lindqvist-Kreuze et al, 2013
SCAR	tomato	Yang et al., 2014

Molecular markers application to improve the resistance of Solanaceae species to some biotic stress factors

Molecular markers application for viruses resistance

Resistance to multiple viruses (tomato mosaic virus, ToMV; tomato spotted wilt virus, TSWV; tomato yellow leaf curl virus, TY-LCV) and additional resistance to *Verticillium* and *Fusarium oxysporum* Schlecht. present in "Anastasia" tomato variety can be detected using both SSR and SRAP marker systems (Ruiz et al., 2005). SSR markers used in the study could be used to discriminate among the three main cultivar types used in the study, but not among cultivars of the same type that had different phenotypes. Nevertheless, all cultivars studied could be differentiated using SRAP markers.

Potato virus Y (PVY) resistance can be classified into two main types of resistance: extreme resistance (ER), which shows either no symptoms or limited necrosis, and hypersensitive resistance (HR), that manifests either local necrotic lesions or systemic necrosis (Solomon-Blackburn & Barker, 2001). Kasai et al (2000) developed SCAR markers linked to Ry_{adg} gene in potato and PCR - based DNA markers linked to two ER genes ($Ry_{sto}$and Ry_{adg}) were used for MAS in potato by Heldak et al. (2007). In pepper resistance gene *Pvr4* was tagged using AFLP markers (Caranta et al., 1999). In the same study, one marker (the closest to *Pvr4* gene) was transformed to CAPS marker in order to aid MAS, especially *pvr* gene pyramiding in a single cultivar.

Yellow leaf curl virus, transmitted by the white fly, causes severe loss of production in tomatoes (Cohen and Lapidot, 2007). Resistance to this virus has been mapped to chromosome 11, to the *Ty-2* gene (Yang et al., 2014).

Molecular markers application for bacteria resistance

Ralstonia pseudosolanacearum causes the bacterial wilt in numerous Solanaceae species (Heyward, 1991). One QTL marker related to bacterial wilt resistance has been identified in tomato on chromosomes 6 using SSR markers (Geethanjali et al., 2010). This QTL has been confirmed and an additional QTL has been identified on chromosome 12 by Wang et al., (2013) using recombinant inbred lines (RILs). Recently, Kim et al. (2018) identified 265 SNPs located in these two QTLs that are responsible for resistance/susceptibility to bacterial wilt. One of these SNP markers located within a functional gene on chromosome 12, *Solyc12g009690.1*, may be used to select varieties resistant to bacterial wilt. In eggplant, resistance QTLs were identified on chromosomes 3 and 6 (Salgon et al., 2017).

Streptomyces scabiae Thaxter causes scarring of the potato tubers surface, reducing their quality and marketability. Enciso-Rodriguez et al. (2018) have identified a novel SNP locus for common scab resistance, mapped in a WRKY transcription factor region, on chromosome IX.WRKY transcription factors are known for their role in controlling systemic and acquired resistance, as they are either activating or repressing genes responsible for defence-related proteins synthesis.

Molecular markers application for fungi resistance

Early blight is caused by the fungus *Alternaria solani* Sorauer. Resistance to this disease is controlled by multiple genes, out of which none is conferring a major resistance (Adhikari et al., 2017). Furthermore, even if some wild species are resistant to early blight, the cultivated varieties that are moderately resistant have undesired traits as low yield and late maturity. Combining the QTLs from different species by

gene pyramiding may produce a variety resistant to early blight (Adhikari et al., 2017).
Arafa et al. (2017) identified two genes on tomato chromosome 6 that could confer resistance to tomato late blight produced by *Phytophtora infestans* Mont., result validated by SSR analysis. For potato, numerous studies on QTL mapping have been performed. QTLs for *late blight* resistance are present on chromosomes I, III, V, VII, VIII, IX, XII (Ghislain et al., 2001; Visker et al., 2003; Mosquera et al., 2016; Santa et al., 2018). The conclusion of the QTL studies was that polygenic resistance is more efficient and durable as opposed to resistance conferred by Rpi-genes (R genes to *P. infestans*), which was less effective. However, the drawback of polygenic resistance in potato is the late maturity associated with the resistance, trait that is not desired by the breeders and growers (Danan et al, 2011). Nevertheless, using SNP markers, Draffehn et al. (2013) were able to select plants that had improved quantitative resistance to late blight, but were not compromised by late maturity. Furthermore, Mosquera et al. (2016) identified SNP markers in potato associated with quantitative resistance to late blight but not linked to late maturity. One of the R genes to *P. infestans* in *Solanum bulbocastanum* Dunal, a wild *Solanum* species, is the *RB* gene, mapped to chromosome 8, using RFLP and RAPD molecular markers (Naess et al., 2000). In a subsequent study, Colton et al. (2006) developed a PCR-based DNA marker for tracking the *RB* gene throughout the breeding process, while transferring this R-gene from *S. bulbocastanum* to *S. tuberosum*.
Root rot and seedling damping off is caused by *Phytophthora capsici* Leonian in pepper. Kim et al (2008) constructed a linkage map using RFLP and SSR markers. They developed SNAP, SSR and CAPS markers to QTL loci responsible to resistance to *P. capsici*.
Toppino et al. (2008) designed CAPS markers to be used for indirect selection of *Fusarium* resistance in eggplant. They initially performed an initially study of resistance inheritance using *Solanum aethiopicum* L. as a resistance donor, and discovered that one gene, *Rfo-sa1*, was responsible for the *Fusarium* resistance. Subsequent Bulk Segregant Analysis with RAPD markers led to the identification of three RAPD markers linked to the resistance trait, that were subsequently transformed to CAPS markers to be used for selection in future studies.

Molecular markers application for pest resistance

Even though potato cyst nematode broad-spectrum resistance in potato was considered to be a complex inherited trait. Van der Voort et al. (2000) demonstrated that actually two loci are responsible for *Globodera pallida* resistance: *Gpa5* and *Gpa6*. Both loci were mapped to chromosomes 5 and 9 using initially an AFLP marker online catalogue. Thereafter, the loci were identified using CAPS markers based on RFLP insert sequences. *Gpa 5* locus is located on chromosome 5 in a region that is also responsible for fungal and viral resistance. *Gpa 6* appears to be is also located in a resistance cluster on chromosome 9, which contains a virus resistance gene in a homologous tomato genome.
Santa et al. (2018) identified 15 QTLs linked to resistance to *Tecia solanivora*, guatemalan potato moth tuber, out of which 10 were identified in the phenotypic field trial and 10 in the storage conditions. Furthermore, seven QTLs out of the total fifteen QTLs identified were related to resistance and eight QTLs were related to susceptibility to *T. solanivora*.
Avila et al. (2019) have identified QTLs for resistance to tomato-potato psyllid (TPP) in tomato wild relatives. Some of the accessions that have shown good resistance to TPP, also shown resistance to the bacterium *Candidatus* Liberibacter solanacearum (Lso).
Sun et al. (2020), using SNP markers, mapped resistance to aphid, *Myzus persicae,* to three QTL loci, two on chromosome 2, and one on chromosome 4. The QTL loci on chromosome 2 are affecting aphid survival and reproduction, whereas the locus on chromosome 4 is affecting aphid reproduction. The fine mapping of the locus on chromosome 2 affecting reproduction identified a DNA region containing resistance genes of the receptor-like kinase family containing a leucine-rich repeat domain (LRR-RLKs).

CONCLUSIONS

During their development, plants must continuously defend against attacks from viruses, bacteria, fungi and pests. Wild plants have developed in time resistance against such attacks, but cultivated plants, since they have been selected through domestication for different traits (yield, colour, taste, size, etc.) have lost resistance/tolerance to pathogens and pests. For instance, compared to the wild species of tomato, that have a large genetic diversity, cultivated varieties of tomato have much less diversity, less than 5% of the genetic variation of the wild species (Bai & Lindhaut, 2007).

Wild plants, relatives of the cultivated ones can be used a reservoir for genetic resistance. The molecular markers can be employed to verify the transfer of the desired DNA regions that control useful traits including resistance/tolerance to biotic stress during the breeding process. Genetic resistance, along with other desirable traits (taste, shape, size, etc.), can be transferred as well by marker-assisted selection from some established traditional cultivars. In order to make this possible, numerous studies to detect which of the cultivars are resistant to one or more pathogens /pests have been performed (Mihnea et al., 2019; Mîndru et al., 2019). Once the cultivars with the desired trait are determined, the breeders may proceed to marker-assisted selection. The marker-assisted selection is not only accelerating the breeding process, but is also making it more efficient and is changing the breeding focus from phenotype selection to gene selection (Bai & Lindhaut, 2007).

Chemical protection of cultivated plants is used successfully against a wide range if organisms (bacteria, fungi, insects, etc.). However, their use has several strong disadvantages: development of pesticide resistance, water, soil and air pollution, disappearance of pollinators, etc. Developing plants with genetic resistance negates the need for chemical protection and subsequently eliminates its negative effects. As novel resistance genes are identified, they should be tagged and used in the creation of new varieties of plants resistant to common pathogens and pests.

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INFLUENCE OF ORGANIC FERTILIZATION ON THE NUTRITIONAL REGIME OF TOMATOES

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Abstract

During the period 2012-2014, in steel-glasshouse on the experimental field of Agricultural University - Plovdiv, was carried out a study about elements of organic tomatoes production technology. The following organic fertilizers have been tested: Evrobio; Osmo Bio garden; Biofa; Orgamax; Agrobiosol; Naturale; Lumbrikompost; Alga 600 PO 2; Hemozim bio 5 N5P3K6; StimAK; Softgard. The organic fertilizers have been introduced in their recommended norms. We have explored the influence of fertilization with organic soil fertilizers and foliar spray on productivity of greenhouse grown tomatoes by late production technology. It was established the nutritional substances were assimilated during the whole vegetation period but with different intensity.More intensive assimilation of N, P, K from the beginning of fruit formation to mass bearing was established in both variants with fertilizers in the soil alone, and with soil fertilizers and foliar sprays.

Key words*:greenhouse tomato, biological production, assimilation of nutritional substances.*

INTRODUCTION

Organic vegetable production, under greenhouse conditions in particular, usually is related to lower yields. Some of the performed studies state this assertion (Pascale, 2004). Most commonly, the studies are done on separate elements of the technology of organic production, or only separate organic fertilizers are tested for root, leaf or combined application (Chapagain & Wiesman, 2004; Gravel et al., 2012; Hidalgo-Gonzales et al., 1998; Kolota & Osinska, 2000; Márquez-Hernández et al., 2013; Tringovska, 2012**;** Yu et al., 2010; Liu et al., 2012; Martins et al., 2010, Nakano, 2003; Surrage et al., 2010; Yildirim, 2007; Pascale et al. 2004). The results in this case are quite multidirectional, too. The number of the studies is low, and in our country we lack surveys on the combined effect of a larger range of organic fertilizers with root, leaf, nutrient reserving and vegetative application under greenhouse conditions.

In order to optimize biological fertilization, it is necessary to establish the absorption of nutrients. In such studies for other conventional vegetable crops - eggplant, tomatoes and peppers (Villora et al., 1988; Boteva & Kostova, 2009; Doikova & Rankov, 2003) state that the absorption of plant nutrients is strongly influenced by the yield and the formed vegetative mass.

To clarify the matters about nutritional regime of the greenhouse tomatoes, an experiment was set when keeping the requirements of organic production.

MATERIALS AND METHODS

For the period 2012-2014 a soil experiment was set to study the elements from the technology for organic tomato production. The effect of fertilization with organic fertilizers and combinations from them on the nutritional regime of the greenhouse tomatoes - grown in accordance with the technology for late production was studied (Kartalov et al., 1979). The experimental work was conducted in the steel-glasshouses in the experimental field of the Agricultural University - Plovdiv with indeterminate tomatoes - sort Fado F_1. The experiment was conducted in geoponic environment under all the requirements for organic production (with application of a complete technology for organic production). A drip irrigation system was used, which is also

used for fertigation with the liquid organic fertilizers. Plant protection was applied with organic agents. Various combinations between 8 organic fertilizers for root fertilization and 3 fertilizers for leaf application were studied. The following 15 variants were studied: 1. N_{44}:P_8:K_{52}; 2. Agrobiosol + Osmo Bio garden + Biofa; 3. Lumbrikompost + Osmo Bio garden + Alga 600 PO 2; 4. Orgamax + Hemozim bio 5 $N_5P_3K_6$ + Biofa; 5. Agrobiosol + Hemozim bio 5 $N_5P_3K_6$ + Biofa; 6. Evrobio + Lumbrikompost + Hydrolysed proteins + Softgard; 7. Orgamax + Lumbrikompost + Stimak+ Softgard; 8. Agrobiosol + Lumbrikompost + Stimak + Softgard; 9. Naturale + Lumbrikompost + Stimak+ Softgard. One variant with mineral fertilization was used as a control: NH_4NO_3, TSP (46% P_2O_5) and K_2SO_4 under optimal levels for greenhouse tomatoes - N_{44}:P_8:K_{52}. Two of the organic fertilizers were applied on vegetative growth through fertigation: StimAK and Hemozim. The organic fertilizers were used in the recommended norms - not vegetative and vegetative (four times - from the beginning of fruit formation every other 15 days). The foliar spray was performed twice with an interval of 10 days, starting three weeks after planting. The plants were grown from seedlings in a heated steel-glasshouse with period for sowing - the first ten days of January and planting in the third ten days of March. The field experiment was setup in 4 repetitions with 14 plants in each repetition (Barov, 1982). The following planting scheme was applied: 40+85+70+85+40 x 42.5 cm with 28000 plants/ha and nutritional area per plant of 3400 cm^2. The plants formed with one stem, the tops were pruned 50 days before the last harvesting. The productivity of the plants was determined as: early yield - up to the fifth pick - kg/m^2; total yield - up to the end of vegetation - kg/m^2. Mineralization of plant material for analysis of P and K was performed using dry ashing and subsequent extraction with 2 M HCl (Chapman & Pratt, 1961). Phosphorus and potassium were then quantified by spectrophotometry and flamephotometry, respectively. Total N was determined by the Kjeldahl method.

Agrobiosolis an organic fertilizer - granular biomass with no additives from conventional materials. Contains dry matter - 95.6%; organic matter - 90.7%; pH ($CaCl_2$) - 3; humidity - 4%; N (total) - 6-8%; phosphates (P_2O_5) - 0.5-1.5%; potassium (K_2O) 0.5-1.5%; C: N 6: 1; CaO 0.21%; MgO 0.05%; Cl, 0.04%; With 1.80%; Zn 6.0 mg/kg; Fe 101 mg/kg; B 7.1 mg/kg; other trace elements and vitamins.

Orgamax is a soil, organic - humic fertilizer made from carefully selected and processed lignites. It is of organic origin and is free from pathogens and heavy metals. With its rich in organic matter and humic substances composition, it improves the chemical properties of the soil (cation exchange capacity), thus making more nutrients in the soil available to the plants, creating better conditions for their assimilation. Suitable for greenhouse, vegetable production. Contains a guaranteed composition of 1% organic nitrogen N; 30% total organic matter (17.4% organic hydrocarbon); 7% humic substances; 8% sulfur (SO_3); 1% iron (Fe); 5-6 pH; 17/1 C/N ratio; 8% max moisture.

Eurobio P 26 N - pro is an organic fertilizer containing P, CaO and the patented N-pro complex. Phosphorus feeding increases with time; calcium neutralizes soil pH and creates a microenvironment that facilitates the absorption of all soil micro and macroelements; By improving soil pH, Eurobio activates the bacterial flora and stimulates the mineralization process primarily of nitrogen. The patented N-pro complex helps to facilitate the mineralization of organic matter in the soil by converting nitrogen into a plant accessible form - nitrate, nitrite and ammonia.

Naturale NPK 8-8-6 contains organic nitrogen 8%; P_2O_5 - 8%; K_2O - 6%; MgO - 2%; Organic Biocarbon - 30%. It is a high quality organic fertilizer, both in terms of raw materials used and in terms of its extremely low humidity level. It is obtained as a result of the exclusive use of organic substances of vegetable origin, bone meal and organic products, which is why it manages to ensure a gradual and continuous supply of nitrogen, thus providing the plants with nutrients throughout the cultivation cycle. Immediate over dosage with nitrogen, leading to strong vegetative growth and weakening of plants, is avoided. It is used in the form of pellets with sizes ranging from 3-4 mm. in diameter and 8-10 mm. length and humidity not exceeding 10%. The pellets produced in this

way are excellent for spreading with all types of fertilizer spreaders and, after being in the soil, quickly disintegrate due to the fact that they absorb up to 4 times more water than their own weight.

LK (Lumbrikompost) N1.71: P3.49: K1.71: Ca 6.25: Mg 2.14 - Organic fertilizer from California worms. Biohumus (lumbricompost) is a product that results from the vital activity of the red California worms (*Lumbricus rubellus* and *Eisenia foetida*), which feed on organic residues. After being processed by the body of the worms, these raw materials change extremely favorably. Worm faeces are high humus fertilizer. Contains a large amount of beneficial bacteria and other microorganisms, many biologically active plant stimulants, vitamins, amino acids and antibiotics added to it during the digestive process of the worm

Osmo Bio garden 6-5-7 (+4) is organic granular fertilizer for general nutrition in greenhouse plants from March to September. Provides fast nourishment and does not burn plants. The special composition of premium materials guarantees a slow release period of 3-6 months and keeps the soil structure in good condition. It is suitable for growing vegetables. The product contains seaweed, which provides the plant with a wide range of essential trace elements. The special formula guarantees remarkable results in the development of each plant in a greenhouse. Fertilizer improves soil structure and fertility. Suitable for organic production.

Alga 600 PO 2 is an organic liquid leaf fertilizer containing N 5%; P_2O_5 4%; K_2O 15%; amino acids > 1%; PGR enzymes (plant hormones); OM organic matter > 15%; pH 8-9. Organic substances contained in Alga 600 PO 2 are a formulation of organic liquid fertilizer, which thanks to its formula acts as a rapidly digestible complex food. Increases the plant's resistance to drought and frost. L-Amino acids together with N and K increase protein synthesis. Stimulates photosynthesis and absorption of nutrients. Promotes the synthesis of sugars (starch). Creates reserves of nutrients (tubers). Increases vitamin content in plants. Acts as an organic catalyst. Has a positive effect on the quantity and quality of production.

Biofa is a natural extract of brown algae used as antistress factor and nutritional supplement in plants. Contains: dry matter -10.89%, pH-7.4, organic carbon - 26.0%, total nitrogen (N) - 0.20%, total phosphorus (P_2O_5) - 0.011%, total potassium (K_2O) - 0.20%, total calcium (CaO) - 0.12%, total magnesium (Mg) - 0.05%, total sulfur (S) - 0.24%. Trace elements in ppm: Cu - 0.81; Zn - 4.10; B - 8.7; Mn - 0.43; Fe - 4.18; Mo- 0.03.

Stimak is a multicomponent, amino acid fertilizer derived from hydrolyzed vegetable and yeast proteins with 30% dry matter.Used as a biostimulator for plants to promote their growth and sustainability. It contains a dry matter of not less than - 44%, of which: organic substance - 82% and amino acids - 35%, total nitrogen (N) - 3.8%, total phosphorus (P_2O_5) - 4%, total potassium- (K_2O) - 4.4%, total calcium (CaO) - 0.035%, total magnesium (MgO) - 0.22%; trace elements (mg/kg): zinc - 75, copper - 4, manganese - 24, boron - 38, iron - 50.

Softgard is a Coftgap limited edition. that contain: N - 5%; P_2O_5 - 4%; K_2O - 3%; Cu (xylene) > 2%; Zn (xelatene) > 1%; Chitosan > 2.6%; OM > 5%; pH 4-6.

RESULTS AND DISCUSSIONS

The amount of nutrients extracted by the yield and vegetative massis different. This is due to the changes in the mineral composition of the plants, the plant mass and the yield (Table 1).

First are the group of variants in which the foliar treatment with Softgard is applied against a background of Lumbrikompost (LK) and Stimak fertigation. The total amount of N, P_2O_5 and K_2O absorbed is highest after the introduction of Orgamax + LK + Stimak + Softgard - 30.72 kg/da, of which 10.43 kg/da N, 6.44 kg/da P_2O_5 and 13.85 kg/da K_2O. They are followed by the plants grown after application of Agrobiosol + LK + Stimak + Softgard - 28.63 kg/da (9.80 kg/da N; 6.44 kg/da P_2O_5; 13.85 kg/da K_2O) and Evrobio + Lumbrikompost + Stimak + Softgard - 25.55 kg/da of which 9.67 kg/da N; 5.73 kg/da P_2O_5; 10.15 kg/da K_2O. Last but not least in this group, the plants fertilized with Naturale + LK + Stimak + Softgard– 25.54 kg/da of which 9.46 kg/da N; 6.38 kg/da P_2O_5; 9.70 kg/da K_2O.

After this group of variants is placed the control variant torus with N_{44}: P_8: K_{52} - 23.97 kg/da, of which 9.08 kg/da N, 5.83 kg/da P_2O_5 and 9.05 kg/da K_2O.

Fewer nutrients from the control have mastered the options fertilized with Osmo organic fertilizer. The highest nutrients in this group were absorbed by plants grown after LK + Osmo + Alga - 21.73 kg/da (8.11 kg/da N; 4.90 kg/da P_2O_5; 8.72 kg/da K_2O). Agrobiosol + Osmo + Biofa fertilizers rank - 23.71 kg/da, of which 7.98 kg/da N, 4.61 kg/da P_2O_5 and 11.12 kg/da K_2O.

At least nutrients are absorbed by the plants after application of Orgamax + Hemozim + Biofa - 20.50 kg/da, of which 6.88 kg/da N; 4.56 kg/l P_2O_5; 9.07 kg/da K_2O.

Changes in the extracted amounts of N, P_2O_5 and K_2O are related to the changes in the accumulated overhead mass per decare. The amount of nitrogen and potassium absorbed from the soil by the stemsand inflorescences is highest when fertilizing with Agrobiosol + Osmo + Biofa - 2.87 kg and 5.64 kg respectively, and phosphorus after fertilizing with Agrobiosol + LK + Stimak + Softgard - 0.95. In organic fertilization, nitrogen is at least after application of Orgamax + Hemozim + Biofa - 2.01 kg, phosphorus after organic fertilization with Evrobio + Lumbrikompost + Stimak + Softgard - 0.64 and potassium after application of Evrobio + Lumbrikompost + Stimak + Softgard- 2.91 kg. In the leaves, the amount of nutrients extracted (13.69 kg) is greatest after the application of Naturale + LK + Stimak+ Softgard, respectively - 5.87 kg of nitrogen, 5.25 kg of phosphorus and 2.57 kg of potassium. The smallest amounts of N and P_2O_5 with the stalks are extracted when fertilizing with Agrobiosol + Osmo + Biofa, 3.70 and 3.27 kg, respectively. The amount of K_2O stems absorbed is at least at control N_{44}: P_8: K_{52}.

Changes in the extracted amounts of nutrients under the influence of applied fertilization and foliar spraw also lead to changes in their ratio (Table 2). In all variants except the control, the proportion of potassium, followed by nitrogen and phosphorus, prevails. Control and Naturale + LK + Stimak+ Softgard show an increase in the proportion of nitrogen at the expense of potassium.

In order to determine the rate of fertilization and the type of fertilizers used in greenhouse tomatoes, it is necessary to know the necessary quantities of nutrients to form a unit of production. The following nutrient quantities were required to form one ton of fruit from the fertilized plants - 0.96 kg to 1.95 kg for nitrogen, 0.58 kg to 1.20 kg for phosphorus and 1.03 kg to 2.59 kg for potassium (Table 3).

After organic fertilization, the highest consumption of nutrients is needed to build one ton of production from the plants grown by the combined application of Orgamax + LK + Stimak+ Softgard - 5.74 kg of which 1.95 kg of nitrogen, 1.20 kg of phosphorus and 2.59 kg of potassium, and the smallest after LK + Osmo + Alga - 2.58 kg of which 0.96 kg of nitrogen, 0.58 kg of phosphorus and 1.03 kg of potassium. In control plants, these amounts are 1.73 kg for nitrogen, 1.11 kg for phosphorus and 1.73 kg for potassium, respectively.

Compared to their total amount, the proportion of potassium is highest and phosphorus is the lowest for all variants.

Table 1. Organic export of nutrients from greenhouse tomatoes, averaged over the period 2012-2014, kg/da

Variants	stems + inflorescences + Roots			leaves			fruits		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
1. N_{44}:P_8:K_{52}	2.90	0.86	5.05	4.94	4.45	1.57	1.25	0.52	2.43
2. Agrobiosol+Osmo+Biofa	2.87	0.91	5.64	3.70	3.27	2.05	1.41	0.42	3.43
3. Lumbrikompost+Osmo+Alga	2.06	0.74	3.95	4.30	3.52	1.81	1.75	0.63	2.96
4. Orgamax+Hemozim+Biofa	2.01	0.82	4.39	3.83	3.28	1.82	1.04	0.46	2.86
5. Evrobio+LK+Stimak+Softgard	2.20	0.64	2.91	5.08	4.16	1.94	2.40	0.93	5.29
6. Orgamax+LK+Stimak+Softgard	2.38	0.73	4.50	4.91	4.44	2.32	3.14	1.28	7.03
7. Agrobiosol+LK+Stimak+Softgard	2.85	0.95	5.36	5.15	4.60	2.41	1.81	0.76	4.75
8. Naturale+LK+Stimak+Softgard	2.39	0.73	4.71	5.87	5.25	2.57	1.20	0.40	2.42

Table 2. Proportion of digested nutrients under the influence of applied fertilization and foliar spray on average for the period 2012-2014

Variants	Organic exports of Nutrients throughout the plant, kg/da			Proportion NPK, %		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
1. $N_{44}:P_8:K_{52}$	9.08	5.83	9.05	37.90	24.33	37.77
2. Agrobiosol+Osmo+Biofa	7.98	4.61	11.12	33.66	19.43	46.91
3. Lumbrikompost+Osmo+Alga	8.11	4.90	8.72	37.33	22.54	40.13
4. Orgamax+Hemozim+Biofa	6.88	4.56	9.07	33.54	22.22	44.24
5. Evrobio+LK+Stimak+Softgard	9.67	5.73	10.15	37.84	22.44	39.72
6. Orgamax+LK+Stimka+Softgard	10.43	6.44	13.85	33.96	20.96	45.08
7. Agrobiosol+LK+Stimak+Softgard	9.80	6.31	12.52	34.24	22.02	43.74
8. Naturale+LK+Stimak+Softgard	9.46	6.38	9.70	37.04	24.97	37.98

Table 3.Nutrients consumed to form 1000 kg of production on average for the period 2012-2014

Variants	Total yield, t/da	Required quantitiesnutrients per tonne of production, kg / da		
		N	P_2O_5	K_2O
1. $N_{44}:P_8:K_{52}$	5.24	1.73	1.11	1.73
2. Agrobiosol+Osmo+Biofa	6.26	1.27	0.74	1.78
3. Lumbrikompost+Osmo+Alga	8.44	0.96	0.58	1.03
4. Orgamax+Hemozim+Biofa	4.92	1.40	0.93	1.84
5. Evrobio+LK+Stimak+Softgard	5.11	1.89	1.12	1.98
6. Orgamax+LK+Stimak+Softgard	5.35	1.95	1.20	2.59
7. Agrobiosol+LK+Stimak+Softgard	7.22	1.36	0.87	1.74
8. Naturale+LK+Stimak+Softgard	7.48	1.27	0.85	1.30

CONCLUSIONS

Organic fertilizers are able to supply the need for basic nutrients for growing tomatoes in steel-glass greenhouses in late production.

The amount of nutrients extracted in 1 decade is the highest after fertilization with Orgamax + LK + Stimak + Softgard - 30.72 kg, of which nitrogen represents 33.96%, phosphorus - 20.96% and potassium - 45.08%.

The proportion of the three nutrients is not significantly affected by the fertilization variants.

The consumption of nutrients (nitrogen, phosphorus and potassium) for the formation of 1000 kg of production is higher in the background, including biological fertilizers LK and Stimak.

The amounts recovered are from 0.96 to 1.95 kg for nitrogen, from 0.58 to 1.20 kg for phosphorus and from 1.03 to 2.59 kg for potassium. The most nitrogen, phosphorus and potassium are extracted by Orgamax + LK + Stimak + Softgard plants.

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INFLUENCE OF ORGANIC FERTILIZATION ON THE PHYSIOLOGICAL BEHAVIOR OF FIELD TOMATOES

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Abstract

During the vegetation period of the year 2019 on experimental field at Agricultural University - Plovdiv it was conducted a physiological study of tomato cultivar (Rugby) with determined growth under the treatment with chemical and organic fertilizers. The purpose of this research was to examine changes of the functional activity of the plant photosynthetic apparatus of variants with different fertilization and different planting dates. It was determined more optimal ratio between photosynthetic active radiation (PAR) and quantum yield (qY-Fv/Fm) of the photosystem II (PS II) in dark-adapted leaves for organic and chemical fertilized variants, compared to no fertilized variant. Minimal fluorescence (Fo) in reaction centers of PS II after dark-adapted of leaves was with highest value at chemically fertilized variant, which means that it did not affect the photosynthetic activity. Significant differences were observed for values of chlorophyll content index (CCI) in the different variants, as most stable average result showed organic fertilized variant for three differing planting dates on experimental field.

Key words*: tomato, organic fertilizer, photosynthesis, chlorophyll content index.*

INTRODUCTION

Climate change is cumulative accepted as a worldwide phenomenon with possibly significant consequences and accompanying with recurrent extreme weather events (Stern, 2006). There has been a strong global awakening during the last few decades regarding the proper management of existing natural resources. Among them, irrigation water is one which becoming costlier due to increasing demand of human population. Simultaneously, the demand for food is also increasing, which has brought more and more land under cultivation and focused the attention on fertilizer and irrigation water. With these certain limitations, one has to turn to non-conventional recourses to meet the irrigation water demand (Khan et al., 2003; Kannan et al., 2005). Organic vegetable production is expanding its area and total production in Bulgaria and many other countries around the world. This growth rate is slower in greenhouse vegetable production due to high investment, operational and production costs. Organic production is regarded as extensive with more manual labour and less productive capacity. There are a number of studies that confirm this view (Van de Venter et al., 1991; Pascale, 2004; Yildirim, 2007; Ünlü and Padem, 2009; Gravel et al., 2012), but most often they are farther in time. Scientific and technological progress has created the conditions and facilitates for the intensification of this specific production area. A wide range of granulated NPK organic fertilizers and liquid fertilizers have been created and are available. Organically produced new varieties have greater biological potential and a greater range of sustainability. All these are prerequisites for intensifying the organic greenhouse vegetable production and making it an alternative to the conventional one. To test this opportunity by applying modern technological solutions with the use of suitable organic fertilizers, we have put in experience the bio-production of field tomatoes during the transition period. The physiological condition of plants and effect of various stressful factors thereon have been studied using chlorophyll fluorescence properties by many researchers (Gomes et al., 2012; Mathur et al., 2014; Kalaji et al., 2016).

Chlorophyll fluorescence is a non-invasive measurement of photosystem II (PSII) activity and is a commonly used technique in plant physiology. The sensitivity of PSII activity to abiotic and biotic factors has made this a key technique not only for understanding the photosynthetic mechanisms but also as a broader indicator of how plants respond to environmental change (Murchie and Lawson, 2013). At physiological temperatures the fluorescence is emitted mainly from chlorophyll *a* of PSII and reflects the primary processes of photosynthesis by light absorption, distribution and transfer of excitation energy and photochemical reactions in PSII. Because of the functional relation of PSII with other components of the photosynthetic apparatus of the chlorophyll fluorescence, it is seen as a proxy for the state of the integral photosynthetic process and the plant organism as a whole (Roháček, 2002). The CCM 200 plus is useful for improving nitrogen and fertilizer management, and is ideal for crop stress, leaf senescence, plant breeding, health determination, and other studies. Furthermore, the affordability and ease of use make it an exceptional teaching tool for botany and plant science courses (Opti-Sciences 2002; Richardson et al., 2002). The purpose of this study was to determine the change in chlorophyll fluorescence and relative chlorophyll content depending on the fertilization applied-organic and mineral, at three planting dates of tomatoes.

MATERIALS AND METHODS

Plant material and growth conditions
Rugby's determinant tomatoes have been planted on three dates: 30/04/2019, 07/05/2019 and 14/05/2019 in the experimental field of the Agricultural University Plovdiv, Bulgaria. The following three variants were envisaged: 1. Controlled-was not apply fertilizer; 2. Fertilizing with mineral fertilizers - ammonium nitrate - 34%, triplesuper-phosphate - 50% and potassium sulphate - 46%; 3. Fertilizing with organic fertilizer - Arkobaleno. Arkobaleno and mineral fertilizers: super-phosphate, potassium sulphate, ammonium nitrate was used in the recommended and optimal doses. The variants were set in 4 replicates with an experimental plot of 20 plants, 16 of which were reported. The fertilizers were introduced with the last tillage before planting. The tomatoes were grown on mulch beds under a planting scheme of 160/50 cm and a plot size of 16 m^2. Irrigation was carried out with a drip system according to the development phase. Organic fertilizer Arkobaleno has the following main characteristics: organic nitrogen (N) - 4.5%, phosphorous anhydrite (P_2O_5) - 3.5%, calcium (CaO) - 5-8%, magnesium (MgO) 0.8-1%, organic carbon of organic origin (C) - 30%, organic substance 55-60%; humified organic matter 12-14%, trace elements Fe, B, Cu, Zn. pH 6-8. Vegetative and phenological manifestations were reported during the growing season. Measurements were taken for the three planting dates, in the fruit maturity phase to determine some leaves physiological parameters.

Chlorophyll fluorescence imaging
Chlorophyll fluorescence transients of tomato leaves were measured using a portable device. PAR-FluorPen FP 110/D manufactured by Photon Systems Instruments Ltd., Czech Republic. The fluorescence measurement protocol uses short (30 μs) measuring flashes to measure zero level fluorescence (F_0) followed by a strong saturating flash [duration 0.8 s, intensity about 3000 μmol m^{-2} s^{-1}] to measure the maximum fluorescence (Fm). After a short dark adaptation, the leaf was exposed to actinic light [1000 μmol m^{-2} s^{-1}] for 4 min. Photosynthetically Active Radiation (PAR) is measured as Photosynthetic Photon Flux Density (PPFD), which is indicated by units of quanta (photons) per unit time per unit surface area. Three strong flashes of saturating light probed the effective quantum yield of PSII during the actinic light exposure (Maxwell & Johnson, 2000; Nedbal et al., 2000). The chlorophyll fluorescence transients were measured on the same day in the morning. The dates of measurement were 14/08/2019 and 10/09/2019 when the tomato plants had reached fruit maturity growth stage. The nine leaves from each variant were dark adapted for about 30 min by detachable leaf-clips prior each measurement. The numeric value of each ChlF parameter (Fv/Fm, PSII, PAR) was determined by integrating it over the measured leaf area.

Physiological estimate of chlorophyll content index (CCI)

Chlorophyll content index of the leaves was measured using a portable apparatus CCM 200 plus, a Chlorophyll Content Meter manufactured by Opti-Sciences, Inc., NH, USA.

The physiological assessment was carried out *in vivo* on the field. Measurements have been made in two dates of a sample of leaves when the tomato plants had reached fruit maturity growth stage.

The dates of measurement were 12/07/2019 and 18/09/2019. Plant measurements were made of each replication of a variants. From each plant there were analyzed 20 leaves by readings on the central part of the leaf.

Statistical analysis

Data are presented as mean values ± Standard error and Student t -test has been used for comparison of mean values from two independent numeric samples. For mathematical data processing it was used Data Analysis tool by program Excel for Windows 10.

RESULTS AND DISCUSSIONS

At the first date of measurement-14/08/2019 the highest average values of quantum yield (Fv/Fm) of dark adaptation leaves were read in third variant-organic fertilizer.

Respectively, the average ratio Fv/Fm which was measured for second variant was e similar with the third variant of treatment. The lowest photosynthetic activity (Fv/Fm = 0.78) was measured for the first variant, as this value indicated for light heat stress (Table 1).

The average values of ratio Fv/Fm of the other variants are close to optimal value for healthy leaves-0.83 (Demmig and Björkman, 1987). Initial fluorescence (F_0) in oxidized reaction centers of PSII after dark adaptation has had the highest value for the second variant.

The heat or low temperature stress increase F_0 (Zlatev & Kolev, 2012; Chen et al., 2018), but in this case it did not affect the photosynthetic activity of the second variant (Table 1).

Measured photosynthetically active radiation (PAR) was higher at two fertilizer variants compare to no fertilizer variant, as ratio between photosynthetically active radiation and quantum yield of dark adaptation leaves was more optimal at the third variant-organic fertilizer.

More effective photosynthetically activity was expressed in the case of the variant - first sow date in all fertilize treatment variants, while more stressed photosynthetically activity (Fv/Fm = 0.71 and F_0 = 4450) was measured at subvariant- no apply fertilizer and second sow date interaction (Table 1).

High temperature reduced the Fv/Fm ratio, indicating that an important portion of the PSII reaction centre was damaged, according to Asada et al., 1998.

Table 1. Results for chlorophyll fluorescence of tomatoes leaves reported at the first date 14/08/2019

Indices	Variant of treatment	First planting date	Second planting date	Third planting date	Average ± SE
Ft=Fo	1. No fertilizer (control)	2840±257.3	4450±398.2	3220±358.2	3503±343.8
Qy=Fv/Fm		0.82±0.009	0.71±0.04	0.80±0.01	0.78±0.03
PAR		108±19.0	60±10.2	108±15.9	92.0±10.2
Ft=Fo	2. Chemical fertilizer	3811±300.2	3932±289.3	4915±411.1	4219±349.5
Qy=Fv/Fm		0.82±0.01	0.79±0.008	0.78±0.009	0.80±0.01
PAR		120±20.0	95±10.2	152±24.3	122.3±11.5
Ft=Fo	3. Organic fertilizer Arkobaleno	3920±273.3	3280±200.2	2990±185.6	3397±274.4
Qy=Fv/Fm		0.82±0.007	0.80±0.009	0.80±0.008	0.81±0.007
PAR		117±13.2	86±8.9	96±12.3	99.7±9.13

Values are means ± Standard errors of mean

At the second date of measurement-10/09/2019 the most optimal ratio between PAR and quantum yield (Fv/Fm) of dark adaptation leaves was read for the second variant, where low PAR value is interacted with high value of quantum yield (Table 2). Initial fluorescense F_0

in oxidized reaction centres of PSII after dark adaptation have again highest value for the second variant, as the obtained result is similar to those reported in the first measurement. Compare to first measurement, in the second measurement there have more significance variations for results of PAR and initial fluorescence. However, the ratio Fv/Fm in all variants is equal (0.82-0.83), as this value indicates for absence of stress (Table 2).

Table 2. Results for chlorophyll fluorescence of tomatoes leaves reported at the second date 10/09/2019

Indices	Variants	First planting date	Second planting date	Third planting date	Average ± SE
Ft=Fo	1. No fertilizer (control)	3790±152.0	4030±133.3	4213±110.3	4011±122.4
Qy=Fv/Fm		0.81±0.008	0.83±0.007	0.83±0.007	0.82±0.007
PAR		455±56.0	574±67.8	676±75.2	568.3± 63.7
Ft=Fo	2. Chemical fertilizer	3751±259.3	5372±489	4380±300.2	4501±333.6
Qy=Fv/Fm		0.83±0.006	0.82±0.007	0.85±0.006	0.83±0.006
PAR		420±65.3	218±35.6	284±42.3	311.7±42.3
Ft=Fo	3. Organic fertilizer Arkobaleno	2820±195.6	3278±237.8	3630±252.3	3243±234
Qy=Fv/Fm		0.81±0.009	0.83±0.008	0.84±0.008	0.82±0.008
PAR		315±20.36	292±15.6	359±25.3	322.0±19.65

Values are means ± SE-standard error of mean.

From the analysis of the results for the reported average values of leaves relative chlorophyll content from the different variants, it can be concluded that there is no significant advantage of planting date variant. An exception is an option organic fertilizer plus second planting date (Table 3). However, the most stable result shows the third variant. In this variant, the amount of chlorophyll pigments is the same at the second and third planting dates, while the other variants had significance lower results in compare to third planting date. This is an indication of a more sustainable development of the plants of organic fertilization variant, differing in the date of planting. Statistical analysis of the average results indicated a significance difference between no fertilized variant and organic fertilized variant to the advantage of the last variant (Table 3). Alves et al. (2018) and Doncean et al. (2013) reported for increase of chlorophyll content at treated with organic fertilizer of tomato plant and tomato seedlings.

Table 3. Results for chlorophyll content index (CCI) of tomatoes leaves reported at the first date 12/07/2019

Variants	First planting date CCI	Second planting date CCI	Third planting date CCI	Average ± SE CCI
No fertilizer (control)	30.5±2.78	31.93±2.43	36.0±2.27	32.81
Chemical fertilizer	33.95±2.63 $^{n.s.}$	33.93 ±2.45 $^{n.s.}$.	39.34±2.93 $^{n.s.}$	35.74 $^{n.s.}$
Organic fertilizer	33.67±2.47 $^{n.s.}$	38.33±2.96 **	38.26 ±3.01 $^{n.s.}$	36.75 *

SE-standard error of mean; n.s.-no significance, *-significance at p=0.05; **-very significance at p=0.01

In Table 4 are not present data for first and second sow date subvariants, because of lack off green material for analyse. Compare to the first reading, when the most stable result showed the third option, in the second data of measured there is a significant difference between the results for no fertilize variant and other fertilize variants (Table 4). It can be concluded that the plants of the different fertilized variants of the third planting date have better photosynthetic activity than the plants of the first variant in the later stage of their development, as well. Fertilization delays degradation of chlorophyll pigments. The organic fertilized treatment variant was not advantage over chemical treatment variant (Table 4).

Table 4. Results for chlorophyll content index (CCI) of tomatoes leaves reported at the second date 18/09/2019

Variants	Third planting dates CCI values±SE
No fertilizer	28.19±2.53
Chemical fertilizer	38.06 ±2.24**
Organic fertilizer	38.85±2.58 **

SE-standard error of mean; **-very significance at p=0.01

CONCLUSIONS

It was determined more optimal ratio between photosynthetic active radiation (PAR) and quantum yield (qY-Fv/Fm) of PS II in dark-adapted leaves for organic and chemical fertilized variants, as compared to no fertilized variant.

More effective photosynthetically activity was expressed in first planting date variant in all fertilized treatment variants, while at the second date of measurement it was not established a statistically significance difference of Fv/Fm value at different planting dates.

Minimal fluorescence Fo in reaction centres of PS II after dark-adapted of leaves was with highest value at chemical fertilized variant.

Significance differences were observed for values of chlorophyll content index (CCI) in the different variants, as most stable average result showed the organic fertilized variant for three differing planting dates on experimental field.

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MICROBIAL AND ENZYMATIC DEGRADATION OF CARBOHYDRATES: A COMPARATIVE INVESTIGATION OF COMPOST VARIANTS

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Abstract

Microbial biomass carbon and enzymes, degradation carbohydrates have been investigated for the composting of various vegetable and animal wastes. A 4-variant scheme for compost piles from plant residues (vine sticks, fruit twigs, leaves, grass slopes) and rabbit fertilizer with different starters (last year's compost and soil) has been prepared. Microbial biomass and enzymes: cellulase, invertase, amylase and catalase have the highest values up to the 7th day of starting the experience. The higher momentary cellulase activity at the beginning of the experiment determines and a higher potential cellulase activity in cultivation of the variants for the first 10 days, as well as a faster rate of cellulose degradation over the entire composting period. Invertase activity values is lower than these of cellulase and amylase activities. The catalase has shown a smaller decrease at the end of the experiment compared to the beginning in comparison with the other enzymes. Microbial carbon biomass and enzyme activities have a main role in the carbohydrates degradation as an integral part of organic matter and are sensitive indicators in the composting process.

Key words*: compost, microbial biomass, enzyme activities.*

INTRODUCTION

Microbial and enzymatic degradation of carbohydrates as a major part of organic matter is essential in the preparation of organic fertilizers to improve soil fertility upon their application in soils. The composting is a controlled microbial oxidation process in which organic biodegradable waste is converted into safe and useful humus-similar products (compost), which has no phytotoxic and pathogenic properties, has been characterized by the presence of humic acids and is applied in the soil as a nourishing organic fertilizer (Weltzien, 1991; Adani et al., 1997; Epstein, 1997; Boulter et al., 2002; Lashermes et al., 2012; Li et al., 2013; Guanghui et al., 2016; Boteva and Yankova, 2017). Microbial characteristic of composting is important for optimizing the process and quality of the final product. Information on the microbial component of compost substrates can be expected to provide valuable information on the factors influencing the process, in order to support the compost variants optimization and accordingly the quality of the final product to be improved (Mondini et al. 1997; 2002; Jedidi et al., 2004; Yankova and Boteva, 2017). An extremely important part is managing the composting process, considering the factors that exert influence on it (Nedev, 2020). Ayed et al. (2007) have found a decrease in microbial biomass C and microbial biomass N in mature compost, which is probably as a result of the decreasing availability of easily degradable substrates as the municipal solid waste composting process progresses. According to these authors, the dynamics of the ratio between the two biomasses suggests a change in the composition of the microbial populations during the composting process from the prevailing bacteria and actinomycetes to the predominant micromycetes. Bouzaiane et al. (2011) concluded that the content of microbial biomass C and N and DNA during the solid waste composting process can be of great benefit for understanding the compost stability status. The basic requirement for the safe use or application of compost in agricultural land is its degree of stability, which implies a stable content of organic matter (Castaldi et al., 2004; 2008; Mondini et al., 2004). In fact, the amount

of microbial biomass cause a significant role for biochemical transformations, for optimization and for the quality of the final product (Mondini et al., 2002; Jedidi et al., 2004). Microbiological activity determines the stability and maturity of the compost and it is expressed in the formation of microbial biomass by mesophilic and thermophilic bacteria, oxygen absorption and CO_2 release, as well as changes in the enzyme activity in the components (Barrena et al., 2008; Cunha-Queda et al., 2007; Jurado et al., 2014).

The evolution of total microbial biomass, Gram+ bacteria, Gram- bacteria, moulds, and enzyme activities (β-glucosidase, cellulase, protease, acid, and alkaline phosphatase) significantly depends on the type of waste (Villar, 2016). According to this author's investigation, it should be paid more attention to the ripening phase in order to optimize composting. Decreases in both the enzyme activity and the microbial community may indicate stability in the maturation of the compost. It is important to monitor microbial communities and their enzymatic activity during the time to determine if and when the compost is stable enough to be applied in the soil, or whether more time or alternative process management is required (Villar, 2016). The time required for the ripening phase is a function of the substrate and the environment and the operating conditions of the facility and it may vary from a few weeks to a year or two (Diaz et al., 2002). This lack of process control can cause environmental problems such as odors and leaching, in addition to adversely affecting the quality of the compost. Castaldi et al. (2008) propose the study of the dynamics of some enzyme activities as an appropriate indicator of stability, although they do not establish a threshold value. However, enzyme activity studies provide information on the degradation of organic matter and metabolic processes during composting, and therefore on product stability. The nature of organic substrates is also an important factor in determining the dynamics and microbial diversity during composting (Klammer et al., 2008; Ryckeboer et al., 2003; Vargas-García et al., 2010). Organic degradation is carried out by different groups of microbial populations. They develop depending on the temperature of the compost mass. Thermophilic and mesophilic groups of microorganisms have been isolated (Bernal et al., 2009). Bacteria prevail at the beginning of composting, moulds are present during the entire process, but dominate at humidity below 35%, at temperatures above 60°C their activity decreases (Shestakov et al., 2018). The decomposition of carbohydrates in compost, as well as other compounds is accomplished by a variety of microorganisms, with thermophilic forms evolving at temperatures of 50-60°C (Antonyan, 2004). Actinomycetes predominate in the process of stabilization and maturation, i.e. participate in the degradation of resistant polymers together with micromycetes. During composting, various microorganisms with cellulolytic-lignolytic activity, such as *Trichoderma viridae*, *Aspergillus niger*, *Aspergillus terreus*, *Bacillus* sp., degrade various animal and vegetable waste products, as well as farm and cattle shed waste. The compost maintains high populations with a higher percentage of Gram-negative microorganisms. All Gram-positive isolates were identified as *Bacillus* sp. (Boulter et al., 2002).

During the composting process, the starting material is transformed by a variety of biological and biochemical processes and, in which enzymes served their purpose (Gupta et al., 2015). In a study by Stutzenberger et al. (1970) the maximum cellulase activity is demonstrated at 65°C, pH 6.0. The cellulose activity of the compost is increased 10 times at logarithmic rate, while the cellulose content is reduced by 50%. According to Kubicek et al. (1998) the rate of hydrolysis of cellulose derivatives by β-glucosidase determines the induction of cellulases. Enzymes also cause an important role in the humification stage. Microorganisms capable to degrade polymers in the constituent mass of composts produce a complex of extracellular enzymes (Jurado et al., 2014). The dynamics of changes in some enzyme activities have been recognized by many authors as biological indicators of compost maturity (Bohacz and Korniłłowicz-Kowalska, 2009; Castaldi et al., 2008). Pursuant to study of Bohacz (2019) the enzymatic activity represented by the study of cellulase, protease, urease and arylsulfatase is

higher during the 10 weeks of composting in compost in small amounts bird feathers and easier available lignocellulosic fraction - mainly grass, pine skin and wood sawdust than in compost containing more difficult-to-degrade lignocellulosic waste (wheat straw, wood sawdust, pine skin) and a higher content of feathers. Microbial diversity (mainly fungus) and enzymatic activity (alkaline phosphatase and β-glucosidase) cause positive effect on the mineralization of phosphorus during the production of phospho-compost (Kutu et al., 2019). Among the 19 enzymes tested in the composting process, Tiquia (2002) found that esterase, valine amino-peptidase and α-galactosidase were the most common enzymes in bird manure, whereas N-acetyl-β-glucosaminidase in pork manure. According to a study by Mondini et al. (2004) microbial carbon biomass decreases throughout the composting period (149 days), while the enzyme activity in wet fractions stabilizes between 50 (β-glucosidase, alkaline phosphatase) and 90 (arylsulfatase, acid phosphatase) days of composting. In composting plant biomass, Wei et al. (2012) have found that cellulase activity shows increasing prevalence in the later stages (24 weeks) of composting, and measured hemicellulase activities, mainly α-arabinosidase and β-galactosidase, were higher in the earlier stages (3 weeks), in response to the availability and absorption of chemically diverse biomass materials. Nakamura et al. (2004) have found that the composting materials in the composting process are dominated by the species *Cerasibacillus quisquiliarum* and *Bacillus thermoamylovorans*, which degrade gelatin and starch. They suggest that these species produce gelatinase and amylase, respectively. The increase in gelatinase is dependent on an increase in the diversity of *Cerasibacillus quisquiliarum,* whereas for *Bacillus thermoamylovorans* and amylase such trend has not been observed. The highest activity of amylase (73-129 U/g) and cellulase (75-148 U/g) has been observed in the beginning of the composting process, and maximum activities of lipase (5-10 U/g) and protease (46-72 U/g) have been established in the middle stage of the process of composting kitchen waste, dried leaves and rice bran (Fan et al., 2015). In the preparation of compost from sewage sludge and straw Niu and He (2014) found that, at the beginning of the compost, cellulase activity firstly has increased and then gradually has decreased and there is a trend to be stable. The activity of catalase is higher in the beginning of the compost and it is stable during the temperature rising, after that it quickly decreased and is maintained at a lower level. According to our previous study, the application of composts from organic waste (compost variants analyzed in this investigation) leads to activation of soil microbiological activity (Malcheva et al., 2019). Antonious (2016) establishes that the recycling of organic waste, its composting and its application leads to increased soil urease and invertase activity.

The purpose of this study is to monitor dynamically the influence of various compost variants on the accumulation of microbial carbon biomass and the activity of enzymes involved in the carbohydrates degradation as part of the organic matter of composts.

MATERIALS AND METHODS

There is a 4-variant scheme of compost piles (V1, V2, V3, V4) with different starters.

The recipes for the variants are presented in Table 1.

Microbiological studies include the determination of microbial carbon biomass by fumigation spectrophotometric method (Cai et al., 2011). The enzyme activity of the compost variants has been presented in dynamics by investigation of: cellulase, amylase, invertase by spectrophotometric method of Gradova et al. (2004) and catalase by a manganometric method (Khaziev, 1976) (instant activities). Potential cellulase activity has been presented at the beginning and end of the experiment (mesophilic phase) and, using a laboratory method according to Khaziev (1976), as in petri dish with 10 cm diameter is poured thick soil about 7 mm on which 3 strips of sterile filter paper are placed with 10/50 mm in size and cultured at 25°C, 60% maximum field moisture capacity has been maintained. The decomposed cellulose area with a standard grid shall be recorded over 10 days. Average values from the three bands are calculated.

Table 1. Recipes of compost variants

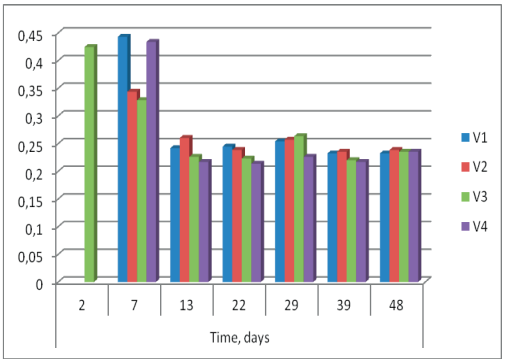
Variants	Materials	Starter
V 1	Brown materials: - Vine sticks- 26.150 kg - Fruit twigs – 10.800 kg Total brown materials: 34.950 kg Green materials: - Grass slope – 21.550 kg	Last year's compost – 3.000 kg
V 2	Brown materials: - Vine sticks – 23.450 kg - Fruit twigs – 10.500 kg Total Browns: 33.950 kg Green materials: - Grass slope – 10.000 kg - Rabbit fertilizer – 9.000 kg Total green materials: 19.000 kg	Last year's compost – 3.100 kg
V 3	Mulberry-tree twigs with leaves - 19.800 kg *The 7th day after preparing the experiment - additional insertion into the bowl, upon turning – 2.050 kg of pure litter for small bugs growing and 1.450 kg of pure twigs; the 13th day – 1.030 kg of litter and twigs.	Soil
V 4	Mulberry-tree twigs with leaves – 28.950 kg (taken from heavy metal contaminated area) *The 7th day after preparing - additional insertion into the bowl, upon turning – 3.050 kg litter for small bugs growing and twigs; the 13th day – 0.200 kg of litter and twigs taken from the same contaminated area.	Soil

Statistical processing of the data include calculating the average value of three repetitions and coefficient of variation (C.V.) by the use of Excel 2010.

RESULTS AND DISCUSSIONS

Study was started one week after the compost pile 1, 2 and 4 is prepared, and on the second day after the compost pile 3 was prepared. Analyzes were repeated upon each turn of the compost (every 7-10 days).

The results of microbial biomass carbon (MBC) dynamics provide an idea of the accumulation of organic carbon with microbial origin in compost heaps. This indicator is important for assessing the degree of degradation of compostable substrates, insofar as the mineralization of organic compounds shows (mainly carbohydrates) in them. The microbial biomass carbon dynamics data are presented in Figure 1.



*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

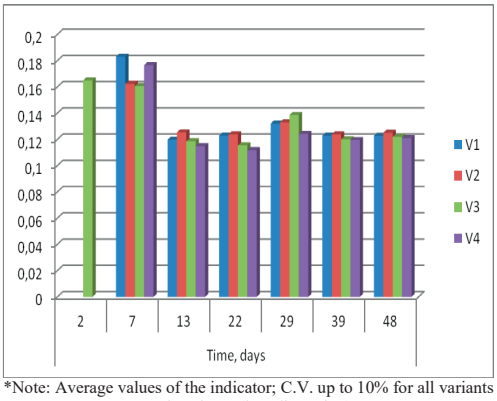
Figure 1. Microbial biomass carbon (mg glucose/g soil)

The data indicate different dynamics of MBC accumulation in the four composting variants, which is determined by the differences in the compost formulations and, accordingly, by the development and activity of the microflora in them. On the 7th day of experience the highest and the amount of biomass carbon to microbial origin at V1 has been established. This amount is about 1 time higher than the same in the other variants. For this variant (V1), the brown (vine sticks, fruit twigs) and green (grass slopes) materials are in the biggest amount - a total of 56.500 kg, and the starter is last year's compost - 3 kg. According to data from our previous study (Malcheva et al., 2018), these vegetable wastes have the highest microbial presence, whereas in variants with different amounts of mulberry twigs with leaves (V3 and V4), microbial diversity and presence are lower. Reducing the amount of green and brown materials and adding rabbit fertilizer at V2 slows the development of microorganisms at the beginning of the experiment (the 7th day), but in the next reporting days their activation is most presented on the 13th day.

In V2 and V3, the amount of MBC decreased more gradually, while in the other variants (V1 and V4) the accumulation of microbial biomass sharply decreased by the 13th day - about 2 times. After that day, the biomass carbon decreases more smoothly until the end of composting, with a slight increase on the 29th day after the experiment is set. For all four composting variants, it is found that on the 39th and on the 48th days the amount of MBC reaches the lowest values. After composting it decreased about 2 times in variants V1 and V 4,

and about 1.4 times in V2 and V3 compared to the amount of MBC in early experiment. The more diverse composition of vegetable waste at V1, the addition of rabbit fertilizer at V2, waste from the heavy metal contaminated area at V3, and the different amount and quality composition of the microflora in the different compost variants are relevant to this trend (Malcheva et al., 2018). Mondini et al. (2004) also establish that microbial biomass carbon decreased throughout the composting period.

According to our previously research (Malcheva et al., 2018), variants 1, 2 and 4 have passed a mesophilic and thermophilic composting phase with the development of mesophilic and thermophilic groups of microorganisms. As long as variant 3 does not go through the thermophilic phase. This fact has an effect on biomass accumulation by the 7th day of the experiment, the lowest value at V3, where only mesophilic groups of microorganisms develop, and in the other variants the activity of mesophiles and thermophiles is higher. After the 7th day, by the end of the experiment, the accumulation of MBC is close to the individual variants. Therefore, not only the amount of microorganisms is a prerequisite for their activity, and respectively for the accumulation of biomass carbon of microbial origin. Other factors also affect the temperature and humidity of the compost, the type and amount of compostable material. The same factors affect the enzyme activity of the microorganisms in the compost. Cellulase activity is presented in Figure 2.

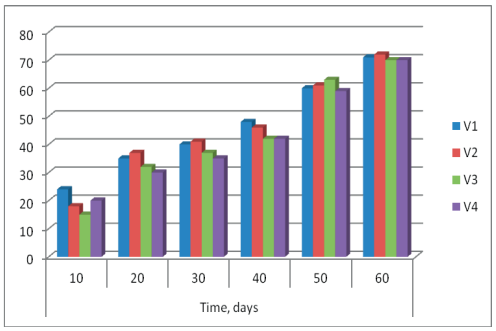


*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

Figure 2. Cellulase activity of compost materials (mg glucose/g soil)

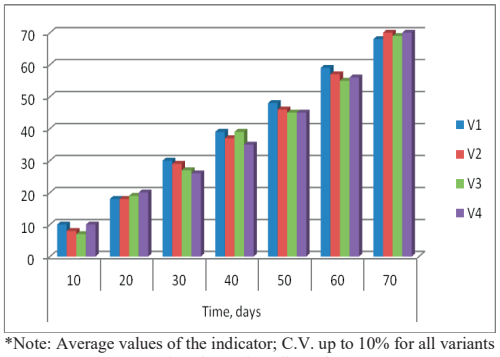
The results for cellulase activity show similar trends as for the accumulation of microbial biomass carbon - higher enzyme values by the 7th day, more sharply decrease in enzyme activity between the 7th and the 13th days, and subsequent a gradual decrease in cellulase values by the end of the experiment, with a slight increase on the 29th day. A similar trend for the highest values of cellulase at the beginning of composting was found by other authors (Niu and He, 2014; Fan et al., 2015). While in a study by Wei et al. (2012) cellulase activity shows an increasing predominance in the later stages (24 weeks) of composting, and the measured hemicellulase activities are higher in the earlier stages (3 weeks), in response to the availability and digestibility of chemically different biomass materials. Again, the enzyme activity at the beginning of the experiment was higher at V1 and V4, and lower at V2 and V3. While at the end of the experiment the cellulase values are close in all variants, the decrease from the beginning is 1.5 times for V1 and V4, and 1.3 times for V2 and V3, i.e. the reduction of cellulase activity during the composting period is less than the decrease in the accumulation of microbial biomass carbon. Despite the lower amount of accumulated microbial biomass, cellulase activity remains higher, which once again confirms the fact that the enzyme activity of microorganisms does not dependent on their amount only, but also MBC, and cellulase activity depends on the development and activity of microorganisms in compost materials. As per our research on the same compost variants (Malcheva et al., 2018), non-spore-forming bacteria and bacilli occupy a major part of the total microflora, while actinomycetes and micromycetes are developed less. Bacteria predominate at the beginning of composting, and moulds are present throughout the whole process, but dominate at humidity below 35% and temperature below 60°C (Shestakov et al., 2018).

Besides instant cellulase values, the potential cellulase activity of composting in dynamics for a period of 60-70 days has been studied. The following Figures 3 and 4 show the results for the cellulose degradation dynamics for the samples as of the 7th day (the beginning of the experiment) and the 48th day (the end of the experiment).



*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

Figure 3. Degradation of cellulose (%) - the beginning of the experiment



*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

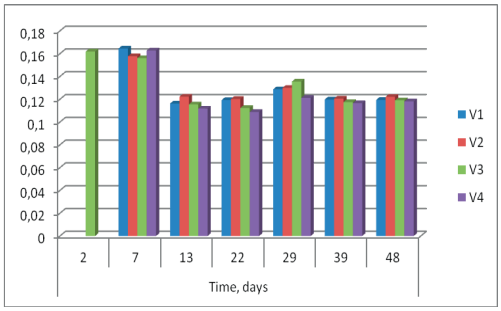
Figure 4. Degradation of cellulose (%) - the end of the experiment

As per Figure 3 and Figure 4 it has been established that higher amount of accumulated microbial biomass carbon and higher instant cellulase activity at the beginning of the experiment also lead to higher potential cellulase activity in cultivation variants as of the 7th day and counting the percentage of degraded area in 10 days for a period of 60 days.

During the first 10 days a higher percentage of degraded area has been established - about 20%, increasing by 10% for the following periods and reporting for 70% of the degraded area in all variants on the 60th day.

Whereas, on cultivation of samples from the day 48, the initial percentage of degraded cellulose is lower and 70% of the degraded area is established on the day 70 of cultivation, which correlates with the lower values found in the spectrophotometric determination of cellulase after the 7th day up to the 48th day.

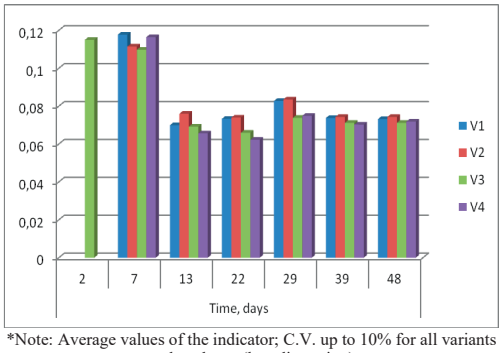
The amylase activity represents the rate of degradation of starch in the compost variants (Figure 5).



*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

Figure 5. Amylase activity of compost materials (mg glucose/g soil)

The data indicate that amylase values are slightly lower than those of cellulase. The trends have been repeated - increased levels of amylase activity on the day 7, a sharp decrease from the days 7 to 13, and a subsequent more gradual decrease in enzyme activity, with a slight increase in values on the day 29. Fan et al. (2015) also establish higher amylase activity at the beginning of composting and lower at the end of the process. Again, at the beginning of the experiment, amylase activity was higher at V1 and V4, and lower at V2 and V3, and at the end of the experiment the enzyme values were close in all variants. Invertase activity has the lowest values (Figure 6).

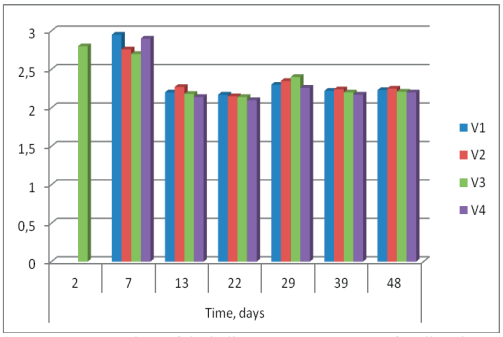


*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

Figure 6. Invertase activity of compost materials (mg glucose/g soil)

The invertase activity is about 1.7 lower than cellulase and amylase activity. Probably the invertase activity is lower, since its producers

are mainly the yeast that get killed when the compost passes through the thermophilic phase. The trends in the dynamics of invertase are the same as in previous enzymes.
Higher invertase activity was found at the beginning of the experiment and lower after the 13th to 48th day.
However, the application of compost from organic residues leads to an increase in soil invertase activity (Antonious, 2016). Cellulose, starch and sucrose are degraded by the β-glucosidase enzyme to hydrogen peroxide, which in turn is degraded to water and oxygen by the catalase enzyme.
The catalase activity of the studied compost variants is presented in Figure 7.



*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

Figure 7. Catalase activity of compostable materials (ml O_2/30 min)

The catalase activity cannot be compared quantitatively with other enzymes due to different enzyme assay methods, but it repeats the trends observed with the previous enzymes. A slight decrease in catalase activity at the end of the experiment towards to the beginning, compared to the other enzymes tested, has been established - 1.3 times for V1 and V4, and 1.2 times for V2 and V3.
Niu and He (2014) also establish a similar trend. According to the study of these authors, the activity of catalase is higher at the beginning of composting and in the period of increasing temperature is stable, then rapidly decreases and is maintained at a lower level.
The catalase values have been also influenced by the presence of catalase of vegetable origin, in addition to microbial origin.

CONCLUSIONS

Biomass carbon with microbial origin in the experienced variants of aerobic active composting has decreased at the end of the experiment compared to the beginning. The accumulation of microbial biomass is most active up to the 7th day of experience set, to a greater extent for the variant with vine sticks, fruit twigs and grass slopes (V1), as well as the variant with mulberry twigs with leaves (V4), at starter soil for both composts. The values of biomass carbon (the 7th day) are lower for the vegetable residue and rabbit fertilizer (V2) variant, as well as for the mulberry twigs with leaves, but in a smaller amount (V3), for last year's compost starter for V2 and soil for V3. MBC at the end of experiment is similar for all compost variants.
Cellulase activity of the compost variants follows the trend with the accumulation of microbial biomass - the highest enzyme values at V1 and V4, and lower at V2 and V3 at the beginning of the experiment (the 7th day), as well as close enzyme values at end of experiment (the 48th day). The higher instant cellulase activity at the beginning of the experiment also resulted in a higher potential cellulase activity in cultivation of variants during the first 10 days, as well as a faster rate of cellulose degradation over the entire composting period.
Amylase and invertase activity are lower than cellulase activity. This trend is more expressed with respect to invertase activity. And these enzymes are established at higher values at V1 and V4, and the lower at V2 and V3 initially, and similar values at the end of the experiment.
Catalase activity has followed the course of the other enzymes in the different composting phases, but in contrast, the catalase has showed a slight decrease at the end of the experiment compared to the beginning. The indicators studied - microbial biomass carbon and enzyme activities cause a significant role in the carbohydrates degradation in organics and are sensitive indicators in the composting process.
The application of the types of compost presented can support to be improved soil fertility and crop production, and to be an important step in achieving sustainable

ecological farming and integrated crop production.

ACKNOWLEDGEMENTS

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THE INFLUENCE OF SUBSTRATE TYPE ON THE PRODUCTION OF ASPARAGUS GROWN IN DIFFERENT ENVIRONMENTAL CONDITIONS

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Abstract

The study was conducted within the Horticulture faculty in Bucharest using the cultivar Argenteuil of asparagus (Asparagus officinalis L.), during 2016-2018. We had grown the plants in three types of substrate, peat, perlite and mixture of perlite and peat and we used six type of fertilizers.
The earliest production was obtained in the greenhouse conditions on all types of substrate compared to the crops grown under field conditions.
The purpose of the study was to see what was the most effective method of getting an early asparagus production.

Key words*: asparagus, substrate, fertilizers, condition of growing.*

INTRODUCTION

Asparagus officinalis L. is native to North America it has acclimatized very well in Romania and in other geographical areas.
It is one of the species highly appreciated for its low calorie content (40 calories), without cholesterol or fat) being rich in minerals, especially in potassium and vitamins. It can be consumed fresh or preserved. In the world there is a growing demand for asparagus, mainly for fresh asparagus but also for frozen and preserved products.
In Europe, asparagus is grown on 66,500 hectares and is one of the most popular vegetables being consumed fresh or processed. In Europe the leading producers are Germany with 25 thousand ha and a production of 130,881 t, Spain with 13 thousand ha and 63,433 t, Italy with 6,863 ha and 46,419 t and Nederland with 20,800 t. China is the leading producer in the world with 57,000 t production in other continents is much lower: Africa (4,800 t), Asia (12,200 t excluding China) and Oceania (3,180 t).
The highest yields of asparagus are obtained in areas with temperate climate in late spring and early summer, but there is a period of when there is no fresh asparagus in temperate climates, which results in import insurance from tropical areas (Jakše, 2008; Nichols, 1992; Wolyn, 2018).
Asparagus is cultivated only on small areas, in individual households but in some countries such as Turkey, production is continuously increasing, at present it is 200 tons (Arman Badur, 2017). Crop technology, soil type and soil can influence the production of asparagus (H. Araki, 2002; Jishi, 2012) Wallace et al., (1993), Taga et al. (1980) showed that keeping the asparagus plants at temperatures of 22ºC led to higher shoots but the plants exposed to temperatures of 28ºC were stimulated for the emergence of the shoots but they were thinner.
Van Os and Simonse (1988) recommend for early production forcing before planting thus harvests are obtained 10 days earlier.
Green spears were produced in winter with warming cultural beds (Haruyama et al., 1985; Koizumi et al., 2002; 2003; 2013). Koizumi et al., 2013; Ku et al., 2007).
W. Chen (2018), also underlines this aspect that lower temperatures during the formation of shoots extends the harvesting period, being different in cultivation.
Some results from two years of experiments show asparagus rhizomes kept from the forcing period during February to July in a chamber at –1°C, then from August to September at 0°C then planted in containers. In each box either two or three rhizomes were placed, and were filled with different growing media, placed on a bench in a heated greenhouse and fertilized

either every one or two weeks. The effects of the different treatments applied were significant (Nicola, Hoeberechts and Fontana, 2003).
Growing asparagus plants in large containers filled with coco peat in greenhouses during the summer months, and forcing in the dark has the potential to provide consumers with a high quality local product year round. By forcing in the dark the plants can be grown at soil level, and the white spears harvested above the ground (Nichols, 2007).
Under field conditions, cultivation of other vegetable crops after deforestation of the asparagus crop, led to high vegetable yields (Asaduzzaman et al., 2013; Young, 1984; Young and Chou, 1985).

MATERIALS AND METHODS

The experiment was made during 2017-2018 in two condition of medium, in greenhouse (in Hortinvest greenhouse) and in field.
We used the Argenteuil cultivar of *Asparagus officinalis* L species.
We used three types of substrate, peat, perlite with 4 mm granulation and mixture of 50% perlite and 50%peatand five types of fertilizers. The fertilized variant was: V1 - control; V2 - Amalgerol; V3 - Formulex; V4 - Vermiplant; V5 - Poco and V6 - Iguana. Amalgerolis a product with effect on the plant and the soil obtained from natural oils, plant extracts and organic carbohydrates.
Vermiplant is a natural liquid, stabilisated, resulted thru product obtained from extraction from composting under the action of earthworms. It contains microelements (barium, zinc, iron, manganese and amino acids), all of which contribute to better growth and development of plants.
POCO is a organic product with contains of: 0.04-0.05% Calcium; 6.30-12.70 mg/l Iodine; 0,50-0,80 mg/l Magnesium; 0.025-0.038 mg/l Nitrogen; 0.50-0.64% Potassium;0.088-0.120% Sodium; 0.028-0.050% Sulf; 0.10-0.12%, Orange oil; 0.04-0.06% rape oil and Organic acids 0.20-0.25%.
Iguana is a 100% organic product with 4% nitrogen, 3% phosphorus, 6% potassium contains: Formulexis a organic product that contains nitric nitrogen 2.19; ammoniacal nitrogen 0.21; phosphorus (P2O5); Potassium (K2O) 3.36; Calcium (CaO) 1.85; ameliorated with Bor 0.0108; Cobalt 0.0006; Copper 0.0025; Iron 0.0526; Manganese 0.0131; Molybdenum 0.0012; and Zinc 0.036.
Planting was done in pots with a capacity of 4 litres filled according to the substrate variants.
I recorded the amount of water and nutrient solution administered. I watched in a dynamic way the vegetative growth, the number of shoots formed. The plants were in year 4 of culture. The care work consisted of watering, fertilization, temperature and light monitoring.
We have correlated the plant mass, fertilizer type and substrate type. The purpose of the study was to identify the best option for obtaining quality asparagus plants.
The results were statistically estimated by the average, median, maximal and minimal values.

RESULTS AND DISCUSSIONS

The temperature was recorded between 1 March and 31 May 2018 that is the period taken into consideration for asparagus culture, both in hot greenhouses and field (Figure 1).

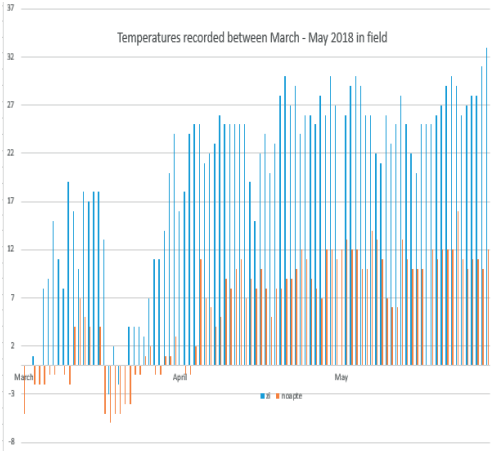

Figure 1. Temperatures recorded between March - May 2018 in field

During the period of emergence and harvest of the asparagus shoots we found that the sum of the registered temperature degrees was 3674ºC in the greenhouse and 2368ºC in the field. The temperature difference between the greenhouse and the field was 1306ºC (Table 1).

Table 1. The temperature recorded during March-May 2018

Harvest period		The sum of the temperature degrees, °C		Differences greenhouse/ field
		Greenhouse	Field	°C
March	Day	620	280	340
	Night	527	-17	544
April	Day	660	747	-87
	Night	558	233	325
May	Day	720	794	-74
	Night	589	331	258
Sum degrees of temperatures		3674	2368	1306

In March, no shoots were harvested at the plants grown in the field. In the greenhouse were harvested between 1 shoot (V1) and 3 shoots (Figure 2).

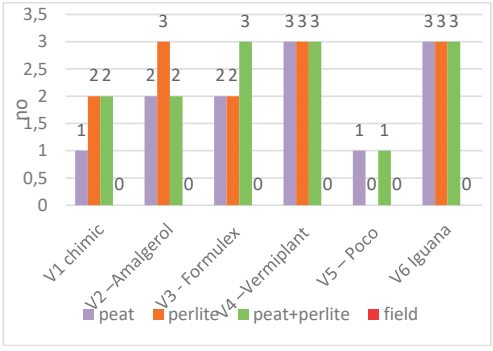

Figure 2. Sprouts harvested in March

In April shoots were harvested from all variants (Figure 3).

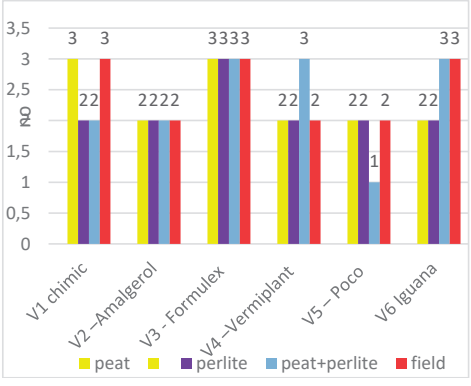

Figure 3. Sprouts harvested in April

In May, at the variant fertilized with Amalgerol no shoots were harvested. But for the rest of the variants only one sprout was harvested at the chemically fertilized variant on the peat, pearl and pearl + peat substrates (Figure 4).

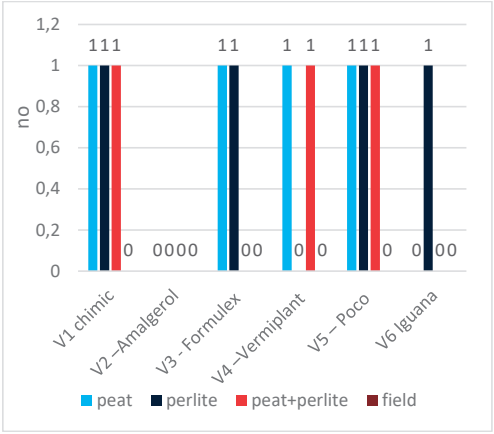

Figure 4. Sprouts harvested in May

The highest number of shoots harvested per square meter was recorded at V4, on peat + pellet substrate (7 shoots/m^2) and the lowest in field cultivated and fertilized with Amalgerol and Poco (Figure 5).

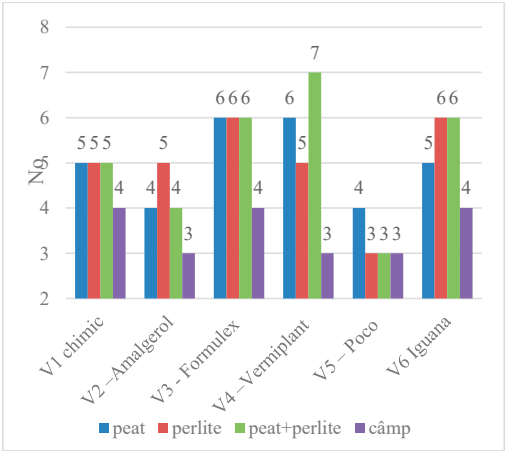

Figure 5. The total number of shoots harvested on square meter

The total number of shoots harvested per square meter in field crops was only three shoots in V2, V4, V5 and four shoots in V1, V3 and V6 If we analyze statistically the total number of shoots from the experimental variants, we found statistically insignificant differences (Table 2).

Table 2. The influence of the number of shoots obtained on the asparagus plants grown in the field

VARIANTS	Shoots (No)	Difference significance (No)	(%)	
V(0) Average	3.50	-0.50	87.50	N
V(1)	4.00	0.00	100.00	Control
V(2)	3.00	-1.00	75.00	N
V(3)	4.00	0.00	100.00	N
V(4)	3.00	-1.00	75.00	N
V(5)	3.00	-1.00	75.00	N
V(6)	4.00	0.00	100.00	N
DL5% =	1.460	DL5% in % =	36.5000	
DL1% =	2.090	DL1% in % =	52.2500	
DL0.1% =	3.020	DL0.1% in % =	75.5000	

In the variant cultivated on peat substrate, in the greenhouse, the total number of shoots collected per 1 m^2 was between 4 shoots at V2 and V5, the difference from the control was only 80%. For variants 3 and 4, a number of 6 shoots/m^2 were harvested, with 20% over the control variant. From a statistical point of view, the differences between the various ones were insignificant (Table 3).

Table 3. The influence of the number of shoots obtained on asparagus plants grown on peat substrate

VARIANTS	Shoots (No)	Difference significance (No)	(%)	
V(0) Average	5.00	0.00	100.00	N
V(1)	5.00	0.00	100.00	Control
V(2)	4.00	-1.00	80.00	N
V(3)	6.00	1.00	120.00	N
V(4)	6.00	1.00	120.00	N
V(5)	4.00	-1.00	80.00	N
V(6)	5.00	0.00	100.00	N
DL5% =	1.460	DL5% in % =	29.2000	
DL1% =	2.090	DL1% in % =	41.8000	
DL0.1% =	3.020	DL0.1% in % =	60.4000	

Analyzing the cultivated variant, on perlite substrate, we found that only V5 presented the smallest number of shoots of only 3 shoots / m^2, the difference being 40% below V1 control. From a statistical point of view the difference was with negatively significant. In the other variants the differences were insignificant (Table 4).

Table 4. The influence of the number of shoots obtained from asparagus plants on the perlite substrate

VARIANTS	Shoots (No)	Difference significance (No)	(%)	
V(0) average	5.00	0.00	100.00	N
V(1)	5.00	0.00	100.00	Mt
V(2)	5.00	0.00	100.00	N
V(3)	6.00	1.00	120.00	N
V(4)	5.00	0.00	100.00	N
V(5)	3.00	-2.00	60.00	O
V(6)	6.00	1.00	120.00	N
DL5% =	1.970	DL5% in % =	39.4000	
DL1% =	2.800	DL1% in % =	56.0000	
DL0.1% =	4.050	DL0.1% in % =	81.0000	

In the variant grown on 50% pearlite and 50% peat substrate, we observed significant positive differences at V4, in which we harvested a number of 7 shoots/m^2. At the V5 were harvested only 3 shoots/m^2, the difference being from a statistically significant negative point of view (Table 5).

Table 5. Influence of the number of shoots obtained on asparagus plants grown on perlite + peat substrate

VARIANTS	Shoots (No)	Difference significance (No)	(%)	
V(0) Average	5.17	0.17	103.33	N
V(1)	5.00	0.00	100.00	Mt
V(2)	4.00	-1.00	80.00	N
V(3)	6.00	1.00	120.00	N
V(4)	7.00	2.00	140.00	*
V(5)	3.00	-2.00	60.00	O
V(6)	6.00	1.00	120.00	N
DL5% =	1.970	DL5% in % =	39.4000	
DL1% =	2.800	DL1% in % =	56.0000	
DL0.1% =	4.050	DL0.1% in % =	81.0000	

Analyzing the influence of the type of fertilizer used according to the type of substrate, we found an influence on the total number of shoots per square meter. Thus, in the case of chemical fertilization the total number of shoots per square meter was five shoots in the case of peat, pearl and mixture 50% peat + 50% pearlite and in the field of four shoots. The correlation coefficient being $R^2 = 0.6$ (Figure 6.).

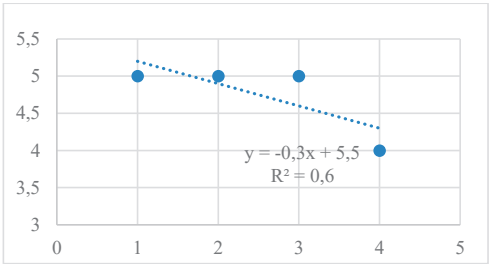

Figure 6. The variant chemically fertilized

In the case of fertilization with Amalgerol, an average number of 3 shoots were harvested, for the variant cultivated in field and 5 shoots for the variant cultivated on the pearl substrate, the correlation coefficient being $R^2 = 0.4$ (Figure 7).

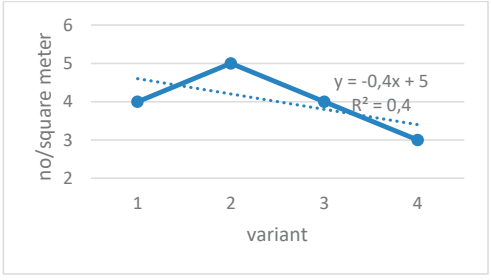

Figure 7 The variant with Amalgerol fertilizer -Number of shoots obtained on square meter

At the variant three, fertilized with Formulex, were obtained 6 shoots per square meter and for variants 1-3 and for the variant cultivated in the field only 4 shoots per square meter. The correlation coefficient being $R^2 = 0.6$.

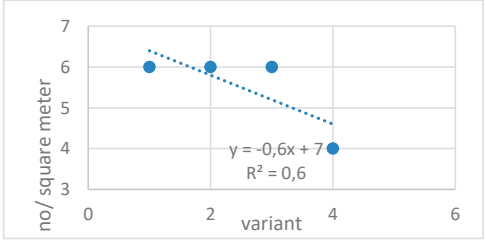

Figure 8 The variant with Formulex fertilizer

In the case of the variant fertilized with Vermiplant, we obtained a greater number of shoots per square meter in the case of the culture on substrates, in the greenhouse. The total number of shoots harvested was 6 shoots at the peat substrate, 5 shoots at the pearlite substrate and 7 shoots at the peat + pellet substrate.

The correlation coefficient being $R^2 = 0.28$ (Figure 9).

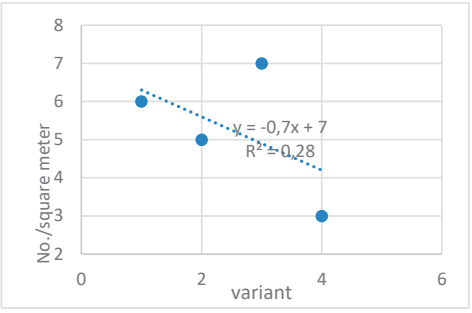

Figure 9. The variant with Vermiplant fertilizer

The application of the Poco product had positively influenced the number of shoots in the case of the variant cultivated on perlite substrate (4 shoots/m^2). The coefficient of correlation being $R^2 = 0.6$ (Figure 10).

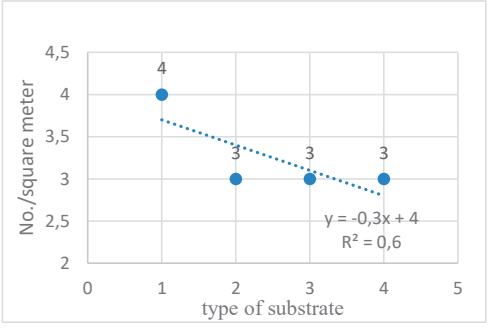

Figure 10. The variant with Poco fertilizer

In the case of variant 6 fertilized with the Iguana product were obtained 6 shoots/m^2 on the substrates of perlite and 50% perlite +50% peat and only 4 shoots on the variant cultivated on the soil in the field. The correlation coefficient being $R^2 = 0.1636$ (Figure 11).

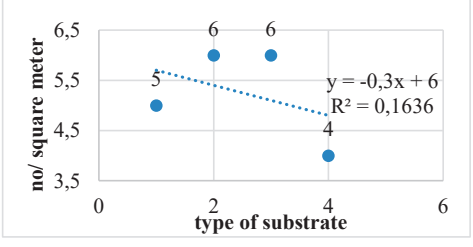

Figure 11. The variant with Iguana fertilizer

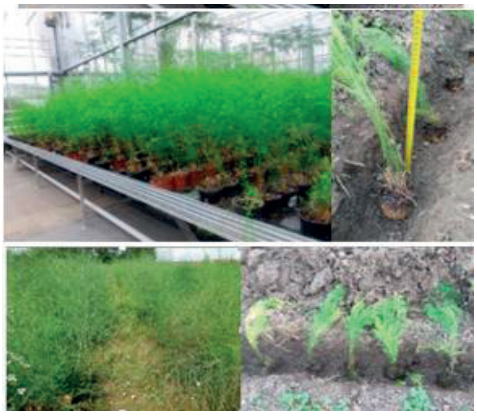

Figure 12. The experiment in greenhouse and in field

If the total number of shoots did not provide us a clear information regarding the experiment, we can see that the mass of the shoots was influenced by the type of fertilization. Thus, in the case of chemical fertilization, we found that the highest amount of shoots was obtained at the variant planted on the perlite substrate in the greenhouse with 98.8 g. As shown in the Table 6, the differences were positively significant at V2 and negatively distinctly significant at V4.

Table 6. The influence of chemical fertilization on the mass of shoots

VARIANTS	Mass (g/m^2)	Difference (g/m^2)	(%)	significance
V(0) average	82.83	-7.80	91.40	O
V(1)	90.63	0.00	100.00	Ct.
V(2)	98.80	8.17	109.02	*
V(3)	78.90	-11.73	87.06	OO
V(4)	63.00	-27.63	69.52	OOO
DL5% =	6.270	DL5% in % =	6.9185	
DL1% =	9.490	DL1% in % =	10.4715	
DL0.1% =	15.120	DL0.1% in % =	16.6838	

In the case of variants fertilized with Formulex we observed differences regarding the total mass of the shoots between 83.63 g / m2 with a very significant negative statistical significance and 135.4 g/m^2 in the variant grown in greenhouse on 50% perlite and 50% peat substrate (Table 7).

The fertilized variant with Vermiplant showed a total average mass of shoots of between 63 g/m^2 with a differences negative distinctly significant. Results over V1 (the control variant) regarding productions were also recorded at variants 2 and 3 with positive distinctly significant, statistical (Table 8).

In the variant to which the Poco product was applied there were no significant differences in production only in the variant cultivated on the substrate of 50% perlite + 50% peat where harvested on average 40.90 g/m^2. At this variance, we recorded statistically a negative distinct significative (Table 9).

Table 7. The influence of Formulex fertilization on the mass of shoots

VARIANTS	Mass (g/m^2)	Difference (g/m^2)	(%)	significance
V(0) average	116.10	-0.08	99.93	N
V(1)	116.18	0.00	100.00	Ct.
V(2)	129.20	13.02	111.20	N
V(3)	135.40	19.22	116.54	*
V(4)	83.63	-32.55	71.98	OO
DL5% =	14.040	DL5% in % =	12.0843	
DL1% =	21.240	DL1% in % =	18.2815	
DL0.1% =	33.830	DL0.1% in % =	29.1178	

Table 8. The influence of Vermiplant fertilization on the mass of shoots

VARIANTS	Mass (g/m^2)	Difference (g/m^2)	(%)	significance
V(0) average	95.72	8.72	110.03	*
V(1)	87.00	0.00	100.00	Ct
V(2)	118.20	31.20	135.86	***
V(3)	114.30	27.30	131.38	***
V(4)	63.40	-23.60	72.87	OOO
DL5% =	8.460	DL5% in % =	9.7241	
DL1% =	12.800	DL1% in % =	14.7126	
DL0.1% =	20.390	DL0.1% in % =	23.4368	

Table 9.The influence of Poco fertilization on the mass of shoots

VARIANTS	Mass (g/m^2)	Difference (g/m^2)	(%)	significance
V(0) average	51.38	-4.13	92.57	N
V(1)	55.50	0.00	100.00	Ct
V(2)	52.50	-3.00	94.59	N
V(3)	40.90	-14.60	73.69	OOO
V(4)	56.60	1.10	101.98	N
DL5% =	5.330	DL5% in % =	9.6036	
DL1% =	8.060	DL1% in % =	14.5225	
DL0.1% =	12.840	DL0.1% in % =	23.1351	

In the case of the fertilized variant with the Iguana product the average mass was 106.0 g/

m^2 in the variant cultivated on perlite substrate, and 110.5 g/m^2 in the variant on 50% perlite and 50% peat substrate, with a significance statistically positive very significant (Table 10).

Table 10. The influence of Iguana fertilization on the mass of shoots

VARIANTS	Mass (g/m^2)	Difference (g/m^2)	significance (%)	
V(0) average	98.89	7.64	108.37	N
V(1)	91.25	0.00	100.00	Ct
V(2)	106.00	14.75	116.16	**
V(3)	110.50	19.25	121.10	**
V(4)	87.80	-3.45	96.22	N

DL5% =	9.570	DL5% in % =	10.4877
DL1% =	14.470	DL1% in % =	15.8575
DL0.1% =	23.060	DL0.1% in % =	25.2712

Figure 13. The asparagus shoots

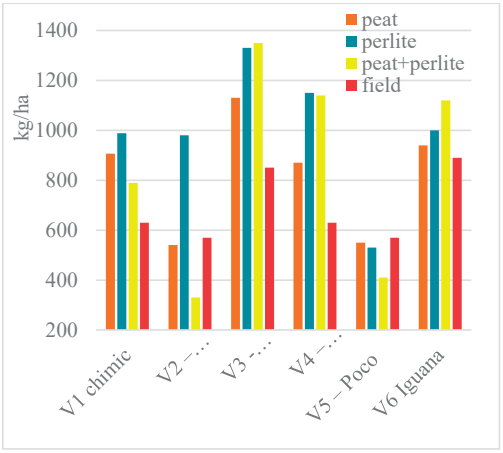

Figure 14. Production evaluated on hectare in the first year

In the Figure 14, the data regarding the evaluation of asparagus production are presented, in the first year of culture. Taking into account the fact that during the first year of cultivation, a small number of shoots are harvested in order to fortify the plant. In the first year of cultivation (after 3 years after planting) on the plant there must be at least one shoot that ensures the fortification of the plant, the production in the first year according to the specialized literature, can be 500-900 g/m^2.
The smallest productions were obtained in the field culture. We noticed that the highest yields were obtained on the perlite and peat substrate.

CONCLUSIONS

During the course of the experiments, regarding the appearance of the asparagus shoots, we recorded in the greenhouse a sum of temperature degrees of 3674ºC and in the field 2368ºC. The temperature difference between the greenhouse and the field was 1306ºC. In March we harvested asparagus shoots only in variants grown in the greenhouse these being different depending on the type of substrate, varying between 1 and 3 shoots. In April, shoots were collected from all varieties cultivated in the greenhouse but also from those cultivated in the field. The number of shoots being between 2 and 3, depending on the variant.

In May, shoots were harvested only from the chemically fertilized variant from the greenhouse, from the variant 3 fertilized with Formulex on 50% peat and 50% perlite substrate, respectively variants 4 and 5. Most shoots were harvested at variant cultivated in the field.

In this experiment we determined that the highest number of shoots harvested per square meter was recorded at V4, on 50% peat + 50% perlite substrate (with 7 shoots/m^2) and the lowest in field cultivated and fertilized with Amalgerol and Poco.

In present study we obtained the smallest productions in the field culture and we noticed that the highest yields were obtained on the perlite and peat substrate, in greenhouses.

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EVALUATION OF THE MAIN PHENOTYPIC AND PHYSICO-CHEMICAL CHARACTERISTICS IN THE NEW GENOTYPES OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) OBTAINED AT V.R.D.S. BUZĂU

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Abstract

Long ago, the Jerusalem artichoke was grown on large areas and almost all households had areas allocated to this crop. Over time, it was gradually removed ending up endangered. The Jerusalem artichoke was called turnip by the Romanian peasants, a mistake later rectified by Romanian specialists demonstrating that it belongs to the Asteraceae family, while the turnip belongs to the Brassicaceae family. The Breeding and Biodiversity Laboratory included the Jerusalem artichoke in its research in 1996, achieving a rich collection of genotypes, three of which have been approved and have distinct phenotypic characteristics: L1 (Olimp) shows white tubers, rich foliage mass composed of stems taller than 3 m; L2 (Rareș) has brown pink tubers, rich foliage mass and heights over 3 m; L3 (Dacic) has pink tubers, reduced foliage mass and stems shorter than 80 cm. During the vegetation period, phenological and biometric determinations were performed. Genotypes L1, L2 and L3 were physical-chemically analysed in the laboratory: the protein content was 1.1% in L1, 1.34% in L2 and 1.42% in L3 and cellulose content ranged from 0.9% in L1 to 0.98% in L3 and 0.99% in L2.

Key words*: biodiversity, breeding, genotype, germplasm, natural sweetener.*

INTRODUCTION

The Jerusalem artichoke belongs to the *Asteraceae* family and it is known botanically as *Helianthus tuberosus* L. It has several names among the folk such as "earth apple, bulbous chervil, rape, winter potato, earth potato, earth turnip, Jerusalem artichoke"; however, the most used name is that of "turnip". Unfortunately, this name was mistakenly embraced by the folk because the Jerusalem artichoke belongs to the *Asteraceae* family and the turnip belongs to the *Brassiceae* family. In Romania, the species is very little known as Jerusalem artichoke.

The plant has its origin in North America, being cultivated since ancient times by a tribe of Indians named topinambas. European explorers introduced it to Europe, where it spread rapidly, both as food for humans and animals (Rebora C., 2008). We have relevant data on its use as food from France, where it received the name of Jerusalem artichoke, gradually expanding throughout Europe, globally registered and known in all botanical nomenclatures under the name of Jerusalem artichoke (Luchian et al., 2017).

The Jerusalem artichoke is a perennial grassy plant with branched stems and can reach up to 2-3 m tall. Leaves are located towards the top of the stalk, flowers are small, bright yellow (Gupta D., Chaturvedi N., 2020).

In the soil it develops a system of rhizomes unequal in size, elongated and varying from nodes to round clusters in the form of small, fleshy tubers, similar to potatoes. The color of the tubers varies from pale brown to pink, red and purple, depending on the climate conditions (Gupta D., Chaturvedi N., 2020).

The plant was introduced in Romania in the 19th century, as a turnip though. There were also hypotheses released by the scientific world that, the species had been in Romania long before the 19th century, arguing that both in spontaneous flora and in households, there was a variety of dwarfed artichoke, called "Dacic". The species adapted very well to the pedoclimatic conditions of our country, even considered as invasive, being encountered in

the spontaneous flora and in the domestic system, almost throughout the entire territory of our country (Gherman N., 2013).
Vegetable Research Development Station (V.R.D.S.) Buzău has been intensively studying this species since 1996. The researches were channeled towards the breeding of the species in order to obtain genotypes with distinct phenotypic expressiveness suitable for several uses: consumption of fresh tubers, industrialization and processing, use of both, the flowering stems and the aerial stems, in the feeding of animals, use of the flowering stems as an energy plant, and the production of pellets. The researches were also extended for the use of the plant for medicinal, melliferous and ornamental purposes. Also, due to the woody stalk and tall height of 2-3m, the Jerusalem artichoke can be used successfully as a protective crop for the more sensitive crops to cold winds or to prevent the evaporation of humidity from the soil (Ciuciuc et al., 2019; Barcanu et al., 2019).
Therefore, the Jerusalem artichoke is a plant with great potential, both in the food industry and as industrial and fuel product.
Tuber production has a high yield, grows better in poor soils than most crops and has a high resistance to diseases and pests, as well as to low temperatures (Rebora, 2008; Drăghici, 2018). This paper aims to evaluate the phenotypic expressivity and biological production potential of three new genotypes of Jerusalem artichoke obtained at Vegetable Research Development Station (V.R.D.S.) Buzău.

MATERIALS AND METHODS

Vegetable Research Development Station (V.R.D.S.) Buzău has a valuable germplasm base of this species, out of which three genotypes were selected, which received code names L1, L2 and L3 and were approved under the names of L1 - Olimp, L2 - Rareș and L3 - Dacic.
The breeding method used to obtain the genotypes was clonal selection.
The crop technology applied was the one specific to Jerusalem artichoke, being very similar to the one specific to potatoes. The land was prepared in the fall and the establishment of the crop was carried out for all three varieties, after the fall of haze, starting with November 10th.
Planting was done on hilling, with a distance of 70 cm between rows and 20-30 cm between plants/row.
The care work consisted of mechanical and manual hoeing for rebuilding the hills, in the spring. Starting with June, three irrigations have been made to fill the water supply required by the plant.
Starting from June 15, mechanical and manual hoeing could no longer be realized, as the flowering stems would have been broken.
During the vegetation period and after the harvest, phenological and biometric observations, as well as sensorial and laboratory analyzes were made.

RESULTS AND DISCUSSIONS

Bellows, it will present the results obtained in the study performed on the three genotypes obtained at Vegetable Research Development Station (V.R.D.S.) Buzău: L1 Olimp (Figure 1), L2 Rareș (Figure 2) and L3 Dacic (Figure 3). These are measurements that have highlighted the main phenotypic characteristics and sensory and physico-chemical analyzes of fresh Jerusalem artichoke tubers.

Figure 1. Olimp variety, with yellow-white tubers

Figure 2. Rareș variety, with pink-brown tubers

Figure 3. Dacic variety, with pink colour tubers and small height

The three genotypes selected for research have distinct characteristics. The first three phenotypic characteristics studied in the Table 1, below are: plant height (m), where first place was occupied by L2 genotype (Rareș) with 2.8 m, while the last place, at plant height characteristic was occupied by L3, Dacic, with only 0.8 m. The next indicator studied was number of stems/plant, where L3, Dacic has the highest value (10 stems/plant), and the smallest value was L1, Olimp with 6 stems/plant (Table 1). The last column from Table 1 is reserved for the distance between the leaves, where the first place is occupied by L1 (6 cm) and the last place by L3 (1.2 cm).

Table 1. The Jerusalem artichoke main characteristics I

Feature	Plant height (cm)			Stems no./ plant			Leaf distance (cm)		
	Limit of variability		Average value	Limit of variability		Average Value	Limit of variability		Average Value
	lowest	highest		lowest	highest		lowest	highest	
Genotype L1 OLIMP	240	260	121	5	7	6	4,00	8,00	6,00
Genotype L2 RAREȘ	260	300	280	6	10	8	4,00	6,00	5,00
Genotype L3 DACIC	60	80	70	8	12	10	0,70	1,70	1,20

In the Table 2 below, were considered for study the following three characteristics as follows: length of the leaf (cm), width of the leaf (cm) and the length of the peduncle (cm). Regarding the length of the leaf, the highest value was registered at L1, Olimp (22 cm), and the lowest value at L3, Dacic (10 cm). The next phenotypical characteristic studied was the width of the leaf, where the highest value was measured at L1, Olimp (16 cm), and the lowest value measured was at L3, Dacic (4 cm).

The last studied characteristics was the length of the peduncle, where the first position was occupied by L1, Olimp with 10 cm, and the last position was occupied by L3, Dacic, with a length of the peduncle of only 1 cm.

Table 2. The Jerusalem artichoke main characteristics II

Feature	Leaf length (cm)			Leaf width (cm)			Peduncle length (cm)		
	Limit of variability		Average value	Limit of variability		Average value	Limit of variability		Average value
	lowest	highest		lowest	highest		lowest	highest	
Genotype L1 OLIMP	20	24	22	12	16	14	8	12	10
Genotype L2 RAREȘ	16	20	18	10	14	12	6	10	8
Genotype L3 DACIC	8	12	10	2	6	4	1	2	1

Continuing, for the three genotypes that were studied, Olimp, Rareș and Dacic, the tubers were subjected to multiple measurements.

First of all, were selected several tubers coming from each and every genotype. Therefore at the Olimp, L1 genotype, the tubers were numbered, weighted and the average weight of the tubers was calculated.

The same method was repeated for the Rareș, L2 genotype tubers, identical measurements were made also to the Dacic, L3, tubers. According to the Table 3, at the genotype Rareș, L2, were obtained the highest values, that is, the biggest tubers, with an average weight of 192 g.

On the other side, the smallest values were occupied by Olimp, L1, with an average weight of 98 g. In the Table 3, were presented, also, the length (cm) and the diameter (cm) of Jerusalem artichoke tubercles, it was highlighted the fact that the genotype Dacic, L3, show the highest value of tuber length (10.2 cm), and the genotype Olimp, L1, show the lowest value in table, regarding the tuber length (7.7 cm). Measures that were made further revealed that the biggest diameter was measured at the genotype Dacic L3, with 5.4 cm, and the smallest diameter was measured at the genotype Olimp L1, with 4.2 cm.

Table 3. Quantitative characteristics at Jerusalem artichoke tubers

Feature	Tuber weight (g)			Tuber length (cm)			Tuber diameter (cm)		
	Limit of variability		Average	Limit of variability		Average	Limit of variability		Average
	lowest	highest	value	lowest	highest	Value	lowest	highest	Value
Genotype L1 OLIMP	54	143	98	5,2	10,3	7,7	3,8	4,5	4,2
Genotype L2 RAREȘ	46	338	192	6,0	13,4	9,7	4,2	6,4	5,3
Genotype L3 DACIC	34	166	100	7,8	12,7	10,2	4,1	6,7	5,4

Accordingly, transposed in a graphic, the first characteristics determined in Table 3, that is, The Average weight of artichoke tubercles is represented in Figure 4.

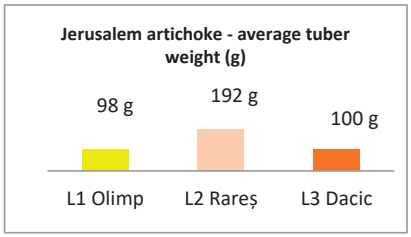

Figure 4. The Average weight Jerusalem artichoke tubers representation

The following step of the study was the sensorial analyses for fresh tubers, that was done for all three genotypes L1 Olimp, L2 Rareş and L3 Dacic. All the measured values are presented in the Table 4.

Table 4. The sensorial analyses of the fresh Jerusalem artichoke tubers

Jerusalem artichoke Variety	INDICATOR					
	Appearance before washing	Appearance after washing	Color	Texture	Taste	Smell
L1 Olimp	tubers of irregular shape and different sizes, with the surface covered by wet dust	tubers of irregular shape (oval, fusiform) of different sizes, 5-10 cm length, and 4-5 cm diameter	specific yellow - white	freshness crispy, juicy	moderately sweetness, characteristic pleasant taste	distinctive
L2 Rareș	tubers of irregular shape and different sizes, with the surface covered by wet dust	tubers of irregular shape (oval, fusiform) of different sizes, 6-13 cm length and 4-6 cm diameter	specific pink-brown	freshness crispy, juicy	weakly sweetness, characteristic pleasant taste	distinctive
L3 Dacic	tubers of irregular shape and different sizes, with the surface covered by wet dust	tubers of irregular shape (oval, fusiform) of different sizes, 8-13 cm length and 4-7 cm diameter	specific pink	freshness crispy, juicy	strongly sweetness, characteristic pleasant taste	distinctive

Therefore, the following indicators were followed: the aspect of the tubers before washing, the aspect after washing, colour of the tubers, the texture, the taste and the smell.

For the first indicator, the aspect of the tubers before washing, the observations made revealed that all three genotypes of Jerusalem artichoke, that is: L1- Olimp, L2- Rareş and L3-Dacic, presents tubers with an irregular forma, sizes and dimensions and the surface of the tubers was covered with wet dust.

The second indicator that was studied, the aspect of the tubers after washing, was analysed after the procedure of cleaning the wet dust and revealed the fact that the tubercle presents different irregularities on the surface of the tubers, of different shapes and forms: oval, spindly, and different lengths and diameters as well, as follows: at genotype L3, Dacic (Figure 5) were obtained the highest values in regards of the length of the tubers that is between 8-13 cm, and at the measurement of the diameters, at the same genotype, L3, Dacic, were measured diameters between 4-7 cm.

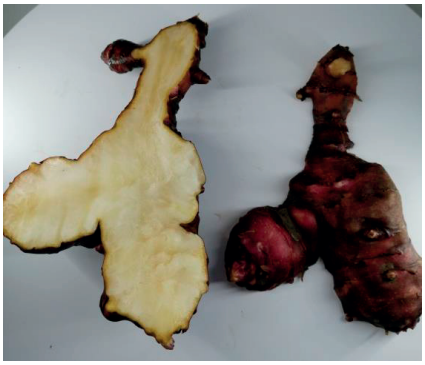

Figure 5. Genotype Dacic, longitudinal section

The smallest values were registered measuring the tubers for the genotype L1, Olimp (Figure 6), that is lengths covered in the interval 5-10 cm, and regarding the diameters, the measured values were found between 4-5 cm.

Figure 6. Genotype Olimp, longitudinal section

At the Jerusalem artichoke genotype L2, Rareş (Figure 7), the tubers length measured value is included in the interval 6-13 cm, and the diameter values that were measured, is included in the interval 4-6 cm.

The third studied characteristic, was the colour of the Jerusalem artichoke tubers where the following aspect were revealed: at the genotype L1, Olimp, as presented in Figure 6, the tubers presents a specific white-yellow colour, at the genotype L2, Rareş (Figure 7), the tubers presents a characteristic pink-brown colour, and at the genotype L3, Dacic (Figure 5), tubers have a specific pink colour.

Figure 7. Genotype Rareş, longitudinal section

The forth characteristic that was studied was the texture of the Jerusalem artichoke tubers, where it was revealed that for all the three genotypes, the texture of each one was crunchy and juicy, with the specification that at Rareş, L2, the texture of fresh tubers was a little bit floury, similar to the raw potato. Also, all the Jerusalem artichoke samples generated a pleasant sensation of freshness at the taste buds level.

The fifth characteristics, in regards of the sensitive analyse, is the taste, and it was revealed that the taste is sweet, nice and characteristic to the Jerusalem artichoke tubers, to be mentioned here that the genotype L1, Olimp has a moderate sweet taste, the genotype L2, Rareş is the less sweet and the genotype L3, Dacic is the sweetest one of the three genotypes.

The last studied characteristic was the smell of the Jerusalem artichoke tubers that revealed the fact that all the tubers coming from the three genotypes, that is L1 Olimp, L2 Rareş and L3 Dacic, as well, present a distinctive smell.

In Table 5 are presented the figures that were obtained after the physical and chemical analyse at the Jerusalem artichoke tubers at all three studied genotypes, that is L1, Olimp, L2, Rareş and L3, Dacic. Therefore, there were studied the following indicators, in percentual form: humidity, total ash, proteins and cellulose.

The first indicator studied physically and chemically was humidity, where the lowest value measured was registered at genotype L3, Dacic 73.72%, meantime the highest value measured was at genotype L1, Olimp, 74.10%.

After that, the total ash was analysed, the lowest percentage was measured at genotype L1, Olimp (72%), and the highest percentage was measured at the genotype L3, Dacic 1.16%.

The third indicator that was studied was the protein percentage, where the genotype L3 Dacic generated the highest value measured that is 1.42%, and the genotype L1, Olimp, the lowest value measured 1.10%.

The last indicator that was analysed physical and chemical for the Jerusalem artichoke tubers was to calculate the cellulose percentage. In this case the highest rate was identified for the genotype L1, Olimp, 0.90%, and the highest value was 0.99%, found at genotype L2, Rareş.

Table 5. Physical and chemical analyse for the fresh Jerusalem artichoke tubers

Crt. No.	Physical-Chemical Indicator	Jerusalem artichoke variety		
		L1 Olimp	L2 Rareş	L3 Dacic
1	Humidity (%)	74,1	75,31	73,22
2	Total ash (%)	0,72	1,14	1,16
3	Protein (%)	1,1	1,34	1,42
4	Cellulose (%)	0,9	0,99	0,98

CONCLUSIONS

The researches were finalised achieving three different Jerusalem artichoke varieties, each one having distinct phenotypical expression.

L1 - present white-yellow colour tubers, rich foliage mass and a height over 3m.

L2 - present pink-brown tubers, rich foliage mass, and height beyond 3m.

L3 - present pink colour tubers, slight spherical, low foliage mass and a height of a maximum 80 cm.

L2 was approved and registered in The Official List of Culture Plants from Romania, starting 2018, being named Rareş.

L1 was approved and is registered in The Official List of Culture Plants from Romania from 2019, and L2, is presently in the last year

of tests and trials, and will follow to be approved and registered in The Official List of Culture Plants from Romania, starting 2020.
Biological material (the tubers), offered promotionally to the farmers, helps to increase the areas cultivated with this specie in Romania, increasing the interest of the farmers and of the consumers as well for this plant. During the study, was followed not only the plant, but the preparation of the specific process of farming of the plant, as well, each farmer receiving not only the biological material, but specific information regarding the farming of artichoke.
The interest for Jerusalem artichoke remains of great actuality, if we consider the valorification of tubers for achieving of functional ingredient with high nutritional value. We are speaking here about the powders achieved from Jerusalem artichoke tubers, and can be used to fortify food products (bakery and pastry products, especially), in order to increase the nutritional and their antioxidant potential, but also as sweetening agent for products, for diabetics. (Catană et al., 2018).
Researches will continue through the enrichment of the germplasm base germplasm of this specie and the support and promotion of the production for this specie and the promotion of the production on a large scale of the new genotypes achieved.

ACKNOWLEDGEMENTS

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MICROELEMENTS STATUS IN CARROT ROOT ACCORDING TO DIFFERENT SALES AND PRODUCTION CHANNELS

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Abstract

Carrot (Daucus carota L.*) is vegetable of Apiaceae family that has a very significant nutritional and health value in human nutrition. Depending on the production system, the mineral composition of the vegetables also differs. Therefore, the aim of this research was to determine the status of microelements in a carrot's root and to compare samples collected at different sales channels on the Zagreb market (Croatia). Carrot sampling was carried out in triplicate in city of Zagreb in 5 retail chains, 5 markets and 5 organic products stores. After digestion of dry plant material with concentrated HNO_3 and H_2O_2 microelements (iron, manganese, zinc and copper) were determined by atomic absorption spectrometry. The results showed differences among carrots sampled at different sales channels and with different production system. Average content of microelements in retail chains, markets and organic stores ranged from 15.34-19.61 mg Fe kg^{-1}, 7.97-9.07 mg Mn kg^{-1}, 14.62-14.87 mg Zn kg^{-1}, 4.38-6.11 mg Cu kg^{-1}. The data obtained from this study showed a statistically greater status of microelements in carrot samples grown in conventional production than in organic ones.*

Key words*: copper, Daucus carota* L.*, iron, manganese, zinc.*

INTRODUCTION

Carrot (*Daucus carota* L.) belongs to the group of the ten most important types of vegetables along with cabbage, tomatoes, onions and peppers (Kantoci, 2014). It provides the human body with a range of minerals and vitamins that have a beneficial effect on eyesight, skin, immunity, digestive and circulatory systems. The most common elements in carrots are potassium and calcium, followed by iron, phosphorus and iodine (Lešić et al., 2004). In addition to vitamin A, carrots also contain B vitamins, vitamins C, D, E and K, and small amount of folic acid (Parađiković, 2009). Disorders of iron metabolism are one of the most common diseases in humans and cover a wide range of diseases with different clinical manifestations like anemia or neurodegenerative diseases (Đokić and Bilandžić, 2012). The World Health Organization (2001) published an estimate that two billion people are anemic in the world, and that 50% of all anemias are caused by iron deficiency. Copper is associated with the formation of red blood cells, therefore deficiency of copper as well as iron causes anemia (Angelova et al., 2011).

Microelements, although required in small quantities, are essential to the plant, meaning that they are needed throughout the life cycle and they perform a function that cannot be replaced by another element. For the plant, iron is essential for chlorophyll synthesis, nitrite and sulfate reduction, nitrogen assimilation, and electron transport (Grabić, 2015). Due to the poor mobility of iron in the plant, its deficiency is manifested by the yellowing of young leaves. According to Brancadoro et al. (1992) plants where iron deficiency occurs accumulate larger amounts of amino acids, nitrates, and organic anions. Unlike other trace elements in the plant that are mainly components of enzymes, manganese acts as an enzyme activator (Burnell, 1988). Manganese has a great influence on photosynthesis, especially on electron transfer in photo system II, and on nitrogen metabolism (Campbell and Nable, 1988). Manganese deficiency in plants most often occurs in the form of chlorosis on young

leaves, while in the case of severe deficiency, brown necrotic spots and fallout of leaves may also occur. Excess of manganese can cause deficiency of Fe, Mo and Mg in plants (Vukadinović and Lončarić, 1998). Zinc in the plant plays a role in enzyme activation and auxin formation, so its deficiency manifests itself in the form of small plant growth (Parađiković, 2009). Copper is essential in plant organism for the synthesis of lignin, which is important for cell wall strenght (Gluhić, 2013), also participates in electron transfer in photosynthesis in the form of plastocyanin, is a cofactor in Cu-Zn superoxide dismutase and polyphenol oxidase, and is important for structural stability of chromosomes (Vidaković-Cifrek et al., 2015). Copper deficiency of the plant is manifested by chlorosis and necrosis of young leaves, fading and twisting of the whole plant. Function of copper in the plant is in the formation of auxin, so its deficiency can affect the small growth of the plant (Parađiković, 2009). In addition, it increases resistance to diseases, drought and low temperatures (Grabić, 2015).

A study from Poland (Bosiacki and Tyksiński, 2009) presents data on the amount of microelements in the dry matter of carrots, showing a comparison of the amount of microelements from the year in which the work was created, and from the 15 years preceding it by Tyksiński et al. (1993). The results of the study show and increase in the amount of iron (35.0 mg Fe kg^{-1}, Tyksiński et al., 1993, 54.4 mg Fe kg^{-1}, Bosiacki and Tyksiński, 2009) and zinc (19.5 mg Zn kg^{-1}, Tyksiński et al., 1993, 22.3 mg Zn kg^{-1}, Bosiacki and Tyksiński, 2009) in carrots, reducing the amount of manganese (10.0 mg Mn kg^{-1}, Tyksiński et al., 1993, 5.2 mg Mn kg^{-1}, Bosiacki and Tyksiński, 2009) by almost half, while the amount of copper (4.2 mg Cu kg^{-1}, Tyksiński et al., 1993, 4.2 mg Cu kg^{-1}, Bosiacki and Tyksiński, 2009) has not changed.

In order to achieve stable and expected yields, increased use of substances containing metals, and the application of essential microelements such as Cu, Zn, Fe and Mn became common agricultural operation on soils less availability of microelements (Grabić, 2015). Conventional production means the development of mechanization and use of mineral fertilizers that have a higher amount of nutrients than organic fertilizers used in organic production.

The term organic farming refers to a specific system of sustainable management in agriculture with the aim of producing healthy food, that is, meeting the relevant social and economic needs while preserving the natural ecosystem and landscape (Pejnović, 2012).

Therefore, the aim of this research was to determine the amount of microelements in the dry and fresh matter of carrots and to compare the samples collected at the Zagreb market at different selling spots.

MATERIALS AND METHODS

Orange root colored carrots were sampled on the different selling spots in of the city of Zagreb.

Sampling was carried out on 04.12.2017 in triplicate in the city of Zagreb in 5 retail chains (RC), 5 markets (M) and 5 organic products stores (OPS). Informations on the method of cultivation were collected orally through personal communication with the salesperson or by the insight into the declaration. The assumption is that all carrot samples from retail chains were grown in the conventional way because they were not specifically labeled as organic products. According to the sellers, carrots from Dubrava and Kvatrić market were fertilized with mineral manure (conventional production method), while carrots from Britanski trg and Dolac markets were fertilized with manure and from Branimir market with sheep manure (organic production method). All samples of carrots from organic stores were grown in an organic way because only organic products are sold in these stores.

The carrot samples were dried at 105 ° C, after which they were grounded and homogenized. Zinc, manganese, iron, and copper, after digestion with concentrated HNO_3 and $HClO_4$ in a microwave oven, were determined by atomic absorption spectrometry-AAS (AOAC, 2015). The dry matter was determined gravimetrically by drying to a constant mass. The samples, collected in triplicate, were analyzed individually and the results showed average values. Statistical data processing followed the variance analysis model (ANOVA). The SAS System for Win program

was used. ver 9.1 (SAS Institute Inc.), and Tukey's significance threshold test (SAS, 2002-2003) was used to test the results.

RESULTS AND DISCUSSIONS

The average value of dry matter in carrot samples ranges from 10.15 to 11.18% (Figure 1). According to Parađiković (2009) amount of dry matter in carrots is in the range of 12-17%, while Kantoci (2014) states 14.5%. The amount of dry matter determined in this study is less than the amount reported in the literature.

The average amount of microelements in the dry matter of carrots is shown in Figure 2. Statistically, highest amount was found for iron (19.61 mg Fe kg^{-1}) at retail chains (RC). The levels of manganese and zinc do not differ significantly from point of sale, while the amount of copper differs and is the largest in organic products stores (OPS) (6.11 mg Cu kg^{-1}). Figure 3 shows the average quantities of microelements in fresh carrot matter by point of sale. Significantly different values were found for iron and copper, while manganese and zinc quantities did not differ significantly between retail chains, markets and organic products stores. The highest amount of iron was found in retail chains (0.19 mg Fe 100 g^{-1} of fresh matter), while the highest amount of copper was found in organic products stores (0.06 mg Cu 100 g^{-1} of fresh matter).

Of all the examined carrot samples, statistically highest amount of iron in dry matter was determined in RC5 and its amount is 24.67 mg Fe kg^{-1} of dry matter and 0.23 mg Fe 100 g^{-1} of fresh matter (Figures 4 and 5). Other retail chains also have high levels of iron (16.26 - 20.49 mg Fe kg^{-1} of dry matter, 0.15-0.21 mg Fe 100 g^{-1} of fresh matter), while markets and organic product stores range from 12.23-18.85 mg Fe kg^{-1} of dry matter and 0.1-0.18 mg Fe 100 g^{-1}, with the exception of the M5 market, which has a higher amount of iron (20.66 mg Fe kg^{-1} of dry matter, 0.2 mg of Fe 100 g^{-1} of fresh matter). Carrots from retail chains generally have higher amount of microelements than markets and organic product stores. The literature reports amount of iron 0.3 mg Fe 100 g^{-1} in fresh matter (USDA, 2018), and in the range of 0.5-2.68 mg Fe 100 g^{-1} in fresh matter (Lešić et al., 2004). In comparison with the results of this study, the amount of iron in dry matter of carrots is less than the amount in the 1993 study (Tyksiński et al.) and 2009 study (Bosiacki and Tyksiński). Possible reason for the low supply of iron in carrots from markets and organic product stores is the ecological way of production and fertilization. A study from Poland conducted between 2011 and 2012 dealt with the controlled cultivation of carrots in a conventional and ecological way. In this study, the amount of iron in carrots ranged from 41.10-61.13 mg Fe kg^{-1} of dry matter in both cultivation methods, and the highest amount of iron was determined in carrots grown without the use of protection against pathogens (Wierzboska et al., 2016). Analysis of carrots fresh matter (mg 100 g^{-1}) from Hisar market in India, found higher amounts of iron (7.7 mg Fe 100 g^{-1}) (Singh et al., 2001) than carrot analysis in Zagreb (0.11-0.2 mg Fe 100 g^{-1}). Accordingly, neither of the maximum amount of iron found in carrots (RC5) is higher than the literature cited. Manganese is a trace element in carrots, so its values do not exceed 0.1 mg Mn 100 g^{-1} in fresh matter, or 11.46 mg Mn kg^{-1} in dry matter (Figures 6 and 7). Statistically lowest value of manganese was determined in carrot from market M1 (0.05 mg Mn 100 g^{-1} of fresh matter, 6.51 mg Mn kg^{-1} of dry matter), and statistically highest in carrot from RC1 (0.1 mg Mn 100 g^{-1} of fresh matter, 11.46 mg Mn kg^{-1} of dry matter). The following maximum values were determined in carrots from the organic product stores OPS3 (0.9 mg Mn 100 g^{-1} fresh matter, 9.99 mg Mn kg^{-1} dry matter) and OPS5 (0.9 mg Mn 100 g^{-1}, 9.57 mg Mn kg^{-1} of dry matter). None of the determined values from this study exceeds the values reported in the literature: 0.14 mg Mn 100 g^{-1} in fresh matter (Parađiković, 2009) and 0.143 mg Mn 100 g^{-1} in fresh matter (USDA, 2018). Also, the amount of manganese in dry matter determined in this study is less than the amount determined in the 1993 study (Tyksiński et al.), but higher than the 2009 study (Bosiacki and Tyksiński). Compared to a study conducted in controlled cultivation in Poland where the lowest amount of manganese (8.70 mg Mn kg^{-1} dry matter) was recorded in carrots from ecological cultivation (Wierzboska et al., 2016), the amount of manganese in carrot samples from organic product stores (OPS)

slightly higher in this study (7.63-9.99 mg Mn kg^{-1} dry matter). Carrot samples collected from a market in India show a significantly higher amount of manganese in fresh carrot matter (1.8 mg Mn 100 g^{-1}) (Singh et al., 2001) than samples collected from Zagreb markets (0.05-0.08 mg Mn 100 g^{-1}).

Zinc amount results show a statistically highest value in M5 carrots of 0.21 mg Zn 100 g^{-1} in fresh matter and 21.9 mg Zn kg^{-1} in dry matter, while the next highest value is from RC5 (0.18 mg Zn 100 g^{-1} of fresh matter, 19.47 mg Zn kg^{-1} of dry matter) (Figures 8 and 9). The lowest value was determined in the sample of carrots on M2 and is 0.09 mg Zn 100 g^{-1} of fresh matter and 10.47 mg Zn kg^{-1} of dry matter. Values from organic products stores (OPS) range from 0.09-0.16 mg Zn 100 g^{-1} of fresh matter, or 11.7-17.29 mg Zn kg^{-1} of dry matter. In the case of zinc, there is no significant deviation from the literature data. Parađiković (2009) states the amount of zinc in carrots of 0.2 mg Zn 100 g^{-1} in fresh matter, and USDA (2018) 0.24 mg Zn 100 g^{-1} in fresh matter. As for the 1993 (Tyksiński et al.) and 2009 (Bosiacki and Tyksiński) studies, the amount of zinc in dry matter in this study is less than the amount of zinc in the studies mentioned. Samples were collected in a 2006 study (Radwan and Salama) to determine the amount of heavy metals (Pb, Cd, Cu, and Zn) in fruits and vegetables in Egyptian markets. The amount of zinc found in carrot dry matter is 6.02-11.1 mg Zn kg^{-1} (Radwan and Salama, 2006). Comparing zinc values in carrots from markets in Zagreb and those in Egypt, a possible reason for the low supply, in both cases, is the way of production and fertilization. The amount of zinc in the dry matter in the Polish study was between 8.22-13.0 mg Zn kg^{-1} (Wierzboska et al., 2016). In that study, conventional and organic farming were compared, where carrots grown with conventional way had 25% more zinc than carrots grown with organic way (Wierzboska et al., 2016). Also in this study the amount of zinc is higher in carrots from retail chains (conventional production) than in carrots from organic product stores.

Copper, like manganese, is a trace element in the carrot. The amount of 0.03 mg Cu 100 g^{-1} in fresh matter is the lowest found in this study, repeated at multiple outlets, and most commonly in markets. Statistically highest values of copper were found in carrots from the OPS4 (0.06 mg Cu 100 g^{-1} fresh matter, 6.69 mg Cu kg^{-1} dry matter) (Figures 10 and 11). On markets, the highest amount of copper was determined in the carrot from the M5 market and it is 0.05 mg Cu 100 g^{-1} in fresh matter and 5.97 mg Cu kg^{-1} dry matter. Regarding retail chains, the highest determined quantity of copper in fresh matter is in carrots from retail chain RC3 (0.05 mg Cu 100 g^{-1} of fresh substance), while for the quantity in dry matter it is highest recorded in carrots from shopping center T1 (5 mg Cu kg^{-1} dry matter). The copper values determined in this study do not deviate from the values reported in the literature, namely 0.05 mg Cu 100 g^{-1} in fresh matter (Parađiković, 2009) and 0.045 mg Cu 100 g^{-1} in fresh matter (USDA, 2018). The amount of copper in the dry matter of carrots in the studies in 1993 (Tyksiński et al.) and 2009 (Bosiacki and Tyksiński) do not differ significantly from the values determined in this study. In a 2006 study (Radwan and Salama), where samples were collected from different markets in Alexandria, Egypt, copper values found in the dry matter of carrots (0.99-2.10 mg Cu kg^{-1}) are significantly smaller than the quantities determined from the markets in this study (3.59-5.97 mg Cu kg^{-1}), while the results of the analysis of copper in fresh carrot matter in the market in India (0.8 mg Cu 100 g^{-1}) (Singh et al., 2001) showed higher amounts of copper than in carrots from Zagreb markets (0.03-0.05 mg Cu 100 g^{-1}). A study from Poland (Wierzboska et al., 2016) comparing conventional and organic carrots growing found the amount of copper in the dry matter of carrots from 3.05-4.74 mg Cu kg^{-1}, also showing that carrots grown ecologically have 10.3% more copper than carrots grown by conventional cultivation. In comparison with the results of this study, the amount of copper in carrots from retail chains (conventional cultivation) (3.5-5 mg Cu kg^{-1}) is not significantly different from the amount of copper in carrots grown in conventional cultivation from Poland (3.05-4.35 mg Cu kg^{-1}) (Wierzboska et al., 2016), while the determined quantities of copper in the dry matter in organic products stores (ecological cultivation) (5-6.69

mg Cu kg^{-1}) from this study are slightly higher than the amount of copper in the dry matter of carrots grown ecologically from research in Poland (3.77-4.74 mg Cu kg^{-1}) (Wierzboska et al., 2016).

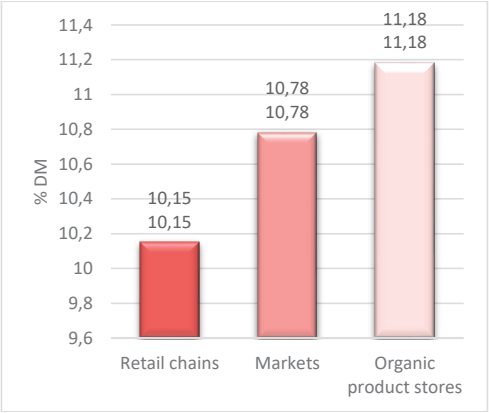

Figure 1. Amount of dry matter (% DM) determined in carrot samples collected from retail chains, markets and organic product stores
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

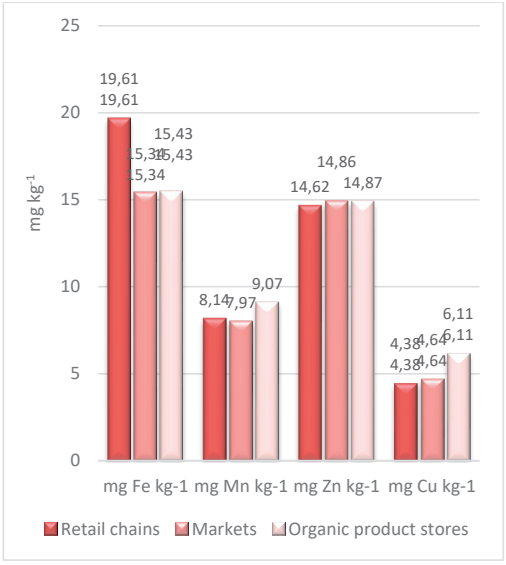

Figure 2. Amount of microelements determined in carrots dry matter (mg kg^{-1}) at different selling points
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

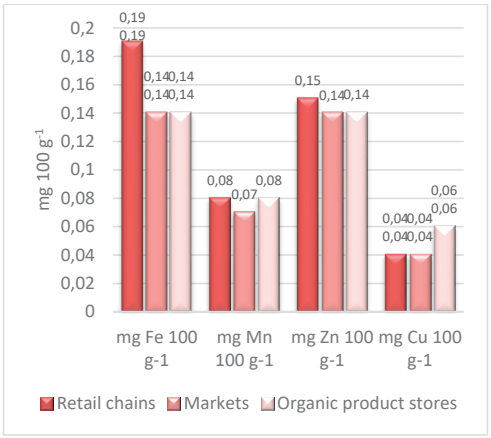

Figure 3. Amount of microelements determined in carrots fresh matter (mg 100 g^{-1}) at different selling points
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

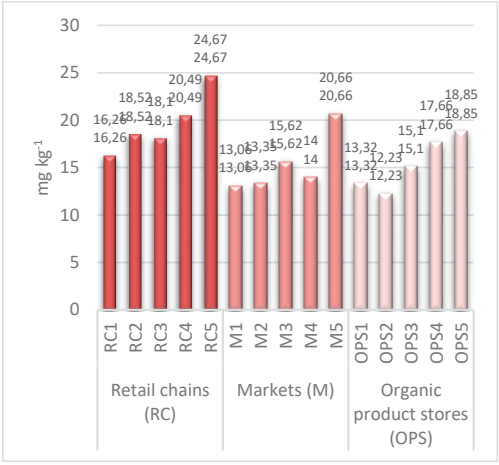

Figure 4. Amount of iron determined in the dry matter of the carrot (mg kg^{-1})
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

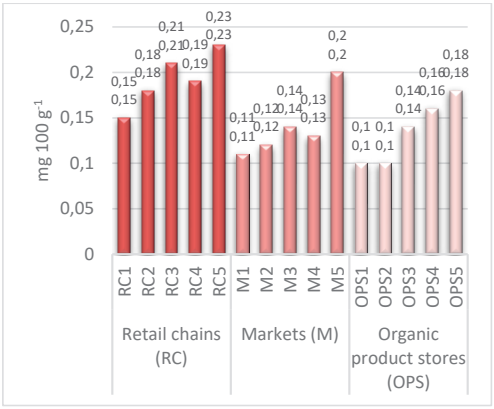

Figure 5. Amount of iron determined in the fresh matter of the carrot (mg 100 g^{-1})
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

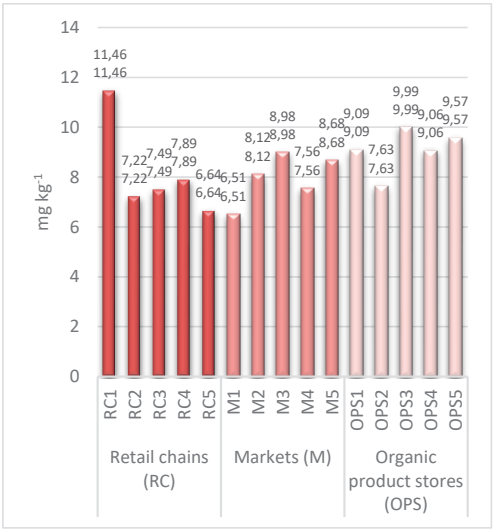

Figure 6. Amount of manganese determined in the dry matter of the carrot (mg kg^{-1})
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

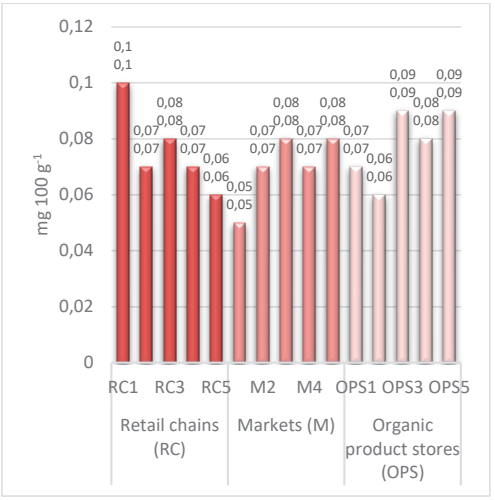

Figure 7. Amount of manganese determined in the fresh matter of the carrot (mg 100 g^{-1})
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

Figure 8. Amount of zinc determined in the dry matter of the carrot (mg kg^{-1})
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

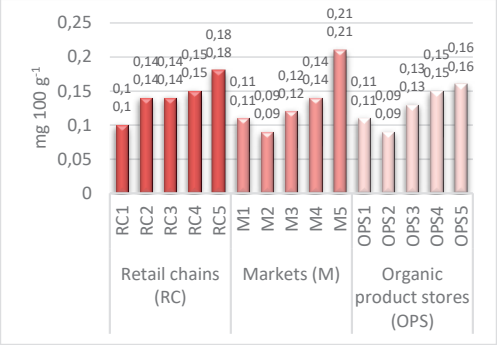

Figure 9. Amount of zinc determined in the fresh matter of the carrot (mg 100 g^{-1})
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

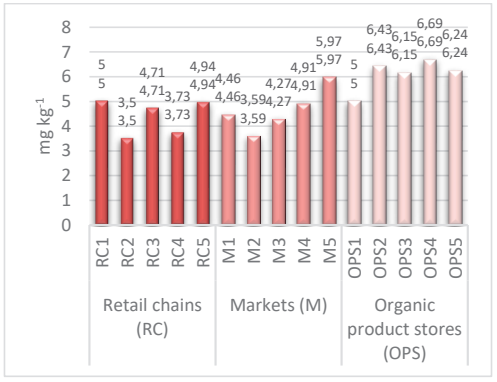

Figure 10. Amount of copper determined in the dry matter of the carrot (mg kg^{-1})
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

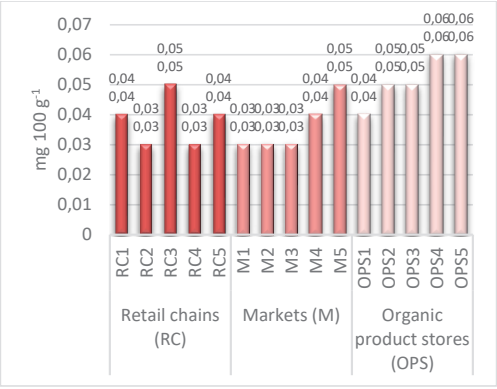

Figure 11. Amount of copper determined in the fresh matter of the carrot (mg 100 g^{-1})
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

CONCLUSIONS

In this study, the quantities of microelements were determined in the orange colored carrot roots, sampled at different outlets in the City of Zagreb.

The amounts of microelements expressed in mg kg^{-1} of the dry matter of the orange colored carrot root were: 12.23-24.67 Fe, 6.51-11.46 Mn, 10.47-21.9 Zn and 3.5-6.69 Cu.

The amounts of microelements expressed in mg 100 g^{-1} of the fresh matter of orange colored carrot root were: 0.1-0.23 Fe, 0.05-0.1 Mn, 0.09-0.21 Zn and 0.03-0.06 Cu.

Differences were identified with consideration to the different selling points. Namely, carrots from retail chains were grown in a conventional way, while most carrots were grown ecologically from markets and organic products stores. Generally, the highest values of microelements are found in carrots from retail chains. Considering that the production conditions are not known (soil type, agricultural practices, fertilization), it can be assumed that the conventional way of producing and using mineral fertilizers containing more nutrients than organic fertilizers is probably reason for higher quanities of microelement in carrots from conventional cultivation.

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IN VITRO EFFECT OF ABAMECTIN FROM *STREPTOMYCES AVERMITILIS* ON THE SURVIVAL OF THE CYST NEMATODES *GLOBODERA PALLIDA, HETERODERA CAROTAE* AND *HETERODERA SCHACHTII*

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Abstract

The effect of an abamectin formulation (Vertimec© EC) was tested against the cyst nematodes Globodera pallida, Heterodera carotae and Heterodera schachtii in an in vitro hatching test. Abamectin (a mixture of two macrocyclic lactones B1a and B1b) is produced by the actinomycete Streptomyces avermitilis. Cysts of the nematodes were extracted from samples of infested soils by the Fenwick can and then subjected to different concentrations of an aqueous solution of the abamectin formulation (0, 1.125, 2.25, 4.5, 9.0, 18.0 and 36 µg/ml) and exposure times from 24 to 384 hours. For each nematode specie batches of 50 cysts were set up and arranged in a growth cabinet (20°C ± 2) according to a randomized block design. Untreated cysts were used as control. There were three replications for each treatment. As natural and artificial hatching agents for H. carotae, H. schachtii and G. pallida were used carrot root leachate, 0.3 mM zinc chloride and 0.6 mM sodium metavanadate aqueous solutions, respectively. Every week emerged juveniles were counted. At the end of the hatching test cysts were crushed and unhatched eggs and juveniles counted. The total number of juveniles emerged in the hatching test were expressed as the percentage of the total egg content of the cysts (hatched + unhatched). From percentage hatch was calculated the mortality of each treatment considering the natural death in the control. Data of percentage mortality were subjected to probit analysis to calculate values of lethal doses (LD) required for 50% egg mortality. At 24 hours exposure, values of LD_{50} for H. carotae, G. pallida and H. schachtii were 9.9, 13.2 and 796 µg/ml, respectively. For the same nematode species at 384 hours exposure LD_{50} decreased at 3.6, 2.9 and 17.6 µg/ml.

Key words*: cyst forming nematodes, lethal doses, nematicidal effect, nematode management.*

INTRODUCTION

A wide range of biologically active substances especially antibiotics and hydrolytic enzymes protecting plant growth from pathogenic fungi and pests are produced by Streptomycetes spp. (Jones and Samac, 1996; Trejo-Estrada et al., 1998; Sasanelli et al., 2016). Among these substances abamectin is known for its insecticidal, acaricidal and anthelmintic activities (Jayakumar, 2009; Poiras et al., 2013). Abamectin is a mixture of macrocyclic lactones (B1a and B1b) isolated from fermentation broths of the actinomycetes *Streptomyces avermitilis* (Burg et al.) Kim and Goodfellow. It is registered as an miticide/insecticide in many countries and it was introduced as bio-pesticide in 1985. Abamectin is commercialised under the names Affirm, Agri-Mek, Avid, Avomec, Dynamec, Vertimec EC and Zephyr depending on the formulations. Jansson and Dybas (1998) reported that avermectins blok the transmittance of electrical activity in nerves and muscle cells by the release of g-aminobutyric acid-like (GABA) neurotransmitters. Abamectin is an effective control method as bionematicide against some plant parasitic nematodes as *Pratylenchus penetrans* Cobb, Sher et Allen (Samac and

Kinkel, 2001), the reniform nematode *Rotylenchulus reniformis* Lindford and Oliveira and the root-knot nematodes *Meloidogyne incognita* (Kofoid et White) Chitw. and *M. arenaria* (Neal) Chitw. (Cayrol et al., 1993; Jayakumar, 2009; Laquale et al., 2014). Although it is known the nematicidal effect of the abamectin on these plant parasitic nematodes, few information is available on the effect of abamectin on the cyst forming nematodes. Therefore, the present in vitro investigation was undertaken to explore the potential nematicidal effect of different doses of an abamectin formulation (Vertimec© EC), applied for different exposure times, in the control of the cyst nematodes *Globodera pallida* (Stone) Behrens, *Heterodera carotae* Jones and *H. schachtii* Schmidt.

MATERIALS AND METHODS

The populations of *G. pallida*, *H. carotae* and *H. schachtii* used in the *in vitro* experiments were obtained from soil samples collected in infested fields at Conversano (Province of Bari, Apulia region, Italy) (40°.57'N, 17°.09'E), Zapponeta (Province of Foggia, Apulia region, Italy) (41°.45'N, 15°.96'E) and Luco dei Marsi (Province of L'Aquila, Abruzzo region, Italy) (41°.96'N, 13°.48'E), respectively. The cysts were collected by the Fenwick can from dried soil (Figure 1).

Figure 1. Apparatus of Fenwick for cysts extraction

For each nematode specie identification was based on morphological parametrs and on the shape of vulva and ano of ten cysts. Slides of vulval cones and juveniles were prepared for nematode identification by microscope observation (Figure 2).

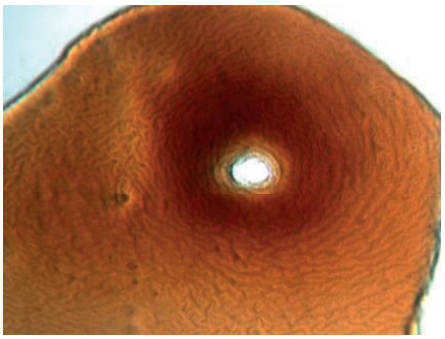

Figure 2. Vulval cone of *Globodera pallida*

Batches of 50 cysts of each nematode specie were placed in 2 cm diam sieves (215 µm aperture). Each sieve was put in a 3.2 cm diam Petri dish (Shepherd, 1986) and all dishes were arranged according to a complete randomised block design in a growth cabinet at 20°C with three replications for each treatment (Figure 3).

Figure 3. Batches of *Heterodera carotae* cysts for the hatching test

A commercial formulation of abamectin Vertimec© EC (a.i. abamectin 18 g/l), normally registered as insecticide and acaricide on stone fruit, citrus plants, flowers and vegetables, was used to prepare different concentrations from 0.0625 to 2 ml c.p./l, in a geometric series, corresponding to from 1.125 to 36 µg a.i./ml. The different concentrations were obtained by dissolving the largest rate of
the commercial formulation in distilled water. Four ml of each test solution, sufficient to cover cysts, were then added to each batch of cysts for different exposure times (0, 24, 48,

96, 192 and 384 hours). Untreated cysts were used as controls. After treatments cysts in each batch were removed from the test solutions and rinsed for 3 times in distilled water and then subjected to an hatching test to verify the nematicidal effect of each treatment (concentration x exposure time).

As hatching agents were used 0.6 mM sodium metavanadate aqueous solution, 3 mM zinc chloride aqueous solution and carrot root leachate for *G. pallida*, *H. schachtii* and *H. carotae*, respectively (Clarke and Sheperd, 1966; Sasanelli and D'Addabbo, 1995). Carrot root leachate was collected from 40 days-old actively growing carrot plants (cv. 92) (30 plants/pot) cultiva 2,500 ml clay pots, by drenching the soil with excess tap water. The leachate was then centrifuged at 1,300 rpm for 5 minutes, stored in plastic bottles and kept in a freezer until required. Only small amounts were kept at room temperature for immediate use (Figure 4).

Figure 4. Preparation of the carrot root leachate for the hatching test of *Heterodera carotae*

Juveniles (J2) emerging from cysts were counted and removed every week over a 10 week period renewing the hatching agents every week, according to an already described methodology (Sasanelli and Di Vito, 1991; Sasanelli and D'Addabbo, 1992). At the end of the experiments cysts were crushed according to Seinhorst and Den Ouden's technique (1966), and juveniles and unhatched eggs were counted. Numbers of J2 emerging weekly were expressed as cumulative percentages of the total egg content of the cysts (hatched + unhatched eggs). The number of extracted J2 per treatments were also expressed as percentage of those in the control and the difference to 100 as percentage mortalities, according to the following formula:

% Mortality = 100 - % hatched J2 where % hatched J2 = (% hatched J2 in the treatment/% hatched in the control) x 100.

The experiments were performed twice. Percentage mortality data were subjected to analysis of variance (ANOVA) and means compared by the Least Significant Difference's test (LSD's Test). Data were also subjected probit analysis (Finney, 1971) to estimate LD_{50} and LD_{90} rates for each exposure time. All statistical analysis were performed using the PlotIt software V. 3.2.

RESULTS AND DISCUSSIONS

The nematicidal effect of the different abamectin concentrations, in a range of exposure time (24-384 hours), on *G. pallida*, *H. carotae* and *H. schachtii* is reported in the Tables 1, 2 and 3, respectively.

For all cyst nematodes a significant increase of mortality was observed with the increase of abamectin dose (at the same exposure time) or exposure time (at the same concentration).

The use of an aqueous abamectin solution of 2.25 µg a.i./ml resulted in a significant increase of *G. pallida* mortality in comparison to that noted the lowest abamectin concentration at 24, 48 and 384 hours exposure (Table 1). An abamectin dose of 4.5 µg a.i./ml was effective for a significant increase of *G. pallida* mortality at 96 and 192 hours exposure compared to the previous used abamectin doses. The highest dose (36 µg a.i./ml) resulted in a *G. pallida* mortality ranging between 66.4 and 93.3% at the different exposures times resulting significantly higher than those observed for 18 µg a.i./ml at 24 and 48 hours (Table 1).

Heterodera carotae egg mortality for an exposure time of 24 hours was not affected by the used abamectin doses with the exception of the highest rate (Table 2). Nematode mortality ranged between 18.5-82.5, 23.4-90.3, 30.1-93.3 and 17.0-91.1% at 48, 96, 192 and 384 hours, respectively (Table 2). More than 50% *H. carotae* mortality was observed from 4.5 µg a.i./ml at 192 hours exposure time (Table 2). At the highest abamectin dose mortality of *H.*

carotae resulted higher than 80% just also at the lowest exposure time (Table 2).

Heterodera schachtii resulted less sensible than the two other cyst nematodes to abamectin aqueous solutions. Fifty % mortality was achieved only at the highest abamectin dose at 384 hours exposure (Table 3). The highest *H. schachtii* egg mortality (75.7%) was observed at 36 µg a.i./ml x 384 hours exposure time (Table 3).

Abamectin doses required to kill 50 and 90% vitality of *Globodera pallida*, *Heterodera carotae* and *Heterodera schachtii* eggs inside cysts treated with different concentrations of abamectin aqueous solutions (1.125, 2.25, 4.5, 9, 18 and 36 µg a.i./ml) were also calculated by probit analysis (Table 4). In the considered range of the exposure times, LD_{50} varied between 2.9-13.2 and 2.5-9.9 µg a.i./ml for *G. pallida* and *H. carotae,* respectively (Table 4). For *H. schachtii* it was not possible to calculate accurate LD values because for the exposure times from 24 to 192 hours mortality was lower than 50% (Table 3 and 4). For the sugar beet nematode 17.5 µg a.i./ml was calculated as LD_{50} at 384 hours exposure time (Table 4).

Based on results *H. schachtii* resulted less sensitive than *G. pallida* and *H. carotae* to abamectin treatments. The carrot cyst nematode resulted the most sensible to the bio-pesticide because its LD_{50} was lower than that calculated for *G. pallida* at each exposure time.

Table 1. Percentage mortality of *Globodera pallida* at different abamectin doses after a range of exposure times

Exposure time (hours)	Abamectin dose (µg a.i./ml)											
	1.125		2.25		4.5		9.0		18.0		36.0	
24	11.0[1]	a[2]	26.3	b	27.3	b	49.0	c	49.7	c	69.0	d
48	9.7	a	24.4	b	33.2	bc	39.5	c	39.1	c	66.4	d
96	13.5	a	28.8	ab	35.1	b	38.9	b	51.9	bc	69.2	c
192	21.4	a	30.0	a	55.0	b	75.5	c	76.4	c	77.3	c
384	9.8	a	50.6	b	75.0	c	84.1	cd	89.0	cd	93.3	d

[1]Each value is an average of six replications from two independent *in vitro* experiments;
[2]Data flanked in each row by the same letters are not statistically different according to Least Significant Difference's test (P≤0.05).

Table 2. Percentage mortality of *Heterodera carotae* at different abamectin doses after a range of exposure times

Exposure time (hours)	Abamectin dose (µg a.i./ml)											
	1.125		2.25		4.5		9.0		18.0		36.0	
24	25.0[1]	a[2]	31.3	a	39.1	a	38.7	a	49.0	a	81.0	b
48	18.5	a	36.0	ab	48.9	bc	52.4	bc	72.9	cd	82.5	d
96	23.4	a	45.0	abc	42.3	ab	73.7	bcd	76.2	cd	90.3	d
192	30.1	a	45.9	ab	62.7	b	86.7	c	90.4	c	93.3	c
384	17.0	a	45.7	b	60.2	bc	72.1	bcd	80.2	cd	91.1	d

[1]Each value is an average of six replications from two independent *in vitro* experiments;
[2]Data flanked in each row by the same letters are not statistically different according to Least Significant Difference's test (P≤0.05).

Table 3. Percentage mortality of *Heterodera schachtii* at different abamectin doses after a range of exposure times

Exposure time (hours)	Abamectin dose (µg a.i./ml)											
	1.125		2.25		4.5		9.0		18.0		36.0	
24	2.9[1]	a[2]	0.0	a	1.2	a	3.6	a	6.8	a	10.7	a
48	2.8	a	5.0	a	5.9	a	11.6	a	31.3	b	33.8	b
96	4.3	a	6.3	a	7.5	a	17.7	ab	30.9	b	32.6	b
192	1.4	a	5.7	ab	12.3	abc	19.2	bc	27.8	bc	34.7	c
384	1.0	a	7.7	ab	11.6	b	55.5	c	57.7	c	75.7	d

[1]Each value is an average of six replications from two independent *in vitro* experiments;
[2]Data flanked in each row by the same letters are not statistically different according to Least Significant Difference's test (P≤0.05).

Table 4. Abamectin doses required to kill 50 and 90% vitality of *Globodera pallida*, *Heterodera carotae* and *Heterodera schachtii* eggs inside cysts treated with different abamectin concentrations (1.125, 2.25, 4.5, 9, 18 and 36 µg a.i./ml)

Exposure time (hours)	LD_{50} (µg/ml)			LD_{90} (µg/ml)		
	Globodera pallida	*Heterodera carotae*	*Heterodera schachtii*	*Globodera pallida*	*Heterodera carotae*	*Heterodera schachtii*
24	13.2	9.9	---[1]	231.0	325.0	---
48	18.1	5.7	---	444.0	75.6	---
96	13.2	3.9	---	316.0	40.9	---
192	4.5	2.5	---	58.3	17.9	---
384	2.9	3.6	17.5	15.5	30.0	61.1

[1]For *Heterodera schachtii* it was not possible to calculate accurate LD values because for the indicated exposure times (24-192 hours) mortality was lower than 50%.

CONCLUSIONS

Results from the *in vitro* experiments clearly demonstrate the efficacy of the abamectin solutions in the control of cysts nematodes, although *H. schachtii* seemed to be less sensible in comparison to *G. pallida* and *H. carotae.* The use of abamectin solutions appears to be a promising tool to use in Integrated Pest Management programs and organic farming. The reduction of eggs viability inside cysts could help growers to reduce soil nematode population density for the following susceptible crops.

However, further studies are suggested to investigate the effect of abamectin in field condition with different types of soils and nematode genera and species.

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AUTOMATIC SORTING OF POTATOES ACCORDING TO THEIR DEFECTS

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Abstract

In this work we proposed an automatic sorting algorithm for potatoes based on computer vision techniques. We performed two types of sorting: one depending on the potatoes size and the other one depending on their quality. We proceeded with the segmentation of the defected areas through global thresholding methods. Then, we extracted some morphological and statistical features from the segmented areas. These features were chosen as inputs for classification algorithms. We trained the SVM, Tree and LDA classification learners implemented in MATLAB Classification Toolbox and evaluated their performance. We concluded that the SVM has classified the potatoes according to their size with a higher success rate. In the case of quality sorting, the LDA method is recommended.

Key words*: image processing, grading, potatoes.*

INTRODUCTION

Potato culture is of great importance worldwide, both from a nutritional point of view and as a raw material in food industry. This aspect is supported by the composition of its nutrients which classifies it as a strategic product for the food industry. Due to the large-scale consumption of this product, precise control of it is recommended.

In Romania, the production of autumn potatoes was about 2 million tonnes, as it was recorded in 2019 (agrotrends.ro).

At present, the inspection and analysis of the quality of food products using techniques based on image processing and computer vision has taken a wide development due to the low cost, and also because it is sustainable and efficient. Plenty of scientific work in the field of image processing provides significant results in terms of inspection and quality analysis of fruits, vegetables, cereals or seeds (Feng et al., 2019; Gursoi 2020; Lurstwut et al, 2017). Image processing in agricultural applications comprises three stages: image enhancement, image feature extraction and image feature classification (Mahendran et al., 2011).

At present, it is necessary to replace the manual sorting with the automated one which is characterized by speed, accuracy and easy to use.

Potato sorting is an essential factor in the context of marketing process, helping to establish a fair price and providing quality products. Also, the sorting process for sprouted potatoes is essential because they generate a high level of solanine, becoming toxic. Potato grading is an important step, both in the harvesting stage and also in post-harvesting operations. Potatoes can be sorted according to several criteria: morphological features (size and volume), variety (colour features), quality (healthy potatoes, unsound potatoes, sprouted potatoes or damaged potatoes).

Computer vision is a complex process that applies a number of steps for the automatic inspection of some images. These steps are intended to extract significant data to control a process or a certain activity. Computer vision has been applied for several goals as: shape classification, defect detection, quality classification and variety identification. Size, which represents the first parameter associated with the quality of a product, can be estimated with computer vision technology starting from the measurements like: area, minor axis length, major axis, diameter, perimeter. Machine vision method applied in works based on size

related feature have been developed by many researchers, such as: Yongsheng et al., 2017; Lopez et al., 2018; Geng et al., 2019; Moallem et al., 2013.

The grading procedure implemented in this work, based on computer vision technique, has as input data images of potatoes of different sizes, belonging to two varieties, both damaged and healthy. The result of processing will classify the potatoes according to their size and their quality.

The colour represents also a good quality indicator of fruits and vegetables.

Image texture reveals information about the intensity of the colours and the spatial arrangement of them within an image. Statistical features are considered to analyse an image texture. Skewness indicator provides information about image surfaces. Glossy and darker surfaces appear to be more positively skewed than lighter and matte surfaces. Kurtosis indicator quantifies the sparsity of data. It establishes if a distribution is peaked or flat.

A lot of current studies have approached the advanced technology of digital image processing in order to indicate the degree of damage attack on some crops, such as: potatoes (Geng et al., 2019; Lopez et al., 2018), cucumbers (Wei et al., 2018), roses (Tuba et al., 2017), oranges (Fouda et al., 2013), apples, grapes and mango fruits disease detection (Sandesh et al., 2017), sugarcane (Prajakta et. al, 2016), cotton (Naik et al., 2015).

Diagnosis of healthy and defected potato images based on image processing and computer's vision recognition can be implemented on a large scale, because it requires a reduced amount of time, lower costs and identifies different classes in which potatoes can be integrated, it is fast and accurate compared to visual observation performed by farmers.

This article aims to classify the potatoes into classes delimited by their size or their degree of quality, i.e. potatoes with certain skin defects or potatoes with intact and healthy skin.

MATERIALS AND METHODS

The main purpose of this work is to classify potatoes according to their size and to identify imperfections on the potato surface (mechanical damage potatoes or sprouted potatoes).

The experiments comprise a lot of 206 potatoes, belonging to two varieties: 65 potatoes of yellow skin variety and 141 potatoes of red skin variety. The samples were selected from the experimental fields UASVM Cluj-Napoca. The potato samples were analysed three months after the storage under favourable conditions of temperature and humidity.

The colour images of potatoes are captured using a mobile phone camera. Images are stored in jpg format and were processed with MATLAB 2018b application. The images quality was improved in the preprocessing step, using some routines implemented in MATLAB. Preprocessing means adjusting the quality of the images for accurate processing of them. We opted for a method based on the direct manipulation of the pixels in the image. We chose a 5x5 median filter to eliminate the noise spots. Median filter is a nonlinear filter that replaces the value of the central pixel by the median of the grey values of neighbouring pixels. The pattern of neighbours is called the "window", which slides, pixel by pixel over the entire image. This type of filtering involves arranging pixels values in ascending order, then calculating the median value and finally assigning the median value to the window central pixel.

MATLAB is highly-performant software for technical computing that integrates computation, visualization and programming in an easy-to-use environment. MATLAB stores images as matrices and each element of the matrix represents a pixel of the image.

In MATLAB, a grayscale image is represented by a matrix of the *m x n* type, where *m* is the number of lines (the number of pixels of the image wide) and n is the number of columns (the number of pixels for the image length).

$$I(m,n) = \begin{bmatrix} a_{11} & \cdots & a_{1n} \\ \vdots & \ddots & \vdots \\ a_{m1} & \cdots & a_{mn} \end{bmatrix},$$

where each element of the image matrix has range values 0 to 255, 0 is the code for black colour and 255 corresponds to white colour. In the concept of image processing, the colour is a powerful descriptor that often facilitates the

identification and extraction of objects from a scene.

The RGB images are represented in MATLAB as a three-dimensional matrix *m x n x p*, where *m* and *n* values were defined for the grayscale images and *p* parameter denotes the plane which can be 1 for the red colour, 2 for green colour and 3 for the blue one.

$$I_R(m,n,1) = \begin{bmatrix} r_{11} & \cdots & r_{1n} \\ \vdots & \ddots & \vdots \\ r_{m1} & \cdots & r_{mn} \end{bmatrix},$$

$$I_G(m,n,2) = \begin{bmatrix} g_{11} & \cdots & g_{1n} \\ \vdots & \ddots & \vdots \\ g_{m1} & \cdots & g_{mn} \end{bmatrix},$$

$$I_B(m,n,3) = \begin{bmatrix} b_{11} & \cdots & r_{1n} \\ \vdots & \ddots & \vdots \\ b_{m1} & \cdots & b \end{bmatrix}.$$

If we intend to process a pixel of a colour image it is necessary to assign a value to each plane.

A binary image represents an image in which each pixel can have a value of 0 or a value of 1. A quick method for generating binary images is the threshold technique. It follows the steps: setting a threshold value, assigning a value of 1 to all pixels with a value greater than the threshold and encoding with a value of zero all the pixels having values smaller than the threshold. Regarding the binary images, their structural characteristics can be easily distinguished.

The features are morphological or quantitative descriptors extracted from an image or from an object image. A pattern is a way of arranging descriptors.

In computer vision technology, the determination of morphological features such as: area, perimeter, Minor Axis Length, Major Axis Length is of particular importance.

Potatoes Classification

Creating a predictive model based on a training data set involves the passing through the following stages: collection of individual data records, records description using their attributes, placing attributes in a lot of classes and, finally the assignment of each record to a class.

In this paper we tested the following supervised classifiers: SVMs (Support Vector Machines), Tree and LDA (Linear Discriminant Analysis).

SVMs is a successfully method applied in the data classification process. A classification task usually involves separating data into training and testing sets. Each instance of the training set contains one target value (i.e. the class label) and some attributes (i.e. features). The purpose of this method is to generate a model, starting from the training data which predicts the target values of the test data given only the test data attributes. The SVMs algorithm output is an optimal hyperplane which categorizes new examples. It can be synthesized in this way: given a training set of instance-label pairs $(x_i, y_i), i = 1, \ldots, l$ where $x_i \in \mathbb{R}^n$ and $y \in \{1, -1\}^l$, the SVMs algorithm aims to determine the solution of the optimization problem:

$$\min_{w,b,\zeta} \frac{1}{2} w^t w + C \sum_{i=1}^{l} \zeta_i$$

subject to $y_i(w^t \phi(x_i) + b) \geq 1 - \zeta_i,\ \zeta_i \geq 0$.

The training vectors x_i are mapped into higher dimensional space by the function ϕ. SVMs find a linear separating hyperplane with the marginal margins in the higher dimensional space. $C > 0$ is the penalty parameter of the error term and $K(x_i, y_i) \equiv \phi(x_i)^T \phi(x_j)$ is called the kernels function. The following basic kernels can be considered: linear kernels, polynomial kernels, radial basis function, sigmoid kernels, gaussian kernels (Hsu et al., 2011). Gaussian kernel depends on the Euclidean distance between x_i and y_i and is based on the assumption that the similar points are close one to each other in the features space, in terms of Euclidean distance.

A Decision Tree is a predictive method based on a branching series of Boolean tests that uses specific facts to make more generalized conclusions.

This method can be compared to a tree where each node represents a feature (attribute), each link (branch) describes a decision rule and each leaf shows an outcome. The idea of this method is to generate a tree for the entire data and process a single outcome at every leaf. Decision algorithms aim to separate the attributes to be tested at the level of each node

and to determine when the separation is best in the individual classes (Patel et al., 2018). There are several variants of this method. In this article we have choose the Fine Tree method.
Linear Discrimination Analysis (LDA) is a supervised technique applied in the pattern classification and machine learning applications. The purpose of this method is to project a data set onto a lower-dimensional space with good class separability in order to remove overfitting and to reduce the computational costs (Li et al., 2005). LDA computes the directions that will represent the axis that maximize the separation between multiple classes.
The classification process was evaluated by K-fold cross-validation, k = 5, implemented in MATLAB environment.
Experiments were conducted on potatoes data set comprising two different classes, depending on the size specific features, such as: area, perimeter, major axis length, minor axis length. The first class includes samples with small dimensions: area less than 12,500 pixels, perimeter less than 400 pixels and the other class contains samples with area larger than 12,500 pixels and perimeter larger than 400 pixels. Samples of this class are considered to be of normal size
The Gaussian SVMs algorithm, Fine Tree algorithm and Linear Discriminant Analysis was trained with a sample of 147 potatoes in order to obtain a classification model. Three different predicted models were generated by these three algorithms.
At this stage, we have prepared the test features data file. It contains 59 samples belonging to the two size categories.
Potatoes included in the test file were pre-sorted by experts starting from quantitative and qualitative factors in order to later verify the accuracy of computer sorting.
The samples data set has been uploaded into MATLAB *Classification Learner Apps,* as input data.
Then, we have performed the classification task on the test data started with the three generated predicted model. The first classification has been made by SVMs algorithm and then Fine Tree and LDA algorithms were applied.
Finally, the accuracy of total sorting was established comparing pre-graded values and Computer Vision Grading. We have quantified the accuracy of classification process as the ratio of correctly recognized sample images to the total number of sample images.

$$Percentage\ accuracy\ (\%) =$$

$$= \frac{correctly\ recognized\ sample\ images}{total\ number\ of\ test\ sample\ images} x\ 100.$$

The performance of the proposed algorithms can be quantified using the FAR indicator (False Acceptance Rate) that represents the percentage in which false acceptance occurs.

Defect Classification

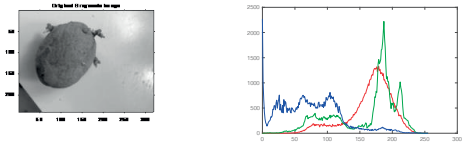
We also have applied computer vision technique in order to identify potato defects.
Many of the defects of the potatoes are identified by colours features. These features allow the classification of image pixels into homogeneous regions which are useful in the image segmentation process.
Two different main colour classes are identified: one that corresponds to undamaged potatoes and one that includes potatoes with various defects. These classes are divided into two sub classes according to potato variety: red skin potatoes variety respectively yellow skin potatoes variety. Thus, the sorting process targets to place the samples in four classes: healthy yellow skin potatoes, unhealthy yellow skin potatoes, healthy red skin potatoes and unhealthy red skin potatoes.
The defects of two potato cultivars are identified starting from colour features included into a training set respectively into a test set. The training set comprises 147 samples belonging to four qualitatively different classes. In the test set we included 59 potatoes, also selected from the four quality classes
Feature selection is an essential step in computer vision context. It contributes to reducing the size of the input vector removing features with insignificant information involved in the process of pattern recognition or classification.
Image texture provides information regarding the spatial arrangement of colours within an image. The computer vision technology can identify a wide range of colour spectrum compared to human vision.

The colours feature of the sample images are analysed to separate defected area from the healthy ones. The colour information in RGB space were extracted from the acquired images. We considered the red (R), green (G) and the blue (B) one component of potato images, then we calculated twelve statistical features of these components in RGB space using a routine written in MATLAB. We calculated the next features: Mean(R), Mean(G), Mean(B), Standard Deviation(R), Standard Deviation(G), Standard Deviation(B), Skewness(R), Skewness(G), Skewness(B), Kurtosis(R), Kurtosis(G), Kurtosis(B). Thus, each potato is represented by twelve features analysed in order to determine its quality.

The extracted statistical features are used as inputs for the SVMs, Tree and LDA, supervised classification routines. These algorithms, implemented into *MATLAB Classification Learner Apps*, were trained on features data file to obtain predictive models for classifying potatoes according to their quality.

The obtained models are then checked starting on a test file containing 59 samples. The output file contains a string that represents the index of the class in which each sample was placed.

We have done defect detection by calculating a threshold value to highlight pixels belonging to the affected regions. We have got the threshold value as the local minimum. The histogram shows how many times a certain intensity occurs in an image (Figure 1.b). The histogram was generated in the MATLAB environment using *imhist* function.



a) b)

Figure 1. Grayscale image (a) and image histogram (b)

In order to separate and evaluate the defected area we applied image segmentation by using *Colour Thresholding* tool implemented in *Matlab2018b Apps*. Figure 2 shows the segmentation results applied for the aim to isolate defected areas.

RESULTS AND DISCUSSIONS

In this article, we processed colour images using the *Colour Thresholder* tool implemented into *Image Processing and Computer Vision Apps* of the MATLAB application. This tool contains a collection of algorithms created for the purpose of binary image processing, image segmentation, colour and morphology manipulation or structure recognition.

Image segmentation is a procedure that partitions an image into independent components that are similar according to a set of predefined criteria, in order to identify objects of interest within it. Due to the high degree of variation of the defects, we tested various global threshold techniques on filtered images. Finally, we chose the one that provided optimal damage detection.

The experimental results of the thresholding image segmentation technique applied in the *RGB* space are shown in Figure 2.

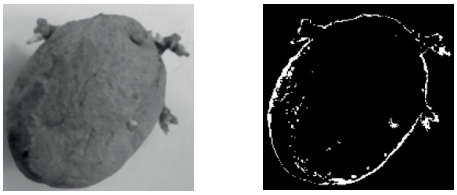


Figure 2. Filtered red skin potato compare to segmentation of defected areas

The statistical features are calculated over the segmented area of each potato tuber.

The next supervised classifiers are tested in this work: SVMs, Tree and LDA.

A comparison between the performance of considered classification methods has been done in terms of accuracy. The Table 1 shows the classification accuracy based on size features and the execution time corresponding to each chosen algorithm.

Table 1. Accuracy of classification process according to size and execution time

	SVMs	Fine Tree	Linear Discriminant
Overall Classification Rate	92%	90%	86%
Execution Time (seconds)	1.86	1.80	0.80

Analyzing the accuracy of the classification results obtained after the application of algorithms dedicated to grading process, we highlight the fact that the SVM algorithm is recommended in the classification according to size. From the point of view of the working speed, the LDA algorithm is distinguished. The FAR indicator for SVMs algorithm is 8.47%.
In order to choose the best algorithm, indicated for quality assessment, we evaluated the accuracy of each implemented method. The results, expressed as accuracy rate are displayed in Table 2. It also contains the time required for the classification process.

Table 2. Accuracy of classification process according to quality and execution time

	SVMs	Fine Tree	Linear Discriminant
Overall Classification Rate	46%	41%	72%
Execution Time (seconds)	1.84	0.97	0.78

The evaluation of the classification according to quality shows that the LDA method is more efficient compared to SVMs and Fine Tree. We conclude that the LDA method provides better detection performances with lowest execution time.
The accuracy of the classification starting from the quality indicator can be improved by cumulating other texture features in the input data file. For example we can choose the features like uniformity, entropy and smoothness.
Overall, in terms of processing time, the LDA algorithm is distinguished by speed. We also remark that the Fine Tree method is faster than the SVM method.
If we aim to simultaneously classify a potato sample both according to the size and from a qualitative point of view, we can combine the morphological features with the statistical ones. Thus, we will integrate the input data into eight distinct classes. The sample data considered into the test file will also be distributed in eight different classes.
Choosing as many attributes as possible, of different types, can considerably improve the quality of the potato tuber classification algorithm. In Europe, agricultural products must respect UNECE (United Nations Economic Commission for Europe) standards in order to be marketed in stores. The standards are set according to precise criteria: quality, color, size, shape, these contributing to the pricing of products. So it is necessary to verify these standards on a large scale in order to sell potatoes.
In conclusion, this type of approach applied to grading potato tubers has provided useful results to develop an automatic system to monitor the quality of potatoes. Computer vision and image processing techniques have been extended on a large scale in the context of artificial intelligence, being able to capture, quickly and precisely, complex features. These features allow a sufficiently accurate, non-destructive evaluation of products, which tends to replace manual checking (inspection) trained by farmers/humans.

CONCLUSIONS

The external quality of agricultural products is a main attribute that contributes to the classification process. It can be quantified in colour, size, shape, texture, visual defects, features that can be extracted directly from images and automatically monitored with computer vision technique.
Therefore, the images taken from the potato tubers provide useful information in identifying their quality. The algorithms applied in the classification process can be basic elements that, integrated in automatic classification systems can bring benefits in the potato industry. Automatic systems significantly reduce the large volume of work done on farms, require a short time, are sufficiently accurate and eliminate the human error involved in the classification process.

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RESEARCH ON PHENOTYPIC AND GENOTYPIC EXPRESIVITY OF BEAN VARIETIES OBTAINED AT VEGETABLE RESEARCH DEVELOPMENT STATION BUZĂU

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Abstract

The pedo-climatic conditions of Romania are favorable for bean cultivation, one of the reasons that, in past, field beans used to occupy large areas in Romania. Although it is a plant that can offer high economic profit to the growers and is a good precursor for other vegetable plants due to nitrogen accumulation, the field bean it's no longer sought and used at its maximum potential as a crop plant. Since 1996, Vegetable Research Development Station (V.R.D.S.) Buzău has been revive the researches in beans, realizing until now a valuable collection of germplasm, grouped by types of growth and directions of use. For this research, three stable genotypes of field bean group were studied. The genotypes showed distinct variability in plant height, the highest value being 61.83 cm (A3) and the smallest value being 38.8 cm (A1). Slightly differences were also registered by the number of pods per plant. The studies have been completed with the registration of the genotype A2 for patenting and approval under the name of "Doina" and the other two genotypes will be proposed for approval.

Key words*: biodiversity, Phaseolus vulgaris, breeding, phenotype, patenting.*

INTRODUCTION

Common dried bean (*Phaseolus vulgaris* L.) is an annual herbaceous plant belonging to the family *Leguminosae* (*Fabaceae*) (Gentry, Howard Scott, 1969).

Hutchinson (1973) and Jones and Luchsinger (1987) mentioned that *Fabaceae* consist of about 440 genera and 12000 species.

Phaseolus vulgaris is one of the most important vegetables from the nutritional and alimentary point of view, being cultivated for dry seeds or unripe fruits (pods) that have a high content of proteins, iron, magnesium, folic acid and complex carbohydrates (Pachico, D., 1993).

Due to the high protein content it successfully replaces meat in vegetarian diets, but most importantly it keeps hunger under control in poor countries (Peters, A., 1993; Schwartz, H. F. and Pastor-Corrales, M.A., 1995). It also contains phytoestrogen which reduces the risk of breast cancer and helps in the treatment of postmenopausal osteoporosis (Shirke S.S. et al., 2009).

Beans first appeared in Europe in the early 16th century, when it was brought from Central America by the Spanish and Portuguese. It was used by Toltecs and Aztecs from ancient times (Wortmann, C.S., 2006). In Romania, beans were introduced in the eighteenth century. In Asia and Africa, dried leaves, stalks and ground pods are used as animal feed (Sperling L. et al., 1996).

Beans are like the other species in the legume family an excellent precursor for the other legume species, having the ability to fix atmospheric nitrogen, thus improving the soil structure. It is recommended to avoid returning to the same soil for a period of 3-4 years, thus reducing the risk of disease and pests. One of the most harmful diseases is *Xanthomonas phaseoli*, a bacterium that is stored and transmitted through the seed. (Lagunovschi-Luchian V and C. Vînătoru, 2016).

In 2018 the area cultivated with dry beans in Romania was 12 231 ha, with a production of 17 298 tons, and worldwide the largest cultivated area is occupied by the United States of America, more precisely 815 850 ha, with a production of 1 700 510 tons (FAOSTAT).

Until now, at the Vegetable Research and Development Station (VRDS) Buzău, five

varieties have been approved and can be found in the Official Catalogue of Romanian Crop Plants (Anisia, Clarisa, Ioana, Maura and Menuet).

MATERIALS AND METHODS

The Laboratory of Genetics, Breeding and Biodiversity from VRDS Buzau has a valuable germplasm collection of *Phaseolus* sp. having over 100 genotypes. A number of 77 genotypes are in an advanced state of breeding. The genotypes will be used as importance source material for breeders to develop new varieties.
The germplasm collection was divided into 3 groups according to the direction of use:
- cultivars with indefinite growth that can be grown in protected areas and field, in a fence system;
- cultivars with determined growth for pods suitable for field crop;
- field cultivars with determined and semi-determined growth for dry grains suitable for field crop;

In the present study were chosen 3 stable genotypes for dry grains: A1- C.A. Rosetti; A2- Doina; A3- Călărași, genotypes that come from localities located in Bărăganului Plain and Buzău Plain.
Phenological, biometric and laboratory measurements were carried out during the vegetation period.

Field work procedure

The research experiences were carried out in the research field of VRDS Buzău, on an alluvial soil.
The applied culture technology was specific to the field bean crop.
Sowing is done in rows, in the first decade of May, more precisely on the 7th, when the soil measure a temperature of over 10ºC for several days in a row. The distance between rows was 45 cm, and between plants/row was 15 cm, using 30-40 kg seeds per hectare.
The pest management was done accordingly to field bean crop and only when economic threshold of harm was exceed.
The negative mass selection was made and all the plants that were not typical were eliminated.

The care works applied were: two mechanical hoeing to keep the soil clean of weeds and loosen, drip irrigation and fertilizers for a good development of the plants.
Harvesting occurred when about 75% of the pods have reached maturity and the seeds are hard.

Observations and recording of data

Vegetative and reproductive growth. The variability of the qualitative and quantitative characters and the correlation between them was made, which is very important for the process and the conservative selection.
The qualitative characters analyzed were: terminal leaflet (shape), terminal leaflet (length of tip), leaf (intensity of green color), leaf rugosity, color of flower, pod (ground color), pod (degree of curvature), pod (shape of curvature), pod (shape of distal part).
The quantitative characters targeted in the study were divided into two groups:
Plant observations: plant height, bush diameter, diameter at stem base, number of main shoots, number of leaves/plant, total leaf length, total leaf width, petiole length, leaf length, leaf width.
Observations of the pods: number of pods per plant, weight of pods per plant at maturity of consumption, weight of pods per plant at physiological maturity, total weight of pods per plant, average weight of pods at maturity of consumption, average weight of pods at maturity physiological, the length of the pod, the width of the pod, the number of berries/ pods, the length of the spur.
For statistical analysis, ANOVA was used, followed by the Duncan test.

RESULTS AND DISCUSSIONS

Throughout the vegetation period, all the three cultivars studied were the subject to phenological and biometric measurements. Thus, the descriptive analysis of the quantitative characteristics are found in Tables 1 and 2, and that of the qualitative characteristics in Table 3.

Mean values and standard deviation regarding

Tabel 1. Quantitative plant characteristics

Plant observations	A1± sd	A2±sd	A3±sd
Plant height (cm)	60.5± 0.7b	38.8±2.8a	61.8±1.6b
Plant diameter (cm)	40.75±1.06a	43.2±0.7a	51.1±1.9b
Stem base diameter (cm)	0.87±0a	0.79±0.1a	0.75±0.1a
The number of main shoots (pcs)	2-3±0.7a	2-3±0.5a	3±0.1a
The number of leaves/plant (pcs)	22±1.4a	22±0.5a	21±0.1a
The length of total leaf (cm)	10.75±1.7a	11.46±0.2a	11.23±2.2a
The width of total leaf (cm)	16±2.1a	15.63±1.9a	13.23±3.1a
The length of petiole (cm)	13.6±0.8a	8.66±1.2ab	10.23± 2.1b
The number of blades/leaf (pcs)	3±0a	3±0a	3±0a
The length of the leaflets (cm)	7.85±1.9a	8.76±0.7a	8.1±1.7a
The width of the leaflets (cm)	5.15±0.9a	5.5±0.8a	5.7±1.6a

SD-standard deviation, different letters means significant differences

Regarding the quantitative characteristics of the three cultivars, significant differences were registered at the height and diameter of the plants and the length of the petiole.

The genotype that registered the highest height was A1 (Figure 1), with 60.5 cm, and the smallest height was at A2 with 39.1 cm.

The diameter of the plant ranged from 40.75 cm at A1 to 51.1 cm at A3.

The length of the petiole had the smallest record on A1 with 8.66 cm and the highest was record by A1with 13.6 cm.

The other quantitative characteristics studied were: stem base diameter, the number of main shoots, the number of leaves/plant, the length of the total leaf, the width of the total leaf, the length of the leaflets and the width of the leaflets have similar values.

Crop detail

Figure 1. A1 C.A. Rosetti

Figure2. A2 Doina

Figure 3. A3 Călărași

Tabel 2. Quantitative pod characteristics

Pod observations	A1±sd	A2±sd	A3±sd
Total number of pods/plant (pcs)	40±5.6b	21±0.5a	34±3.7b
Number of pods/plant at harvest maturity (pcs)	8±0.7a	13±4.5a	12±3.6a
Number of pods/plant at physiologic maturity (pcs)	32±4.9b	8±5a	22±1.5b
Total weight of pods/plant (g)	170±0c	83.33±11.5a	133.33±5.7b
Weight of pods/plant at harvest maturity (g)	20±0a	46.66±20,8a	46.67±15.2a
Weight of pods/plant at physiologic maturity (g)	150±0c	36.67±20,8a	86.66±20.8b
Means weight of a pod at harvest maturity (g)	2.67±0.2a	3.51±0,3b	3.84±0.1b
Means weight of a pod at physiologic maturity (g)	4.85±0.4a	5.06±1,4a	3.91±0.9a
Length of pod at harvest maturity (cm)	11.5±1.2a	11.02±0,5a	11.21±0.7a
Width of pod at harvest maturity (cm)	1.2±0.1a	119±0,1a	1.10±0.08a
Length of pod at physiologic maturity (cm)	14.55±0.4b	12.11±0,1a	12.59±0.9a
Width of the pod at physiological maturity (cm)	1.26±0,04a	1.19±0,04a	1.17±0.03a
Number of bean/pod (pcs)	8±0b	6±0.5a	7±0.5a
Length of distal part (cm)	1.07±0,2a	1.02±0.02a	1.06±0.1a

SD-standard deviation, different letters means significant differences

The highest number of pods per plant was registered by A1 with a number of 40 pods and A2 had 21 pods. A1 has a concentrate ripening, from a number of 40 pods/plant, 32 pods have reach physiological maturity. Contrariwise, A2 reaches maturity in a slowly manner, from 21 pods/plant, 8 pods have reach physiological maturity at the measurement time.

Accession A1 had recorded the highest total weight with a value of 170 g and from it 150 g were held by the pods that are at physiological maturity, resulting in an average weight of the pod of 4.85 g. At the same time, the lowest total weight was held by the genotype A2 (83.33 g), and the weight of the pods of physiological maturity was 36.67 g, resulting in an average weight of the pod of 5.06 g.

As for the rest of the quantitative characters of the pods, they have very close values, mentioning that for all the remaining characters the highest value was recorded by genotype A1. UPOV descriptors were used to determine the qualitative characters (Table 3).

The leaves of the 3 cultivars analyzed are similar in shape and size. Small differences were observed in the color of the leaves, varying from light green in the case of A3 (Figure 3), to medium-dark green in the case of A2 (Figure 2).

The degree of curvature of the pods varies from weak on accession A2 to strong on accession A1. The shape of the curvature differs on genotype A2, being convex (Figure 5), the other genotypes have a concave shape (Figures 4 and 6).

Figure 4. A1 C.A. Rosetti

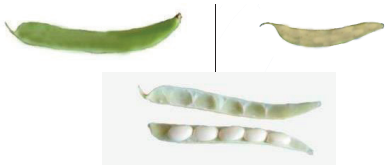

Figure 5. A2 Doina

Figure 6. A3 Călărași

Tabel 3. Qualitative characteristics

Characteristics	A1	A2	A3
Terminal leaflet (shape)	Triangular	Triangular	Triangular
Terminal leaflet (length of tip)	Short	Medium	Medium
Leaf(intensity of green color)	Medium	Medium-dark	Light
Leaf rugosity	Weak	Weak	Weak
Color of flower	White	White	White
Pod (ground color)	Light yellow	Light yellow	Light yellow
Pod (degree of curvature)	Strong	Weak	Medium
Pod (shape of curvature)	Concave	Convex	Concave
Pod (shape of distal part)	Acute to truncate	Acute to truncate	Acute to truncate
Seed color	White	White	White

Figure 7. Different stages development on Doina cultivar

Seed development is the final stage in the life of annual legumes, the seed weight is primarily composed of proteins and carbohydrates, which are derived from amino acids and sugars that are translocated from source tissues (Weber et al., 1997). The weight of thousand grains (TKW) varied from A2 with a weight of 378 g to 488 g at A1.

The length of seed had the highest value at A3 and the smallest one was recorded by A2 (Table 4).

Tabel 4. Seed characteristics

Seed observations	Unit	A1	A2	A3
TKW	g	488.2	378	421
The medium weight of a seed	g	0.49	0.39	0.43
Length of seed	mm	14.97	13.88	15.35
Width of seed	mm	8.43	8.53	8.52

CONCLUSIONS

The studied accessions showed differences amongst themselves and this can be useful for the future breeding program. The genotypes showed distinct variability in plant height, the number of pods per plant, the weight of the pods and Thousand Kernel Weight.

The studies have been completed with the registration of the genotype A2 for patenting under the name of "Doina" and the other two genotypes will be proposed for approval.

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GREENHOUSE PERENNIAL WALL-ROCKET CROP AS INFLUENCED BY MULCHING AND FERTILIZATION PRACTICES

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Abstract

This research was carried out in North-Eastern Romania in order to evaluate the effects of mulching and fertilization on the yield of fresh leaves for the perennial wall-rocket crop - Diplotaxis tenuifolia (L.) D.C. grown in the tunnel. Comparisons were made between three variants of soil mulching: non-mulched (NM), mulched with white polyethylene (WLDPE) and mulched with black polyethylene (BLDPE) and three variants of fertilization: non-fertilized (NF), chemical fertilization (Ch) and application of microorganisms (M). The best combinations in terms of production were obtained by the variants WLDPE x Ch (25.3 t·ha^{-1}) and WLDPE x M (23.9 t·ha^{-1}), productions that were also supported by the highest Leaf Area Index (LAI) and the highest weight of the dry matter. The highest chlorophyll content was recorded by the NM x Ch variant. Following the experiments carried out, both mulching with white film and chemical fertilization determined good production results, but the combination of the two factors led to the phenomenon of synergism, generating the highest yields. For the fall harvest cycle, the species behaves well in the North-East of Romania conditions.

Key words*: Diplotaxis tenuifolia (L.) D.C., Leaf Area Index, perennial wall-rocket, physiological and biometrical analysis.*

INTRODUCTION

Diplotaxis tenuifolia (L.) D.C. - the perennial wall-rocket is a species belonging to the *Brassicaceae* (*Cruciferae*) family (Warwick and Sauder, 2005). The specific common name "perennial wall-rocket" - perennial rocket, is currently preferred for differentiating it from other species of the *Diplotaxis* genus (Caruso *et. al*., 2018). The species is currently cultivated in many agricultural areas, especially in Italy, where it covers an area of about 4000 ha (Bonasia *et al*., 2017). Here it is known under the common name "rucola selvatica" (wild rocket), as opposed to the name "rucola cultivata" (cultivated rocket), which refers to the species *Eruca sativa* Mill. (sin *Eruca vesicaria* L.) (Parsons and Cuthbertson, 1992, cited by Caruso *et al*., 2018).

Up until two decades ago, *Eruca sativa* was the dominant species being cultivated, while *Diplotaxis tenuifolia* was mostly harvested from the spontaneous flora. The extension of the areas cultivated with perennial rocket in the last two decades is due to its fine and succulent leaves that correspond to the consumers' requirements (Bell and Wagstaff, 2019), the leaves of this species being rich in mineral elements and antioxidants (Caruso *et al.*, 2019a).

Diplotaxis tenuifolia is a perennial species that regenerates easily after harvesting by cutting the leaves 3-5 cm above the soil surface. Besides the harvesting-related reasons, the cosmopolitan distribution of the *D. tenuifolia* species results from its easy adaptability and propagation (Hurka *et al*., 2003; Acar *et al*., 2019).

The economic interest for the cultivation of perennial rocket has increased as a consequence of the progressive distribution of ready-to-eat salads, the so-called "fourth-

generation vegetables", an efficient marketing model of keeping the freshness and the typical smell of the leaves, thus increasing the duration of storage, storage and availability on the market (Bonasia *et al.*, 2017).

In our country, this species is not known in the native specialized literature. In this context, the *purpose* of this paper is to evaluate the effects of mulching and fertilization on some physiological and production characteristics for a perennial wall-rocket grown in protected spaces (in protected crop).

Based on these first results, research perspectives may open to highlight the value of this species and, possibly, to promote it within the vegetable assortment of our country.

MATERIALS AND METHODS

Experimental Design

Preliminary research on the *Diplotaxis tenuifolia* L. (perennial wall-rocket) species was carried out in 2019, in a polytunnel on an area of 130 s.m., from the experimental field of the vegetable growing discipline belonging to USAMV Iasi, on an anthropic cambic chernozem soil with 2.86% organic matter; pH 7.2; 2.8 g·kg^{-1} N; 32 mg·kg^{-1} P, 218 mg·kg^{-1} K.

The evolution of temperature, atmospheric humidity and dew point, measured at the plant level during the experimental period, for the autumn crop cycle, are presented in Figure 1.

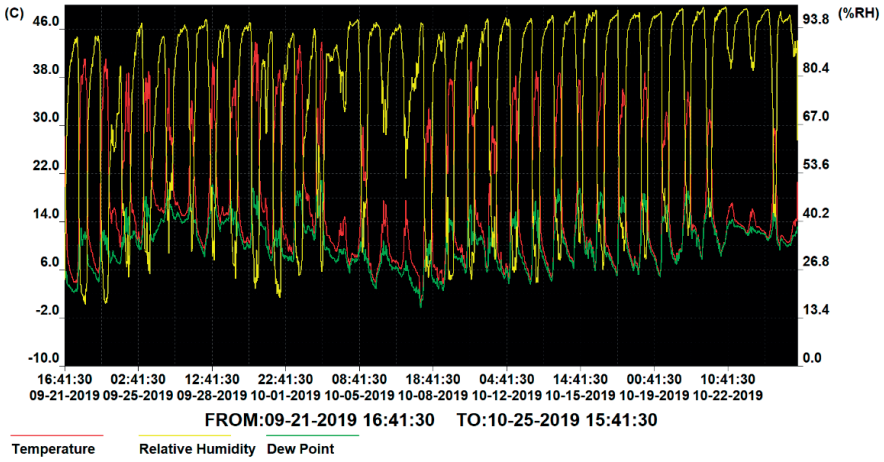

Figure 1. Trend of temperature, relative humidity and dew point inside of polytunnel

The experimental protocol set up the establishment of a bifactorial experiment with three replications (Figure 2). The first experimental factor was mulching with three gradations: non-mulched (NM), mulched with white polyethylene film (WLDPE) and mulched with black polyethylene film (BLDPE), both 60 µm thickness, and the second factor was the type of fertilization with three gradations: non-fertilized (NF), chemical fertilization (Ch) and application of microorganisms (M).

The fertilization treatments were:

1. Control treatment (NF), without the application of fertilizers.

2. Chemical fertilization (Ch) applied at 360 kg·ha^{-1} Cristaland®, a solid chemical fertilizer. The composition of this fertilizer was: 30% total N of which ammoniacal N 2% and ureic N 28%; 10% water soluble P_2O_5; 10% water soluble K_2O; 2% water soluble MgO.

3. Biological fertilization (M) were applied at 80 kg·ha^{-1} Micoseeds MB®, a microgranulated fertilizer based on microorganisms. It predominantly contains spores of arbuscular mycorrhizal fungi (AMF), based on *Glomus* spp. and enriched with *Beauveria* sp. and *Metarhizium* sp.



Figure 2. Aspects of the perennial wall-rocket experiment

On the land preparation, the soil was organically fertilized with 500 kg·ha^{-1} Orgevit®, a chicken fertilizer on granular form, 90% dry matter, 65% OM, 4% N, 3% P_2O_5, 2.5% K_2O, 1% MgO, 0.02% Fe, 0.01% Mn, 0.01% B, 0.01% Zn, 0.001% Cu, 0.001% Mo. The soil was ploughed and hoed, arranged in 100 cm wide raised beds. The soil mulching was performed in accordance with the experimental protocol.

The sowing performed in order to produce the seedlings was carried out on August 22nd in alveolar trays with a volume of 31.3 cm^3. The planting of the 24-day seedlings was carried out on September 19th, at distances of 20 cm by 20 cm between plants, 80 cm between beds. The result density was 14.3 nests per square meter (Caruso *et al*., 2019b). The same time with the planting process, the fertilization was also performed, according to the experimental protocol. In the vegetation period, during the first crop cycle, the maintenance works were applied, according to the specialized literature (Caruso *et al*., 2018): the weed control was carried out by two weeding and one hoeing works, irrigation being done by dripping. Leaf harvesting was performed by cutting 3-5 cm above the cotyledons to allow efficient regeneration of the vegetative apex (Schiattone *et al.*, 2018). The harvesting moment was achieved when the first leaves forming the rosette reached the maximum size, respectively on October 22nd.

CCI Determination

Assimilatory pigment content was measured with a non-destructive portable chlorophyll content meter CCM-200 plus, from the Opti-Science Company, performing 50 readings on undamaged wall-rocket leaves for each experimental variant, the results being expressed in CCI (Chlorophyll Content Index).

Yield and Biometrical Determinations

Leaf samples were collected from each experimental plot, which were sent immediately to the laboratory for a series of determinations to be made, such as: weight and number of marketable leaves, their length and width, leaf area, dry matter content.

The leaf area index (LAI) was determined using the Li-3100 Area Meter, produced by LI-COR, inc. Lincoln, Nebraska, USA. The leaves' dry biomass was measured by weighing the samples after dehydrating the fresh leaves in a MOV-112F oven, produced by SANYO Electric Co., Ltd, Japan, whose temperature was set to 70°C, until reaching a constant weight of the leaves.

Statistical Analysis

The production results were processed using the least significant differences method (LSD), and for the other parameters analyzed the data were statistically processed by ANOVA and the mean separation was performed through Tukey's test at 0.05 probability level, using the SPSS software version 20. The results were reported as means ± standard deviation.

RESULTS AND DISCUSSIONS

The average leaf production ranged from 17.7 t·ha^{-1} in the case of the non-mulched (Ct) variant and 23.0 t·ha^{-1} in the case of the variant mulched with white film (Table 1). The difference of 5.3 t·ha^{-1}, respectively 29.24% recorded by the variant mulched with white film, is considered positively significant compared to the non-mulched variant, while the difference of 1.1 t·ha^{-1}, respectively 6.21% recorded by the version mulched with black film is insignificant compared to the control variant of the experiment.

Table 1. Results obtained regarding the influence of mulching and fertilization on the perennial wall-rocket yield

Experimental factor	Yield			Differences significance
	t·ha^{-1}	% vs. Control	Differences from Control	
Mulch type				
NM	17.7	100.00	0.0	Ct
WLDPE	23.0	129.24	5.3	*
BLDPE	18.8	106.21	1.1	ns
LSD 5% = 3.5 t·ha^{-1}; LSD 1% = 5.8 t·ha^{-1}; LSD 0.1% = 10.9 t·ha^{-1}				
Fertilization type				
NF	17.3	100.00	0.0	Ct
Ch	22.9	132.37	5.6	***
M	19.2	110.98	1.9	*
LSD 5% = 1.5 t·ha^{-1}; LSD 1% = 2.2 t·ha^{-1}; LSD 0.1% = 3.1 t·ha^{-1}				

Ct – Control; ns - no statistically significant difference
NM – non-mulched
WLDPE - white polyethylene film
BLDPE - black polyethylene film
NF – non-fertilized
Ch – chemical fertilization
M – biological fertilization

Table 2. Results regarding the influence of the mulching x fertilization combination on the perennial wall-rocket yield

Experimental variant	Yield			Differences significance
	t·ha^{-1}	% vs. Control	Differences from Control	
NM x NF	15.7	100.00	0.0	Ct
NM x Ch	21.2	135.03	5.5	***
NM x M	16.2	103.18	0.5	ns
WLDPE x NF	19.7	125.48	4.0	**
WLDPE x Ch	25.3	161.15	9.6	***
WLDPE x M	23.9	152.23	8.5	***
BLDPE x NF	16.5	105.10	0.8	ns
BLDPE x Ch	22.3	142.04	6.6	***
BLDPE x M	17.5	111.46	1.8	ns

LSD 5% = 2.7 t·ha^{-1}; LSD 1% = 3.7 t·ha^{-1}; LSD 0.1% = 5.3 t·ha^{-1}
Ct – Control; ns - no statistically significant difference
NM – non-mulched
WLDPE - white polyethylene film
BLDPE - black polyethylene film
NF – non-fertilized
Ch – chemical fertilization
M – biological fertilization

Regarding the type of fertilization, the chemically fertilized variant registered a yield increase of 5.6 t·ha^{-1}, respectively 32.37% compared to the non-fertilized variant, this difference being very significant. The variant on which microorganisms were applied registered a significant yield increase compared to the control variant, respectively of 1.9 t·ha^{-1} (10.98%).

Regarding the combined influence of the two studied factors, mulching x fertilization (Table 2), the yields obtained for the perennial wall-rocket crop in the first harvesting cycle (autumn) ranged between 15.7 t·ha^{-1} in the NM x NF variant and 25.3 t·ha^{-1} in the case of the WLDPE x Ch variant. Compared with the control variant (NM x NF), all the combinations of factors achieved yield increases with different degrees of significance. The highest yield increases, with very significant differences from the control variant, were registered for the variants: WLDPE x Ch - 61.15%, WLDPE x M - 52.23%, BLDPE x Ch - 42.04%, NM x Ch - 35.03%. Positive differences distinctly significant from the control variant were registered for the WLDPE x NF combination, with a yield increase of 25.48%. The other variants registered yield increases compared to the control variant, but they were non-significant.

Compared with the results obtained in our experimental field, Caruso *et al*., 2019c, reported yields between 11.0 t·ha^{-1} for the non-mulched variant and 12.5 t·ha^{-1} for the variant mulched with standard black film LDPE, 45 µm thick. Schiattone *et al*., 2018, mentioned yields between 10.5 and 17.9 t·ha^{-1}, these being registered in the first harvest cycle (December) at a low nitrogen level, respectively in the third cycle (March) in the case of a high level of nitrogen.

The highest yield value (23.0 t·ha^{-1}) achieved in the case of mulching with white film, is supported by the highest value of leaf area (3.27 m^{-2}·m^{-2}), by the highest quantity of dry matter (421.0 g·m^{-2}), as well as the highest average leaf weight (1.28 g), these production indicators having significant values compared to the non-mulched variant. At the same time, the yield increase was also influenced by the number of leaves/nest (126.6 leaves/nest), even though it presented non-significant values compared to the non-mulched variant (Table 3).

In the case of fertilization, the variant to which chemical fertilizers were administered determined the highest production of leaves, being supported by the highest values of leaf area (3.30 m^{-2}·m^{-2}), of dry matter (375.6.0 gm^{-2}), the number of leaves/nest (126.2 leaves/nest) and the average leaf weight (1.27 g). These indicators show significant values compared to the control variant (Table 3).

The average length and width of the leaf, presenting non-significant values both to the control variant and to the other variants, did not influence the yield achieved, for any of the studied factors.

In the case of combination between those two factors, the highest yields obtained by WLDPE x Ch (25.3 t·ha^{-1}) and WLDPE x M (23.9 t·ha^{-1}) variant are correlated with the highest values of leaf area (3.51 m^{-2}·m^{-2}), of dry matter (466.8 g·m^{-2}, respectively 454.9 g·m^{-2}) and of the number of leaves/nest (136.8, respectively 141.7 leaves/nest), these indicators having significant values compared to the control variant. The average leaf weight, although it had significantly higher values for the two variants above compared with the control, was outweighed by the WLDPE x NF variant which presented the highest average leaf weight (1.36 g/leaf).

The average values of leaf length and width were non-significant, as they did not influence the yields obtained from the combinations of the two factors (Table 4).

Similar results to those obtained in our experiment were also reported by Schiattone *et al*., 2018, the leaf area values ranging between 1.88 m^{-2}·m^{-2} in the first crop cycle and 3.32 m^{-2}·m^{-2} in the third crop cycle. Caruso *et al*., 2019a,c, reported in their works lower values of the leaf area in the first crop cycle, of 1.36 m^{-2}·m^{-2}, respectively 1.40 m^{-2}·m^{-2}.

For Schiattone *et al*., 2018, the average number of leaves ranged from 4.6 leaves/plant during the 1st crop cycle to 16.2 leaves/plant during the last one. The leaf length increased from 14.8 cm in the first cycle, to 20.1 cm in the last cycle, while the width varied from 1.4 cm in the second and third crop cycles up to 1.84 cm in the first cycle. The above mentioned results are lower compared to those obtained in our study.

Caruso *et al*., 2019c, mentioned a number of leaves between 119.0 leaves/nest in the winter cycle and 155.3 leaves/nest in the spring cycle, while in our study the obtained values were between 101.2 and 136.8 leaves/nest. Also, in the same article, Caruso *et al*. reports an average leaf weight between 0.51 g and 0.8 g, the values being lower than those obtained during our research (Tables 3 and 4).

Table 3. Productivity indicators for the perennial wall-rocket crop based on the type of mulching and fertilization

Experimental factor	LAI (m^2·m^{-2})	Dry matter (g·m^{-2})	Number of leaves/nest	Average length/leaf (cm)	Average width/leaf (cm)	Average weight/leaf (g)
Mulch type						
NM	2.76±0.16 b	290.3±8.60 b	112.6±3.68 ns	25.82±0.29 ns	5.95±0.43 ns	1.10±0.16 b
WLDPE	3.27±0.41 a	421.0±69.30 a	126.6±22.08 ns	25.14±0.23 ns	5.50±0.07 ns	1.28±0.09 a
BLDPE	3.04±0.38 ab	320.3±34.62 b	115.4±9.57 ns	24.90±0.27 ns	6.35±2.05 ns	1.13±0.10 b
Fertilization type						
NF	2.74±0.12 b	308.1±30.04 b	108.6±6.63 b	25.58±0.49 ns	5.37±0.48 ns	1.13±0.21 b
Ch	3.30±0.33 a	375.6±84.62 a	126.2±10.72 a	25.16±0.38 ns	5.53±0.07 ns	1.27±0.03 a
M	3.03±0.42 ab	347.9±92.86 ab	119.9±18.90 ab	25.13±0.55 ns	6.90±1.61 ns	1.11±0.07 b

Within each column, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$.
NM – non-mulched
WLDPE - white polyethylene film
BLDPE - black polyethylene film
NF – non-fertilized
Ch – chemical fertilization
M – biological fertilization

Table 4. Productivity indicators for the perennial wall-rocket crop based on the mulching x fertilization combination

Experimental variant	LAI ($m^2 \cdot m^{-2}$)	Dry matter ($g \cdot m^{-2}$)	Number of leaves/nest	Average length/leaf (cm)	Average width/leaf (cm)	Average weight/leaf (g)
NM x NF	2.60±0.31 b	282.7±18.37 c	114.1±21.30 ab	26.13±0.67 ns	5.81±0.18 ns	0.98±0.13 d
NM x Ch	2.92±0.34 ab	299.6±48.46 c	115.3±5.86 ab	25.57±1.50 ns	5.61±0.71 ns	1.28±0.05 ab
NM x M	2.74±0.22 ab	288.6±27.27 c	108.4±11.11 ab	25.75±1.24 ns	6.43±0.69 ns	1.05±0.03 cd
WLDPE x NF	2.80±0.36 ab	341.3±38.69 bc	101.2±7.50 b	25.39±0.98 ns	5.43±0.45 ns	1.36±0.05 a
WLDPE x Ch	3.51±0.20 a	466.8±48.94 a	136.8±13.46 a	25.09±1.55 ns	5.49±0.87 ns	1.29±0.06 ab
WLDPE x M	3.51±0.15 a	454.9±37.36 ab	141.7±2.52 a	24.95±1.89 ns	5.57±0.69 ns	1.18±0.06 abc
BLDPE x NF	2.81±0.11 ab	300.3±20.54 c	110.3±7.02 ab	25.21±1.00 ns	4.87±0.37 ns	1.04±0.01 cd
BLDPE x Ch	3.48±0.40 a	360.3±43.67 abc	126.4±12.81 ab	24.81±1.70 ns	5.49±0.88 ns	1.23±0.08 abc
BLDPE x M	2.83±0.38 ab	300.3±64.70 c	109.4±17.59 ab	24.69±1.61 ns	8.69±3.80 ns	1.11±0.08 bcd

Within each column, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$.
NM – non-mulched
WLDPE - white polyethylene film
BLDPE - black polyethylene film
NF – non-fertilized
Ch – chemical fertilization
M – biological fertilization

The highest content of chlorophyll pigments was identified both in the case of the non-mulched variant and in the chemically fertilized version. By contrast, both the variant mulched with black film and the non-fertilized variant recorded the lowest values of photosynthetic pigments (Figure 3).

In the case of the combination of the two factors (Figure 4), the highest content of chlorophyll pigments was registered in the NM x Ch variant, thus generating yields with very significant positive differences compared to the control variant. Similarly, the combination between mulching with black film and chemical fertilization recorded the second highest value in terms of chlorophyll pigments content, generating, as in the case of the non-mulched x chemical fertilization variant, leaf yield supported at a statistically significant level compared to the control variant.

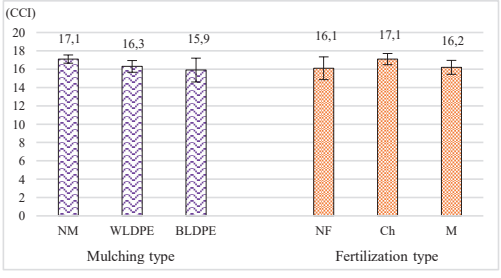

Figure 3. Chlorophyll pigments content in perennial wall-rocket leaves expressed as CCI, as influenced by mulching and fertilization.
Values associated to different letters are significantly different according to Tukey's test at $p<0.05$

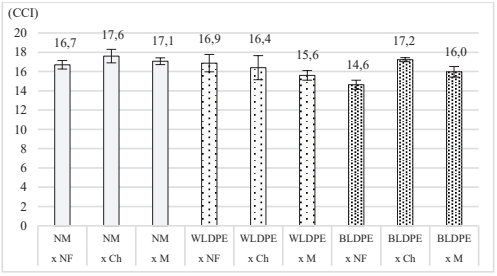

Figure 4. Chlorophyll pigments content in perennial wall-rocket leaves expressed as CCI, as influenced by the interaction between mulching and fertilization.
Values associated to different letters are significantly different according to Tukey's test at $p<0.05$

CONCLUSIONS

Following the experiments carried out on the *Diplotaxis tenuifolia* (L.) D.C. species, in the autumn harvesting cycle, both the mulching with white film and the chemical fertilization determined good yield results, but the combination of the two factors led to the phenomenon of synergism, generating the highest yields. In the autumn harvesting cycle this species behaves well in the conditions of North-Eastern Romania, the results being comparable to those from other research.

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BEHAVIOUR OF THE SWEET PEPPER AT SHORT-TERM STORAGE, DEPENDING ON CROP FERTILIZATION SCHEDULE

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Abstract

The scientific paper presents the impact of some technological production sequences (nutrition regime) and capitalization (storage conditions) on the quality and its maintenance capacity during short-term storage of sweet pepper. Two pepper varieties "Minthos" and "Boni" were supplied by an experimental vegetables farm. They were fertilized with different amounts of fertilizers, both varieties in three variants. The experimental storage was conducted at the Institute of Research and Development for Processing and Marketing of Horticultural Products "Horting" Bucharest in two technological variants: at ambient temperature (T=23-25^{0}C, RU=70-75%) and at refrigerant temperature (T=10°C, RU=80-85%) for the period of 10 and 20 days, respectively. Organoleptic properties, biochemical compounds, quantitative and qualitative losses during storage were analysed. Best results regarding the quality and storage capacity were obtained at "Minthos" variety. Best fertilization variant was represented by 200:150:80 kg NPK/ha. Cold storage was considered the most efficient option for peppers short-term storage.

Key words: *biochemical compounds, Capsicum annuum, organoleptic properties, storage losses.*

INTRODUCTION

Sweet peppers come from South and Central America and belong to the Solanaceae family. They were introduced into Europe for the first time at the beginning of the 16th century. The name "pepper" was given by Europeans when Christopher Columbus brought the plant to Europe. The terms bell pepper, sweet pepper, mild pepper or capsicum are often used for any of the large bell-shaped peppers, regardless of their color (green, yellow, orange, red, purple, brown or black). They are widely used because of their nutritional value, aroma, strong pungency and colour (Yaldiz et al., 2010). Due to their importance gradually increased, the peppers became ones of the most consumed vegetable crops worldwide (Téllez-Pérez et al., 2012). In addition, the food industry employs them widely as colouring and flavouring agents in sauces, soups, processed meats, lunches, sweetmeats and alcoholic beverages (Bogusz Junior et al., 2011). Sweet peppers are distinguished by a high content of vitamin C which is higher than of many other types of fruits and vegetables (www.greenfood.ro/ardeiul/).

Pepper is considered the second source of vitamin C after parsley, 200 g of pepper offering the daily amount needed by an adult. In addition to vitamin C, pepper also contains vitamin A, this combination representing a «deathly cocktail» for free radicals. These vitamins do not allow cholesterol deposit and, therefore, protect the body against heart diseases, offering protection against tumors, glaucoma and arthritis, as well (Ciofu et al., 2003).

The achievement and up keeping of the horticultural products quality depend on a blend of factors that interfere in all the cropping technology and capitalization, starting with variety selection and crop manage-ment until harvesting, conditioning and delivery (Jobling, 2012; Gherghi et al., 1977; Ryall and Lipton, 1972). The vegetables varieties have different particularities as regard a shorter or longer period of storage (Jamba and Carabulea, 2002; www.concordfoodcoop.documents/ncg_ produce_storage_guide_for_web.pdf.37;www. halfyourplate.ca/wpcontent/uploads/2014/12/ cpma_fruits_and_vegetables_storage_guide_final2. pdf). The shor - term storage capacity of

the peppers depends not only on the variety, but also on the quality of the raw material intended for storage (Tsegay et al., 2013; Lim et al., 2007; Lill and King, 1999; Popescu, 1978) and on the storage conditions (Raffo et al., 2008; Abdel-Maksoad et al., 1975; www.agrimedia.ro/articole/recoltarea-conditio-narea-si-pastrarea-legumelor; www.vegetablegardenplanner.com/how to-store/bell-pepper; www.naturefresh.ca/how-to-store-vegetables; www.chilipeppermadness.com/preserving-chili-peppers/how-to-store-pepper).

The chemical composition of peppers which determines the level of biochemical processes during storage and, therefore, the storage capacity is strongly influenced by the fertilization regime (Hameed et al., 2015; Bosland and Votava, 2000).

The doses of mineral or organic fertilizers influence the chemical composition of the fruits whith effects on the storage capacity (Ion, 2004; Salunkhe and Kadam, 1998; Meir et al., 1995).

As concern the capitalization technologies, it is recommended to apply those technological storage procedures that will determine the inhibition of the physiological and biochemical processes of fruits, leading to the up keeping of their commercial value for a longer period (Titisina et al., 2019; Burzo et al., 2005; Lill and King, 1999; Burzo, 1986).

The purpose of the present paper is to evaluate the achievement and quality mainte-nance of *Minthos* and *Boni* sweet pepper varieties, depending on the fertilization schedule and storage conditions.

MATERIALS AND METHODS

The sweet peppers were supplied by an experimental vegetable farm situated in Dobrogea, Romania. The technological conditions of culture and quality achievement were verified.

Tri-factorial experience was organized, with the following experimental factors:

A: variety:

- A_1: Minthos;
- A_2: Boni;

B: nutrition level: three graduations of fertilization have been applied, as is follows:

- B_1: 100 N + 100 P_2O_5 +50 K_2O kg a.s./ha;
- B_2: 200 N + 150 P_2O_5+80 K_2O kg a.s./ha;
- B_3: 300 N + 200 P_2O_5 +70 K_2O kg a.s./ha

C: storage conditions:

- C_1: warm (ambient temperature): T=23 - 25ºC ;RU=70-75%;
- C_2: cold (refrigerant temperature): T=10ºC, RU=80-87%

Where: T=temperature (ºC); RU=relative (air) humidity (%).

Minthos F1 is an early sweet pepper hybrid recommended for protected or open field cultivation. The plant has a high resistance to stress and good fruiting capacity. The fruits have an average weight of 200 g, are large, glossy, waxy, with a particular commercial aspect (www.wikipedia.org/wiki/bell_pepper; www.syngenta.ro/minthos-f1; www.zki.ro/blog/sortimentul-de-ardei-gras-de-tip-blocky/). Their color changes gradually from light green to yellow and then red to physiological maturity (Figure 1).

Boni is a sweet pepper heirloom with indeterminate growth, intended for open field cultivation. It is resistant to TMV (Tobacco mosaic virus) and is noticed for its good overall stressors resistance. It tolerates well the variations of temperature, drought and sunburn. *Boni* is a stable variety, with uniform aspect (truncate,) and an average weight of 140-180 g, fleshy, sweet, with a light yellow-red ripening at physiological maturity (*xxx*-2003; www.seminte-demetra.ro/product/boni/; www.royalsluis.com/hun/catalog/paprika/boni) as is shown in the Figure 2.

Figure 1. *Minthos* sweet pepper

Figure 2. *Boni* sweet pepper

The organoleptic determinations and bio-chemical analyses of the main components were made at two key moments:

- immediately after harvesting, just before storage;

- at the end of the storage period.

As regard the biochemical compounds, were analysed: the soluble dry matter using refractometric method (expressed in Brix/ refractometric degrees) soluble carbohydrates using Bertrand titrimetric method (expressed in percentage), titratable acidity (expressed in malic acid/100 g) and vitamin C using titrimetric method (expressed in mg/100 g). Also, the fruits weight losses (quantitative losses) and their depreciation (qualitative losses) during storage were quantified.

The evaluation of the organoleptic quality was achieved by carrying out the fruits sensory testing, using a grading scale from 1 to 100. Tasting sheets were used which included a number of three criteria of appreciation (aspect, firmness, taste), with different share in the general notation, depending on their importance: the aspect represents 15%, firmness 35%, and taste 50%. Depending on the score obtained, five quality classes are distinguished, as follows: very good (80-100 points), good (60-79 points), satisfactory (40-59 points), sufficient (20-39 points) and insufficient (0-19 points).

During storage, a daily control of the thermo-hydric factors in the storage rooms was carried out, in order to ensure the accomplishement of optimum conditions for up keeping the peppers quality. At the same time, the capacity to keep up the peppers quality was evaluated, including the monitoring of different storage pathogens.

RESULTS AND DISCUSSIONS

a. Organoleptic quality

The results of the organoleptic evaluation are presented in the Figures 3-5, pointing out that:

- immediately after harvest, *Minthos* obtai-ned a higher score than *Boni*, due to its attrac-tive aspect, good firmness and pleasant taste. The best variant of fertilization was B_2 (93.20 points), followed by B_3 (91.35 points) and B_1 (89.5 points). *Minthos* peppers at all fertiliza-tion variants obtaneid "very good" grading.
- after 10 days of warm storage (ambient temperature) the sensory properties of *Minthos* peppers remained at high parameters at B_2, getting “very good” grading. At B_1 and B_3 the score was quite lower and they got “good” grading, due to the aspect, taste and firmness depreciation. Storing the peppers at 23-25ºC conducted of getting fewer points than fresh crops.
- after 20 days of cold storage (refrigerant temperature), *Minthos* peppers maintained their nice aspect, great firmness and pleasant taste, recieving “very good” grading. The best fertilization variant was B_2 with 88.20 points.
- immediately after harvest, *Boni* peppers got a little bit lower score than *Minthos,* but high enough to receive “very good” grading at all fertilization variants (Table1).
- after warm storage, the sensory properties of *Boni* peppers remained at high parameters only at B_2, getting “very good” grading (81.33 points). B_1 and B_3 got a quite lower score and they received “good” grading.
- after cold storage of *Boni* peppers, B_2 and B_3 received “very good” grading, while B_1 received only “good” grading.

b. Biochemical composition

Analyzing the data presented in the Figures 6-9, it is found that the values of the biochemical indicators for *Minthos* variety and its evolution during storage varied upon the fertilizer’s doses applied to the crop.

Thus, the content in the soluble dry matter, expressed in Brix/refractometric degrees (ºR) was between 5.6% (B1) and 6.6% (B2).

Best values were obtained at B2, as regard the carbohydrates content, (4.35%) and vitamin C (198.68 mg/100 g), as well.

At harvest, B1 obtained the smallest values as regard all analyzed biochemical compounds.

High temperature during warm storage determined the intensification of biochemical processes in fruits, so that, after 10 days of warm storage, the content of the soluble dry matter increased, while the soluble carbohydrates and malic acid decreased in a higher rate, in comparison with other storage methods.

Lower temperature during cold storage conducted in slowing down the biochemical processes of the peppers. Thus, the soluble dry matter increased by 5, 86%, compared to the time of harvest, while the carbohydrates content

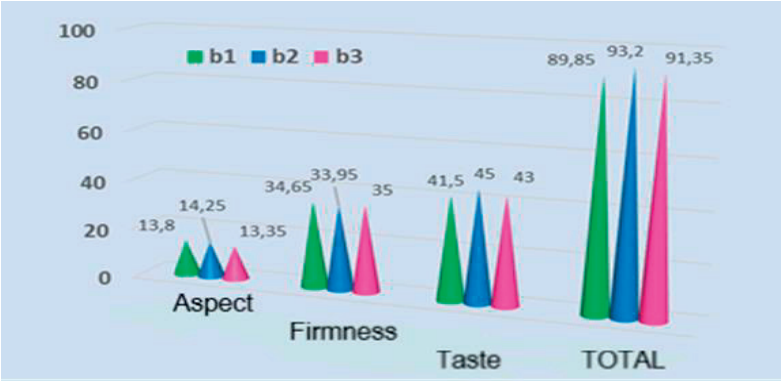

Figure 3. Sensory evaluation of *Minthos* after harvest

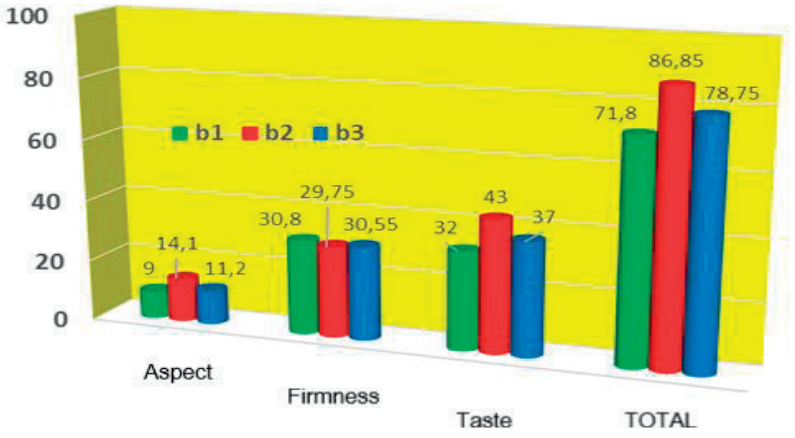

Figure 4. Sensory evaluation of *Minthos pepper* after ambient storage

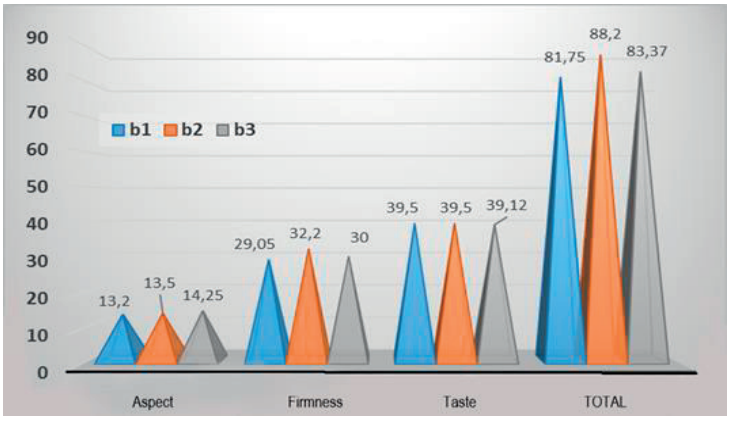

Figure 5. Sensory evaluation of *Minthos* pepper after cold storage

Table 1. Sensory evaluation of *Boni* sweet pepper

Determination time Total score/indicator score/ grading	Sensory evaluation/fertilization variant		
	B_1	**B_2**	**B_3**
At harvest			
- total score	**83.33**	**87.99**	**86.33**
- aspect	10.50	12.00	11.00
- firmness	29.50	32.66	30.33
- taste	43.33	43.33	45.00
- grading	very good	very good	very good
After ambient storage			
- total score	**70.07**	**8133**	**79.00**
- aspect	12.50	13.00	11.00
- firmness	26.80	35.00	28.00
- taste	30.77	33.33	40.00
- grading	good	very good	good
After cold storage			
- total score	**79.00**	**86.66**	**84.66**
- aspect	11.00	14.00	11.00
- firmness	28.00	32.66	30.33
- taste	40.00	40.00	43.33
- grading	good	very good	very good

decreased by 1.19%; titratable acidity decreased by 3.85% and vitamin C decreased by 9.5%, compared to harvesting time.

In the Table 2 are shown the biochemical indicators values of *Boni* peppers and their evolution during warm and cold storage.

B_2 was the best variant of pepper fertilization which determined the greatest biochemical values. The dynamics of biochemical processes development follows near some characteristics as in the case of *Minthos* sweet pepper (Figures 6-9).

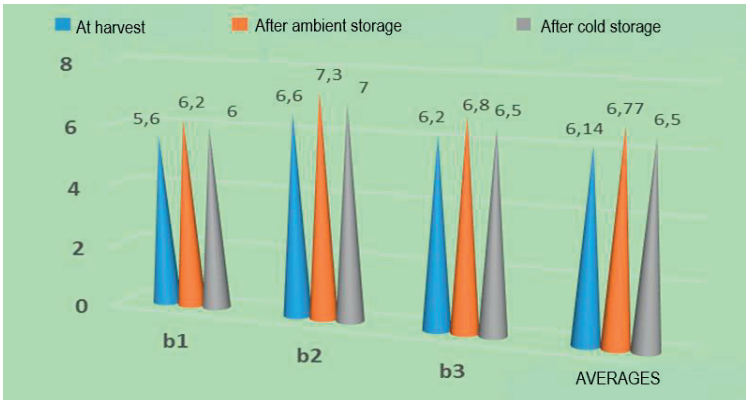

Figure 6. *Minthos* soluble dry matter content (^{0}R)

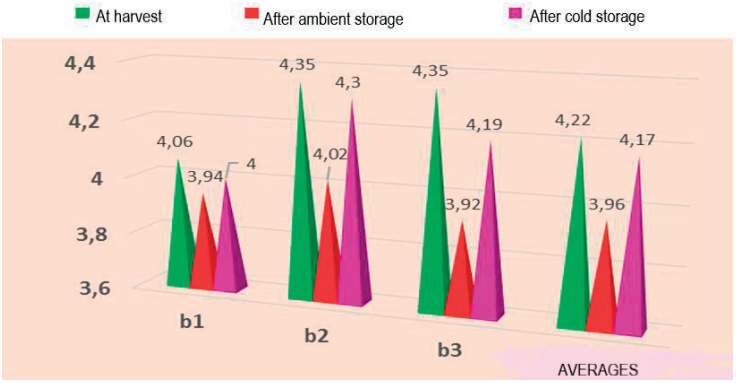

Figure 7. *Minthos* carbohydrates content (%)

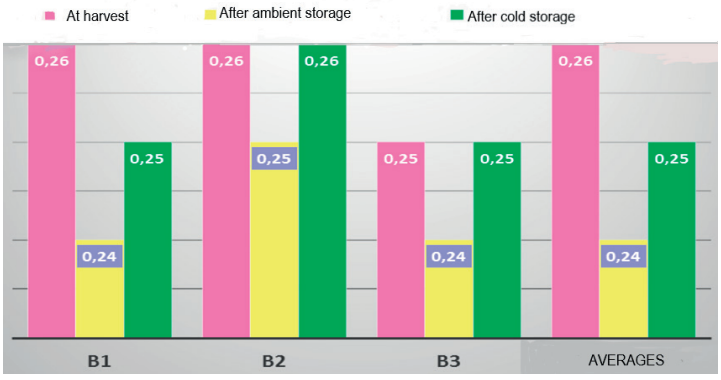

Figure 8. *Minthos* titratable acidity (malic acid/100 g)

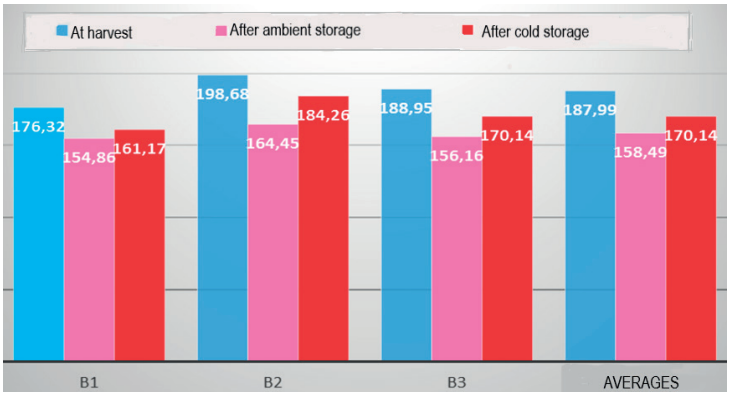

Figure 9. *Minthos* vitamin C content (mg/100 g)

Table 2. Main biochemical compound at harvest and after storage of *Boni* sweet pepper

Determination time/ Biochemical indicator	Variant			
	B_1	**B_2**	**B_3**	**Averages**
At harvest				
- soluble dry matter (^{0}R)	4.90	5.60	5.20	5.24
- carbohydrates (%)	3.49	3.99	3.61	3.70
-titratable acidity (malic acid/100 g)	0.26	0.27	0.26	0.26
-vitamin C (mg/100 g)	199.82	208.94	204.82	204.53
After ambient storage				
- soluble dry matter (^{0}R)	5.20	6.0	5.80	5.66
- carbohydrates (%)	2.99	3.34	3.24	3.19
- titratable acidity (malic acid /100 g)	0.24	0.25	0.25	0.25
- vitamin C (mg/100 g)	164.12	175.34	176.28	171.92
After cold storage				
-soluble dry matter (^{0}R)	5.00	6.0	5.5	5.50
-carbohydrates (%)	3.26	3.76	3.50	3.51
- titratable acidity (malic acid /100 g)	0.26	0.25	0.25	0.25
- vitamin C (mg/100 g)	180.93	191.16	188.76	186.95

c. Quantitative and qualitative losses

From the research carried out, it appears that the **best fertilization variant** of *Minthos* and *Boni* sweet peppers, concerning their storage losses, in both technological methods, **was B_2**, followed by B_3 (Table 3).

At **cold storage**, *Minthos* pepper total losses were: 11.33% at B_1, 9.92% at B_3 and 9.17% at B_2, the average losses being of 10.14%.

The values of *Boni* pepper total losses at cold storage were: 13.64% at B_1, 11.15% at B_3 and 10.26% at B_2, the average losses being of 11.69%. B_2 recorded the smallest losses to both sweet pepper varieties.

Pepper losses during **warm storage** (ambient temperature) for 10 days are high at all three variants of fertilization, due to the weight loss and deterioration, as well. Thus, the total losses are very high at the variant B_1 (16.44% - *Minthos* and 19.11% - *Boni*), followed by the variant B_3 (14.39% - *Minthos* and 18.26% - *Boni*). The variant B_2 recorded quite less losses (12.87% - *Minthos* and 17.92% - *Boni*).

The variety level are: 14.57% total losses, 3.55% weight losses and 11.02% depreciation losses of *Minthos* variety and 18.43% total losses, 4.43% weight losses and 14.01% depreciation losses of *Boni* variety.

The causes which determine very high percentages of pepper losses are the infections installed prior to harvest, produced by the *Xanthomonas vesicatoria* (bacterial spot) and the attack produced by *Erwinia carotovora* and *Botrytis cinerea* (two fungi which produce wet rot, respectively dry drying) during harvest and transport.

By using the refrigeration storage method, the pathogens development was slowed down and the total losses were greatly reduced, at all three variants of fertilization.

Table 3. Pepper losses during short - term storage

Variety	Fertilization variant	Total losses		Weight losses		Depreciation losses	
		10°C	23°C	10°C	23°C	10°C	23°C
Minthos	B_1	11.33	16.44	1.68	4.96	9.65	11.48
	B_2	9.17	12.87	0.43	2.53	8.74	10.34
	B_3	9.92	14.39	0.33	3.16	9.59	11.23
	Average	**10.14**	**14.57**	**0.82**	**3.55**	**9.33**	**11.02**
Boni	B_1	13.64	19.11	2.00	5.64	11.64	13.47
	B_2	10.26	17.92	1.09	3.48	9.17	14.44
	B_3	11.15	18.26	1.83	4.15	9.32	14.11
	Average	**11.69**	**18.43**	**1.64**	**4.43**	**10.04**	**14.01**

CONCLUSIONS

The quality of the sweet peppers and their maintenance capacity have varied according to the fertilizer doses applied to the crop and to the environmental storage conditions, especially, the temperature.

Among the fertilization variants, the variant B_2 promoted the best quality and storage capacity.

Of two storage methods (ambient tempera-ture and refrigerated room) better results were obtained in case of the second method, at which the smallest losses during storage were recorded, because low temperatures inhibit or slow down the fungi & molds growing rate - specific to each species of vegetables and slow down the rate of biochemical processes during their storage. Therefore, the depreciation losses were greatly reduced.

The content of sweet peppers as regard the main biochemical indicators (soluble dry matter, soluble carbohydrates, organic acids, vitamin C) varied according to the crop fertilization schedule.

The best results were obtained at variant B_2 - the sweet peppers were fertilized in the dose of 200 N + 150 P_2O_5 +80 K_2O kg/ha.

During storage, the peppers biochemical composition recorded variations. Thus, as the soluble dry matter content increased, the soluble carbohydrates & vitamin C content and acidity level decreased. The intensity of these processes differed according to the storage conditions.

The biodegradations of carbohydrates and organic acids are influenced by the storage temperature which is one of the essential factors that regulate the speed of ripening processes.

As higher the storage temperature is, as pronounced the biodegradation appears.

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APPLICATION OF THE MATHEMATICAL MODEL HJ-BIPLOT TO IDENTIFYING THE LINKS AMONG BIOACTIVE COMPOUNDS OF THE TOMATO VARIETY PANEKRA

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Abstract

Samples were collected from the Plovdiv, Bulgaria. The aim of this study was to is to compare the similarity and remoteness of different irrigation regimes: 1. Irrigation regime (50% of irrigation rate) and 50% fertilization; 2. Irrigation regime (75% of irrigation rate) and 50% fertilization; 3. Optimal irrigation regime (M-100%) with 50% fertilization; for the variety Panekra during the period 2016-2018 year and to identify the link among bioactive compounds of tomatoes through the mathematical model HJ-biplot. An important indicator of the quality of tomatoes is the content of total dyes. The highest values were found in tomato farmed irrigation regime (50% of irrigation rate) where total dyes attained up to 4.95 mg/100 g. The β-carotene content determines the orange color of the tomato fruit. The good combination of the two components (lycopene and β-carotene) with an antioxidant effect determines hybrids of tomatoes such as hybrids of high biological value. In the present study the content β-carotene ranges from 0.26 to 0.35 mg/100 g. The content of β-carotene is higher at a 50% of irrigation rate

Key words*: irrigation regimes, tomatoes, Panekra, HJ-biplot, Bulgaria.*

INTRODUCTION

The prevalence and widespread use of tomatoes is due primarily to the excellent food, taste and technological qualities of the fruit.

The chemical composition of fruits fluctuates quite widely, depending on the variety, the region, the growing conditions and the farming practices applied. However, the nutrient content of tomatoes depends mainly on genetic and ecological factors, and Javanmardi et al., 2008 consider maturation. Irrigation is also an agricultural practice that can influence the final content of these parts in the tomato fruit (Dumas et al., 2003). Water supply is limited globally and there is a growing need to reduce the amount of water used during irrigation practices (Zegbe-Domlnguez et al., 2003). The water deficit has a positive influence on the dry matter content, (Shao et al., 2014) and lycopene (Dumas et al., 2003).

The influence of irrigation practices on the processing tomato quality has not been sufficiently studied yet. Here are many studies related to the effects of quantity and frequency of irrigation on the quality of tomatoes (Machado et al., 2005; Zegbe-Dominguez et al., 2003) but still this question is relevant given the content of antioxidants in tomatoes. Examining the influence of water deficit there are established parameters of water deficit and productivity of irrigation water which result in stable yields and high quality (Lahoza et al., 2016; Nangare et al., 2016). Favati et al., 2009 evaluate the relationship between all quality parameters in tomatoes and seasonal water for irrigation. Tested and evaluated are the effects of different irrigation regimes, taking into account the physical and chemical characteristics of fruit and content of antioxidants. In recent years, as a result of expanding knowledge about the benefits of carotenoids for health, there is a significantly increasing attention of researchers to food taste and antioxidant properties of tomato fruits. A plurality of medical research has shown that dietary intake of lycopene-rich food limiting cases of some oncological and cardiovascular diseases, cataracts and the like. Color is principally associated with the lycopene content of tomato and is generally considered the most important attribute determining the product quality (Garcia et al., 2005).

The purpose of this paper is to compare the similarity and remoteness of different irrigation regimes the variety Panекra and to identify the link among bioactive compounds of tomatoes through the mathematical model HJ-biplot.

MATERIALS AND METHODS

Tomato Sampling

For the purpose of the study there were used test results of different irrigation regimes in tomato cultivar Panекra during the period 2016-2018 year (Figure 1).

Figure 1. Panекra

The Panекra variety is offered for early production in steel-glass and polyethylene greenhouses. The hybrid has many habitats, with houses between restoration and a good balance between vegetative and generative development. It has great potential for obtaining average yield height. The areas are large, up to very large (over 300 g.), solid, black in color, multicameral without parenchymal tissue inside the fetus. It is resistant to tobacco mosaic virus, Verticillium, Fusarium, mold list and nematode.

The experiment is based in the region of Plovdiv with geographical coordinates 42° and 09′ north latitude and 24° and 45′ East GMT (GPS). The experiment based on the block method on a flat surface in scheme 110 + 50 + 35 with the size of the parcel plot of 10 m^2 (Barov, 1982).

1. Irrigation regime (50% of irrigation rate) and 50% fertilization; 2. Irrigation regime (75% of irrigation rate) and 50% fertilization; 3. Optimal irrigation regime (M-100%) with 50% fertilization.

Greenhouse production is intense and it requires the application of high doses of fertilizers. For the purposes of the experiment, different doses of basic fertilization and feeding during the growing season were tested. The main fertilization was carried out with P 230 kg/ha in the form of P_2O_5) and K 250 kg/ha (as K_2SO_4). Feeding through vegetation was performed with N 500 kg/ha as NH_4NO_3) and K 660 kg/ha as KNO_3) according to the experimental methodology. Submission of irrigation water was realized with the drip irrigation system. During the three years of the study various watering rates have been implemented depending on the requirements of culture and the length of the growing season.

Sample Preparation

Each sample was weighed, and the weight was divided by the number of tomatoes to give the weight per tomato. Then, three tomatoes were selected at random from each sample for analysis. They were hand-rinsed with ultra-pure water, shaken to remove any excess water, and gently blotted with a paper towel. A small portion (approximately 3 g weight) of each tomato was cut and put into 10 mL of 3% H_3PO_4 as subsamples for the ascorbic acid assay. The tomatoes were then mixed and homogenized to homogeneous puree. A fraction of this puree was desiccated at 105°C, homogenized again, and stored in a polyethylene tube at room temperature until assay for metals and protein. The rest was immediately stored in a polyethylene tube at -80°C in order to avoid enzymatic changes and for the measurement of the other chemical variables.

Analytical Methods

All assays were performed in triplicate, and the results expressed per fresh weight (FW). Moisture was determined using the oven-drying method (AOAC, 2006). The residue was heated at 550°C for 24 h for the ash determination (AOAC, 2006). Organic

nitrogen was determined by the Kjeldahl method (AOAC, 2006), and the protein concentration estimated using 6.25 as the conversion factor. All assays were performed in triplicate, and the results expressed per fresh weight (FW). Sugars were determined as described by Galdon et al., 2009. In the determination of mineral elements were determined by Atomno absorption spectrometer "AAnalyst 800 with graphite furnace HGA" Company "Perkin Elmer". The tomato samples were previously acid-digested in nitric acid, in accordance with the procedure described by Hernandez Suarez et al., 2008. Ascorbic acid was determined in the individual tomatoes using the 2,6-dichlorophenolindophenol titration procedure (AOAC, 2006). The lycopene concentration was determined spectrophotometrically at 503 nm following extraction in 20 mL of acetone: ethanol: hexane (5: 5: 10 v/v) in the dark, in accordance with the method described by Fish et al., 2002. Organic acids were determined by HPLC as described by Hernandez Suarez et al., 2008. β-carotene was determined by HPLC as described by Hernandez Suarez et al., 2007.

Statistical Analysis

Statistical Software

All statistical computing, analysis and all charts were performed with the statistical software R program Version 3.5.2.

The log-ratio transformation

In order to apply standard statistical multivariate analysis, the raw compositional data were subjected to a clr-transformation. This transformation is symmetric with respect to the compositional parts, and maintains the same number of components as the number of parts in the composition. Its advantage is that it is an isometric transformation of the simplex with Aitchison metric onto a subspace of real space with the ordinary Euclidean metric (Egozcue et al., 2003; Aitchison et al., 2002; Aitchison, 1986). The clr-transformation is given by the expression:

$$clr(x) = \left[\ln \frac{x_1}{g_m(x)}, \ln \frac{x_2}{g_m(x)}, \ldots, \ln \frac{x_D}{g_m(x)} \right],$$

where: $x = (x_1, x_2, \ldots, x_D)$ is a compositional data vector; x_i are the chemical variables all of which must be expressed in the same units; $g_m(x)$ is the geometric mean of that compositional data vector.

HJ-Biplot Method

The statistical technique used was the HJ-Biplot multidimensional data classification technique (Galindo, 1986), which is a variant of the Biplot graphic display proposed by Gabriel (Gabriel, 1971). This technique makes it possible to plot the rows and columns of the data matrix as points on a low dimension vectorial space. It has been shown theoretically (Galindo, 1986) that the quality of plotting both for individuals and for ical technique has been demonstrated in other studies (Castela et al., 2010; Lodoño et al., 2007; Galante et al., 1991). Also, the discriminatory power of this statistical technique has been demonstrated in other studies (Castela et al., 2010; Lodoño et al., 2007; Galante et al., 1991).

The HJ-biplot is a joint representation in a low-dimensional vector space (usually a plane) of the rows and columns of a data matrix X, using markers (points/vectors) $j_1, j_2, \ldots, j_n$ for its rows and $h_1, h_2, \ldots, h_p$ for its columns. The markers are obtained from the usual singular value decomposition (SVD) of the data matrix $X = U\Sigma V^T$, where U is formed by the eigenvectors of the matrix XX^T, V by the eigenvectors of the matrix X^TX, and Σ is a diagonal matrix containing the singular values (i.e., the square roots of the non-zero eigenvalues of both XX^T and X^TX), taking as rows the marker rows of $J = U\Sigma$ and as columns the marker rows of $H = V\Sigma$, in the appropriate dimensions. Thus, the matrix X is formed by clr-transformed data, and then double-centered through its SVD to ensure that the components are analyzed on a ratio scale (Aitchison, 2002) in the appropriate dimensions.

A biplot of compositional data consists of the elements shown in Figure 2 (Pawlowsky-Glahn et al., 2015; Aitchison et al., 2002):

- The similarity (Sij) between two samples or individuals is taken to be an inverse

function of their distance, in such a way that closer samples are more similar.

- The centroid represents the center-of-gravity formed by the geometric mean of the compositional parts used in the clr-transformation.
- The ray provides information on the variance of the corresponding log-ratio with respect to the geometric mean (g_m):

$$\mathrm{var}\left(\ln\frac{x_i}{g_m(x)}\right)$$

The square root of this expression is the standard deviation of the clr-transformed variable Xi, and is represented by the length of the ray. The cosine of the angle (α) between two rays represents the approximate correlation coefficient between the corresponding variables.

The length of a link between the vertices of two rays represents the standard deviation of the log-ratio between the associated compositional parts, with the corresponding variance being defined as:

$$\mathrm{var}\left(\ln\frac{x_i}{x_j}\right).$$

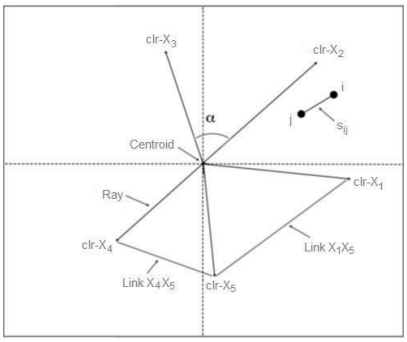

Figure 2. Elements of a compositional biploot (Pawlowsky-Glahn et al., 2015; Aitchison et al., 2002)

Angles between links provide information on the relationships between pairs of variables. If two links intersect at a right angle then this is an indication that the pairs of variables are possibly uncorrelated (in Figure 2, links X_1X_5 and X_4X_5), while if they are parallel (or the angle is obtuse) then the pairs of parameters may be strongly correlated (in Figure 2, links X_1X_5 and X_4X_3).

Coincident vertices or short links mean that the two variables are linearly proportional, so that the two parts involved can be assumed to be redundant. If a subset of links is collinear, this might indicate a possible one-dimensional variability.

RESULTS AND DISCUSSIONS

Tables 1 give the results of the influence of irrigation regime on the chemical composition of the tomato fruit (Panекra) (proximate composition, mineral elements, organic acids, and antioxidant compounds).

The highest values were found in tomato farmed irrigation regime (75% of irrigation rate and 50% fertilization) where ascorbic acid attained up to 33.58 mg/100 g. Ascorbic acid contents in tomato farmed irrigation regime (50% of irrigation rate and 50% fertilization) declined to 27.84 mg/100 g. A similar trend was noted in Vasileva et al., 2016, in an analysis of the influence of potassium fertilization.

An important indicator of the quality of tomatoes is the content of total dyes. The two main groups of pigments in the fruit of tomato carotenoids and chlorophylls but the final color is determined by the total quantity and ratio of different carotenoids (Danailov, 2012). The highest values were found in tomato farmed irrigation regime (50% of irrigation rate and 50% fertilization) where total dyes attained up to 4.95 mg/100 g. Total dyes contents in tomato farmed irrigation regime (100% of irrigation rate and 50% fertilization) declined to 3.07 mg/100 g.

Lycopene is a phytochemical from the group of carotenoid pigments. Its antioxidant activity predetermines the interest in increasing its content in tomatoes. Researchers have found that lycopene accounts for between 75 and 83% of the total content of pigments in tomatoes (Gould, 1992; Abushita et al., 1997) making it an important biochemical quality indicator. According to some authors the content of lycopene is varietal characteristic and fruit of tomatoes respond to fertilization with potassium by increasing the content of antioxidants (Hartz et al., 2005; Henry et al., 2008).

Table 1. Proximate and mineral elements composition (mean ± standard deviation, data expressed in fresh weight, FW) of tomato samples

Irrigation regime (50% of irrigation rate) and 50% fertilization	Proximate Composition (% FW)			
	Compound	2016	2017	2018
	Moisture	92.84 ± 0.52	93.41 ± 0.46	93.11 ± 0.36
	Ash	0.72 ± 0.07	0.74 ± 0.11	0.73 ± 0.08
	Protein	0.69 ± 0.09	0.67 ± 0.06	0.67 ± 0.09
	Fructose	1.35 ± 0.11	1.36 ± 0.15	1.35 ± 0.18
	Glucose	1.44 ± 0.12	1.43 ± 0.22	1.43 ± 0.23
	Mineral Elements (mg/kg FW)			
	P	264 ± 28	283 ± 31	261 ± 27
	K	2820 ± 325	3025 ± 342	2870 ± 328
	Mg	135 ± 15	141 ± 18	137 ± 16
	Organic Acids and Antioxidant Compounds (mg/100 g FW)			
	Ascorbic acid	27.84 ± 0.48	32.61 ± 0.56	29.88 ± 0.68
	Titratable acidity	0.32 ± 0.09	0.39 ± 0.11	0.21 ± 0.06
	Total dyes	4.95 ± 0.32	3.89 ±0.28	4.95 ± 0.31
	Lycopene	4.63 ± 0.18	3.79 ± 0.15	5.00 ± 0.21
	β-carotene	0.32 ± 0.08	0.35 ± 0.07	0.34 ± 0.08
Irrigation regime (75% of irrigation rate) and 50% fertilization	Proximate Composition (% FW)			
	Moisture	93.21 ± 0.38	93.85 ± 0.43	93.54 ± 0.35
	Ash	0.69 ± 0.08	0.71 ± 0.13	0.70 ± 0.08
	Protein	0.71 ± 0.10	0.69 ± 0.08	0.71 ± 0.09
	Fructose	1.34 ± 0.12	1.36 ±0.11	1.34 ± 0.14
	Glucose	1.42 ± 0.11	1.44 ± 0.22	1.42 ± 0.18
	Mineral Elements (mg/kg FW)			
	P	248 ± 25	265 ± 27	245 ± 21
	K	2654 ± 307	2863 ± 328	2680 ± 305
	Mg	127 ± 12	132 ± 16	129 ± 14
	Organic Acids and Antioxidant Compounds (mg/100 g FW)			
	Ascorbic acid	30.57 ± 0.46	33.58 ± 0.52	29.88 ± 0.62
	Titratable acidity	0.3 ± 0.08	0.4 ± 0.12	0.25 ± 0.07
	Total dyes	4.58 ± 0.18	3.47 ± 0.25	4.57 ± 0.28
	Lycopene	3.92 ± 0.15	3.47 ± 0.14	4.64 ± 0.25
	β-carotene	0.30 ± 0.07	0.28 ± 0.08	0.29 ± 0.08
Optimal irrigation regime (M-100%) with 50% fertilization	Proximate Composition (% FW)			
	Moisture	93.53 ± 0.44	94.21 ± 0.48	93.92 ± 0.41
	Ash	0.68 ± 0.08	0.70 ± 0.12	0.69 ± 0.09
	Protein	0.70 ± 0.11	0.68 ± 0.09	0.68 ± 0.08
	Fructose	1.34 ± 0.11	1.36 ± 0.15	1.33 ± 0.16
	Glucose	1.43 ± 0.12	1.44 ± 0.21	1.39 ± 0.21
	Mineral Elements (mg/kg FW)			
	P	241 ± 27	256 ± 28	241 ± 23
	K	2574 ± 285	2728 ± 195	2570 ± 258
	Mg	119 ± 14	127 ± 17	123 ± 11
	Organic Acids and Antioxidant Compounds (mg/100 g FW)			
	Ascorbic acid	32.31 ± 0.41	30.17 ± 0.39	30.43 ± 0.38
	Titratable acidity	0.32 ± 0.08	0.32 ± 0.08	0.22 ± 0.08
	Total dyes	4.11 ± 0.15	3.07 ± 0.13	4.19 ± 0.11
	Lycopene	3.37 ± 0.12	3.05 ± 0.11	4.29 ± 0.11
	β-carotene	0.27 ± 0.08	0.26 ± 0.09	0.28 ± 0.08

According to the FAO, the lycopene content ranges from 7 to 13 mg/100 g (Rath et al., 2009). In the present study the content ranges from 3.05 to 5.0 mg/100 g. Table 2 shows that the content of lycopene is higher at a 50% of irrigation rate. The water deficit has a positive influence on the content of lycopene as well as Dumas et al., 2003.

The β-carotene content determines the orange color of the tomato fruit. The good combination of the two components (lycopene and β-carotene) with an antioxidant effect determines

hybrids of tomatoes such as hybrids of high biological value (Pevicharova et al., 2012). In the present study the content β-carotene ranges from 0.26 to 0.35 mg/100 g. The content of β-carotene is higher at a 50% of irrigation rate. Our results are in line with those of Mozafar, 1994. β-carotene content in fruit increases with increasing levels of K, Mg, Mn, B, Cu and Zn. Phosphorus may also increase the fruit concentration of phytochemicals such as ascorbic acid, flavonoids and lycopene (Dorais et al., 2008).

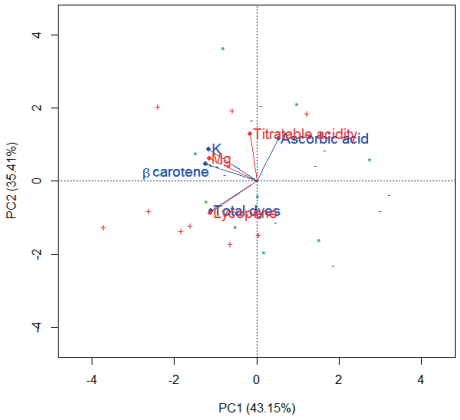

Figure 3. Standard HJ-biplot

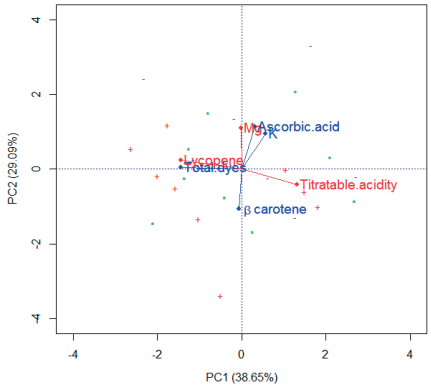

Figure 4. Compositional HJ-biplot of the chemical composition data

It is important to emphasize how the problem of spurious correlations is resolved with compositional statistics. By way of illustration, a standard HJ-biplot (Figure 3) and a compositional HJ-biplot (Figure 4) were constructed for K, Mg, Ascorbic acid, Titratable acidity, Total dyes, Lycopene and β-carotene. The HJ-biplot was coustructed with data expressed as percentages, and the compositional HJ-biplot with centred log-ratios, in both case with a double-centring transformation of the matrix. The angles between the vectors approximate the correlations between variables in such a way that small acute angles are associated with variables that are strongly positively correlated, obtuse angles close to 180° with variables that are strongly negatively correlated, and right angles with uncorrelated variables. One observes different correlations between the variables, and different percentages of the variance explained in each biplot, with an overall fit of 78.56% in the HJ-biplot and 67.74% in the compositional HJ-biplot.

Regarding compositional HJ-biplot (Figure 4), samples near the centroid have values similar to the geometric mean for each component, while those far from the centre can be diagnosed as having a large relative variation with respect to the geometric mean for the clr-transformed chemical variables that the points are near. In the case of the rays, Lycopene and Total dyes has the greatest variation with respect to theirs mean, while the smallest variation corresponds to K. The samples are mainly located near the rays of Lycopene, Total dyes, β carotene and Titratable acidity.

The acute angles for Total dyes/Lycopene and Ascorbic acid/Mg indicate positive correlations, a correlation that is especially strong in the case of Total dyes. This is coherent with the shortness of the Total dyes/Lycopene link. In order to quantify the relationship, the values of the variance given in Table 2 must be considered. For the Total dyes/Lycopene log-ratio, the "variance corresponds to a standard deviation of 0.0067. Since this value is expressed on a logarithmic scale, it must be back-converted to a Euclidean scale, i.e., 0.0398. Thus, an almost linear increase in the lycopene content is due to the Total dyes content of the tomato fruit. However, Ascorbic acid is more strongly correlated with the ascorbic acid than the Mg content, with an estimated standard deviation of 0.01436.

In the case, of β-carotene/Ascorbic acid, the obtuse, close to 180°, angle between their rays indicates a possible strong negative correlation.

Right angles mean uncorrelated variables, which here are Titratable acidity/Ascorbic acid and Total dyes/β-carotene.

Table 2. Variation array of functional compounds

	K	Mg	Ascorbic acid	Titratable acidity	Total dyes	Lycopene
Mg	0.0047					
Ascorbic acid	0.0128	0.01436				
Titratable acidity	0.0837	0.0996	0.0936			
Total dyes	0.0306	0.0288	0.0385	0.1619		
Lycopene	0.0303	0.0273	0.0402	0.1742	0.0067	
Beta carotene	0.0377	0.0426	0.0701	0.1256	0.0590	0.0610

CONCLUSIONS

An important indicator of the quality of tomatoes is the content of total dyes. The highest values were found in tomato farmed irrigation regime (50% of irrigation rate and 50% fertilization) where total dyes attained up to 4.95 mg/100 g. Total dyes contents in tomato farmed irrigation regime (100% of irrigation rate and 50% fertilization) declined to 3.07 mg/100 g.

The β-carotene content determines the orange colour of the tomato fruit. The good combination of the two components (lycopene and β-carotene) with an antioxidant effect determines hybrids of tomatoes such as hybrids of high biological value. In the present study the content β-carotene ranges from 0.26 to 0.35 mg/100 g. The content of β-carotene is higher at a 50% of irrigation rate.

Compositional Data Analysis (CoDA) refers to the analysis of data, which have been defined as random vectors with strictly positive components whose sum is constant.

Using this novel statistical technique, linear relationships or links between the variables were identified that are different from those provided by the usual correlation coefficient studies.

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RESEARCH ON PHENOTYPIC AND BIOCHEMICAL VARIABILITY IN NEW GENOTYPES OF *PERILLA FRUTESCENS*

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Abstract

Perilla frutescens originates in Asia and is widely grown in China, Japan, South Korea, Vietnam and India. China is considered to be the center of origin; therefore, in some areas of Asia, Perilla Nanking or Sisho is considered a sacred plant. In Europe, Perilla sp. has entered rather late, appreciated for its economic properties, and used for its oils or as an ornamental plant, although, it occupies quite small areas.In Romania, the plant was studied after 2010 by the Breeding and Biodiversity Laboratory Buzau, obtaining three genetically stable genotypes from which two genotypes are the subject of this work. The genotypes taken in the study were phenotypically analysed, while biochemical laboratory analyses were also performed. Regarding the content of volatile oil, there were no differences between genotypes (0.4 mL/kg), while the content in total polyphenols has variations from 3.94 to 6.26%; the contents in flavones ranged from 1.19% to 0.49, and the content in pectin also varied from 7.68 to 11.29%.

Key words*: breeding, biodiversity, volatile oil, polyphenols, flavones.*

INTRODUCTION

The genus Perilla is part of the Lamiaceae family, also called Labiatae, or the mint family, with 236 genera and more than 7000 species, is the largest family of the Lamiales order. *Perilla frutescens* (L.) Britt. originates from Asia being widely grown in China, Japan, South Korea, Vietnam and India. China is considered to be the primary gene center, in some areas of Asia, Perrilla Nanking or Sisho is regarded as sacred plant. The largest cultivated area is found in Korea. In Europe, Perilla sp. has entered rather late, appreciated for its economic properties, and used for its oils or as an ornamental plant (Nitta et al., 2003), although, it occupies quite small areas. Perilla sp. is divided according to the morphology of the plant and its use in two varieties: *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa. P. frutescens* var. *frutescens* is used to obtain oils and is known in the countries of East Asia as Ren in China, Dllggae in Korea and Egoma in Japan. Only in Korea the leaves are consumed. Perilla seeds are used like sesame seeds from ancient times in China, Korea and Japan (Lee and Ohnishi 2001, 2003; Nitta et al. 2003). *P. frutescens* var. *crispa* is part of Chinese herbs, and is called Cha-jo-ki in Korea, Shiso in Japan and Zisu in China (Lee and Ohnishi 2001, 2003). It is also used as a condiment for pickles in Japan. In conclusion, these two species have been an important crop in East Africa since ancient times. (Lee and Ohnishi 2003; Nitta et al. 2003). In southern China, the leaves of crispa variety are used mainly because of its medicinal properties (Tan et al. 2012; Wang and Guo 2012; Wei et al. 2015). For instance, the leaves have detoxifying properties and have been used for cooking crab and fish for over 2000 years (Yu șicolab. 2016). In addition, seeds and leaves of crispa varieties have been considered effective in the treatment of cough, common cold, asthma and digestive problems (Yu et al. 2016). Another study (Asif M., 2011, Lands William E. M., 2005) suggested that Perilla oil is known to have one of the highest concentrations of omega-3 fatty acids and it is beneficial for human health and for preventing various diseases, such as cardiovascular disease, cancer, inflammatory and rheumatoid arthritis.

Taxonomic studies have been the foundation of genetic resources management in many aspects. Research on the plant taxonomy is based on comparing the agro-morphological characteristics. Thus, until now, characteristics such as leaf size, seed size, plant height, number of branches, colour of flowers and leaves, degree of pubescence and aroma of the plant are used to differentiate *frutescens* variety from *crispa* variety. Of these, the seed size is considered the most reliable characteristic that distinguishes *P. frutescens* var. *frutescens* from *P. frutescens* var. *crispa* and *P. frutescens* var. *frutescens* the wild variety. Rao and Hodgkin (2002) suggested thatmorphological analyses are dependent on environmental factors.

Perilla frutescens in pedoclimatic conditions of Romania is an annual plant. *Perilla frutescens* is a plant with multiple uses, appreciated for its therapeutic and food uses, but is also valued for its ornamental and aromatic purpose.

In Romania, the plant was taken into study after 2010 at the Breeding and Biodiversity Laboratory, Buzau, obtaining until now a number of three genetically stable genotypes and two of them made the subject of this work. The research was extended in collaboration with University of Agronomic Science and Veterinary Medicine of Bucharest.

The studied genotypes were analysed from a phenotypic point of view, at the same time biochemical laboratory analyses were performed for two varieties, crispa variety and frutescens variety.

MATERIALS AND METHODS

In the study were used two genotypes that have distinct genotypic characteristics, one is *P. frutescens* var. *crispa*, with reddish purple leaves and *P. frutescens* var. *frutescens* with green and reddish side leaves.

The crop technology used: was sowing in the first decade of March, in plastics pots with 70 cubes with a volume of 50 mL/cube in a mixture of peat and sand. The planting was made at the beginning of May, and the planting scheme used was 70 cm between the rows and 35 cm between plants. A special care was made using one mechanical hoeing and two manual hoeing for loosen soil and weed. Throughout the plant season, no chemical treatments were applied for management of diseases and pest. So far, no pathogens have been identified to endanger the crop.

One of the main objectives of the study was to observe the adaptability process in pedoclimatic conditions of Romania. It is known that only varieties that acclimatize can survive to produce progeny from which a new population may become established.

During the vegetation period, biometric and phenological observations were made. Another main objective of this study was to performed laboratory analyses in order to establish the chemical composition of the studied varieties. The volatile compound was done using gas chromatography on fresh leaves and shoots on 23rd of September.

The content of flowers and seeds in volatile oils, polyphenols, flavones, antioxidants, pectin, amino acids, total lipids and calcination residue was made on 4th October.

RESULTS AND DISCUSSIONS

Perilla frutescens var. *crispa* (Figure 1) has a strong, fibrous-branching root that exploits a large volume of soil. The plant height can reach, on average, up to 2meters, with a diameter at the base of the stalk measuring, on average, 2.8cm. The stalk is flexible in the vegetative stages, but it lignifiesduring the senescence period. The number of main stems varies between 16 and 18.

Figure 1. *Perilla frutescens* var. *crispa* crop detail

The plant diameter has varied from 1.10-1.40meters. The leaves are simple, opposite, with broad oval shape pointy ends, serrated margins and a long petiole, or even absent on the leaves on the top (Figure 2). The leafstalks

measure, on average a length of 9.12 cm. The length of leaf in averaged, measured 16.12 cm, and the width was 10.24 cm. The leaves have a strong anthocyanin colouring. It has been shown that the red overtones are mainly due to the presence of malonilshisonin (Meng L. et al., 2006).The average number of leaves per main stem was 155 leaves.

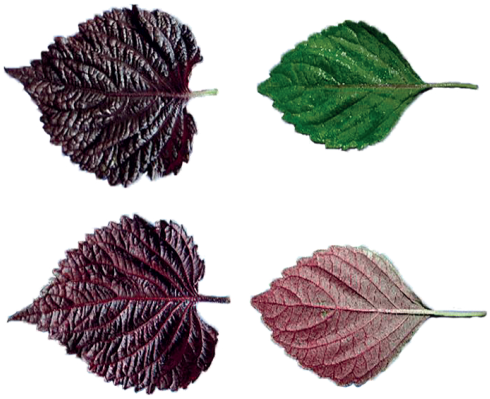

Figure 2. Different types of leaves belonging to *P. frutescens* var. *crispa* (left) and *P. frutescens* var. *frutescens* (right)

The beginning of flowering began on 17th of August. The plant is self-pollinating, but is preferred by insects, especially bees, due to the pleasant smell (Figure 3). The seeds have a grey colour and in one gram can be found 213 seeds.

Figure 3. Flowers of *P. frutescens* var. *crispa*

Perilla frutescens var. *frutescens* (Figure 4) has a strong root, which exploits a large volume of soil. The stalk is coloured green, with a height of up to 2 meters am with a plant diameter, on average, of 1.33 m. The leaves are green, coloured purple on the side. The average size of the leaflethas a length of 15.93 cm and the width has a value of 9.87. The average number of leaves on the main shoot was 149 leaves.

Figure 4. *Perilla frutescens* var. *frutescens* crop detail

The flowers appear in the middle of August, grouped in racemes, and have a white colour. The seeds have a grey colour and the mass of one thousand seeds is 4.26 g (Figure 5).

Figure 5. Seeds of *Perilla frutescens* var. *frutescens*

Following the results, it is recommended to grow the Perilla species in the field in a well drain soil; it does not require high soil fertility.
Being a new acclimatized plant,during the vegetation period, no diseases and pests were registered.
Leaves and shoots of *Perilla frutescens* var. *frutescens* and *crispa* were harvested during the vegetation period and were subjected to chemical analyses. The analyses were performed on the fresh material and were determined by gas chromatography-mass spectrometry analysis. In Tables 1 and 2 are shown the volatile compounds of both Perilla species.

Table 1. Volatile compound of *P. frutescens* var. *frutescens*

Compound	RT	Area %	Peak Area	Peak Height	S/N
3 Octenol	23.64	0.30	10557125	3057045	4108.03
Linalool	26.21	0.76	26410282	7791242	10469.82
Cariophylene β	27.27	4.10	142334173	39113802	52560.85
Cariophylene α	29.03	0.51	17604740	4906300	6593.05
Germacrene	29.97	0.13	4382556	1167577	11568.98
Hexanoyl furan	30.47	2.16	74893076	20620856	27710.16
Cas 16076-65-9	30.85	0.35	12206287	3469820	4662.72
Hexanoyl furan	32.10	88.58	30764663044	73178087	983361.87
Farnesoljhvc	34.11	0.10	3551027	911590	1224.99
Trimethylpropylsilane	35.17	0.40	13720037	3900422	5241.36
Cariophyleneoxid	35.70	0.29	9904768	2721065	3656.55
Cariophyleneoxid	35.88	1.31	45586931	12253122	16465.66
Nerolidol	37.08	0.32	11152956	2033704	2732.88
Spathulenol	38.65	0.40	13735148	363560	4885.17
Cadinol α	46.94	0.10	3558601	519375	697.93
Phytol	47.18	0.20	6870902	1471880	1977.90

The result of the study suggested that there were differences in volatile compounds among the *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* grown in Buzau site. Chemical polymorphism indicated that genetic and environmental factors should be considered to ensure consistent quality. Xie et al. (2012) also states that the yield, chemical compositions and bioactivities may be influences by genetic and environmental factors. The quality of the volatile compounds is directly influenced by the relationships between the variation in chemical composition and the bioactivity.

Table 2. Volatile compound of *P. frutescens* var. *crispa*

Compound	RT	Area%	Peak Area	Peak Height	S/N
Perillene	22.74	0.95	36088223	10347198	15206.46
3 Octenol	23.62	0.31	11616928	3380426	4967.68
Linalool	26.20	1.31	49516537	14551536	21385.25
Cariophylene β	27.26	2.96	112324437	30744782	45227.27
Cariophylene α	29.02	0.20	7617124	2087524	3067.87
Germacrene	30.46	0.98	37031232	10277782	15104.45
Hexanoyl furan	30.85	0.29	10854233	3093804	4546.72
Isoledene	31.09	0.10	3754014	954715	1403.07
Hexanoyl furan	32.09	90.87	3444728173	783466079	1151398.34
Trimethylpropylsilane	35.16	0.30	11333517	3176587	4668.38
Cariophyleneoxid	35.69	0.20	7714819	2105306	3094.00
Cariophyleneoxid	35.88	0.97	36632271	9869422	14504.31
Nerolidol	37.07	0.16	5923843	1077019	1582.81
Spathulenol	38.64	0.10	3968968	980909	1441.56
Cadinol α	40.64	0.18	6673555	1797402	2641.50
Phytol	47.18	0.13	4913071	903840	1328.30

The flowers and seeds of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* were studied for the chemical content in: volatile oil, total polyphenols, flavones, antioxidants, pectin, amino acids, lipids and calcination residue.
Based on the chemical properties, these active compounds in Perilla could be classified either as hydrophilic (phenolic compounds, flavonoids, anthocyanin) or hydrophobic (lipophilic) ones (volatile compounds, triterpenes, phytosterols, fatty acids, tocopherols and policosanols).
Perilla is an important oleaginous plant; the seeds contain about 45% oil and most are loaded with unsaturated fatty acids. The laboratory analysis showed that the lipid content in seeds ranged from 25.36% to 33.79% (*P. frutescens* var. *frutescens*).

Phenolic compounds are frequently occurring in Perilla plant. They have a wide structural variability with a broad range of pharmacological activities.
Some of these products have been studied and proven to be an efficient source of phenolic antioxidants. A recent study (Gai F. et al., 2017) showed that a greater accumulation of phenolic component occurs at the complete flowering stage.In the present work, the content in total polyphenols varied quite from 3.94%, in *P. frutescens* var. *frutescens* to 6.26% in *P. frutescens* var. *crispa.*
The total flavone content also registered quite large differences between the studied genotypes, thus, *P. frutescens* var. *frutescens* had a value of 0.49%, and in *P. frutescens* var. *crispa* content in flavone was 1.19%. Recent studies show that flavonoids from *P. frutescens* have a strong antioxidant activity and have pharmacological properties (Peticilă et al., 2019)
In Table 3 are presented the results of chemical analyses of flowers and seeds of studied Perilla varieties.

Table 3. Chemical compositions of flowers and seeds of Perilla sp.

Compound	***Perilla frutescens* var. *crispa***	***Perilla frutescens* var. *frutescens***
Volatil oil (mL/kg)	0.4	0.4
Total polyphenols (%)	6.26	3.94
Total flavones (%)	1.19	0.49
Antioxidants (%)	12.01	8.76
Pectin (%)	7.68	11.29
Amino acids (%)	0.51	0.41
Total lipids in seeds (%)	25.36	33.79
Residue calcination	7.0	7.5

Antioxidant content recorded the highest value on *P. frutescens* var. *crispa*, with a content of 12.01%, and *P. frutescens* var. *frutescens* had a value of 8.76%.
In terms of pectin content, the highest value was recorded by *P. frutescens* var. *frutescens* and had a value of 11.29%, and to *P. frutescens* var. *crispa* was 7.68%.
The content in volatile oils had the same value of 0.4% for both varieties studied.

CONCLUSIONS

The seeds and seedlings offered promotionally at Vegetable Research Development Station Buzau, Romania attest that the Perilla sp. can be grown throughout the territory of our country both in greenhouse conditions and also in the field. This species may be soon become a niche crop, expanding throughout the country.
The acclimatized and genetically stabilized genotypes will be forward to ISTIS Bucharest for approval and patenting.

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COMPARATIVE EVALUATION OF THE ECONOMIC EFFICIENCY BY THE APPLICATION OF BIOPRODUCTS FOR FERTILIZING WITH BIOLOGICAL GROWING OF MIDDLE EARLY TOMATOES

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Abstract

The experimental work was carried out during the period 2014-2016 at the experimental field of Technical University of Varna. There have been tested biological products for fertilizing: Biosol, Biofa and Emosan with determinant tomatoes, variety Kopnezh F_1, being grown with two plans - single-row and two-row scheme. The purpose of the research is to be realized an evaluation of the economic efficiency by the fertilizing and way of growing of middle early tomatoes in the conditions of biological agriculture. The highest profitability and rate of profitableness average for the period of research is established with combined fertilizing with Emosan + Biofa, respectively 2,00 % for the one-row and 2,18 % for the two-row scheme. There have been established higher values of the total incomes with the two-row scheme of growing, which is due to the higher realized yield. The total income with variety Kopnezh F1 average for the period is from 8 226.30 BGN/da for the control up to 10 724,70 BGN/da for the combination of Emosan + Biofa with the two-row scheme of growing. The high values of the economic indexes, in combination with the high average yield, determine the feeding up with Emosan, put independently or combined with Biofa with both schemes of growing as economically justified and appropriate for biological production of middle early tomatoes.

Key words*: bioproducts, economic evaluation, growing, organic production, scheme, tomato, yield.*

INTRODUCTION

During the last several years the biological production is one of the sectors, which develops with accelerated rates. The development of the bio-sector emphasizes not only on the production of safe foodstuffs, but also provides opportunity for sustainable income of the population, combining the traditions, innovations and environment protection (Dimitrakiev et al., 2016). There is a social effect in the same time as additional incomes of the population are created and new job positions are opened in comparison to the traditional production (Nikolova, 2013; MZH, 2016).

The pursuit of a high economic effect of the put into fertilizers contradicts to the requirement for environmental claims of the agricultural activity. One of the ways for combining the economic efficiency with the environmental claims is the introduction of alternative systems for fertilizing with organic origin, as well as the usage of biological methods for fight with the pests and diseases. Authors prove that the manure, BactoFil B and Lumbrical are an alternative with the biological production of broccoli, but the mineral fertilizers have higher economic effect (Dintcheva, 2013; Borisov & Dintcheva, 2014).

During the last years in countries with developed bio-agriculture the emphasis is put on perfection of the models for fertilizing, based on a more efficient usage of the nutrients and decrease of the quantities put in the soil (Nakano et al., 2003; Pascale et al., 2004; Kolota & Osinska, 2000; Plamenov and Naskova, 2017). Alternatives are sought; ecologically sound solutions for maintaining the nutrient regime, which conform to some of the basic requirements of the vegetable farming - biological control of the soil fertility and realization of an economic effect (Martins, 2010; Tringovska, 2012; Márquez-Hernández, 2013; Naskova, 2017). The vegetable farming is a specific branch where there is a clearly expressed seasonality in the generating of incomes during the farm year (Borisov et al., 2014). This determines lower turnover of the capital, which is invested in this type of

business, higher instability in the financial condition of the farm, as well as the difficult realization of new technological solutions, bringing additional risk for the farmer (Koprivlenski and Dirimanova, 2010). All this determines the Bulgarian farmer's strong reserve towards the biological production and the difficult acceptance of the decision for conversion of the traditionally underlying technology of production in his farm. Having equal other conditions, one of the important motivators for the farmers to realize a transition to the biological production is the economic effect of it (Nikolov et al., 2013). There are researches, which prove that the biological production in Bulgaria has a perspective for development (Mitova, 2010; Mitova, 2011), provided that the farm owners are convinced that it gives them competitive advantage.

For the purpose of organic farming, allelopathy can be an important element in balancing the relationship between density and weeds, pests, diseases and cultivars. Valcheva et al. (2019) establish that the applied concentrations of parsley, carrot, dill and onion extracts had stimulating, inhibiting or indigenous effect on tomato seed germination, growth and accumulation of dry biomass. Positive allelopathic influence have been observed on the height, the number of leaves and the root length of pepper plants for the extracts of roots by marigold and basil (Vlahova, 2014; Vlahova & Yoveva, 2014).

The research purpose is to be analyzed the economic effect of the application of biological products for fertilizing with moderately early tomato production, grown with different planting schemes.

MATERIALS AND METHODS

The experimental work is developed during the period 2014-2016 on carbonate black earth, in the experimental field of department "Plant-growing" at Technical University - city of Varna. One Bulgarian variety determinant tomatoes has been tested with three bio products for fertilizing with two growing schemes in the conditions of biological production. The following bio products for fertilizing have been tested within the research: Biosol, Emosan and Biofa with determinant tomatoes, variety Kopnezh F_1 with two growing schemes: 160/25 cm (single-row band) and 120+40/35 cm (two-row band) at the conditions of the biological production.

The following variants have been included in the research with purpose establishing the effect of the bio fertilizers brought in with the tomatoes:

1. Control - unfertilized;
2. Biosol - 100 kg/da - put in the soil single time, before planting;
3. Biosol - 100 kg/da (soil) + Biofa - (0.3-0.5%) - applied to the leaves: phenophases mass flowering and beginning of fruit formation;
4. Emosan - 20 L/da, put in the soil locally, two times: 15 L/da after planting and 5 L/da in phase mass flowering;
5. Emosan - 20 L/da (soil) + Biofa (0.3-0.5%) - applied to the leaves: phenophases mass flowering and beginning of fruit formation.

The economic evaluation is determined by the means of the indexes (Bogoev et al., 2002): Average realization price - BGN/kg; Prime cost - the proportion between the spent production expenses and the realized crop - BGN/t; Total income - BGN/da; Total expenses - BGN/da; Total income - BGN/da; Profit - BGN/da; Rate of profitability - the proportion of the total income towards the material expenses, %; Rate of profitableness - the proportion of the profit towards the total expenses, %.

RESULTS AND DISCUSSIONS

One of the main questions with inclusion of the biological fertilizing in the technology for moderately early field production of tomatoes is what is its efficiency and which variants of fertilizing are economically profitable, paying and remunerative.

The values of the index total income are directly bound with the quantity of the total crop and the average realization price.

Average for the period 2014-2016 with variety Kopnezh F_1 the realized total income is 8001.60 BGN/da for the control up to 10040.90 BGN/da for the combination of Emosan + Biofa with the single-row scheme of growing (Table 1). The formation of the total income follows the peculiarities of formation of the total income. The control with the two schemes

of growing has the lowest total income for the period of the research - 6261.74 BGN/ da (single-row scheme) and 6486.44 BGN/da (two-row scheme). The highest total income have variants, with which the total income is also the highest. With variety Kopnezh F_1 the highest total income is realized with the variant Emosan + Biofa 7939.54 BGN/da, followed by Emosan 7777.74 BGN/da with the single-row scheme of growing. Higher total income is realized with combined application of the bio products Emosan + Biofa (8623.34 BGN/da), with the two-row scheme, followed by the variant with the independent application of Emosan - 8370.44 BGN/da. Higher values of the total incomes have been determined with the two-row scheme of growing, which is due to the higher realized crop.

The total income average for the period of the experimental work is from 8226.30 BGN/da for the control up to 10724.70 BGN/da for the combination of Emosan + Biofa.

Table 1. Economic results from the fertilization of tomatoes

Variant Yeild, kg/da		Average price, BGN/kg	Total earning, BGN/da	Total expenses BGN/da	Total income, BGN/da
Single-row scheme of growing					
Control	2667.20	3.00	8001.60	1739.86	6261.74
Biosol	3038.50	3.00	9115.20	2163.86	6951.34
Biosol + Biofa	3177.50	3.00	9532.50	2343.86	7188.64
Emosan	3240.50	3.00	9721.60	1943.86	7777.74
Emosan + Biofa	3346.50	3.00	10040.90	2101.36	7939.54
Two-row scheme of growing					
Control	2742.10	3.00	8226.30	1739.86	6486.44
Biosol	3206.40	3.00	9619.20	2163.86	7455.34
Biosol + Biofa	3364.80	3.00	10094.40	2343.86	7750.54
Emosan	3438.10	3.00	10314.30	1943.86	8370.44
Emosan + Biofa	3574.90	3.00	10724.70	2101.36	8623.34

The total income average for the period of the experimental work is from 8226.30 BGN/da for the control up to 10724.70 BGN/da for the combination of Emosan + Biofa.

The impact of the average realization price is less, which is permanent for the separate variants. The spent material expenses are of big significance with the formation of the total income. Determining for them are the prices of the fertilizers, which are used with the tomato growing, which are between 30 and 50%, followed by the labour expenses with the plants growing.

The prime cost of the produce depends on the resources used in its production and the spent total expenses. It is lowest with the variants Emosan with single-row scheme and Emosan+Biofa with the two-row scheme (Figure 1). The changes in the prime cost of the produce influence the modification of the profit, which is manifested by the modification of the total income and the total expenses.

The profit is a summary index, determining the economic benefit, depending on the way of fertilizing, type and dose of the used fertilizers. Unidirectional tendencies in the profit formation are determined average for the period 2014-2016. The highest profit with variety Kopnezh F_1 is realized with combined fertilizing of Emosan + Biofa (3969.77 BGN/da), as the increase in comparison with the control is with 26.8%. High profit is also realized with the variant Emosan (3888.87 BGN/da) with single-row bed.

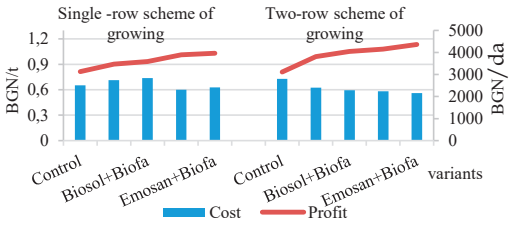

Figure 1. Profit and cost in cultivation of determinant tomatoes

The rate of profitability and the rate of profitableness are indexes, which describe more completely the economic advisability of organization, bringing out and realization of a certain production. The rate of profitability and the rate of profitableness are indexes, which describe more completely the economic advisability of organization, bringing out and realization of a certain production.

Average for the period 2014-2016 all tested variants have high rate of profitability, which shows that the growing of tomatoes as per technology for moderately early field production and the usage of biological fertilizers is economically justified (Figure 2).

The lowest rate of profitability has the control, and the highest has the variant with the

combined soil and leaf fertilizing with Emosan + Biofa. The rate of profitableness is also the highest with the same variant. The quantity of both indexes is determined by the realization of a bigger profit, on the grounds of achieved bigger increase of the total crops. The rate of profitability with the variants of independent fertilizing with Biosol and Emosan is commensurable with the one of variants with soil and leaf fertilizing.

The tendency for high profitability and profitableness with the two-row growing scheme is confirmed. The results are unidirectionalwith the obtained for the single-row scheme.

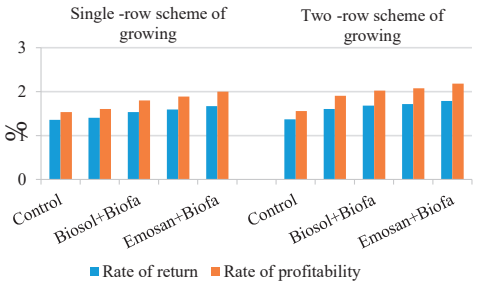

Figure 2. The rate of return and profitability in cultivation of determinant tomatoes

CONCLUSIONS

The biggest economic efficiency of biological tomato production is determined with the combined application of Emosan and Biofa, notwithstanding the growing scheme.

The values of the economic indexes in combination with the obtained crop, determine the fertilizing with the bio product Emosan, brought in independently or combined with Biofa, as economically justified and appropriate for biological production of moderately early tomatoes.

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STUDIES AND RESEARCH ON THE SPECIES AND VARIETIES OF HOSTA IN CULTIVATION

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Abstract

Throughout the time there have been numerous researches on the particularities of growth, flowering, hybridization, fertilization of species, varieties and cultivars of Hosta. The present article presents a brief summary of some research conducted in recent years on Hosta. The purpose of these studies is to evaluate the behavior of Hosta as perennial plants, to identify the different colours of the flowers due to the presence of anthocyanins in the tepals, the presence of volatile oils in flowers, the presence of macro and micro minerals in the leaves, to investigate the possibility of storing the seeds of Hosta, to establish the flowering period, the nectar production and flower pollination. The current review addresses the following issues: Growth, flowering and leaf character variation of Hosta; Anthocyanins of the genus of Hosta and their impacts on tepal colors; Analysis of the variation in scent components of Hosta flower by HS-SPME and GC-MS; Nectar and polen production and insect visitation on ornamentals from the genus Hosta Tratt. (Asparagaceae); Analysis of essential macro-micro mineral content of twelve Hosta taxa. Seed storage longevity of Hosta sieboldiana (Asparagaceae).

Key words: *anthocyanins, nectar, pollen, scent, tepal.*

INTRODUCTION

Hosta is considered one of the most important and most popular perennial flowers, used especially as a decorative plant for its beautiful leaves. Hostas are mainly grown for their beautiful foliage that comes with a broad range of leaf shapes, colors, sizes and textures (Mehraj & Shimasak, 2017). The multitude of varieties, hybrids and cultivars makes Hosta a permanent source of new studies for those who love these species and for researchers. The technology of Hosta plant cultivation is relatively easy, as the plant has no particular demands on environmental factors. More experiments are needed to discover new aspects of Hosta plant culture, which presents us with such a rich assortment of varieties and cultivars.

Hosta is a plant originating from Japan, China and Korea and it was first introduced in Europe in the late 1700's and in the US in the mid-1800's (Greenfell & Shadrack, 2004). The number of currently known species is about 43, and of the cultivars over 2500 (Șelaru, 2007). The names used in Romanian are "autumn lily" or "August lily", in French "Funkia" or "Hosta", in English "Funkia", "Corfu lily" or "day lily" (Băla, 2007). The scientific name of Hosta is also used as a popular name. The most widespread species are: *Hosta plantaginea* Aschers. (syn. *Funkya subcordata* Spreng.), *Hosta sieboldiana* Engl. (syn. *Hosta glauca* Stearn.), *Hosta fortunei* (Hort.) Engl., *Hosta undulata* (Hort.), *Hosta lancifolia* Engl. (syn. *Funkia japonica* Voss.), *Hosta albomarginata* Hook. (syn. *Hosta sieboldii* Aschers) (Toma, 2009). In Japan and China, new species and varieties are still undiscovered.

Hosta plants have a wide spread in parks and gardens, due to their preference for shade and semi-shade. This foliage plant can be grown for various purposes: for a position by itself, for edging, for borders, and for filling an entire flower bed (Noordhuis, 1995). *Hosta longipes* (Fr. et Sav.) *Matsumura* (*Liliaceae*), widely distributed throughout Korea, China, and Japan, is an edible vegetable in Korea. It has long been used as a traditional Korean medicine for treating cough, sputum, laryngopharyngitis, burns, swelling, snake bites and inflammation (Kim et al., 2014).

The plant grows in the form of leaf bushes with a height of about 60 cm and a diameter of 30

cm (Toma, 2009). It has rhizomes or stolons on the ground, lanceolate or ovate leaves (Toma, 2003). The colour of the leaves in the wild species is predominantly green, but there are species and cultivars with leaves having blue, yellow, gold and white colour. The leaves may have a single colour or may be variegated, with a white border or white streaks in the middle part of the leaf. The flowering takes place in the summertime, between June and July. The colour of the flowers is white, pink or lavender. The growth of the plant is relatively slow, especially for the dwarf varieties and for the varieties having foliage that is variegated or of a colour other than green (Șelaru, 2007).

Hosta is an unpretentious plant. It prefers loose, moist, humus-rich soils, sunny exposures, but it also grows in semi-shade and even shade (Băla, 2007).

In order to be adapted, all the wild species need the winter coldness, with temperatures below 4°C for several weeks in order to get an adequate rest (Greenfell, 2004).

Hosta species are vegetatively propagated by the division of the bush. The seed propagation is practiced more in the improvement work, for creating new varieties (Toma, 2009). The mother plants that undergo the division process must be at least four or five years from the last split. The optimum period for the division of the bush is autumn, in October, but the spring period (April) is not excluded, immediately after the plants start their vegetation (Toma, 2009). Sowing is less practiced. In vitro propagation is the most commonly used method for the production of planting material (Șelaru, 2007).

GROWTH, FLOWERING AND LEAF CHARACTER VARIATION OF HOSTA

The researches carried out by Mehraj & Shimasaki in 2017 use as a material and tracking method the following: 12 Hosta plant taxa, five plants from each one of those, studying their evolution of growth, flowering and variation of leaf characters during two years, 2015 and 2016. The 12 taxa are: *Hosta sieboldiana, Hosta alismifolia, Hosta sieboldii, Hosta longissima, Hosta tardiva, Hosta longipes* var. *gracillima, Hosta nakaiana, Hosta kikutii* var. *caput-avis, Hosta kikutii* var. *polyneuron, Hosta longipes* var. *expira, Hosta kiyosumiensis, Hosta montana,* marked as T1-T12 (Figure 1). The rhizomes were planted in pots and the culture substrate was enriched with 320 mg/L N; 210 mg/L P and 300 mg/L K, slightly acidic fertilizer. The observations made referred to the height of the plants, the number of leaves, the surface of the leaves, the chlorophyll content, the length of peduncle, the length of the inflorescence and the number of flowers in inflorescence. Chlorophyll was measured with the SPAD meter. Significant differences were determined using Tukey's HSD test ($P < 0.05$).

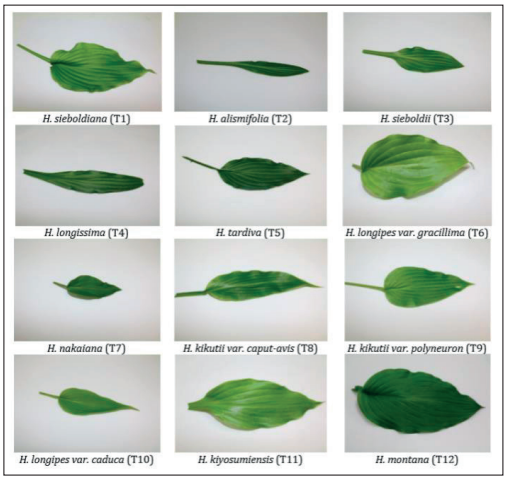

Figure 1. Pictorial presentation of leaves of the *Hosta taxa* (after Mehraj & Shimasaki)

After these researches carried out in 2015 and 2016, Mehraj and Shimasak obtained the following result and discussions: the highest plant was *H. montana* (year 2015: 73.17 cm and year 2016: 71.72 cm), and the smallest plant was *H. alismifolia* (year 2015: 22.93 cm and year 2016: 20.46 cm). The height of the plants decreased in the second year.

The maximum number of leaves was at *H. kikutii* var. *caput-avis* (13.8/plant in 2015 and 12.8/plant in 2016), and the minimum number of leaves was recorded in *H. longipes* var. *gracillima* (3.78/plant in 2015 and 3.02/plant in 2016). The number of leaves decreased in the second year was higher in *H. longipes* var. *gracillima* (20.11%), *H. sieboldiana* (19.92%) and *H. montana* (16.95%).

The highest leaf area was recorded in *H. montana*, followed by *H. sieboldiana*, *H longipes* var. *gracillima* and *H. kiyosumiensis*, and the smallest leaf surface in *H. alismifolia*. The percentage reduction of leaf area in the two years was considered insignificant.

H. tardiva recorded the highest chlorophyll content (75.44% in 2015 and 74.14% in 2016) and the minimum chlorophyll content in the leaves was found in *H. longipes* var. *gracillima* both in 2015 (34.89%) and in 2016 (33.02%). The percentage reduction in chlorophyll content was considered insignificant.

H. montana had the longest petiole (81.10 cm in 2015 and 79.70 cm in 2016). The minimum length of the peduncle was found in *H. longissima* (71.90 cm and 70.30 cm in 2015, respectively 2016). The percentage reduction of the peduncle length was very small.

The maximum length of the inflorescence was found in *H. tardiva* and the minimum length in *H. kiyosumiensis,* both years. The percentage reduction was considerable in all taxa.

The highest number of inflorescences was in *H. sieboldiana*, and the least was found in *H. kiyosumiensis*. The percentage of inflorescence reduction was high.

The authors of the research also commented on the number of leaf nerves and the shape of the leaves.

ANTHOCYANINS OF THE GENUS OF HOSTA AND THEIR IMPACTS ON TEPAL COLORS

The researches made in 2012 by Liu et al., use the following as a material and method of tracking: the biological material was composed of 86 variants (six species and 80 cultivars). The flowers were harvested in the open flower stage in 2010 and then stored at - 40°C. The composition of anthocyanins was determined by high performance liquid chromatography coupled to diode array detection (HPLC-DAD) and high performance liquid chromatographyc with elecro spray ionization and mass spectrometry (HPLC-ESI-MS).

To measure the colour of the tepals, a colorimeter (NF333 spectrophotometer) was used. Anthocyanins were quantitatively determined by HPLC-DAD using a Dionex HPLC system (Dionex Corp., Sunnyvale, CA, USA) equipped with a P680 pump, an UltiMate 3000 autosampler, a TCC100 thermostated column compartment and a Dionex PDA-100 detector.

Following these researches conducted between 2010-2012, Liu et al. obtained the following results and discussions: initially, the researchers identified 9 anthocyanins in a purple cultivar, *Hosta nakaimo,* and in a species of white flowers, *Hosta montana*. Nina Liua et al. found the following anthocyanins: delphinidin 3,5-O-diglucoside (Dp3G5G), cyanidin 3,5-O-diglucoside (Cy3G5G), petunidin 3,5-O-diglucoside (Pt3G5G), peonidin 3-O-rutinoside-5-O-glucoside (Pn3Ru5G), malvidin 3-O-rutinoside-5-0-glucoside (Mv3Ru5G) malvidin 3,5-O-diglucoside (Mv3G5G), petunidin 3-O-rutinoside (Pt3Ru), peonidin 3-O-rutinoside (Pn3Ru), malvidin 3-O-rutinoside (Mv3Ru).

The violet variants had a higher level of anthocyanins and correlated with the pigments Mv3Ru5G, Mv3G5G, Pt3G5G and Dp3G5G, and the variants with white flowers had a lower level of anthocyanins and correlated with the pigments Pt3Ru, Pn3Ru, and M Pn3Ru5G. Mv3G5G and Mv3Ru5G dominated the purple flowers.

Nina Liu et al., find that anthocyanin contents were different among the selected 86 hostas. There were discovered nine anthocyanins in *Hosta* "Grand Master", a cultivar with purple flowers, three anthocyanins in the species with white flowers *Hosta montana* and three anthocyanins in *H. montana*. They identified four anthocyanins (delfinidin, cyanidin, petunidin and malvidin) of 3,5-O-diglucoside, 2 anthocyanins (peonidin and malvidin) of 3-O-rutinoside-5-O-glucoside and 3 anthocyanins (petunidin, peonidin and malvidin) of 3-O-rutinozide.

ANALYSIS OF THE VARIATION IN SCENT COMPONENTS OF HOSTA FLOWER BY HS-SPME AND GC-MS

During this research made in 2014 by Liu et al., the following material and method were used: as a plant material, they used six species and 40 Hosta cultivars and determined the composition and content of volatile floral compounds by

headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS). Volatile compounds in different stages of development and different floral organs were analyzed. HS-SPME analysis was performed with SPME fiber 85 carboxen polydimethylsiloxane (CAR-PDMS), equipped with a manual SPME support.
After this research, Liu et al., obtained the following result and discussions: they identified over 70 volatile compounds. The predominant components were terpenoids, mainly myrcene, limonene and linalool. Linalool has emerged from these researches as being the component with character impact of Hosta flowers. Among these volatiles, 48 fragrance compounds, of which 2-ethenyl-1,1-dimethyl-3-methylenecyclohexane and 1,3,5,5-tetramethyl-1,3-cyclohexadiene were identified and have not been previously reported.
Among the fragrant varieties there were slight differences in perfume detected by the human sensory evaluation. In *Hosta plantaginea* and *Hosta* "Summer Fragrance" predominated linalool. *Hosta* "Royal Standard", *Hosta* "Tortilla Chip" and *Hosta* "So Sweet" had high levels of myrcene, lemonene and linalool, and in *Hosta* "Diana Remembered" and *Hosta* "Moonlight Sonata", ocimene and linalool.

NECTAR AND POLEN PRODUCTION AND INSECT VISITATION ON ORNAMENTALS FROM THE GENUS HOSTA TRATT. (ASPARAGACEAE)

The researches made between 2012-2014 by Bożek et al. use as material the following Hosta species and cultivars: *H. sieboldiana* Engler, *H. capitata* Nakai, *H. crispula* Maekawa, *H. fluctuans* Maekawa, syn. *H. sieboldiana* var. *fluctuans* hort., *H. undulata* var. *univittata* Miquel (Hylander), syn. *H. univittata*.
The phenology of flowering, the production of pollen and nectar were studied, observing the biology of flowering, the nectar secretion, the pollen production, the shape and size of pollen grains (Figure 2) and the groups of visiting insects. The phenology of flowering was documented for two years, using the method described by Denisow, establishing the flowering time and the duration of the flowering period. The number of inflorescences on the flower stems and the number of flowers on the plant was determined.
The nectar production was determined over two years, by collecting it using the pipette method (Jabłoński, 2002). The nectar harvesting was repeated three, five times, during the blooming period, collecting four – six samples each time. The total sugar concentration was measured with an Abbe refractometer. The amount of sugars produced per 10 flowers (in mg) and per 10 m^2 (in g) was determined.
Pollen production was determined using the ether-ethanol method described in detail by Denisow (2011). The mass of pollen produced was calculated for 10 flowers (in mg) and for 10 m^2 (in g). Observations on the dimensions of the pollen grains were performed using a Nikon Eclipse 200 microscope.

Figure 2. Light micrographs of the pollen grains of *Hosta capitata* in a. equatorial view b. distal polar view and c. proximal polar view; insect visitors d. *Apis mellifera*, e. *Halictus* sp. (after Małgorzata Bożek)

Insects visits were recorded between 7:00 and 18:00, at intervals of two, three hours on random plots, for three consecutive days, during the full flowering phase of the species. During each observation census (three to six min), the total number of visiting insects was recorded.
After this researches, Bożek et al. obtained the following result and discussions: *Hosta sieboldiana* was the first to flower, followed by *H. fluctuans*, *H. capitata*, *H. crispula* and *H. undulata* var. *univittata*.

The flowering lasted the least time in *Hosta crispula* (16-20 days) and the longest time in *H. capitata* (25-36 days). In 2014, compared to 2012 and 2013, the flowering period was advanced by 10-14 days.

H. undulata var. *univittata* and *H. crispula* recorded the smallest number of flowers in inflorescences and *H. sieboldiana* the highest number.

Hosta flowers have been observed to attract insects. The pollen is released from anthers in the first hours of the flower life cycle. The highest amount of pollen was recorded in *H. capitata*, and the smallest in *H. undulata* var. *univittata*. The average pollen production per 10 m^2 was 0.24 g (*H. undulata* var. *univittata*) and 9.53 g (*H. capitata*). The pollen granules of the Hosta species were bilaterally symmetrical.

ANALYSIS OF ESSENTIAL MACRO-MICRO MINERAL CONTENT OF TWELVE *HOSTA TAXA*

During this research made in 2017, Mehraj et al. use as material and method the following: as biological material, the leaves of 12 taxa of Hosta namely *Hosta sieboldiana, H. alismifolia, H. sieboldii, H.longissima, H. tardiva, H. longipes* var. *gracillima, H. nakaiana, H. kikutii* var. *caput-avis, H. kikutii* var. *polyneuron, H. longipes* var. *caduca, H. kiyosumiensis* and *H. montana*. The rhizomes of the 12 *Hosta taxa* were planted in the field for 1 year, then they were transferred into pots, four plants for each cultivar/variety, with a total of 48 plants. The culture substrate was supplemented with 320 mg/L N, 210 mg/L P and 300 mg/L K, without using any additional fertilizer. Leaves were collected, washed with tap water, rinsed with distilled water, dried in an oven, powdered and placed for six hours in the desiccators to remove moisture.

Then the procedures described by Ikeda (1980) for sample preparation and acid dissolution were followed. The mineral content of the samples was determined using Induced Coupled Plasma Spectroscopy (Japan), Plasma spectroscopy equipped with an automatic module for macro minerals (K, P, Ca and Mg) and micro minerals (Fe, Zn, Mn and Cu).

After this researches made in 2017, Mehraj obtained the following result and discussions: K content was 4.05% in *H. alismifolia*, 3.87% in *H. montana* and 3.23% in *H. sieboldii*. The highest P content, 0.34%, was recorded in *H. nakaiana*, followed by *H. tarvida* with 0.29%, *H. montana* with 0.23% and *H. sieboldii* with 0.21% P. The Ca content, significantly higher was observed in *H. sieboldii* (1.15%), and the lowest in *H. montana* (0.17%). The highest Mg content was recorded on the leaves of *H. nakaiana* (794.12 ppm), followed by *H. alismifolia* (767.37 ppm), *H. montana* (606.68 ppm) and *H. sieboldii* (603.95 ppm).The highest content in Mn was observed in the leaves of *H. longissima* (133.77 ppm). Zn content was highest in the leaves of *H. nakaiana* (334.52 ppm), followed by *H. longissima* (322.08 ppm), *H. montana* (294.92 ppm) and *H. alismifolia* (284.78 ppm). In terms of Cu content, the leaves of *H. longissima* (5.95 ppm), *H. nakaiana* (5.62 ppm) and *H. montana* (5.52 ppm) were noted. Fe content was highlighted in the taxa of *H. sieboldii* (251.95 ppm) and *H. alismifolia* (206.41 ppm).

Leafy vegetables from spontaneous flora have a higher content in macro and micro minerals compared to growing vegetables (Pradeepkumar et al., 2013). *H. montana* and *H. sieboldii* are plants whose leaves are commonly consumed in Japan.

SEED STORAGE LONGEVITY OF *HOSTA SIEBOLDIANA* (ASPARAGACEAE)

During this research made in 2015, Kanazawa et al., used as material and method the following: mature and immature seeds by *H. sieboldiana* were harvested from two areas of Japan. The first area, where the seeds were ripe, was noted with YA, and the second area, with immature seeds, was noted AS. Immediately after the harvesting, the seeds were stored at temperatures of - 20°C and 5°C, at a humidity of about 65%. The seeds were also dried to about 10% or 5% of the moisture content (MC). The study was conducted for more -than four years and tracked the maturity and the effects of seed storage, the moisture value and the temperature during storage. The

storage was done in three variants: ~ 65% (the initial MC of seeds on collection), ~ 10% (dry) and ~ 5% (very dry). The seeds were divided into three groups: the first group was sealed in polyethylene bags immediately after collection and then stored at 5°C and - 20°C, with a content of 64% to 66% MC (moisture content). In the other two groups, the seeds were first dried for 9 to 18 days and then stored at both 5°C and - 20°C. During storage, germination tests were performed every three months. The moisture content of 180 seeds was evaluated gravimetrically, by drying the seeds for 16 hours at 105°C.

After this researches, Kanazawa et al., obtained the following result and discussions: the mature seeds of YA had an initial germination of 82%; the immature seeds from AS had an initial germination of only 18%. During storage, YA seed viability decreased significantly.

Seeds with a high MC of 65.7% died rapidly at both 5°C and - 20°C.

Seeds with 9.6% MC, stored at - 20°C, lost their viability much faster than seeds with 9.6% MC and 4.5% MC kept at 5°C.

The viability of the stored seeds with 7.7% humidity (MC) increased both at 5°C and at - 20°C and decreased faster than that of the stored seeds with 5% MC.

At the same humidity (MC 5% or 7.7%), the viability increased in equal percentages for the two temperatures, but decreased much faster at - 20°C than at 5°C.

Seeds of *H. sieboldiana* survived at the 5% moisture content and the temperature of - 20°C. For both mature and immature seeds, decreasing the moisture content increased the longevity of the seeds.

With a high moisture content (MC) of ~ 65%, the seeds of *H. sieboldiana* have lost all viability within five months.

The dried seeds of *H. sieboldiana* (~ 5% to ~ 10% MC) lost their viability faster at - 20°C than at 5°C.

During storage, immature seeds (AS) of *H. sieboldiana* continued their maturation. When maturation was completed, the germination rates decreased faster at higher MC and at lower temperatures.

CONCLUSIONS

As a result of their researches made in 2017, Mehraj and Shimasaki reached the following conclusions: over the course of two years, it is noted that the 12 varieties and cultivars of Hosta behaved well as perennial plants, even though some traits diminished in the second year, such as the number of leaves and the flowering of the plants.

Mehraj and Shimasaki's conclusion was that the division of the bushes at Hosta improves the plant's appearance, but repeated division leads to a decrease in the size of the leaves and of the flowers and it is recommended that the separation of the plants must be done only at the maturity of the plants.

As a result of their researches made in 2012, Liu et al., reached the following conclusions: the tepals of the Hosta genus present colour variations, but these are insignificant. There were differences between the purple flowers and the white flower variants. In terms of anthocyanin content, the highest values were obtained for *Hosta longipes* (purple species). Hosta varietes with purple flowers, *Hosta* "Kabitan", *Hosta* "Spritzer", *Hosta lancifolia, Hosta* "Grand master" and *Hosta* Antioch contain all 10 anthocyanins identified.

The results concured the hypothesis according to which the dominant positions of Mv3G5G and Mv3Ru5G in the synthesis of anthocyanidin glycosides were responsible for the purple color in the Hosta flowers.

As a result of their researches made in 2014, Liu et al. reached the following conclusions: the fragrant components were noticeable in the open flower stage, but decreased greatly after complete flowering. 39 of the 40 Hosta plants analyzed have no distinct perfume. Part of the 39 varieties emitted a light scent that can only be felt in the proximity of the flowers.

Regarding the presence of the perfume in different stages of the flowering and in different floral organs, the strong emission of the perfume took place in the open flower stage and decreased after flowering. Regarding the differences in perfume between tepals, stamens and pistil, there were not significant variations found.

As a result of their researches made in 2012-2014, Bożek et al. reached the following conclusions: the Hosta species taken into study continue to bloom continuously starting from the second decade of June until the first week of August. The flowers of the inflorescences had a longevity of 20-38 hours. The number of flowers in inflorescence did not change during the study years.
H. sieboldiana, H. fluctuans, H. crispula and *H. undulata* var. *univittata* were sought mostly by bumblebees, and the flowers of *H. capitata* were visited mostly by bees.
The phenotypic features of the flowers prevent access to the nectar and restrict the visit of insects. Although total sugar mass and pollen production are low in Hosta species, the flowers can complement summer pastures, especially for bumblebee.

As a result of their researches made in 2017, Hasan Mehraj et al. reached the following conclusions: -the leaves of *H. sieboldii* have a content of 1.15% Ca, higher than STFC-2015 (The Standard tables of food composition in Japan - 2015) and higher than the studies made in different countries for various wild edible plants. Hosta is asparagus-like; the young leaves, the petiole and the sprouts are edible. *H. sieboldii* and *H. alismifolia* are good sources of Fe, *H. nakaiana* and *H. longissima* of Zn. The results of Hasan Mehraj et al.'s research showed that Hosta leaves have a higher mineral content than asparagus and that they represent a very good source of minerals. *H. alismifolia, H. sieboldii, H. nakaiana, H. longissima, H. montana* may be recommended for their K, Ca, Fe, P, Mg and Zn content.

As a result of their researches made in 2017, Yumiko Kanazawa et al. reached the following conclusions: on a short term, the storage of *H. sieboldiana* seed may be feasible, but the cryogenic storage would be considered a more efficient method for the long-term storage of these seeds. The state of maturity had significant effects on the longevity of the seed storage. The longevity of the immature seeds was shorter than that of the mature seeds stored under the same storage conditions.

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A HISTORY OF ORCHIDS. A HISTORY OF DISCOVERY, LUST AND WEALTH

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Abstract

Orchidaceae is the second largest families of flowering plants. There are approximately 900 orchid genera comprising between 28,000-32,000 species of orchids. The relationship between orchids and mankind is complex. The history of orchids' discovery goes hand in hand with the history of humanity, encompassing discovery and adventure, witchcraft and magic, symbolism and occultism, addiction and sacrifice, lust and wealth. Historically, the Chinese were the first to cultivate orchids as medicinal plants, more than 4000 years ago. Gradually, records about orchids spread, reaching the Middle East and Europe. Around 300 B.C., Theophrastus named them for the first time orkhis. In 1737, Carl Linnaeus first used the word Orchidaceae to designate plants with similar features. The family name, Orchidaceae was fully established in 1789, by Antoine Laurent de Jussieu. In 1862, Charles Darwin published the first edition of his book, Fertilisation of Orchids. Darwin considered the adaptations of orchid flowers to their animal pollinators as being among the best examples of his idea of evolution through natural selection. Orchidology was on its way. During the 18th and the 19th centuries, orchids generated the notorious Orchid Fever where orchid-hunters turned the search for orchids into a frantic and obsessive hunt. Ever since, orchids have conquered the world, becoming a much-desired, multibillion-dollar commodity.

Key words*: orchids, history, cultivation, medicinal, micropropagation.*

INTRODUCTION

EXOTIC, BIZARRE, MYSTERIOUS BLOSSOMS IN ROMANTIC FARAWAY PLACES. ORCHIDS

Since ancient times they have exerted a fascination and power of seduction which has increased over the centuries, sparking a range of emotions from wonder and curiosity to admiration and passion. Orchids or daydream flowers as they sometimes have been known, are a symbol of magnificence in the world of flowering plants. By the incredible luxuriousness of their forms, the splendour, variety and harmony of their colours and the diversity and refinement of the traps they display to attract pollinating insects, orchids can be singled out as having attained a peak of beauty and perfection unrivalled in the Plant Kingdom. The combination of their mysterious beauty, amazing colours, strange growth habits, intricate shapes and hypnotic perfumes has ensured a long-lasting obsession for orchids (Hawkins-Tillerson, 2007). The strange tuberous forms of the roots of the terrestrial species and the unusual ways of life and features of the exotic species have led various cultures and civilisations to award orchids sacred and magical virtues. The relationship between orchids and mankind is complex. The history of orchids encompasses discovery and adventure, witchcraft and magic, symbolism and occultism, addiction and sacrifice, lust and wealth. In their quest for world domination, after colonising all the six continents and almost every habitat on Earth, orchids decided to shadow the history of human kind throughout the millennia, from antiquity to modern times.

ANTIC CIVILISATIONS

For thousands of years human civilisations have regarded the art of healing as having been sent from the divine. The remedies of traditional medicine were passed on and practised by knowledgeable lay people and by trained practitioners such as wise women, shamans or priests. As soon as writing was

developed and culture had progressed sufficiently, numerous records of religious and magical writings accompanied by lists of herbs were inscribed onto thousands of clay tablets and rolls of papyrus, these documents becoming the first Herbals (Budge Wallis, 1927). In ancient Egypt, Mesopotamia, China, India and Europe, herbals were among the first literature produced, containing the medical wisdom of the day accumulated by herbalists, apothecaries and physicians. Herbals were collections of names, descriptions and illustration of plants that included their medicinal, culinary, toxic, hallucinatory, aromatic and magical properties as well as the legends associated with them. It would be foolish to deny the fact that in ancient times medicine and magic were almost inextricably linked, yet despite the use of magic, the compilers of the oldest herbals had a very real knowledge of primitive traditional medicine.

MESOPOTAMIAN CIVILISATION

Although human settlements had probably existed for millennia, traditional medicine developed between the Tigris and Euphrates rivers during the period of the great Mesopotamian civilisations, beginning with the Sumerian (c. 5200-2000 B.C.), followed by the Akkadian, Assyrian, Babylonian and Persian Empires (Retief & Cilliers, 2007). The first written mention of the use of orchids comes from two clay tablets of recipes found in the Royal Library of the King of Assyria, Ashurbanipal (668-626 B.C.), in the ancient city of Nineveh (Price, 2001). The recipes were elaborate and often called for rare ingredients. Various grains and vegetables were used, and there were lists of dates, apples, figs, pomegranates, grapes, apricots, mulberries and melons as well as 'saffron and orchid root, truffles and mushrooms [which] were harvested for the table' (Budge Wallis, 1927). The orchid roots are likely to have been species of the tuberous *Orchis* genera, as well some of the edible species of the *Disa, Habenaria* and *Eulophia* genera, all of which were widely spread across Northern Africa and Central Asia (Pant, 2013). These recipes reveal the fact that orchids were familiar to the ancient Sumerians, at least as food. Since we know that a drink flavoured with saffron has been made from the tubers of various kinds of *Orchis* and *Eulophia* orchids for over four millennia, it is quite possible that the Sumerians may have also used them for this purpose. Today this drink is known as Salep, the Middle Eastern beverage (Sezic, 2002). Also, in ancient Mesopotamia, young mothers used for the first time, various orchid species to stimulate lactation (Jacquet, 1994).

ANTIC CHINA

Historically speaking, the Chinese were the first to fully document and leave written certification of their use of orchids as medicinal plants, starting almost 4000 years ago (Yang L., 2008; Teoh, 2016). Their interest in orchids started with the legendary Shennong, an emperor and scholar who lived between 4000-2500 years ago and who is considered to be the father of Traditional Chinese Medicine (Yang S., 2005). The 'Shen Nong Ben Cao Jing' (The Divine Husbandman's Classic of Materia Medica) is a Chinese book on ancient medicinal plants attributed to the scholar, probably written around 2700 B.C. (Yang, 2005). This is considered to be the earliest Chinese Pharmacopoeia, and includes 365 medicines derived from minerals, plants and animals. Species such as *Bletilla striata* (pai-chi), *Dendrobium nobile* (shih-hu) and *Gastrodia elata* (chih-chien) were mentioned for the first time as being utilised in Chinese medicine (Bulpitt et al., 2007). The Chinese word for orchids, 'lan' or 'fragrant', has appeared in Chinese herbal medicine for centuries, although in many ancient writings, 'lan' was used as a general reference to all fragrant plants rather than an exclusive reference to orchids (Bulpitt, 2005). Orchids were first collected from the wild and cultivated in the private gardens of nobles during the Wei (220-265 C.E.) and Chin Dynasties (265-317 A.D.). In Chinese manuscripts dated 290-370 C.E., there are some direct referrals to orchids, the most common species being *Cymbidium ensifolium, Dendrobium moniliforme* and *Vanda tessellata*. Wang Kuei Hsueh wrote 'Wang Shi Lan Pu' (Wang's Treatise on Orchids) in 1247, and described the cultivation of 37 new species including some *Cymbidium* species (Hew, 2001). Although orchid cultivation was

common during the Song Dynasty, it became very popular during the Ming (1368-1644 C.E.) and Ching (1644-1911 C.E.) Dynasties. In the royal palaces and parks, the Chinese gardeners used to cultivate *Cymbidium*, *Vanda* and *Aerides* species suspended in baskets. During his travels in China in 1613, a Jesuit missionary, Álvaro de Semedo (c. 1585-1658), saw these hanging orchids and referred to them as 'diao hua' or 'hanging plants', saying that they possessed the peculiar property of 'growing suspended in the air' (Arditti, 1992).

INDIA'S ANCIENT AYURVEDIC MEDICINE

Along with China, India has had a long tradition in herbal medicine dating back several thousand years, having its roots in magical and occult practices. This led to a system of health care known as Ayurvedic medicine, which comes from the Sanskrit word Ayurveda meaning the 'knowledge of life'. The oldest known Ayurvedic texts are the Suśrutha Saṃhitā and the Charaka Saṃhitā, which contain 700 descriptions of medicinal plants and their usage. They were appendices of the Hindu texts the Rig Veda and the Atharva Veda, where several orchids, *Dendrobium, Eulophia*, *Habenaria, Orchis* and *Vanda,* were mentioned to be used as medicinal plants (Panda & Mandal, 2014).

ANTIC GREECE

In Greece, the early compilers of herbals based much of their knowledge on the translations of the ancient Egyptian and Mesopotamian medical and medicinal writings (Jacquet, 1994; Bulpitt, 2005). The Greek physician Hippocrates (460-377 B.C.) is often called the Father of Medicine. He infrequently used herbal remedies, preferring to treat with diet, physical therapy and rest, he did use species of Mediterranean orchids in some of his cures, which have been identified as being from the *Orchis* genus. Written references to Mediterranean orchids were also made by the Greek philosopher Theophrastus (371-287 B.C.). Two of his works, 'Historia Plantarum' (Enquiry Into Plants) and 'Causis Plantarum' (On the Causes of Plants), are in existence today, having been translated into Latin in the middle of the 15th century. In the 9th book, which dealt specifically with medicinal herbs and their uses, Theophrastus described a plant with two little ovoid tubers, which he referred to as *orkhis*, the ancient Greek word for the mammalian testicle (Bulpitt, 2005). He used this word to denominate some of the terrestrial Mediterranean orchid species that had similar root characteristics. The name of the family *Orchidaceae* has origins in the same word, and to this day, the species Theophrastus described are known by the same genus name, such as *Orchis maculata, Orchis mascula* and *Orchis simia*. Pedanius Dioscorides (40-90 C.E.), a Greek physician, pharmacologist and surgeon with the Roman army, compiled the information about the many useful plants he had found on his travels into 'De Materia Medica' (On Medical Materials), a 5-volume encyclopaedia, written between 60-70 C.E. The book contained approximately 600 species of medicinal plants, two of which were species of orchids, *Orchis mascula* and *Orchis militaris*. Interestingly, the text also recorded the Dacian and Thracian names for about 60 species of medicinal plants. Dacians were the ancient inhabitants of the present-day countries of Romania and Moldova, which are located in and around the Carpathian Mountains and to the west of the Black Sea.

In 77 C.E., Gaius Plinius Secundus or Pliny the Elder (23-79 C.E.), the Roman naturalist and philosopher, described numerous species of orchids and indicated their medicinal qualities in his treatise 'Naturalis Historia' (Natural History), a synthesis of the information contained in about 2000 scrolls and it includes myths and folklore, written between 77-79 C.E.

ARABIC TRADITIONAL MEDICINE

Arabic traditional medicine originally developed from a combination of the magic-based medicine of the Bedouins with the medical traditions of the major ancient civilisations. One of the most famous 11th Century Arabic medical treatises was 'The Canon of Medicine' (1025 C.E.) by Persian philosopher and medic Ibn Sīnā (980-1037 C.E.). Ibn Sīnā was most commonly known in the West by his Latinised name, Avicenna. The book described the use of several exotic orchids in herbal cures. One of the most widely used beverages of the Middle East around that time

was Sahlab or 'sahlep', the name of which derived from 'th-thaeleb' or 'hasyu al-tha'lab', which in Arabic means 'fox testicle'. This ancient, highly nutritious and healing drink, also mentioned in the Persian medicinal works, was made from the tubers of various *Orchis* species, particularly from those of *Orchis mascula* and *Orchis militaris* that grew in abundance across the Middle East and Asia (Subedi et al., 2013; Bulpitt, 2005).

THE GOTHIC ERA AND THE MIDDLE AGES – SUPERSTITIONS AND WITCHCRAFT

Astrology, astronomy, medical knowledge, alchemy and magic from the ancient philosophers of the great scholarly cities of the Middle East, Greece and Rome were circulated throughout society. The Swiss-German physician, astrologer and philosopher Philippus Aureolus Theophrastus Von Hohenheim, later known as Paracelsus (1493-1541), developed the 'Doctrine of Signatures' further in his treatise 'Liber de Imaginibus' (Eternal Imagination), by describing the properties of all animals, plants and minerals and assuming associations between their shapes, colours, forms and curative properties. Perhaps due to this long-standing belief that the larger tubers of orchids had stimulatory, generative and curative benefits for the male genitalia, some of the philosophers of the Middle Ages imagined that these plants grew from the drops of semen which fell to earth in places where animals or birds came together to breed. Medieval medicine and healing practices were reverting to their roots in pagan rituals and traditional herbal folk medicine. Witches were thought to use orchid tubers in their philtres or love potions, the fresh, firmer tuber being given to promote true love, and the withered one to reduce passion, perpetuating the belief in the aphrodisiac properties of orchids. They added magical elements to the prescriptions for the treatment of diseases, much of the knowledge about plants being usually recorded in legends. In Western Europe the first witch hunts, in which large numbers of people were tried and convicted of witchcraft, were held in France and Germany in the 15th century. In the 16th century witch mania spread to England and Scotland. Eventually the more educated people gradually stopped believing in witches and during the 18th century it finally became fashionable to regard witchcraft as a superstition.

RENAISSANCE - THE 'EARLY MODERN' IDEAS

During the 16th century, new publications dealing with orchids became increasingly common in Europe. In 1539, Hieronymus Bock (1498-1554), a German botanist and Lutheran minister, firmly reiterated the belief in the spontaneous generation of orchids from animal semen in 'Das Kräuterbuch' (Book of Herbs). In 1542, the German physician and botanist Leonhart Fuchs (1501-1566), mentioned over 400 plants in 'De Historia Stirpium Commentarii Insignes' (Notable Commentaries on the History of Plants), and also described various *Dactylorhiza* species.

William Turner (1508-1568), the father of English botany, was an early English herbalist who studied medicine in Italy. His three-part 'A New Herball' (1551) described over 200 species native to England, among which are mentioned species of the *Orchis* genera and their uses in the treatment of alcoholic gastritis. In 1597, the English naturalist John Gerard (1545-1612) published the 'General Historie of Plantes', which became known as 'Gerard's Herbal'. Gerard mentioned several types of *Orchis*, calling them *Satyrion feminina* or 'Fox-Stones'. His approach had an extensive impact on medicine in early North American colonies, the early English settlers taking the 'Herbal' to North America. Many species that Gerard had described were also introduced into the New World from England, including the Lady's Slipper Orchid *Cypripedium calceolus* (Cribb, 2014; Higgins & Alrich, 2016). In 1640, the English botanist John Parkinson (1567-1650) published 'Theatricum Botanicum' (Theater of Plants - An Herball of Large Extent), which was a compendium of about 3800 plants and the largest herbal ever produced in the English language. In this he described a wide range of orchids, putting them into several different classes and separating those with oval tubers from those with palmate tubers. He also noted that orchids produce dust-like seed after the flowers fade and are therefore unlikely to be spontaneously generated from the semen of

animals or birds. In the 17th century, the English botanist, herbalist, physician and astrologer Nicholas Culpeper (1616-1654) took the 'Doctrine of Signatures' theory as common knowledge in his book the 'Complete Herbal' (1652-1653), and maintained a common belief in the magical and medicinal powers of orchids. He wrote that the many *Orchis* species could be separated into two main groups, those with two round tubers and those with a hand-shaped or palmate tuber - such as the *Dactylorhiza* species, perpetuating the idea of the 'lustful power' of orchids.

THE FIRST CONTACTS WITH THE NEW WORLD

After the first contact with the Bahamas by Christopher Columbus (1451-1506) in 1492, the numerous successive expeditions taken to what later became known as the New World focused the interest and actions of the Europeans towards the colonisation and exploitation of this new continent. 'The Libellus de Medicinalibus Indorum Herbis' (Little Book of the Medicinal Herbs of the Indians), was written in Mexico, in 1552, and described the medicinal properties of 250 medicinal herbs used by the Aztecs (Safford, 1912). It was translated from Aztec Nahuatl into Latin by the Aztec Indian Juan Badianus (1484-1552), who offered the translation as a present to Charles I the King of Spain (1500-1558). This became known as the 'Badianus Manuscript'. One of the most well-known orchids, *Vanilla planifolia*, was described in great detail and its nutritional properties indicated. During the 1600s Europeans also began to be introduced to the flora of distant new lands discovered in Africa, the Far East and Australasia. In 1688 the very first specimen of the distinctive red *Disa uniflora* was brought from South Africa. John Ray (1627-1705) described this flower as 'the loveliest orchid from Africa' in his work 'Plantarum History' (History of Plants). The earliest Western books specifically on orchids did not appear until the 'Herbarium Amboinense' was written by the blind Georg Eberhard Rumphius (1627-1702). 'Herbarium Amboinense', a catalogue of the plants of the island of Amboina in modern-day Indonesia, was published posthumously in 1741, 39 years after his death, after a series of disasters (Veldkamp, 2011; Kull, 2002).

CARL LINNAEUS AND THE BINOMIAL NOMENCLATURE

The avalanche of new species of plants coming into Europe without the assistance of a unique classification and notation system created a lot of confusion, with each botanist using a system different from the others. Towards the middle of the 18th century, the need for a unique system to classify plants became imperative. The Father of Modern Ecology and Taxonomy was Carl Linnaeus (1707-1778), a Swedish botanist, physician and zoologist, also known as Carl von Linné after his ennoblement in 1761. Linnaeus wrote three major works, all of which were regularly revised and updated. These were 'Systema Naturae', 'Genera Plantarum' and 'Species Plantarum'. In 1737, in 'Genera Plantarum' (The Genera of Plants), Linnaeus first used the word Orchidaceae to designate the entire orchid family. At that time, he described only eight orchid genera, one of which was *Orchis*, the name that had been coined by Theophrastus almost 2000 years before. 'Genera Plantarum' was the first step towards a universal standardized biological nomenclature. By 1753, Carl Linnaeus had published 'Species Plantarum' (The Species of Plants), the work that is now internationally accepted as the beginning of the modern botanical nomenclature known as Binomial Nomenclature, this being the first coherent identification of plants by a genus name followed by a specific name (Jarvis & Cribb, 2009). By the time the 10th edition of 'Systema Naturae' had been brought out in 1758, Linnaeus had classified 4400 species of animals and 7700 species of plants, among which a hundred different species of *Epidendrum* were mentioned, a name which was loosely given to all of the tropical orchids known at that time (Müller-Wille & Reeds, 2007). The Orchidaceae family name became fully established when the French botanist Antoine Laurent de Jussieu (1748-1836) published his own work in 1789, also called 'Genera Plantarum'. De Jussieu continued to use the term 'Orchidaceae', gathering together under this name all the plants with similar morphological features that had been described

up to that date. Towards the end of the 18th century, research on orchids had progressed well. The classification of orchids had passed through numerous revisions, and the number of genera they had been divided into, had increased.

THE ORCHID-HUNTERS AND THE GREAT EXPLORATIONS OF THE NEW WORLDS

The search for new lands and the study and collection of new species of flora and fauna attracted many noted botanists and adventurers. Great travelers such as the French admiral Comte Louis Antoine Bougainville (1729-1811), the British explorer and cartographer James Cook (1728-1779) and the experienced French navy officer Jean François de Galaup, Comte de La Pérouse (1741-1788) discovered and explored Australasia, New Guinea, Polynesia and the Easter Islands. After his first voyage to South America and Australasia (1768-1771), Captain James Cook made a presentation on preserving the health of his crew to the Royal Society in London (Biskup, 1987). During the 18th century, the most common fatalities on long sea voyages were due to scurvy, a debilitating illness caused by lack of Vitamin C in the diet. In addition to scrupulous cleanliness, fumigation and a selection of other measures, which included fresh vegetables and meat, he had used Salep, a nutritious ancient drink made from the ground dried roots of several types of orchid including *Orchis militaris, Orchis (Anacamptis) morio*, *Orchis mascula* and *Orchis simia* as part of the diet on his ship (Cook, 2010). By the end of the 18th century, many new species of exotic orchids have been introduced to Europe, not only from Australasia and the Pacific but also from China, the Antilles and the Americas. Unfortunately, very few orchids survived the conditions in which the sailing ships navigated. Improper storage conditions in dark and unaired stowage, predation by rats, and lack of the fresh water that was critical to the survival of these plants would all have taken their toll. Despite this, the stories of the sailors and explorers, as well as the specimens of unusual plants they brought back, heightened the interest in orchids and fired the imagination of the Europeans. A passion developed for these exotic plants (André, 1995). Explorers and gentlemen of fortune travelled to the tropics to search for orchids which had never been seen before, as well as to collect specimens of those that had become popular back home and would fetch the highest prices. They penetrated deep into the heart of these new lands and the search for orchids turned into a frantic and obsessive hunt (Orlean, 2000). Neither the presence of pirates or cannibals nor the danger of earthquakes and hurricanes deterred the orchid-hunters of the 18th and the 19th centuries. They were confronted with excessive moisture and heat, the constant threat of tropical diseases, insect bites, venomous reptiles, the poisoned arrows of the native people and the weapons of their European competitors. Thus, many of them paid for their desire to become rich and famous with their lives. Once the rarest species were found, whole areas were often stripped. Specimens that could not be loaded onto the ships due to lack of space and those that could not be transported from the regions where they had been discovered were destroyed in vast quantities, either in order to avoid falling into the hands of competitors, or to further enhance the value of those being shipped back. Once the plants arrived home, they were sold at exorbitant prices, enriching the most important European botanical collections and gardens. The orchid hunt also had catastrophic consequences for the existence of some of the species. Many habitats were seriously damaged or destroyed during this time, causing numerous species to become endangered or driven to extinction. This obsession with orchids became known as the Orchid Fever (Hansen, 2001). Numerous naturalists, horticulturalists, physicians and adventurers over the centuries have been commemorated by having orchids named after them. For example, the genus *Goodyera* was named after the 17th century English botanist John Goodyer (1592-1664). Goodyer translated and revised many important works including 'Dioscorides' 'De Materia Medica' and 'Gerard's Herbal'. He was so well thought of that a stained-glass window in his local church even now bears a commemorative inscription about him and his family coat of arms. An important figure of the 18th and 19th centuries was the English botanist, orchidologist and taxonomist John Lindley

(1799-1865). In a later work, 'The Genera and Species of Orchidaceous Plants' (1840), Lindley described thousands of species and hundreds of new genera, many of which are still in accepted use today. Lindley was particularly fascinated by orchids and on his death left an unfinished book, 'Folia Orchidaceae', which is considered a classic on the Botany and Taxonomy. Lindley is considered the Father of Orchidology. In 1818, Sir William Cattley (1788-1835), a merchant and horticulturalist, was unpacking a shipment of plants that he had received from Brazil when he found some half-dead tendrils. Under his attention, these grew into a beautiful orchid, which was given the name *Cattleya labiata* in 1824, in honour of its rescuer. One of the foremost German orchidologists of the 19th century, Heinrich Gustav Reichenbach (1823-1889), described thousands of species of orchids and has many orchids named after him using the species epithet *reichenbachii*. A German taxonomist, Friedrich Richard Rudolf Schlechter (1872-1925) was responsible for the first systematic classification of orchids, used up until a few decades ago. His volume 'Die Orchidaceen von Deutsch Neu Guinea' (The Orchids of the German New Guinea) described around 1400 species of orchids from Papua New Guinea, 1102 of which were new to science. In South America, the Brazilian botanist João Barbosa Rodrigues (1842-1909) was recognised as an important scientist and artist. He published 'New Varieties and Species of Orchids' (1877-1881) on the orchids found in the Amazon basin area. His magnificent orchid watercolours and illustrations of 700 species of rare Brazilian orchids have since been published in 'Iconographie des Orchidées du Brésil' (1996).

ROBERT BROWN & CHARLES DARWIN - THE GIANTS OF MODERN BIOLOGY

During the 18th and 19th centuries a dichotomy developed between the practitioners of herbal medicine and regular physicians. This was an essential and pivotal point in the expansion of scientific knowledge, since it opened the way for the rigours of objective scientific enquiry lead by proof.

In 1833, the Scottish botanist Robert Brown published a pamphlet called 'Observations on the Organs and Mode of Fecundation in Orchideae and Asclepiadaceae'. The article contained the observation that the pollen of orchids, when placed on the stigma, emitted long tubes which could be traced down into the ovary. Having closely observed the leaves of *Cypripedium* orchids under the microscope, Brown also noted the existence of a peculiar dark, circular structure within the cells, like a single circular areola, which he called the 'nucleus'. It was the first time that the word 'nucleus' had been used for this intercellular organelle in cell biology. Some years earlier, Brown had observed under the microscope the irregular motion of tiny particles of orchid pollen grains in water. This phenomenon became known as Brownian Movement (1827).

Although earlier botanists had described the structure of orchid flowers and observed visits by insects, the nature and variations of pollination mechanisms in orchids were first fully appreciated by the English naturalist, explorer and geologist Charles Darwin (1809-1882). In 1862, Darwin published the first edition of his book named 'Fertilisation of Orchids, with the subtitle On the Various Contrivances by Which British and Foreign Orchids Are Fertilised by Insects and on the Good Effects of Intercrossing'. This was his first essential contribution to the understanding of the strategies used by orchids to ensure propagation by cross-fertilisation (Cameron, 2011). Darwin also explained how complex ecological relationships could result in the co-evolution of orchids and insects. He considered the adaptations of orchid flowers to their animal pollinators as being among the best examples of his idea of evolution through natural selection, considering their floral structures '*as perfect as the most beautiful adaptations in the Animal Kingdom'* (Yam, 2009).

ORCHID CULTIVATION AND THE MYSTERIES OF SEED GERMINATION

The dust-like seeds of orchids were first observed and drawn by the Swiss naturalist Konrad Gessner (1516-1565), sometime between 1540 and his death in 1565. The earliest published description of a cultivated orchid in Europe can be traced to the herbal of Rembert Dodoens (1568), which illustrated a Slipper Orchid that had been drawn by Joannes

Vreccomtus, who had grown the plant in his garden in Brussels (Mathé, 2016). In England the first published record of an orchid in garden cultivation was made in 1597 by the author of 'Gerard's Herbal', John Gerard, this also being the Lady's Slipper Orchid *Cypripedium calceolus* (Cribb, 2014). An English botanist of Scottish descent, Philip Miller (1691-1771) mentioned several orchids in his first edition of the 'Gardeners Dictionary' (1731), including three species of European *Cypripedium*, which he had cultivated in the Chelsea Physic Garden while he was Curator.

Towards the end of the century, he described twenty species of *Orchis* native to the British Isles in the 8th version of 'The Gardeners Dictionary' (1768). He explained that transplanting orchids from the wild frequently failed because each species needed a particular habitat to survive. During the 19th century, many orchidologists had achieved some success by planting seeds in the soils the plants had been transported in from their places of origin. Gradually horticulturists began to concern themselves with the conservation and cultivation of these orchids, using greenhouses to reproduce tropical climatic conditions. Recorded horticultural attempts to germinate orchid seeds date to the mid-1800s (Jacquet, 1994). Sir Joseph Paxton (1803-1865) was the first grower to build and use different greenhouses with improved conditions of sunlight, ventilation, watering and humidity for cultivating species of orchids that came from different habitats. The first reported germination of orchid seeds was *Prescottia plantaginea*, from the Horticultural Society Garden in Chiswick, England, around 1830, but it was not until 1849 that the first detailed and substantiated paper on seed germination was published, this by David Moore (1807-1879), Director of the Glasnevin Botanic Gardens in Ireland. In 1820, there were just 130 species of exotic orchids grown in England, but by 1830 their number had exceeded 400. Orchidology was on its way. But despite the intense interest and much experimentation, the secrets of orchid seed germination through mycorrhizal fungi continued to remain a mystery.

NOËL BERNARD (1874-1911) - 'THE GREATEST HOPE OF FRENCH BOTANY'

During his short but productive career, Noël Bernard shed much light on the nature of the endophytic fungi found in orchids and their importance in the survival of these plants. His major discovery was the symbiotic germination of orchid seeds. This is where a soil fungus provides water, mineral nutrients and carbon to the seedling, compensating for the lack of seed reserves typical of the Orchidaceae. In 1899 Bernard was 25 years old, still studying for his doctorate and also doing his National Service in Melun when he discovered the dead inflorescence of the achlorophyllous orchid *Neottia nidus-avis* while walking in the nearby forest of Fontainebleau. In a letter to a friend about his find, he wrote that the *Neottia nidus-avis* flower was '*accidentally buried in the soil, under a layer of dead leaves. In the spring, the seeds, still inside the fruit, had not been released, but started to germinate'*. Under the microscope, Bernard saw subterranean orchid seedlings and protocorms that '*no botanist's eye had seen before'*. He also observed the fungal mycelial filaments associated with them, and became convinced that the fungus was providing nutrition. It was common knowledge at that time that orchids were generally infected by mycorrhizal fungi, but Bernard's work was to establish once and for all that the relationship was necessary for the survival of the plants. On January 26th, 1911, very weak but fully conscious until his last hours, Noël Bernard lost his long battle with tuberculosis. He was only 37 years old. In a note published after his death, Bernard reported on the fungicidal capacity of orchids. He wrote: '*even a relatively limited infection of the orchid Himantoglossum hircinum is sufficient for the orchid's tubers to acquire fungicidal capacity'*. Bernard suggested that the orchid killed the fungus in a controlled manner, allowing it to survive in the soil during the winter and to re-colonise the plant the following spring. Antifungal compounds from orchids, such as hircinol, loroglossol and orchinol, were later discovered because of these observations (Selosse et al., 2011).

FROM GERMINATION TO INDUSTRIAL CLONING

Working in parallel with Bernard, in 1904 German botanist Hans Burgeff (1883-1976) germinated orchid seed in agar inoculated with the right species of mycorrhizal fungus. Although Noël Bernard had not formed a practical and reliable method for asymbiotic germination in 1904, the direction of his research had allowed others to do so (Arditti, 1979). In 1922, the American biologist Lewis Knudson (1884-1958) used an improved culture medium to reproduce germination in the absence of fungi. This he called the asymbiotic method. The Knudson Method and Knudson culture medium were named after the American (Arditti, 2010). In 1932, Burgeff, and independently, Carlos Cappelletti in 1935, cultured different fungi in association with various genera of orchid seeds. In 1949, Professor Gavino Rotor (1917-2005) at Cornell University made the first real attempt at propagating an orchid from *Phalaenopsis* genus by tissue culture methods. This was based on vegetative or meristematic propagation. Using Rotor's culture method, an orchid nursery owner Hans Thomale was the first to culture stem-tip explants of *Dactylorhiza maculata* in Germany in 1956. When the new plantlets formed and started to develop, he stated: '*This is a form of vegetative multiplication whose potential cannot be overlooked'*. Meristematic cloning methods were later used industrially by hundreds of horticultural companies and research laboratories worldwide (Arditti, 1984; 2000).

ORCHID HYBRIDS - AN INCOMMENSURABLE TREASURE

John Dominy (1816-1891), a British horticulturist working for James Veitch, achieved the first artificially bred intraspecific orchid hybrid: *Calanthe* × *dominyi (Calanthe sylvatica* × *Calanthe triplicata),* which flowered in 1856. Then in 1861, Dominy hybridised two different genera, *Goodyera* (*Ludisia*) *discolour* × *Dossinia marmorata*, which generated *Goodyera* × *Dominii* (Veitch, 1906). This was the first ever intergeneric orchid hybrid, or as Charles Darwin once called them: '*strange crosses between distinct genera'*. Over his lifetime, Dominy raised only 25 hybrids. In 1892, it was created the first tri-generic hybrid, *Sophrolaeliocattleya veitchiana*. Just one year later, in 1893, one of the most popular natural orchid hybrid in the world, *Vanda* 'Miss Joaquim' var. Agnes, was disclosed to the orchid community in Singapore by its discoverer, Miss Agnes Joaquim (1854-1899) (Arditti, 1984; Ridley, 1893). At the 1899 Singapore Flower Show, the orchid hybrid *Vanda* 'Miss Joaquim' var. Agnes was the main highlight. Ms. Agnes had lived just long enough to see her orchid win first prize and be publicly recognised for her achievement. Suffering from cancer, she died less than three months later. For its resilience and year-round blooming quality, *Vanda* 'Miss Joaquim' var. Agnes was chosen on the 15th of April 1981 to become Singapore's National Flower (Johnson & Wright, 2008).

THE 20TH CENTURY SURRENDERS!

At the beginning of the 1900s, the scientific interest in describing new species continued, and plants were still collected in large numbers in order to be sent to Europe, mostly to botanical gardens and wealthy amateurs. The requirements of botanical gardens as well as the considerable earnings from the trade of orchids generated a proliferation of horticultural companies in England, the Netherlands, Belgium, Denmark, Germany, France and the United States. Orchids became a social symbol of luxury and wealth in the West much as they had been in the Far East centuries before. Orchids that had once been reserved for the wealthy elite could at last be afforded by the masses. Orchid sales became gigantic business. The topmost orchid cultivator in the world is currently the United States, particularly California, where hundreds of thousands of plants are sold every year. In Europe, the most important orchid cultivation areas are in the Netherlands, Germany and France, which together produce hundreds of thousands of specimens a year. There is fierce competition from Southeast Asian countries like Thailand and Singapore where the trade in orchids triggers annual sales of tens of millions of dollars.

"Orchidelirium is beyond addiction and beyond hope", revealed in 2006, William

Langley, journalist with The Telegraph newspaper.

Avid orchid collectors worldwide have a craving for new specimens that just cannot be satisfied. Today, there is a massive market for illicit plants and it seems the rarer and more valuable the find the better. Many of these collectors rely on buying specimens from people who have smuggled the orchids across borders without a permit and have obtained them directly or indirectly from the wild. Often the fact that a plant has been secretly brought into the country under wraps adds greatly to the appeal. Of course, this means that the collected plants are even more sought-after and expensive. Carlo Blistery, legal counsel to the American Orchid Society, says: '*There are people who are kind of romanced by the idea that a plant has been smuggled in. It adds to the attraction and they want to have it, no matter what'*. It cannot really be known how many illegally collected orchids are traded every year on the black market, although it has been estimated at over £6 billion (Langley, 2006). The supply routes criss-cross the world and in many ways the market resembles the international trade in drugs and arms. Eric Hansen, the author of 'Orchid Fever', recalls a conversation with an otherwise down-to-earth neighbourhood flower grower who told him: '*You can get off alcohol, drugs, women, food and cars, but once you're hooked on orchids you're finished. You never get off orchids. Never'* (Langley, 2006). Nevertheless, there is currently a longstanding debate about whether collection from the wild is always harmful. The argument is that world class facilities such as the Royal Botanic Gardens at Kew and numerous private horticulturalists may in fact be doing the world a service by propagating and preserving plants that are threatened by habitat destruction, so using the trade as a conservation tool. It is a Catch-22 situation.

But paradoxically, orchid smuggling let to new orchid discoveries in more recent years.

In 2002, American orchid dealer and entrepreneur Michael Kovach bought three magnificent, large flowered pink and purple slipper orchids from a roadside flower seller in Peru. Kovach smuggled one of the plants into the United States in his suitcase to Marie Selby Botanical Gardens in Sarasota to get the orchid identified. The stunned experts confirmed that this was a species of the genus *Phragmipedium* previously unknown to science. They published the first scientific description of the flower within 8 days, calling it *Phragmipedium kovachii* to the honour of its discoverer. Following an investigation Kovach was arrested and fined for breaking the international CITES treaty prohibiting the movement of wild orchids across borders. He was fined and put on probation, narrowly avoiding a prison sentence. Federal officials charged the Marie Selby Gardens and one of its employees with illegally possessing the plant. On returning to Peru, Kovach found to his dismay that all of the wild plants, some 500 or so specimens, had already been dug up and removed from the area by plant poachers. Meanwhile, wild specimens were reportedly being sold to collectors for up to $10,000 each. Despite the illegality of the actions, the intrepid back-stage story and many calls for his name to be removed, Kovach's surname currently remains as the species epithet for this orchid (Yoon, 2002). *Phragmipedium kovachii* is considered to be the most important orchid species to have been found in the Neotropic Ecozone in the last 100 years. Orchid specialists say the newcomer is a multimillion-dollar commodity that will enable breeders to produce a novel array of Slipper Orchid hybrids, which beauty will defy imagination (Shapira, 2002).

In 2004, an established pharmacist and head of a pharmaceutical company, Sian Tiong Lim (1974-2014), had a permit to bring certain orchids into Britain from Malaysia, but attempted to bring some rare orchids into the country hidden among the others. He was discovered with several wild-collected orchids in his luggage on his arrival at Heathrow airport in England. Among the 126 specimens found in the consignment, there were several species threatened with extinction in the wild. Experts from the Royal Botanic Gardens at Kew who were called in to identify the plants. They were so rare, that one orchid expert at Kew had never ever seen them before. Lim was imprisoned for 4 months, which, it was said, nearly destroyed his life. He was also struck off from practising pharmacy for bringing the profession into disrepute. The scientist sadly passed away in 2014 aged 40, killed in a

cycling accident in Richmond Park, London (Morgan, 2014).

Orchidelirium is clearly alive and well!

As fashions change sometimes certain species go out of favour for a while, but for their beauty, fragrance and endless variety, orchids have been delightful companions not only for orchid lovers worldwide, but for mankind throughout history. There is little doubt that this most beautiful and intriguing member of the Plant Kingdom will remain a firm favourite, now and in the future (Guérin, 2004). The only question is, will we still have wild orchids growing in their natural habitats, or will the only orchids we have left be growing under artificial conditions? Perhaps only time will tell.

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ANALYSIS OF THE PRODUCTION OF BULB FLOWERS IN THE REGION OF STARA ZAGORA, BULGARIA

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Abstract

The purpose of the research was to explore the possibilities of growing bulb flowers - tulip, hyacinth, lily and to make an economic evaluation of the production. The research was conducted in the region of Stara Zagora, Bulgaria. The total arable land was 0.1 hectares. The direction of production for tulip and lily was for cut flowers and for hyacinth - for potted plant. As a result of the study was found that among the three cultivated flowers, the highest was the profitability for lily - 77.21%. Lily production was 4.8 times more profitable than tulip production. When growing tulip and lily for cut flowers, net income in the first year was the lowest due to the cost of seedlings. In the following years, the profit increased and the average of net income for one year over three-year growing period was: for lily - 142,850 euro.ha^{-1}; tulip - 5,580 euro.ha^{-1} and for hyacinth 877 euro/1000 pots.

Key words*: bulb flowers, income, Bulgaria.*

INTRODUCTION

Flower farming is a relatively new branch of horticulture in Bulgaria. The favorable soil and climate conditions and Bulgarians' love for flowers result in their cultivation in almost every household and garden.

The industrial production of flowers began in the mid 60's with the building of the first steel-and-glass greenhouses in Velingrad. In 1988, greenhouse flower production accounted for 86.1% of all flower production. In 1970, there were 28.5 hectares of heated steel-and-glass greenhouses. By 1975 that area had grown to 56.8 ha, rising to 96.7 ha in 1978, to 130 ha in 1980, and reaching 148 ha in 1990. An analysis conducted in the field of flower farming shows that between 1970 and 1988, areas planted with flowers kept increasing, and investments generated high return (Denchev and Bachev, 1991). Apart from greenhouses, a large portion of flower types were grown in outdoor areas, which were estimated to cover 144.3 ha of arable land in 2003. Over 7 million units of cut flowers were produced, as well as 2.7 million ornamental flowers and shrubs, and 399,000 pot plants. Flowers were produced in 970 farms, of which 809 were owned by private individuals. The production of cut flowers was comprised of 655 farms, 497 of which were implementing flower production in outdoor areas, while 290 produced greenhouse products. 183 farms specialized in pot plant production. In 2007, there were 500 farms which grew flowers and ornamental shrubs, utilizing a total area of 166 hectares (Ministry of Agriculture and Food, 2008). In comparison with 2003, the production area had shrunk by an negligible margin. The production of pot flowers and ornamental shrubs increased. Implementation of flowers grown in pots saw a rise.

In 2012, flowers and ornamental shrubs were grown in 231 professional farms. The area of the used arable land was 366.8 ha. Cultivation facilities took up 6% of the total area. 61% of farms in 2012 belonged to private individuals. 2,194,800 units of cut flowers and 2,215,100 units of pot flowers were produced in 2012. Of bulb types, 50,200 bulbs were produced, as well as 138 kg baby bulb. Out of the 10,062,200 ornamental flowers and shrubs produced for the decoration of parks and gardens, the highest percentage belongs to ornamental shrubs with 54%, followed by annual flowering species with 37%. 2,061,500 cut flowers were implemented, as well as 1,595,400 pot plants, 7,420,900 ornamental flowers and shrubs. Out of those, 26.1% were for export.

According to statistical data (Ministry of Agriculture and Food, 2016) after 2012 the production and arable land used for flower farming in Bulgaria remained within these values, with no measured growth. A negligible decline in greenhouse production of flowers could be observed, while outdoor areas remained the same, primarily used as seed plots. Sales shrunk by approximately 30% due the insufficient financial capacity of Bulgarians. The reason is, flowers are viewed not as essential goods but rather as a luxury, and that becomes more evident during a unfavorable financial conditions. It is difficult to predict what changes might come in the coming years or long-term, but we hope for positive change.

The purpose of this study was to explore the possibilities for growing basic bulbous flower species by identifying the most important components of the cultivation technology and make an economic assessment of production.

MATERIALS AND METHODS

Methodology of research

The study was conducted in the region of Stara Zagora, Bulgaria on leached cinnamon forest soil. The total area of arable land used was 0.1 hectares, divided into plots with separate flower types as follows:

1. Tulip (genus *Tulipa*) - 0.03 ha;
2. Lily (genus *Lilium*) - 0.04 ha;
3. Hyacinth (*Hyacinthus orientalis* L.) - 1,000 pots.

All of the above-mentioned species are bulb flowers and the bulbs were planted manually. Inter- and intra-row spacing was determined by the size of the bulbs and the aboveground part, as well as the specific requirements of the separate species and types.

In the production of tulips and lilies, the direction taken was for cut flowers, and in the case of hyacinth it was pot plants.

Agricultural techniques for growing flower crops were studied, including tillage, plant protection, fertilization, density of planting, and the planting-to-harvest period. An economic analysis was made for an area of one hectare as the difference between the revenue and the production costs of flowers and production lines. The economic evaluation of production of three types of bulb flowers in a private family business is defined by the difference between the revenue from flower sales and the costs.

The profitability ratio is defined as the ratio of profit to revenue and is expressed in (%).

Types and cultivars of grown bulb flowers were:

- Tulip - 'Darwin' and 'Triumph' (Figures 1, 2);

Figure 1. Tulip cultivar 'Darwin'

Figure 2. Tulip cultiar 'Triumph'
/www.amazon.com/Mixed-Triumph-Tulips/

- Lily - types from Oriental and Asian forms (Figures 3 and 4);
- Hyacinth - 'Anna Marie' and 'Blue Star' (Figure 5).

Agrometeorological conditions of the area

The arable land where the experiment was conducted is located in the climate zone of Central Southern Bulgaria. According to the climatic delineation of the country, this region is part of the European Continental region, and

the Transitional Continental sub-region. In regards to rainfall and air temperature, the region is considered favorable for the cultivation of flowers.

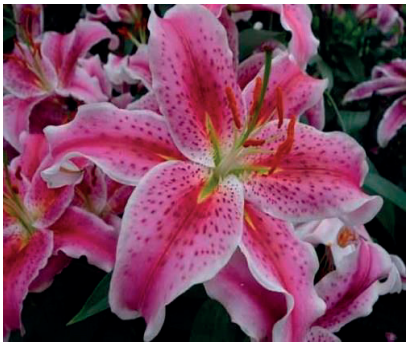

Figure 3. Lily - Oriental form

Figure 4. Lily - Asian form

Figure 5. Hyacinth cultivars 'Blue Star' and 'Anna Marie'

Climate conditions in the region are defined by the cold northern influence, due to the relatively low height of the Stara Planina and Sredna Gora mountain ranges (The Balkan Mountains), and the existence of a southern orographic border, the Rhodope Mountains, which keep southern influences at bay to a degree.

The climate is transitional continental with a Mediterranean influence, and is significantly milder than Northern Bulgaria's climate. Some subtropical characteristics can be observed. Winter is relatively mild with frequent snowfalls, though the snow cover is not persistent. There are 28-31 snow days, starting from the second half of November until the beginning of March. Temperatures reach their lowest in January. Spring begins in the beginning of March. Temperatures rise above 5°C, starting from March 4th-7th. Summer is dry and hot. Average temperature in July is 23.2° C, and 23°C in August. Due to the frequent influx of tropical air masses in July and August, maximum temperatures can exceed 40°C. Autumn is dry, warm and prolonged. The transitional nature of the climate is even more obvious in the annual rainfall distribution. The annual sum of rainfalls is insufficient, at 471 mm (Table 1).

Table 1. Climate characteristics of the region of village Malka Vereya, average for 1978-2018

Month	Average annual air temperature, °C	Monthly sum of rainfall, mm
January	1.2	30.4
February	2.6	27.7
March	6.7	34.9
April	11.8	36.9
May	17.0	54.3
June	21.3	53.2
July	23.7	44.4
August	22.9	41.0
September	18.7	34.0
October	13.3	28.2
November	7.7	42.2
December	3.3	43.9
Annual	12.51	471.1

Two precipitation maximums are observed: one in summer - during May and June; and one in winter - during November and December. The difference between summer and winter rainfalls is small. This equalization is at the expense of winter rainfalls, which increase while summer rainfalls dwindle. The precipitation minimum occurs in August-September. Periods of drought begin earlier, in July, and are lengthier than those in the temperate climate zone. They can last 90-100 days, until the end of October.

Soil characteristics

The soil is of the leached cinnamon forest type. This is a widely spread soil type in Bulgaria. The soil depth profile is 75-120 cm. The soil type is characterized by a well-defined differentiation between the humus and illuvial horizons. The humus horizon has low depth of 25-30 cm, has cinnamon color, and has compacted-to-dense structure. The illuvial horizon is deep (up to 70-80 cm), clayey, compacted, reddish-brown. The carbonates are washed to a depth of 80-100 cm, with the carbonate layer being significantly less pronounced than in typical cinnamon forest soils.

The soil is more clayey than typical cinnamon soils but contains less humus. The total stock of humus in the 1-meter layer varies widely, but 40-45% of it is located in the top 20-30 cm. The content of physical clay is from 58.4 to 60.5%. It contains approximately 2% humus in surface horizon. The soil is poor on nitrogen and phosphorus and rich on potassium.

The physical properties of these soils are not particularly favorable - great connectivity, poor water permeability, swelling when moistened, and if dried they harden and form a solid soil crust. Their natural fertility is poor, but good yields can be obtained with good agricultural technique. These soils are suitable for cultivation of flowering species, field crops, vineyards, tobacco and perennials.

Soil characteristics show low to medium mineral nitrogen storage, low mobile phosphate storage, and medium storage of absorbed potassium. The soil reaction is slightly acidic.

In the case of agricultural production without irrigation, the soil in the area is categorized as good for growing bulb flowers.

RESULTS AND DISCUSSIONS

For tulips, the recommended inter-row distance was approximately 15 cm, and the intra-row distance was 10 cm. The planting depth was 8 cm. The planting scheme for tulip and lily in the field experiment is presented in Table 2.

Table 2. Planting scheme for tulips in Malka Vereya

Flower species	Inter-row spacing cm	Intra-row spacing, cm	Planting depth, cm	Number of bulbs/ 1000 m^2	Number of cultivated bulbs
Tulip	15	10	8	66,500	19,950
Lily	25	15	10	26,700	10,680

The data in Table 3 shows that the highest percentage of production costs was the cost of planting material, which was 96.6% of total costs for tulips and 97.1% for lilies on average for the three-year period. The cost of lily planting material was higher than that of the tulips due to the higher price of lily bulbs (0.46 Euro/unit), compared to tulip bulbs (0.13 Euro/unit). Fuels and pesticides comprised a smaller percentage of the costs. The area was fertilized with manure, potassium sulfate and ammonium nitrate.

Table 3. Costs for the production of cut flower tulips and lilies, Euro/hectare

Period	Bulbs	Fuel	Fertilizer	Pesticides	Total cost
Tulip					
I year	8,500.00	25.50	102.2	7.60	8,635.30
II year	-	25.50	51.10	7.60	84.20
III year	-	25.50	51.10	-	76.60
Average	2,833.30	25.50	68.10	5.10	2,932.00
Lily					
I year	12,286.35	25.50	102.2	10.20	12,424.20
II year	-	25.50	76.70	10.20	112.40
III year	-	25.50	76.70	10.20	112.40
Average	4,095.50	25.50	85.20	10.20	4,216.40

It was vegetatively enriched with leaf fertilizer, once for tulips and twice for lilies. Pesticides were used to treat bulbs and the lilies were sprayed against aphids once in the beginning of vegetation.

The data shows that over the course of one year the production of tulips in a 0.1 ha area cost 2932.00 EUR while lilies cost 1284.4 EUR more, due to the higher price of the planting material.

The cost of the 1,000 units of hyacinth, which were grown in pots, accounted for 41.4%, and as such had the highest percentage, of the total production costs (Figure 6). Electricity costs (22%) were the second most prominent, while pesticides had the smallest percentage.

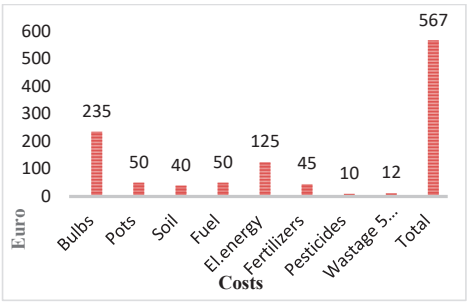

Figure 6. Total production costs of hyacinth, euro

Table 4 depicts the year-by-year and the 3-year-period economic evaluation of production of the three bulb flowers in a private family business in Bulgaria.

It is evident from the (selling price 0.61/unit). For tulips, during the first year of production the net income was negative.

One approach, which reduced losses during the first year, was the distribution of the product at a highe price during periods of high market demand.

In regards to the pot-grown hyacinth with a selling price of 1.53 EUR/unit, the net income was 60% of the revenue, sitting at 877.38 EUR, which places it between lilies and tulips.

Data that revenue from lilies was significantly higher (selling cost 0.77 EUR/unit) compared to those of tulips.

Table 4. Economic evaluation of the production of bulb flowers, euro/hectare

Flower species	For cut flowers		For pot flowers		Net income
	Revenue	Costs	Revenue	Costs	
Tulip					
I year	3,671.10	8,635.7			- 4,964.60
II year	3,488.00	84.40			3,403.60
III year	3,313.20	76.70			3,236.50
Average	3,490.80	2,932.3			558.50
Lily					
I year	19,454.70	12,424.4			7,030.30
II year	18,485.25	112.5			18,372.75
III year	17,562.80	112.5			17,450.30
Average	18,501.00	4,216.50			14,284.50
Hyacinth					
Average	-	-	1,457.20	579.8	877.40

The profitability ratio, which is defined as the ratio of net income to revenue, shows that most profitable was the production of lilies for cut flowers with 77.21%, while the profitability was lower for tulips at 16.00%. Pot production of hyacinth was also characterized by a high profitability with 60.21% (Table 5).

Table 5. Profitability ratio of basic bulb flowers, %

Flower species	Profitability, %
Tulip	16.00
Lily	77.21
Hyacinth	60.21

CONCLUSIONS

Based on the conducted analysis of the growing of bulb flowers - tulip, lily, hyacinth, in the area of the village of Malka Vereya, municipality of Stara Zagora, Bulgaria it can be concluded that over the three-year period lilies had the highest profitability with 77.21%, followed by hyacinth and tulips. The production of lilies was 4.8 times more profitable than that of tulips.

Net income was lowest in the first year of growing tulips and lilies for cut flowers, even dipping in the negative for tulips due to the initial costs of planting material in the first year. During the following years the gross profit increased, reaching a peak in the third year.

The soil and climate characteristics of the region show that conditions are favorable and basic bulb flower types can be produced successfully.

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COMPARATIVE STUDY OF FLOWER MORPHOLOGY AND FLOWERING PHENOLOGY IN SOME *HEMEROCALLIS* HYBRIDS

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Abstract

The study effectuated during the period between 2014 and 2020 at seventeen Hemerocallis cultivars ('Arctic Snow', 'Aten', 'Black Prince', 'Bumble Bee', 'Campfire Embers', 'Cartwheels', 'Chicago Cardinal', 'Chicago Picotee Memories', 'Cologne Rocket', 'Custard Candy', 'El Desperado', 'Frans Halls', 'Mikado', 'Pandora's Box', 'Spits Beauty', 'Stella de Oro', 'Strawberry Candy') held in the collection of the University of Agricultural Sciences and Veterinary Medicine of Iasi had as purpose the analysis of some ornamental interest features, including of the flowers and of the phenology of the flowering. The results showed that from a morphometric point of view, the studied hybrids differ between them regarding the total size of the flowers and of the floral components. Also, the dimension, the colour and the form of the tepals differ in the cultivars, and also at the same cultivar (between the outer and the inner tepals). The flowering time at the Hemerocallis hybrids cultivated at Iasi was of approximately one month, except for the 'Arctic Snow' cultivar (approximately 3 weeks) and 'Stella de Oro' (7-8 weeks, through repeated prolonged flowering). The period in which most of the cultivars are blooming coincides with the month of July (with extensions in the last decade of June and the first decade of August).

Key words*: daylily, flower, morphometry, flowering, phenology.*

INTRODUCTION

Hemerocallis L. (daylilies) genus comprises about 20-30 species, native to Himalaya, East Europe, China, Japan and Korea (Toma, 2009; Chung and Kang, 1994; Lin et al., 2020). Other authors claims that the genus *Hemerocallis* consists of 14 wild species; 11 of them originated from China (Liu et al., 2017; Hsu et al., 2011). Numerous nomenclatural and taxonomic problems exist within the genus which have been attributed to the diverse factors (many species were described from cultivated plants of unknown origin, because the extreme differences in appearance between living plants and dried herbarium specimens, also many species of *Hemerocallis* are so variable ecologically and morphologically that a proper species concept requires morphological, ecological, and biosystematic studies (Chung and Kang, 1994; Kang and Chung, 2000). Placed in the Liliaceae family by Linnaeus (1753), recently, under Angiosperm Phylogeny Group IV (2016), it has been moved to the family Asphodelaceae. Many species and cultivars of the genus *Hemerocallis* are popular perennial plants, used along borders, wide edgings, or in mass plantings (https://plants.ces.ncsu.edu/plants/hemerocallis-hybrida/), widely grown in gardens in Asia, Europe and North America for their attractive flowers, ability to adapt to a wide range of soils and resistence to unfavourable conditions such as light or water deficiency climates (Yang et al., 2012; Chung and Kang, 1994; Kang and Chung, 2000; Podwyszyńska et al., 2015). As of May 2018, there are nearly 89.000 registered cultivars (https://daylilies.org).

Daylilies are perennials plants having short rhizomes, tuberous roots (globose, fusiform, or oblong) and basal, sessile, linear leaves. Flowers are large, hypogynous, of short duration (bloom for only a day) (Cantor et al., 2007; Chen and Noguchi, 2000). The floral coating is simple (perigon) petaloid, composed from 6 tepals disposed on two cycles. The base of the tepals is intergrown and it forms the perigonal tube, and at the upper part, over the perigonal tube, the tepal is free (perigonal lacinia, perigonal lobe). The androceum is made of 6 stamens shorter than the perigon, and the gynaeceum is made of an

inferiorly disposed ovary and an erect, thin style, longer than the stamens. At the base of the perigon lays the receptacle (with the superior ovary) and the short pedicel (Chen and Noguchi, 2000; Sîrbu and Paraschiv, 2005). Morphological characters of da flowers were performed for a taxonomic study of native species (Hwang and Kim, 2012; Chen and Noguchi, 2000; Krestova and Nesterova, 2013) or to re-evaluate the systematic position (Yan et al., 2017), biosystematic studies and evaluated phenotypic and genome size (Podwyszyńska et al., 2015; Kawano and Noguchi, 1975), evaluating how the tepal color, floral scent and floral morphology are selected by pollinators (Hirota et al., 2013) etc. Also, studies on the phenology of flowering in daylilies were performed in order to understand the circadian system that controls the closing and opening of flowers (Ren et al., 2019), the blooming and withering time in natural populations or taxonomical identity of native taxa (Hasegawa et al., 2006; Kawano and Noguchi, 1975), to classify floral fragrance (Jiao et al., 2016) etc. Similar studies have been performed on other perennial flowering species, such as *Gladiolus* (Cantor et al., 2007; Horţ et al., 2015), *Dahlia* (Ciobanu et al., 2017), *Iris* (Crișan et al., 2018), *Narcissus* (Cantor et al., 2013), *Paeonia* (Cazan et al., 2018).

The aim of this work is to study some characters of flowers and flowering phenology in the *Hemerocallis* cultivars under the cultivation conditions of north-eastern Romania (in Iasi).

MATERIALS AND METHODS

Seventeen daylilies cultivars ('Arctic Snow', 'Aten', 'Black Prince', 'Bumble Bee', 'Campfire Embers', 'Cartwheels', 'Chicago Cardinal', 'Chicago Picotee Memories', 'Cologne Rocket', 'Custard Candy', 'El Desperado', 'Frans Halls','Mikado', 'Pandora´s Box', 'Spits Beauty', 'Stella de Oro', 'Strawberry Candy') were used in this study and were field grown at the collection site of Faculty of Horticulture, University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania (47°11′31″ N, 27°33′20″ E latitude, in temperate-continental climat with excessive nuances, chernozem cambic soil with sandy-loam texture and pH 7.8).

The investigations were carried in period 2014-2020. All plants were grown under the same management of fertilization, watering, soil and disease control.

Establishment of experimental cultures in the field was made during the autumn of the year 2013, with biologic material purchased from the company HEGEDE KFT (Hungary), sold as plants in pots. The experience included 17 variants (each cultivar representing a variant) distributed in randomized blocks with three repetitions (10 plants/repetition). The examination of the morphological characters and phenology was made on the plants from the collection. The qualitative characters studied were the colour of the flowers, the form of the tepals (depending on the proportion between length and width), the duration and the period of the flowering. The quantitative characters studied were: the length and width of the exterior and interior perigonal lobes, the diameter of the perigonal tube and its length (including the ovary). The results were compared to the average of the variants (considered control), and the interpretation was made using the analysis of the variance, with the LSD test (Saulescu and Saulescu, 1967).

The symbols used to indicate the significance of the differences from the control are: ns = non significant; o/x = negative/positive significant difference; oo/xx = negative/positive distinct significant difference; ooo/xxx = negative/ positive very significant difference.

RESULTS AND DISCUSSIONS

The daylilies hybrids from the experimental variants have flowers which are different in form, dimension, colour. In this paper, a series of quantitative (the dimensions of the perigon and the dimensions of the pedicel) and qualitative (the form and colour of the tepals, the period and the duration of the flowering) features of the flowers from the studied cultivars are analyzed.

From the components of the flower, the perigonal lobes (inner and outer), the perigonal tube and the pedicel were morphometrically.

At the level of the lobes the length and the width were determined, both for the outer and for the inner ones, taking into consideration the morphological differences of the two tepals categories. Also, the report between the length

and the width of the lobes was calculated, the obtained value being an indicator of their form.
The length of the outer lobes varied between 10 and 3.8 cm (Table 1). Although at this feature most of the cultivars had values over 8 cm, the average of the variants was 7.34 cm because of some cultivars with shorter tepals (‘Pandora´s Box’ with 3.8 cm, ‘Bumble Bee’ and ‘Stella de Oro’ with 4.6 cm, ‘Custard Candy’ with 6.4 cm and ‘Strawberry Candy’ with 6.5 cm), which situated under the threshold with 11.5% up to 48.4%. The differences from the threshold were non significant at the cultivars with the length of the outer lobes of 7.2-7.8 cm (‘Black Prince’, ‘El Desperado’, ‘Frans Halls’, ‘Spits Beauty’).
The width of the outer perigonal lobes (Table 1) also represented a variety feature. The dimension varied between 1.3 cm (the variants V_{14} – ‘Pandora´s Box’ and V_{16} – ‘Stella de Oro’) and 3.3 cm (V_1 – ‘Arctic Snow’). The cultivar which registered the maximum values was the only one which fitted, statistically, in the category of very significant positive differences from the threshold, while, at the opposite pole, with very significant negative differences were the two cultivars with the narrowest tepals (‘Pandora´s Box’ and ‘Stella de Oro’). Seven cultivars came close to the average of the variants (2.39 cm) and the differences were non significant. With statistically ensured differences in a positive way were ‘Chicago Picotee Memories’, ‘Custard Candy’, ‘El Desperado’ and ‘Spits Beauty’, and in a negative way were ‘Black Prince’, ‘Bumble Bee’, ‘Frans Halls’, ‘Pandora´s Box’ and ‘Stella de Oro’.

Table 1. Dimensions of the outer perigonal lobes

Variants (cultivars)	Length			Width		
	Average (cm)	Relative values (%)	Difference (±cm)/ Significance	Average (cm)	Relative values (%)	Difference (±cm)/ Significance
V_1 - ‘Arctic Snow’	8.0	108.97	0.7^{xx}	3.3	138.18	0.9^{xxx}
V_2 - ‘Aten’	8.0	108.97	0.7^{xx}	2.3	96.31	-0.1^{ns}
V_3 - ‘Black Prince’	7.6	103.53	0.3^{ns}	1.7	71.18	-0.7^{00}
V_4 - ‘Bumble Bee’	4.6	62.66	-2.7^{000}	1.8	75.37	-0.6^{0}
V_5 - ‘Campfire Embers’	10.0	136.22	2.7^{xxx}	2.6	108.87	0.2^{ns}
V_6 - ‘Cartwheels’	8.9	121.23	1.6^{xxx}	2.8	117.24	0.4^{ns}
V_7 - ‘Chicago Cardinal’	8.7	118.51	1.4^{xxx}	2.7	113.05	0.3^{ns}
V_8 - ‘Chicago Picotee’	8.5	115.79	1.2^{xxx}	2.9	121.43	0.5^{x}
V_9 - ‘Cologne Rocket’	8.5	115.79	1.2^{xxx}	2.2	92.12	-0.2^{ns}
V_{10} - ‘Custard Candy’	6.4	87.18	-0.9^{000}	3.2	133.99	0.8^{xx}
V_{11} - ‘El Desperado’	7.8	106.25	0.5^{x}	3.0	125.62	0.6^{x}
V_{12} - ‘Frans Halls’	7.2	98.08	-0.1^{ns}	1.9	79.56	-0.5^{0}
V_{13} - ‘Mikado’	8.2	111.70	0.9^{xxx}	2.0	83.74	-0.4^{ns}
V_{14} - ‘Pandora´s Box’	3.8	51.76	-3.5^{000}	1.3	54.43	-1.1^{000}
V_{15} - ‘Spits Beauty’	7.5	102.16	0.2^{ns}	3.0	125.62	0.6^{x}
V_{16} - ‘Stella de Oro’	4.6	62.66	-2.7^{000}	1.3	54.43	-1.1^{000}
V_{17} - ‘Strawberry Candy’	6.5	88.54	-0.8^{00}	2.6	108.87	0.2^{ns}
Average (control)	***7.34***	***100.0***	***0***	***2.39***	***100.0***	***0***
$LSD_{5\%}$ = 0.5 cm $LSD_{1\%}$ = 0.6 cm $LSD_{0.1\%}$ = 0.9 cm		$LSD_{5\%}$ = 0.4 cm $LSD_{1\%}$ = 0.6 cm $LSD_{0.1\%}$ = 0.8 cm				

The report between the length and the width of the lobes gives clues referring to their form. At the outer tepals, the more elongated forms (the big values of the report between length and width) are in cultivars ‘Black Prince’, ‘Mikado’, ‘Cologne Rocket’, ‘Campfire Embers’, ‘Frans Halls’, ‘Aten’, ‘Stella de Oro’, ‘Cartwheels’, ‘Chicago Cardinal’, and more rounded forms in ‘Custard Candy’, ‘Arctic Snow’, ‘Spits Beauty’, ‘Strawberry Candy’, ‘Bumble Bee’, ‘El Desperado’ (Figure 1).

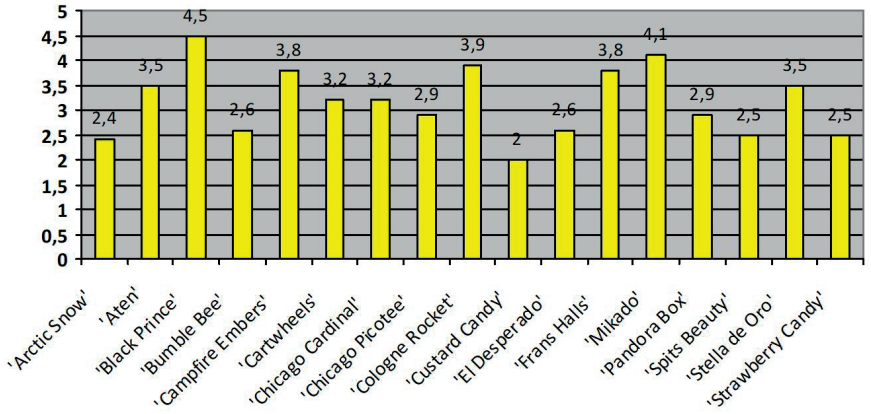


Figure 1. Ratio between the length/width of the outer lobes

Similar determinations were also made at the inner perigonal lobes, respectively their length and width. The length of the inner perigonal lobes (Table 2) situated at an average of the variants of 7.51 cm.

From the point of view of the differences from the control, the significances are similar to those from the outer lobes, except for the cultivar 'Black Prince' (with significant differences at the length of the outer tepals, but very significantly positive at the length of the inner tepals) and at the cultivar 'Mikado' (with non significant differences at the length of the inner tepals, but very significantly positive at the length of the outer tepals).

Table 2. Dimensions of the inner perigonal lobes

Variants (cultivars)	Length			Width		
	Average (cm)	Relative values (%)	Difference (±cm)/ Significance	Average (cm)	Relative values (%)	Difference (±cm)/ Significance
V_1 - 'Arctic Snow'	8.2	109.16	0.7^{xx}	5.0	131.99	1.2^{xxx}
V_2 - 'Aten'	8.3	110.49	0.8^{xx}	3.3	87.11	$-0,5^{0}$
V_3 - 'Black Prince'	8.4	111.82	0.9^{xxx}	2.7	71.27	$-1,1^{000}$
V_4 - 'Bumble Bee'	4.9	65.23	-2.6^{000}	3.1	81.83	$-0,7^{000}$
V_5 - 'Campfire Embers'	10.3	137.12	2.8^{xxx}	3.6	95.03	$-0,2^{ns}$
V_6 - 'Cartwheels'	8.9	118.48	1.4^{xxx}	4.1	108.23	0.3^{ns}
V_7 - 'Chicago Cardinal'	9.3	123.81	1.8^{xxx}	4.2	110.87	0.4^{x}
V_8 - 'Chicago Picotee Mem'	8.7	115.82	1.2^{xxx}	5.1	134.63	1.3^{xxx}
V_9 - 'Cologne Rocket'	8.6	114.49	1.1^{xxx}	3.5	92.39	-0.3^{ns}
V_{10} - 'Custard Candy'	6.2	82.54	-1.3^{000}	4.7	124.07	0.9^{xxx}
V_{11} - 'El Desperado'	8.0	106.50	0.5^{x}	4.9	129.35	1.1^{xxx}
V_{12} - 'Frans Halls'	7.3	97.18	-0.2^{ns}	3.1	81.83	-0.7^{000}
V_{13} - 'Mikado'	7.8	103.84	0.3^{ns}	3.3	87.11	-0.5^{0}
V_{14} - 'Pandora´s Box'	4.0	53.25	-3.5^{000}	1.8	47.52	-2.0^{000}
V_{15} - 'Spits Beauty'	7.5	99.84	0.0^{ns}	4.7	124.07	0.9^{xxx}
V_{16} - 'Stella de Oro'	4.6	61.24	-2.9^{000}	3.1	81.83	-0.7^{000}
V_{17} - 'Strawberry Candy'	6.7	89.19	-0.8^{00}	4.2	110.87	0.4^{x}
Average (control)	***7.51***	***100.0***	***0***	***3.79***	***100.0***	***0***

$LSD_{5\%}$ = 0.5 cm
$LSD_{1\%}$ = 0.6 cm
$LSD_{0,1\%}$ = 0.8 cm

$LSD_{5\%}$ = 0.4 cm
$LSD_{1\%}$ = 0.5 cm
$LSD_{0.1\%}$ = 0.7 cm

The width of the inner perigonal lobes is a feature with pretty big fluctuations at the analyzed cultivars (Table 2). The average value of the variants was 3.79 cm, with variations from 1.8 cm (‘Pandora´s Box’) up to 5.1 cm (‘Chicago Picotee Memories’). Wider lobes, with higher values than 4.7 cm and with very significantly positive differences had the variants V_8 – ‘Chicago Picotee Memories’, V_1 – ‘Arctic Snow’, V_{11} – ‘El Desperado’, V_{10} – ‘Custard Candy’ and V_{15} – ‘Spits Beauty’. With the narrowest inner lobes (under 3.1 cm) were the variants V_3 – ‘Black Prince’, V_4 – ‘Bumble Bee’, V_{12} – ‘Frans Halls’, V_{14} – ‘Pandora´s Box’, V_{16} – ‘Stella de Oro’.

At the inner tepals, the report between the length and the width had values higher than 2 in ‘Black Prince’, ‘Campfire Embers’, ‘Aten’, ‘Cologne Rocket’, “Mikado’, ‘Frans Halls’, ‘Cartwheels’, ‘Chicago Cardinal’, and a smaller report at ‘Custard Candy’, ‘Stella de Oro’, ‘Bumble Bee’, ‘El Desperado’, ‘Arctic Snow’, ‘Spits Beauty’, ‘Strawberry Candy’ (Figure 2).

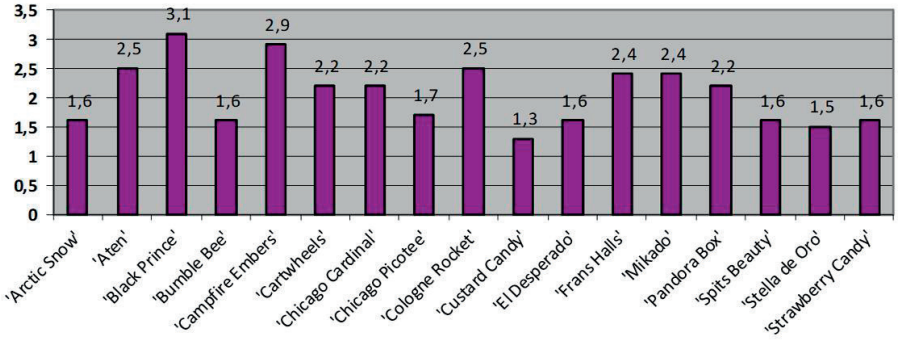


Figure 2. Ratio between the length/width of the inner lobes

The perigonal tube is another floral element which completes the morphology of the flower at daylilies, through two more important features, the length and the diameter. At the determination of the length of the perigonal tube was also included the portion occupied by the ovary.

The length of the perigonal tube was remarked with bigger differences than the average of the variants (2.18 cm) at some of the varieties (Table 3). Thus, ‘Aten’ and ‘Black Prince’ exceeded the control with 55.8%, respectively 32.9%, and ‘Campfire Embers’ with 19.1%. But, ‘Bumble Bee’, ‘Pandora´s Box’ and ‘Stella de Oro’ situated under the value of the control with 22.1-35.8%. The other 11 variants had values of the analyzed feature around the average and with non significant differences.

The diameter of the perigonal tube (expressed in millimetres, due to its reduced dimensions) registered an average of the variants of 5.6 mm, the maximum values being at the cultivar ‘Chicago Picotee’ (7.8 mm), and the minimum ones at ‘Pandora´s Box’ (3.8 mm). The cultivars ‘Arctic Snow’, ‘Custard Candy’, ‘Chicago Cardinal’, ‘Cologne Rocket’, ‘Chicago Picotee Memories’ and ‘El Desperado’ got remarked through a thicker perigonal tube, with the diameter between 6.7 and 7.8 mm, while, in the case of the cultivars ‘Black Prince’, ‘Bumble Bee’, ‘Frans Halls’, ‘Stella de Oro’, ‘Mikado’ and ‘Pandora´s Box’, the perigonal tube was thin, with a diameter of 3.8-4.2 mm (Table 3).

From the biometric evaluation of the floral elements, we can calculate the total (maximum) length of the perigon, as the sum of the maximum length of the lobes and the length of the perigonal tube (including the ovary).

In Figure 3 these results are presented graphically, and it shows the fact that the longest flowers are at ‘Campfire Embers’ (12.9 cm). With big values of the length of the flowers, over 10 cm, are also the cultivars ‘Arctic Snow’, ‘Aten’, ‘Black Prince’, ‘Cartwheels’, ‘Chicago Cardinal’, ‘Chicago Picotee Mem.’, ‘Cologne Rocket’, ‘El Desperado’, ‘Mikado’. The flowers with a short perigon (5.4-6.6 cm) are those from ‘Bumble Bee’, ‘Pandora´s Box’ and ‘Stella de Oro’.

Table 3. Dimensions of the perigonal tube

Variants (cultivars)	Length			Diameter		
	Average (cm)	Relative values (%)	Difference (±cm)/ Significance	Average (mm)	Relative values (%)	Difference (±mm)/ Significance
V_1 - 'Arctic Snow'	2.2	100.81	0.0^{ns}	6.7	119.64	0.1^{xxx}
V_2 - 'Aten'	3.4	155.80	1.2^{xxx}	5.3	94.44	-0.3^{ns}
V_3 - 'Black Prince'	2.9	132.88	0.7^{xxx}	3.9	69.64	-1.7^{000}
V_4 - 'Bumble Bee'	1.7	77.90	-0.5^{000}	4.1	73.21	-1.5^{000}
V_5 - 'Campfire Embers'	2.6	119.14	0.4^{xx}	5.7	101.79	0.1^{ns}
V_6 - 'Cartwheels'	2.3	105.39	0.1^{ns}	5.6	100.00	0.0^{ns}
V_7 - 'Chicago Cardinal'	2.1	96.23	-0.1^{ns}	7.4	132.14	1.8^{xxx}
V_8 - 'Chicago Picotee Mem.'	2.3	105.39	0.1^{ns}	7.8	139.29	2.2^{xxx}
V_9 - 'Cologne Rocket'	2.0	91.64	-0.2^{ns}	7.4	132.14	1.8^{xxx}
V_{10} - 'Custard Candy'	2.0	91.64	-0.2^{ns}	6,7	119,64	1.1^{xxx}
V_{11} - 'El Desperado'	2.0	91.64	-0.2^{ns}	7.1	126.79	1.5^{xxx}
V_{12} - 'Frans Halls'	2.1	96.23	-0.1^{ns}	4.2	75.00	-1.4^{000}
V_{13} - 'Mikado'	2.0	91.64	-0.2^{ns}	4.0	71.43	-1.6^{000}
V_{14} - 'Pandora´s Box'	1.4	64.15	-0.8^{000}	3.8	67.86	-1.8^{000}
V_{15} - 'Spits Beauty'	2.4	109.97	0.2^{ns}	5.8	103.57	0.2^{ns}
V_{16} - 'Stella de Oro'	1.5	68.73	-0.7^{000}	4.2	75.00	-1.4^{000}
V_{17} - 'Strawberry Candy'	2.2	100.81	0.0^{ns}	6.0	107.14	0.4^{ns}
Average (control)	***2.18***	***100.0***	***0***	***5.6***	***100.0***	***0***

$LSD_{5\%}$ = 0.3 cm
$LSD_{1\%}$ = 0.3 cm
$LSD_{0,1\%}$ = 0.5 cm

$LSD_{5\%}$ = 0.5 mm
$LSD_{1\%}$ = 0.7 mm
$LSD_{0.1\%}$ = 1.0 mm

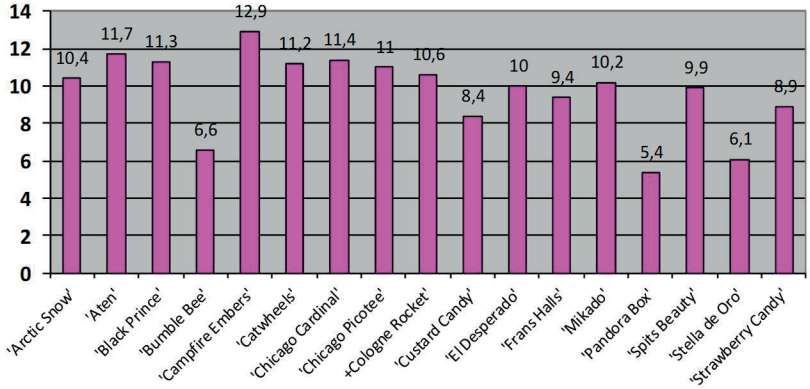
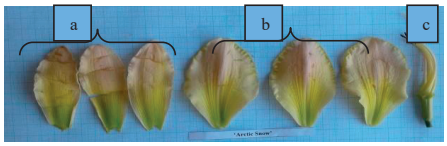
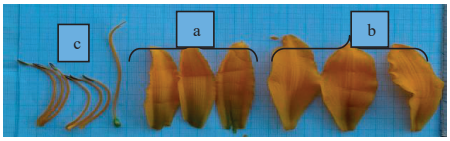


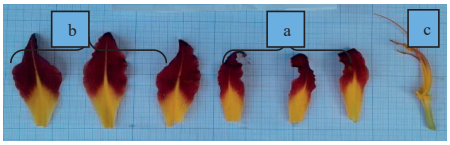
Figure 3. Total length of the perigon (cm)



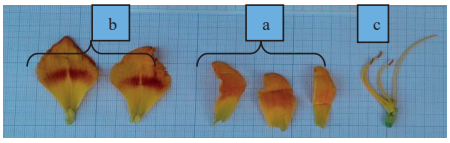
‘Arctic Snow’



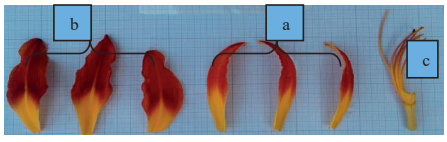
‘Aten’



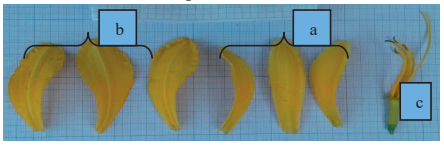
‘Black Prince’



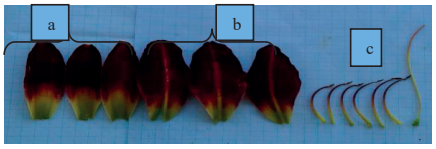
‘Bumble Bee’



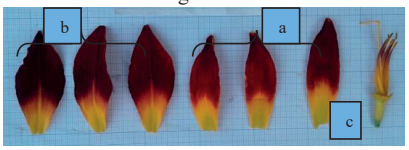
‘Campfire Embers’



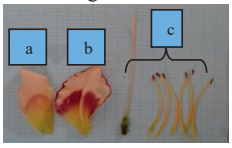
‘Cartwheels’



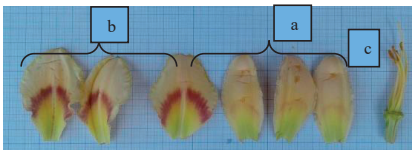
‘Chicago Cardinal’



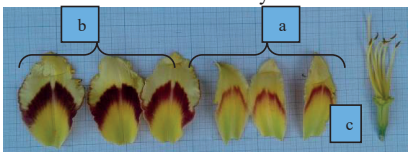
‘Cologne Rocket’



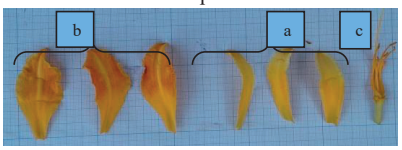
‘Chicago Picotee Memories’



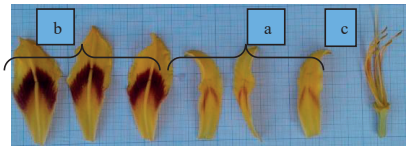
‘Custard Candy’



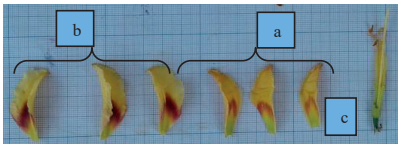
‘El Desperado’



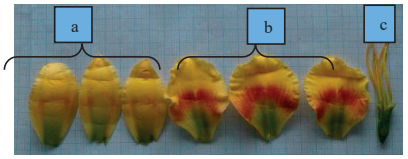
‘Frans Halls’



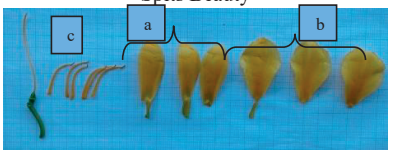
‘Mikado’



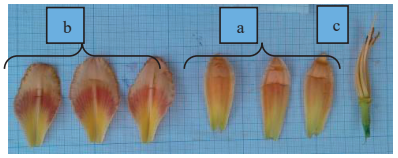
‘Pandora´s Box’



‘Spits Beauty’



‘Stella de Oro’



‘Strawberry Candy’

Figure 4. Components of the flowers in daylilies cultivars (original photo): a) outer perigonal lobes; b) inner perigonal lobes; c) stamens, pistil, perigonal tube, pedicel

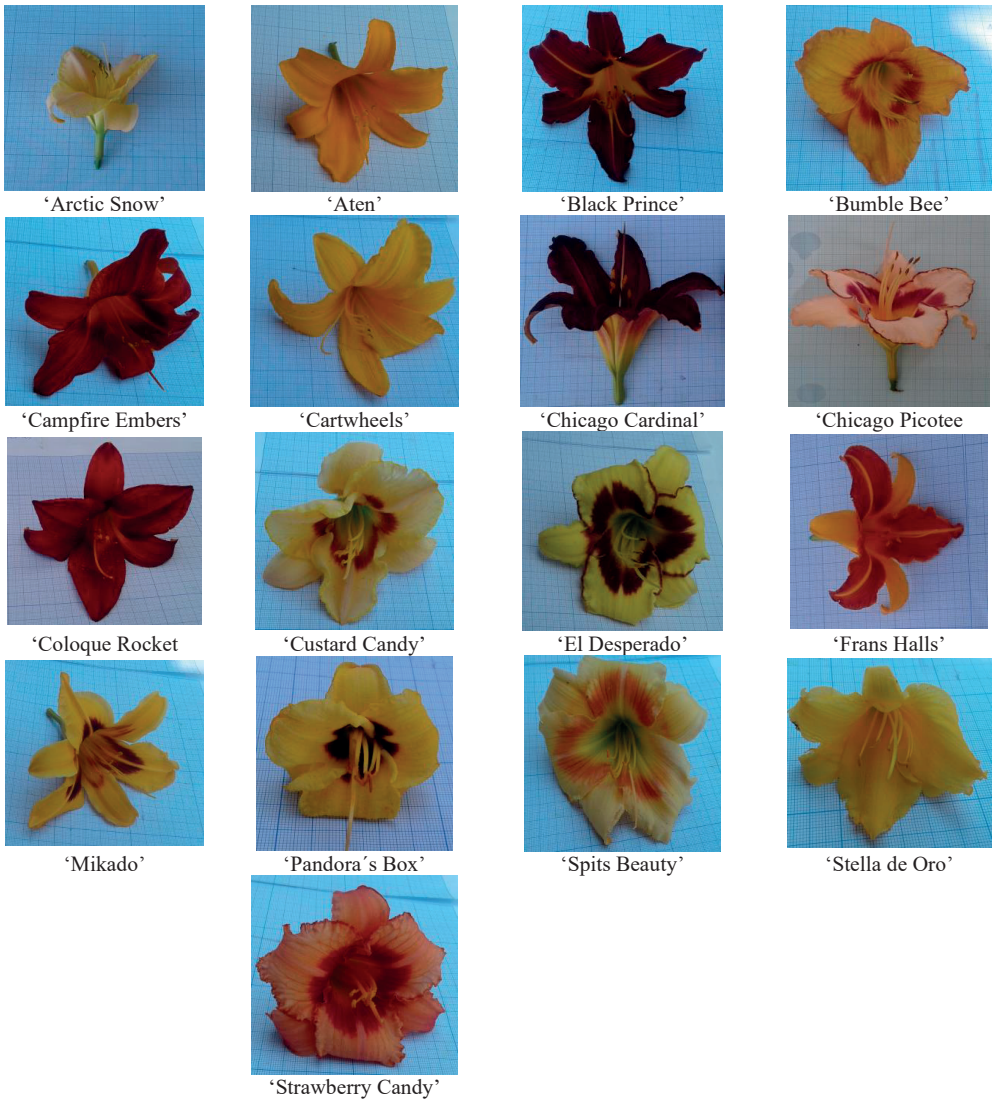


Figure 5. Aspect of the flowers in daylilies cultivars

The daylilies hybrids present a remarkable range of colours, the only colours which are not present being the pure white and the blue. The tepals can be in one colour, two colours (the outer tepals differently coloured from the inner ones) or they may present variations given by the colour of the “neck” of the flower’s cup, and also by the patch from the central area of the tepals, which compose a ring in close or powerfully different shades from the main colour of the tepals (Figure 5). Depending on the colour of the tepals, the analyzed cultivars can be grouped as follows:

- tepals in approximately one colour or with variations very close to the basic colour: ‘Arctic Snow’ (cream-coloured or light yellow tepals, eventually with a slightly green neck); ‘Aten’ (yellow-orange tepals), ‘Stella de Oro’ (powerful bright yellow tepals);
- tepals without patch, but with the neck of the cup in contrasting colours: ‘Black Prince’ (red-purple tepals, with black reflexes and a yellow-golden neck), ‘Campfire Ember’ (red-scarlet or red-orange tepals with a yellow-golden neck), ‘Chicago Cardinal’ (bright red tepals and yellow-

green neck), 'Cologne Rocket' (red tepals and yellow golden neck of the flower);
- tepals with patch to the median area, where they form a contrasting ring: 'Bumble Bee' (yellow-golden tepals, with a contrasting red-brown or brown ring), 'Chicago Picotee Memories' (cream- tepals with violet patch), 'Custard Candy' (bright yellow tepals with a brownish ring), 'El Desperado' (yellow-butter tepals, with an obvious violet or purple ring), 'Mikado' (yellow tepals and a red-brick center of the cup), 'Pandora´s Box' (yellow-cream to ivory tepals, with a purple ring), 'Spits Beauty' (yellow tepals, with a pink-red ring), 'Strawberry Candy' (pink to pink-salmon tepals, with red ring);
- bicolored tepals (different colours of the outer and inner tepals): 'Frans Halls' (golden outer tepals, red-orange inner tepals).

The decor duration of the ornamental plants represents one of the major interest features concerning their use. From this point of view, the most appreciated are the plants which have the capacity to decorate for a longer period of time, regardless the way in which the decor is provided. It is well known that the plants with flowers may decorate through flowers, leaves, flowers and leaves, fruits, aspect, etc. The daylilies species and cultivars are known as plants which decorate both through the flowers arranged in composed inflorescences, and also through the linear leaves, slightly bent, elegant, which ensure the maintenance of a beautiful aspect of the bushes since early spring until late in the autumn, when the coldness comes. The decorative effect provided not only by the flowers, but also by the leaves, represent an advantage for the daylily, if we take into account that the flowering period is of approximately one month (in June-August); during the rest of the vegetation period, since early spring until autumn, the leaves are the ones that remain as the ornamental element.

Although the meteorological conditions from the analyzed period (especially the spring and summer beginning temperatures) registered differences from one year to another, the main phenophases of the daylilies varieties fitted in quite close intervals, with very little variations, from one year to another. In the years 2014-2020, the averages of the temperatures from March, when the summer daylilies start in vegetation, are, in most cases, over the multi-annual average (3.9^0C), with values between 1.9 and 7.2^0C (the biggest difference being registered in 2020, when the average temperature of March was 11.1^0C). Only in 2018 the average temperature of March was under the multi-annual average (with 2.8^0C). Also, April has the same tendency of exceeding the multi-annual temperature which characterizes the conditions from Iasi (10.5^0C), but with smaller differences (0.2-4.8^0C). The months of April from 2015 and 2017 were colder. Regarding the precipitations form March and April, in March were quantities of precipitations which exceeded the multi-annual only in 2015, 2017 and 2018, in other years the weather was drier. The biggest deficit was registered in 2019, with only 8.1 mm as opposed to 30.9 mm the multi-annual average. April was more dry, especially during the last three years, when the precipitations had a total of 6.4 mm in 2018, 6.9 mm in 2019 and 1.8 mm in 2020, as opposed to the multi-annual of 46.1 mm. With all these thermal and precipitations fluctuations, the phenology of daylilies took place within close limits, without major deviations. Thus, during the seven years of study, the start in vegetation at all the varieties took place in the interval 15-20 of March.

Differences between the varieties appear at the other phenophases, but at the same variety the starting of the phenophases fits within the limits of a maximum of one week. The periods in which the floral buds rods appear may be grouped as follows:
- 22-28 March - for the varieties 'Stella de Oro', 'Chicago Picotee Mem.';
- 5-10 June - for the varieties 'Campfire Embers', 'Custard Candy', 'Pandora´s Box', 'Spits Beauty', 'Strawberry Candy';
- 10-15 June - for the varieties 'Cartwheels', 'Chicago Cardinal';
- 12-18 June - for the varieties 'Bumble Bee', 'Cologne Rocket', 'Mikado';
- 16-20 June - for the varieties 'Arctic Snow', 'Black Prince', 'El Desperado';
- 20-25 June - for the varieties 'Aten', 'Frans Halls'.

One of the most important phenophases from an ornamental point of view is represented by the flowering. The start of the flowering defines the varieties as being early-flowering or late-flowering. At the analyzed varieties, the period of flowering lasted since the beginning of June until

the beginning of July. The most early-flowering was 'Stella de Oro', with a flowering from the first decade of June (1-10 of June), sometimes even from the last part of May, followed at approximately one week by 'Chicago Picotee Memories', varieties from the first group depending on the period of formation of the floral buds rods. During the period 20-27 of June, more cultivars start their flowering, namely the ones from the second group: 'Campfire Embers', 'Custard Candy', 'Pandora´s Box', 'Spits Beauty', 'Strawberry Candy'. Starting with 23-25 of June 'Bumble Bee', 'Cologne Rocket' and 'Mikado' start their flowering, and after the 1st of July, the rest of the varieties ('Arctic Snow', 'Black Prince', 'El Desperado', 'Aten', 'Frans Halls').

The flowering ended during the interval form the half of July until the first decade of August.

The cultivar 'Chicago Picotee Memories', one of the varieties with early flowering, is the first to end its flowering, around the 12th-15th of July. The cultivar 'Stella de Oro' which, although it fits in the first group of flowering, after the first wave of flowering keeps on flowering until after the 1st of August (but with a reduced number of flowers). With an abundant flowering until the first decade of August were observed the varieties 'Aten', 'Frans Halls', 'Chicago Cardinal', 'Black Prince', 'El Desperado'.

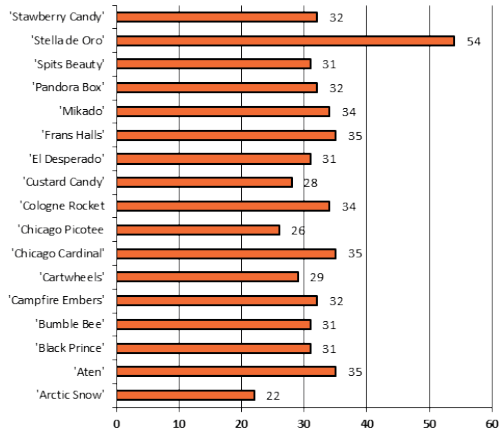


Figure 6. Flowering time of daylilies cultivars

The average of flowering time, respectively since the opening of the first flowers and until the complete fading of the last flowers varied between 22 and 54 days (Figure 6). But if we consider that 'Stella de Oro' registers 54 days of flowering through the extension with the second stage, generally weaker, we may notice that the common duration for the 17 varieties does not exceed 34-35 days. The shortest flowering period (22 days) was that of 'Arctic Snow'; a flowering duration of up to 30 days had 'Cartwheels', 'Chicago Picotee Mem.' and 'Custard Candy'. The longest flowering duration was that of 'Aten', 'Chicago Cardinal', 'Frans Halls'. The period in which most of the cultivars are flowered coincides with the month of July, with extensions in the last decade of June and the first decade of August. Also, as it was mentioned, the longest period of the vegetation season the plants decorate through their leaves.

CONCLUSIONS

At all the analyzed hybrids differences between the dimensions of the interior and the exterior lobes were noticed. The biggest differences were regarding the width, the exterior lobes being always wider than the interior ones (1.4-1.8 times, and at 'Stella de Oro' 2.4 times). The length registered smaller differences (0.2-0.8 cm), and in three cases (the cultivars 'Cartwheels', 'Spits Beauty' and 'Stella de Oro') the interior and the exterior lobes had the same length.

The total length of the perigon varied between 5.4 cm ('Pandora´s Box') and 12.9 cm ('Campfire Embers').

The exterior lobes fitted, at most of the cultivars, in spear-shaped or spear-shaped-elongated forms, with the proportion length/width over 3-4. More rounded (ovate) forms, with values of the proportion length/width 2-2.6 were registered at 'Custard Candy', 'Arctic Snow', 'Spits Beauty', 'Strawberry Candy', 'Bumble Bee', 'El Desperado'. The form of the interior lobes was more similar to the ovate one, the proportion between length and width being from 1.3 to 2.5 (except for the 'Black Prince' and 'Campfire Embers' cultivars with 3.1, respectively 2.9).

Bigger differences in colour between the interior and the exterior tepals were noticed, generally, at the cultivars with two coloured cultivars ('Frans Halls') and at those characterized through the presence of the median patch, more intensely coloured at the

interior ones (‘Bumble Bee’, ‘Chicago Picotee Memories’, ‘Custard Candy’, ‘El Desperado’, ‘Mikado’, ‘Pandora´s Box’, ‘Spits Beauty’, ‘Strawberry Candy’).

The flowering time at the daylilies hybrids was of approximately one month, shorter (approx. three weeks) at the ‘Arctic Snow’ cultivar. At ‘Stella de Oro’ cultivar, although approximately 7-8 weeks were registered until the last flowers faded (due to the repeated flowering), the quality of the flowering in the second part of the interval was much diminished.

The period in which most of the cultivars were blooming coincides with the month of July (with extensions in the last decade of June and the first decade of August).

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VEGETATION HABITATS MAPPING IN VĂCĂREȘTI NATURAL PARK

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Abstract

The study aims to identify and map the types of vegetation habitats with the indication of tree and palustrine species which had spontaneously developed in the last 30 years in Văcărești Natural Park in Bucharest. The park is surrounded by a concrete dam. The research was carried out on the surface of 156 ha within the dam. The vegetation mapping was done through visual analysis and interpretation of the satellite maps. The classification of the 11 identified habitats was made according to 2 major criteria: the tree coverage (groves, dense groups, isolated groups, herbaceous layer) and the percentage of soil moisture, evaluated according to the type of vegetation encountered (mesophytes, mesohygrophytes, hygrophytes). The results present the spatial distribution and percentage cover of each type of plant habitat and also their percentage cover regarding specifically soil moisture and vegetation structure, the value and the variation of EVI (Enhanced vegetation index) and NDVI (Normalized difference vegetation index). The mapping provides data for future studies regarding the ecological effect and for developing a conservation management plan for one of the largest urban wetlands in Europe.

Key words*: Urban wetland assessment, Văcărești Natural Park, Vegetation habitats mapping.*

INTRODUCTION

The main interest of the study is to identify and map the typologies and vegetation units of Văcărești Natural Park, declared the first urban natural area of Romania in 2016, by government decision (Guvernul României, 2016).
This naturally young ecosystem, located in South-Eastern Bucharest, has been formed inside a concrete dam of an abandoned reservoir during the last 30 years. The area is also one of the largest urban wetlands in Europe. The main objective of this study is to provide a basis in the developing of the park's management plan. The study will provide a zoning of the vegetation habitats found in Văcărești Natural Park. The management plan will use this mapping to include other elements, such as a tree inventory, plant species identification, management of the existing water covered areas, animal habitats and other necessary works to conserve and develop the biodiversity of this protected area.
This study provides the first mapping in a protected urban wetland in Romania of the distribution of two main types of vegetation: tree and swamp vegetation. The results will be useful for a closer evolution monitoring of the dendrological and palustrine vegetation, as well as of the ecological succession in general.

MATERIALS AND METHODS

The mapping of the areal of Văcărești Natural Park regarding the trees and palustrine vegetation was developed on the basis of the retrieved information from several similar studies. (Anastasiu et al., 2017; Doniță et al., 2005; Stoican et al., 2014). Therefore, 11 habitat types were identified, each of them include 1 to 3 dominant species of trees/palustrine herbaceous plants. This means that individuals belonging to these species occupy a significant land coverage compared to the other species identified in a specific habitat.
The study comprises the following components:
1. Main habitats general mapping plan;
2. Individual sheets for each habitat;
3. Inventory statistic with the habitat data (areas and statistics);
4. Differential plans of habitats and statistics (vegetation structure, soil humidity, EVI, NDVI, vegetation dispersion).

The habitats were classified by 2 main axes:
A. Vertical axis - the land cover degree (groves, dense groups, isolated groups, herbaceous layer)
B. Horizontal axis - the degree of soil moisture, evaluated according to the type of vegetation encountered (mesophytes, mesohygrophytes, hygrophytes) (Figure 1).

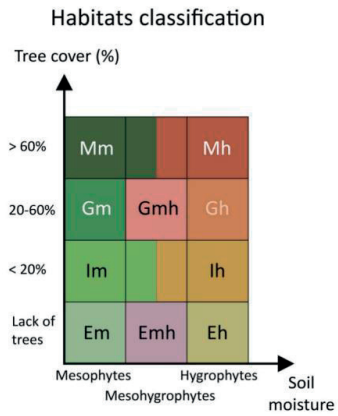

Figure 1. Habitats classification in Văcărești Natural Park according to tree cover and soil moisture degree

Each habitat was marked with a specific symbol e.g. Gm for dense mesophyte groups. The uppercase letters indicate the tree cover degree, while the lowercase show the type of vegetation according to the level of soil moisture. Based on this classification, the resulting habitats (Figures 1 and 2) are:
1. Mm - Mesophyte groves of *Juglans regia*, *Ulmus minor* and *Prunus* spp.;
2. Gm - Dense mesophyte groups of *Juglans regia*, *Ulmus minor* and *Prunus* spp. with mesophyte steppe vegetation;
3. Im - Mesophyte steppe communities with isolated groups of *Juglans regia*, *Ulmus minor* and *Prunus* spp.;
4. Em - Herbaceous mesophyte steppe communities;
5. Gmh - Mesohygrophyte groups of *Salix* spp. with *Prunus* spp. and *Ailanthus altissima*;
6. Emh - Herbaceous mesohygrophyte steppe communities;
7. Mh - Hygrophyte groves of *Salix* spp. and *Populus* spp. with *Phragmites* sp.;
8. Gh - Dense hygrophyte groups of *Salix* spp. and *Populus* spp. with *Phragmites* sp.;
9. Ih - Hygrophyte communities of *Phragmites australis* with isolated groups of *Salix* spp.
10. Eh - Herbaceous hygrophyte communities of *Phragmites* sp. and *Typha* spp.;
11. Aquatic habitat - Danubian communities of *Lemna minor, Lemna trisulca, Spirodella polyrhiza* and *Wolffia arrhiza*;
The herbaceous mesophyte and mesohygrophyte communities will be mapped in a further study.
The methodology comprised the following steps:
- Field studies for habitat type identification, taking into account the dominant species encountered in each area; the species were identified using local scientific literature (Ciocîrlan, 1990; Iliescu, 2003) and previous studies conducted in Văcărești Natural Park (Anastasiu et al., 2017; Doniță et al., 2005; Stoican et al., 2014);
- Tree canopy and palustrine vegetation mapping through satellite images from 2019;
- Classification and mapping of the habitat types, based on field and satellite assessment, considering the dominant species found in each area, the vegetation covering degree and the moisture level of the soil (Figure 1);
- Checking of the preliminary mapping results on the field and final editing of the general mapping plan (Figure 2);

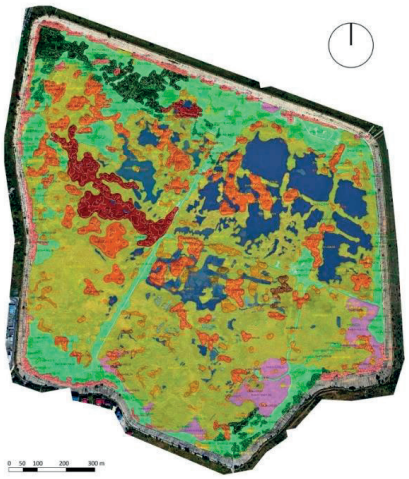

Figure 2. Habitats distribution in Văcărești Natural Park. General plan

- Elaboration of the habitats percentage cover sheet (Figure 3);

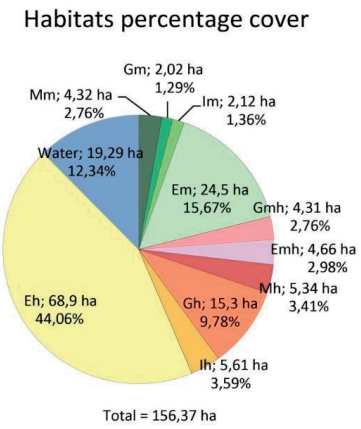

Figure 3. Habitats percentage cover in Văcărești Natural Park

In order to present the results of the study, the following specific mapping plans were elaborated:

a) Tree canopy cover (stratification and structure of the vegetation) (Figure 4);

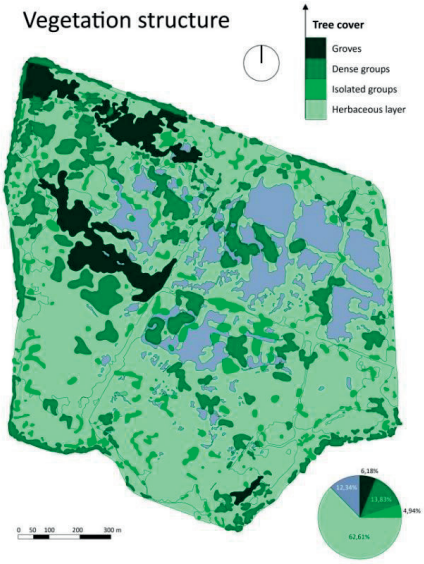

Figure 4. Vegetation structure in Văcărești Natural Park

b) Classification of vegetation zones according to soil moisture (Figure 5);

c) EVI analysis (Enhanced Vegetation Index - evaluation of the density of vegetal biomass mapped by remote sensing - minimum 0; maximum 0.7) (source: agrimonitoring.com, July 2019) (Figure 6);

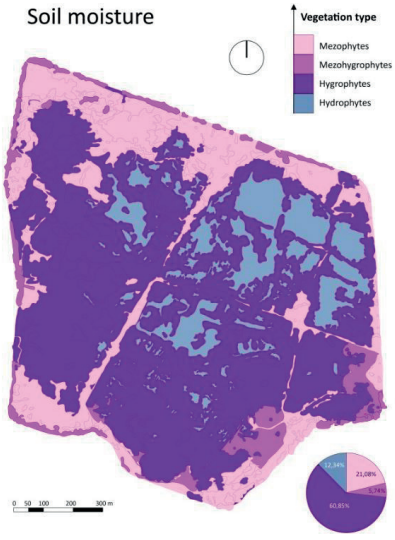

Figure 5. Vegetation type depending on soil moisture in Văcărești Natural Park

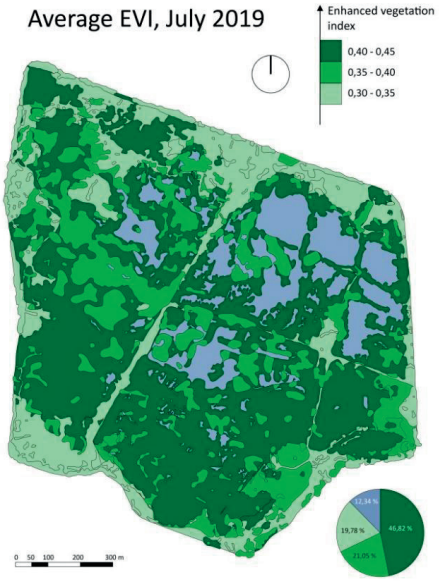

Figure 6. Average EVI in Văcărești Natural Park, July 2019

d) NDVI analysis (Normalized Difference Vegetation Index - evaluation of the amount of chlorophyll mapped by remote sensing - minimum 0; maximum 0.9) (source: agrimonitoring.com, July 2019) (Figure 7);

e) Vegetation dispersion (the predominant type of plants propagation encountered in each habitat) (Figure 8).

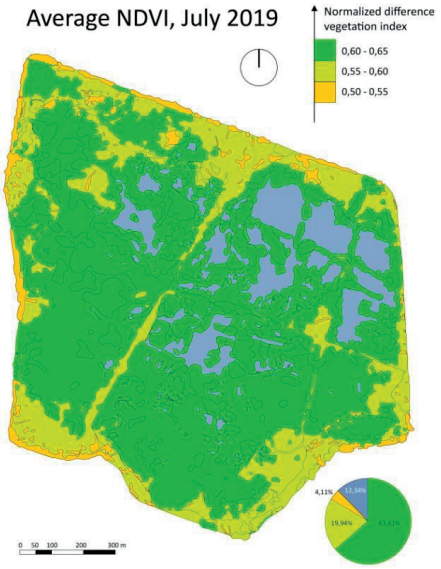

Figure 7. Average NDVI in Văcărești Natural Park, July 2019

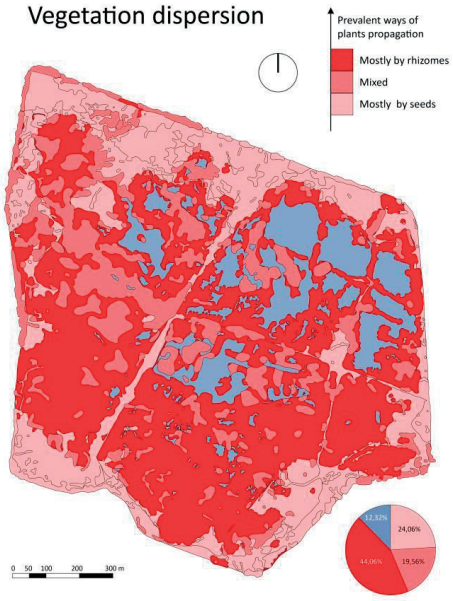

Figure 8. Vegetation dispersion in Văcărești Natural Park

RESULTS AND DISCUSSIONS

The results following the analysis, revealed data about the main native and exotic species, the main invasive dendrological species and data about EVI and NDVI indices, regarding the layering of the vegetation levels.

The main native species are: *Ulmus pumila, Juglans regia, Prunus spinosa, Prunus cerasifera, Fraxinus angustifolia, Acer pseudoplatanus, Acer platanoides, Populus alba, Populus nigra.* Other notable species*: Populus × canescens* and *Malus domestica.*

The main exotic species are: *Ailanthus altissima, Acer negundo, Salix babylonica, Salix matsudana 'Tortuosa', Elaeagnus angustifolia, Gleditsia triacanthos, Morus alba, Fraxinus pennsylvanica, Fraxinus americana, Celtis australis.* Other notable species*: Catalpa bignonioides, Celtis occidentalis, Prunus armeniaca, Prunus persica, Prunus cerasus, Robinia pseudoacacia.*

Invasive arborescent species were identified in isolated groups inside the park and are mainly composed of *Ailanthus altissima* and *Acer negundo*. Other present species with invasive behaviour, but who do not form isolated groups yet, are: *Elaeagnus angustifolia*, *Salix babylonica* and *Salix matdusanda 'Tortuosa'.*

The palustrine vegetation is predominantly composed of two native water grasses species: *Phragmites australis* and *Typha angustifolia* located mostly in the Eh habitat, which has the highest coverage in the park's area: 68.9 ha (44.06%) (Figures 2 and 3)

More than 90% of this surface is covered with *Phragmites sp.* Although this, we can identify also palustrine vegetation of hygrophyte grasses that are found at the base of the tree groups in the Mh, Gh, Ih and Gmh habitats. The predominant species as a component of these groups is *Phragmites australis*, which is also prevalent in the rest of the park.

In terms of vegetation stratification, the herbaceous level is the prevalent layer (over 62%), the tree layer being divided into different components like: groves, dense groups or isolated groups occupying less than 25% of the total area of the park (Figure 4).

The EVI and NDVI analysis between 2015 and 2019 reveals that these indicators have maximum values in the summer (June-July) and shows insignificant differences, due to the comparison of this situation in the last 4 years in the studied months. The values obtained in July 2019 on most of the park's area are above average (NDVI > 0.60, EVI > 0.35), which confirms a high density of biomass and a good state of vegetation health. The values are higher

especially in the areas covered with *Phragmites* sp. and in the case of dense groups and groves of trees (Figures 6 and 7).

CONCLUSIONS

As a general conclusion, there is a high level of plant biodiversity, with a high share of palustrine vegetation composed mainly of *Phragmites australis.* We also observed a tendency of expansion of the dendrological vegetation, which mainly includes species of *Salix, Ulmus* and *Juglans*. Also, the emergence of new isolated groups and the extension of the mature groups reveals a tendency to increase the dense deciduous trees habitats in the detriment of the grassland vegetation. The species identified in the park are mainly native, representative for the spontaneous flora encountered in urban environments in South-Eastern Romania.

In order to continue the research of the young and atypical ecosystem of Văcărești Natural Park and to carry out an integrated management plan for the area, we consider that the following studies should be conducted:

- Analysis of the evolution of the identified habitats (with emphasis on the groves and groups of tree vegetation and palustrine vegetation) over the last 30 years based on satellite maps;
- Assessment of the evolution of water surfaces and level differences by seasons and years;
- Individual inventory of tree specimens;
- Mapping of the herbaceous flora in the Emh and Em areas;
- Soil studies throughout the park;

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PARTIAL RESULTS ON THE LIFETIME OF FLOWERS IN VASES OBTAINED BY USING DIFFERENT SOLUTIONS OF SOME HERBACEOUS PEONY CULTIVARS

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Abstract

The present work is of a necessary topicality and is of major importance in that it presents observations and results that contribute to maintaining the flower life in vases for as long as possible, thus helping the herbaceous peony cultivators as cut flowers, flowerings but also people who love this flower for keeping and decorating peony flowers as long as possible. This research study brings to light the results regarding the use of traditional solution recipes in pots water for the longest storage of flowers in pots. The material is represented by cultivars: Festive Maxima, Dorren, Pink Giant, Kansas. In this research, 17 recipe solutions were used to keep flowers in vases. As research methods, biometric measurements, determinations and visual observations were used during the research period. The biometric measurements were made by the following indicators: phenophase in which the buds are; diameter of the buds; diameter of the flower stem; length of flower stems. The visual observations were made by the following indicators: flowering phenophases (semi-open bud, flower opening, total flower opening, flowering completion, petal shaking) and flower vase maintenance (days). Different results were observed regarding the lifetime of the flowers in pots depending on the cultivar used. This resulted in a minimum duration of storage of flowers in pots of 5 days until a maximum duration of storage of flowers in pots of 11 days from the moment of harvesting from the plant.

Key words*: cut flowers, peony, solutions, vases.*

INTRODUCTION

This paper is of great importance, being also topical, due to the fact that it presents notes and remarks that contribute to the flowers' keeping alive in vases as long as possible, thus helping the growers of herbaceous peony as cut flower, florists', and the people who love this flower, both with the commerce with cut flowers and the prolonged preservation of these flowers.

This research study makes known the results regarding the use of the traditional solution recipes in the vase water, in order to preserve the flowers in vases as long as possible. The quality of the cut flowers depends mainly on the flower form, color, fragrance and the mechanical resistance of the inflorescence stem. In recent years the peony has become more and more popular globally as cut flower. Millions of cut peony stems are sold annually to different countries and regions by means of auctions held in Holland. Yet, the natural blooming period of the peony is short, a fact that affects seriously its industrial development. The commune method of prolonging its economic viability consists in its keeping at a low temperature after cutting, yet the storage time is limited (approximately 20-30 days on the average) and its quality is not stable after its storage. Therefore, it is necessary to understand the effects of the cut peony storing at low temperature and the mechanisms that form its basis, so as to improve the future post-harvest technologies.

The cut flowers are shoots detached from the mother-plant. After harvest, the link to the mother-plant is separated, and the flower shoot can no longer be supplied with water and nutrients from the mother-plant. However, the normal physiologic metabolism continues. The storage at low temperatures, which can be divided into dry storage (DS) and wet storage (WS), delays the senescence, prolongs the flowers' lifetime and reduces the caused damage to the bacteria of the flower sprays (Eason și colab., 2002; Shahri și colab., 2011).

It has been reported that some cut flowers are suitable for DS, such as roses (*Rosa hybrida* L.), whereas others are suitable for WS, that is lisianthus (*Eustoma grandiflorum* Salisb.), zinnia

(*Zinnia elegans* Jacquin) and freesia (*Freesia* × *hybrida* Bailey) (Ahmad et al., 2012). However, it is usually acknowledged that WS is mostly used for short-term storage (S-tS), while DS is more suitable for long-term storage (L-tS) (Ahmad et al., 2012; Reid, 2002).

During the post-harvest stage, the energy supply (mainly through starch hydrolysis and sucrose) is vital for the blooming of the flowers, and the lack of saccharides diminishes seriously the quality of the cut flowers (van Doorn, 2004). Generally, the starch can be hydrolyzed into saccharide in order to release energy, and the sucrose can also be formed in the starch to store energy (Fernie et al., 2002; Roby et al., 2002). Sucrose phosphate synthase (SPS, EC 2.4.1.14) is a key enzyme for the catalysis of the sucrose biosynthesis and affects positively the sucrose and the starch in the plants, during the bidirectional process (Grof et al., 1998; Hashida et al., 2016).

Saccharose is one of the main photosynthetic items, which is synthetized in cytosol and then transported to the plant cells, and that being hydrolyzed into glucose and fructose, so as to release energy (Gifford et al., 1984). The saccharose can be hydrolyzed by two different enzymes *in vivo*: (i) sucrose synthase (SUS, EC 2.4.1.13) and (ii) invertase (INV; EC 3.2.1.26). SUS is associated with the saccharose that is hydrolyzed reversibly into UDP-glucose and fructose in the presence of UDP (Fallahi et al., 2008; Xu et al., 2012). In some plants, SUS plays an essential part due to the sucrose that enters the cellular metabolism, and it also can be implied in the biosynthesis of the cellular wall through the supply of UDP-glucose (Craig et al., 1999; Ruan et al., 2003). INV is associated with the irreversible hydrolysis of the saccharose into glucose and fructose. INV has proved to play the main parts in the development of the plants, as well as the resistance to biotic and abiotic tensions (Kulshrestha et al., 2013; Qian et al., 2016; Ruan, 2014; Tauzin and Giardina, 2014). INV can be further divided into three functions depending on the cell location, namely cell-wall invertase (CWIN), vacuolar invertase (VIN) and cytoplasmic invertase (CIN). The WINs play essential parts in the phloem discharge, the VINs have major roles regarding the sugar accumulation and the cell expansion, and the CINs are necessary for the development of the reproduction and the roots (Ruan, 2014; Wan et al., 2018).

In a research study, J. Xue et al. (2019) (Postharvest Biology and Technology, 155, 11–19), on "Evaluation of dry and wet storage regarding life quality of cut flowers kept in vases based on the metabolism adjustment of starch and sucrose" concludes the following:

- In comparison with the WS treatment, DS has improved significantly the life quality of the cut flowers of herbaceous peony kept in vases "Yang Fei Chu Yu" after both treatments S-tS and L-tS.
- For the S-tS treatment, the main factors that contribute to that model can be the faster consumption of starch during the storage and the sucrose hydrolysis at the beginning of the vase period.
- For L-tS treatment, the greater level of glucose and fructose at the end of the storage stage can contribute more to the higher quality of the flowers.
- The subsequent transcriptional analysis of nine representative genes for starch and the sucrose metabolism (including two SPSs, two SUSs and five INVS) have supported these conclusions.
- Based on these results, DS is recommended for refrigeration storage. This recommendation occurs regardless the storage period, as long as the storage period remains within the accepted period.

MATERIALS AND METHODS

The research was carried out in the town of Singureni, Giurgiu County, in the personal household, in 2019. To perform this experiment a space for research was organized. The research material used in this experiment consists of peonies of some cultivars of herbaceous peony in their own gardens, as well as peonies from the University collection. The experiment was carried out in May 2019.

As research methods we used biometric measurements, findings and visual observations during the research period.

The biometric measurements were performed using the following indicators:

- Phenophase of buds;
- Diameter of buds;
- Diameter of floral shoot;
- Length of floral shoots

The visual observations were performed using the following indicators:

- Blooming phenophases (half-bloomed bud, flower blooming, complete blooming of flower, blooming closure, petal falling);
- The period of flower keeping in vase (day).

This research is two-factor experiment and consists of two elements: factor A and factor B. Factor A consists of the herbaceous peony cultivars: Festiva Maxima, Dorren, Pink Giant, Kansas. Factor B is represented by the solutions used to preserve the flowers in vases: aspirin, starch, sugar, white vinegar, vodka, hydrogen peroxide, lemon juice, mouthwash, sodium bicarbonate, carbonated juice, mineral water. By combining the two factors, 17 variants were found, which are presented in Table 1.

Table 1. Experimental variants of the research carried out in May 2019

Variaty	Cultivars	Solutions to preserve flowers
V1	Festiva maxima, Dorren, Pink Giant, Celebrity	Aspirin 1 pc/1 l of water
V2	Festiva maxima, Dorren, Pink Giant, Celebrity	3 drops of starch + 1 spoonful of sugar/1 l of water
V3	Festiva maxima, Dorren, Pink Giant, Celebrity, Kansas	3 spoonful of sugar + 2 spoonful of white vinegar/1 l of water
V4	Festiva maxima, Dorren, Pink Giant	3 – 4 drops of vodka/1 l of water
V5	Festiva maxima, Dorren, Pink Giant	1 spoonful of hydrogen peroxide/ 1 l of water
V6	Festiva maxima, Dorren, Pink Giant, Celebrity	Lemon juice 250 ml
V7	Festiva maxima, Dorren, Pink Giant, Kansas	1/4 spoonful of starch/1 l of water
V8	Festiva maxima, Dorren, Pink Giant, Kansas	1 spoonful of vinegar+ 3 spoonful of sugar + a few drops of starch/ 1 l of water
V9	Festiva maxima, Dorren, Pink Giant, Kansas	1 spoonful of vinegar + 2 spoonful of sugar + 1 pc of aspirin/1 l of water
V10	Festiva maxima, Dorren, Pink Giant, Kansas	1 spoonful of mouthwash + 1 spoonful of sugar/1 l of water
V11	Festiva maxima, Dorren, Pink Giant, Kansas	1 spoonful of hydrogen peroxide + 1 spoonful of apple vinegar + 1 spoonful of sodium bicarbonate/ 1 l of water
V12	Festiva maxima, Dorren, Pink Giant, Kansas	2 spoonful of lemon juice /250 ml of water
V13	Festiva maxima, Dorren, Pink Giant, Kansas	250 ml carbonated juice + 250 ml water + ½ spoonful of starch/1 l of water
V14	Festiva maxima, Dorren, Pink Giant	250 ml carbonated juice
V15	Festiva maxima, Dorren, Pink Giant	Mineral water
V16	Festiva maxima, Dorren, Pink Giant	1 part of juice + 3 parts of water + 2-3 drops of starch
V17	Festiva maxima, Dorren, Pink Giant	100 g sugar/1 l of water

Picture 1. Aspect of the cut flowers at the moment of flowers' introductions into the vases with solutions belonging to some herbaceous peony cultivars, May 2019

The research material used, as well as the studied characteristics of the cultivators' flowers are represented in Table 2.

Table 2. The characteristics of the peony cut flowers kept in vases with different preservation solutions

No.	Cultivar	Length of floral shoot (cm)	Diameter of floral shoot (mm)	Phenophase of the buds at harvest	Diameter of buds (mm)
1	Festiva maxima	50-60	4-7	Colored, closed bud	25
2	Dorren	50-60	3-6	Colored, closed bud	23
3	Pink Giant	40-50	4-5	Colored, closed bud	20
4	Celebrity	30	3-4	Colored, closed bud	17
5	Kansas	50	4-5	Colored, closed bud	19

Picture 2. Aspect of flowers belonging to some cultivars of herbaceous peonies, at the moment of their introduction into vases with different solutions, 2019

Picture 3. Aspect of buds belonging to some cultivars of herbaceous peonies, at the moment of their introduction into different solutions, 2019

Picture 4. Aspect of cut herbaceous peonies at their introduction into vases with different solutions, 2019

RESULTS AND DISCUSSIONS

This paper, with the help of the observations, measurements and findings performed during the research, led to some conclusions regarding the blooming phenophases of the herbaceous peony cultivars, the petal aspect after blooming, but especially the lifetime of the flowers kept in vases with different solutions (blooming duration).

The results obtained on the blooming phenophases are presented separated for each cultivar.

The blooming phenophases for the cultivar Festiva maxima and the lifetime of the flowers in vases are presented in table 3. It can be observed that variant V3 has a period of 5 days in vase until the flower blooming. It was noticed that the maximum lifetime of the flowers in vases was of only 5 days, and the maximum lifetime of the flowers in vases for V4 was of 11 days, respectively a lifetime period of de 10 days with variants V7 and V8.

Table 3. Blooming phenophases for the cut peony flowers kept in different solutions, cultivar Festiva maxima, May 2019

Variant/ phenophase	Date of flowers' introduction into vases	Half-bloomed colored bud	Blooming of flowers	Complete blooming of flowers	End of blooming	Aspect of petals	Blooming duration (days)
V1	20.05	22.05	23.05	24.05	30.05	falling	8
V2	20.05	22.05	23.05	24.05	29.05	falling	7
V3	20.05	23.05	25.05	26.05	01.06	falling	8
V4	20.05	21.05	22.05	23.05	01.06	falling	11
V5	20.05	22.05	23.05	24.05	27.05	falling	5
V6	20.05	22.05	24.05	26.05	31.05	falling	8
V7	20.05	22.05	24.05	25.05	02.06	falling	10
V8	20.05	22.05	23.05	24.05	01.06	falling	10
V9	20.05	22.05	23.05	24.05	30.05	falling	8
V10	20.05	22.05	24.05	25.05	31.05	falling	8
V11	20.05	22.05	23.05	24.05	30.05	falling	8
V12	20.05	22.05	23.05	24.05	31.05	falling	9
V13	20.05	21.05	22.05	23.05	29.05	falling	8
V14	20.05	21.05	22.05	23.05	28.05	falling	7
V15	20.05	21.05	22.05	23.05	30.05	falling	9
V16	20.05	22.05	23.05	24.05	31.05	falling	9
V17	20.05	21.05	22.05	23.05	30.05	falling	9

Having analyzed the data presented in Table 4 on the flowers' duration in the vases for the Dorren cultivars, it can be noticed a period of 2 or three days of the herbaceous peony buds in vases till the blooming beginning. Also, it can be noticed a minimum lifetime of the peonies in vases at the variants: a lifetime of 7 days for the variants V1, V4, V11, and V12. The maximum lifetime noticed for the Variant V7 of peonies in vases of 11 days, followed by variants V13, V15, V16 with a lifetime of only 10 days.

Table 4. Blooming phenophases for the cut peonies kept in different solutions, cultivar Dorren, May 2019

Variant/ Phenophase	Date of flowers' introduction into vases	Half-bloomed colored bud	Blooming of flowers	Complete blooming of flowers	End of blooming	Aspect of petals	Blooming duration (days)
V1	20.05	22.05	23.05	24.05	29.05	petal wilting	7
V2	20.05	21.05	22.05	23.05	29.05	falling	8
V3	20.05	21.05	22.05	23.05	30.05	falling	9
V4	20.05	22.05	23.05	24.05	29.05	falling	7
V5	20.05	21.05	22.05	23.05	30.05	petal wilting	9
V6	20.05	21.05	22.05	23.05	30.05	petal wilting	9
V7	20.05	21.05	22.05	23.05	01.06	falling	11
V8	20.05	21.05	22.05	23.05	30.05	petal wilting	9
V9	20.05	21.05	22.05	23.05	29.05	falling	8
V10	20.05	21.05	22.05	23.05	29.05	falling	8
V11	20.05	22.05	23.05	24.05	29.05	petal wilting	7
V12	20.05	22.05	23.05	24.05	29.05	falling	7
V13	20.05	21.05	22.05	23.05	31.05	falling	10
V14	20.05	21.05	22.05	23.05	30.05	falling	9
V15	20.05	22.05	23.05	24.05	01.06	falling	10
V16	20.05	21.05	22.05	23.06	31.05	falling	10
V17	20.05	21.05	22.05	23.05	30.05	petal wilting	9

Having analyzed the data presented in Table 5 on the lifetime of the flowers in vases for the cultivar Pink Giant, it can be noticed a duration of 1 and 2 days in vase of the herbaceous peony buds till the blooming start. Also, it can be noticed a minimum lifetime of 8 days for the

peony flowers in vases of the variants: V6 and V12. It was noticed a maximum lifetime period of 11 days of the peony buds in vases for the variants V5, V8, V9, V10, V11 and V15, followed by a period of only 10 days for the variants V2, V4, V13 and V14.

Table 5. Blooming phenophases of cut peonies kept in different solutions, cultivar Pink Giant, May 2019

Variant/ phenophase	Date of flowers' introduction into vases	Half-bloomed colored bud	Blooming of flowers	Complete blooming of flowers	End of blooming	Aspect of petal	Blooming duration (days)
V1	20.05	22.05	23.05	24.05	31.05	falling	9
V2	20.05	22.05	23.05	24.05	01.06	falling	10
V3	20.05	21.05	22.05	23.05	30.03	falling	9
V4	20.05	22.05	23.05	24.05	01.06	falling	10
V5	20.05	21.05	22.05	23.05	01.06	falling	11
V6	20.05	22.05	23.05	24.05	30.05	falling	8
V7	20.05	21.05	22.05	23.05	30.05	falling	9
V8	20.05	21.05	22.05	23.05	01.06	falling	11
V9	20.05	21.05	22.05	23.05	01.06	falling	11
V10	20.05	21.05	22.05	23.05	01.06	falling	11
V11	20.05	21.05	22.05	23.05	01.06	falling	11
V12	20.05	22.05	23.05	24.05	30.05	falling	8
V13	20.05	21.05	22.05	23.05	31.05	falling	10
V14	20.05	22.05	23.05	24.05	01.06	falling	10
V15	20.05	21.05	22.05	23.05	01.06	falling	11
V16	20.05	21.05	22.05	23.05	30.05	falling	9
V17	20.05	21.05	22.05	23.05	30.05	falling	9

It can be observed from the data presented in Table 6 on the lifetime of peony flowers in vases for the cultivar Kansas that the blooming of flowers took place after 1 and 2 days since their introduction in vases with different solutions. Continuing the analysis of Table 6, it can be noticed a minimum lifetime of 7 days for the peony flowers, variant V13, and a maximum period of 10 days to keep peony flowers for the variants V10 and V12.

Table 6. Blooming phenophases of the cut flowers kept in different solutions, cultivar Kansas, May 2019

Variant/ phenophase	Date of flowers' introduction into vases	Semi-open colored bud	Blooming of flowers	Complete blooming of flowers	End of blooming	Aspect of petal	Blooming duration (days)
V3	20.05	21.05	22.05	23.05	29.05	falling	8
V7	20.05	21.05	22.05	23.05	30.05	falling	9
V8	20.05	21.05	22.05	23.05	29.05	falling	8
V9	20.05	22.05	23.05	24.05	30.05	falling	8
V10	20.05	22.05	23.05	24.05	01.06	falling	10
V11	20.05	22.05	23.05	24.05	30.05	falling	8
V12	20.05	22.05	23.05	24.05	01.06	falling	10
V13	20.05	22.05	23.05	24.05	29.05	falling	7

After analyzing the data presented in Table 7 on the lifetime of the flowers kept in vases for the cultivar Celebrity, it can be noticed that there is a period of 1 and 2 days in vases of the herbaceous peony buds till the blooming start. Also, it can be noticed a minimum lifetime of 6 days for the peony flowers kept in vases for variant V1. It was noticed a maximum lifetime of 8 days for peony flowers kept in vases, variants V3 and V6, followed by a lifetime period of only 7 days for variant V2.

Table 7. Blooming phenophases of the cut peonies kept in different solutions, cultivar Celebrity, May 2019

Variant/ phenophase	Date of flowers' introduction into vases	Half-bloomed colored bud	Blooming of flowers	Complete blooming of flowers	End of blooming	Aspect of petals	Blooming duration (days)
V1	20.05	21.05	22.05	23.05	27.05	falling	6
V2	20.05	22.05	23.05	24.05	29.05	falling	7
V3	20.05	21.05	22.05	23.05	29.05	falling	8
V6	20.05	21.05	22.05	23.05	29.05	falling	8

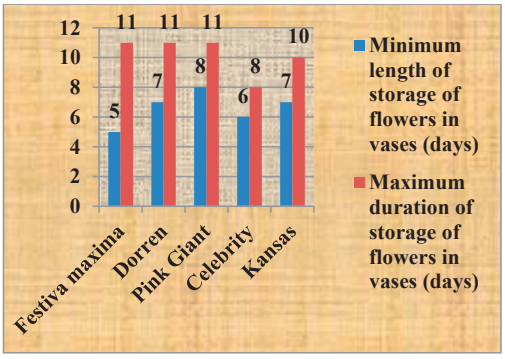
Picture 5. Aspect of flowers kept in vases with different solution, variants V1-V3, May 2019

Picture 6. Aspect of flowers kept in vases with different solutions, variants V4-V7, 2019

Picture 7. Aspect of flowers kept in vases, blooming of flowers, variants V1-V11, May 2019

Picture 8. Aspect of flowers in vases, blooming of flowers, variants V12-V17, May 2019

Observing the data in Picture 9 on lifetime of the flowers kept in vases with different solutions, it results a minimum lifetime period between 5 day for cultivar Festiva maxima, and a period of 8 days for the cultivars Pink Giant and Celebrity, as well as a lifetime period of the flowers between 8 days for the variant Celebrity and 11 days for the cultivars Festiva maxima, Dorren and Pink Giant



Picture 9. Lifetime period of the flowers kept in vases of some herbaceous peony cultivars

Picture 10. Aspect of flowers kept in vases, blooming of flowers, variants V1-V3, May 2019

Picture 11. Aspect of flowers in vases, blooming of flowers, variants V3-V6, May 2019

Picture 12. Aspect of flowers kept in vases, complete blooming of flowers, variants V1-V11, May 2019

Picture 13. Aspect of flowers kept in vases, flower complete blooming, V1-V11, May 2019

Picture 14. Aspect of blooming in vases, flower complete blooming, V12-V17, May 2019

Picture 15. Aspect in blooming in vases, complete flower blooming, variants V15-V17, May 2019

Picture 16 Aspect of flowers, blooming end and petal falling, May 2019

Picture 17. Aspect of flowers in vases, blooming end and petal fallings, May 2019

Picture 18. Blooming end and petal falling, May 2019

CONCLUSIONS

The research carried put led to the following conclusions:

- For cultivar Festiva maxima, the minimum period to preserve the flowers in vases was of 5 days for V5, and the maximum period to preserve the flowers in vase was of 11 days for V4 and 10 days for V8.
- For cultivar Dorren, the minimum period to preserve the flowers in vases was of 7 days for V1, V4, V11, V12, and the maximum period of flower preservation in vases was of 11 days for variant V7, followed by a period of only 10 days for the variants V13, V15, V16.
- For cultivar Pink Giant, the minimum preservation period was as follows: 8 days for the variants V6 and V12, and the maximum period of flower preservation in vases was of 11 days for the variants V5, V8, V9, V10, V11 and V15, followed by a period of only 10 days for the variants V2,V4,V13 and V14.
- For cultivar Kansas, the minimum flower preservation period was of 7 days for variant V7, and the maximum peony preservation period was of 10 days for variants V10 and V12.
- For cultivar Celebrity, the minimum flower preservation period was of 6 days for variant V1, and the maximum peony preservation period was of 8 days for the variants V3 and V6, followed by a period of only 7 days for the variant V2.
- The best solutions to preserve flowers in vases were those for variants V11, V10, V7, V8, V13, and V15.
- On a smaller scale, the traditional solutions can be recommendedto preserve the flowers in vases for as long as possible.

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STUDY REGARDING THE INFLUENCE OF SUBSTRATE TYPE ON SOME PARAMETERS OF GROWTH OF GERBERA SEEDLINGS

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Abstract

The study was conducted at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, the greenhouses Hortinvest. In the experiment I followed the influence of some factors on the germination of gerbera seeds. We used 12 types of substrate, V1-100% peat with pH 5.5; V2 - 100% peat with pH 4; V3 - 100% Perlite; V4 - 50% Perlite + 50% peat with 5.5 pH; V5 - 50% Perlite + 50% peat with 4 pH; V6 - 100 vermiculite; V7 - 50% vermiculite + 50% peat with pH 5.5; V8 - 50% vermiculite +50% peat with 4 pH. V9 jiffy seven peat pot; V10- pot with perlite; V11- pot with vermiculite and V12 pot of grodan, I tested the capacity of emergence at seeds stored in different condition. The varieties of gerbera were analized, regarding the percent of emergences and growth rate of the seedlings. The best variants with the highest percentage of seeds emerged were those where we used peat and mixed of perlite and peat with a pH of 5.5.

Key words: *Gerbera jamesonii, substrate, fertilizers, condition of growing.*

INTRODUCTION

Gerbera, is a species belonging to the *Asteraceae* family that comprises about 40 species and is also known as the "African daisy". *Gerbera jamesonii* is appreciated both as a potted flower but especially as a cut flower. This species is originary from South Africa, it has elegant flowers with single, double or multiple petals and come in various sizes and colors including white, yellow, orange, red and pink. The flower diameter is between 9-13 cm and there are four different classes of Gerbers with a single flower, semi-double flower, double flowers, and spider flowers. We can remark that each class delineates the number, position, and type of petals. The leaves of the gerbera are lobed, or pinnate, and often toothed.

Gerbera requires a lot of sun and grows in optimal conditions at temperatures of 20-21ºC. Different varieties of gerbera have different nutrient requirements depending on the stage, Savvas and Manos (1999), EunJoo et al. (2001), Savvas et al. (2003).

Gerbera can be propagated by seeds, vegetatively or *in vitro*. Vegetative propagation of gerbera plants gave better results compared to seed propagation (Nazma et al., 2012).

Propagation of gerbera by seed or in vitro (Pierik et al., 1973; 1975; Kanwar and Kumar, 2008), from a commercial point of view, is much longer, lasting, and growers prefer the method of dividing the bushes (Schiva, 1975; Reynoird et al., 1993).

In some varieties the propagation coefficient is very low and seed multiplication ensures an earlier production (Krause, 1988; Lisiecka, 1988; Mayer, 1992).

Usually, the plant of gerbera can be generally propagated both vegetatively by rhizome divisions and cuttings and generatively by seeds. This method by seeds produces a higher number of regenerants, the technique results in varied-regenerants and their performances (Kanwar and Kumar, 2008; Rukmana, 1995; Draghici et al., 2016).

One of the commonly used methods is clonal propagation via tissue culture works are importantly addressed in producing a large number of plants, uniform, vigorous and pathogen free in a short time (Mohammed and Azzambak, 2014).

The improvement of the species and the creation of new varieties of gerbera has been and is in the attention of many researchers (Cantor and Chis, 2006).

Seed wetting reduces the germination and emergence time of gerbera seeds (Harris et al., 2001). There is a serious problem in seed germination and uniform growth of gerbera seedlings (Tjia, 1984; Cockshull, 1985; Moe et

al., 1996) due to temperature fluctuations during nursery preparation (Farooq et al., 2004), which negatively affect flowering synchronization to all plants. So far, the increasing of germination of gerbera seeds induced by seed priming has been observed it has been observed.
William J. Carpenter and Eric R. Ostmark (1995) state that temperature and humidity affect the germination of gerbera seeds (*Gerbera jamesonii*). Also, keeping the seeds at temperatures from 5ºC to -5ºC or -10ºC did not significantly influence germination. Other authors recommend for seed germination temperatures of 21-24ºC (Ball, 1991)

MATERIALS AND METHODS

The experiment was carried in greenhouse conditions (in Hortinvest greenhouse). We used seeds of mix cultivars.
We used 12 experimental substrate variants: V1- 5.5 peat; V2- peat 4; 2 mm perlite V3; V4 mixture 50% peat 5.5, + 50% 2 mm perlite; V5 mixture 50% peat 4.0 + 50% perlite 2 mm; V6 vermiculite; V7 mixture 50% peat 5.5, + 50% vermiculite; V8 mixture 50% peat 4.0 + 50% vermiculite; V9 pots peat jiffy seven; V10- pots filled with perlite; V11- pots filled with vermiculite; V12 mineral wool pots
For each experimental variant we used 20 gerbera seeds. The seeds were 25 days old after harvest. I sowed seeds exposed for 5 days at a temperature of 20ºC and 4ºC.
The seeds exposed at 20ºC and 4ºC, respectively were sown in two variants, on the surface of the substrate without coating and with seed coating. The seeds were sown with the tip up, and for the variants where we covered the seeds we used a layer of about 0.5 cm substrate. The temperature in the greenhouse was maintained at 25ºC during the day and night until sunrise, then in the vegetation it was reduced to 20ºC during the day and 18ºC at night.
The seeds were sown according to the experimental substrate variants. Wetting was performed with nutrient solution with EC 0.5 and pH 5.5. We followed the percentage of emergence, the growth of seedlings in dynamics, the number of leaves in gerbera seedlings. Only the variants that responded very well and had well-formed leaves were retained. After planting in pots I watched the number of leaves, the appearance of flower buds and the number of flowers on the plant.
Data were analyzed statistically according to analysis of variance technique using analytical software and treatment means were compared using Tukey's test (Steel et al., 1997).

RESULTS AND DISCUSSIONS

The experiment in which we used seeds 25 days after harvest.
If we follow the total percentage of seeds sprouted after 8 days from sowing, we found that the variant in which the seeds had 25 days from harvest, sown on peat substrate with pH 5.5 showed the highest emergence of 92.0%.
A high percentage of emergence was also observed in the variant in which the seeds were kept in conditions of 20ºC, this being 87% when the seeds were covered at sowing. The lowest germination percentage was recorded for variant 5 (mixture 50% peat 4.0 + 50% perlite 2 mm) of 72% for seeds kept at 20ºC and uncovered. In the case of seeds stored at a temperature of 20ºC and sown in peat pots jiffy seven (V9) we recorded the lowest germination rate of 51% at 25 days old stored at 20ºC.
If we follow by comparison the germination percentage of the seeds kept in conditions of 20ºC in the variants with uncovered seeds and covered with a layer of substrate we found that the seeds in the case of the uncovered variant emerged faster, after 4 days after sowing.

Figure 1. Aspect of some experimental variants

Depending on the substrate, the percentage was 24% for V2 and V12 and 52% for V4 and V7. (Table 1). In the case of variants were seeds do not covered with substrate, after 4 days no seed emergence was recorded on any substrate variant, but after 6 days from sowing we noticed that in the uncovered variant the germination percentage was higher compared to the variant in which the seeds were covered.

After 6 days, the percentage of sprouted seeds was between 88% (V6) and 52% (V2) for the variant without covering the seeds with substrate. In the case of variants in which the seeds were covered with a mixing layer, the seeds emerged after 6 days in a percentage of 32% (V2) and 68% (V7) (Tables 1 and 2).

Analyzing the data from a statistical point of view, we find, in Table 1, that, compared to the control variant (peat substrate with pH 5.5), the meanings are negative distinctly very significant at V2, V5 and V9, and, insignificant at V3, V8, V10, V11 and V12.

At the same time, in the variant with coating of seeds, we counted from a statistical point of view very significant negative meanings at V2, V4, V5, V7, VV8, V9 and V12, compared to the control variant (Table 2)

Table 1. The evolution of seed germination at the variant without coating of seeds (seeds stored at 20°C)

Variants	After 4 days	After 6 days	After 8 days	Diferences		Significance
	%	%	%	%	% to Ct.	
V1	48	78	92	0.00	100.00	Control
V2	24	52	76	-16.00	82.61	OOO
V3	44	86	88	-4.00	95.65	N
V4	52	83	86	-6.00	93.48	OO
V5	32	68	72	-20.00	78.26	OOO
V6	44	88	87	-5.00	94.57	O
V7	52	84	87	-5.00	94.57	O
V8	44	84	88	-4.00	95.65	N
V9	32	68	78	-14.00	84.78	OOO
V10	48	87	89	-3.00	96.74	N
V11	48	86	88	-4.00	95.65	N
V12	24	72	89	-3.00	96.74	N
Average	41	78	85	-7.00	92.39	OO
			DL5= 4.110 DL5% in % = 4.4674 DL1% = 5.600 DL1% in % = 6.0870 DL01% = 7.540 DL01% in %= 8.1957			

Table 2. The evolution of seed germination at the variant with coating of seeds (seeds stored at 20°C)

Variants	After 4 days	After 6 days	After 8 days	Differences		Significance
	%	%	%	%	% to Ct.	
V1	0	61	87	0.00	100.00	Control
V2	0	32	66	-21.00	75.86	OOO
V3	0	37	75	-12.00	86.21	OO
V4	0	42	73	-14.00	83.91	OOO
V5	0	47	68	-19.00	78.16	OOO
V6	0	66	75	-12.00	86.21	OO
V7	0	68	73	-14.00	83.91	OOO
V8	0	33	47	-40.00	54.02	OOO
V9	0	46	51	-36.00	58.62	OOO
V10	0	66	77	-10.00	88.51	OO
V11	0	67	76	-11.00	87.36	OO
V12	0	37	66	21.00	75.86	OOO
Average	0	50.17	69.5	-17.50	79.89	OOO
			DL5%= 6.960 DL5% in % = 8.0000 DL1%=9.470 DL1% in% = 10.8851 DL01%=12.750 DL01%in%=14.6552			

If we follow by comparison, the percentage of seed germination, kept at 20ºC, with that of seeds kept at 4ºC, we estimate that, in case of seed storage at 4ºC and then sown on different types of substrate, the percentage of seed germination was zero after 4 days, in both variants without coverage and with seed coverage. After 6 days from sowing, for the variant without seed cover, the percentage of seedlings emergented was 32% at V12 and 48% at V3 and V4. In the case of the variant with seed cover at sowing, at V2, no seed emerged, and at V11 only 67%.

In the variant with seed cover, with substrate, the percentage of seeds sprouted was lower compared to the variant not covered with substrate, the percentage of sprouted seeds being 66% in V7 and V10 and only 47% in V4 and V9 (Tables 3 and 4).

At the variant without coating of seeds, we found an insignificant significance at V2, from a statistical point of view but in the most variants, we also found negative distinctly very significant, from a statistical point of view (Table 3). In the case of seed cover (seeds stored at 4°C) we noticed from a statistical point of view, very significant positive meanings, at V7 and V10 (Table 4).

Correlations made between the type substrate and the percentage of seeds sprouted after 6 days from sowing indicated minimum values of 52% for the variant stored at 20ºC and uncovered and 47% for the variant stored at 4ºC and covered with substrate. The highest percentage of emergence of 88% was recorded in the seed stored at 20ºC and uncovered with a standard deviation of 10.9461 and the lowest in the stored at 20ºC and covered, with a standard deviation of 4.9909 (Table 5).

Correlations made in order to see the influence of seed treatment conditions (stored at 20°C and 4°C, respectively), and substrate types, indicated a significant relationship to the variant with seeds stored at 4oC and then sown on substrates without cover the seed (Table 6).

After 8 days, the variant without seed cover, the lowest percentage of emergence was 47% recorded at V12, and the highest at 73% at V1.

Table 3. The evolution of seed germination at the variant without coating of seeds (seeds stored at 4ºC)

Variants	After 4 days	After 6 days	After 8 days	Diferences		Significance
	%	%	%	%	% to Ct.	
V1	0	47	73	0.00	100.00	Control
V2	0	37	58	-15.00	79.45	OOO
V3	0	48	68	-5.00	93.15	N
V4	0	48	57	-16.00	78.08	OOO
V5	0	41	61	-12.00	83.56	OOO
V6	0	37	63	-10.00	86.30	OO
V7	0	36	54	-19.00	73.97	OOO
V8	0	41	64	-9.00	87.67	OO
V9	0	41	47	-26.00	64.38	OOO
V10	0	42	52	-21.00	71.23	OOO
V11	0	42	51	-22.00	69.86	OOO
V12	0	32	47	-26.00	64.38	OOO
Average	0	41	57,92	15.08	79.34	OOO
			DL5% = 6.250 DL5% in % = 8.5616 DL1%= 8.500 DL1% in % = 11.6438 DL01%=11.450DL01% in %=15.6849			

Table 4. The evolution of seed germination at the variant coating of seeds (seeds stored at 4ºC)

Variants	After 4 days	After 6 days	After 8 days	Diferences		Significance
	%	%	%	%	% to Ct.	
V1	0	11	58	0.00	100.00	control
V2	0	0	51	-7.00	87.93	OO
V3	0	21	56	-2.00	96.55	N
V4	0	42	47	-11.00	81.03	OOO
V5	0	47	54	-4.00	93.10	N
V6	0	66	63	5.00	108.62	N
V7	0	66	66	8.00	113.79	**
V8	0	33	52	-6.00	89.66	O
V9	0	46	47	-11.00	81.03	OOO
V10	0	66	66	8.00	113.79	**
V11	0	67	64	6.00	110.34	*
V12	0	37	48	-10.00	82.76	OOO
Average	0	42	56	-2.00	96.55	N
			DL5% = 5.050 DL5% in % = 8.7069 DL1% = 6.880 DL1% in % =11.8621 DL01% = 9.260 DL01% in %= 15.9655			

Table 5. Standard deviation at the variants recorded at 6 days after sowing

Variable	Variants	Min.	Max.	Mean	Std. deviation
a.	12	52.00	88.00	78.00	10.9461
b.	12	32.00	48.00	41.00	4.9909
c.	12	32.00	68.00	50.17	14.4148
d.	12	47.00	66.00	56.00	7.3113

a. stored at 20 degrees C and uncovered; b. stored at 20 degrees C and covered; c. stored at 4 degrees C and uncovered; d. stored at 4 degrees C and covered

Table 6. Correlation matrix at the variants

Variables	stored at 20ºC and uncovered	stored at 20ºC and covered	stored at 4ºC and uncovered	stored at 4ºC and covered
a.	**1**			
b.	0.326	**1**		
c.	0.529	-0.016	**1**	
d.	0.571	-0.047	**0.851**	**1**

a.stored at 20 degrees C and uncovered; **b**. stored at 20 degrees C and covered; **c.** stored at 4 degrees C and uncovered; **d.** stored at 4 degrees C and covered

Values in bold are different from 0 with a significance level alpha=0,01

Analyzing the influence of seed storage treatment at 4oC and substrate type and emergence percentage, we found a significant positive correlation, the correlation coefficient being $R^2 = 0.7234$ between the variant stored at 4 degrees C and covered and the variant stored at 4 degrees C and uncovered (Figure 2).

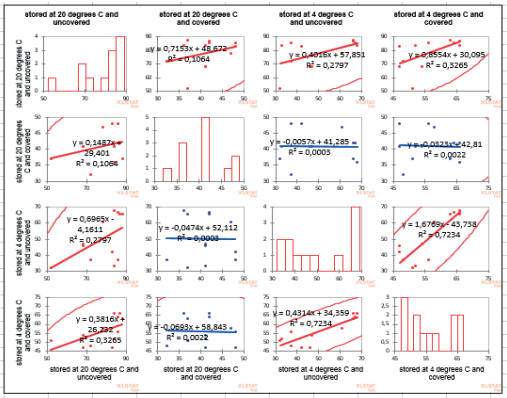

Figure 2. The influence of seed storage treatment, substrate type and percentage of emergence

The experiment in which we used seeds 25 days after harvest.

Gerbera seeds, 80 days old, stored at 20ºC and 4ºC had a very low germination in the case of sowing without cover. If we covered the seeds at sowing the percentage of emergence was zero.

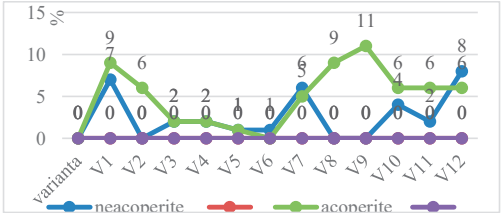

Figure 3. The percentage of the seeds emerged in the variants with seeds of 80 days after harvest

The number of leaves after 45 days from emergence in the variants stored at 20ºC covered and uncovered and stored at 4ºC covered and uncovered was different from one variant to another. Thus, we noticed the highest number of leaves per plant in V1 - peat substrate 5.5, in the variant without coating, this being 10.25 leaves / plant and 9.75 in V1 with coating. In the variant where we kept the seeds at a temperature of 4ºC the number of leaves per

plant was 8.25 leaves/plant in the uncovered variant and 6.55 leaves in the covered variant (Figure 4).

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
stored at 20 degrees C and uncovered no. leafes	10,25	5,50	6,25	7,25	5,50	6,75	6,00	5,75	5,25	6,25	5,25	5,75
stored at 20 degrees C and covered no. leafes	9,75	3,75	5,25	6,75	5,00	5,75	5,50	5,75	5,00	6,00	5,50	5,50
stored at 4 degrees C and uncovered no. leafes	8,25	4,25	5,25	6,65	4,75	5,00	5,75	5,50	5,25	5,75	5,50	4,25
stored at 4 degrees C and covered no. leafes	6,55	3,25	4,00	4,75	4,00	4,25	5,25	5,00	4,75	5,50	5,00	3,00

Figure 4. Number of leaves on plant

Analyzing the number of leaves per plant we noticed that there was a positive relationship (R^2=0.3191) depending on the type of substrate (Figure 5).

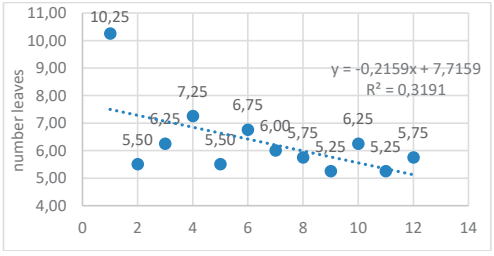

Figure 5. Influence of substrate type on the number of leaves formed on the plant in the variant with seeds stored at 20ºC and uncovered

At the time of the observations, 50 days after emergence, for the variant in which the gerbera seeds were covered with a thin layer of substrate, we noticed that the plants had a smaller number of leaves/plant and the substrate it had very little influence on the formation of leaves on the plant (R^2 = 0,0817) (Figure 6).

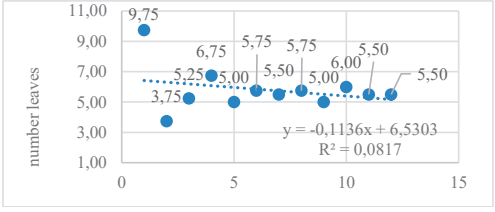

Figure 6. Influence of substrate type on the number of leaves formed on the plant in the variant with seeds stored at 20ºC and covered

In the variant where the seeds were kept at 4oC, and at sowing they were not covered, the plants after 50 days formed the smallest number of leaves, being between 4.25 leaves at V2 and V12 and 8.25 leaves at V1. Among the experimental variants we noticed relatively small influences (R^2=0.1479). We noticed between the experimental variants relatively small influence (R^2 = 0.1479) (Figure 7).

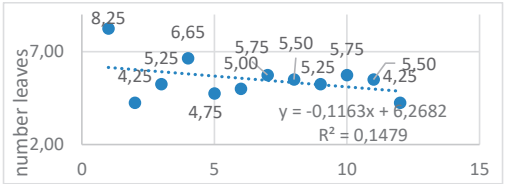

Figure 7. Influence of substrate type on the number of leaves formed on the plant in the variant with seeds stored at 4ºC and seed uncovered

In the variant where the seeds were kept at 4ºC, and when sown they were covered, the plants after 50 days formed a number of leaves of 3 at V12 and 6.55 leaves at V1, between the experimental variants was a relatively small influence (R^2 = 0,0127) (Figure 8).

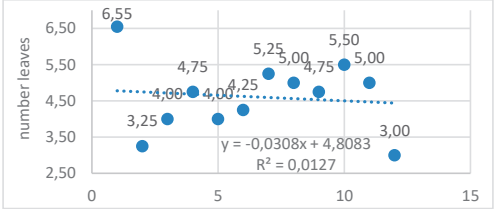

Figure 8. Influence of substrate type on the number of leaves formed on the plant in the variant with seeds stored at 4ºC and seed covered

Figure. 9. Aspect of gerbera plant in V1 - variant with seeds kept at 20ºC, uncovered - on peat substrate with pH 5.5 and V2 - peat with pH 4.5

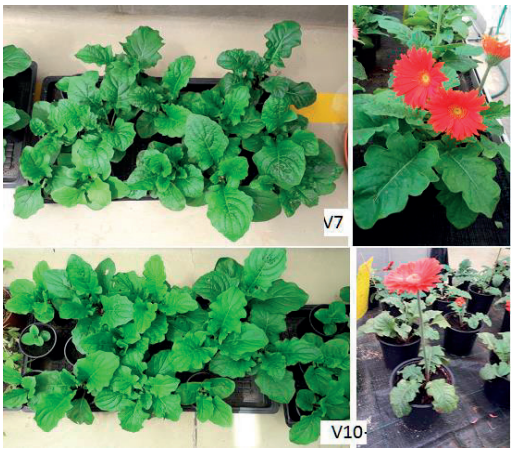

Figure. 10. Aspect of gerbera plant - variant with seeds kept at 20ºC, uncovered V7 and V10

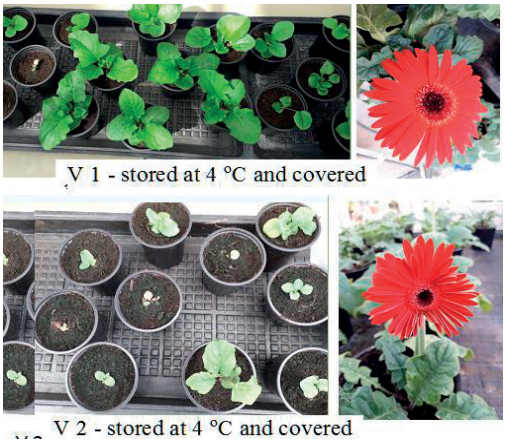

Figure 11. Aspect of gerbera plant in V1-variant with seeds kept at 4oC, covered seeds with substrate, on peat substrate with pH 5.5 and V2 -peat with pH 4.5

CONCLUSIONS

Gerbera seeds with aged of 25 days after harvested showed a higher viability compared to seeds aged 80 days after harvest. The highest percentage of sprouted seeds was recorded in the variant with seeds kept at 20ºC and not covering the seeds with a layer of substrate. Also, the seeds emerged in a percentage of 87% in the case of the variant with covering the seeds after sowing with a layer of substrate. The culture substrate influenced the germination of seeds but also the growth of gerbera plants. The number of leaves on the plants was different depending on the treatment made to seeds also by the type of substrate.

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THE INFLUENCE OF SUBSTRATE TYPE ON GROWTH AND FLOWERING OF GERBERA PLANTS

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Abstract

The study was made in greenhouses conditions on three gerbera cultivars, 'Dune', 'Blind Date' and 'Balance'. We analyzed the effect of five type of substrates on growth and flowering of gerbera. The types of substrate used were following: peat is acidic both with pH 4 and with pH 5.5 peat with pH 4, peat with pH 5.5, Perlite, 50% Perlite + 50%, peat with pH 4.0 and 50% Perlite+ 50% peat with pH 5.5. The best results was obtained when we used the substrate 50% Perlite+ 50% peat with pH 5.5 *regarding number of flowers, flower height, flower diameter, shoot diameter, showed significant difference among growing media.*

Key words*: Gerbera jamesonii, substrate, fertilizers, condition of growing.*

INTRODUCTION

Gerbera (*Gerbera jamesonii* Bolus), *Asteraceae* family is a species originally from southern Africa and Asia (Gao and Hind, 2011; Francielly et al., 2016**).** The flower is very elegant, and this impresses through the diversity of forms and colours of the inflorescences. Worldwide, gerbera is considered one of the most ornamental flowers cultivated both as cut flower and potted plant.

The growers used different methods for multiplicated from seeds, transplants and micropropagation (Pablo et al. 2002).

In recent years, the cultivation of gerbera for cut flowers or potted has gained great economic importance for floriculture (Santos et al., 2015; Francielly et al., 2016; Toma et al., 2019**).**

Cultivation on different inert substrates is the most modern method of gerbera culture, with very favorable results in terms of productivity, plant health and production quality. Awang et al. (2009) mentioned that suitable growing substrates are essential for quality flower production as these affect development and maintenance of plant rooting system. Noureen et al. (2010), Ahmad et al. (2012) shows that substrates are used for growing seedlings, plant propagation of gerbera. Different types of substrate are used for growing gerbera as coconut fiber (coco peat), rock wool, perlite, vermiculite, sand, expanded clay or different organic substrates, (compost cow, zeolite, pumice, sand etc. (Khalaj, 2007; Fakhri et al., 1995). Gerbera cultivated in a soilless system is currently practiced with the aim of increasing production and reducing costs (Maloupa et al., 1993).

Jesiotr et al. (1975) recommend with good results the pine bark compost when mixed with other types of substrate such as sphagnum peat.

Peat is the most widely used substrate for potted plant production in the nurseries and accounts for a significant portion of the materials used to grow potted plants (Marfa et al., 2002; Ribeiro et al., 2007). Since the last few years, coco peat, also known as coir dust or coconut mesocarp has been considered as a renewable sphagnum peat substitute for the use in horticulture (Yau and Murphy, 2000).

Perlite is an inert substrate providing excellent drainage of the medium and aeration of rhizosphere (Özçelik, 1997; Enache et al., 2019). Performance of plants of gerbera 'Dafne' cultivar grown in 100% coconut fibre substrate was attributed to their strong capacity to accumulate Fe in the aerial part under alkaline conditions and to maintain a better plant nutritional status, higher P and Mg (Hamid et al., 2016; Aung et al., 2017). The

researchers concluded that the use of coconut fibre substrate could provide a useful tool to improve alkalinity tolerance of gerbera plants under $NaHCO_3$ stress.
Culture in peat-perlite (1: 1) mixture produced better or similar yield and flower quality compared to soil. The performance of gerberas in perlite culture was intermediate while in pumice was the lowest, though satisfactorily (Fakhri et al., 1995).
The accumulation in the substrate of a high concentration of elements in the area of roots influences the quality of the flowers (Toma et al., 2017). These remain small, undeveloped.
The use of super-absorbent polymers as well as organic worm products improves the physico-chemical properties of growth media, Cocopeat, Perlite, Vermiculite leading to plant growth and flower yield in gerbera cv. 'Yosemite' (Verma et al., 2019; Drăghici and Jerca, 2017).
Both high and low temperatures and light in summer and winter influence the production and quality of gerbera flowers (Berninger, 1979; Aragón et al., 1984; Panter et al., 2016).
The objective of this study was to determine the effect of different substrates on growth and yield of gerbera under an open soil-less production system. We studied the effect of five types of substrate on growth and flowering of gerbera.

MATERIALS AND METHODS

The experiment was carried in greenhouse conditions at University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania. We used three gerbera cultivars: 'Dune', 'Blind Date' and 'Balance'.

Figure 1. 'Dune', 'Blind Date' and 'Balance'

The types of substrate used for every cultivars were following: V1 - peat with pH 4; V2 - peat with pH 5.5; V3 - perlite 100%; V4 - 50 % perlite + 50%, peat with 4 pH; and V5 - 50% perlite + 50% peat with pH 5.5. We used 3 plants/replicates on every variant in block randomized. Planting was done in pots with a capacity of 4 L filled with substrate according to experimental variants. We recorded the amount of water and nutrient solution administered, we watched in dynamics the vegetative growth of the plants, the number of leaves and the height as well as the number of flowers formed on the plants. The care work consisted of watering, fertilization, temperature and light monitoring. Plants were fertilized with a same nutrient solution. Electrical conductivity of nutrient solution was 1.6 mS and pH 5.5.
In a period of 8 months, some quality and quantity characteristics of plants and flowers were recorded such as number of leaves, number of flower, flower stem height, flower diameter, stem diameter. All data for growth and flowering parameters were recorded and processed statistical using analysis of variance and means were compared by Duncan's Multiple Range Test (Steel et al., 1996). The temperature from greenhouse was 20-33°C and relative humidity were 50-60%.

RESULTS AND DISCUSSIONS

In May, the number of leaves on the plants was different depending on the variety and the type of substrate. The lowest number of leaves was recorded for the 'Dune' variety of 3.5 leaves on the perlite substrate and the highest for the 'Balance' variety grown on the perlite substrate (Figure 2).

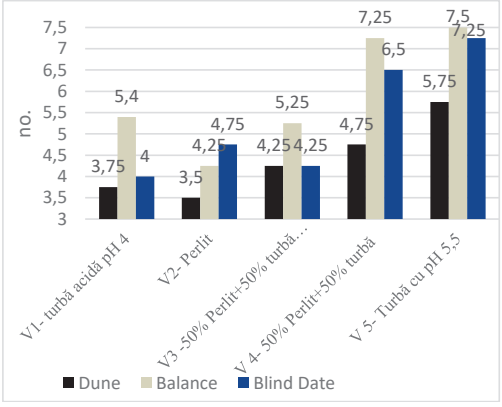

Figure 2. Number of leaves on plant in May

Figure 3. 'Dune' gerbera plants - in Mayâ

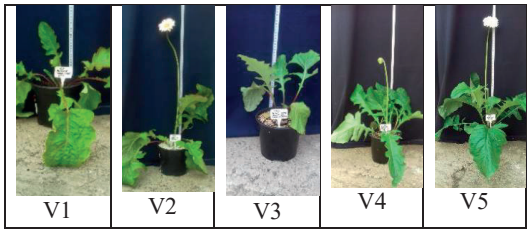

Figure 4. 'Balance' gerbera plants - in May

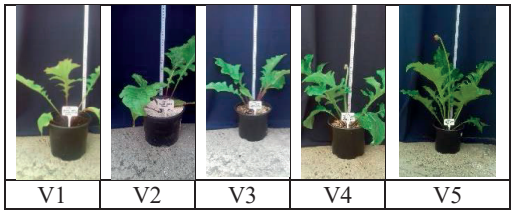

Figure 5. Gerbera 'Blind Date' plants - in May

If we analyze the behavior of the varieties according to the type of substrate we found that in the case of peat substrate with pH 4 (V1) for the 'Balance' variety the lowest average total number of leaves was 9 on plant with a percentage of 78.03% below the average of the varieties calculated on each substrate variant. In the case of variant 5 we also found that the 'Dune' variety showed the highest number of leaves compared to the 'Blind Date' and 'Balance' varieties (Table 1).

Table 1. Number of leaves on plant

Variants	Dune	Blind Date	Balance	Average	Dune	Blind Date	Balance
	No.	No.	No.	No.	Percent to average		
V1	12.6	13	9	11.53	109.25	112.72	78.03
V2	12.25	8	10.5	10.25	119.51	78.05	102.44
V3	12.2	6	5.5	7.90	154.43	75.95	69.62
V4	16.6	10	8	11.53	143.93	86.71	69.36
V5	20.2	7	12	13.07	154.59	53.57	91.84

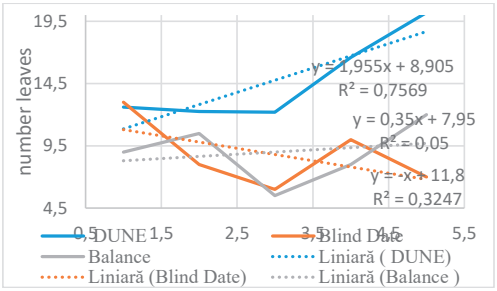

Figure 6. Number of leaves on plants

In the cultivated varieties, the correlations made between the number of leaves formed on the plant and the type of substrate showed insignificant relations in the case of the 'Balance' variety (R^2 = 0.05) and very significant in the case of the 'Dune' cultivar (R^2 = 0.7569) (Figure 6).

Based on the data recorded in the experimental variants regarding the number of leaves formed on plant we found that the varieties behaved differently when grown on different substrates. Thus, analyzing the total number of leaves, we noticed that in the 'Dune' variety a higher number of leaves were formed in the case of all substrate variants, these being 12.2 leaves at V3 and 20.2 leaves at V5. In the case of this variety we found that compared to V5 taken as a control, all experimental variants showed a lower number of leaves of 60.4% in V3 and 82% in V4. In the 'Balance' cultivar we also noticed that as a percentage the number of leaves in the experimental variants was below the control variant. In the case of the 'Blind Date' cultivar, we noticed that the largest number of leaves was formed in plants grown on acid peat substrate, the percentage being 85.7% above the control variant (Table 2).

Table 2. The number of leaves on plant and the percentage differences to the control variant

Type of substrate	'Dune'		'Blind Date'		'Balance'	
	No.	% to control	No.	% to control	No.	% to control
V1	12.6	62.4	13.0	185.7	9.0	75.0
V2	12.3	60.6	8.0	114.3	10.5	87.5
V3	12.2	60.4	6.0	85.7	5.5	45.8
V4	16.6	82.2	10.0	142.9	8.0	66.7
V5	20.2	100.0	7.0	100.0	12.0	100.0

V1 - peat pH 4; V2 - Perlite; V3 - 50% Perlite+50% peat; V4 - 50% Perlit+50% peat; V5 - Peat pH 5.5 (Ct)

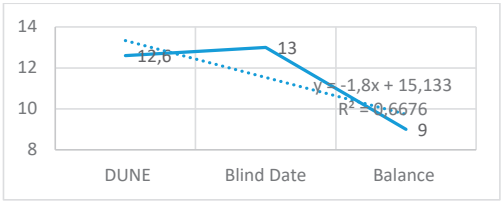

Figure 7. Influence of the peat with pH 4 substrate on the number of leaves formed on the plants

Analyzing the influence of the type of substrate on the average number of leaves formed on cultivars we found a significant correlation of $R^2 = 0.6676$ (Figure 7).

In the case of V2, perlite substrate, we found smaller relationships between cultivated varieties, this being $R^2 = 0.1678$ (Figure 8).

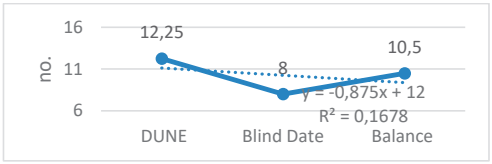

Figure 8. Influence of perlite substrate on the number of leaves formed on the cultivars

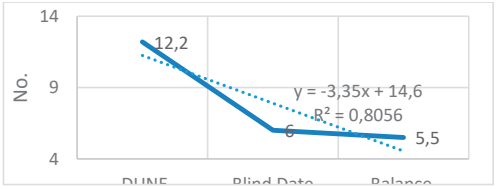

Figure 9. Influence of 50% Perlite+50% acid peat substrate on the number of leaves formed on the cultivars

Correlations between the number of leaves formed on plants at all cultivars grown on substrate of 50% Perlite+50% peat 4 pH and 50% Perlite+50% peat 5.5 pH indicated significant relationships of $R^2 = 0.8056$ at V3 respectively of $R^2 = 0.9129$ at V4 and slightly significant at V5 of $R^2 = 0.3785$ (Figures 9, 10 and 11).

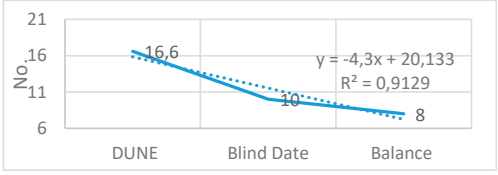

Figure 10. Influence of substrate with 50% Perlite+50% Peat with pH 5.5 on the number of leaves formed on the cultivars

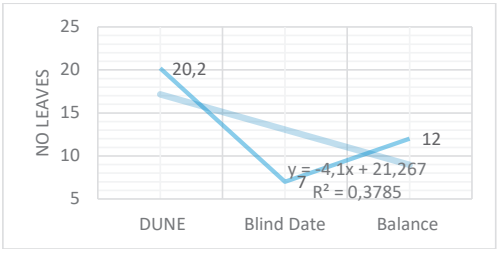

Figure 11. Influence of substrate - Peat with pH 5.5 on the number of leaves formed on the cultivars

The length of the leaves varied between 27.24 cm for the 'Dune' variety at V1 and 31.11 cm at V5. In the 'Blind Date' variety, the leaves were between 31 cm at V3 and 46 cm at V4. At the 'Balance' cultivar, the length of the leaves was 34 cm at V1 and 44 cm at V4. (Table 3).

By varieties, there were slightly significant relationships to the 'Dune' variety $R^2 = 0.4469$ (Figure 12).

Table 3. The average length of gerbera leaves on cultivars and the percentage compared to the average on variant

Variant	Dune	Blind Date	Balance	Average	Dune	Blind Date	Balance
	cm	cm	cm	cm	%	%	%
V1	27.24	34.00	38.00	**33.08**	82.35	102.78	114.87
V2	29.59	46.00	39.00	**38.20**	77.47	120.43	102.10
V3	26.51	31.00	48.00	**35.17**	75.38	88.14	136.48
V4	30.13	45.00	39.00	**38.04**	79.20	118.29	102.51
V5	**31.11**	**38.00**	**44.00**	**37.70**	**82.51**	**100.79**	**116.70**

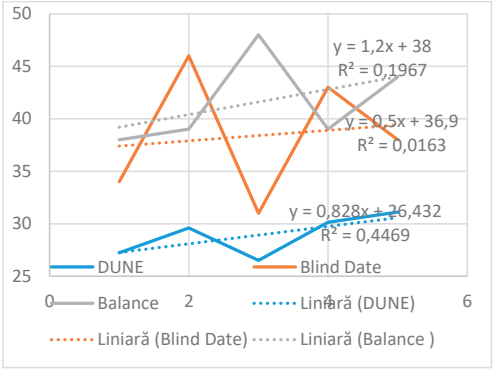

Figure 12. The influence of the substrate and the cultivars on the length of the leaves

Analyzing the average number of flowers formed on plant in the three cultivars, we found that they reacted differently depending on the type of substrate. We noticed that the 'Dune' cultivar grown on acid peat substrate pH 4 (V1) showed the lowest number of flowers formed on plant of 0.4 flowers / plant followed by the 'Blind Date' variety with 1.0 flowers / plant at V3 and the 'Balance' cultivar behaved very well on this type of substrate forming on average 4.5 flowers / plant (V1).

All gerbera cultivars grown on perlite (V2) substrate formed a large number of inflorescences on plants of 4.4 flowers in the 'Dune' variety, 5.3 flowers in the 'Balance' variety and l6.2 flowers in the 'Blind Date' variety. Analyzing the influence of the substrate type, we found that the correlation coefficients were for the 'Blind Date' cultivar R2 = 0.3535, for 'Balance' 0.0066 and for 'Dune' $R^2 = 0.2392$ (figures 13).

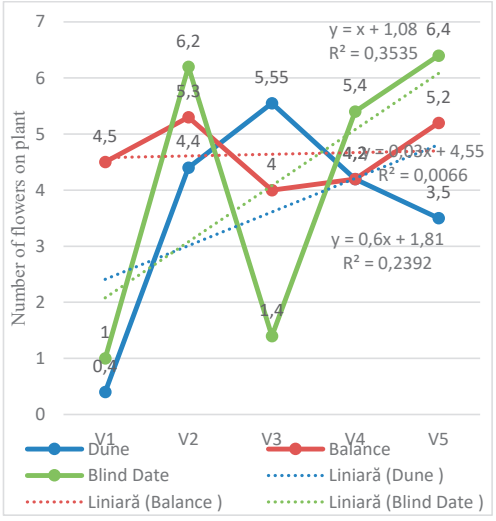

Figure 13. The influence of the type of substrate on the number of flowers per plant

In the 'Dune' variety, the number of flowers formed on plant in the first year of cultivation between May and October was on average 0.4 flowers at V1 with a difference statistically distinct negative very significant. Differences positive distinct very significant were registered at V3 where the highest number of flowers on plant of 5.5 was obtained where the percentage compared to the control was with 58.57% higher (Table 4).

Table 4. Number of flowers on plant at 'Dune' cultivar

VARIANT	Number (no.)	Difference (no.)		Significance (%)
V(0) Average	3.61	0.11	103.14	N
V(1)	0.40	-3.10	11.43	OOO
V(2)	4.40	0.90	125.71	*
V(3)	5.55	2.05	158.57	***
V(4)	4.20	0.70	120.00	N
V(5)	**3.50**	**0.00**	**100.00**	**Control**

DL5% = 0.800 DL5% in % = 22.8571
DL1% = 1.170 DL1% in % = 33.4286
DL0.1% = 1.750 DL0.1% in %= 50.0000

In the case of the 'Balance' cutivar, the total number of flowers on plant was 4.0 in V3 with a statistically negative significance and 4.5 flowers in V1 with a statistically insignificant significance.

Table 5. Number of flowers on plant at 'Balance' cultivar

VARIANT	Number (no.)	Difference (no.)		Significance (%)
V(0) Average	4.64	-0.56	89.23	N
V(1)	4.50	-0.70	86.54	N
V(2)	5.30	0.10	101.92	N
V(3)	4.00	-1.20	76.92	O
V(4)	4.20	-1.00	80.77	N
V(5)	**5.20**	**0.00**	**100.00**	**Control**

DL5% = 1.040 DL5% in % = 20.0000
DL1% = 1.520 DL1% in % = 29.2308
DL01% = 2.280 DL01% in %= 43.8461

In the case of the 'Blind Date' cultivar the number of flowers in average formed on plant was 6.4 flowers at V5 control and 1.4 flowers at V3 respectively 1.0 at V1, these indicating significantly distinct negative from statistical point of view (Tables 6).

Table 6. Number of flowers at 'Blind Date' cultivar

VARIANT	Number (no.)	Difference (no.)		Significance (%)
V(0) average	4.08	-2.32	63.75	OOO
V(1)	1.00	-5.40	15.63	OOO
V(2)	6.20	-0.20	96.88	N
V(3)	1.40	-5.00	21.88	OOO
V(4)	5.40	-1.00	84.38	O
V(5)	6.40	0.00	100.00	control
DL5% =	0.780	DL5% in % =		12.1875
DL1% =	1.140	DL1% in % =		17.8125
DL01% =	1.710	DL01% in %=		26.7188

In the case of the 'Dune' variety, we found that, for the substrate variant where we used peat

(V1), no flowers formed in May, June, July and August, but only in September. The height of the floral stem was 33 cm. In the case of the rest of the substrate variants, we recorded heights of 40.5 cm at V5 and 54.5 cm at V2. The lowest heights of floral stems were recorded at V4 - a mixture of 50% perlite + 50% peat with pH 5.5 in July, August and September. Compared to the 'Balance' and 'Blind Date' varieties, the 'Dune' variety obviously reacted to the high values of temperatures during July-August above 34ºC and low relative humidity below 50%. It should be noted that on the peat substrate with a pH of 5.5 the flower stalks had heights of 40.5 cm in May and 60 cm in August (Figure 14).

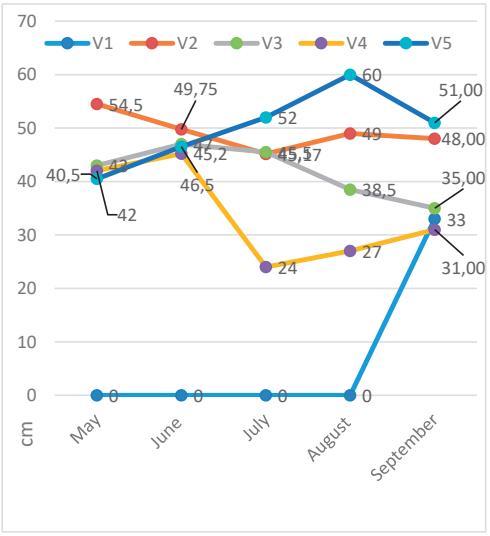

Figure 14. Inflorescence height during May-September at 'Dune' cultivar

In the case of the 'Balance' variety, we found that the height of the floral stem was relatively constant in May to September in the case of substrates V5, V3 and V1 and varied greatly in the perlite substrate (V2). It should be noted that at V1 substrate - acid peat with a pH of 4 flower stalks had the lowest heights of 12 cm in August and 14 cm in July (Figure 15).

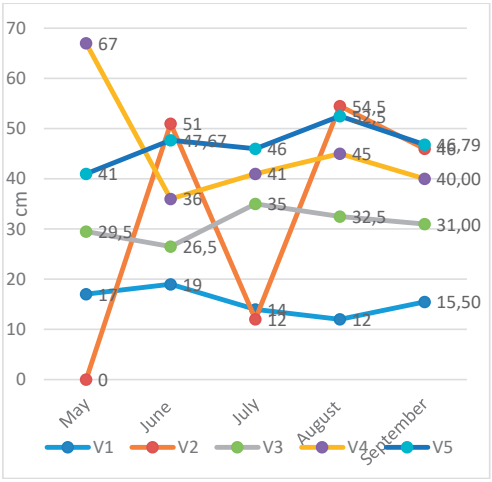

Figure 15. Inflorescence height during May-September at 'Balance' cultivar

In the case of the 'Blind Date' cultivar no flowers formed in May on all types of substrate also in June on V1, in July. In variant 3, no flowers were registered in July and August. In the case of this variety the longest floral stems were obtained. 52.66 cm at V4 in July and smallest of 24.6 cm at V2 in June (Figure 16).

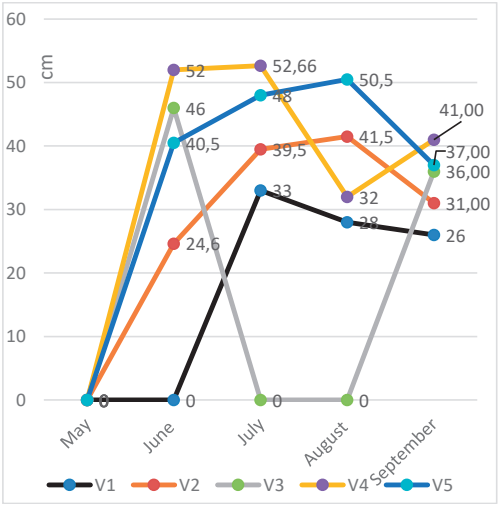

Figure 16. Inflorescence height during May-September at 'Blind Date' cultivar

The table 7 shows the average data on the height of the floral stem by variety and the type of substrate.

Table 7 Influence of inflorescence height on varieties and months and type of substrate

Cultivar	Var.	May	June	July	Aug.	Sept.
'Dune'	V1	0	0	0	0	33
'Balance'	V1	17	19	14	12	15.50
'Blind Date'	V1	0	0	33	28	26
'Dune'	V2	54.5	49.75	45.17	49	48.00
'Balance'	V2	0	51	12	54,5	46
'Blind Date'	V2	0	24.6	39.5	41.5	31.00
'Dune'	V3	43	47	45.5	38.5	35.00
'Balance'	V3	29.5	26.5	35	32.5	31.00
'Blind Date'	V3	0	46	0	0	36.00
'Dune'	V4	42	45.2	24	27	31.00
'Balance'	V4	67	36	41	45	40.00
'Blind Date'	V4	0	52	52.66	32	41.00
'Dune'	V5	40.5	46.5	52	60	51.00
'Balance'	V5	41	47.67	46	52.5	46.79
'Blind Date'	V5	0	40.5	48	50.5	37.00

We notice that on the peat substrate with pH 4 (V1) the 'Balance' variety behaved best but the height of the floral stems was the lowest.

On the perlite substrate (V2) the 'Dune' cultivar behaved very well presenting floral stems in all months. On the substrate of 50% perlite and 50% acid peat the 'Dune' presented the largest floral stems and the 'Balance' formed smallest floral stems of 26.5 cm in June and 32.5 cm in August but 'Blind Date' formed floral stems of 46 cm in June and 36 cm in September. In the case of substrate 50% peat with pH 5.5 + 50% perlite (V4), the flower stalks had lower heights in July and August for the '*Dune*' variety and heights of 41 cm in July and 67 cm in May. The 'Blind Date' variety presented floral stems in June-September of 32 cm in August and 52.66 in July. The best substrate variant for stem height was presented by variant 5 (peat with pH 5.5).

The 'Dune' cultivar formed uniform inflorescences, with a diameter between 9.5 cm and 9.6 cm in variant 3. The lowest values were recorded for the substrate type of V4 (Figures 17, 18).

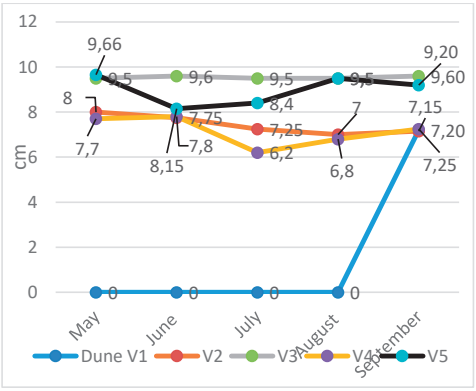

Figure 17. The diameter of inflorescences at 'Dune' cultivar

Figure 18. 'Dune' cultivar

In the 'Balance' variety, the diameter of the flowers showed the lowest values in May (5.75 cm) in the case of cultivation on acid peat substrate but also in the rest of the months, it being between 7 cm in June and 8 cm in the July and August. On the perlite substrate, the diameter of the flowers was approximately uniform, being between 9.15 cm in June and August (Figures 19, 20).

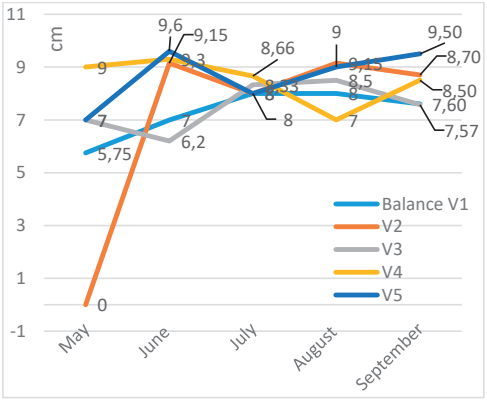

Figure 19. The diameter of inflorescences at 'Balance' cultivar

Figure 20. *'Balance'* *'Blind Date'*

In the case of the 'Blind Date' cultivar, we noticed flowers with a larger diameter compared to the rest of the analyzed cultivars. We obtained flowers with a large diameter between 9.33 cm in August and 10.38 cm in September, in variant 4. The culture substrate had influenced the diameter of the flower it varied from one month to another (Figure 21).

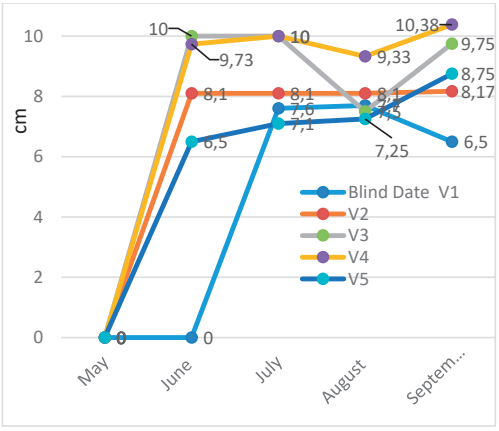

Figure 21. The diameter of inflorescences at 'Blind Date'

CONCLUSIONS

The number of leaves on the plants was different depending on the variety and the type of substrate. It was lower in gerbera plants grown on perlite substrate but also in 'Dune' varieties grown on all types of substrate in the early stages of growth. After 9 months of cultivation, the lowest number of leaves was recorded in the 'Balance' variety between 5.5 leaves at V3 and 12 leaves at V5. Also, the number of leaves per plant at 'Blind Date' was lower compared to the 'Dune' variety. At the 'Dune' variety we recorded the highest number of leaves at V5 of 20.2 leaves and 12.25 leaves at V2.

The length of the leaves varied from cultivar to cultivar. In the 'Dune' variety the number of leaves was 26.51 cm at V3 and 31.11 cm at V5. The 'Blind Date' variety presented leaves with a length between 31.00 cm at V3 and 46 cm at V2. In the case of the 'Balance' variety, the leaf length was the largest between 38 cm at V1 and 48 cm at V3.

The number of flowers per plant was relatively constant for the 'Balance' variety in May-September, being between 4 flowers / plant at V3 and 5.2 flowers/plant at V5. The 'Blind Date' variety showed the greatest reaction to the type of substrate.

The height of the flower stalk was different depending on the variety, but it differed a lot in some varieties depending on the very high temperatures during the summer.

The diameter of the flowers was smaller for the 'Dune' variety in the case of variant 4 and almost constant for V5. In the 'Balance' variety, the diameter of the flower was 5.75 at V1 and 7.6 cm at V5. Flowers with the largest diameter were obtained from the 'Blind Date' variety.

The culture substrate had influenced the diameter of the flower it varied from one month to another.

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POSSIBILITIES FOR *EX SITU* CONSERVATION OF BULGARIAN ENDEMIC *BETONICA BULGARICA* DEGEN. & NEIČ.

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Abstract

The Bulgarian endemic Betonica bulgarica Degen. & Neič. is a protected species included in the Red Data Book of the Republik of Bulgaria vol.1. Plants and fungi. On the territory of "Sinite Kamani" Natural Park it forms populations in Ablanovo area, Slancheva polyana area, Upper lift station area and Karandila area. Main threats to the populations in the Park are the anthropogenic impact, difficult seed reproduction, soil erosion processes and the spread of eagle fern (Pteridium aquilinum) in the border areas of the population in Slancheva polyana area. According to evaluations, both in-situ, and ex situ conservation measures should be included for the protection and stabilization of populations of Betonica bulgarica. The aim of this study was to develop a technology for growing species from mature seeds in laboratory conditions. In order to realize the objective, a vast amount of literature was studied and biennial field research of the populations in the "Sinite Kamani" Natural Park was conducted. The developed technology is successful and fully complies with the environmental conditions of the natural habitat of the species in the Park. Using it, the species has been successfully propagated in the scientific laboratories of the Faculty of Agriculture at Trakia University from mature seeds to 6-8 leaves (phenophase) for replenishment of the populations. The technology can be used to replenish other natural populations of Betonica bulgarica in Bulgaria. In order to determine the effectiveness of the application of this measure for ex-situ conservation is necessary to continue observations after introduction of the plants grown in laboratory conditions, to follow their adaptation and further development, and if necessary to protect them and stabilize their condition.

Key words*: Betonica bulgarica Degen. & Neič., "Sinite Kamani" Natural Park, ex situ conservation.*

INTRODUCTION

The Bulgarian endemic *Betonica bulgarica* Degen. & Neič. is a species protected by the Biological Diversity Act (2002), included in the Red Data Book of the Republic of Bulgaria vol. 1. Plants and fungi in the category "endangered" (Genova, 2011). Main threats for the populations are trampling, grazing or collection of the aboveground and underground parts of the plant for medicinal purposes (Koeva, 1984; 1989; Genova, 2011). Genova (2011) reported that the conservation of the species *Betonica bulgarica* in the country would require studying the biology and ecology of the species, determining the numbers and area of the populations and the possibilities for cultivation, as well as collecting seeds for the National Seed Genebank in Sadovo.

The present study is part of project No 5103020-15-658 "Restoration of the habitats and conservation of the biological diversity in "Sinite Kamani" Natural Park. One of the aims of the project is *ex situ* conservation of protected and endemic plant species, located on the territory of "Sinite Kamani" Natural Park, in order to stabilize their populations.

According to preliminarily conducted biennial field research one of the species, whose conservation in the Park requires the use of *ex-situ* measures for conservation, is *Betonica bulgarica.* On the territory of "Sinite Kamani" Natural Park, the Bulgarian endemic was first described by Grozeva et al. (2004) with one population in Ablanovo area. Later three more populations were registered - in Slancheva polyana area, near Upper lift station and east of Microyazovir in Karandila area (Grozeva et al., 2014). The assessment of their condition showed that main threats for the species on the territory of the Park were: anthropogenic impact, difficult seed reproduction, the spread of eagle fern in the border areas of the

population in Slancheva polyana area, and erosion processes (Grozeva et al., 2014).
The aim of this study was to develop a technology for growing *Betonica bulgarica* from mature seeds in laboratory conditions in order to replenish and stabilize the populations of the species in "Sinite Kamani" Natural Park.

MATERIALS AND METHODS

For the development of the technology for growing *Betonica bulgarica* were used various literary sources (Hayek, 1929; Yankulov, 1964; 2000; Medicinal plants 2001; Grozeva et al., 2004; Panayotova et al., 2014) and data from conducted observations and performed analyses related to the realization of project No 5103020-15-658 "Restoration of the habitats and conservation of the biological diversity in "Sinite Kamani" Natural Park.
The morphological characteristic of the species corresponds with the one indicated in the Flora of the Republic of Bulgaria (Koeva, 1989) and the Red Data Book of the Republic of Bulgaria vol.1 (Genova, 2011).
The laboratory analyses were conducted in the scientific laboratories of the Faculty of Agriculture, Trakia University - Stara Zagora.
The mature seeds were taken from a population of the species in Ablanovo area after receiving a permit from the Minister of Environment and Water. In accordance with the permit conditions, 250 seeds were given for storage in the National Seed Genebank in Institute of Plant Genetic Resources "Konstantin Malkov" - Sadovo.
To prepare the soil for planting were used the data from the soil analyses of each population (Grozeva et al., 2014).
All actions related to the collecting of mature seeds, growing them in laboratory conditions and returning the grown plants in the natural populations of the species in "Sinite Kamani" Natural Park are in compliance with the Protected Areas Act (PAA, 1998), the Biological Diversity Act (BDA, 2002) and Ordinance No 8.

RESULTS AND DISCUSSIONS

Morphology and biology of the species

Betonica bulgarica is a perennial plant from the family *Lamiaceae* (Figure 1). It has a horizontal rhizome. The stem is erect, four-edged, with no branches, covered with bristles facing down.
The leaves are opposite each other. Their form is oblong ovate, heart-shaped at base, coarsely crenate to crenate-dentate at the edge, on both sides pubescent. The leaves at the base have stalks longer than the lamina, the 2-3 pairs of stem leaves have short stalks and the upper leaves are sessile. The inflorescence has concise and densely spiked raceme, rarely the lowest is separate. Bracts are of equal length to the calyx, lanceolate at the top, barbed at the edge and the veins - fibrous. The calyx is 8-9 mm long, almost bare or bare only at the base, from the middle upward - densely fibrous with long, white, hard bristles; the tube is 5 mm long; the teeth - narrowly triangular, 3-4 mm long, hairy at base, bare upward with a long apical spine.

Figure 1. *Betonica bulgarica* - general view (photo N. Grozeva)

The petal is pink-purple, layered with white bristles; the tube is narrow, slightly curved, 9-10 mm long, the upper lip flat or slightly bulging, entire or furcate at the apex, 4 mm long, the lower lip trilobite, 6 mm long, the middle part is repand and large at the edge, the lateral parts are small and ovate. There are 4 stamens with fibrous handles.

The fruit is coenobium, thereof named tetraeremum based on nutlets number. The nutlets are brown, triangular, elongated, 4 mm long, 2 mm wide, the outside almost flat, the edges with narrow wings, which at the top edge go into irregularly toothed membranous appendage.
Flowering in July-August, fruiting in August-September. The pollination is entomophilous. The species reproduces by seeds and vegetatively.

Natural habitat
The species inhabits Central and East Balkan range and Thracian lowlands on open grass lands in the oak and beech forest belts at an altitude from 540 to 1500 m (Genova, 2011; Asyov et al., 2012; Grozeva et al., 2014). It grows on Chromic Luvisols, Eutric Cambisols and Rendzinas, moderate structured soils with crumb structural aggregates and on soil texture from sandy clay loam to silty clay loam. It grows successfully both in acidic soil reaction with pH values (H_2O) = 4.23, and in slightly acidic to neutral reaction with pH values (H_2O) of 6.01 to 7.04. It dwells on soils with low mineral nitrogen content, poorly to well stocked with available forms of phosphorus and with high available potassium content. The presence of carbonates in the soil does not affect negatively the development of the species.

Environmental requirements
Betonica bulgarica is a sun-loving plant but can endure some shade. It prefers open sunlit meadows near forests. It is a cold-hardy plant. It can endure low temperatures during the winter-spring period. The species isn't particularly demanding regarding soil moisture - it's drought-tolerant but it can develop well in more humid places. It has no specific requirements for the soils, as long as they have good drainage. It can grow on Chromic Luvisols, Eutric Cambisols and Rendzinas, moderate structured soils with crumb structural aggregates and on soil texture from sandy clay loam to slity clay loam, as well as stony and sandy ground. It prefers soils with neutral or lightly acidic reaction, however our studies (Grozeva et al., 2014) show that it can grow successfully in acidic soil reaction.

Actions for growing *Betonica bulgarica*
The species propagates by seeds and vegetatively by dividing the root.
In laboratory condition it is propagated by seeds. The seeds are collected the previous autumn from the natural habitats of the plant. Isolation bags are placed on the racemes after the flowers wilt (Figure 2 A). The isolators are collected after the seeds mature, when the stems have fully dried and the fruit separate by themselves (Figure 2 B). The racemes are shed from the isolators (Figure 2 C) and the seeds checked for mechanical and biological mixes (Figure 2 D).
The seeds are cleaned manually.
To determine the seed sowing qualities a mean sample is taken according to the method of split middle diagonal sample.
The appearance of the batch of seeds is inspected for colour, smell, shine, etc. The health status of the seeds regarding diseases and pests is determined through the use of microscopic technology (Figure 2 E). The seeds collected from *Betonica bulgarica* should be stored in tightly closed, if possible sealed containers labeled with the necessary information for the population, date and year of acquiring the seeds (Figure 2 F), in order to ease their future use. Additionally, the net/gross weight or number of seeds can be indicated, as well as the name and address of the person responsible for the labeling and storing.
The moisture of the seeds is of vital importance. They will retain good sowing qualities if they are well dried and stored in appropriate places and low positive temperature from 1 to 5 °C and air humidity at around 50-55% is maintained. The seed qualities will worsen if a significant increase or drop in the air temperature is allowed. In order to remove the excess moisture, immediately before storing the seeds, they must be spread on a sheet of paper and sun dried for 1-2 hours on dry and sunny days with temperature of the air in the shade around 20°C. At this temperature they are not at risk to lose germination. It is best to store them in cloth or paper bags. If the seeds are well dried, they can also be stored in well closed, fully dry on the inside glass containers (jars, bottles), filled 2/3 of their volume or in well-closed box. Beads or an entire packet of Silica gel can be placed in the containers, in

order to suck up the moisture if any occurs. This way the valuable seeds can be preserved for longer without disturbing their sowing qualities. It's preferable that during the period of storage the containers should be opened only if necessary, for example to check the health status of the seeds. Frequent opening or change of containers can lower the sowing qualities and germination characteristics of the seeds. If there is the faint odour of mold or a weird smell, when opening the containers, this is a sign for a disease or unfitness of the seeds. For longer term storage the seeds can be stored in a fridge, sealed well, at temperature between 2-5°C.

The soil for sowing the seeds is prepared according to the data from the soil samples, taken from the natural habitats of the species. Before placing it pots or plastic containers, it should be made friable and sieved to remove rocks and large lumps Figure 2 G).

Betonica bulgarica has no special requirements regarding the soil fertility and can be grown with no fertilizer but for better development of the plants it is best to use organic or mineral fertilizers. From the organic ones decomposed manure can be used which should be mixed with the soil before planting of the seeds, ratio soil: fertilizer = 2: 1. From the industrial fertilizers - superphosphate 60-80 kg/ha, potassium sulfate 40-60 kg/ha and others can be used, added to the soil before planting, and a nitrogen fertilizer in amount of 100-120 kg/ha like ammonium nitrate or a different fertilizer is applied in the soil during the preparation of the soil and like water solution when watering the pots several times after planting the seeds. Complex foliar fertilizers containing the necessary macroelements also have a good influence on the growth and development. The exact amount of fertilizer can be determined according to the results of the soil sample analyses and the condition of the plants.

The seeds are sown from the middle of January to the beginning of March. Due to the prolonged slower germination period and low germination (35%), there are two possibilities for sowing - sowing the mature seeds at 1-1.5 cm in the soil (Figure 2 H) or sowing germinated in Petri dishes seeds (Figure 3 A, B). Panayotova et al. (2014) found that *B. bulgarica* was characterized by a prolonged period of germination and low rate of germination - 35.0%.

Figure 2. A - placing of isolators; B-C - collecting of isolators and shelling of seeds mature; D - seeds free of mechanical pulp; E - checking the health status of the seed; F - packaging and labeling; G - soil preparation for sowing; H - sowing the seeds matur

Sowing the germinated seeds reduces the costs for preparing the fertilizer-soil mix and the care until sprouting, including the use of nitrogen fertilizer. After planting the seeds must be watered with water or water solution of nitrogen fertilizer. The differences in the development of the plants in both types of sowing have not been discovered as the sown germinated seeds go through a period of adaptation, and the directly sown seeds sprout during that time (Figure 3 C).

The care during vegetation is related to ensuring optimum conditions for the development of the plants. The soil is made arable and periodically watered. Plant nutrition can be done with combined fertilizers two or three times, during the cotyledon phase (Figure 3 D), the formation of four (Figure 3 E), and 6-8 true leaves (Figure 3 F). Any weeds which appear should be removed manually. Economically important diseases and pests

were not discovered but if needed it can be used authorized plant protection products.
The plants are grown in laboratory conditions until they form a rosette of 6-8 leaves (Figure 3 G). The plants should be returned to their natural habitats during autumn and/or spring (Figure 3 H). The planted young plants should be observed and watered, if they are dug out by wild boars - they should be pressed into the soil again until their condition stabilizes.

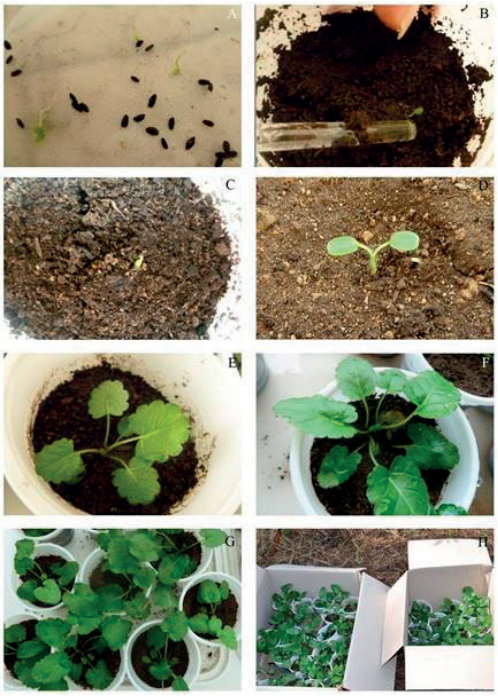

Figure 3. A - germination of seeds in Petri plates; B - planting seeds germinated; C - germination of seeds sown; D - stage cotyledons; E - 4-5 leaves stage; F-G - 6-8 leavesstage; H - plant prepared to return to their natural habitats

CONCLUSIONS

The technology developed for growing the Bulgarian endemic *Betonica bulgarica* from mature seeds to 6-8 leaves phenophase in laboratory conditions was successful and can be used to replenish the natural populations of the species in Bulgaria. In order to determine the effectiveness of the application of this measure for *ex*-situ conservation is necessary to continue observations after introduction of the plants grown in laboratory conditions to the Natural park, to follow their adaptation and further development, and if necessary to protect them and stabilize their condition.

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STUDY OF THE INFLUENCE OF DIFFERENT SOWING PERIODS ON THE PHENOLOGICAL AND DECORATIVE CHARACTERISTICS OF *VERBASCUM THAPSUS* L.

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Abstract

Verbascum thapsus L. is a wild plant in Bulgaria and has very good decorative qualities. The purpose of this study was to explore the possibility of using Verbascum thapsus as an ornamental plant. The investigation was conducted during the period 2017-2019. Seeds of wild plants were collected in the area of Plovdiv. The seeds were sown on 4 dates - the beginning of June, July, August and September. Vitality, germination and germination energy of the seeds were studied. The phenological and ornamental characteristics were recorded. The plants with the sowing of seeds in the beginning of July have the best decorative behaviour - the largest flowers, the largest number of flowers and the longest flowering period.

Key words*: Verbascum, seed propagation, cultivation, germination, ornamental plant.*

INTRODUCTION

Verbascum thapsus L., the family *Scrophulariaceae*, is a biennial, perennial or, rarely, an annual plant with a deep tap root. It is native to Eurasia and Africa (Muzik, 1970; Fuller and Barbe, 1985). In its first year it produces a low vegetative rosette up to 60 cm in diameter. Basal leaves are oblong-obovate to obovate lanceolate, 10.2-30.5 cm long, 2.5-12.7 cm wide and covered with woolly hairs. Stem leaves are elliptic lanceolate, decurrently alternate and decrease in size towards the apex. The rosette overwinters and a 50-180 cm flowering stem develops in the succeeding growing season. The flower stem is longitudinally ridged by the bases of decurrent leaves and is densely woolly with branched hairs (Watson, 1977; Baskin and Baskin, 1981; Gross and Werner, 1982). The inflorescence is a spikelike raceme 20-50 cm long and approximately 3 cm in diameter. It is usually very dense; rare axillary racemes may arise from the upper leaves. The sessile flowers are usually one per axil with pedicels less than 2 mm and slightly irregular with rotate corollas. The calyx consists of 5 lanceolate or ovate sepals, 7-9 mm long with caudate tips. The corolla is 20-25 mm broad consisting of five yellow or white petals and the seeds ripen from August to September (Gross, 1984; Salisbury, 1942). The plant is suitable for sandy, loamy and clay soils and prefers well-drained soil (Spencer, 1957). It can grow in very alkaline soils with pH - acid, neutral and basic (Brewer, Watson, and Gray, 1976; Gross, 1981). It cannot grow in the shade, prefers a sunny position. The plant can tolerate strong winds but not maritime exposure. It is an easily grown plant (Kivilaan and Bandurski, 1981; Semenza, Young, and Evans, 1978). Hybridizes with other members of this genus, though the progeny are usually sterile (Daar, 1983; Gross, 1984). A very ornamental plant with attractive foliage, flowers or blooms, it often self-sows, especially on dry calcareous soils (Gross, 1980). The plant attracts birds and butterflies. Edible, invasive, naturalizing, suitable for dried flowers (Jones and Stokes 1984; Semenza, Young, and Evans, 1978; Gross and Werner, 1978).

There are many decorative types and varieties of *Verbascum* (Lade et al. 1974). The wild species of the *Verbascum* genus, are characterized by valuable decorative and ecological qualities. They are distinguished by their toleration to soil and climatic conditions, drought tolerant, relative pests and disease free

(Westcott, 1960; USDA, 1953; 1960; 1984) and are very well adapted to the conditions in Bulgaria. Therefore, the purpose of this study was to explore the effect of different sawing dates on seed quality, as well as on phenological and ornamental characteristics of *Verbascum thapsus* L.

MATERIALS AND METHODS

The seeds were collected in October from previously identified wild plants of *Verbascum thapsus* L. near the city of Plovdiv. The sowing was carried out from June to September of the respective year in 4 dates in containers, the soil mixture was suitable for sowing flower and vegetable seeds. With the emergence of 3-4 true leaves, the plants were picked outdoors at 25 cm between the spacing and 20 cm between the rows. The absolute mass of 1000 seeds was according ISTA (2013), vitality, germination, germination energy and length of embryonic root were determined. Phenological characterristics traced the beginning and mass emergence of seedlings, the appearance of cotyledons, the appearance of the first true leaf and the appearance of 4 true leaves. The decorative characteristics studied were the height and diameter of the inflorescence, the diameter of the flower and the number of flowers in the inflorescence. The results were processed by analysis of variance.

RESULTS AND DISCUSSIONS

Table 1 presents the results of studies of the seed quality of *Verbascum thapsus*. The absolute mass of 1000 air-dried seeds was 0.072 g. This indicator is influenced by the size and fulfillment of the seeds, as well as by the climatic conditions in the growing area. These data are a criterion for the ecological plasticity of a species and its suitability for acclimatization to the conditions of an area. The vitality of the seeds determines their potential ability to germinate. In the seeds used in this experiment, the vitality was 78.91%. Germination is the most important indicator of the suitability of seeds to form normal sprouts under optimal conditions over a period of time. In *Verbascum thapsus*, seed germination was determined at 7 days - 82.48% (Table 1).

Table 1. *Verbascum* seed quality averages 2017-2019

Absolute mass per 1000 seeds (g)	Vitality, %	Germination, %	Germination energy, %	Embryonic root length (cm)
0.072	78.91	82.48	73.18	1.07

Germination energy indicates the percentage of normally germinated seeds under optimum germination conditions within a period shorter than that for germination. In *Verbascum thapsus* L., the germination energy was determinated for 5 days and was 73.18%, indicating that the seeds germinate jointly and give strong and viable seedlings and, respectively, more viable plants. The average embryonic root length of *Verbascum thapsus* L. is 1.07 cm, which is also evidence of seed viability and usability.

The phenological characteristics of the plants are presented in Table 2. The seeds sown on 08.08 appeared first - variant 3, seeds sown at the beginning of July, September and June, 1.7, 2.4 and 4.8 days, respectively. However, the mass emergence occurs first at sowing in early July - 13.7 days after sowing, followed by sowing in August, July and September. The appearance of cotyledons, the appearance of the first and fourth true leaves (Figure 1) follows the trend observed in the mass emergence indicator - these phenophases first occur in the seeds sown in early July, followed by sowing in August, June and September.

Table 2. *Verbascum* phenological behaviour averaged over the period 2017-2019

Variants	Beginning of emergence (days)	Mass emergence (days)	Occurrence of cotyledons (days)	Occurrence of the first true leaf (days)	Occurrence of the fourth true leaf (days)	Duration of flowering of a single flower (days)	Duration of flowering of the whole plant (days)
1. Sowing 4.06.	11.5	19.2	15.8	27.9	37.9	7.4	72.1
2. Sowing 6.07.	8.4	13.7	9.7	18.4	27.3	7.2	91.4
3. Sowing 8.08.	6.7	16.9	11.7	21.8	30.5	7.4	77.2
4. Sowing 1.09.	9.1	21.8	19.3	31.4	-	-	-
LSD (p=0.05%)	0.7	1.3	2.4	7.5	6.8	0.1	12.5

The plants obtained from the seeds sown in September do not at all enter the “emergence of a fourth true leaf” phase. All plants from this date of sowing die in winter. The sowing time does not affect the duration of flowering of the

individual flower - 7.4; 7.2; 7.4 days respectively for the three sowing periods. The duration of flowering period of the whole plant is greatest for the plants obtained from sowing in July - 91.4 days. The flowering period of plants obtained from sowing in August and June was with 15.5% and 21.1% shorter, respectively. The sowing time of the seeds had a significant effect on the decorative appearance of *Verbascum thapsus* L. (Table 3). The height of the inflorescence is greatest for plants obtained from seeds sown in July - 61.8 cm. The height of the inflorescence of plants obtained from seeds sown in August is 11.1 cm lower and 21.1 cm lower than the height of the inflorescence of those obtained from sown in June.

Table 3. *Verbascum* ornamental characteristics averages 2017-2019

Variants	Height of inflorescence (cm)	Inflorescence diameter (cm)	Flower diameter (cm)	Number of flowers in inflorescence
1. Sowing 4.06.	40.7	2.47	0.31	134.5
2. Sowing 6.07.	61.8	2.50	1.75	384.5
3. Sowing 8.08.	50.7	2.51	0.65	211.7
4. Sowing 1.09.	-	-	-	-
LSD (p=0.05%)	9.6	0.07	0.35	143.5

The diameter of the flower is a determinant of the decorative qualities of the plants. The largest are the flowers of the plants obtained from sown in July seeds - 1.75 cm, followed by those obtained from sowing in August and June - respectively by 1.10 cm and 1.44 cm smaller. The strongest influence is the sowing time on the number of flowers in the inflorescence. The plants obtained from sowing in July form 384.5 flowers with 172.8 pcs. and 250.0 pcs. more than those obtained from sowing in August and June, respectively by 65.1% and 44.9% respectively. The sowing time does not affect the diameter of the inflorescence, it varies within very small limits - from 2.47 cm for plants obtained from sowing in June to 2.51 cm for plants obtained during sowing in August.

Figure 1. Phenophases: emergence (left), first true leaf (middle) and fourth true leaf (right)

CONCLUSIONS

1. It was found that the seeds collected in October from wild plants *Verbascum thapsus* L. have very good qualities - 78.91% vitality; germination - 82,48% and germination energy - 73,18% and can be used for production of propagating material.
2. The best phenological characteristics have the plants obtained from sowing seeds in July. The same plants have the longest flowering period of the whole plant. The sowing time does not affect the duration of flowering period of the individual flower.
3. The values of the height of the inflorescence, the diameter of the individual flower and the number of flowers in the inflorescence are highest for the plants obtained from sowing in July. For obtaining of plants with high ornamental characteristics and long flowering period, it is recommended to be used this sowing term.
4. *Verbascum thapsus* L. plants can be successfully used as ornamental plants in outdoor gardening.

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STUDY ON PHENOLOGICAL BEHAVIOURS OF *DAHLIA VARIABILIS* HORT. IN OVERWINTERING OF TUBEROUS ROOTS IN THE SOIL

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Abstract

Dahlia is used for landscaping parks and gardens as well as cut flower. Because the dahlia comes from Central America (Mexico), it does not tolerate the cold temperatures of the winter. That is why the tuberous roots are planted in April, and removed in October. The tuberous roots are stored for 6 months in a dark and ventilated place where the temperature does not fall below 0^0C. Recent changes in the agro-climatic environment and preliminary studies have naturally led to the conclusion that it is possible the tuberous roots of to be left without removal and storage. The purpose of this study is to identify damage or lack thereof in overwintering the tuberous roots in the soil. Three cultivars were used -'Vitus', 'White Ball', 'Dark Red'. In overwintering plant the sprouting started with 5-7 days earlier. The growth rate was faster with overwintering plants, the most pronounced being that of the Dark Red cultivar. The wintering plants enter the phenophase beginning and mass flowering 11 to 14 days earlier. Flowering of the individual flower and the whole plant is 38-45% longer in wintering plants.

Key words*: dahlia, tuberous roots, overwintering, phenological behaviours.*

INTRODUCTION

Dahlia is a common species used both for outdoor landscaping and for cut flower (Nikolova N., 1999). Mariña J. L. (2015) points out that dahlia has great potential as a cut flower species due to the huge variety of shapes and colors and relatively easy reproduction. It belongs to the group of perennial flowers (Tafradzhiyski O., Ivanova, V., 1999). In most studies, light is the main factor influencing its development and especially on flowering, but other environmental conditions also have a significant influence. In this regard, Malik, S.A. et al. (2017) study the effect of growth regulators on the growth and flowering of dahlia, finding that the diameter and number of inflorescences and the shelf-life of the cut state are maximal when treated with 4000 ppm chlormequat. The phenological manifestations of dahlia in connection with the use of growth regulators were also examined by Khan F.U. et al. (2003). The productivity of some late-planting varieties on calcareous soils is studied by Mishra, H. P. et al. (1990) and found that in terms of plant height, number of buds, days from planting to full flowering, quality and size of cut flowers, the 'Kenya' variety, followed by 'Kelvin Rose', 'Black Out', and 'Vigor', is most appropriate. Growing technology under our conditions provides for the planting of tuberous roots in the second half of April and removal at the end of September. The tuberous roots are stored for 6 months in a dark and airy place where the temperature does not fall below 0°C. Due to the recent changes in the agro-climatic climate and preliminary studies, it has been found that the tuberous roots damage in the autumn is not negligible. The main objective of this study is to investigate the wintering of dahlia tuberous roots in the soil.

MATERIALS AND METHODS

The study was conducted in the Dendrologycal Park of Agricultural University - Plovdiv, in the period 2017-2018. Three varieties of dahlia were used: from the low 'Vitus' variety; the medium ones are 'White Ball' and the tall ones are 'Dark Red'. At the beginning of the experiment, tuberous roots of nearly the same length and diameter were selected. Each lump had a part of the old stem. Planting took place in the second half of April. During the first

year, the plants were grown according to the technology adopted for the country, without taking into account the vegetative and generative manifestations. In the second half of October, the plants of the control variants were removed, cleaned and stored in a dark, ventilated area where the winter temperature did not fall below 0°C. In the second half of April of the following year, the cleaned tuberous roots were planted. The stem of plants of the experimental variants were cut to a height of 10 cm. In the second half of October, during the first year of cultivation, tuberous roots were left in the soil for the next growing season. During the second vegetation of plants, both control and experimental variants, vegetative and ornamental manifestations were studied. For the climatic conditions in Plovdiv, January is the month with the lowest temperature. For the studied period the average air temperature for 2017 was -6^0C, and for 2018 -4^0C. It would be good in future studies to monitor the soil temperature.

RESULTS AND DISCUSSIONS

The growth rate of the stem in plants grown by wintering or traditional technology is shown in Figures 1, 2 and 3. It is traced from the end of March (emergence) to the first week of September (when the growth processes gradually subside).

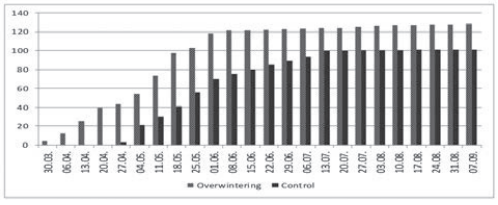

Figure 1. *Dahlia variabilis*, cv. 'Dark red' growth rate (cm)

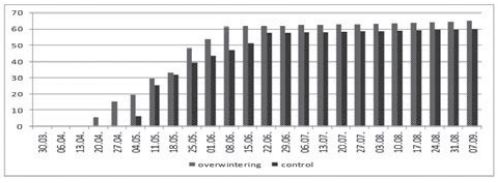

Figure 2. *Dahlia variabilis*, cv. 'White ball' growth rate (cm)

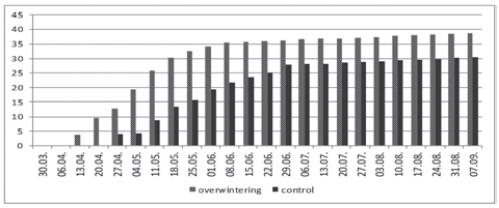

Figure 3. *Dahlia variabilis*, cv. 'Vitus' growth rate (cm)

In the 'Vitus' variety, winter-growing plants begin their development in the middle of April, while those grown under traditional technology - at the end of the same month, i.e. about two weeks later.

The data show that in the initial stages of their development the plants grow quite rapidly, with differences in the one-week reporting period ranging from 0.2 cm to 4.8 cm for the control and from 1.5 to 6.6 cm for the experimental plants. The rapid increase continues until the last week of June for plants grown using traditional technology and until the first week of the month for wintering plants, i.e. overwintering plants outperform this indicator by about two weeks with plants grown using traditional technology. This actually shows that overwintering plants accumulate vegetative mass faster, i.e. earlier they reach a decorative appearance, and that the period of decorativeness in them is longer.

With the 'White Ball' variety, the maximum of the growth processes reaches in the first week of June, with an increase ranging from 3.6 cm to 18.0 cm over a one-week period. For traditional-grown plants, the maximum increase is in the third week of June or about a week later. The increase in the one-week period ranges from 3.6 cm to 19.3 cm. In both types of plants, the growth processes decrease over the next 11-13 weeks and stop during the first week of September.

At the latest, the maximum occurs in the growth processes of plants grown using traditional technology of the 'Dark Red' variety - the second week of July, when the plants reach a height of 100.1 cm. For wintering plants, the first week of June is the period with the most intense growth - the plants reach a height of 121.5 cm, and the weekly growth is from 2.8 to 19.3 cm. For both experimental and control plants, the period up to the first week of September is 8 weeks and the other 13 weeks is a period of weak to stunted growth, with weekly increments of 0 to 1.3 cm.

Data on the phenological manifestations of dahlia plants grown differently are presented in Table 1.

Table 1. Phenological behaviours on emergence of *Dahlia variabilis* (date)

Variants cv. / Indicators	'Vitus'		'White Ball'		'Dark Red'	
	control	overwintering	control	overwintering	control	overwintering
Beginning	27.04.	13.04.	04.05.	20.04.	27.04.	30.03.
Mass	18.05.	27.04.	25.05.	04.05.	25.05.	13.04.
Simultaneously emergence (days)	22	15	22	15	29	14

The onset of emergence depends on both the variety and the type of cultivation. Of the three varieties studied, the earliest plants emerge from the 'Dark Red' variety - 30.03. winterized and 27.04. grown by traditional technology. After them emerge the 'Vitus' plants - 13.04. for the winter winners and 27.04. for control plants. And, at the latest, the 'White Ball' plants start sprouting - on 20.04 respectively. and 04.05. Wintering, on the other hand, has a strong positive effect on the onset of emergence. Overwintered plants of all three varieties enter this phase earlier - 'White Ball' with 15, 'Vitus' with 17, and 'Dark Red' 27 days earlier. There is a similar trend in the next phase - mass emergence. Here, the results are even more emphatic - the difference between wintering plants and plants grown using traditional technology is from 21 days in the 'White Ball' variety, 22 days in the 'Vitus' variety, to 43 days in the 'Dark Red' variety. The emergence friendship shows the duration of the period from onset to mass emergence. This period is of utmost importance both in the use of dahlia for landscaping parks and gardens and in planting for the production of cut flowers. In terms of this indicator, pre-winter plants have the advantage - germination friendship is 15 days for the 'Vitus' and 'White Ball' varieties and 14 for the 'Dark Red' variety. The emergence rate of plants grown using traditional technology for the 'Vitus' and 'White Ball' varieties is 46.6% lower, and 107.2% for the 'Dark Red' variety.

Data on the duration of budding and flowering in dahlia are presented in Table. 2.

Table 2. Phenological behaviours during flowering of *Dahlia variabilis* (days after emergence)

Variants cv. / Indicators	'Vitus'		'White Ball'		'Dark Red'	
	control	winterized	control	Winterized	control	winterized
Beginning of budding	66.7	60.5	94.8	89.7	74.5	70.7
Mass budding	71.8	63.8	99.1	92.5	81.4	75.6
Beginning of flowering	77.8	64.7	101.2	93.9	79.7	74.5
Mass of flowering	108.8	97.8	133.2	124.5	118.9	104.5
End of flowering	178.3	211.5	163.2	199.5	142.5	178.8

Phenophase 'onset of budding' occurs at the earliest in the 'Vitus' variety - 60.5 days after emergence in wintering plants and 66.7 days in plants grown using traditional technology.'Dark Red' is the next variety to form flower buds - 70.7 days after emergence in winter and 74.5 days in control plants. The 'White Ball' variety is the variety that most recently forms flower buds - 89.7 and 94.8 days after emergence, respectively. A similar trend is observed in the phenophase 'mass budding' - the 'White Ball' variety, followed by the 'Dark Red' and finally the 'Vitus' variety, first entered this phenophase. Regarding the wintering of tubers in the soil, plants grown using traditional technology enter this phenophase 5.8 days, 6.6 days and 8.5 days later than the wintering plants, respectively, for the 'Dark Red', 'White Ball' and 'Vitus' varieties.

The beginning of flowering starts first with overwintered plants of the 'Vitus' variety - 64.7 days after emergence, and at the latest in control plants of the 'White Ball' variety - 101.2 days after emergence. Overwintered plants enter this phenophase earlier than those grown under traditional technology in all three varieties tested - the biggest difference is in the 'Vitus' variety - 13.1 days in favor of the winterers, 7.3 days in the 'White Ball' variety and 5.2 days at 'Dark Red'.

Overwintering of tubers in soil and on mass flowering has a strong positive effect. This phenophase occurred 14.4 days earlier in the 'Dark Red' variety, 11.2 days earlier in the 'Vitus' variety and 8.17 days earlier in the 'White Ball' variety.

The end of the flowering period in dahlia depends on both the variety and the environmental conditions, as the plant usually blooms in the fall until the first frosts fall. The longest flowering plants are winter plants of the 'Vitus' variety - 211.5 days, with 33.2 days more than the plants grown according to traditional technology. 36.3 days later, the flowering of 'Dark Red' and 'White Ball' varieties for wintering plants is over.

The duration of flowering is an important indicator for both the individual flower and the whole plant (Table 3).

Table 3. Duration of flowering period for *Dahlia variabilis*

Variants cv. / Indicators	'Vitus'		'White Ball'		'Dark Red'	
	control	winterized	control	winterized	control	winterized
Separate color (days)	3.7	5.4	5.9	11.3	4.1	6.8
Whole plant (days)	35.9	94.5	33.6	81.6	33.1	73.5

It is crucial for the decorative qualities of the plant. The flowering time of the individual blossoms varies from 3.7 days in traditional cultivated 'Vitus' plants to 11.3 days in winterized 'White Ball' plants. The individual flowers of the wintering plants bloom by 1.7 days, 5.4 days, 2.7 days more

than those grown using traditional technology in the three varieties studied - 'Vitus', 'White Ball' and 'Dark Red'. The duration of flowering of the whole plant in the 'Dark Red' variety is 45% longer. The 'Vitus' and 'White Ball' varieties are 37.9% and 41.2% respectively.

CONCLUSIONS

1. Plants obtained by overwintering tuberous roots in the soil have a faster growth rate. The maximum in their growth is at the beginning of June. Plants grown using traditional technology grow fastest in late June and early July.
2. 'Dark Red' overwintering plants emerge and develop at the earliest, and at the latest - grown traditional technology 'White Ball' plants. Emergence is best with overwintering plants - they sprout massively within 14-15 days. With 46.6-107.2%, the emergence rate is less in plants grown using traditional technology.
3. The duration of budding is not affected by the mode of cultivation of the plants. This is probably due to the fact that the dahlia belongs to the plant group of the short day. However, the formation of flower buds depends on the variety - it forms the 'Vitus' flower buds, and the 'White Ball' variety at the earliest. Phenophase onset and mass flowering occur 1-2 weeks earlier in plants derived from wintering tubers. The flowering period is 18.6 - 22.4% longer for overwintering plants.
4. There is a very strong positive effect of wintering on the flowering duration of both the individual flower and the whole plant. The duration of the individual flower is 1.7 to 5.4 days longer, while that of the whole plant - by 37.9-45% more.

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EVALUATION OF VEGETATIVE DEVELOPMENT AND DECORATIVE BEHAVIORS OF SOME GLADIOLUS (*GLADIOLUS HYBRIDA* L.) VARIETIES UNDER BULGARIAN CONDITIONS

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Abstract

The main goal of the present study was to establish the most appropriate gladiolus variety for growing under Bulgarian conditions. The experiment carried out in the Experimental fields of Agricultural University - Plovdiv with five gladiolus varieties: Purple flora, Priscilla, Plum tart, Oscar and Green star. The corms were planted in March. The phenological phases of sprouting, the appearance of the inflorescence stalk, beginning and end of flowering were observed. During the vegetation, the most important vegetative behaviours of the plant as high of plant, the diameter of the stem, number of leaves, length of inflorescence stalk and numbers of fully developed flowers and undeveloped flowers per plant were established. The coluor of the flowers was also registered. A Green star and Purple flora varieties have the strongest vegetative development, resulting in the formation of the highest plants, with the largest diameter and number of leaves. The highest decorative value of all tested genotypes, under the conditions of Bulgaria, indicated the Purple flora variety. Positive correlations are established between the height of the plant and the number of leaves and also for the length of inflorescences stalk and the number of developed flowers.

Key words*: flower crops, flowering, decorative value, phenophases, corms.*

INTRODUCTION

Gladiolus is one of the main flower crops, widely distributed for both cultivations of cut flowers and inclusion in outdoor landscaping groups. The diversification of the varieties in this crop is significant, and in this regard, it is important to carry out research to identify suitable varieties depending on the specific climatic and soil conditions. One successful way to get new genotypes according to Denisa et al. (2012) is an inter-species hybridization. As a result, the breeders from the Cluj-Napoca, Romania region have received over ten new well-established hybrids suitable for outdoor cultivation. Pragya et al. (2010) studied an extremely large collection of 54 genotypes of gladiolus. Based on the study of nine major morphological features and the application of 225 random amplified polymorphic DNA (RAPD) markers amplified with 25 arbitrary primers, they estimate the genetic relationships between the selected genotypes. In Bulgaria is also carried out breeding programs with gladiolus. Ivanova et al. (2019) reported for new Bulgarian gladiolus variety named Iva and emphasized that this variety excided the other traditional cultivars from this flower crop and has very high decorative values.

Meira et al. (1990) examined *in vitro* regeneration by using the explants from the inflorescence of the gladiolus stalks and established the new plantlets within 6-7 weeks. The regeneration depended on the levels of growth substances added to the basal medium and the best combination for organ initiation is observed in the medium with 10 ppm naphthalene-acetic acid and 0.5 ppm of kinetin.

By the experiments conducted by Kamble et al. (2004) with nine varieties of gladiolus, the earliest development and flowering is manifested the Snow White variety. The emergence was established in 58.20 days and flowering began at 66.70 days. Good results were also noted for the American Beauty variety. The latest development was observed in the cultivar Cultivars Magic. Shillo and Halevy (1996) emphasize the importance of specific climatic conditions for the normal development of gladiolus. An important factor, according to the authors, is the presence of optimum light, especially sensitive the plants

are in phase 4-6 sheets. The researcher emphasized that insufficient light in the initial periods delayed development and delayed flowering, however, many test varieties subsequently overcome the delay.
Kaninski et al. (2012), intending to establish the impact of the specific conditions of the area, investigated different periods of the planting and established that, at later dates, flowering and plant development are significantly more rapid. In this scope, a similar investigation carried out also Ivanova et al. (2016) and established that the best period for the planting of gladiolus is the middle of April. Through a study of the timing of planting and the impact of GA3 Suman et al. (2012) indicate that the best results are obtained with the application of the gibberellic acid in a concentration of 100 ppm.
The main goal of the present study was to establish the most appropriate gladiolus variety for growing under Bulgarian conditions.

MATERIALS AND METHODS

The experiments were carried out during the period 2017-2019 in the Experimental field of the Agricultural University of Plovdiv Bulgaria with the following five gladiolus cultivars: Purple flora, Priscilla, Oscar and Green star. The corms were planted during the last decade of March by the scheme 25 x 25 cm. The approved technology of gladiolus cultivation, developed by the Institute for Decorative and Healing Plants (Bistrichanov et al., 2008) has been implemented. During the vegetation, the necessary agro-technological practices were applied. The experiments were performed in four replicates, in a randomized method, with a plot size of 3 sq.m. and 48 plants were included.
The following phenophases were reported: emergence, beginning of the inflorescence stalk, beginning and end of flowering. The appearance of these phenophases was established when they occurred in 10% of the plants in a given phase (Dimova and Marinkov, 1999).
The diameter, height and weight of the corms before planting were measured on 15 corms. At the end of flowering the plant height, stem diameter, number of leaves, number of developed flowers, number of undeveloped flowers, as well as the color of flowers were established. Measurements were made on ten plants of each replicate. The percentage of developed plants towards the total planted corms number was also determined.
The data obtained were subjected to analysis of variance (ANOVA) using software package SSPS, developed by IBM Corporation (https://en.wikipedia.org/wiki/SPSS).
Due to the similarity of the trends in the obtained results, the presented data are averaged values from the three-year studies.

RESULTS AND DISCUSSIONS

The genotypic differences between the studied varieties of gladiolus according to the morphological features of the corms were observed about the planting material (Table 1). The differences between the highest and lowest diameter of the corm of studied varieties are 1.0 cm. The values of this index ranged from 2.9 cm for the Oscar and Green star varieties to 3.9 cm for Purple flora. Smaller differences within 0.7 cm are reported for the corm height. It is the highest for the Plum tart variety, followed by the Oscar, 2.7 cm and 2.6 cm respectively, and the smallest one is for Priscilla. The most significant differences in corm morphology were observed for weight. This difference reaching to 13.42 g. Values range from 11.97 g (Green star) to 25.39 g (Purple flora). The results about the weight of corms are with statistical significance with the exception between Purple flora and Plum tart and also between Oscar and Green star.
The data of the phenological observations are presented in Table 2. The difference in the sprouting period between the tested varieties is relatively small and ranges from 27 to 31 days after the planted of the tubers. The earliest sprouting was reported for Priscilla on day 25, followed by Oscar on 27 days.

Table 1. Morphological features of the gladiolus corms

Varieties	Diameter (cm)	Height (cm)	Weight (g)
Purple flora	3.9	2.4	25.39
Priscilla	3.4	1.9	15.82
Plum tart	3.7	2.7	23.17
Oscar	2.9	2.6	12.38
Green star	2.9	2.1	11.97
LSD p = 5.0%	0.4	0.6	3.4

The most delayed sprouting was observed in the Plum tart variety, and it's was reported on 31 days. More variation is recorded in the next phenophase, the appearance of the inflorescence stalk. Earlier this phase was established in Purple flora, 18 days after sprouting. The differences between the earliest developed stalks, in the mentioned variety and the latest, formed one in Green star is 14 days. As vegetation progresses the differences between the studied genotypes increase. This is very clearly observed in the phase of the beginning of flowering. The earlier appearance of the inflorescence stalk in Purple flora also caused earlier flowering, as early as 90 days. The flowering is the latest for Priscilla, with a difference of 23 days from the earliest variety. A positive characteristic of the Purple flora variety, compared to the other varieties, is the later end of the flowering. Although the initial phases undergo more rapidly, the flowering reaches 182 days after planting or lasts 92 days. The shortest flowering period of 63 was observed for the Oscar variety, ending at 168 days from planting, i.e. the flowering period is only 63 days. The longest flowering occurs at Plum tart to 191 days or it in the period from 83 days. Ivanova et al. (2019) point out that for the conditions of Bulgaria the average duration of the flowering of gladiolus is within 50-60 days. This indicates that the tested varieties in this article there are longer flowering, which also makes them more promising for outdoor cultivation.

Table 2. Phenological beahviors of different gladiolus varieties (days after planting)

Varieties	Sprouting	Appearance of the inflorescence stalk	Beginning of the flowering	End of the flowering
Purple flora	28	46	90	182
Priscilla	25	48	113	178
Plum tart	31	52	108	191
Oscar	27	49	105	168
Green star	29	60	109	185
LSD p = 5.0%	3.1	2.3	2.8	2.1

There are no differences between the varieties in terms of the percentage of the developed plants (Figure 1).

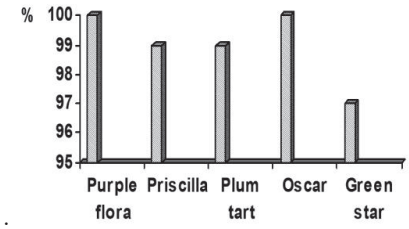

Figure 1. Percentage of the developed plants

Almost 100% is the development of plants in all varieties. Only Green star has a slight deviation and this percentage reaches 97. This may be due to the higher adaptability of these varieties under growing conditions.

Morphological features are important for a more complete assessment of plant development and genotype response (Table 3). The most essential indicator in this regard is the height of the plants.

The significance of this sign and as a decorative value also is emphasized by Kumari et al. (2011) and by Ivanova et al. (2019).

The highest plants were measured in the Green star variety - 132.1 cm. With the lowest height is characterized Oscar variety, with 31.9 cm less than the previous one. The plants of Purple flora are also relatively high. Statistical significance of the data was established.

Another vegetative feature describing plants of gladiolus is the diameter of the stem. This feature ranges from 1.7 cm (Plum tart) to 2.6 cm (Green star). The exceeding between the highest and the lowest values is 34.62%. Changes between the varieties are also observed for the number of leaves. With the highest number of leaves are the plants from Green star variety (9.3), followed by this one of Purple flora (9.0). The significantly smaller numbers of leaves developed the Priscilla and Plum tart varieties, respectively 6.5 and 7.2, or 43.07% and 29.16% lower than the values of this feature in Green star variety. The development of the leaves depends on strongly by the height of plants. This is confirmed from the established correlation coefficients that for many of the studied varieties determinate are high and positive correlation, except Plum tart and Green star, where it is positive but middle. The differences are mathematically proven.

Table 3. Morphological characteristics of the plant of several gladiolus varieties

Varieties	Height of the plant (cm)	Diameter of the stem (cm)	Number of leaves	r
Purple flora	127.8	2.5	9.0	0.66
Priscilla	120.4	1.9	6.5	0.77
Plum tart	115.2,	1.7	7.2	0.35
Oscar	100.2	2.3	8.0	0.68
Green star	132.1	2.6	9.3	0.48
LSD p = 5.0%	4.1	0.6	1.0	

r - correlation coefficient between height of plant and number of leaves

The most important elements for describing varieties of flower crops are their decorative features (Table 4). The length of the inflorescence stalk plays an essential role in determining its quality and decorative behaviours. For the Green star variety, it indicates the highest value among the genotypes tested and reaches 36.6 cm. Very close to it are the gladiolus of Purple flora with the height of the stalk from 36.5 cm. The inflorescence stalk in Plum tart (24.4 cm) and Priscilla (27.3 cm) are significantly lower. The data of the height of the inflorescence stalk are statistically significant, except for those between Purple flora and Green star.

The decorative value of flowering species is primarily determined by the number of flowers developed. This number is the highest for Purple flora - 14.5. It is 16% lower for Green star, with 12.5 pcs flowers. At the least flowers were developed the plant from Priscilla and Plum tart. The number of flowers is directly related to the length of the inflorescence stalk, which is very clear in the last two mentioned genotypes, which were noted that they are with the lowest inflorescence stalk. This tendency is observed also by the established correlation coefficients between this parameter and the number of flowers. The high and positive correlation is determinate for Purple flora, Plum tart and Oscar with r = 0.63, r = 0.68, and r = 0.67, respectively. For the other two varieties, the correlation is also positive but middle. The genotypic response in gladiolus is also appropriate to determine depending on the number of the set but undeveloped flowers. The least undeveloped flowers are count in Plum tart variety - 2.9 pcs. However, it must emphasize that this variety has formed a few fully developed flowers and the part of undeveloped is high, reaching 31.86%. With the low number of a developed and high number of undeveloped flowers is characteristic also Priscilla variety and the portion of undeveloped is 52.22%. The most abundant flowering, as mentioned above has Purple flora variety, and part with it the share of undeveloped flowers is the lowest - 22.06%, followed by Green star - 28.0%. A very important feature is the colour of the flowers, as it completely determines the decorative value of the gladiolus. The colour diversity of the flowers in tested variety is wide. The predominant are the flowers with purple colour and hue. It should be noted that lime-green colour is less common, as is the case with Green star, making it a non-standard and therefore very interesting and beautiful compared to the widespread gladiolus varieties.

Table 4. Decorative behaviors of the gladiolus varieties

Variety	Length of the inflorescences stalk (cm)	Number of developed flowers	Number of undeveloped flowers	r	Colour of flowers
Purple flora	35.5	14.5	3.2	0.73	Dark purple
Priscilla	27.3	9.0	4.7	0.47	White-pink
Plum tart	24.4	9.1	2.9	0.68	Purple-violet
Oscar	31.0	11.2	3.6	0.67	Velvety red
Green star	36.6	12.5	3.5	0.43	Lime-green
LSD p = 5.0%	2.4	1.2	0.6		

r - correlation coefficient between height of plant and number of leaves

CONCLUSIONS

The corms between the different gladiolus genotypes differ in morphological features. With the largest diameter and weight are characterized by those of the Purple flora variety.

The tested varieties of gladiolus are varied slightly on the term of sprouting, and more significant differences there are in the appearance of inflorescence stalk and the flowering. The longest flowering period is in Plum tart variety, from 83 days.

A Green star and Purple flora varieties are with the strongest vegetative growth, resulting in the development of the highest plants, with the biggest diameter and number of leaves.

The highest decorative value of all tested gladiolus genotypes, under the conditions of Bulgaria, was manifested by Purple flora variety, which is with the highest length of the inflorescence stalk and the number of developed flowers and a low proportion of undeveloped flowers.

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MERLINIUS BREVIDENS (SIDDIQI, 1970) AND *MERLINIUS NOTHUS* (SIDDIQI, 1970) NEMATODES DETECTION AND IDENTIFICATION IN FLOWERING PLANTS GROWN IN GREENHOUSE

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Abstract

Merlinius brevidens is detected and identified in the root systems of the Chrysanthemum flowering plants in a greenhouse from Ploieşti, Prahova, Romania; also other species existent in Romania, namely Merlinius nothus was found and identified in Strelitzia plants in a greenhouse from the same locality. The morphobiometrical characteristics mostly used for the separation of the two species involve the body and stylet length, shape of head, the tail shape, the tail and its type, the number of annules found in the head and tail areas, the position of the vulva (V), and the ratio c' in females case. This is the first report for Romania of Merlinius brevidens.

Key words*: Merlinius brevidens and Merlinius nothus, detection and morfobiometrical identification in flowering plants.*

INTRODUCTION

The stunt nematodes are a broadly distributed group of genera and species distributed all over the world in agricultural soils and uncultivated land. Of the 267 now valid species, few have been proven as being pathogenic, but about 8% are known ectoparasites (Anderson and Potter, 1991). Many are part of a complex of plant parasite nematodes in soil around crop plants, they can be thought of a stress factor to their hosts (Anderson and Potter, 1991).

The *Merlinius* genus was introduced by Siddiqi in 1970, these species were previously accommodated in the *Tylenchorhynchus* genus, due to the fact they had six incisures in the lateral fields. Tarjan in 1973 gave a key data and diagnosis of the genus and species of Tylenchorhynchina and discussed with Siddiqi about some characters. În 1973 Tarjan agreed with Siddiqi (1970) that the presence of six incisure in the lateral fields is a consistent and easily recognizable character, so the genus *Tylenchorhynchus* becomes *Merlinius* genus. Handoo et al., (2007) agrees with both Siddiqi (1970) and Tarjan (1973) because their actions led to an easier management of this complex and large group of nematodes. Today the genus *Merlinius* comprises 32 valid species distributed worldwide as parasites of large plants varieties Handoo et al., (2007). The history of the genus *Merlinius* was discussed by Hooper (1978), Fortuner and Luc (1987) including *Merlinius* in the Telotylenchinae subfamily, and subsequently in the Belonolaimidae family. Anderson and Potter (1991) included the genus *Tylenchorhynchus*, *Merlinius* and *Amplimerlinius* in a review of significant species for agriculture, they also presented a good historical past of this nematodes group taxanomy. In 1998, Brzeski included all species of the genus *Merlinius* in *Geocenamus* and gave a key for 19 species and a compendium for 77 species. He concluded that the genus *Geocenamus* may be a collective group, which could be divided into separate genera, but the study of the cephalic structure of several species under electron microscope were necessary before reaching to a conclusion. (Handoo et al., 2007).

As a result of these changes, the development and compilation of a dichotomous key for all species belonging to the *Merlinius* genus has become increasingly difficult.

Many of the species that were included in the *Merlinius* genus were placed in new genera by

different specialists and several species belonging to other related genera were either moved or given other synonyms. The most important character used in distinguishing this genus is the number of lateral lines, comprising species with three or six lateral lines.
The general objective of research is identification of phytoparasitic nematodes fauna in flowering plants grown in Romania. In the present research have been identified two species of *Merlinius* with six lateral lines which are part of the *Merlinius* genus. In 1997, Popovici referred to the existence of the species *Merlinius nothus* in Romania but are not data regarding to description of this species. The description of two species of *Merlinius*, *Merlinius brevidens* and *Merlinius nothus,* based on the morphobiometric characters of these species, is provided.

MATERIALS AND METHODS

The soil samples were taken from the same greenhouse, one from *Chrysanthemum* crop and the other from *Strelitzia* crop. Geographical coordinates (GPS) are: $44^0$56′02.82′′N; $26^0$02′49.18′′ E.
The samples were taken from the root system of the plants and the amount of soil taken was about 500 ml for each cultivated flowering plant variety.
The nematodes density found was approximately 15 specimens for the first species and 20 specimens for the second detected species, for about 500 ml of soil analysed for each flowering plant variety.
The extraction of nematodes from the soil for both species of detected nematodes was done using the COBB method or the buckets method. After the nematode's extraction and recovery from the soil, followed their pre-identification with the Leica DMLB MZ 12.5 stereomicroscope and identification with DIC microscope.
The identification of the species and their measurements were carried out on fresh material in case of *Merlinus brevidens* and permanent slides for *Merlinius nothus*, having a greater number of specimens. In order to make permanent slides the nematodes were stored in a buffer solution of water and formaldehyde and then were placed into glycerine, in order to draw morphometric observations and measurements (Hooper, 1986). The measurements of the analysed samples were done using a Zeiss Axio Imager 2/430004 - 9902 microscope, supplied with Zeiss Axiocam 506 - 426556 digital camera and a Zen 2.6 (Blu edition) incorporated software.

RESULTS AND DISCUSSIONS

Merlinius brevidens (Allen, 1955; Siddiqi, 1970) a new record for Romania

= *Tylenchorhynchus brevidens* (Allen, 1955)
= *Geocenamus brevidens* (Allen, 1955; Brzeski, 1991)

Description
Figure 1 (a-f), Table 1
Female:

The body is straight to strongly arched when relaxed, with the width in the middle between the following values 21.4 (20-23); with fine annules that are about 1µm wide or less.
The lateral lines occupy about a third of the diameter of the body, they present six incisions. The excretory pore (n = 10) has between 105-119 µm, with an average of (110.9±5.0) µm, located at the posterior of the head and at the level of the oesophageal basal bulb. The hemizonide is located in front of the excretory pore at a distance of 1-3 µm from this. The deirids are present, localized in the excretory pore, but most often they are not seen because of the cuticle. The head is not tall, is rounded, continuous or slightly set off against the body, being demarcated by five or six annules. The stylet is 15.7 µm long (15-17), with rounded knobs, 3.5-4.5 µm wide, sloping posteriorly. The oesophagus is 141 (132-147) µm length. The vulva is between 54-59 (56.1±1.3) percent of the body length.
The ovaries extend to the anterior part of the body close to the basis of the oesophagus in pregnant females. The tail is tapered, curved, between 48-65 (53.8 ±5.4) µm, (n = 10) in length. The tail is marked by the presence of 39-62 fine annules, cuticular inclusions being particularly dense at the tip of the tail. Phasmids near or slightly posterior to the middle of the tail.

In case of this species, no males were present in the examined soil.

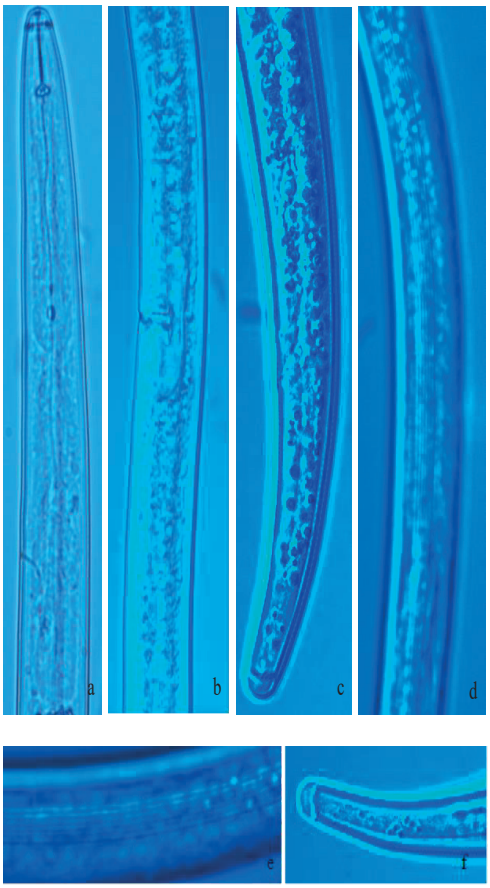

Figure 1 (a-f) *Merlinius brevidens*: (a) Anterior region of female; (b) Vulval region; (c, f) Female tail; (d, e) Lateral field showing six incisures

Merlinius nothus (Allen, 1955; Siddiqi, 1970), existent species in Romania:

= *Tylenchorhynchus nothus* (Allen, 1955)
= *Geocenamus nothus* (Allen, 1955; Brzeski, 1991).

Description
Figure 2 (a-e), Table 1

The female is between the following values n = 11, 747-884 (807.2 ±14.1) µm; the stylet is 15-19 (17.2±1.7) µm is fine to medium, its cone is slightly longer than the shaft, the knobs are elongated to a certain extent having a diameter of 4.5 µm. The cuticle is distinctly annulation, the annules being rounded by 1.1-1.8 µm wide. The lateral lines have six incisions. The head is shorter than the adjacent body, it is rarely continuous, the annules are 6 in number. The deirids are present and located at the level of the excretory pore. The cephalic skeleton is weak. The genital tract can spread to a longer part of the body than the intestine. The vulva has a small cavity between 54-58 (56) percent of the the body length. The sperm tank is off set when it is filled with sperm and can be bilobed. The tail is tapered, having 40 to 46 annules, rounded at the tip, in most cases it is smooth, rarely striated. Tail length for n = 11, (34-55 µm) 47.6 ±7.0. The phasmids are distinct and located in the middle of the tail.
No males of this species were detected in the examined soil samples.

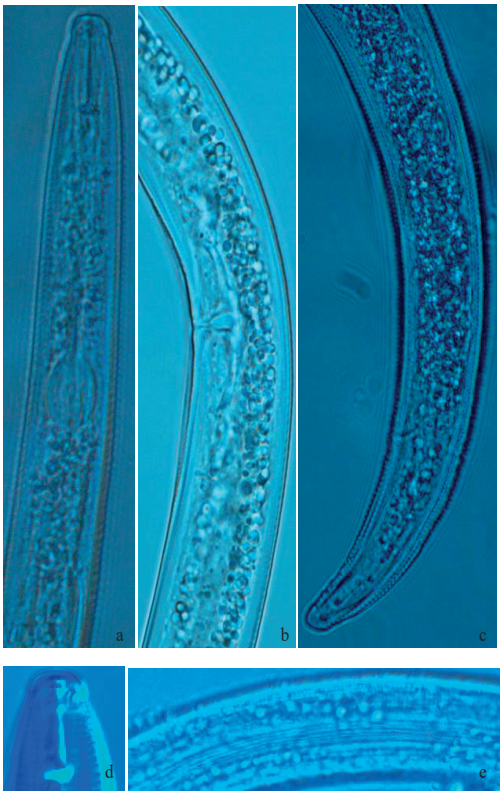

Figure 2 (a-e). *Merlinius nothus:* (a, d) Anterior region of female; (b) Vulva and reproductive structures; (c) Tail region; (e) Lateral field showing six incisures

Merlinius brevidens species detected in *Chrysanthemum* flowering plants is somehow

longer, its length being comprised between (579-778 vs. 540-690 µm).
The stylet length has values comprised (15-17 vs. 14-16 µm), being to a certain extent higher than the original described species.
The vulva is positioned between (54-59 vs. 52-58 %) of the body length. The ratio c' is between 3.6 and 4.3 µm. The annules in the head area are 5 to 6 and the tail ones are 50 to 60. Generally, the morphology of the populations of the two recovered nematode species has similarities that are close to the originally described species.

Table 1. Morphometric characters of the species *Merlinius brevidens* (Allen, 1955; Siddiqi, 1970) and *Merlinius nothus* (Allen, 1955; Siddiqi, 1970) collected from South Romania

Species:	*Merlinius brevidens*		*Merlinius nothus*
Host plants	*Chrysanthemum*		*Strelitzia*
Locality:	Ploieşti		Ploieşti
Characters/ ration[b]	Females	after Allen,1955	Females
n	10	11	11
L (µm)	667.8 ± 61.0 (579 – 778)	540 - 690	807.2 ± 14.1 (747 – 884)
a	31 ± 1.9 (27 - 34)	23 – 27	29.9 ± 2.8 (26 – 32)
b	4.7 ± 0.3 (4.2 – 5.2)	4.2 – 5.2	5.0 ± 0.1 (4.7 – 5.3)
c	12.3 ± 0.6 (11.6 – 13.7)	11 – 13	17.2 ± 1.4 (15 – 24)
c'	4 ± 0.2 (3.6 – 4.3)		2.5 ± 0.07 (1.7 – 3)
V %	56.1 ± 1.3 (54-59)	52 – 58	56 ± 0 (54 – 58)
Stylet length	15.7 ± 0.6 (15 – 17)	14 – 16	17.2 ± 1.7 (15 – 19)
Pharynx length	141 ± 5.8 (132 – 147)		159.5 ± 5.6 (149 – 176)
Excretory por	110.9 ± 5.0 (105 – 119)		133.2 ± 3.5 (127 – 150)
Max. body diam.	21.4 ± 0.9 (20 – 23)		26.9 ± 2.1 (25 – 29)
Anal body diam.	13.9 ± 1.8 (12 – 18.5)		18.4 ± 2.1 (14 – 27)
Tail	53.8 ± 5.4 (48 – 65)		47.6 ± 7.0 (34 – 55)

The abbreviations of the measured characters from table 1 were defined in Siddiqi (2000).

CONCLUSIONS

Merlinius brevidens is reported for first time in Romania in a crop of *Chrysanthemum* flowering plants grown in greenhouse.
The second detected species *Merlinius nothus* was listed for Romania by Moldovan et al, 2007, but in this study, we identified for first time the nematode in association with *Strelitzia* plants.
The species were found in crops of flowering plants grown in the greenhouse from Southern part of Romania, in the Ploiesti region, Prahova county, in autumn of the year 2019.
Both species are plants ectoparasite pests that develop all stages of development in the soil.
Several symptoms were reported in the normal growth and development of plants, which had a smaller growth, showing symptoms of decreased resistance and abnormal development.
The present study was mainly referring to the detection and identification of nematode species harmful to cultivated plants. In order to

provide data on the pathogenicity, biology and dynamics of the populations of these species, more investigation is required.

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ORNAMENTAL PLANTS SPECIES FROM SPONTANEOUS FLORA IN OLTENIA REGION, ROMANIA

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Abstract

The spontaneous flora of Oltenia brings together about 2/3 of the existing plants in Romania. The diversity of climatic, relief and soil conditions has led to this high phytodiversity. Many of the spontaneous plants that vegetate in this part of the country have a high ornamental potential (ex. Acanthus balcanicus, Adonis vernalis, Aster tripolium subsp. pannonicus Fritillaria meleagris, Galanthus elwesii, Hesperis pycnotricha, Limonium tomentellum, Salvia sclarea, etc.). They can be used to decorate green spaces in the urban and peri-urban areas of the cities of Oltenia. The urbanization and agriculture practiced in the last period of time are the perfect pair that leads to the decrease of the phytodiversity and to the reduction of the area of many species of plants. In this paper it is desired to bring to the attention of all those interested in the floral diversity of the spontaneous ornamental plants of Oltenia, highlighting those that are better suited to the conditions offered by urban and peri-urban areas in this part of the country. In order to highlight the chromatic diversity offered by these species, an analysis of the decorative parts is made and finally the advantages of using spontaneous flora in the decoration of green spaces are presented.

Key words*: spontaneous plants, green spaces, urban areas, Oltenia*

INTRODUCTION

The flora and vegetation studies in Oltenia are numerous. These are presented as monographs targeting the flora and vegetation of a specific territory (Păun, 1966; Zaharia, 1972; Popescu, 1974; Roman, 1974; Maloș, 1977; Cârțu 1979; Costache, 2005; Niculescu M., 2009; Răduțoiu D., 2006, 2008) or of scientific papers including floristic inventory (Buia et Popescu, 1952; Buia, 1959; Buia et Păun, 1960a, 1960b) or some plant associations summary (Borza, 1943; Ciurchea M, 1963, 1965; Cârțu M., 1968, 1971; Păun, 1985, Popescu, 1979, 1988, 1996; Răduțoiu et Costache, 2012).

Sporadic data are also found in specialized ”flora” published by various authors (Prodan, 1939; Săvulescu et al., 1952-1976; Beldie, 1977, 1979; Ciocârlan, 2000, 2009; Sârbu et al., 2013, Răduțoiu et Ștefănescu, 2017).

Suburban and exurban growth are affecting biodiversity in many places once thought of as too remote to attract such levels of development (Miller & Hobbs, 2002).

Studies on the attractiveness of different types of urban green spaces were conducted in Germany (Mathey & Rink, 2010).

Witting (2011) mentions that the colorless flowers ("gray") in urban and peri-urban places and a low percentage (maximum 30%) compared to the rest of the spontaneous species present in other places.

MATERIALS AND METHODS

Oltenia is one of the historical provinces of Romania, located in the south-west part of it. The floristic diversity of this area is justified by the variety of soil types, climate and relief. A significant part of the plant species vegetating on Oltenia territory are successfully used as ornamental in urban and peri-urban areas. In order to realize a floristic inventory with the species that present ornamental value, numerous trips in the field were made, in different phenophases, to notice all the development stages necessary for proper identification. The plant species are presented by botanical family, in the systematic order of Professor V. Ciocârlan determinator (2009).

After completing the inventory, laboratory analysis has been made consisting in plant species grouping according to the colour of the decorative parts. This analysis was

schematically represented in order to observe the weight of each colour.

RESULTS AND DISCUSSIONS

After numerous trips and laboratory analyses, a floristic inventory was created with spontaneous plants which are decorative through at least one of their component parts, totalling 189 taxa: Phylum Pteridophyta, Fam. Aspleniaceae: *Matteuccia struthiopteris* (L.) Tod.; Phylum Spermatophyta, subphyl. Pinophytina, Fam. Taxaceae: *Taxus baccata* L.; Subphyl. Magnoliophytina, Cl. Magnoliopsida, Fam. Ranunculaceae: *Hepatica nobilis* Schreb., *H. transsilvanica* Fuss., *Clematis integrifolia* L., *Ranunculus constantinopolitanus* (DC.) D'Urv., *Adonis vernalis* L. (Figure 1.), *A. aestivalis* L.;

Figure 1. *Adonis vernalis* from the periphery localities Fântânele (Dolj county)

Fam. Papaveraceae: *Papaver rhoeas* L., *P. laevigatum* Bieb., *P. dubium* L.; Fam. Fumariaceae: *Corydalis cava* Schweigg. et Körte, *Fumaria schleicheri* Soy.-Willem., *F. officinalis* L., *F. rostellata* Knaf, *F. densiflora* DC.; Fam. Ulmaceae: *Ulmus glabra* Huds., *U. procera* Salisb., *U. minor* Mill.; Fam. Molluginaceae: *Mollugo cerviana* (L.) Ser.; Fam. Caryophyllaceae: *Scleranthus perennis* L., *Minuartia glomerata* (M. Bieb.) Degen, *Minuartia setacea* (Thuill.) Hayek, *Sagina apetala* Ard. subsp. *erecta* (Hornem.) F. Herm., *Arenaria procera* Spreng., *Stellaria holostea* L., *Cerastium glomeratum* Thuill., *Gypsophila muralis* L., *G. paniculata* L., *Saponaria officinalis* L., *Vaccaria hispanica* (Mill.) Rauschert, *Kohlrauschia prolifera* (L.) Kunth, *K. saxifraga*, *Dianthus armeria* L., *D. trifasciculatus* Kit. in Schultes subsp. *parviflorus* Stoj. et Acht., *D. carthusianorum* L., *Silene latifolia* Poir. subsp. *alba* (Mill.) Greuter et Burdet, *S. conica* L., *S. armeria* L., *S. vulgaris* (Moench) Garcke, *S. borysthenica* (Gruner) Walters, *Lychnis coronaria* (L.) Desr. (Figure 2), *L. flos-cuculi* L., *L. viscaria* L., *Agrostemma githago* L., *Herniaria incana* Lam., *Spergularia rubra* (L.) J. et C. Presl.; Fam. Crassulaceae: *Sedum acre* L., *S. sexangulare* L.; *Parnassia palustris* L.; Fam. Rosaceae: *Potentilla anserina* L., *P. reptans* L., *P. argentea* L., *Filipendula vulgaris* Moench, *Sanguisorba officinalis* L., *Rosa gallica* L.; Fam. Fabaceae: *Genistella sagittalis* (L.) Gams,

Figure 2. *Lychnis coronaria* from the periphery localities Vârvoru de Jos (Dolj county)

Genista tinctoria L., *Laburnum anagyroides* Medik., *Sarothamnus scoparius* (L.) W. D. J. Koch, *Chamaecytisus albus* (Hacq.) Rothm., *Ch. hirsutus* (L.) Link subsp. *leucotrichus* (Schur) A. et D. Löve, *Anthyllis vulneraria* L., *Dorycnium herbaceum* Vill., *Lotus corniculatus* L., *Colutea arborescens* L., *Coronilla varia* L., *Onobrychis viciifolia* Scop., *Lathyrus aphaca* L., *L. sphaericus* Retz.; Fam. Lythraceae: *Lythrum salicaria* L., *L. virgatum* L. Fam. Cornaceae: *Cornus sanguinea* L.; Fam. Aceraceae: *Acer saccharinum* L., *A. platanoides* L., *A. pseudoplatanus* L.; Fam. Anacardiaceae: *Cotinus coggygria* Scop.; Fam. Oxalidaceae: *Oxalis corniculata* L., *O. fontana* Bunge; Fam. Linaceae: *Linum flavum* L., *L. uninerve* (Rochel) Jáv., *L. austriacum* L.; Fam. Polygalaceae: *Polygala vulgaris* L., *P. comosa* Schkuhr,; Fam. Araliaceae: *Hedera helix* L.; Fam. Apiaceae: *Eryngium planum* L., *Orlaya grandiflora* (L.) Hoffm., *Smyrnium perfoliatum*

L.; Fam. Paeoniaceae: *Paeonia peregrina* Mill.; Fam. Hypericaceae: *Hypericum perforatum* L.; Fam. Tiliaceae: *Tilia tomentosa* Moench; *T. platyphyllos* Scop., *T. cordata* Mill.; Fam. Malvaceae: *Malva sylvestris* L., *Althaea officinalis* L., *Abutilon theophrasti* Medik., *Hibiscus trionum* L. (Figure 3); Fam. Violaceae: *Viola tricolor* L., *V. canina* L., *V. odorata* L., *V. alba* Bess.; Fam. Cistaceae; *Helianthemum nummularium* (L.) Mill.; Fam. Brassicaceae: *Erysimum odoratum* Ehrh., *Hesperis matronalis* L., *H. pycnotricha* Borbás et Degen, *Rorippa sylvestris* (L.) Bess., *Alyssum montanum* L. subsp. *gmelinii* (Jord. et Fourr.) Thell., *Calepina irregularis* (Asso) Thell.; Fam. Resedaceae: *Reseda lutea* L.; Fam. Salicaceae: *Salix alba* L.,

Figure 3. *Hibiscus trionum* - flower detail

S. fragilis L., *Populus nigra* L.; Fam. Primulaceae: *Primula vulgaris* Huds., *P. veris* L. subsp. *columnae* (Ten.) Maire et Petitmengin, *Lysimachia vulgaris* L., *L. punctata* L.; Fam. Gentianaceae: *Gentiana cruciata* L., *Gentianopsis ciliata* (L.) Ma; Fam. Apocynaceae: *Vinca minor* L.; Fam. Oleaceae: *Fraxinus ornus* L., *F. excelsior* L., *Ligustrum vulgare* L.; Fam. Convolvulaceae: *Convolvulus cantabricus* L.; Fam. Boraginaceae: *Cerinthe minor* L., *Lithospermum purpuro-caeruleum* L. (Figure 4),

Figure 4. *Lithospermum purpuro-caeruleum*

Cynoglossum officinale L., *C. hungaricum* Simonk.; Fam. Lamiaceae: *Teucrium polium* L. subsp. *capitatum* (L.) Arcangeli, *T. chamaedrys* L., *Melittis melissophyllum* L., *Salvia sclarea* L. (Figure 5), *S. aethiopis* L., *S. nemorosa* L., *S. pratensis* L.; Fam. Scrophulariaceae: *Verbascum phoeniceum* L., *V. chaixii* Vill. subsp. *orientale* (M. Bieb.) Hayek, *Linaria vulgaris* Mill., *Veronica chamaedrys* L., *V. teucrium* L., *Digitalis grandiflora* L., *D. lanata* Ehrh., *D. ferruginea* L., *Rhinanthus rumelicus* Velen.; Fam. Solanaceae: *Physalis alkekengi* L.; Fam. Acanthaceae:

Figure 5. *Salvia sclarea* - inflorescence detail

Acanthus balcanicus Heywood et I.B.K. Richardson; Fam. Campanulaceae: *Campanula persicifolia* L., *C. rapunculus* L., *C. glomerata* L. (Figure 6), *C. rapunculoides* L.; *Jasione*

Figure 6. *Campanula glomerata* - inflorescence detail

montana L.; Fam. Caprifoliaceae: *Viburnum opulus* L.; Fam. Dipsacaceae: *Scabiosa ochroleuca* L.; Fam. Asteraceae: *Solidago virgaurea* L., *Bellis perennis* L., *Anthemis austriaca* Jacq., *A. tinctoria* L., *Achillea ochroleuca* Ehrh., *Leucanthemum vulgare* Lam., *Tanacetum corymbosum* (L.) Sch.-Bip., *Doronicum hungaricum* (Sadl.) Rchb., *Xeranthemum annuum* L. (Figure 7), *Tragopogon orientalis* L. (Figure 8); Cl. Liliopsida, Fam. Liliaceae: *Fritillaria meleagris* L., *F. orientalis* Adams, *Erythronium dens-canis* L. subsp. *niveum* (Baumg.) Buia et Păun, *Muscari neglectum* Guss. ex Ten., *Ornithogalum boucheanum* (Kunth) Asch., *O. pyramidale* L., *O. pyrenaicum* L., *Asparagus tenuifolius* Lam., *Ruscus aculeatus* L., *Convallaria majalis* L.; Fam. Alliaceae: *Allium vineale* L. (Figure 9), A. *scorodoprasum* L.; Fam. Amaryllidaceae: *Sternbergia colchiciflora* Waldst. et Kit.,

Figure 7. *Xeranthemum annuum* from dry meadows

Figure 8. *Tragopogon orientalis* - inflorescence detail

Figure 9. *Allium vineale* - inflorescence detail

Leucojum vernum L. (Figure 10), *Galanthus nivalis* L., *G. elwesii* Hook., *Narcissus radiiflorus* Salisb.; Fam. Iridaceae: *Iris variegata* L., *Crocus reticulatus* Steven, *C. flavus* Veston; Fam. Orchidaceae: *Orchis morio* L., *O. coriophora* L., *O. simia* Lam., *Anacamptis pyramidalis* (L.) Rich. Fam. Poaceae: *Lolium perenne* L., *Cynosurus cristatus* L., *C. echinatus* L., *Melica transsilvanica* Schur (Figure 11).

Figure 10. *Leucojum vernum* - flower detail

Figure 11. *Melica transsilvanica* - general aspect

If we consider the decorative part of an analysed taxa, we can observe the presence of a wide range of colours (Figure 12).

The analysis of the flowering period for the inventoried taxa highlights the predominance of summer plant species (124 species) (Figure 13). These are followed by vernal species with 33 taxa and those that can be used throughout the entire vegetation period (29 species).

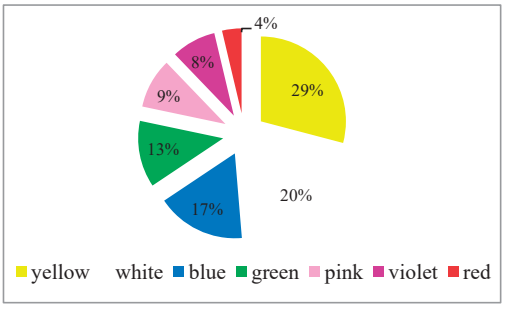

Figure 12. Representation of the chromatic variability

The rest are autumn flowering plant. The predominance of the species that bloom in summer is an advantage if we consider the climatic conditions for this part of Romania.

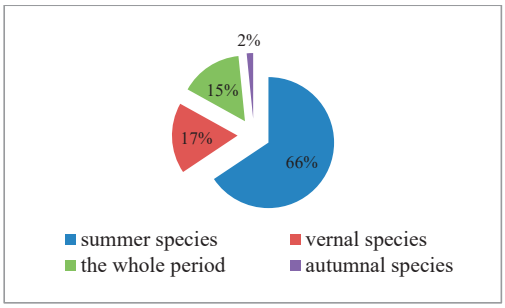

Figure 13. Graphic representation of the periods when the plants can be used for ornamental purposes

Knowing the requirements regarding the humidity index of the plants in the floristic inventory, we can say that the variability of this index is also reflected in the different climatic conditions on Oltenia territory. (Table 1). The dominance of xero-mesophilic and xerophilic elements explains the low values of the humidity index and the high ones of the thermal index in this part of the country.

Table 1. Moisture index analysis

No. crt.	Humidity scale	Number of taxa
1.	mesoxerophilic	91
2.	mesophilic	44
3.	xerophile	33
4.	mesohigrophile	14
5.	higrophile	5
6.	eurifite	2

CONCLUSIONS

From the analysis of the floristic inventory that brings together vascular plants that can be successfully used as ornamental plants in the urban and peri-urban areas of the main cities in Oltenia, the following conclusions can be drawn:

- the decorative parts from most of the selected species are represented by: the floral cover (corolla - most of them, calyx - *Physalis alkekengi*, perigonium - *Fritillaria meleagris*), inflorescences - *Acanthus balcanicus*, *Anacamptis pyramidalis*, *Digitalis ferruginea*, *Hesperis matronalis* etc.), the whole plant (*Ruscus aculeatus*, *Lolium perenne*, etc.)
- the chromatic variety is large: yellow, white, blue, pink, lilac, green and red.
- the plants that can be used have a wide ecological spectrum in green spaces in urban or peri-urban areas.

- these cover all plant layers (trees - *Tilia* sp., *Acer* sp., *Populus* sp., *Salix*, shrubs - *Cotinus coggygria*, *Ligustrum vulgare*, *Cornus sanguinea*, herbaceous - most of them).
- the advantages of using spontaneous flora species are numerous: low costs, minimal maintenance, ensuring long-term chromatic variability, etc.

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HERĂSTRĂU'S MAIN SOUTHERN AXIS. WHAT (EMBLEMATIC) IMAGE TO RESTORE?

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Abstract

Herăstrău is the largest public historical park in Bucharest that has developed in several phases during two main periods. All phases have left different imprints on its layout, leading to a hybrid and unclear image, but also on its undeniable values that list it as an historical monument. Lack of clear urban policies and protection of the historical park have led to the alteration of its emblematic images and perspectives during the last 30 years. The most important and iconic image of the park is represented by the central axis of the southern part, leading from Charles de Gaulle square towards the lake. Therefore, this paper will analyse its evolution and transformation in time, in order to find a better response to its present and urgent issue: Herăstrău's restoration plan. Following a detailed historical landscape study looking at all design phases, this paper will present the main values of the park subjected to legal protection and will bring in a perhaps radical solution for the restoration of the axis and its central perspective.

Key words*: Herăstrău, central/main axis, historical development, evaluation, restoration.*

INTRODUCTION - SHORT HISTORY OF HERASTRAU PARK

Before being a park, Herăstrău was a marsh that ran slowly and lazily along the Colentina. It had, though, already become a place for locals to visit. Lieutenant Colonel Papazoglu (1891) described the banks of the Colentina during springtime: "the Herăstrău marsh started towards the north side, on who's banks Constantin Ipsilante erected a hut higher up on the hill, in 1780, for his lady. He would sit in the pavilion with his fellow noblemen, while his wife sailed on the marsh with her ladies-in-waiting in a beautiful boat while a group of musicians sang for them. Floreasca Lake was named as such because of the Floreasca family who lived on its banks in grand villas and houses, given as dowry by *băneasa*[1] Anica Hereasca, and in whose villages people worked bleaching material and cutting wood at the mill. Not far from there starts the Bănesii Grove, the property of *ban* Ghica, which he left to his widower, the *băneasa*, whose name remains to this day. At this grove, the Bucharest elite would celebrate every 1st of May. It is there that the melodies of the cuckoo birds, nightingales, turtledoves and larks could be heard, alongside the 'baa's' of the lambs and sheep and the songs of their herders, the bagpipes of the gardeners, the fiddler's violin, and, during the Phanariot age, the drums, pan flutes and the oriental violins which would caress the public".

In 1816, the Austrian Baron Ludwig von Stürmer (Parusi, 2005) remarked during his visit to the capital that the locals' favourite wandering haunts were the Mavrogheni, Herăstrău, and Colentina (the Ghica family's Tei estate) alcoves, and the Elefterie Garden in the Dâmbovița meadow. Doctor Felix (1892) mentions the existence of a number of springs with good water (that come out of chalk) in Herăstrău.

The 1935 Master Plan, created by Duiliu Marcu, G.M. Cantacuzino, Roger Bolomey, I.Al. Davidescu and T. Rădulescu include Herăstrău as a large park that starts alongside Kiseleff Street and grows around the Colentina River, for which work on its regularisation had already begun. The sanitation and improvement project undertaken by Nicolae Caranfil (director of the Bucharest Municipal Plant - U.C.B.) had already been approved by the

[1] Ban - title and function of great governor in Țara Românească (Wallachia) after 15th century, it is the higher rank of boyar (DEX). Băneasă – the wife of the Ban, became the toponymical of Băneasa

Municipal Council on the 21st of February, 1932, with the Ministry of Public Works giving their final approval on the 1st of July 1935. Preliminary construction had, however, already begun in October-November 1933 with the creation of the artificial reservoir in Buftea which could hold up to 9.600.000 cubic metres of water (and which would be finalised in May 1935).

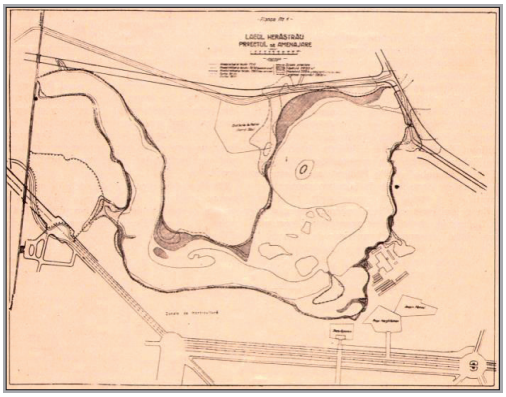

Figure 1. Improvement plans for Herăstrău Lake (Strunschi, 1932)

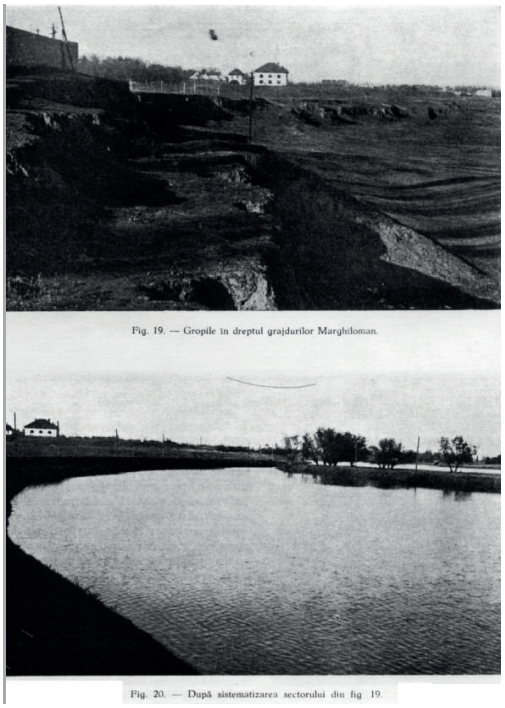

Figure 2. Sanitation project of Coletina - west shore of Herăstrău (U.C.B., 1936)

In 1934, during Dem I. Dobrescu mandate as Mayor, the development of the Băneasa and Herăstrău lakes were finalised (Figures 1, 2).

MATERIALS AND METHODS

Herăstrău Park and its beginnings - a historical analysis

In 1910 and 1912, two laws referring to the creation of a public park on the shores of the Herăstrău Lake were published without any results. The law from 1912 foresaw the establishment of a national park with a surface of 210 hectares. An ulterior forecast proposed that the Herăstrău estate be enlarged and the surface area be expanded to 820 hectares, without taking into account the surface area of the lake (Sfințescu, 1933). Today, Herăstrău Park has 187 hectares.

In 1915, the Master Plan accounted for the establishment of the national park, which was illustrated in the Bucharest Guide of 1923, created by Pântea (Figure 3), and was present in Bucharest's administrative division plan of 1929 as well, but only on the west side of the lake. The guide's example shows the plan of the new park drawn all the way along Kiseleff Street, on the shores of the Floreasca Lake, reporting Old Herăstrău Park and Fronescu Park (around the Bordei Park). According to the Bucharest administrative division plan, the park on the Herăstrău shore is defined as the National Park.

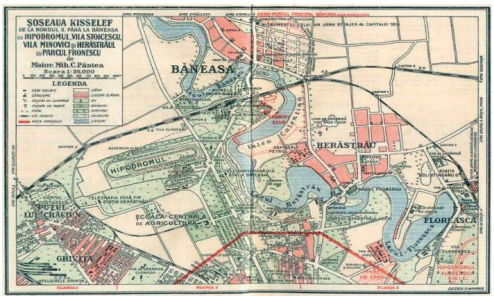

Figure 3. Green area placed along Kiseleff Street - 1923. The National Park is not yet mentioned (Pântea, 1923)

Taking all these into account, C. Argetoianu stated, on February 28th, 1934, during the conference held at the Carol Foundation in the framework of a series organised by the

Association for Bucharest's Urbanism (Asociația pentru Urbanistica Bucureștilor), about the stringent necessity of the construction of the National Park, construction which was never undertaken following his departure from the Ministry of the Interior. This leads us to believe that construction had begun earliest in 1935, under the coordination of Fr. Rebhun (Olteanu, 2002).

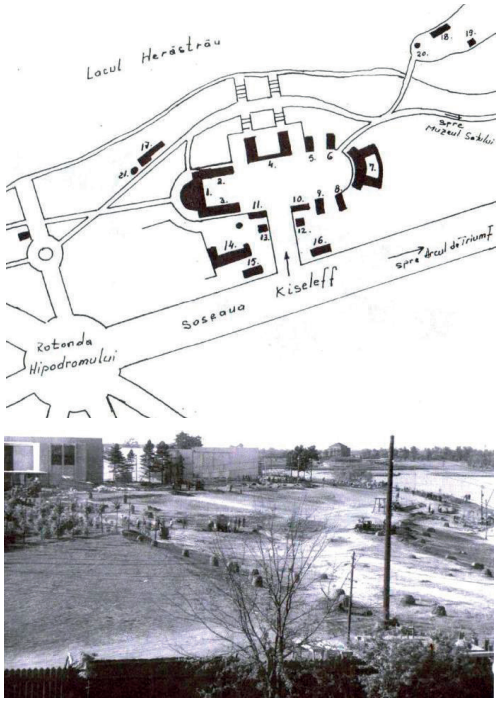

Figure 4. Exhibition plan for the Bucharest's Month festival and the development of the esplanade and lakeshore - 1936 (Dușescu et al., 2016)

The development of the Herăstrău Park was intrinsically tied to a series of public events in Bucharest, with every step of its construction being tied to a major festival. The organisation of the Bucharest's Month Festival (Luna Bucureștilor) in 1936, in the western area of the park, along Kiseleff Street (following the 1935 edition when Carol I Park was modernised), was decided upon given that the landscape development plan in the area had already begun, which, at the time, covered 60 hectares, and was already a point of attraction for locals. In this way, a series of improvements, built following the plans of architect Octav Doicescu, and flora composition following the indications of Friedrich Rebhun, are linked to the organisation of the Bucharest's Month Festival from 1936. On this occasion, the whole west side of the park was developed, along with Rose Island and the Village Museum. This intervention also included the Miorița Fountain. More important, however, is the esplanade of the exhibition along the former School of Horticulture, whose stairs lead to the lakeshore and are still in place today (Figure 4).

The construction of the southernpart of Herăstrău Park

If in 1936 the Bucharest Month Festival was organised in the new National Park, the 1939 edition, then when the festival was again organised within the current Herăstrău Park, the emphasis was taken away from Kiseleff Street in favour of Jianu Square. Here, efforts were made on a grand scale, with old areas requiring demolition, and whose former streets are still discernible within the park's structure. Simultaneously, the new profile of Boulevard Prezanwas established, with the route of the old street being transformed into a path in the park that still exists today (Figures 5, 6).

An actual demolition and urban restructuration project is to be brought to light, leading to the construction of the south side of the park. Evident also is the maintenance of the existing infrastructure of former road, included as paths throughout the park, arguably an economic decision.

Figure 5. Prezan Blvd and former route of the street integrated in the park (photo: Willy Pragher - http://willypragher.blogspot.com/)

At the same time, urban E-V connections are also established which follow former roads sections (Figure 6).

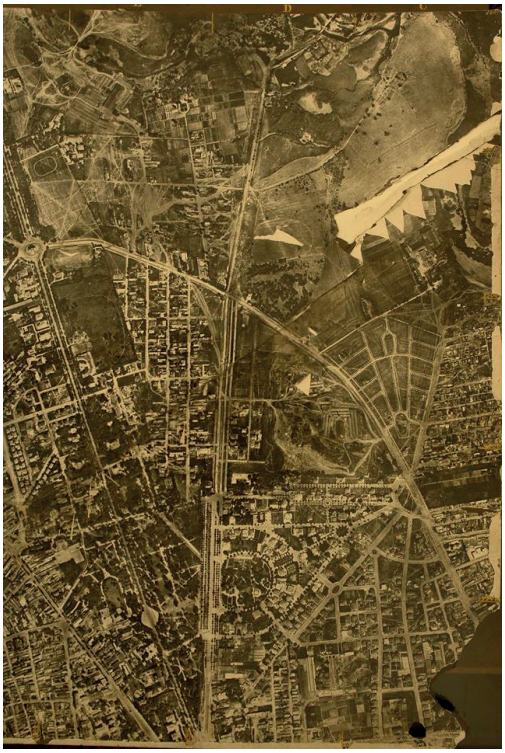

Figure 6. Former route of Prezan Blvd, and the existing neighbourhood within the park's current boundaries cca. 1933-1937 (Romanian Academy Library - BAR)

As for the park layout, things are unclear. The results of archival research do not clarify the project on which the construction of the south side of the park was based, a possibility being, however, a hybrid arising out of several incentives and political motivations of the time (such as the partial preservation of the road system). The mix of several proposals and economical decisions is resulting out of historical and present plan analyses. One plan created by Friedrich Rebhuhn can be found in the Central National Historical Archives (Figure7), probably referencing a study in its early stages, created before the completion of the lake's restoration and the consolidation of its banks due to the shape of the lake which appears in a vastly different form from not only the one finally achieved, but also by all variations set forth by Nicolae Caranfil (Caranfil, 1936).

The project proposed by Rebhuhn includes the eastern bank as part of the park, this being connected with the rest by a bridge that takes the former road leading to the Petrol Block refinery. Closer to the current image of the park is the plan proposed by Emil Pinard and published in "Urbanismul" in 1933 (Figure 8). This one seems to have served, at least partially, as the basis for the 1939 construction and includes the system of boulevards which intersect a *étoile* plaza from where the central axis of the park takes off. This was an integral part of a new urban structure inspired by the Parisians. Both projects obviously rely on a visual relationship with the lake along an axial perspective "to infinity".

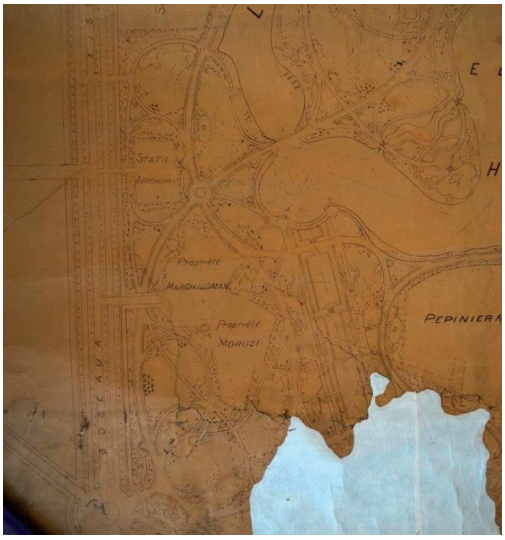

Figure 7. Herăstrău Park plan, development proposal. (National Historical Archive - Fritz Rebhuhn Fund)

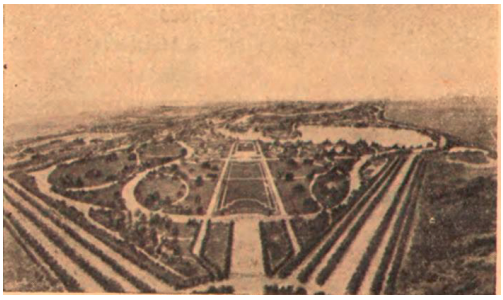

Figure 8. Emil Pinard's project for the National Park (Sfințescu, 1933)

For the Bucharest's Month Festival of 1939, the works for the exhibition pavilions as well as

for other facilities were entrusted to the architect Horia Creangă. It establishes the central pavilion and the courtyard of honour, located in the center of the newly developed area, in the proximity of the new E-V axis and placing two large lawns, one facing Jianu Square (presently Charles de Gaulle Square) and the other facing the lake (Figure 9). Thus, Creangă's proposal contradicts the visions of the two landscapers pursuing the axial visual relationship between Jianu Square and the lake, blocking a direct view and consequently generating two distinct paths, of different characters. The pavilion was organised around courts of honour that opened to the lake, in a diving perspective (Figure 10).

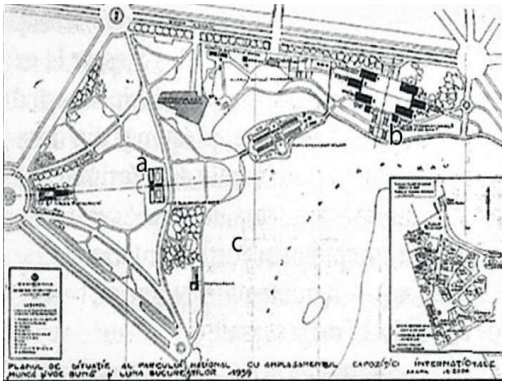

Figure 9. General organizational plan for the Bucharest Month Festival, 1939 (Sion, 2012)

The entrance to the park from Jianu Square, was, during the 1939 exhibition, marked by the presence of a double row of "vernacular caryatids" representing peasants from the Muscel and Mehedinți region (Figure 11) made by Constantin Baraschi and called Restoration Alley - Aleea Restaurației (King Carol II in 1930). It was demolished immediately following the festival. At the end of the alley, the Modura fountain was erected, also made by Constantin Baraschi (Figure 12).

It stayed there for some time, after which first the statue, and then the socket from the basin was removed. The basin of the fountain has, however, permanently remained in its original position. In removing the statuary ensemble, the park once again cleared the way to the Square, reintegrating the visual axis of the park into the general urban composition (Figure 13).

After the demolition of the pavilion, it was possible to restore the perspective "to infinity" towards the lake.

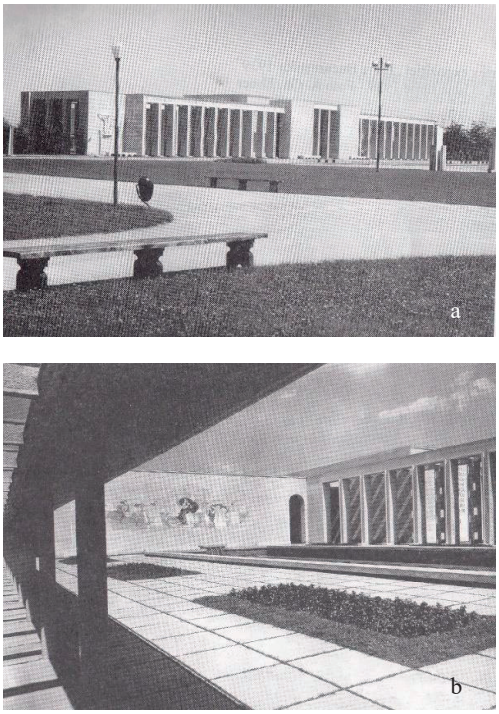

Figure 10. Central pavilion, arh. Horia Creangă (Dușescu et al., 2016)

Both the caryatids and Modura illustrated a nationalist discourse that is hardly relevant today, the reconstruction after 1990 of the Restoration Alley being difficult to explain both in terms of political discourse and the principles of restoration. The reconstruction of the caryatides took place in 2005-2006 by the sculptor IonelStoicescu. He also reconstructed the statue of Modura, the original of which can presently be found in the Bellu Cemetery, at the tomb of EllyBaraschi-Xenakis, the sculptor's wife. The caryatid originals can also be found in the Bellu cemetery, built in bronze, like the Modura.

Apart from construction dedicated to the Bucharest Month Festival, garden landscaping was also carried out. In addition to the large lawns, massive groups of trees have been planted that define the spatial structure of the park to this day (Figures 14). Besides the poplars and willows that marked the shores ofthe lake or the alignments and groups of

oaks, maples, linden trees, ashes, tufts, etc. Nicolae Caranfil mentions the bringing and acclimatisation of pine trees in the park arranged along the Colentina River during 1936-1937.

Figure 11. Restoration Alley (Aleea Restaurației) in 1939 (Dușescu et al., 2016)

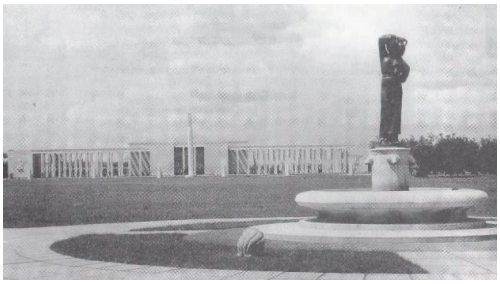

Figure 12.Modura Fountain during Bucharest's Month Festival, 1939 (Dușescu et al., 2016)

Figure 13. Adolf Hitler Square and thepark entrance ca. 1941 (Simetria Magazine Archives)

Figure 14. Aerial Image of Herăstrău in 1966 (http://fostulbucuresti.github.io/#13/44.4410/26.0745/!a6)

Subsequent interventions and reconfiguration of the central axis

It is not certain when the demolition of the Horia pavilion took place, but it is clear that the arrangements related to the two courtyards of the building (Figures 10 b, 14) were maintained for a long while, namely the basins, pavements and flower beds integrated in the current central esplanade from the intersection of the two major axes of the southern area (Figures 15, 16).

Figure 15. The plan of the exhibition pavilion of the Bucharest Month Festival, 1939 superimposed with the current image of the central esplanade as in the Screenshot Google Earth 8/27/2018 (Sion, 2012, Google Earth, ©2018 DigitalGlobe / © 2018 Google)

Part of them have subsequently disappeared or been severely altered over time.

Today, there are still present only the basins whose former natural stone kerbs are covered by concrete planters and the interiors are painted in bright blue (Figure 17 a). Only the basin on the esplanade at the entrance to the park remembers (through it's regularly colours changed fence) its original image (Figure 17 b).

Two major moments of intervention emerge over time. The first, at the beginning of the 1950s, is also related to the organisation of the World Festival of Youth and Students in 1953, in Bucharest (Festivalul Mondial al Tineretului și Studenților - that included just the communist bloc), which led, among other things, to the reconfiguration of the central ground floor by introducing the suggested axial composition and by the plans of Rebhuhn and Pinard (Figures 8 and 9).

Figure 16. Images of the basins on the esplanade before and after addition of concrete planters (Georgescu family archives by courtesy of Oana Pîrvu)

Figure 17. a. The basins on the central esplanade covered by planters; b. the fenced basin at the park entrance (personal archives)

An image from the inaugurationof the new park of culture and rest I.V. Stalin on the 1st of May, 1951 (attended by Gheorghe Gheorghiu-Dej, Vasile Luca, I.I. Kavtaradze, Petru Groza, Ana Pauker, Petre Borilă, Gh. Apostol, C.I. Parhon, and Şt. Voitec) is catch, besides the slight alignments of poplars that today flank the central ground floor and the rockery located inside the basins of Horia Creangă (Figure 18 a).

Figure 18. Central Axis in 1951 and 1970.
(a- http://redescoperaistoria.ro/ Online communism photo collection - Photo #EA028,
b-http://orasulluibucur.blogspot.com)

Also during this period, the park was expanded in the North-East area, the summer theatre was built and other new facilities for the working class. Adaptation to the new "popular" taste and especially to the taste of party and state leadership brings with it the plethora of "ornamental" plants, foliage and floral arrangements.Rică Marcus mentioned in 1958 "two pavilions - library, three pavilions for exhibitions, buffets, refreshment kiosks, docks, a restaurant, a large group of entertainment facilities, three corners reserved for children, with swings, cabinets, buggies, etc. The plantations were greatly enriched with trees and flowering shrubs. Countless ridges exaggerated

by many mosaics were made especially around the constructions, beside the statues, etc." (Marcus, 1958). In that vein, "of the plantations remade in the southern area, we deem the ridge from the entrance of Stalin Square successful, from where it would be good to remove the 'vessel' made of flowers that has a dimension completely out of proportion and which is of questionable taste and very expensive." (Marcus, 1958). The alignment of the poplar trees created a huge green room whose entire dynamic was oriented towards the lake and the diving perspective towards it (Figure 18 b).

A second moment, in the same aesthetic line that denied the modernism of the initial arrangements, is the achievement of the Expoflora flowers exhibition, at the initiative of the park administration. Its arrangement, including the construction of the two pavilions, of which the one at the top of the slope destroys the symmetry of the central axis, was attained in 1970. In the same period, the alleys that cross the ridges were rebuilt and raised so that its focal point is presently fragmented (Figure 19 a).

Figure 19. a. The symmetry of the axis severely affected by the resin plantations and the Expoflora pavilion marked in red and the green carpet crossed by the transverse alleys marked in yellow (picture taken by me in 2006 and still featuring the incongruous vegetal clock); b. The axis (in yellow) destroyed by the inadequate realisation of / The Expoflora upper pavilion, located asymmetrically in relation to the visual axes of the initial layout of the park (Tudora et al. 2018)

Another catastrophic intervention that accompanied the new passion for flowers was the destruction of the modernist basins, as they were covered by planters, poorly built both in terms of materials and design (Figures 16 b, 17 a).

The establishment of the Expoflora was accompanied by the new fashion of the ceramic vessels that adorn the entire central axis (Figures 18 b, 20 a). On the other hand, despite all the insertions made at the Expoflora, the descending perspective towards the lake remained open between the 70s-90s.

All the problems mentioned in the text are highlighted in the pictures using Adobe Photoshop CS3.

Figure 20. a. Ascending perspective from Expoflora to Aviatorilor Sq.; b. Perspective on the lake in Expoflora (a. *www.orasulluibucur.ro*, b. DMI archive)

New reality post 1990

After 1990, unsuccessful interventions carried out in the absence of specialists certified by the Ministry of Culture (although Herăstrău is included in the List of Historical Monuments with code 832-B-II-aA-18802, which requires that any intervention be based on a project developed by an MC certified specialist and with the input of the National Commission of

Historical Monuments) led to the rapid and aggressive destruction of the image of the park.

Figure 21.The main access from Charles de Gaulle Square - both directions of perspective with planters and fences (a.), aggressive lightning system around Modura (b.) and the floral clock located in the axis and the conifers on the sides and background, the buildings on the Bucharest-Ploiești Road (c.) (Tudora et al., 2018)

From decisions such as the reconstruction of the Restoration Alley (Aleea Restaurației) to poor management, a series of unsuccessful planting initiatives, but also the construction in the vicinity of the Scânteii House, led to the irreparable destruction of the axial perspective towards the lake and to the northern part of the park (Figure 21). The destruction of poplar alignments, the location of the upper pavilion of the Expoflora and the generation of longitudinal and transverse visual partitions led to the destruction of the simple and clear image of the central "green room".

The central esplanade, a key element of the improvements made in 1939, was transformed into a fair in where a number of statues are chaotically placed, temporary attempts at *arstopiaria*, various kiosks and stalls, blue basins, yellow garbage cans, green bicycle lanes, pink flowers, etc (Figure 22). The situation is all the more serious as the current state of the central esplanade severely affects **the perception of the spaces along the** transverse axis of the park.

Figure 22. Central Esplanade today (personal archives)

In turn, the Expo flora was transformed from the flowering meadow that had been arranged in the 70s into a collection of floral patterns bordering on kitsch, where new ornamental trees have been planted that block the view towards the lake (Figure 23, 24). The pavilions are in a state of advanced degradation and are closed to the public, thus becoming useless for the Expoflora. The end of the axial route ends with a distressed perspective towards the opposite banks of the lake, dominated by the presence of new buildings that rise above the top of the trees as well as the kiosks and docks transformed into terraces (Figure 25).

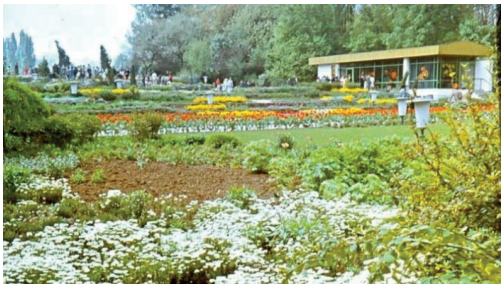

Figure 23. Expoflora image from 1972 (http//www.xplorio.ro)

Figure 24. Current image of the Expoflora area with plant inserts (especially trees) blockingthe view (blue mark) towards the lake (Tudora at al. 2018)

Figure 25. Perspective of the horizon irretrievably affected by the buildings (marked in red) risingabove the flora in the background (Tudora et al., 2018)

RESULTS AND DISCUSSIONS

Following the analysis of the way in which the central axis has evolved over time, from a compositional point of view and its architectural quality, but especially of the historical importance of its constituent elements, the values are summarised in Table 1. The data in the table represent details on segments of the axis of the values taken from the Landscape Study conducted by myself in the frame of RPR_birou de studii contemporane (bureau of contemporary studies) in 2018. The evaluation takes into consideration the original project and its evolution along the time.

Table 1. Constitutive elements of the central axis evaluation - based on historical criteria

Constitutive elements of the park	Pre 1935	1935-1940	1950-1953	1965-1975	1990-Prezent	Historic value
Charles de Gaulle entrance esplanade	-	high	high	high	high	high
Aleea Restaurației	-	high	medium	medium	medium	medium
Central ridge / green room	-	-	high	high	high	high
Central esplanade	-	high	high	high	medium	high
Diving lawn / Expoflora	-	high	high	high	medium	high
Lakeshore / perspective towards the northern part	high	high	high	high	high	high

Figure 26. Evaluation of perspectives in the southern area of Herăstrău (Tudora et al., 2018)

Given the irremediable nature of the destruction of part of the park's iconic values that occurred after 1990, such as the destruction of the flora borders on the each side of the lake, a restructuring and reconfiguration of the central axis composition is more than necessary. The situation of the inner perspectives within the central axis of the southern area is analysed in Autocad (Figure 26).

The aforementioned analyses break into the directions of intervention on the central axis that aim less to a restoration of one phase of development but to an integrated vision that respect and recover the main elements of each phase that, meantime, became emblematic.

Thus, on the section between Charles de Gaulle Square and the Central Esplanade, the following actions are required:

- the removal of ornamental and design elements that do not conform to the modernist project;
- the relocation of statues and monuments in a coherent spatial and stylistic structure;
- realignment and regeneration of the poplar trees found in the green room;
- correction measurements and vegetation management plans;
- restoration of all elements from the 1939 project; assessment of the possibility of rebuilding the central courtyard of honour. (RPR_birou de studii contemporane, 2018).

Regarding the Flora Expo area, including its lateral zones between the Colentina perch and the Summer Theatre, the following priorities and intervention measures are required:

- restructuring the plant composition according to the conclusions of the axial perspective study;
- moving the pavilion from the upper level of the perch to a position symmetrical with the pavilion from the lower level; restoration of valuable landscape elements.

All this intervention should be correlated with those required by the others areas of the park, mainly the two free-composition zones that juxtapose to the main axis. Thus, the visual enclosure of the green room is also ensured by the vegetation (and its management) in the proximity areas, which should be taken into account.

CONCLUSIONS

The southern part of Herăstrău Park is the result of different stages of evolution. Each stage of its evolution was marked by the political discourse of the moment, the main ones being the expression of two the dictatorial periods (fascist and communist) but also the "opening" period of the 60s and 70s. The democratic period, after 1989, is marked, from Herăstrău park point of view, only by aggressive and disrespectful interventions, marked by kitsch and lack of vision. Due to the lack of a coherent management plan of the park the entire vegetation is suffering today, most of the trees being mutilated by radical cuts called (in the local administration's erroneous language) "toaletare".

Despite this reflection of dictatorial discourses in the architecture of the park the result was one of an outstanding and almost neutral modernity, due, on one hand, to the appreciation for modern architecture during the fascist period and, on another, to the shy, but important, insertions and details of the short period of modernity in the communist era.

At the architectural and landscape language level, the single strong reminder of the two dictatorial phases are the public art monuments, scattered around the park. Thus, the relocation of statues and monuments, which has been analysed in detail in aforementioned landscape study, but weren't presented in the present article because of the limited space, should follow this political periods that generated from the very beginning their initial location in the park. The entire green room should be transformed in a monumental alley, presenting the statues of the main Romanian writers that are presently spattered around the park, while Aleea Restaurației shall be subjected to a *mise-en-valeur* project.

The reconstruction of Creangă pavilion depends of the quality of archive documents to be found. Detailed plans could lead to a real reconstruction while the lack of document might impose a architectural reinterpretation based both on architectural and landscape elements. Nevertheless the three basins still standing in the park have to be restored.The proper reconstruction of the pavilion or an architectural / land-art / landscape

reinterpretation of the pavilion not only aims to recover the historical image of the park but also to regain a visual coherence of the two main areas of the axis: the green room spatial structure and the perspective from the city into the park, that now ends in a destructured space and the Expoflora area. The restructuration of the entire space and the visual relation are presented in Figure 27, realised in Lumion.

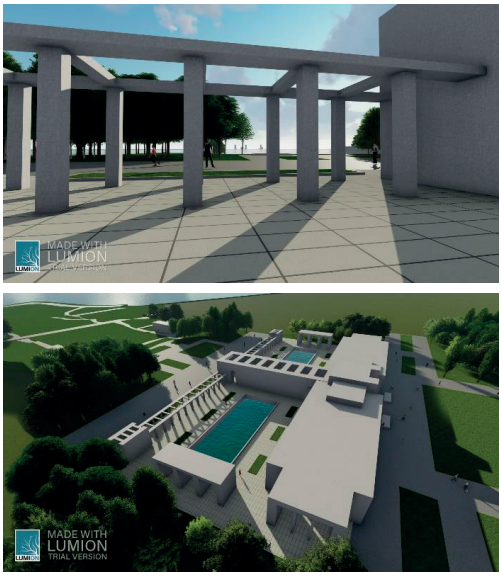

Figure 27. The spatial and visual impact of the possible reconstruction of Creangă's pavilion (Images: Iasmina Petre - diploma project in landscape architecture USAMV Bucharest - work in progress)

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The research work was carried out by RPR_birou de studii contemporane in 2018 as landscape preparatory study for the Urban Zonal Plan regarding the protection zone for Herăstrău park, project coordinated by S.C. Synergetics Corporation for ALPAB. The study was lead on the entire surface of the park, the present article focusing only on one of its emblematic elements. Therefore, the lack of detailing in some plans presented in the frame of the article. The team, coordinated by the author of the article, was formed by landscape architects: Diana Culescu, Mihai Culescu, Ștefan Ivanov, Mariana Nițu, Oana Pîrvu and landscape architecture students from USAMV Bucarest collaborating with RPR_birou de studii contemporane: Andreea Chiriac, Răzvan Dumitru, Giovani Luca, Raluca Mihai, Iasmina Petre, Anca Strugariu, Ioana Șerbulea.
The study will hopefully be followed by a regulation plan for the entire park.

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MISCELLANEOUS

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PHYSICO-CHEMICAL ANALYSIS OF CRANBERRY USING FT-IR SPECTROSCOPY

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Abstract

In the last years several wild fruits were considered as valuable sources of bioactive molecules as vitamin C, benzoic acid, anthocyanins etc. These compounds have a series of health benefits. For better pharmaceutical use of the plants, it is important to study them from the chemical and compositional point of view. Cranberry species are largely represented in the European, Asia and North America flora and various parts of the fruits are used in traditional medicine. The fruits are rich in anthocyanin, flavonoids, polyphenol, vitamin C and acids such as ascorbic acid. In the last year, FT-IR spectroscopy has been introduced as a very efficient and non-destructive analytical tool for the reliable way to determine the functional groups of three compenents

Key words*: cranberry fruit (Vaccinium macrocarpon), FT- IR (Fourier transform spectroscopy).*

INTRODUCTION

Increased interest in medicinal plants has been demonstrated a plethora of valuable resources of naturally occurring polysaccharide-polyphenolic conjugates with many health benefits (Veskoukis et al., 2019). Shrub genus Vaccinium which belongs to Ericaceae family is comprised from over 450 species and mainly commercially varieties are known as *Vaccinium macrocarpon*, *Vaccinium oxycoccos* and *Vaccinium vitis-idaea* (Brown et al., 2012). Cranberry (*Vaccinium macrocarpon*) is categorized as small northern fruit, historically native to North American and Northern Europe. Fruits are red-colored having spherical shape berries (Narwojsz et al., 2019).

These barriers have been traditionally processed into various products such as juice, sauce, sweetened dried cranberries and also for medicinal use. Cranberry berries reported being rich in sugar (4-7%), citric acid, malic acid, provitamin A, vitamin B1, B2, vitamin C, benzoic acid and anthocyanins comprising peonidin-3-galactoside, which have been implicated in health benefits associated with the consumption of cranberry (Narwojsz et al., 2019).

Despite chemical composition of fruits, cranberry leaves are rich in tannins, flavonoids which are considered strong antibacterial, antimycotic, antioxidant substances, and can be used for their antiseptic properties because of high arbutin content (Ahmad et al., 2019; Nebu and Walsh 2019). The antiseptic and antifungal properties of the fruits may be due to polyphenols, benzoic acid derivates and anthocyanins, which efficacy was proved in vitro and in vivo studies (Kraus et al., 1979; Stambergova et al., 1985; Veskoukis et al., 2019). Plants are the primary source of dietary phenolic compounds (Vinson et al., 2008) that have been suggested to be responsible for many health benefits including anticancer activity (Sun and Liu 2006), antioxidant capacity that can prevent low-density lipoprotein oxidation, thrombocytes platelet aggregation and confer protection against cardiovascular diseases (Abbaszadeh et al., 2019). The consumption of cranberry fruits has been associated with reduced risks of chronic diseases including lung dysfunctions and thrombotic strokes (Manganaris et al., 2014). Cranberry phenolic profile includes simple phenolic acids, flavonoids which containing anthocyanins, proanthocyanidins (PACs) as well as flavonols

(Gregoire et al., 2007). Polyphenolic-rich Vaccinium macrocarpon extracts provide antibacterial activity against numerous *listeria* species (Diarra et al., 2020).

Cranberry leaves and fruits are suggested for treating all forms of renal infections and consumption of cranberry juice (CBJ) is reputed to be effective against urinary tract infections (UTI) (Foo et al., 2000). This phenomenon is attributed to the ability of cranberry phenolics, A-type proanthocyanidin (PAC) oligomers to inhibit adhesion of *Escherichia coli* which is usually responsible for UTI cases (Howell et al., 2005). Moreover, *in vitro* study shows that propolis and cranberry powders sufficiently extended the anti-adherent activity of the proanthocyanidins by reducing the adherence of *E. coli* towards epithelial cells (Lavigne et al., 2011). Association of cranberry and propolis supplementation significantly decreases the occurrence of UTIs during the first 3 months and delays the onset of cystitis episode (Bruyère et al., 2019).

Phenolics from CBJ previously showed a rich antioxidant capacity regulating cholesterol and other biochemical parameters, also cranberry juice polyphenolics stimulate nitric oxide synthase mediated in vasodilatation in a rat model system and have been implicated in the human lipoprotein profile regulation (Ruel et al., 2006). Cranberry flavonols, anthocyanins, and proanthocyanidins have been previously characterized via HPLC (White et al., 2010). Some attempts for cranberry compounds isolation such as flavonol glycosides by semi-preparative –HPLC have been reported as not quantitatively technique (Yan et al., 2002; Gregoire et al., 2007). However, other reports to our knowledge regarding cranberry chemical analysis provide quantitative yields (Wilson et al., 2008) including Fourier transform infrared spectroscopy (FT-IR) analysis (Andronie et al., 2019). FT-IR spectroscopy proved to be a suitable and efficient method for the analysis of biologically active molecules derived from plant powders (Namiesnik et al., 2013; Galarraga-Vinueza et al., 2018).

Therefore, the scope of this study was to analyze and obtain comparisons among molecular structures of *Vaccinium macrocarpon* after application of different drying temperatures. The berries from Romanian flora were analyzed by using vibrational spectroscopy techniques (FT-IR) and Photochem assay. Tools that recently have been described as useful for food and pharmaceutical industries due to the qualitative description of secondary metabolites and polysaccharide – polyphenolic conjugates derived from plant extracts.

MATERIALS AND METHODS

Biological material

The samples were obtained from fresh cranberry fruits at full maturity harvested from four points (1 - 46°40'54.6"N 23°05'46.1"E; 2 - 46°40'48.9"N 23°05'32.2"E; 3 - 46°40'32.1"N 23°05'48.3"E; and 4 - 46°41'19.3"N 23°05'27.2"E), located in Mărișel commune, Cluj County, Romania. The fruits were harvested in early September and preventively washed with water. The samples were dried in the air-oven at different temperatures for different periods of time at 40 °C for 4 days (sample 1), 70 °C for 3 days (sample 2) and 90 °C for 2 days (sample 3), respectively. For the liquid samples, we used juice from fresh cranberries and from frozen cranberries at -15 C° for 2 days.

FT-IR spectroscopy

The powdered forms of the fruit samples were prepared from cranberries dried at different temperatures.

The sample from the FT-IR spectrum was obtained from 0.005g of fruits used without further purification. FT-IR spectra were performed in the absorbance whit a Jasco FT-IR-4100 spectrophotometer using KBr pellet technique. When radiant energy is equal to the vibrational frequency of the molecule, it realizes the absorption and vibration. Absorption intensity for each frequency of vibration is monitored by a detector. Specific footprint is a specific combination between molecular vibration and rotational vibration and has a great significance to identify specific molecules.

The sample was prepared using calcinated potassium bromide as a matrix material and was mixed at a proportion of 3 mg of the sample to 200 mg KBr. Then the mixture was condensed in 15 mm die at a pressure equal to 10 t till 2 min.

Measurements were carried out on the infrared scale of 650-4000 cm^{-1} and a spectral resolution was set at 4 cm^{-1} and all spectra were acquired over 256 scans.
The spectral data were analyzed using Origin 6.0 software (Figures 1 and 2). These spectra were analyzed by comparing the obtained vibrational bands with those of similar functional groups from the literature.

RESULTS

The FT-IR spectrum was used to identify the functional groups of the active components present in the sample, based on the peak's values in the region of IR radiation.
The presence of flavonoids, pectin, proanthocyanidins, tannins, carotenoids, sugars, fruit acids such as ascorbic acid, malic acid, and citric metabolites which possess antioxidant activity was reported, according to FT-IR analysis. The peak characteristics for asymmetric stretching vibration of $–CH_2$ is corresponding to 2927 cm^{-1} (Santana et al., 2016) and the absorption bands for the carboxyl group may be found at 1742 cm^{-1} (Pancerz et al., 2019). Moreover, these groups are presented in all obtained spectra showing clear intensity for al samples. Significant discrepancies in intensity between all of the samples were observed in the area 1650-1220 cm^{-1}, corresponding to the oscillation of the carbonyl C=O group of fructose and aldehyde CH=O of glucose (Saha et al., 2007; Vardin et al., 2008).

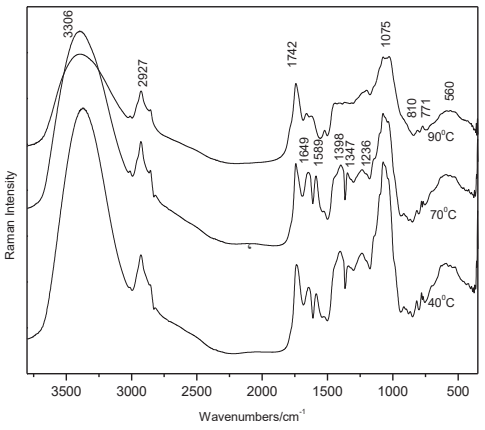

Figure 1. FT-IR spectrum of cranberry fruits (*Vaccinium vitis idaea*) dried at different temperatures

The band located at 1236 cm^{-1}, it is more intense for samples with lower drying temperature and this seems to be linked to the prediction of the presence of ester carbonyl groups.
The analysis of FT-IR spectra showed that each particular polysaccharide has a specific band, with a maximum detected within 1200-1000 cm^{-1} region, which is assigned to stretching vibration of (C-OH) side groups, and the glycoside bond (C-O-C) vibrations in polysaccharide chains, as it can be observed in Figure 1 (Pawlaczyk et al., 2008). The bands identified at 1075 cm^{-1} specific to this group are much more intense in the case of the spectra from sample were fruits was dry a temperature at 90^0 compared to the one obtained for 70^0 and 40^0 .This finding may suggest that the cranberry have a lower polysaccharide content when was dry for more temperature (90^0C).
Another interesting region is a characteristic of various types of fruits, and it is represented by bands in the range from 850-760 cm^{-1}, which correspond to the specific oscillation of the anomeric region of carbohydrates, or C-H deformation. The region is described by considerable differences between particular samples, which evidences a significant change of bond conformation (glycoside bond) (Samborska et al., 2018).
The absorbance of bands at 553-633 cm^{-1} indicates C-O-O and P-O-C bending of aromatic compounds such as phosphates.
Anthocyanin is an integrated molecule, which particularly eases the transport of electrons through its structure (Cramer et al., 2011).
It can be seen that the absorption peaks of the wild fruits at around 560 cm^{-1} are demonstrating the presence of three anthocyanin pigments (Ramamurthy and Kannan, 2007).
Figure 2 shows a comparison between fresh cranberry juice and fruits which were frozen at a temperature of 15 °C over 2 days. It can be observed that both FT-IR spectra showed a band at a location of 1650 cm^{-1} that is attributed to the vibrational C = O bond from amide I. In addition, this region was more intense in the spectrum obtained from the fresh juice sample.
Furthermore, the band from the 1075 cm^{-1} region is specific to the polysaccharides and showed an elevated vibrational intensity in

fresh cranberry juice compared to the frozen fruits. This suggests that the polysaccharides content decreases after exposure of the fruits to the thermal processes.

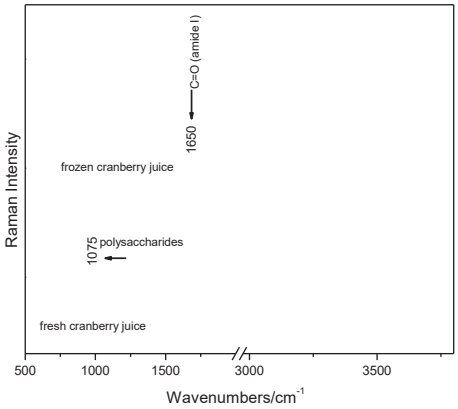

Figure 2. FT-IR spectrum from fresh and frozen cranberry juice (CBJ)

CONCLUSIONS

The acquired FT-IR spectral data is suggesting that the curative and protective properties of the fruits begin to decrease due to the physical-chemical changes occurred in the fruits as the temperature increases (the drying process).

Based on the obtained results, it can be concluded that the FT-IR spectroscopy is a reliable instrumental technique for the determination of mean components in wild fruits.

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INVESTIGATION THE QUANTITY AND QUALITY OF ESSENTIAL OIL OF *ARTEMISIA VULGARIS* L.

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Abstract

Artemisia genus strong and aromatic smell is due mainly to high concentrations of volatile terpenes, constituents of their essential oils and extensive studies have been done on this issue. In this research, the analysis of the essential oil from the quantitative and qualitative point of view has been carried out for leaves, stems and flowers of Artemisia vulgaris L. species in Romania. The oil has been extracted during the flowering period, by hydro distillation and analyzed by gas chromatography–mass spectrometry (GC-MS). The obtained results emphasized the presence of some major chemical compounds in leaves, such as eucalyptol, germacrene D and β-pinene. Flowers contained higher amounts of eucalyptol and borneol, while in stems, especially beta-pinene and eucalyptol have been determined. The common chemical compounds in the three vegetative organs, were β-pinene and eucalyptol, for which different values have been recorded. The highest amount of β-pinene has been recorded in the volatile oil extracted from stems (11.9%), followed by leaves (4.49%) and flowers (3.02%), respectively.

Key words: *Asteraceae, chromatography, essential oils, flowers.*

INTRODUCTION

The genus *Artemisia (Asteraceae*) consists of about 500 species, occurring throughout the world. *Artemisia vulgaris* L. (commonly known as mugwort) is a perennial herb widespread throughout temperate regions of the Northern Hemisphere. The plant is also known as an aggressive weed. *A. vulgaris* exhibits wide morphological and physiological variability. Fresh plants have a strong odor, and a spicy-bitter taste (Judzentiene et al., 2016). *Artemisia* has a vast range of biological activities including antimalarial, cytotoxic, antihepatotoxic, antibacterial, antifungal and antioxidant activity (Bora et al., 2011). The large genus *Artemisia* L., from the tribe Anthemideae, comprises important medicinal plants which are currently the subject of phytochemical attention due to their biological and chemical diversity. *Artemisia* species, widespread throughout the world, are one of the most popular plants in Chinese traditional preparations and are frequently used for the treatment of diseases such as malaria, hepatitis, cancer, inflammation and infections by fungi, bacteria and viruses. (Abad et al., 2012).

Essential oils make a major contribution into the plant's biological activity as well. For that reason the chemical composition of mugwort oils has been investigated in several studies (Judžentienė et al., 2006; Zhigzhitzhapova et al., 2016; Pandey et al., 2017; Janaćković et al., 2019). The strong and aromatic smell of some species of *Artemisia* genus is due mainly to high concentrations of volatile terpenes, constituents of their essential oils, especially in leaves and flowers. Williams et al. (2012) states that the major constituents of essential oil extracted from *A. vulgaris* L. are: Germacrene D (25%), Caryophyllene (20%), α - Zingiberene (15%) and Borneol (11%), while the buds are rich in 1,8-Cineole (32%), Camphor (16%), Borneol (9%), and Caryophyllene (5%). The essential oils from *A. vulgaris* can be used for various medicinal purposes (Malik et al., 2019). As reported, the essential oils from *A. vulgaris* showed bactericidal and fungicidal properties against *Staphylococcus aureus* and *Candida albicans*, respectively. Instead, any anthelmintic activity of essential oil against *Haemonchus contortus* was observed.

The objective of this study was to analyze the volatile oil from a quantitative, as well as qualitative, point of view.

MATERIALS AND METHODS

The research was carried out on *Artemisia vulgaris* plants (stems, leaves, flowers), harvested from spontaneous flora - Vitănești (Teleorman County) from Romania, in a fresh state. The aerial parts of the plant were collected during full blossoming. The extraction and analysis of the volatile oil were implemented within the Faculty of Horticulture, Bucharest. Fresh herbal parts of the collected plant were subjected to hydrodistillation for 3 h using a Singer-Nickerson equipment to extract the oil. The separation and identification of components has been carried out using an Agilent 6890 GC coupled with a 5973 Network single quadruple mass spectrophotometer detector in Electron Ionization (EI) mode and a 7673 injector. A capillary column 452 DB-5 (25 m length x 0.25 mm i.d. and 0.25 µm film thickness) and helium as carrier gas (1 mL/min) were used (Bădulescu et al., 2010). The initial oven temperature was 50°C for 8 min, then a 4°C/min ramp to 280°C. The NIST spectra library was used for to identify the essential compounds, and the Kovats indices were verified using an alkane mixture purchased from Sigma-Aldrich.

RESULTS AND DISCUSSIONS

Regarding the volatile oil content, the same amount of oil was found for leaves and flowers (0.10 mL/100 g). The determinations made in the stems revealed that they had such a low content of volatile oil that it had to be captured in hexane, in order to be chemically analyzed. After the volatile oil, extracted from the vegetative organs of *Artemisia vulgaris* species, had been analyzed, it was found a greater number of chemical components in the volatile oil obtained from flowers (31), and in the case of leaves and stems, the number of chemical components was lower (29, respectively 23).
The common chemical compounds, in all three vegetative organs, were beta-pinene and eucalyptol, which recorded different values. The highest amount of beta-pinene being recorded was in the volatile oil extracted from stems (11.93%) (Figure 1), in the volatile oil extracted from leaves and flowers, the amount of beta-pinene decreased (4.49% and 3.02%, respectively) (Figure 3 and Figure 5).

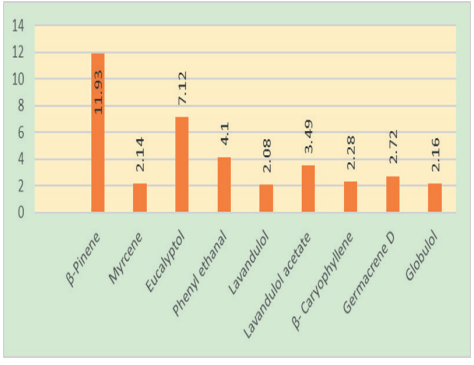

Figure 1. Major chemical compounds of stems, %

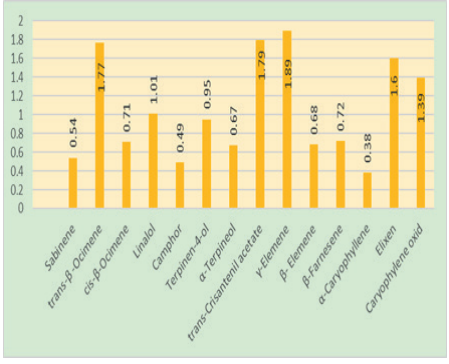

Figure 2. Minor chemical compounds of stems, %

The eucalyptol ranged from 6.29% to 12.39% in the volatile oil extracted from, leaves, stems and flowers, most of it being found in the volatile oil which was taken from flowers. Eucalyptol (1,8-cineole) is one of the major essential oils in *Artemisia vulgaris* (Jiang et al., 2019). The amount of eucalyptol (6.29%) in the leaves is approximately equal to that of germacrene D (6.42%). In the essential oil extracted from flowers the amount of eucalyptol (12.39%) is equal to that of borneol (12.39%) (Figure 5). Eucalyptol has been used as an antibacterial and expectorant (Giamakis et al., 2001), and as an anti-inflammatory (Juergens et al., 1998) or antihypertensive agent (Lahlou et al., 2002). As for the data

presented in Figures 1 and 3, it can be noticed the presence of some common substances in the three organs, determined in significant quantity, such as germacren D, myrcene and sabinene. With regard to germacrene D, a higher quantity is observed in the volatile oil extracted from the leaves (6.42%), and in the case of myrcene and sabinene, a higher content is observed in the volatile oil obtained from flowers (4.70-4.24%) and approximately equal amounts of myrcene from stems and leaves (2.14-2.17%). It seemed that the borneol was determined in greater quantity only in the volatile extract which was taken out of flowers (12.39%).

Figure 3. Major chemical compounds of leaves, %

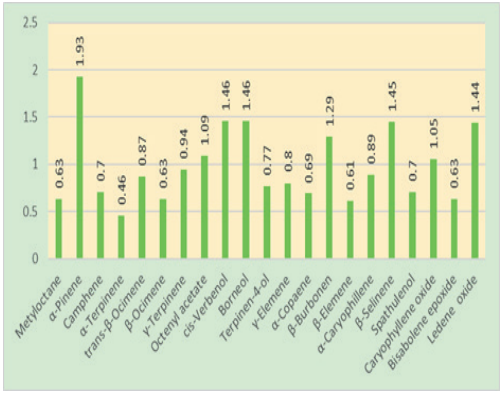

Figure 4. Minor chemical compounds of leaves, %

The major constituents of the oil from plants of *Artemisia vulgaris* collected from different places were reported to be α-pinene, menthol, beta-eudesmol, and spathulenol (Alizadeh et al., 2012, Iran- aerial plants), germacrene D and β-caryophyllene (Burzo et al., 2008, Romania - aerial plants), camphor, α-thujone, germacrene D, camphene, 1,8-cineole and β-caryophyllene (Govindaraj et al., 2008, fresh leaves were collected from greenhouse-grown plants), sabinene, β-pinene, 1,8-cineole, artemisia ketone, cis and trans-thujone, chrysanthenyl acetate, germacrene D and caryophyllene (Judžentienė et al., 2006, Lithuania- aerial plants), 1,8-cineole, camphor, α-terpineol (Thao et al., 2004, Vietnam - leaf and flower), camfor, camphene, α-thujone, 1,8-cineo, γ-muurolene, and β-caryophyllene (Govindaraj et al., 2013, vitro raised stems), caryophyllene, germacrene D and humulene (Malik et al., 2019, Brazil - aerial parts, before the onset of flowering). The quality and yield of essential oils from *Artemisia* species is influenced by the harvesting season, fertilizer, soils pH, the choice and stage of drying conditions, geographic location, chemotype or subspecies, choice of plant part or genotype, or extraction method (Abad et al., 2012).

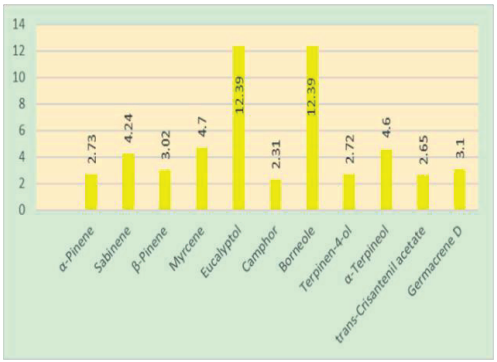

Figure 5. Major chemical compounds of flowers, %

Regarding the minority substances identified in the composition of the volatile oil, it can be stated that there were some significant differences, depending on the studied organ: thus, trans-β-ocimene, caryophyllene oxide, β-elemene, γ-elemene, terpinen 4-ol and α-caryophyllene were present in the stems (Figure 2) and leaves (Figure 4), whereas camphene, α-terpinene, γ-terpinene, α-copaene, β-selinene and spathulenol were found in leaves (Figure 4) and flowers (Figure 6), γ-elemene, cayophyllene oxide, β-elemene, terpinen-4-ol were found in stems, leaves and flowers.

It is also noted the existence of compounds which were characteristic of each organ, such as: trans-linalool, β-farnesene, elixen in the

composition of the volatile oil derived from stems, methyl-octane, β-ocimene, octenyl-acetate, cis-verbenol, β-bourbonene, bisabolene epoxide, ledene oxide in leaves and α-thujene, α-phellandrene, isopropenyl methyl cyclohexen-1-ol, α-terpinolene, isopropenyl methyl-bicyclo-hexane-2-ol, dimethyl-hexahydro benzofuran, isogeraniol, and bornyl acetate in flowers (Figure 6).

Figure 6. Minor chemical compounds of flowers, %

CONCLUSIONS

The studies carried out by the researchers indicate that the volatile oil extracted from *Artemisia* plants contains chemical compounds of particular importance in the treatment of various diseases. The data obtained in this study showed a remarkable quantitative and qualitative variation of constituents in the oils obtained from different plants organs. To the major constituents belonged β-pinene, eucalyptol (1,8-cineole) and borneol. Most of these constituents dominated in mugwort oils in other countries.

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COMPARATIVE ANALYSIS BETWEEN MUSHROOMS *LACTARIUS PIPERATUS* AND *AGARICUS BISPORUS* (CHAMPIGNON) USING FT-IR SPECTROSCOPY

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Abstract

In the last years the consumers are in favour of edible mushrooms due to the nutritious value and potential medical value because that are rich in proteins, vitamins, mineral elements.
Also, mushrooms contain terpenoids, carbohydrates and very important for organism, antioxidants.
Lactarius piperatus and Agaricus bisporus are two well-know mushrooms which have a high nutrition and many benefits for health. Therefore, a lot of mushroom is a very interesting subject for many studies and analyzes.
In this sudy, a rapid method using Fourier transform infrared (FT-IR) spectroscopic was established for analysis and characterized a principal components of two types of mushrooms.
Vibrational spectral techniques, FT- IR, offer several advantages in the context of current research and using this techniques we can identify molecular components in the samples studied. Following the analysis of the two spectra, we can observe an increased intensity of the characteristic absorption peaks belong to proteins (1655 cm^{-1}), polysaccharides (1000-400 cm^{-1}) and amino acids.

Key words: *Lactarius piperatus*, *Agaricus bisporus (champignon), FT-IR (Fourier transform infrared) spectroscopy*.

INTRODUCTION

Natural products with antioxidant activity are used to aid the endogenous protective system, increasing interest in the antioxidative role of nutraceutic products (Kanter, 1998). Concerning this, the antioxidants in human diets are of great interest as possible protective agents to help human body reduce oxidative damage.

Oxidation is essential to many living organisms for the production of energy to fuel biological processes, proceeding in lipids with polyunsaturated fatty acids, and generating reactive oxygen species (ROS) such as hydroxyl radicals (Halliwell and Gutteridge, 1989).

A multitude of natural antioxidants have already been isolated from different kinds of plant materials such as oilseeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs (Ramarathnam et al., 1995). Mushrooms become attractive as a functional food and as source for the development of drugs and nutraceuticals.

Mushrooms are highly nutritious food sources, which can be cultivated on cheap and usually readily available raw materials.

The term "mushroom" describes the reproductive structure of fruiting body of a fungus (Berch et al., 2007). Mushrooms belong to the kingdom of fungi, a group very distinct from plants, animals and bacteria. In the last years, there has been sustaining increase of commercial interest in mushrooms in pharmaceutical and food industries due to their wide usages as both food and medicine in many countries in this world (Sanmee et al., 2003).

The mushrooms have been associated to the life of human. Mushrooms are potent source of

biologically active substances which have beneficial effect on human health.

For example, most wild-grown mushrooms are rich in polysaccharides, proteins, amino acids, vitamins, and minerals, which can provide a high nutritional value for health (Ulziijargal and Mau, 2011; Kalač, 2009).

Many, if not all, mushroom species contain polysaccharides which may boost human immune system. In the last years much research have been done on biologically active substances originated from Basidiomyceteae which have beneficial effect of health and help in the treatment of many disease (Smith et al.,2002; Lindequist et al., 2005; Rajewska et al., 2004). Amoung these compounds, polysaccharides seem to play the most important role due to their anticancer. *Agaricus bisporus* (the white button mushroom, champignon) is the most commonly cultivated and consumed mushroom in Western Europe and North America. *Agaricus bisporus* is well known to mycophagists as the common "button mushroom" of commerce. The common grocery store form of *Agaricus bisporus* is completely white, but in recent years the mushroom industry has developed brown strains of the species, which it markets as "crimini" and "portobello" mushrooms (the distinction is simply that the portobellos have been allowed to mature past the button stage).

It contains high levels of proteins, carbohydrates, minerals (potassium, iron and phosphorus) and vitamins (vitamin C, niacin and thiamine), and is low in fat and calories (Clarke, 2007; Wu et al., 2006).

Analysing *L. piperatus* for constituents such as moisture, fat, proteins, ash and carbohydrates, Barros et al. (2007) showed that while protein and unsaturated fatty acid levels increased with the fruiting body maturity stage, the carbohydrate and saturated fatty acid content decreased. The maturity of the mushroom stage had little effect on individual sugar composition.

Methanol extracts from *Lactarius piperatus* have been investigated for antimicrobial activity. Using agar disk diffusion assays, *L. piperatus* revealed antimicrobial activity against *Escherichia coli, Proteus vulgaris*, and *Mycobacterium smegmatis*, but did not show any antagonistic effect against the yeast *Candia albicans* (Dulgar et al., 2002).

Several studies described by us (Barros et al., 2007; Ferreira et al., 2007; Turkoglu et al., 2007) report a correlation between the mushrooms antioxidant activity and their phenolic content. However, none of the existent reports on mushrooms antioxidants composition and antioxidant activity indicated the stage of development of the fruiting bodies selected for the studies.

In recent years, FT-IR spectroscopy has been introduced as a very efficient and non-destructive analytical tool for the reliable way to determine the functional groups of mushrooms compenents.

Fourier transform infrared (FT-IR) spectroscopy is one of the most widely used methods to identify chemical compounds and elucidate chemical structure. FT-IR technique is applied to detect compositional differences between samples on the basis of vibrations of various chemical groups at specific wave lengths of the spectrum (400-4000 cm^{-1}). Thus, FT-IR spectroscopy is used as a rapid and accurate method to detect natural compounds in food industry, and is often approached as a simple and fast alternative to other laborious methodologies, with minimum sample preparation.

The literature is mentions researches using FT-IR transform infrared and ultraviolet (UV) spectroscopies coupled with data fusion for discriminationn of *Boletus* mushrooms from seven different geographical area in Yunnan Province (Sen et al., 2019).

In another study, cultivated *Wolfiporia extensa* colleted from six regions in Yunnan Provice of China were analyzed by FT-IR and ultra-fast liquid chromatography (UFLC) in order to invstigate the differences and similarities in different origins and parts (Ly et al., 2016).

The use of the FT-IR technique has also been reported for the analysis of food matrices (Andronie et al., 2016), but literature mentions lots of works that involve the use of this technique and its great potential to be used in a large variety of other research fields (Andronie et al., 2019; Keseru et al., 2016).

In the present study it was investigated a principal components of two type of mushrooms powder using FT-IR technique.

The range of 1800-400 cm^{-1}, which exhibited major characteristics of mushroom samples was selected for analysis.

MATERIALS AND METHODS

In this research, we analyzed different varieties of mushrooms according to the analytical information obtained from dried mushrooms by means of Fourier transform infrared spectroscopy (FT-IR). One common varieties of mushrooms species *Lactarius piperatus* were collected at full maturity harvested from one point (1 - 46.6997° N, 23.5650° E), located in Făget commune, Cluj County, Romania.

Another species of mushrooms, *Agaricus bisporus,* were purchased from the local Romanian market. The mushrooms were washed with tap water and then was dried at 40^0C for 24 h. The dried mushrooms samples were crushed using a commercial blender.

The sample from the FT-IR spectrum was obtained from 0.003 g of mushrooms used without further purification. FT-IR spectra were performed in the absorbance whit a Jasco FT-IR-4100 spectrophotometer using KBr pellet technique. The sample was prepared using calcinated potassium bromide as a matrix material and was mixed at a proportion of 3 mg of the sample to 200 mg KBr. Then the mixture was condensed in 15 mm die at a pressure equal to 10 t till 2 min. Measurements were carried out on the infrared scale of 650-4000 cm^{-1} and a spectral resolution was set at 4 cm^{-1} and all spectra were acquired over 256 scans. The spectral data were analyzed using Origin 6.0 software (Figures 1 and 2). These spectra were analyzed by comparing the obtained vibrational bands with those of similar functional groups from the literature.

RESULTS AND DISCUSSIONS

In this study, FI-IR spectra of two type of mushroom species *Lactarius piperatus* and *Agaricus bisporus* (champignon) were obtained and average spectra are presented in Figures 1 and 2. These spectra can give overall and comprehensive metabolic fingerprints of *Lactarius piperatus* and *Agaricus bisporus* mushrooms.

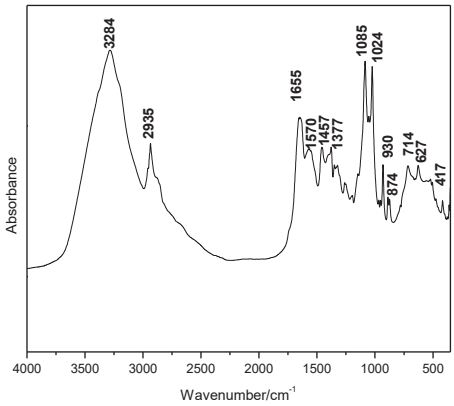

Figure 1. FT-IR spectrum for *Agaricus bisporus* (champignon)

The band at 3284 cm^{-1} which may be caused by strong water absorption mainly represents the O–H stretching (Hirri et al., 2016). The region of 3100-2800 cm^{-1} is mainly related to fatty acids and the obvious absorption peak at 2920 cm^{-1} in this range expresses stretch of methylene group of lipid (Zhao et al., 2015).

The peack present in spectrum characteristic from champinion at 2935 cm^{-1} shows a stretching vibration of the $–CH_3$ group. In addition, a weak peak around 2851 cm^{-1} may be caused by pyranose ring (Mohaček-Grošev et al., 2001).

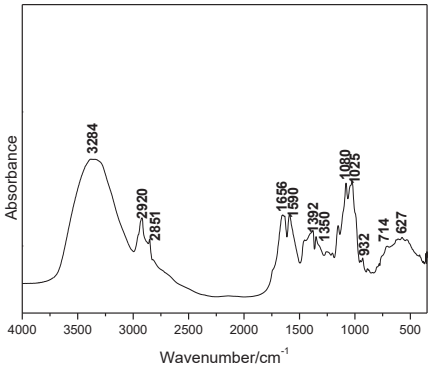

Figure 2. FT-IR spectrum for *Lactarius piperatus*

According to previous literatures, the band of 1700-1000 cm^{-1} is the dominating region which is attributed by organic material in macrofungi (Nie et al., 2007).

Peaks around 1655 cm^{-1} present C=O, C=N and N-H, which may be the result of proteins amide II. Also, the peak around 1570 cm^{-1} and 1590 cm^{-1} can be to protein amide II absorption (Zervakis et al., 2012; Fischer et al., 2006).

The O-H bending and $=CH_2$ groups are present around 1392 cm^{-1} and it belongs to polysaccharides and proteins.

The region of 1720-1480 cm^{-1} is highly relevant to protein substances. Both major peaks around 1085 and 1024 cm^{-1} are assigned to C–C stretching which is attributed as structures of chitin in spectrum of champinion (Nie et al., 2007). This bands appear shifters at 1080 and 1025 cm^{-1} in the spectrum obtained from *Lactarius piperatus* mushrooms whit a lower intensity.

The chitin is the main structural polysaccharide compounds in mushrooms and therefore, the region of 1200-1050 cm^{-1} mainly corresponds to the absorptions of carbohydrate (Mellado-Mojica et al., 2001).

Peaks in the region of 1000-400 cm^{-1} mainly belong to polysaccharides, such as β-D-glucan and pyranose from of glucose. For the above reasons, the characteristic absorption peaks belong to proteins, polysaccharides and amino acids (Sen Yao et al., 2018).

CONCLUSIONS

FT-IR spectroscopy could provide quantitative information concerning the functional groups of mushroom components.

The vibrational analysis allowed differentiation of mushrooms species according to the protein content.

Analyzing the spectra obtained from the two types of fungi we could notice a decrease in intensity of the bands characteristic of proteins in the case *Lactarius piperatus* mushrooms. This suggests that the *Lactarius piperatus* mushrooms have less protein than they do *Agaricus bisporus*

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OPTIMISATION OF OIL EXTRACTION FROM HALOPHYTE SP. SEEDS

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Abstract

The present study is part of a comprehensive study dedicated to the cultivation of halophytes species on salt affected soils, aiming on new value chains development from obtained biomass. The work is conducted on the monitoring the degree of soil purification, the biomass production and seeds yield, and their chemical composition. In this context, the paper contains information related to the oil content of Portulaca sativa sp. seeds, in order to produce 2nd generation biofuels. Extraction of oil from seeds via traditional method (Soxhlet method) and accelerated solvent extraction (ASE) were carried out. ASE method was applied because requires small quantities of solvent, sample and operating time. ASE appear to be the most suitable method and the optimal conditions were: pressure - 10.34 MPa, temperature - 105ºC, residence time - 10 min, solvent - petroleum ether, extraction ratio of 1:40, dynamic extraction time - 30 min, and 0.3 g diatomaceous earth. The highest oil recovery achieved was 33.4%.

Key words*: accelerated solvent extraction, extraction parameters, Portulaca sativa.*

INTRODUCTION

Global climate change, water scarcity, and availability of nutrients in the soil (Guyer et al., 2018) are the main problems affecting all-natural resources availability (Borsai et al., 2018). Simultaneously, the population of the globe and its needs are constantly increasing, while resources are becoming less and less. Therefore, a sustainable approach is needed, targeting both need and consumption.

One of the sectors that needs attention, other than the main one for food, is energy. Energy demand is increasing along with population growth, urbanization, and living standards. As is known, the main categories of fuels are petroleum, natural gas, coal, natural gas, biofuels, nuclear energy, hydro energy, and other sources like solar, wind, geothermal. Of this, fossil fuels remain the main source of energy (Khan et al., 2014). The use of vegetable oils (palm, soya bean, sunflower, peanut, and olive oil), as fuel for engines dates long ago, and is promoted again. It is a renewable source and the ideal solution for global energy demands (Shereena & Thangaraj, 2009).

For now, the annual worldwide oil production is close to 135 Mt including palm, soybean, and rapeseed oils (Jolivet et al., 2013).

One of the answers to the hypotheses presented above is represented by halophyte plants. Using halophytes as crops would lead to reduce water consumption in agriculture and can be ideal for areas with water availability (Koyro et al., 2011). Some halophyte species (grasses, shrubs, and trees, etc.) can remove the salt from salt-affected soils through salt excluding, excreting, or accumulating (Hasanuzzaman et al., 2014; Karakaş et al., 2017).

Seeds of many halophytes (*Portulaca* sp., *Salicornia bigelovii*, etc.) contain appreciable

quantities of oil and could be used as source of energy. Biofuels obtained using halophyte biomass could be a feasible alternative to conventional ones. The main advantage of using halophytes is that they don't compete with food (in terms of agricultural soil and water resources). Other aspect is the low impact on environment (Hameed & Khan, 2011). Non-edible biodiesel crops are expected to use lands that are largely unproductive and those that are located in poverty areas (Ahmia et al., 2014).

Portulaca sativa (Family Portulacaceae) is a possible candidate for this purpose. Is an annual plant widespread both in temperate and tropical regions. *Portulaca* is a grassy plant with freshly stems, succulent leaves, yellow or white small flowers and small black seeds (Rahimi et al., 2019).

Besides its pharmacological potential (Al-Shedd et al., 2015), *Portulaca* is highly adaptable to dry and saline conditions, being a strong candidate for areas with dry conditions and salty soils (Yazici et al., 2007).

Seeds are brown to black, oval, and tiny. A single plant may produce 240.000 seeds, and can germinate even after 5 - 40 years (Okafor et al., 2014).

Regarding the chemical composition of the oil from the seeds, related to the likelihood of obtaining biodiesel, the specialized literature is scarce. Thus, the main components found in *P. oleracea* are fatty acid content of the oil which was found to be composed of unsaturated (79%) and saturated (20.7%) compounds (Sodeifian et al., 2018).

MATERIALS AND METHODS

For this study were used two extraction methods: Soxhlet technique and accelerated solvent extraction.

The most popular solid-liquid extraction technique is called Soxhlet technique, which is practically carried out in an extraction apparatus of the same name (Figure 1). Soxhlet extraction is usually applied to solid or ″semi-solid″ samples. The samples to be analysed are first crushed and brought into a very fine powder form to enlarge the surface of contact in the extraction process. This method was used for various purposes, like vegetable oils (Dutta et al., 2011), flavonoids (Kaleem & Ahmad, 2018), anionic surfactant removing (Rowe, 2010), foods (Hammond, 2003), phenols (Luque de Castro & Priego Capote, 2010) etc. This method is based on a large difference between the boiling points of the solvent and those of the extracted analytes.

Figure 1. Soxhlet extraction system

Based on this property the extract is brought to a boiling temperature of the solvent, which will condense into a refrigerant and return to cartridge containing sample extract. By performing several extraction cycles, the efficiency of the process can be controlled so that the extraction efficiency is maximum.

However, two main drawbacks can occur in the Soxhlet extraction technique. The first refers to the fact that the extract is exposed throughout the process to the boiling temperature of the solvent, which, if very high, may affect some analytes in the sample, thermally labile. The second disadvantage is the low concentration in the end, due to the large amount of solvent used.

Accelerated solvent extraction (ASE, Figure 2) is an extraction method based on the use of high temperature and pressure to accelerate the dissolution kinetics and to break the analytical-matrix interaction bonds. For this reason, this method is also called liquid pressurized (solvent) extraction. In addition, increasing the temperature reduces the viscosity of the solvent, which makes it easier to penetrate the solid matrix of the sample. In this way, the extraction time is reduced from tens of minutes

to a maximum of several minutes, and the extraction samples can be in small quantities. This type of extraction is recommended by the U.S. EPA for the extraction of solid samples from the environment like dioxins and furans, herbicides (Guzzella & Pozzoni, 1998), Organochlorinated Pesticides (OCPs), Organophosphorus Pesticides (OPPs), Persistent Organic Pollutants (POPs), (Hubert et al., 2001), Polybrominated Diphenyl Ethers (PBDEs), Polychlorinated Biphenyls (PCBs) (Brandli et al., 2006), etc. Also, the method is applied in fats and food safety domains (Abdul Mottaleb & Sarker, 2012).

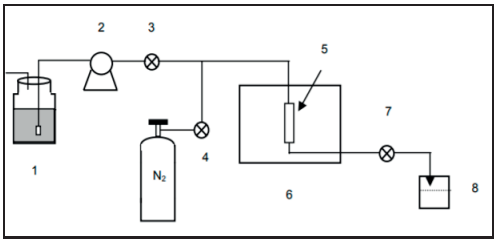

Figure 2. Components of an ASE system: 1 - the tank; 2 - pump; 3 - pump valve; 4 - purge valve; 5 - the extraction cell; 6 - thermostat enclosure; 7 - static valve; 8 - collection vial

The seed sample was minced to 1-2 mm. For accelerated solvent extraction method, the sample was mixed with diatomaceous earth. The sample: diatomaceous earth ratio used was 4: 1. ASE 350 model equipment was used (Figure 3).

Figure 3. ASE 350 system and extraction cell

For Soxhlet method, the sample: extraction solvent ratio was 1: 40. Extraction variants for accelerated solvent extraction (ASE 350) are presented in Table 1. In static ASE, the sample was extracted with solvent at elevated temperature and pressure conditions without any outflow of solvent (Mandal et al., 2015).

Table 1. ASE and Soxhlet extraction variants

Extraction solvent	Petroleum ether 100%	Petroleum ether 100%	n-Hexane	n-Hexane
ASE 350				
Temperature	105°C	105°C	80°C	80°C
Static time	10 min	10 min	10 min	10 min
No. of cycles	3	6	3	6
Washing volume	100%	100%	60%	60%
Purge time	60 sec	60 sec	60 sec	60 sec
Whole seeds	1.2 g	-	-	-
Sample 1	1.2 g	-	-	-
Sample 2	5 g	5 g	5 g	5 g
Soxhlet				
Sample 3	10 g	20 refluxes	15 min/reflux	

The advantage of the Soxhlet system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled (Nafiu et al., 2017).

RESULTS AND DISCUSSIONS

The factors which influence the oil extraction yield were: solvent of extraction, temperature, extraction time and liquid/solid ratio. Thus, optimal conditions for oil seeds extraction were obtained as follows: temperature 105°C, static time 10 min, and the number of cycles 3. Under these conditions the best value obtained for the oil extraction yield was 33.39 ± 0.013%. The obtained results are similar with those found by Petropoulos et al. (2020), 33.7% and 38.1%, respectively. Compared to ground seeds, the use of whole seeds, did not give positive results. The results obtained according to the extraction variants are presented in Table 2.

Table 2. Oil content in *Portulaca* sp. seeds

Variants	g oil/ sample	g oil %
Whole seeds	0	0
1.2 g shredded seeds, petroleum ether, 3 cycles	0.4008	33.4±0.013
1.2 g shredded seeds, n-hexane, 3 cycles	0.0994	8.25± 0.083
5 g shredded seeds, petroleum ether, 3 cycles	0.423	8.46±0.416
5 g shredded seeds, petroleum ether, 6 cycles	0.4586	9.17±0.383
5 g shredded seeds, n-hexane, 3 cycles	0.4214	8.43±0.378
5 g shredded seeds, n-hexane, 6 cycles	0.4994	9.98± 0.035
10 g shredded seeds, petroleum ether	1.671	16.71±0.273

Regarding Soxhlet extraction, the amount of oil obtained from the sample was 1.67 g. According to the obtained results found by Hoseini et al. (2019), it can be concluded that *Portulaca* weed seeds are a suitable source for biodiesel production.

Regarding the extraction solvent, the best variant was the one in which petroleum ether was used. The results are similar with those found by Uoonlue and Muangrat (2019) on *Camellia sinensis*.

Regarding the use of a larger quantity of sample 5 g and even if was kept the same number of cycles and the same solvent it was found that the amount of oil obtained was smaller than that obtained in the initial version of 1.2 g.

Among the experiment, the most influential parameter was solvent type. Although solvent sample ratio is reported to have no significant effect, this aspect has been shown to be partially demonstrated, the amount of solvent can be greatly reduced. Taking as reference other experiments on other types of samples, each optimized method is unique to the plants.

However, regarding purity, advanced extraction technology such as ASE should be considered.

CONCLUSIONS

Comparing the two extraction methods used, we concluded that the oil content was higher for ASE method than Soxhlet (33.4% vs 16.71%). The extraction ratio was the same 1: 40. The differences between the 2 methods were related to the extraction time, the volume of the solvent and the amount of sample used. Thus, for the Soxhlet vs ASE method the extraction time was 5 h-1 h, the volume of the solvent 400 ml-100 ml, and the sample amount was 10 g-1.2 g.

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AN OVERVIEW ON BLUE LIGHT BENEFITS ON PLANTS PHYSIOLOGICAL PERFORMANCES AND ON PLANT PRODUCTS QUALITIES

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Abstract

Light is one of the external factors of crucial importance for the plants growth and development in normal environmental conditions, as well as in the case of stress factors incidence. It is important not only the presence of light, because, the quality or the light spectra plays a major role throughout the all ontogenetic cycle of the plant. Therefore, especially in the current period, the use of light emitting diodes (LEDs) in indoor farming systems is one of the technological procedures applied in order to modify the light spectral composition, to regulate plants growth and last but not least to obtain value-added crops that are high in nutritional or nutraceutical contents. In view of the above, the purpose of this review is to present some of the newer results regarding the effects of blue light emitted by diodes (LEDs) on the physiological parameters of plants during vegetation period, which result in quantitative yield improvement, but also in terms of nutritional quality, including its preservation during the post-harvest period.

Key words*: light spectra, LEDs, physiological effect.*

INTRODUCTION

Light is one of the external factors of crucial importance for plants growth and development in normally environmental conditions (Smith, 1982), as well as in the case of stress factors incidence (Hoffman et al., 2015; Klem et al., 2019; Kovtun et al., 2019).

Under natural conditions of life, the amount and quality of light vary over the course of a day (Spitschan et al., 2016), but also with the unfolding of the seasons (Menzel and Fabian, 1999). The effects of these changes are not only related to the photosynthesis (Kreslavski et al., 2018), but also the light signalling pathways are interconnected that mediate the acclimatization to the various environmental conditions, or modulation of growth and development processes, thanks to the influence of the pathways to achieve physiological and biochemical processes (Kami et al., 2010; Paik et al., 2019).

Besides the cultivation of plants in the open field (Dunea et al., 2019), light effects are also important for in vitro cultures (Manivannan et al., 2017), greenhouses cultures (Pennisi et al., 2019), tunnels (Dou et al., 2017), including post-harvest effects (Jensen et al. (2018).

It is important not only the presence of light, because, the quality or the light spectra plays a major role throughout the all ontogenetic cycle of the plant, including germination, photomorphogenesis and floral induction (Smith, 1982). At the same time, the spectral composition of light has significant effects on the response of plants to the action of different stress factors, either abiotic (Courbier and PieriK, 2019; Kovtun et al., 2019) or biotic (Kim et al., 2013; Ballester et al., 2017).

In protected spaces (but not only), besides the possible use of different sources of additional lighting, to improve climate control, there are also effects on solar radiation due to climate screens and nets (including in the case of fruit tree plants growth in orchards) (Zoratti et al., 2015; Asănică et al., 2017) and their light transmittance is affected by the properties of the material used (Bastias et al., 2012;

Martinez-Gutierrez et al., 2016; Kotilainen et al., 2018).

In order to reduce the costs, at the same time with the increase of the yield and the enhance of the quality of the harvested products in a plant factory, Chang et al. (2016) noticed that it is necessary to implement an intelligent system for the automatic control of the lighting, so called "cloud-based lighting management system", considered to be of interest in the future for use in greenhouses.

Therefore, especially in the current period, the use of light emitting diodes (LEDs) in indoor farming systems (e.g. vertical farms) is one of the technological procedures applied in order to modify the light spectral composition, to regulate plants growth and development (Pennisi et al., 2019) and last but not least to obtain value-added crops that are high in nutritional or nutraceutical contents (Tan et al., 2019).

As Morrow (2008) emphasised, among the advantages of LEDs in lighting techniques in horticulture are: their ability to control the spectral composition, the production of very high light levels with low radiant heat output and the possibility to maintain their proper functioning for many years, without needing by their replacement.

Consequently, there is a major interest in this issue, not only from a scientific point of view, but also for practical reasons, as evidenced by the recent synthesis prepared by Zheng et al. (2019), with reference to horticultural plants (vegetables or ornamentals).

Moreover, not only can the quantity of the production obtained under such conditions be increased, but also its quality can be significantly improved by lighting supplemented by an adequate spectral composition (Kim et al., 2011; Bian et al., 2016).

Thus, manipulation of light quality in horticulture via photo-selective netting or films is applied with a view to improve not only the yield, but also quality (including chemical composition of cultivated plants (Yahia and Carillo-Lopez, 2019). Because the environmental changes have a negatively impact on the vegetables yield and quality, nowadays, but also in the future, indoor farming under LED lighting can be a sustainable farming system under controlled environment (Scheelbeek et al., 2018).

In view of the above, the purpose of this review is to present some of the newer results regarding the effects of blue light emitted by diodes (LEDs) on the physiological parameters of plants during vegetation period, which result in quantitative yield improvement, but also in terms of nutritional quality, including its preservation during the post-harvest period.

Blue light emitting diodes (LEDs) effects on plant physiology

Theoretically, the effects of the presence of light and its spectral composition are well known even on seed germination and seedling growth. Some plants seeds do not germinate under light condition, with other words, these are negatively photoblastic (light-induced dormancy), as is also the *Cleome gynandra*, a vegetable used in South Africa. However, the use of blue light has been shown to promote germination (<=35%), while red light had an inhibitory effect (<=8%). Moreover, the treatment with the blue light associated with the application of organic bio stimulants has determined biochemical changes in the seeds, such as an increase in the content of protein and carbohydrates, as well as increased activities of alpha-amylase, superoxide dismutase and catalase, which favoured seeds germination and seedlings growth (Nemahunguni et al.,2019).

Fantini et al. (2019) demonstrated the key role of cryptochrome in tomatoes, starting from the influence on seed mass, following the ability to control the early growth of seedlings, respectively the hypocotyl elongation and the root development of young plants. On the other hand, by "*in vitro*" studies, Burescu et al. (2015) reported that green light stimulated the spruce seed germination and plant growth, while the hypocotyl elongation has been inhibited by blue light.

The presence of specific photoreceptors, as light quality "radars" (Courbier and Pierik, 2019) that capture informational cues from sunlight (e.g. the phytochrome-PHY, cryptochrome-CRY, and phototropins -UVR8 that perceive red/far-red, blue/UV-A, and UV-B light, respectively (Wang et al., 2015; Podolec and Ulm, 2018; Fantini et al., 2019), as well as the existence of interconnected

signalling networks allow the coordination of photomorphogenesis responses (Doroshenko et al., 2019) and regulation at the plant level of numerous molecular and physiological reactions (Kreslavski et al., 2018; Paik et al., 2019) during the ontogenetic cycle. Besides possible influences in terms of morphological (van Ieperen, 2012) and growth changes (Brazaitytė et al., 2010; Zheng et al., 2018), the researchers' interest was focused on the effects on the functioning of the photosynthetic apparatus, including modifications regarding the chloroplast, the content of assimilatory pigments, but also on the efficiency of the photosystems (PS) located in the tilacoidal membranes (Zheng et al., 2018). For instance, blue light has been shown to have multiple effects, both when applied with red light, in different ratios, but also if it is provided alone.

Blue light contribution during the early steps of photomorphogenesis (the early stages of the seedlings de-etiolation), as well as the cytokinin (CK) dependent greening, has been demonstrated in *Arabidopsis thaliana* L. In this respect, Doroshenko et al. (2019) proceeded to inactivate CRY1, CRY2 and HY (components of signalling due to blue light), which caused the delay of chlorophyll accumulation. In contrast, the application of cytokinin resulted in acceleration of de-etiolation and an increase in chlorophyll fluorescence.

From the morphological view point, internode or petiole elongation growth, as well as leaf expansion directly impact light absorption and consequently plant productivity via photosynthesis process. Leaf deformations and epinasty due to light quality may determine a reduction of the biomass yield caused by a reduction of light interception. More, in the case of ornamentally plants, their final ornamental value decrease (van Ieperen, 2012).

Cryptochrome also has major effects on adult plants, as demonstrated in tomatoes (Fantini et al., 2019), when in the absence of functionally cryptochromes (CRY1a and CRY2), an acceleration of flowering time was induced, and the possible explanation was that the process was mediated by repression SELF PRUNING (SP)5G gene, previously proven as an inhibitor of tomato flowering. The authors concluded that the two types of cryptochromes should be named “master controllers” of tomato development, with reference to their influence on plant architecture, on flowering time and also on the tomato fruits composition. Consequently, they can represent successful molecular targets for the manipulation of the physiological processes in this species.

The alleviation effects of blue light on the so is named “red light syndrome” were noticed by Miao et al. (2019) on cucumber plant exposed to red light for a longer period of time. This negatively effect of continues red light was described by a reduction of the photosynthetic capacity, an unresponsive stomatal conductance, as well as low value for the maximum quantum yield of chlorophyll fluorescence (F-v/F-m). The blue light dependent alleviation was done by changes in chloroplast ultrastructure, as well as nutrient accumulation. The use of red (R) and blue (B) LEDs (with an RB ratio <3) in the case of the lettuce culture (using a ten layer vertical farming system) had the effect of increasing the photosynthetic quantum efficiency, the increase of the stomata conductance (associated with the increase of their densities, although their size was smaller), while the leaves chlorophyll and flavonoids content was lower.

There was also registered an increase of water use efficiency (WUE) up to 75 g FW L^{-1} and energy use efficiency (EUE) (up to 91 g FW $kW^{-1}h^{-1}$) in the case of a ratio of RB = 3, as well as a high production (45 g $plant^{-1}$), generating a potential land surface use efficiency (SUE) of 3110 g m^{-2} d^{-1} for indoor cultivation of basil (Pennisi et al., 2019).

In the case of spinach, Agarwal et al. (2018) highlighted that the use of blue monochromatic light caused the photosynthetic apparatus to deteriorate as a result of the photooxidation process. In contrast, there was an elicitation of the antioxidant defence system, both of the enzymatic and non-enzymatic type, but, nevertheless, the growth of the plants was below an optimal level, when there was not used a proper ratio between red and blue light.

On the other hand, for *Fagus sylvatica* L., Košvancová-Zitová et al. (2009) showed that a ratio of 3/1 (B/R radiation) caused a faster induction of photosynthesis, corresponding to an increased sensitivity of the electron transport process to the quality of light, resulting in a faster activation of the enzyme ribulose-1,5-

bisphosphate carboxylase/oxygenase (RuBisCO) and a reduction of non-photochemical quenching (NPQ) loss. Besides this, no kinetic dependence of the opening of the stomata on the quality of the incident light was observed.

Studies conducted by Zheng et al. (2018) also highlighted the combined effects of light intensity and its quality on *Chrysanthemum* plants. Regarding the application of 100% blue light using LED, at a low intensity (40 micromoles m^{-2} s^{-1}), this caused a decrease of the leaf thickness, respectively in the case of an increased intensity (100 micromoles m^{-2} s^{-1}), also there were observed favourable effects on the evolution of leaf anatomy. At the same time, the reduced light intensity induced a decrease in the value of the stomatal index and implicitly of the stomatal density, while in the white light and in the case of the red + blue combination, the stomata surface increased. Light quality had an effect on the efficiency of photosynthesis, expressed by chlorophyll fluorescence. Moreover, the blue light applied to a high intensity positively influenced the accumulation of biomass, compared to the red monochromatic light.

In the case of some ornamental species (*Cordyline australis* - monocotyledonate; *Ficus benjamina* - dicotyledonate, evergreen leaves; *Sinningia speciose* - dicotyledonate, deciduous leaves) grown in the pot, exposed to LED for 8 weeks, the addition of blue light has been shown to be essential for both normal leaf anatomy development and efficient photosynthetic activity (Zheng and Van Labeke, 2017). Thus, the blue light increased the leaf thickness of *C. australis* and *F. benjamina*, as well as the palisade tissue thickness of the in *S. speciosa*, which made possible a better absorption of the light, but also an increase of photosynthetic quantum efficiency at the PSII level. In has been also found that the stomata conductance was higher in the blue light, compared to the red light, in relation to a higher stomata index and / or a higher stomata density, even though the degree of openness of the stomata was not influenced by the light quality.

The effects of light quality are not only limited to the influence on plant physiology during greenhouse cultivation, as it is usually considered, but, as Jensen et al. (2018) noticed, post-harvest physiological changes may also occur. Thus, in basil grown under various types of light radiation (in different ratios) and exposed after harvesting at low temperature conditions it was found that an increased ratio in favour of blue light had negative effects, whereas in the case of the green light the effects were positive from this point of view. The authors' explanation was that an increase in the ratio in favour of blue light led to an increase in stomata density, contrary to the effects of green light. Thus, in basil, the connection between the leaves water retention capacity and post-cultivation chilling tolerance was highlighted.

Regarding the influence of coloured polyethylene nets (red, blue, pearl, black and no net), with a 50% shading coefficient on basil plants, the studies carried out by Martinez-Gutierrez et al. (2016) showed that although indicators such as photosynthetically active radiation (PAR) and the temperature had lower values, compared to the control, the quality expressed by the oil content (ranged from 0.6 up 0.7%) was improved by red, pearl, blue nets and control. On the contrary, the black net reduced the oil content by 34.3%, as against the control (no net).

The use of coloured nets with the aim of changing the microclimate and to provide protection against stress factors has also proved useful in fruit trees (Bastias et al., 2012; Asănică et al., 2017). They assured a manipulation of the photosynthetic and morphogenetic processes. Thus, in the Fuji apple tree variety of 3 years old, the red and blue nets (40% photo-selective) reduced by 27% the photosynthetically active radiation, compared to the control. At the same time, the rate of photosynthesis and the total leaf area were higher by 28%, respectively 30% in the case of the blue net, which demonstrates its positive effect on the net carbon assimilation, as well as on the total dry matter accumulation.

Also, in the case of cultivation of two varieties of strawberries in the low tunnels covered with foil of different colours, there were found differences according to variety regarding the vegetative growth, productivity and quality of the fruit. In contrast, coloured sheets (red, blue, yellow and green) did not significantly improve

the studied indicators. So, the use of commercially available transparent and opaque sheets was recommended for further use (Henschel et al., 2017). On the other hand, Nadalini et al. (2017) noticed that in the case of protected strawberry cultivation systems (soilless culture) and blue LED treatment, the accumulation of biomass (especially at the root and crown level) was favoured, a better fruit set was ensured and implicitly a greater final yield was obtained, as against the control.

The effects of light quality are registered not only on the morphology, physiology and biochemistry of the upper part of the plant, but also on the root (Klem et al., 2019), which influences not only the development of the root system, but also its resistance to the action of certain stress factors (e.g. drought resistance). In barley roots, if in the case of white light, it was stimulated the accumulation of secondary compounds with defence effect (e.g. proline, jasmonic acid), respectively the production of abscisic acid (ABA) was reduced, in blue light, Γ-aminobutyric acid (GABA), sorgolactones and others secondary metabolites were accumulated, whose osmolytes activity, antioxidants or growth regulators activities highlight the role of blue light as inductor of the protective mechanisms against abiotic and biotic stress factors.

The effects of light quality are recognized even in the case of "*in vitro*" cultures, as Manivannan et al. (2017) emphasised. The quality of carnation plants propagated "*in vitro*" was improved in both blue and red light (applied for 8 weeks). Both treatments significantly increased the growth, photosynthetic parameters and content in mineral nutrients, compared to conventional growth.

Beneficial effects were demonstrated too by Enache and Livadariu (2016) on *Artemisia dracunculus* L. seed, when there were applied different light treatments (white, red, blue or green) using LEDs, over a photoperiod of 16 hours, for seven days. In controlled cultivated conditions there were registered different reactions as regard as the germination process, development of sprouting plant elements (cotyledons and hypocotyl) and in accumulation of the fresh weight, also of the dry weight of biological material.

Blue light benefits on secondary metabolism and biochemical compounds accumulation

Light quality has important effects not only on plant morphologies or intensity of some on the processes specific to the primary metabolism, but also on the secondary metabolism (Kim et al., 2011; Bian et al., 2016; Lee et al., 2016; Doerr et al., 2019).

Therefore, in addition to the primary metabolism, changes in secondary metabolism can lead to positive effects in terms of increasing antioxidant activity (Raiciu et al., 2018), as one of a defence mechanism that protect plant species from stresses factors incidence (Kim et al., 2013), as well as improving the nutritional and nutraceutical qualities of the obtained products (Sucupira et al., 2012; Dou et al., 2017).

In addition to the effects on temperature and morphology, changes in secondary metabolism were noted by Doerr et al. (2019) after the exposure of the species *Plectranthus scutellarioides* (*Solenosternon scutellarioides, Coleus bluntel*) to different lamp systems, including LEDs. LEDs favoured strain elongation, possibly due to a greater amount of red light in the spectrum component, but at the same time, the leaf temperature was lower, which favoured the production of rosmarinic acid and flavone glycosides relative to the dry mass. At the same time, the presence of a large amount of blue radiation in the light spectrum has led to the formation of thicker leaves, and consequently the accumulation of a larger quantity of secondary substances at the unit of leaf surface.

The different effects of blue light have been studied especially in the case of vegetables plants, both in terms of the influence on the physiology of the plants during the vegetation period, with implications regarding the productivity and quality of the crop, but also in relation to possible effects regarding the post-harvest behaviour.

It has been proven that the lettuce quality can be improved by reducing the nitrate content. In this context, Bian et al. (2016) reported that by enhanced the duration of the illumination and the pre-harvest spectral composition, this trait can be modified. Thus, the plants exposure for 24 h at red:blue LED light (R:B = 4:1) with an intensity of 200 micromoles $m^{-2}s^{-1}$ determined

the marked decrease of the nitrate content and besides it led to the increase of free-radical scavenging activity, as well as to increase the content in phenolic compounds. A promotion of the nitrogen assimilation, a favour of the accumulation of above ground biomass, as well as an improved nutritional quality (including a reduction of nitrate accumulation) in the lettuce was assured by a light ratio of 4R/1B, explained by the effects on the enzymes involved in the metabolism of nitrogen (nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase and glutamate dehydrogenase) (Zhang et al., 2018).

A combination of RB light improved the nutritional quality at *Brassica* vegetables (Chinese cabbage and kale) by a better production of polyphenols and flavonoids. Thus, the content in glucoraphanin, glucobrassicin, polyphenols and anthocyanins were improved by culture techniques based on the use of LEDs, as demonstrated by Lee et al. (2016). Also, Kopsell and Sams (2013) showed that in broccoli microgreen tissues, pre-harvest application for the short duration of the blue light, significantly improved the nutritional quality expressed by increasing the content in carotenoids (e.g. β- carotene, violaxanthin), glucosinolates, essential micronutrients (e.g. copper, iron, boron, manganese, molybdenum, sodium and, zinc) as well as essential macronutrients (e.g. calcium, phosphorus, potassium, magnesium and sulfur), which are very useful for consumers.

The highest concentrations of the proteins, polyphenols and flavonoids of sprouts of hemp (*Cannabis sativa* L.) were determined in aseptically culture conditions, by illumination with blue LED, as compared with another two light spectra (red and green) and the control (sunlight), in the study initiated by Livadariu et al. (2019).

Obtaining high quality horticultural products can be achieved by applying biotechnologies based on the use of LED devices, as highlighted by studies conducted by Kang et. (2019), on Chinese cabbage seedlings. Blue light has been shown to increase the accumulation of ascorbic acid (AsA) by activating the expression of the gene involved in AsA biosynthesis. At the same time, there was an increase in antioxidant activity, by activating major reactive oxygen species (ROS) - scavenging antioxidant enzymes.

For soilless cultivated strawberries, Nadalini et al. (2017) determined that the use of blue LED light did not induce changes regarding the main characteristics of the fruit quality, even if in colour, it was weaker and lower level of pelargonidin-3-glucoside has been registered.

In the post-harvest period, blue light led to increased colour index, respiration rate and ethylene synthesis in strawberries stored at 5^0C, increased the activity of antioxidant enzymes (superoxide dismutase, catalase and ascorbate peroxidase), while superoxide anion levels, hydrogen peroxide, and malondialdehyde were kept low. In addition, the free radical-scavenging ability has been improved (Xu et al., 2014).

Also, the results of the studies by Huang et al. (2018) regarding the ripening of bananas in the post-harvest period led to the recommendation of the LED light source as a chemical-free strategy intended to shorten the ripening period. There has been registered a faster de-greening and flesh softening, as well as the increase of the ethylene biosynthesis, and respectively, an intensification of the respiration process, especially due to blue LED. Furthermore, exposure to LED light favoured the accumulation of ascorbic acid, total phenols, and total carbohydrates in banana fruit.

Improvement of the physiological performance (intensification of photosynthesis, enhancement of photochemical efficiency of PSII, decrease of non-photochemical quenching - NPQ, accumulation of flavonols in epidermal cells) of plants exposed to UV stress has been demonstrated by research carried out by Hoffman et al. (2015). The blue light in high amount triggered biochemical and physiological processes meant to ensure the acclimatization and recovery of pepper plants exposed to stress.

The beneficial effects of additional illumination with blue light have also been shown for potato plants exposed to saline stress (Kovtun et al., 2019), possibly due to the accumulation of carotenoids and proline, substances that act as non-enzymatic antioxidants, during the defence reactions. Thus, following the study on the tolerance capacity of potato plants to chlorine salinity, it was found that the protective effect

of the blue light was based on its ability to stimulate the accumulation of organic compounds with low molecular weight and antioxidant activity.
Also, in the case of buckwheat sprouts (known as rich food in nutritional elements destinated for human food, zootechnical industry, apiculture and human medicine) a highest antioxidant activity was induced by illumination with blue LED light (Livadariu & Maximilian, 2017).
In the reinforcement of the aforementioned, also come the results of Kim et al. (2013), who emphasized that tomato seedlings treatment with blue-LED light resulted in an increase of proline content in leaves and stem by about 296% and 127%, respectively, compared to the application of broad-spectrum white LED (BSWL), while in red and green light, the proline content significantly decreased. Also, the total phenolic compounds in leaves and stems significantly increases (1.3-fold, and 1.2-fold, respectively) as against to BSWL conditions.
The inhibition effect of blue LED light on *Botritys cinerea* development on tomato should be explained at least in part by enhanced proline accumulation and antioxidative processes. On the same note, possible effects regarding the elicitation of the resistance of citrus fruits to the attack of the most important pathogen in the post-harvest period (*Penicillium digitatum*) by the blue LED have been studied by Ballester et al. (2017). Although an increase in the content of scoparone phenylpropanoid was determined, it didn't turn out to be the critical factor in inducing resistance by blue LED. At the same time, although there has been an increase in the production of ethylene, it has not been implicated in the elicitation of resistance.
As Alsanis et al. (2019) recently mentioned in a bibliographic synthesis, it is obvious the need to provide artificial lighting to improve the physiological performances of the plants in order to obtain maximum profitability for ornamental plants and to secure the production of vegetables and berries in greenhouse conditions, with low energy consumption and at low costs. Alternative biotechnological measures include light emitting diodes, whose proper use has already been established in urban farms, respectively plant factories. The authors highlight not only the influence of such technology on the plants biotic and abiotic environmental conditions, but also the impact on the plant-microorganism interactions (thereby understanding pathogens, such as bacteria and fungi, control biological agents and respectively, the phyllobiome).

CONCLUSIONS

The presence of light represents a primordial condition for the life of plants, considering first of all its involvement in the unfolding of the greatest phenomenon of nature, photosynthesis. The unfolding under optimum conditions of the whole ontogenetic cycle also depends on the quality of the light or the spectral composition, both under the conditions of cultivation of the plants under field conditions, but, especially under controlled conditions (e.g. "*in vitro*" cultures) and/or protected spaces (e.g. greenhouses, vertical farms etc.).
Besides the general physiologically known effects that blue light has on plant functioning, the use of blue light emitted by diodes has proven to be one of the appropriate technological procedures for modifying the spectral composition of light, in order to regulate the growth process, as well as obtaining products (especially horticultural products in the classical sense, microgreens and herbs) with high added value, characterized by increased nutritional and nutraceutical content.
In addition to these beneficial effects, maintaining products postharvest quality and / or enhancing features that define this attribute (including increased tolerance to abiotic and biotic stressors) may be due to the specific effects of blue light.
Given the experimental results obtained in the field, especially nowadays, when climate change negatively impacts vegetables production, under environmentally controlled conditions, the use of light emitted diodes seems to be a promising technological measure.

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MAPLES (GENUS *ACER* L.) OF "ALEXANDRU BELDIE" HERBARIUM

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Abstract

Herbaria provide useful information for studies related to anatomy, morphology, systematics and taxonomy of plants. In Romania, sixteen herbaria collections exist, the one hosted by "Marin Drăcea" National Institute for Research and Development in Forestry being among them. The aim of this paper was to provide an overview of the representatives of genus Acer of "Alexandru Beldie" Herbarium (Code: BUCF). Each sample provided the following information: the scientific and popular names of the species, the taxonomic classification of the species, the place of collection, the date of collection, summary data about the biotope of the harvested plant and the name of the person who collected the plant. Every sample was evaluated and the degree of conservation was assessed. 333 paper sheets and thirty one taxa belonging to genus Acer were sampled. The collection contains several subspecies and varieties of some of the most common maple species across Romania (e.g. field maple), and also some rare species native to China or Japan. The vast majority of samples are in a good conservation phase, representing a valuable database for future interdisciplinary research.

Key words*: Aceraceae, biological material, herbaria, maple, plant specimens.*

INTRODUCTION

A herbarium contains a collection of dried plants specimens (vouchers) labeled and stored in an organized manner that is used for a broad range of scientific studies (Enescu et al., 2018).
In order to be preserved from fungal infestations and consumption by larvae and insects, the samples are treated with chemical compounds, such as mercury dichloride ($HgCl_2$) (Cabassi et al., 2020). In the last years, herbaria provided primary data source for several studies related to anatomy and morphology, anthropology, biogeography, climate change, ecology, economic botany, ethnobotany, environment variation, genetics, history of plants, phylogenomics, systematics, taxonomy and teaching (Enescu et al., 2018; Jang et al., 2020; Nevill et al., 2020; Sugita et al., 2020).
Particularly, herbaria were used for quantitative assessment of accumulation of certain metals (*e.g*. cobalt, manganese, nickel and zinc) into specific parts of hyperaccumulator plants (Do et al., 2020) or to study the chemical diversity of preserved plants (Resende et al., 2020).
Sixteen herbaria exist in Romania. Among them, one of the oldest is the herbarium founded in 1882 by Professor Dimitrie Brândza that is hosted by "Dimitrie Brândza" Botanical Garden, University of Bucharest (Urziceanu et al., 2017). After half a century, Professor Alexandru Beldie founded another representative collection, which nowadays is hosted by "Marin Drăcea" National Institute for Research and Development in Forestry. "Alexandru Beldie" Herbarium (Code: BUCF) incorporates a collection of 60.000 samples of preserved specimens of trees, shrubs, mosses, lichens, ferns and plants (Enescu et al., 2018) harvested preponderantly from mountain areas (Deleanu et al., 2019).
Al. Beldie, Al. Borza, P. Cretzoiu, C.C. Georgescu, M. Haret, N. Iacobescu, I. Morariu, S. Paşcovschi, M. Petcuț, I. Pop, I. Prodan and E.I. Nyarady were among the Romanian botanists who contributed to the collection (Dincă et al., 2017).
Due to their importance, diversity and large distribution range across Romania, the representatives of Genus *Acer* L. (maples) are

very well represented within BUCF and in other herbaria worldwide.

Maples are distributed in the Northern Hemisphere, in Europe, North America, North of Africa, but especially in China (Asadi et al, 2019; Li et al., 2019). Being a species-rich genus, several opinions among taxonomists regarding the exact number of species exist. According to several studies (Chen, 2010; Contreras et al, 2018; Suh et al., 1996; Suh et al., 2000) the number of maple species varies from 129 to 200 or even more (Harris et al., 2017). Among them, there are several species with restricted distribution range, such as *Acer yangbiense* Y.S. Chen & Q.E. Yang and *A. griseum* (Franch.) Pax in China (Tao et al., 2020; Wang et al., 2017; Zhao et al., 2011), *A. pseudowilsonii* Y.S. Chen in Thailand (Chen, 2010) *A. miyabei* Maxim. in northern Japan (Saeki et al., 2018) or *A. binzayedii* Rehder in Jalisco State, Mexico (Vargas-Rodriguez, 2017). Other species, such as box elder (*A. negundo* L.), are considered to be invasive (Abramova, 2019).

Several maple species have high ornamental value, such as Amur maple (*A. ginnala* Maxim.) (Yang et al., 2020) or *A. pseudoplatanus* "Atropurpureum" (Kostić et al., 2019) being widely used in gardens, as hedges or as urban trees. For example, along Timișoara Boulevard from Bucharest, one third of the total tree species is represented by five maple species, silver maple (*A. saccharinum* L.) and Norway maple (*A. platanoides* L.) being the most common ones (Badea et al., 2016).

Some maple species have other uses, such as in afforestation of degraded lands (Drzewiecka et al., 2019; Enescu, 2015), treating a wide range of diseases (Bi et al., 2016) and/or providing wood and sap (Bilek et al., 2016).

Last but not least, maples are regarded as key forest elements (Mohtashamian et al., 2017) with interesting reproductive models that include monoecy, dioecy, trioecy, andro-monoecy, androdioecy, and andropolygamy (Rosado et al., 2018). Thanks to their importance in forestry and due to the fact that superior genetic material is needed, 79 seed sources of sycamore maple (*A. pseudoplatanus* L.) were recently designated (Rebrean et al., 2019) across Romania and other studies for the establishment of a new seed orchards are ongoing (Marin et al., 2012).

The purpose of this paper was to provide an overview of the representatives of genus *Acer* of "Alexandru Beldie" Herbarium.

MATERIALS AND METHODS

Each labeled sample provided the following data: the scientific and popular names of the species, the taxonomic classification of the species, the place of collection, the date of collection, summary data about the biotope of the harvested plant and the name of the person who collected the plant.

Every sample was evaluated and the degree of conservation was assessed by using the following scale: 1 = well preserved plant (whole plant) properly attached to the sheet, 2 = plant detached from the sheet with detached, but existing parts, 3 = plant detached from the sheet with missing parts and 4 = detached and fragmented plant with over 50% missing parts (Enescu et al., 2018).

RESULTS AND DISCUSSIONS

A total of 333 paper sheets and thirty one taxa belonging to Genus *Acer* were sampled, namely pointed-leaf maple (*A. argutum* Maxim.), *A. austriacum* Tratt (sin. *A. campestre* infrasp. *campestre*), Campbell's Maple (*A. campbellii* Hook. f. & Thomson), field maple (*A. campestre* L. *sensu lato* or *A. campestris* L.), silver maple (*A. dasycarpum* Enhr.), devil maple (*A. diabolicum* Blume ex K.Koch), Amur maple (*A. ginnala* Maxim.), Iranian maple (*A. insigne* Boiss.), *A. italum* Pax (sin. *A. opaluss* ssp. *Italum*), *A. laetum* C.A.Mey (sin. *A. cappadocicim* Gled.), smooth maple (*A. laevigatum* Wall.), bigleaf maple (*A. macrophyllum* Pursh), *A. martini* Jordan [sin. *A. monspessulanum* subsp. *Martini* (Jordan) P. Fourn.], Montpellier maple (*A. monspessulanum* L.), box elder (*A. negundo* L.), *A. obtusatum* Waldst. & Kit. Ex Willd. [sin. *A. opalus* subsp. *Obtusatum* (Waldst. & Kit. Ex Willd.) Gams], *A. opulifolium* Vill. (sin. *A. opalus* Mill.), palmate maple (*A. palmatum* Thunb.), striped maple (*A. pensylvanicum* L.), yellow-paint maple (*A. pictum* Thunb.), Norway maple (*A. platanoides*

L.), sycamore maple (*A. pseudoplatanus* L.), red maple (*A. rubrum* L.), sugar maple (*A. saccharum* Marshall), silver maple (*A. saccharinum* L.), *A. semenovii* Regel et Herd. [sin. *A. ginnala* subsp. *semenovii* (Regel & Herder) Pax or *A. tataricum* subsp. *semenovii* (Regel & Herder) A.E. Murray], mountain maple (*A. spicatum* Lam.), Tatar Maple (*A. tatarica* L. or *A. tataricum* L.), Manchurian striped maple (*A. tegmentosum* Maxim) and other five undetermined taxa.

Almost three quarters of samples consisted in biological material of field maple (38%), Tatar maple (17%), sycamore maple (10%) and Norway maple (7%), respectively.

Among the 30 sampled *A. campestre sensu stricto* biological materials, two originated from France and one from Bulgaria, while the rest were collected from forests across its distribution range in Romania, such as: Slobozeanu Mare Forest and Trei Izvoare Forest (Buzău County), Comana Forest (Giurgiu County), Stejeret Forest (Dâmbovița County), Tufele Grecului Forest, Băneasa Forest and Râioasa Forest (Ilfov County) and Casa Verde Forest (Timiș County), respectively. The oldest one was sampled in 1882, in Nancy (France).

Another 95 *A. campestre sensu lato* individuals were sampled across well-known forests across Romania. For example, from Ciolpani Forest (Ilfov County), the fallowing taxa were collected: *A. campestre* ssp. *eucampestre* var. *lobatum* f. *affine*, *A. campestre* ssp. *eucampestre* var. *normale* f. *hederifolium* H. Braun., *A. campestre* ssp. *hebecarpum* var. *normale* f. *molle*, *A. campestre* ssp. *leiocarpum* var. *normale* f. *hederifolium* H. Braun and *A. campestre* ssp. *marsicum* var. *subtrilobum* f. *hungaricum*, respectively.

Another example is Lunca Stăneşti Forest (Vâlcea County), where *A. campestre* ssp. *eucampestre* var. *normale* f. *leiophyllum*, *A. campestre* ssp. *leiocarpum* var. *normale* f. *lasiophyllum* and *A. campestre* var. *normale* f. *lasiophyllum* were identified.

Last, but not least, other two examples of famous forests among specialists, both located in Tulcea County, are represented by Ciucurova Forest and Valea Fagilor Forest, where *A. campestre* ssp. *marsicum* var. *subtrilobum* and *A. campestre* ssp. *hebecarpum* var. *marsicum* f. *subtrilobum* were sampled.

Among the less common identified taxa within herbarium, two originated from Japan, namely pointed-leaf maple (Figure 1) and devil maple (Figure 2).

Figure 1. Sample of pointed-leaf maple

Maple specimens included in the herbarium were sampled between 1842 and 1996. Among the oldest ones, there were five samples of sycamore maple collected by P. Cretzoiu in 1842. Two of them were well-preserved, while three were detached from the sheet with detached, but existing parts.

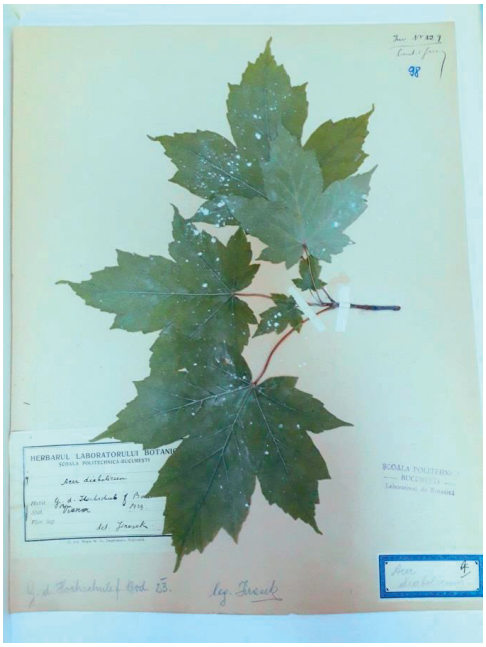

Figure 2. Sample of devil maple

More than half of the biological material (for 315 samples out of the total of 333, this information was also available) was sampled between 1941 and 1960, with the peak in 1948, with 62 samples (Figure 3).

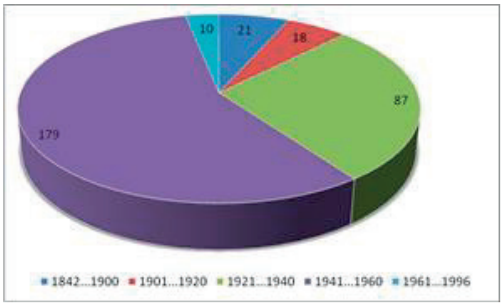

Figure 3. Timeframes of sampling the biological material

As regards their conservation status, more than a half (*i.e.* 57%) of the sampled biological material was well preserved, the whole plant being properly attached to the sheet. Another 36% were detached from the sheet with detached, but existing parts and only a few had missing parts (Figure 4).

"Alexandru Beldie" Herbarium contains also some species with interesting characteristics among the maples, such as smooth maple (Figure 5). This species, native to China, has pinnately veined simple and evergreen leaves.

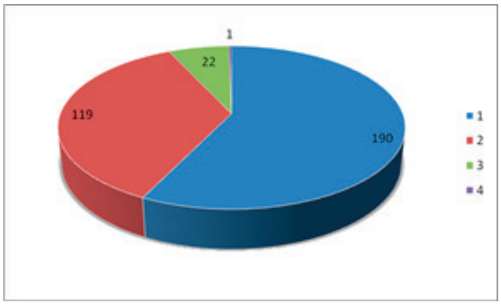

Figure 4. Shares of degrees of conservation of the sampled biological material

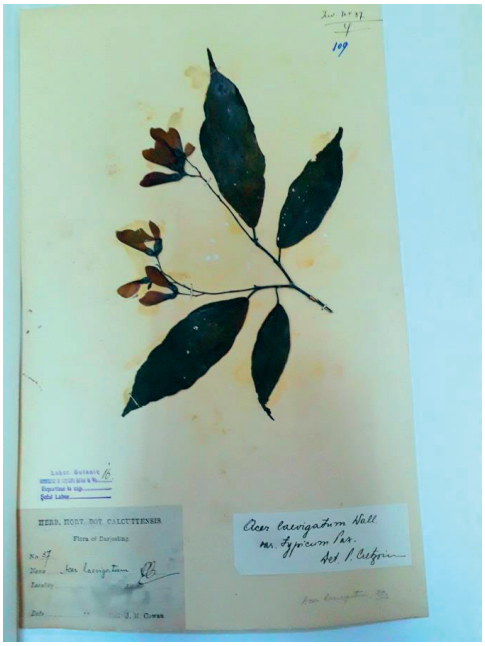

Figure 5. Sample of smooth maple

CONCLUSIONS

In spite of the fact that most of sampled biological material was preserved seven or eight decades ago, or even more, the vast majority of them are in a good conservation phase.

The diversity of maple species from "Alexandru Beldie" Herbarium is high, the collection containing even very rare or endemic species sampled from different countries across Asia.

The collection also provides information regarding the leaf morphological diversity of some of the most common maple species (*e.g.* field maple) that represents a solid database for further research. Moreover, the herbarium contains different subspecies or varieties of the same species that were sampled from the same forest stands that could represent a good starting point for designation of new forest genetic resources, especially in the current context of climate change.

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LEGUME-RHIZOBIAL SYMBIOSIS OF THE PANNONIAN CLOVER VARIETY ANIK USING COMPLEX MICROELEMENTS AND GROWTH REGULATORS

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Abstract

The article presents the scientific results of the influence of complex microelement fertilizers and growth regulators on the formation of the parameters of the symbiotic activity of agrocenoses and the productivity of the Pannonian clover variety Anik. During exogenous seed treatment with micronutrient fertilizers and bacterial preparations, the number and weight of active clover nodules of the first year of use in relation to control increased by 28.8-154.9 million units/ha and 77.2-240.8 kg/ha, respectively. With foliar application, the most effective method is double follicular treatment of Pannonian clover plants in the regrowth and budding phase with Megamix-Nitrogen complex fertilizer. The highest indicators of the symbiotic apparatus of the Pannonian clover crops were formed using tank mixtures of the Corsair herbicide with the drug Albit and Siliplant; according to the experimental variants, they amounted to 93-101 million units/ha of active nodules with a mass of 467.3-504.0 kg/ha. The effectiveness of the anti-stress effect of the drugs Albit and Siliplant is observed with a 50% reduction in the dose of the herbicides Corsair and Agritox.

Key words*: nitrogen fixation, Pannonian clover, biological products, microelements*.

INTRODUCTION

In solving the problem of increasing the production of feed and increasing their energy saturation, a significant role is assigned to perennial herbs. Therefore, it is relevant to organize adaptive fodder production through the introduction of new species that have ecological plasticity, longevity, high forage qualities, are distinguished by stable seed production, high winter resistance, heat and drought tolerance, and increase soil fertility. (Watson, 2012; Komainda et al., 2019).

In the Middle Volga region (Russia), a promising forage crop is the Pannonian clover (*Trifolium pannonicum* Jacq.), characterized by high ecological plasticity and adaptability, productive longevity of 10-12 years, drought resistance, winter hardiness, and resistance to diseases and pests, has stable seed production, increases soil fertility, and is valuable as a predecessor and a honey plant (Kshnikatkina et al., 2018).

An important element of modern agricultural technologies is growth regulators, complex micronutrient fertilizers and bacterial preparations that provide plants with deficient micronutrients and help to increase their resistance to environmental stress factors and pathogens (Watson, 2012; Ruiz-Navarro, 2019).

Of scientific and practical interest is the use of complex liquid mineral fertilizers with a rich composition of micro and macro elements and bacterial preparations to increase the productivity of the Pannonian clover variety Anik, which determined the relevance of the research (Mikula et al., 2020).

MATERIALS AND METHODS

The purpose of the research was to study the treatment of seeds with bacterial preparations and microelement fertilizers on the production process of the Pannonian clover. Experimental studies were conducted in 2014-2016. On the experimental field of Penza State Agrarian University (Penza, Russia).

The soil of the experimental plot is leached chernozem, heavy loam. The humus content in the arable layer is 6.5%, pH (KCl) - 5.2, the degree of saturation with bases - 80.8-82.3%, the availability of mobile forms of molybdenum, boron, manganese, copper, zinc and cobalt is low. Meteorological conditions during the years of research: 2014 - State Customs Committee - 0.59 units; 2015 - State Customs Committee - 0.5 units; 2016 - State

Customs Committee - 1.21 units. The object of research is the Pannonian clover (*Trifolium pannonicum* Jacq) variety Anik.

The area of the accounting plot is 10 m^2. Agrotehnika is generally accepted in this region. Inoculation of seeds with bacterial preparations was performed on the day of sowing. Application rates recommended by manufacturers.

RESULTS AND DISCUSSIONS

Seed treatment before sowing by growth regulators has a multifunctional effect, since the seeds at the time of germination have high plasticity and are susceptible to changes in environmental conditions.

Our experiments have found that when treating clover seeds with the studied drugs, field germination according to the test variants increased on average by 0.6-13.9% over three years. The highest germination rate of 80.2% was observed during seed treatment with the Gumariz biopreparation together with the MegaMix-Semen microelement fertilizer, the excess in relation to the control was 13.9%, the plants were preserved for harvesting - 13.5%. The greatest winter-hardiness was noted during the processing of seeds Megamix-Seeds and Gumarizom. So, in the first year of use, the winter hardiness of clover plants was 94.6-99.8% according to the experimental variants (control 89.4%). After wintering, the largest number of clover plants (238.2 pcs/m^2) remained in the Gumariz + Megamiks-Semen variant, the excess in relation to control was 85.9 pcs / m^2 (10.4%).

When treating seeds with the agrochemicals Albite, Megamix-Seeds, Humate K/Na separately and together with Humaris, the Pannonian clover plants formed a more powerful root system, the mass of dry roots according to the experimental variants was 1.26-1.98 t/ha, which is 0.39-1.11 t/ha exceeds the control option.

The results of the analysis of the linear growth of the clover showed that in the 1st year of life, taller plants were formed in the variant with seed treatment with the Megamix-Semen preparation together with Gumariz. On average, over three years, the height of plants at the end of the growing season was 32.8 cm, in control - 10.2 cm. A similar trend continued in subsequent years.

The analysis of the formation of the symbiotic activity of the agrocenosis of the Pannonian clover 1st year of use showed that the largest number of active nodules and their mass formed in the budding phase during seed inoculation with the Gumariz biological product together with the Megamix-Semen microelement fertilizer. On average, over two years, the total number of nodules during the budding phase of the agrocenosis of the Pannonian clover 1st year amounted to 248.6 million units/ha with a mass of 992.6 kg/ha, active nodules - 219.3 million units/ha with a mass of 876.9 kg/ha, which exceeds the control variant by 3.9 and 2.87 times, with inoculation with rhizotorfin - by 1.8 and 1.3 times respect-tively. A similar regularity in the formation of the parameters of symbiotic activity was also observed in the agrocenoses of the Pannonian clover 2nd year of use (Table 1).

It was found that bacterial preparations and microelement fertilizers have a significant effect on the formation of the leaf surface. The most intensive growth of the leaf surface is noted during the budding phase when treating clover seeds with Gumariz jointly with Megamix-Seeds. The area of leaves in the first year of use during the budding phase was 51.5 thousand m^2/ha, which exceeds the control indicators by 24.1 thousand m^2/ha (87.9%), with inoculation of seeds with risotorfin - by 14.7 thousand m^2/ha (71.5%).

The highest values of photosynthetic potential (PP) and net productivity of photosynthesis (NPP) were noted when treating clover seeds with the micronutrient fertilizer Megamix-Seeds together with Gumariz PP - 2.14 million m^2d/ha, NPP - 4.83 g/m^2, in the risorotin + Megamix version Seeds - 2.02 million m^2dn/ha and 4.69 g/m^2 a day, when treated with Megamix-Seeds - 42.8 million m^2dn/ha, AF - 1.85 million m^2d/ha, NPP - 3.95 g/m^2 a day , in the control indicators the FP - 1.02 m^2d/ha, NPP - 1.92 g/m^2 a day. Moreover, in relation to the indicators of the 1st year of use, the parameters of photosynthetic activity of the 2nd year of use increased by 14.9-15.7.

Optimization of production conditions positively influenced the accumulation of clover dry matter (Table 2).

Table 1. The number and weight of active nodules of agrocenoses the Pannonian clover in the budding phase

Option	1st year (2015-2016)		2nd year (2016)	
	number of tubers, (mln. pcs/ha)	the mass of tubers (kg/ha)	number of tubers, (mln. pcs/ha)	the mass of tubers (kg/ha)
No processing (control)	53.7	328.4	67.2	410.2
Risotorfin + Albit	183.5	657.4	229.5	821.7
Rizotorfin + Megamix-Seeds	208.6	823.5	260.7	1029.4
Risotorfin + Humate K/Na	185.7	685.6	232.5	857.1
Gumariz + Albit	201.5	863.5	252.4	1079.4
Gumariz + Megamix-Seeds	219.3	876.9	264.5	1096.1
Gumariz + Gumat K/Na	204.3	802.6	255.4	1003.2

Table 2. The productivity of the Pannonian clover, 2015-2016

Option	The collection of green mass, t/ha		Seed productivity, kg/ha	
	1st year	2nd year	1st year	2nd year
No processing (control)	21.3	23.2	359.2	431.2
Risotorfin + Albit	37.2	40.2	685.2	822.3
Rizotorfin + Megamix-Seeds	40.6	44.3	703.8	896.2
Risotorfin + Humate K/Na	36.6	39.1	678.9	814.7
Gumariz + Albit	42.1	45.9	720.8	865.3
Gumariz + Megamix-Seeds	47.5	51.8	776.4	931.7
Gumariz + Gumat K/Na	40.8	44.5	714.7	857.6
HCP_{05}	2015 - 3.4; 2016 - 3.1	2016 - 2.4	2015 - 25.8; 2016 - 22.6	2016 - 3.34

On average, over two years, the yield of green mass of the Pannonian clover 1st year of use, according to the experimental options, was 28.7-47.5 t/ha, dry weight - 7.18-11.87 t/ha. The greatest productivity of clover was obtained during the processing of Megamix-Seeds seeds together with Gumariz: green mass - 47.5 t/ha; dry matter - 11.87 t/ha, feed units - 8.54 t/ha, digestible protein - 1.32 t/ha, exchange energy - 95.48 GJ/ha (Table 2).

In the complex treatment of seeds with the Gumariz bio-preparation, together with microelement fertilizers, the most favorable conditions were formed for the formation of structural elements and seed productivity of the Pannonian clover. Thus, the number of generative shoots varied from 4.14 to 4.68 million units/ha, seeds in the head ranged from 41 to 46 pcs., The number of seeds per plant from 170 to 215 pcs., The productivity of an individual plant ranged from 0.67 to 0.90 g, weight of 1000 seeds - from 3.94 to 4.18 g. The average yield of clover seeds of the Pannonian clover 1st year of use for two years amounted to 528.3-776.4 kg/ha in the experimental variants, and in the control - 359.2 kg/ha. The most productive were clover agrocenoses during complex seed treatment per year of sowing with the Gumariz biological product together with micronutrient fertilizers, the seed yield was 714.7-776.4 kg/ha, which is 2.0-2.2 times higher than the control. The maximum seed yield of 776.4 kg/ha was obtained in the variant Gumariz + Megamix-Seeds. In the second year of use, the seed productivity of clover increased and amounted to 634.3–931.7 kg/ha in the experimental variants. The highest yield of clover seeds was obtained on the Gumariz + Megamiks Seeds variant - 931.7 kg/ha, in the control - 431.2 kg/ha.

The use of micronutrient fertilizers for foliar top dressing contributed to an increase in the parameters of symbiotic activity, the value of which depended on the type of preparations and the timing of their use. It has been established that the most effective method is double treatment of clover plants in the growth and budding phase with the complex fertilizer Megamix-Nitrogen. So, the total number of tubers was 378-395 million units/ha, weight - 1485-1625 kg/ha. The number of active tubers in the budding phase - 319-348 million units/ha, weight - 1238-1355 kg/ha, in the

control variant - 108-130 million units/ha and 361-426 kg/ha, respectively (Table 3).

The best indicators of the productivity of the photo-synthetic apparatus were formed during the double treatment of vegetating plants of clover of the Pannonian clover microelement fertilizers in the phase of regrowth and budding. When foliar application with Azosol 36 Extra, the area of the assimilating surface of agrocenoses of the Pannonian clover 4-9th year of use was 70.8-77.8 thousand m^2/ha, photo-synthetic potential - 2.09-5.15 mln m^2d/ha the net productivity of photo-synthesis is 4.36-5.57 g/m^2 a day. When used for feeding Megamix-Profi PP - 2.34-2.95 mln m^2dn/ha, NPP - 4.42-5.63 g/m^2 a day and the preparation Humate K/Na - PP - 2.20-2.90 mln m^2d/ha, NPP - 4.15-5.36 g/m^2 a day. The crops of the Pannonian clover worked most productively during leaf dressing with Megamix-Nitrogen micronutrient fertilizer, the leaf area was 72.6-79.5 thousand m^2/ha, and the NPP was 2.36-3.06 million m^2/day, NPP - 4.47-5.68 g/m^2 a day.

The optimization of the mineral nutrition of the Pannonian clover plants by foliar feeding with complex microelement fertilizers positively influenced the formation of productivity (Table 4).

Table 3. The number and weight of active tubers of agrocenosis of the Pannonian clover

Factor A - the drug	Factor B – processing phase	Year of use					
		4	5	6	7	8	9
Without treatment (k)		130 426	108 418	119 410	122 401	115 389	112 361
Azosol 36 Extra	regrowth	231 969	209 951	220 932	223 913	216 885	213 651
Azosol 36 Extra	budding	241 978	219 959	230 940	233 921	226 893	223 657
Azosol 36 Extra	regrowth + budding	257 1003	235 984	246 965	249 946	242 917	239 577
Megamix-Profi	regrowth	270 1162	248 1140	259 1118	262 1095	255 1062	252 782
Megamix-Profi	budding	284 1173	262 1150	273 1128	276 1105	269 1071	265 788
Megamix-Profi	regrowth + budding	300 1204	268 1181	279 1158	282 1135	275 1100	271 815
Megamix - Nitrogen	regrowth	320 1308	282 1283	299 1258	302 1232	295 1195	291 1098
Megamix - Nitrogen	budding	329 1319	291 1294	308 1269	311 1243	304 1205	300 887
Megamix - Nitrogen	regrowth + budding	348 1355	340 1329	337 1303	330 1277	323 1238	319 1286
Humate K/Na	regrowth	211 882	173/ 865	190 848	188 831	186 805	182 592
Humate K/Na	budding	232 888	194 871	211 854	209 837	207 811	203 594
Humate K/Na	regrowth + budding	243 1000	205 981	222 962	220 943	218 914	214 619

*Note: the numerator is the number of tubers (million units/ha)
the denominator is the mass of nodules (kg/ha)

Table 4. Productivity of the Pannonian clover seeds by years of use

Factor A - the drug	Factor B - processing phase	Year of use					
		4	5	6	7	8	9
Without treatment (k)		459.3	436.3	413.4	381.2	321.5	252.6
Azosol 36 Extra	regrowth	575.5	495.6	517.9	477.8	402.8	316.5
Azosol 36 Extra	budding	627.9	596.8	565.1	521.2	439.5	345.3
Azosol 36 Extra	regrowth + budding	791.6	752.9	712.4	657.0	554.1	435.4
Megamix-Profi	regrowth	523.7	497.5	471.2	434.7	366.7	290.0
Megamix-Profi	budding	565.2	536.9	508.7	469.1	395.6	310.9
Megamix-Profi	regrowth + budding	684.6	650.4	616.1	568.2	479.2	376.5
Megamix - Nitrogen	regrowth	542.6	515.6	488.3	450.4	379.8	299.4
Megamix - Nitrogen	budding	579.2	550.3	521.4	480.7	405.4	318.6
Megamix - Nitrogen	regrowth + budding	746.5	709.2	671.8	603.9	522.6	410.6
Humate K/Na	regrowth	520.8	494.8	468.7	432.3	364.6	86.4
Humate K/Na	budding	548.9	521.5	494.2	455.6	384.2	301.9
Humate K/Na	regrowth + budding	609.0	578.6	548.1	505.6	426.3	335.1
HCP_{05} Factor A		35.4	32.3	30.4	28.1	30.9	21.4
Factor B		43.2	45.4	38.6	37.1	36.4	32.8
Factor AB		68.3	71.2	67.9	70.4	66.3	68.2

The most productive agrocenoses of the Pannonian clover 4-9th year of use were when feeding with Megamix-Nitrogen complex fertilizer.

When feeding clover grass stands twice during the growing and budding phase, the yield of dry matter was 10.68 t/ha, feed units - 7.45 t/ha, digestible protein - 1.21 t/ha, exchange energy - 92.7 GJ. In relation to the control variant, the collection of dry matter increased by 3.27 t/ha (44.1%).

The yield of clover seeds of the Pannonian clover 4-9th year of use with foliar fertilizing with complex fertilizers according to the experimental variants was 286.4-791.6 kg/ha (control - 252.6-459.3 kg/ha).

The highest seed yield was obtained by double treatment of the grass stand with clover with Azosol 36 Extra in the regrowth and budding phase - 435.4-791.6 kg/ha, which exceeds the control variant by 182.8-332.3 kg/ha

A similar pattern is observed during the feeding of vegetation of the Pannonian clover microelement fertilizers Megamix-Profi, Megamix-Nitrogen and Humate K/Na.

The yield of the Pannonian clover seeds when fertilizing with Megamix-Nitrogen microelement fertilizer was approximately on par with that of Azosol 36 Extra (316.5-791.6 kg/ha) and varied from 299.4 to 746.5 kg/ha.

The highest indices of the crop structure elements were formed during the double feeding of the seed grass-standing the Pannonian clover microelement fertilizer Megamix-Nitrogen: the number of generative shoots was 7.13 million units/ha, the number of seeds in the head was 55 pcs. 399 plants per plant, plant productivity 1.84 g, 1000 seeds weight 4.61 g, control - 5.68 million units/ha, 38 units, 216 units, 0.8 g and 3.78 g,

relatively. Almost equivalent indicators of the elements of the crop structure were formed when the fertilizer of the Pannonian clover 4-9th year of using Megamix-Nitrogen was double-fed.

The greatest economic effect was obtained by treating the seeds of clover of the Pannonian clover bacterial preparation Gumaris with Megamix-Seeds, while cultivating the seeds, the profitability was 185.0%, and for fodder purposes - 147.9%.

CONCLUSIONS

Thus, the use of microelement fertilizers and bacterial preparations for exogenous seed treatment significantly increased the indicators of symbiotic nitrogen fixation, photosynthetic activity and productivity of clover of the Pannonian clover variety - Anik.

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THE EFFECT OF LED-S EMITTED LIGHT TREATMENTS ON SPROUTING MUNG BEAN (*VIGNA RADIATA* L.)

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Abstract

Mung bean (Vigna radiata L.), is a plant species from Fabaceae family and represented a very good source of antioxidants and polyphenols, having also an important nutritional value. This paper presents the results obtained by treatments with white (W), red (R) or blue (B) light emitted of LED-s, on mung bean (Vigna radiata L.) sprouts. The parameters evaluated were the number and fresh weight of sprouts mung bean (Vigna radiata L.), obtained by application of the LED treatments. The reference was represented by sprouts mung bean (Vigna radiata L.), illuminated with sunlight. Biochemical studies included determination of concentration of polyphenols, proteins, flavonoids and antioxidant capacity. The white (W) LED treatments on sprouts mung bean (Vigna radiata L.), produced an important number of sprouts while the red (R) LED induced the high fresh weight of mung bean (Vigna radiata L.) sprouts. The content of polyphenols, flavonoids and the antioxidant capacity was significantly higher by treatment with white (W) light LED. The reference contains a higher content in proteins than the samples illuminated with LED emitted light.

Key words: *antioxidant capacity, flavonols, LED-s, sprouts, polyphenols, proteins, Vigna radiata* L.

INTRODUCTION

Mung bean (*Vigna radiata* L.), is an annual herbaceous plant from the major group of Angiosperms, included in genus *Vigna*, family *Leguminosae* (http://www.theplantlist.org/tpl1.1/record/ild-29556).

Archaeobotanical record from many archaeological sites of India, indicate that mung bean (*Vigna radiata*), was presented from 2500 BC to this days (Fuller, 2007). In our days, mung bean (*Vigna radiata*), is one of the most cultivated plant in India, Myanmar, China, Pakistan, Bangladesh, Cambodia, Thailand, Sri Lanka and Australia (Rawal & Navarro, 2019). The quality and size of mung bean (*Vigna radiata* (L.) R. Wilczek), are depending of various abiotic (e. g., waterlogging and salinity), and biotic stresses (e. g., viral and fungal diseases) (Rawal & Navarro, 2019). This species presents a variable short-duration vegetable period from 70-110 days (Lambrides & Godwin, 2007) to 90-120 days (Lim, 2012) or 65-80 days (Rawal & Navarro, 2019), and is cultivated from 0 to 1850 m altitude (Lim, 2012). The mung bean is a species very abundant in nutritive elements (polysaccharides with antioxidant activities, radicals scavenging activity, immunoregulatory and immunomodulatory activities and peptides with antioxidant activity) and also in active principles phenolic acids (1.81-5.97 mg rutin equivalent/g - hydroxycinnamic and hydroxybenzoic acid), flavonoids (1.49-1.78 mg catechin equivalent/g - anthocyanins, flavonols, flavones, isoflavonoids), and tannins (1.00-5.75 mg/g) - Hou et al., 2019. The mung bean contains also balanced nutrients, including protein, dietary fiber, minerals, vitamins, and significant amounts of bioactive compounds (Gan et al., 2017). Furthermore, mung bean protein is easily digestible, as compared to protein in other legumes (Mubarak, 2005 and Yi-Shen, 2018) and high protein content with hypoallergic properties.

Mung bean is one of the most consuming plant in all the world. The seeds of mung bean are eaten whole, as flour (Lim, 2012) or sprouts (Lambrides & Godwin, 2007; Lim, 2012; Tang et al., 2014). The mung beans sprouts contents an increased vitamin C concentration, an enhanced total phenolic compounds and flavonoids, quercitin 3-O-glicoside and

antioxidant capacity 6 times higher than that of mung bean seeds.

As a result, it is useful to obtain sprouts of mung bean (*Vigna radiata* L.), through the method of care to reduce the negative influence of stressors of a biotic and / or abiotic nature.

The goal of our research, was to notice the effect of lighting with white (W), red (R) or blue (B) light emitted of LED-s and sunlight (S, reference) on mung bean sprouts (*Vigna radiata* L.).

MATERIALS AND METHODS

The biological material consisted of mung bean seeds (*Vigna radiata* L.), from two varieties: from China (TRS brand) M1 and Myanmar (RAPUNZEL brand) M2. Mung bean seeds (*Vigna radiata* L.) in the dormant phase present an oval shape, were colored in brownish green. The seeds from China had a larger diameter (0.4-0.5 cm) compared to those from Myanmar (0.3-0.4 cm). The main distinguish element of the two categories of seeds was the type of agriculture used to obtain them, respectively: organic agriculture for M1 variety or conventional agriculture M2 variety.

The experimental variants used in the experiment were the following:

- V1 = M1 + treatment by white color LED-s – emitted light ;
- V2 = M1 + treatment by red color LED-s – emitted light ;
- V3 = M1 + treatment by blue color LED-s – emitted light;
- V4 = M1 + treatment by sunlight, control (S)/reference variant;
- V5 = M2 + treatment by white color LED-s – emitted light;
- V6 = M2 + treatment by red color LED-s – emitted light;
- V7 = M2 + treatment by blue color LED-s – emitted light;
- V8 = M2 + treatment by sunlight, control/reference variant.

Groth conditions and plant material: Lighting treatment were performed in controlled environmental conditions.

The seeds of mung bean (*Vigna radiata* L.), were sterilised using a 2.5% sodium hypochlorite solution for 5 minutes; washed 10 minutes for 3 times with sterile distilled H_2O (Badea & Săndulescu, 2001; Cachiță-Cosma et al., 2004). The seeds were watered and germinated in sterile condition on gauze soaked in sterilized distilled H_2O (Enache & Livadariu, 2016), to obtain sprouts of mung bean. The seed were placed in food casseroles (transparent plastic, sterile and provided with a lid); and germinated under light LED treatment (white, red - Li et al., 2014 - or blue), The lighting emitted by LED-s or sunlight was performed for 8 days, photoperiod of 16 h, at a temperature of 23°C ± 2°C/photoperiod and 20°C ± 2°C/dark period. The technical characteristics of LED-s are: voltage 220 V, power 18 W and light flux 435 lm (Livadariu & Maximilian, 2017).

Morphological measurements (fresh weight) rates (number of sprouts/time) and quantification of proteins, flavonoids, polyphenols, antioxidant capacity was analyzed.

The protein extraction was performed by grinding the sprouts tissue in 50 mM potassium phosphate buffer, 0.05% β-mercaptoethanol, 0.5 mM (DIFP) diisopropyl fluorophosphat – protease inhibitor, pH = 6.8 (1.0 g/0.5 ml, dry weight/buffer) at 4°C for 24 hours. The extract was centrifuged 18.000 rpm for 20 min. and the supernatant was used for protein assay. The protein concentration was carried out using Bradford method (Bradford, 1976). For the polyphenols, flavonoids content and antioxidant capacity assay was used methanolic extracts. The polyphenols concentration was evaluated using Folin - Ciocalteu reagent (Mihailović et al., 2013). The results were expressed as mg gallic acid equivalent / g fresh weight. The antioxidant capacity was carried out according to Marxen et al. (2007), using DPPH (2,2-diphenyl-1-picrylhydrazyl) and a calibration curve with Trolox as antioxidant standard. The antioxidant capacity was expressed as mM Trolox/g fresh weight. The flavonoid compounds were estimated using Zhishen et al., 1999, modified method with aluminum chloride. The absorbance of the mixtures was measured at 510 nm. A calibration curve with rutin was used. The flavonoids concentration was expressed as mg equivalent rutin/g fresh weight.

Statistical procedures. Each experimental variant were consisted from twenty mung bean

(*Vigna radiata* L.) seeds/variety M1/M2. All analysis were performed in triplicate. The data have been statistically analyzed and the standard deviation of mean was calculated. The rate, the fresh weight of sprouts (A), polyphenols, flavonoids, proteins content, and antioxidant capacity were determined.

RESULTS AND DISCUSSIONS

A. Determination of the rate and the fresh weight of two variety of mung bean (M1 and M2) (*Vigna radiata* L.) sprouts by treatment with white (V1/V5), red (V2/V6), blue (V3/V7) LED-s or sunlight (V4/V8) - reference variant (control)

A1. Determination of the rate of mung bean (Vigna radiata L.*) sprouts*

According to the experimental data presented in Figures 1 and 2, the mung bean seeds (*Vigna radiata* L.) begin to germinate in the second day of the experiment.

In the second day, under the influence of the white light treatment emitted by LED-s (V1 and V5) the highest number of sprouts was obtained, both M1 (Figure 1) and M2 (Figure 2) varieties in comparison with all variants analyzed (V2-V4 and V6-V8).

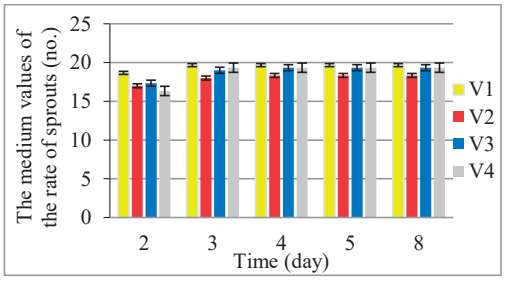

Figure 1. The rate of mung bean (*Vigna radiata* L.) sprouts (no.), for experimental variaty M1 illuminated by LED

Also, on the second day, the smallest number of sprouts, was registered: in the case of M1 seeds under the influence of sunlight treatment (V4) (Figure 1), and in the case of M2 seeds, under the influence of the blue light LED treatment (V7) (Figure 2).

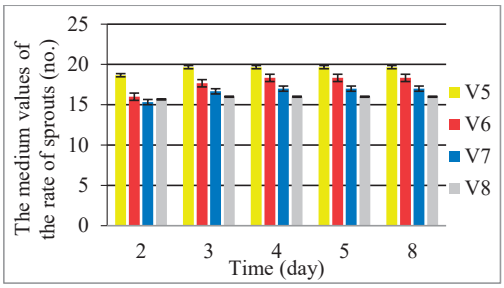

Figure 2. The rate of mung bean (*Vigna radiata* L.) sprouts (no.), for experimental variaty M2 illuminated by LED

On the third day, it can be observed the highest number of sprouts from M1 and M2 varieties, also under the influence of white light treatments emitted by LED-s (V1 and V5). But, unlike the second day, the smallest number of sprouts was recorded for M1 seeds under the influence of the red light emitted by LED-s (V2) and for the M2 seeds, under the influence of sunlight treatment (V8). In the fourth and fifth day were recorded minor differences in the number of sprouts, stabilizing on the eighth day. These results complement those obtained (Livadariu et al., 2019), the treatment with blue LED, induced a superior rate of sprouts in comparison with others variants, red and green LED-s light.

A2. Determination of the fresh weight of mung bean (Vigna radiata L.*) sprouts*

According to the graphic (Figure 3), it can be observed that the values of fresh weight of mung bean sprouts (*Vigna radiata* L.) have the lowest value (2.5 g) for the generated sprouts from M2 seeds, under the influence of sunlight (V8). The highest value (9.05 g) was registered for sprouts illuminated with red light emitted by LED-s, from M1 seeds (V2). Thus, it can be noticed a clear difference of the average values of the fresh weight of mung bean sprouts (*Vigna radiata* L.) between M1 and M2. Other authors Park et al., in 2019, found that red light-irradiated canola (*Brassica napus*) sprouts being significantly higher than those of sprouts exposed to white and blue LEDs.

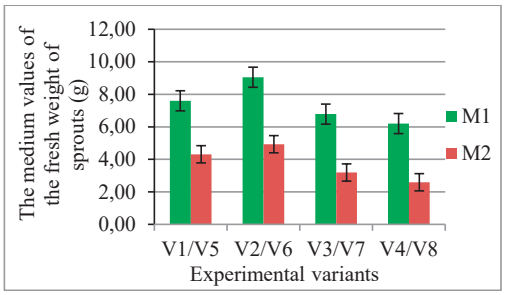

Figure 3. The fresh weight of mung bean (*Vigna radiata* L.) sprouts (g), for experimental variant from M1 variety and M2 variety

B. Biochemical analyses

B.1. Determination the protein content in mung bean sprouts illuminated with LED

The biochemical analyses regarding the content of proteins in mung bean sprouts illuminated with LED for M1 and M2 variety emphasized that the sample represented by reference variant (sunlight) had the higher concentration. The treatment of sample from M1 and M2 varieties illuminated with LED did not induce an increase of protein concentration (Figure 4).

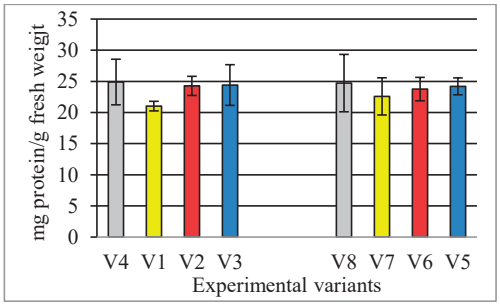

Figure 4. The protein concentration in M1 and M2 varieties illuminated by LED

The white LED produces a higher concentration in M2 variety (V7) while red LED induced a higher content in protein in M1 variety (V2). The values of proteins concentration are very close for M1 and M2 varieties illuminated with blue LED (V3, V5).

There are not data in the literature regarding the effect of different types of illuminated LED on sprouts. However, there are some information regarding the sprouts treatment with the light traditional bulb which induced a higher protein concentration in compared to those illuminated by LED light, by 17% respectively, to obtain plants with increased protein content the light should be used with an increased proportion of wavelengths 600-780 nm (as in the case of traditional bulbs) (Fiutak et al., 2018). Our previous study (Livadariu et al., 2019), on effect of LED treatment have shown that the illumination with blue light LED produced the highest protein concentration for hemp sprouts compared with green and red LED.

B2. Determination the polyphenols concentration in mung bean sprouts illuminated with LED

The higher concentration of polyphenols in mung bean sprouts was determined by illumination with white LED light for both varieties M1 and M2 (V1 and V5) (Figure 5).

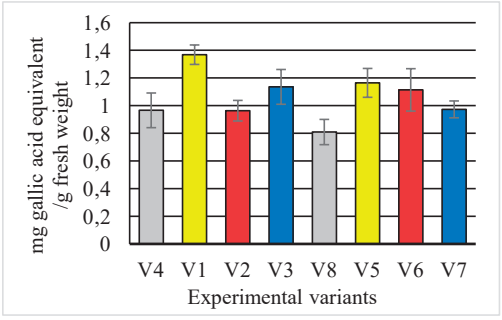

Figure 5. The polyphenols concentration in M1 and M2 varieties illuminated by LED

The effect of red LED illuminated the mung bean sprouts induced a higher concentration of polyphenols in M2 variety comparative with content of polyphenols in M1 variety, while the blue LED treatment produced a stronger effect in M1 variety. The white LED treatment induced an effect similar with blue LED illuminated mung bean sprouts. Similar results obtained by Matysiak & Kowalski, 2019, who mentioned that the phenols concentration was enhanced by illuminated with white LED lighting compared to the control in basil green leaves, lamb's lettuce and garden rocket leaves, but was unaffected in basil purple-leaves.

B3. Determination the flavonoids concentration in mung bean sprouts illuminated with LED

The treatment with white LED on mung bean sprouts determined a high biosynthesis of flavonoids for M1 (V1) and M2 (V5) varieties (Figure 6). Estimating the effect of type LED it can be observed that the white and blue light

was similar revealing a higher content in flavonoids for M1 variety. The effect of red LED illuminated the mung sprouts was significant in flavonoids concentration for M2 variety. Other studies (Matysiak & Kowalski, 2019) emphasized that plants growth under blue LED light had a content in flavonols (a class of flavonoids) higher than in the case of leaves from basil, lamb's lettuce and garden rocket, as compared with white LED light treatment.

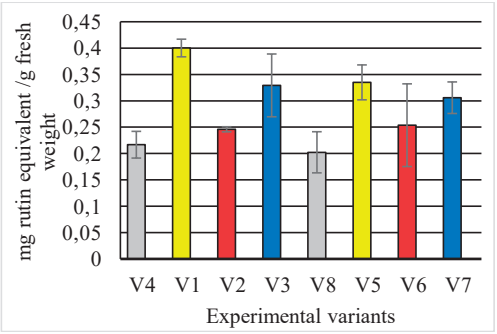

Figure 6. The flavonoids concentration in M1 and M2 varieties illuminated by LED

B4. Determination of antioxidant capacity in mung bean sprouts illuminated with LED

A significant increase in antioxidant capacity was observed by illuminated the mung sprouts with white LED (V1 and V5) for both M1 and M2 varieties (Figure 7).

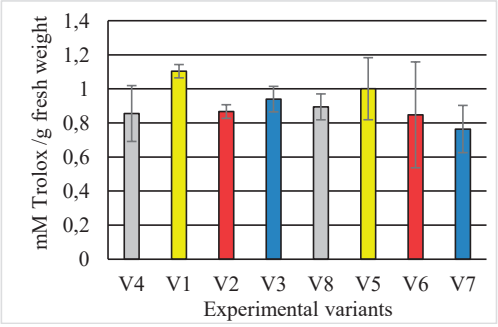

Figure 7. The antioxidant capacity in M1 and M2 varieties illuminated by LED

Comparing the effect induced by each LED type white, red and blue was observed the following: the white and blue LED produced an increase of antioxidant capacity for M1 variety. The treatment with red LED has positive effect for M1 (V2) and M2 (V6) variety of mung bean sprouts.

Comparing the effect of red LED on each mung bean variety we conclude that in M2 variety was stimulated a biosynthesis of polyphenols and flavonoids but the antioxidant capacity was similar for both varieties. In the similar manner, the effect of blue and white LED amplified the accumulation of polyphenols, flavonoids and antioxidant capacity.

Our results differ from those of Wu & al. (2007), that noticed the antioxidant capacity of pea seedlings, after 96 h of radiation by various LED lights, was significantly enhanced by red light radiation.

CONCLUSIONS

Spouts development, morphogenesis, growth, and secondary metabolite synthesis are significantly affected by light quality and the lighting spectrum. Regarding comparatively the M1 and M2 varieties, the highest average values of the number of sprouts were recorded under the influence of white lights emitted by LED-s, while the highest average values of the fresh weight of sprouts were recorded under the red-light treatment emitted by LED-s. In our study, white LED light was found to be suitable for biosynthesis of polyphenols, flavonoids and antioxidant capacity.

Analyzing the results obtained, we observed a direct correlation between the highest concentration of polyphenols, flavonoids and antioxidant capacity and the rate of mung bean sprouts induced by treatment with white LED.

The experimental results obtained from testing light treatments emitted by LED-s for achieving mung bean sprouts (*Vigna radiata* L.) from seeds obtained through organic agriculture (M1) or conventional agriculture (M2), recommend further research for growing sprouts, because, predominantly, there were recorded better experimental values under the influence of treatments with white, red or blue light emitted by LED-s, compared to the sunlight treatment.

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SOME ASPECTS OF THE ANATOMICAL FEATURES OF THE MEDICINAL PLANT *AGASTACHE FOENICULUM* (PURSH) KUNTZE (*LOPHANTHUS ANISATUS* (NUTT.) BENTH.)

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Abstract

Agastache foeniculum (Pursh) Kuntze (Lophanthus anisatus (Nutt.) Benth.) is an aromatic plant used for its medicinal and nutritive properties. It is also is a melliferous and ornamental plant. For the first time, the acclimatization of new species, such as Agastache foeniculum (Pursh) Kuntze, was made in 2010 at the Buzău Institute for Research and Development in Vegetable Growing, Romania. In 2019, Agastache foeniculum (Pursh) Kuntze was studied at the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The current study is dealing with the leaf and stem anatomy of Agastache foeniculum specimens obtained from seeds provided by the Buzău Institute. The anatomical analyses were made at different parts of the specimens. The leaf analyses showed the presence of glandular and non-glandular trichomes in the upper and lower epidermis. The trichomes were observed in the epidermis of the stem, as well.

Key words: *leaf and stem anatomy; trichomes.*

INTRODUCTION

Agastache foeniculum (*Lophanthus anisatus* (Nutt.) Benth.) - common name Blue Giant Hyssop, Giant Hyssop, Fragrant Giant Hyssop, Anise Hyssop, Wild Anise, Lofant popular¸ is a member of the *Lamiaceae* family. It belongs to this very large family whose species are to be found all over the world. The *Agastache* genus comprises 22 species of perennial aromatic medicinal herbs. *Agastache foeniculum* (Pursh) Kuntze is known as a medicinal and flavouring spice plant. Extract from giant hyssop shows significant bioactivity: antimicrobial, antiviral, antimutagenic, antiproliferative, antiatherogenic, cytotoxic for cancer cell, anti-inflammatory, antioxidant, relaxant activity on the contractions a.o. (Yashika et al., 2013; Sánchez-Recillas et al., 2014; Mazza and Kiehn, 1992; Mostafa et al., 2018; Omidbaigi and Sefidkon, 2003; 2004; Zielińska and Matkowski, 2014; Mihaylova et al., 2013; Duda et al., 2015; Hashemi et al., 2017; Ivanov et al., 2019). The results obtained from antimicrobial screening revealed that essential oil of *Agastache foeniculum* possesses inhibitory activity against *Staphylococcus aureus*, *Curtobacterium flaccumfaciens*, *Listeria monocytogenes*, *Bacillus subtilis*, *Salmonella* sp., *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*, while *Enterococcus faecalis* remained unaffected (Ivanov et al., 2019). The phytochemical compounds in different extracts could be possibly used in the pharmaceutical industry and also in the production of natural cosmetics. The leaves and flowers of *A. foeniculum* have been reported to be used in cakes, ice creams, and sweets, and can be added as fresh ingredients to salads and desserts (Van Hevelingen, 1994; Vogelmann, 1983; Hopkinson et al., 1994; Lima, 1986; Tucker, 1994).

At the same time, the insecticide effect of *Agastache foeniculum* has been reported (Kim Soon-Il, 2003; Ebadollahi et al., 2010;

Ebadollahi, 2011; Ebadollahi et al., 2013; Zielińska and Matkowski, 2014).

An overview of *Agastache* research has been made by Ayers and Widrlechner, 1994, as well as by Fuentes-Granados et al., 1998, but does not include anatomy-related data.

The anatomical features of the vegetative organs are important in the characterization of *Lamiaceae* taxa (Metcalfe & Chalk, 1950; Abu-Asab & Cantino, 1987; Kahraman et al., 2009; 2010). The glandular trichomes and their distribution, the stomatal distribution together with other anatomical features provide significant taxonomic information (Werker, 1985; 2006; 2010; Tirillini et al., 1997; Dinç & Öztürk., 2008; Celep et al., 2014; Ascensao & Pais, 1998; Baran et al., 2010; Mota et al., 2013; Duarte & Lopez, 2007; Corsi & Botega, 1999; Van Horne & Zopf, 1948; Svidenko et al., 2018; Gul et al., 2019).

The *Lamiaceae* family comprises many species known for their medicinal and economic importance, due to the production of essential oils in the glandular trichomes (Serrato-Valentini et al., 1997; Rodrigues et al., 2013). There is sparse data regarding the anatomy of the species under study world wide (Schulz, 1899; Gurtovenko et al., 2018). *A. foeniculum* has not been studied anatomically either, although taxonomists need such studies for a correct classification of the *Lamiaceae* species as well as for finding any possible anatomic modifications that are adaptations of the studied species to the local conditions in Romania.

MATERIALS AND METHODS

The seed material (Aromat de Buzău) originated from the Buzău Institute for Research and Development in Vegetable Growing, Romania. The studies showed that this species has adapted very well and can be successfully grown in Romania (Vânătoru et al., 2015). Plants of *A. foeniculum* (*Lophanthus anisatus*) were planted in the experimental field of the Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV-Bucharest). This material was sectioned by hand using razor blades to obtain semipermanent and permanent slides for microscopic studies. The cross-sectioned plantlets presented 4 internodes. Fresh leaves, stems and petioles were collected for anatomical study. Thereafter the sections were clarified with chloral hydrate for 24 hours, then washed and stained with carmine alaunate and green iodine (Săvulescu and Hoza, 2011; Georgescu et al., 2015). Analyses and observations of these cross-sections were performed at the Center for the Study of Food and Agricultural Products Quality at USAMV-Bucharest. Photos were taken and measurements were made using the Leica DM1000 LED, the Leica DFC295 Video Camera and the Leica S8 APO Stereo Microscope, Optika Microscope, as well as a Sony photocamera. Photos were taken using light microscope with different magnifications.

RESULTS AND DISCUSSIONS

Metcalfe and Chalk (1972) stated that stems of many genera and species of the family Lamiaceae are quadrangular with well-defined collenchyma in the four angles. The stem of this plant ehxhibits the general characteristics of the *Lamiaceae*.

The great diversity of plant trichomes has interested botanists by their adaptive and taxonomic values. The morphology and distribution of glandular trichomes are often applied as taxonomic characters at subfamiliar level in *Lamiaceae* family (Abu-Asab & Cantino 1987; Cantino, 1990).

In this paper, the authors analyze the structure of the vegetative organs (leaf, petiol and stem) of *A. foeniculum* (Figure 1), trying to describe the constant and particular histo-anatomical features of the species. The anatomical studies on stem, petiole and leaf cross-sections are presented, along with leaf surface sections. Glandular trichomes are important taxonomic features in the *Lamiaceae* family (Xiang et al., 2010). Trichomes are considered relevant in comparative systematic investigations and in morpho-diagnosis (Metcalfe & Chalk, 1950; 1988). Peltate glandular trichomes with essential oil are localized on the adaxial and abaxial leaf surfaces.

Figure 1. *Agastache foeniculum*

Werker (1993) mentioned, considering a functional viewpoint, that glandular trichomes produce essential oils which apparently protect the plant against herbivores and pathogens and attract pollinators to the floral parts.

Stem anatomy

The stem has a square shape, with four corners and four faces between them (Figure 2).

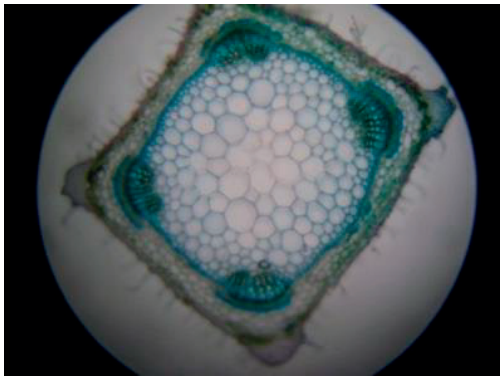

Figure 2. Stem *anatomy*

The epidermis is composed of one oval-shaped or circular cell layer 9.55-9.95 µm thick, a 2.82-4.51 µm cuticle with non-glandular trichomes about 137.8-327.4 µm long, as well as glandular trichomes 172.6-174.6 µm long. Just inwards of the epidermis within each corner there is a region of angular collenchyma cells, 36.25-52.78 µm thick. Cambium is running around the stem and connects the vascular bundles. Inwards of the cortex there is vascular tissue. In the corners, the vascular tissue is made up of prominent vascular bundles (phloem 19.46-22.67 µm, xylem (vessels) 7.13-16.92 µm). The parenchyma tissue has small cells 42.37-46.23 µm and larger cells 74.83-85.69 µm (Figure 3). Pith appears in the centre of the stem.

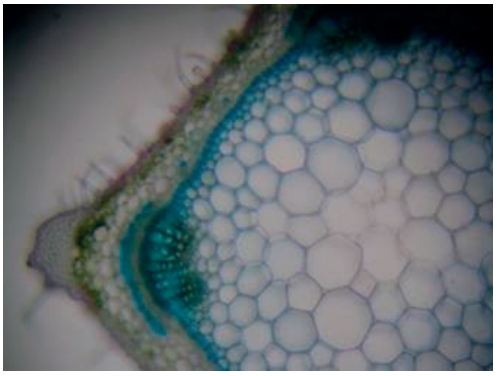

Figure 3. Stem *anatomy*- details

Petiole anatomy

In cross section (Figure 4), the petiole's adaxial and abaxial epidermis consists of a single layer of rectangular and oval cells. The epidermis presents glandular and non-glandular trichomes (Figure 5). There is also cuticle, sclerenchyma tissue, parenchyma tissue with cells about 16-45 mm and a vascular bundle. The non-glandular trichomes are multicellular and long, or unicellular. The glandular trichomes are of the capitate type. Capitate trichomes consist of 1-2 stalk cells and 2 head cells.

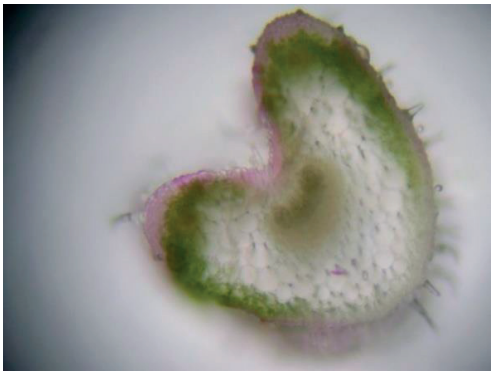

Figure 4. *Petiole* anatomy

Chlorenchyma cells are especially seen at the abaxial side, and vascular bundles are located in the parenchyma tissue. The petiole features 3 vascular bundles, with a large arc-shaped bundle in the middle and a single small vascular bundle in each corner. The median vascular bundle is surrounded by parenchyma tissue.

Figure 5. Petiole epidermis - glandular and non-glandular trichomes

Leaf anatomy (Figures 6 and 7)
The lower epidermis of the leaf is made up of one layer of cells, ranging from 12.38 to14.42 µm, on top of which there is a 3.26-5.08 µm thick cuticle.
The lower epidermis has many glandular and non-glandular trichomes and stomata. Trichomes are considered relevant in comparative systematic investigations and morpho-diagnosis (Metcalfe Chalk, 1988).

Figure 6. Leaf anatomy

The upper epidermis presents one layer of 9.86-13.78 µm thick cells, on top of which there is a 3.08-3.70 µm thick cuticle as well as glandular and non-glandular trichomes (Figures 7, 8).

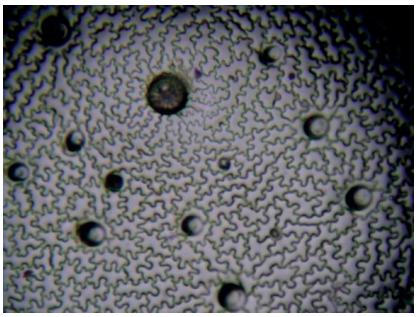

Figure 7. Upper epidermis with glandular trichomes

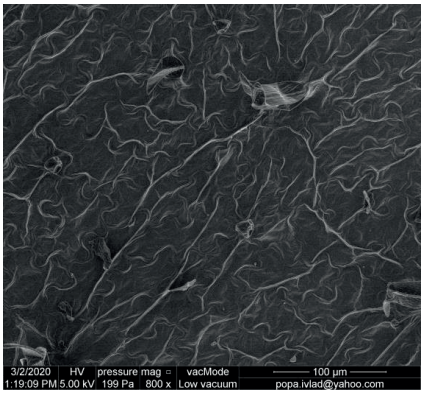

Figure 8. Upper epidermis (SEM)

The glandular (353.2-352.6 µm) and non-glandular trichomes (530-247.5 µm) are present on both the adaxial and the abaxial surfaces.
The glandular trichomes (peltate and capitate) are formed of one basal cell one or two stalk cells, and one head.
The head of mature capitate glandular hairs has two cells (Figure 9).

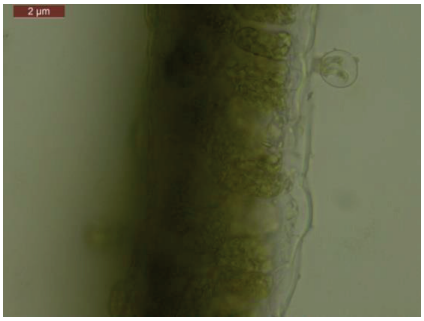

Figure 9. Upper epidermis with glandular trichomes

The leaf width is 657-688 µm. The dorsoventral mesophyll comprises one layer of 350.3 µm long palisade cells and spongy tissue. The cells of the spongy tissue are roundish, with intercellular spaces. The midrib is prominent on the lower side of the leaf and its epidermis has glandular and non-glandular trichomes; the midrib has also parenchyma tissue and a vascular bundle (Figure 10).

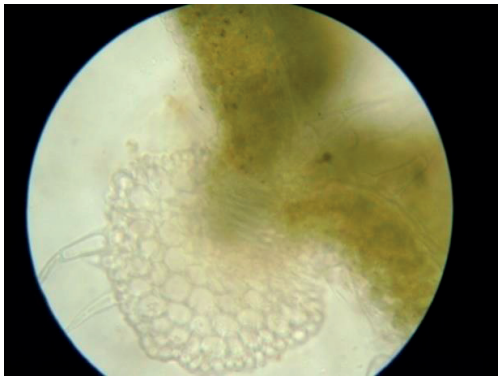

Figure 10. Midrib

On both epidermal surfaces there are glandular and non-glandular trichomes similar to those existing on the stem. Stomata are present in the lower epidermis (Figures 11, 12). The blade, in surface view, has epidermal cells with sinuous contours, with glandular and non-glandular hairs.
(Figures 13, 14, 15).

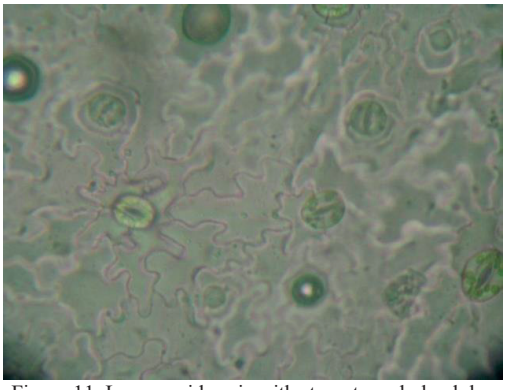

Figure 11. Lower epidermis with stomata and glandular trichomes

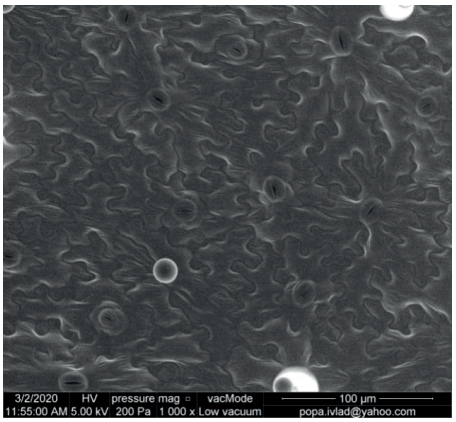

Figure 12. Lower epidermis with stomata and glandular trichomes (SEM)

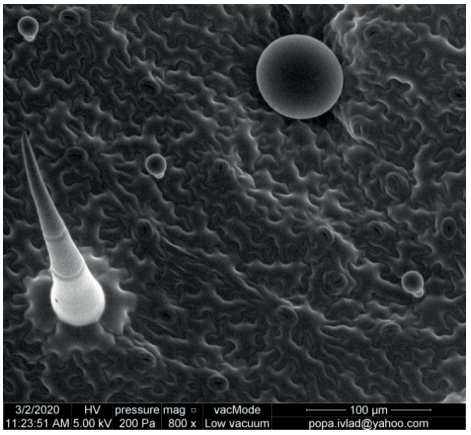

Figure 13. Lower epidermis with trichomes (SEM)

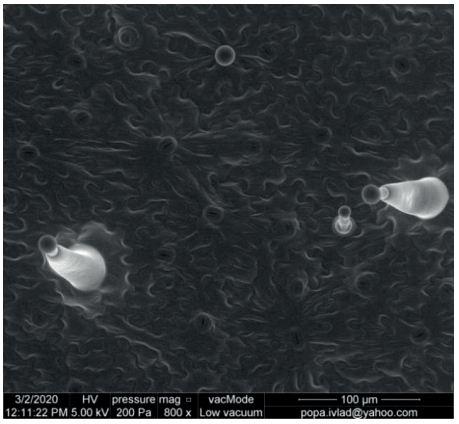

Figure 14. Lower epidermis with stomata and glandular trichomes (SEM)

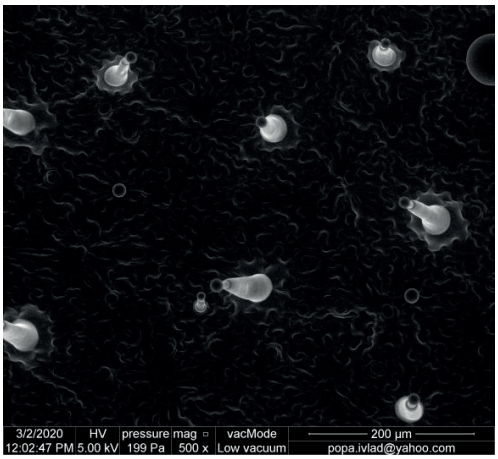

Figure 15. Lower epidermis with and glandular trichomes (SEM)

CONCLUSIONS

This investigation shows that the assembled anatomical characters of *Agastache foeniculum* helps in the identification of this medicinal species.

The micromorphological and anatomical characteristics are of great interest and significance to the discussion of the taxonomy of the species.

The glandular trichomes are important taxonomic features for the *Lamiaceae* family.

The anatomical studies we performed on stem and leaf cross-sections as well as on leaf surface sections demonstrate for the first time the anatomy of the vegetative organs of specimens growing in Romania.

The microscopic observations made on leaves, petioles and stems of *A. foeniculum* show the presence of many types of non- glandular and glandular trichomes.

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SOIL ENZYMES - BIOINDICATORS OF SOIL HEALTH

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Abstract

Nowadays, soil health is a key element in achieving agriculture sustainability. Worldwide organic farming is being increasingly promoted as a sustainable alternative to conventional farming because it can solve the problems associated with the usage of agrochemicals by long-term use of soil resources. Soil enzymes have been suggested as bioindicators of soil health because they are a measure of soil microbial activity, strictly related to organic matter decomposition and nutrient cycles and easy to measure. The purpose of this study was to measure the enzymatic activity (alkaline and acid phosphatase, amylase, cellulase and catalase) from the rhizosphere of organic and conventional soils. These biological indicators were correlated with some physio-chemical parameters such as humidity, pH, total nitrogen and organic carbon. The results showed a significant difference between the two soils with different management systems regarding the enzymatic activity, long-termed pesticide application having a negative effect on the soil enzymes. The results were strongly correlated with pH, total nitrogen and organic carbon.

Key words*: amylase, cellulase, organic soil, phosphatase.*

INTRODUCTION

Nowadays, organic farming has an important role in producing healthy food through exclusion of applications of synthetic chemicals. It is more suited to human metabolism, in full correlation with environment preservation. One of the main goals of organic farming is the production of fresh and authentic agri-food products, respecting the natural and environmental factors (Srinivasa Rao et al., 2017). One important aspect in obtaining healthy products consists in monitoring and measuring the ecosystem status of soils in order to maintain and increase the soil quality. Studies have shown that a long-term soil organic management practices with wastes application led to an increase in the urease, dehydrogenase, polyphenol oxidase and peroxidase activities in soil (Senicovscaia, 2014).

On the other hand, continuous application of pesticides can lead to soil pollution threatening, influencing the soil microorganisms and enzymes and thereby, affecting soil fertility (Lopez et al., 2002; Cycon et al., 2006).

Therefore, soil management has an important impact on the chemical, physical and biological properties of the soil and on the subsequent growth of plants. In addition, soil management affects microorganisms and microbiological processes by changing the quantity and quality of plant remains, which are the primary source of soil organic matter (Blońska et al., 2017).

A better understanding of the dynamics of microbial activity and mineralization of soil nutrients from plant residues is essential to quantify the potential benefits for soil and crop quality due to the changes introduced in the soil-plant system by using organic fertilizers (Mihalache et al., 2015).

Compost application is important in establishing and maintaining soil organic matter to a certain level in organic farming. Chang et al. (2007) found that soil enzymes activities, as well as other microbial properties increased significantly in compost-treated soil compared with chemical fertilized soils.

Soil fertility and productivity depend on the content of organic matter, which is an important reservoir of nutrients in the nutrient cycle (Steiner et al., 2007) and can improve the physical, chemical and biological properties of soils (Bhattacharya et al., 2010). The process of decomposition of organic matter in the soil takes place with the participation of soil

microorganisms and enzymes (Schimel and Bennett, 2004).
The suggested bio-indicators for monitoring soil quality were: soil microbial biomass, nutrient cycling, community structure and biodiversity, soil animals, plants and soil enzymes (Killham, 2002).
Soil enzymes are necessary catalysts for decomposition of soil organic matter and nutrient cycling, influencing the energy transformation, environmental quality and agronomic productivity. Further, soil enzymes can provide early detection of changes in soil health because they respond to soil management changes and environmental factors faster than other soil quality parameters (Srinivasa Rao et al., 2017).
A better understanding of the role of this soil enzymes activity in the ecosystem can provide a unique opportunity for an integrated biological assessment of soils due to their crucial role in several soil biological activities, their ease of measurement, and their rapid response to changes in soil management practices (Sherene, 2017).
Phosphatases are a group of enzymes that are capable to catalyze the hydrolysis of esters and anhydrides of phosphoric acid. In soil, these enzymes are believed to play an important role in phosphorus cycles because they can be correlated to phosphorus stress and plant growth. Besides being good indicators of soil fertility, phosphatases play key roles in the soil system (Dick et al., 2000).
Amylase is a starch hydrolyzing enzyme, consisting of α-amylase and β-amylase. The activity of amylase can be influenced by some factors such as cultural practices, type of vegetation, environment, soil types (Rose and Roberts, 1970) and plants by directly supplying enzymes from their residues or indirectly providing substrates for the synthetic activities of microorganisms (Sherene, 2017).
Cellulases are the group of enzymes that catalyze the degradation of cellulose, the most abundant organic compound on the Earth. The activities of cellulases in agricultural soils are affected by some factors such as temperature, soil pH, humidity, the chemical structure and the location of organic matter in the soil profile horizon, the quality of organic matter/plant debris and soil mineral elements and the trace elements from fungicides (Srinivasulu and Rangaswamy, 2006).
Catalase decomposes peroxide and its activity depends on organic oxygen concentration, microbe biomass, changes in carbon dioxide, and dehydrogenase, amidase, glucosidase and esterase activities in soils. Thus, they are an important indicator of soil fertility and aerobic microorganisms (Burns, 1982). Catalase stability is very high in soil and the enzymatic activity is significantly correlated with the organic carbon content (Alef and Nannipieri, 1995).
The aim of this study was to examine the influence of different management systems (organic and conventional) on the enzymatic activities registered in soil. The enzymes analysed were acid and alkaline phosphatase, catalase, amylase and cellulase.
These enzymatic parameters were correlated with some physio-chemical parameters such as humidity, pH, total nitrogen and total carbon.

MATERIALS AND METHODS

Soil

For this study, three types of soil with different management systems were collected from the same area from a depth of 0-20 cm: conventional soil, organic soil and soil under conversion management. On the soil during the conversion period (year I) were cultivated: autumn cabbage and pumpkin. The ecological soil is certified, on its surface being cultivated basil, beans and okra. These cultures were organically treated with milk and garlic. Conventional soil was used for growing beans (current year), celery and flowers.

Enzyme assays

The soil samples were analyzed fresh and were kept in plastic bags in the refrigerator at 4°C.
Acid and alkaline phosphatase method was based on the spectrophotometric determination of p-nitrophenol released after incubation of the soil with an artificial substrate, p-nitrophenyl phosphate (p-NPP) and Modified universal buffer (MUB) (pH = 11 for alkaline phosphatase and pH = 6.5 for acid phosphatase), for 1 h at 37°C (Tabatabai and Bremner, 1969). $CaCl_2$ 0.5 M and NaOH 0.5 M were added for colour development, after

incubation. The samples were homogenized, filtered and the yellow colour was measured spectrophotometrically at the wavelength of 400 nm.
The method used to evaluate cellulase activity was based on the determination of reducing sugars by the method of Deng and Tabatabai (1994) using 3,5-dinitrosalicylic acid (DNS) as a reagent at the wavelength of 640 nm. The samples were incubated 24 h at 50°C in the presence of 0.05 M acetic acid - acetate buffer, pH 5.5 and 1% carboxymethylcellulose solution (CMC).
The assay used for soil amylase measurement was developed by Cole in 1977. The soil samples were incubated with acetic acid acetate - buffer 2 M (pH = 5.5) and 2% starch at 37°C for 24 h. After incubation the samples were centrifuged, and the reducing sugars were determined using 3,5-dinitrosalicylic acid (DNS). The absorbance was read at the wavelength of 546 nm.
Catalase activity was measured by back-titrating residual H_2O_2 with $KMnO_4$ (Johnson and Temple, 1964; Roberge, 1978). The soil samples were homogenized with distilled water and 0.3% hydrogen peroxide solution. The mixture was shaken for 20 min and then 1.5 mol/L H_2SO_4 were added. Afterwards the solution was filtered and titrated using 0.02 mol/L $KMnO_4$.

Physio-chemical methods

The soil samples were dried in the atmosphere and sieved through a 1 mm sieve. The pH assay was performed using 10 g soil and 25 ml of distilled water, to obtain a 1:2.5 (w/v) soil-water extract. The conductivity was measured using a 1:5 (w/v) soil-water. The soil moisture was determined in the oven at 105°C for 24 h.
Total nitrogen from soil was measured using the Kjeldahl method (Saez-Plaza et al., 2013). The Kjeldahl method consisted of three steps: digestion (the decomposition of nitrogen in organic samples utilizing 98% H_2SO_4 and catalysts), distillation (the ammonia content of the digest was determined by distillation with excess 35% NaOH in 25 ml of 4% solution of H_3BO_3) and titration (with 0.1 M HCl in presence of 2-3 drops of indicator).
The determination of organic carbon in the soil was measured based on the Walkley-Black method of humid oxidation of chromic acid. The oxidizable material from the soil was oxidized by the solution of $K_2Cr_2O_7$ 1 N. The reaction was favored by the heat generated by the addition of two volumes of H_2SO_4 over the volume of bichromate. The rest of the bichromate was titrated with iron sulphate solution in the presence of indicator Ferroin, the titrated volume being inversely proportional to the amount of C present in the soil sample (Barlett et al., 1994).
Enzymatic and chemical parameters were statistically evaluated by the determination of variance and correlation analysis, using Microsoft Excel 2016 tools. The linear correlation was considered significant for a significance level p = 0.05.

RESULTS AND DISCUSSIONS

The chemical and biochemical parameters considered in this study provided information on differences in soil quality and fertility between organically and conventionally managed systems. In fact, organic soils have been characterized by higher carbon mineralization, higher enzymatic activity, increased soil nutrients (N) and energy (higher total carbon content). These findings suggested that environmentally managed soils could be considered more conservative systems.
The highest activities of acid and alkaline phosphatase, cellulase, amylase and catalase were recorded in organic soil, which were characterized by the highest accumulation of soil organic matter (Table 1).

Table 1. Physio-chemical parameters of soils

Soil Type	Moisture (%)	Conductivity (µS/cm)	pH	C (%)	N (%)
Conventional soil	15.9	195.6	8.8	1.63	0.519
Soil in conversion	11.5	144.8	8.2	2.00	0.801
Organic soil	13.9	159.9	7.4	2.86	0.943

The content of organic carbon and total nitrogen were higher in the soil with organic management, compared to those in the soil under conventional agriculture.
The obtained results for total organic carbon were similar with the results obtained by Hábová et al. (2019), which comparing soils under organic and conventional farming

systems pointed out that total organic carbon values were higher in organic farming (2%). These findings were in concordance with Marriott and Wander (2006) study, where total organic carbon was higher in organic soil and this increase was strongly correlated with the values obtained for total nitrogen, which were as well higher compared with conventional farming.

Conventional soil was characterized by a moderately alkaline pH compared with the organic soil were the pH was weakly alkaline.

From the point of view of the enzymatic activity, significantly higher values were observed in the organic soils compared to the conventional one for all the enzymes analyzed (Table 2).

Cellulase activity in soil was strong correlated with total carbon ($r = 0.99$, $p<0.05$) and total nitrogen ($r = 0.85$, $p<0.05$). The same observation were noticed regarding the amylase activity, having a strong correlation with the two chemical parameters ($r = 0.96$, respectively $r = 0.98$; $p<0.05$).

Table 2. Enzymatic activity for the three types of soil with different management systems

Enzymes analysed	Soil type	Enzymatic activity
Cellulase (µg glucose/ g soil/24h)	Conventional soil	0.184 ± 0.013
	Soil in conversion	0.281± 0.002
	Organic soil	0.750 ± 0.146
Amylase (µg maltose/ g soil/24h)	Conventional soil	0.796 ± 0.148
	Soil in conversion	1.372 ± 0.054
	Organic soil	1.892 ± 0.158
Acid phosphatase (µg p-nitrophenol/g soil/h)	Conventional soil	124.46 ± 7.54
	Soil in conversion	177.63 ± 9.86
	Organic soil	287.99 ± 10.92
Alkaline phosphatase (µg p-nitrophenol/g soil/ h)	Conventional soil	331.61 ± 16.06
	Soil in conversion	376.41 ± 14.38
	Organic soil	499.39 ± 22.72
Catalase (mmol H_2O_2/ g soil/h)	Conventional soil	0.105 ± 0.010
	Soil in conversion	0.156 ± 0.010
	Organic soil	0.262 ± 0.015

Cellulase and amylase activities were significantly higher ($p<0.05$) in the soil with organic management compared with conventional soil (Figure 1).

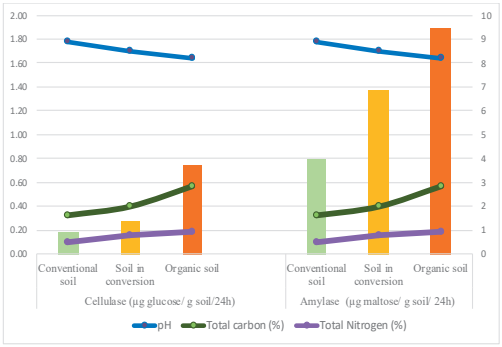

Figure 1. Activity of cellulase and amylase in soil

Similar results were obtained by Balota et al (2004), where was highlighted that amylase and cellulase activity in soil were lower in conventional farming system compared with organic management.

Regarding acid and alkaline phosphatase, the results were higher in organic management and were strongly correlated with total carbon ($r = 0.99$; $r = 0.99$, $p<0.05$) and total nitrogen ($r = 0.92$; $r = 0.89$, $p<0.05$) (Figure 2).

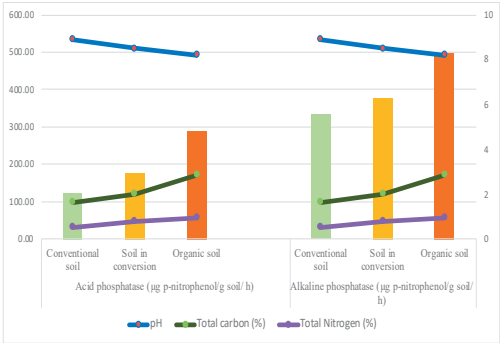

Figure 2. Activity of acid and alkaline phosphatase in soil

Several studies have compared soil enzymatic activities responsible with nutrient cycle under organic and conventional system. They suggested that organically managed fields had a greater enzymatic activity that conventionally managed fields (Garcia-Ruiz et al., 2008; Fliessbach et al., 2007; Melero et al., 2008). These results were in concordance with the results obtained in this study, demonstrating that long-term application of agrochemicals on soil can lead to a strong decrease of enzymatic activity. They have shown that organic soils were characterized by a greater acid and

alkaline phosphatase activity compared with the conventional management where the phosphatase activity was lower.

Catalase activity (Figure 3) from conventional soil was significantly lower ($p<0.05$) than the activity registered in organic management, being positive correlated with the other parameters (total C with $r = 0.92$ and total N with $r = 0.99$; $p<0.05$).

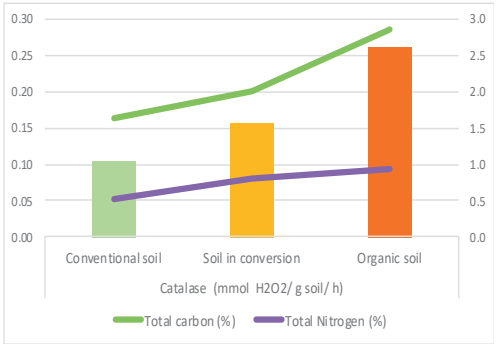

Figure 3. Activity of catalase in soil

Alef and Nannipieri (1995) reported as well in their study that catalase activity was very stable in organic soil and showed a significant correlation with the content of total organic carbon, resulting a higher activity in organic soil.

CONCLUSIONS

The results of this study confirmed that activities of cellulases, amylase, acid and alkaline phosphatase and catalase can be used to obrain a preliminary indication of some of the physical and chemical properties of soil, thus, improving agricultural soil management strategies. Significant differences between organic and conventional soils enzymatic activity were noticed.

Conventional soils contained significantly less organic matter, which could lead in weaker soil structure and lower enzymatic activity. Low activities of the cellulases, amylase, acid and alkaline phosphatase and catalase were observed in the conventional cultivated soils, whereas higher activities of these enzymes were obtained in organic soils.

Due to their importance in soil organic matter degradation, nutrients cycle, environmental quality and agronomic productivity, enzymes can be suggested as bio-indicators of soil health. On the other hand, the results from this study confirmed the usefulness of assessing enzymes activities in order to evaluate the differently managed soils.

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THE EFFECT OF DIFFERENT FERTILIZATION UPON THE GROWTH AND YIELD OF SOME *LAVANDULA ANGUSTIFOLIA* (MILL.) VARIETIES GROWN IN SOUTH EAST ROMANIA

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Abstract

Lavandula angustifolia (Mill.) is a Mediterranean aromatic shrub known since ancient times for its multiple uses. Over the past decade, this species' large-scale cultivation attracted the attention of local farmers, highlighting the necessity of analyzing and improving those crop management practices that can ensure high lavender yields in different growing conditions. Consequently, this paper aimed to analyze the behavior and yield recorded, under the influence of different organo-mineral fertilization levels, by four varieties of Lavandula angustifolia (Mill.), grown during 2017-2019, in the climatic and soil conditions of southeast Romania (Moara Domnească, Ilfov). In the first year of the crop (I), when mineral fertilization $N_{60}P_{60}K_{60}$ was applied, all researched lavender varieties recorded superior values in terms of flower yield and vegetative development compared to other fertilizations. Vera variety obtained the highest yield for this type of fertilization with a value of 798.3 kg/ha. For the second (II) and third-year (III) of the crop, both the perennial character of the species and a longer availability of soil nutrients ensured by organic fertilization determined higher values of the biometric parameters and superior flower yields. Under organic fertilization (manure 30 t/ha), the highest yields were recorded by Vera, with values of 4,877.3 kg/ha in 2018 (II) and 6,411.2 kg/ha in 2019 (III).

Key words*: lavender, flower yield, biometric parameters, mineral fertilization, organic fertilization.*

INTRODUCTION

Lavender (*Lavandula angustifolia* Mill.) is an aromatic shrub with Mediterranean origins, grown for therapeutic, ornamental, or nutritional purposes. Due to its essential oil content and high-quality (Mac and Harris, 2002; Ion et al., 2008; Sönmez et al., 2018; Kara and Baydar, 2018; Giray, 2018), this shrub is mostly used in the cosmetics and perfumes industries.

In the food industry, lavender flowers are used for tea (Yukes and Balick, 2010), as well as for tinctures and infusions (Smetan, 2018). Various extracts from lavender also help preserve the food or give flavor to bakery products (Jianu et al., 2013; Śmigielski et al., 2013).

The literature identifies 48 species of lavender. For commercial purposes, several species, part of the subgenus *Spica*, are used and recognized for their oil production: *L. angustifolia* (*L. angustifolia* Mill., also known as *L. officinalis*), *Lavandula intermedia* (lavandin or *L. hybrida* L.) and *Lavandula latifolia* (*L. spica*) (Yukes and Balick, 2010; Duskova et al., 2016; Sönmez et al., 2018).

The quantity and quality of lavender oil depend mainly on the species and variety but are influenced by both environmental conditions and crop technology applied upon cultivation and by distillation methods (Parkash and Singh, 2013).

Mineral and organic fertilization are the most important factors of lavender's crop technology as it ensures stable and high yields (Maganga, 2004; Silva et al., 2017; Skoufogianni et al., 2017). Fertilization can be applied in both, autumn and spring (Racz et al., 1970).

Macronutrients (N, P, K) are recommended for lavender crops due to their positive influence on plants' development, inflorescence yield and oil quality (Şekeroğlu and Özgüven, 2008; Yukes and Balik, 2010; Chrysagyris et al., 2017). Also, macronutrients can be used in processes such as photosynthesis and transpiration (Camen et al., 2016). Matysiak and Nogowska (2016) recommend crop fertilization management to be established to protect the environment, due to the increased risk of nitrate pollution.

This research aims to enrich scientific data on lavender cultivation in Romania, where research on *L. angustifolia* (Mill.) grown in field conditions is relatively scarce.

MATERIALS AND METHODS

Field experimental design

Lots of field research was carried out in the experimental farm Moara Domnească (Ilfov county), a subunit of UASVM Bucharest. The soil type was a chromic luvisol, with a clay loam texture (TT), a pH of 7.68 (slightly alkaline) and a humus content of 2.49% (0-20 cm depth).

The design of the field experiment was a Latin Rectangle, with four repetitions and the factors displayed as randomized blocks. Lavender (*L. angustifolia* Mill.) plants were provided by local producers in Onești, Bacău county and Bonțida, Cluj-Napoca county.

Lavender varieties: *Sevstopolis* (a_1), *Vera* (a_2), *Hidcote* (a_3), *Buena Vista* (a_4) were treated with the following fertilization types: $N_0P_0K_0$ (**Ct**; b_1), $N_{60}P_{60}K_{60}$ (b_2), CAN: calcium nitrate N_{60}+$CaO_{15,5}$+$Mg_{11,1}$ (b_3), $N_{30}P_{30}K_{30}$+15 t/ha manure (b_4) and 30 t/ha manure (b_5).

To determine plants behavior in terms of vegetative development, biometric measurement as plants' height and vigor were conducted. In terms of yield, data regarding the number of floral stems and the yield of fresh flowers per plant and per hectare.

Crop establishment

Main soil tillage was carried out in autumn by ploughing the soil 30 cm deep. Mineral and organic fertilizers were applied before planting, in the spring of 2017. Fertilizers were incorporated into the soil at seedbed preparation.

Lavender varieties were planted manually on March 29, 2017, at a density of 16,667 plants/ha, using healthy, rooted seedlings, that were trimmed before planting.

A drip irrigation system was installed after planting to complete in the first two years of the crop (2017 and 2018) the amount of water provided by rainfall, with an irrigation volume of 200 m^3/year (average irrigation).

Mechanical weed harrowing (between rows) and manual weed harrowing (along the rows) were carried out each year, with a higher persistence upon crop establishment (2017) on perennial weed species as: *Sorghum halepense*, *Agropyron repens* and *Convolvulus arvensis*, to efficiently control them in the following years.

Climatic conditions

During the growing season of lavender varieties, temperature (Table 1) in each of the three years of research, had higher values than the specific multiannual average

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Table 1. Climatic conditions in research period of the research period (Moara Domnească, 2017-2019)

Cliamatic factor	Year	Months								
		March	April	May	June	July	August	September	October	Average/ Sum
Average monthly temperature (˚C)	2017	15	16.5	18.6	22.4	26.4	24.2	19.1	11.5	19.2
	2018	3.7	16.4	19.7	22.5	23	24.1	19.2	13.8	17.8
	2019	9.5	11.3	17	23.1	22.6	24.3	19.1	12.6	17.4
	1961-2007	4.8	11.1	16.7	20.4	22.3	21.4	16.6	10.7	15.5
Rainfall (mm)	2017	44.5	90	47.3	46.8	105.2	37.1	37	70.9	478.8
	2018	0.2	0	0	53.2	107.6	2	28.9	10.4	202.3
	2019	31.2	78.4	148.2	109.4	76	2.4	4.8	41.1	491.5
	1961-2007	40	46.9	66	77	67.7	57.4	52.9	41.6	449.5

Compared to the multiannual value, average temperature from March to October had an increase between 1.9ºC (2019) and 3.7ºC (2017).

For 2017 and 2019 the amount of rainfalls was similar to the multiannual average of March-October (449.5 mm). In 2018, precipitation amount during the growing season (202.3 mm)

was -247.2 mm lower than the multiannual average. In April and May, there was no rainfall (Table 1).

Statistical analysis

ANOVA (analysis of variance) was carried out for the statistical analysis of data.

RESULTS AND DISCUSSIONS

Lavender varieties development

Of the four lavender varieties grown in South East Romania, Vera had, in each of the three years of crop development, the highest height and number of floral stems per plant (Table 4).

Plants heights (Table 2) from this variety ranged from 41.3 cm (Ct) to 49.1 cm ($N_{60}P_{60}K_{60}$), in the first year of the crop (I), and reached values between 69.2 cm (Ct) and 82.4 cm (Manure30) by the third year (III) of the crop. At the opposite end, Buena Vista variety had the smallest values in terms of plants height with values ranging from 33.6 cm (Ct, Ist year of the crop) to 62.8 cm (Manure30, IIIrd year of the crop). Sevstopolis variety obtained, for the three years of the crop, average heights between 52.7 cm (Ct) and 62.1 cm (Manure30), while for Hidcote the average height of plants varied from 45.5 cm (Ct) to 55.0 cm (Manure30) (Table 2).

The number of floral stems (Table 3), of these two varieties, was between 55.7 stems/plant (Hidcote, Ist year of the crop) and 729.0 stems/ plant (Sevstopolis, IIIrd year of the crop). The highest number of floral stems was recorded by Vera in the IIIrd year of the crop. The lowest number of floral stems was obtained by Buena Vista in each of the three years of the crop.

Upon crop establishment (Ist year), the highest values of the morphological characters (number of floral stems, height) were obtained for the mineral fertilization $N_{60}P_{60}K_{60}$. Thus, for this treatment, plants height was between 39.8 cm (Buena Vista) and 49.1 cm (Vera), and the number of floral stems was between 53.1 stems/plant (Buena Vista) and 122.0 stems/plant (Vera). In the following years of the crop (IInd and IIIrd) a positive influence was observed when organic fertilizers were used, allowing, through slow release of macronutrients, a longer availability of nutrients for plants.

Table 2. Height (cm) of *Lavandula angustifolia* (Mill.) varieties (Moara Domnească, 2017-2019)

Fertilization	Sevstopolis				Vera				Hidcote				Buena Vista			
	2017	2018	2019	**Avg***	2017	2018	2019	**Avg***	2017	2018	2019	**Avg***	2017	2018	2019	**Avg***
$N_0P_0K_0$ (Ct))	38.4	56.3	63.4	**52.7**	41.3	59.1	69.2	**56.5**	34.6	49.3	52.7	**45.5**	33.6	44.6	49.1	**42.4**
$N_{60}P_{60}K_{60}$	45.1	63.2	70.1	**59.5**	49.1	65.4	75.1	**63.2**	42.3	57.7	58.4	**52.8**	39.8	50.3	55.7	**48.6**
CAN	40.6	61.5	68.5	**56.9**	42.7	63.6	72.5	**59.6**	36.5	54.2	56.1	**48.9**	35.7	48.6	52.6	**45.6**
$N_{30}P_{30}K_{30+}$M15**	42.7	64.8	76.3	**61.3**	45.5	67.3	77.2	**63.3**	39.4	58.6	62.6	**53.5**	37.2	52.8	59.3	**49.8**
Manure30***	39.8	66.7	79.8	**62.1**	43.2	71.0	82.4	**65.5**	35.8	61.4	67.8	**55.0**	34.6	57.2	62.8	**51.5**

Avg. - average of the period 2017-2019;15 t/ha bovine manure;***30 t/ha bovine manure*

Table 3. Number of floral stems per plant of *Lavandula angustifolia* (Mill.) varieties (Moara Domnească, 2017-2019)

Fertilization	Sevstopolis				Vera				Hidcote				Buena Vista			
	2017	2018	2019	**Avg***	2017	2018	2019	**Avg***	2017	2018	2019	**Avg***	2017	2018	2019	**Avg***
$N_0P_0K_0$ (Ct))	65.6	323.0	452.0	**280.2**	72.4	484.0	560.0	**372.1**	55.7	303.9	423.5	**261.0**	53.1	280.8	362.6	**232.2**
$N_{60}P_{60}K_{60}$	106.5	390.4	530.8	**342.6**	122.0	538.8	637.7	**432.8**	102.8	364.7	501.7	**323.1**	98.1	333.4	446.5	**292.7**
CAN	76.9	348.0	488.6	**304.5**	86.1	511.6	604.0	**400.5**	70.3	332.9	479.8	**294.3**	66.7	305.3	431.9	**267.9**
$N_{30}P_{30}K_{30+}$M15**	84.8	454.9	599.1	**379.6**	93.0	631.7	702.1	**475.6**	79.5	425.4	560.2	**355.0**	72.4	408.8	510.8	**330.6**
Manure30***	74.6	588.7	729.0	**464.1**	80.1	769.9	813.2	**554.4**	66.6	487.3	618.9	**390.9**	61.8	477.4	541.1	**360.1**

Avg. - average of the period 2017-2019;15 t/ha bovine manure;***30 t/ha bovine manure*

Thus, for organic fertilization with bovine manure (Manure30) plants height ranged between 57.2 cm (Buena Vista, IInd year of the crop) and 82.4 cm (Vera, IIIrd year of the crop).

The number of floral stems varied from 477.4 stems/plant (Buena Vista, IInd year of the crop) to 813.2 stems/plant (Vera, IIIrd year of the crop).

Yield analysis as influenced by fertilization

Along with its contribution to plants vegetative development in the first year of the crop (I), application of different doses and types of fertilizers (mineral and organic) ensured an increase of fresh flowers yields, both upon the establishment of the crop (I) and in the following two years (II, III). Analyzing the average yield obtained during 2017-2019, by the four lavender varieties (Table 4) it was observed that compared to the unfertilized variant (Ct), where fresh flowers yield was 1,933.9 kg/ha, the complex mineral fertilization ($N_{60}P_{60}K_{60}$) determined a significant yield increase ($p < 0.05$) of 392.4 kg/ha, while the use calcium nitrate (CAN) generated a distinctly significant increase ($p < 0.01$) of 675.6 kg/ha. The highest increase of the average yield, very significant in statistical terms ($p < 0.001$), was obtained for the organic fertilization Manure30 (1,590.6 kg/ha). Compared to control, for the organo-mineral treatment ($N_{30}P_{30}K_{30}$ + M15), the average yield also had a very significant increase, by 943.7 kg/ha. In the first year of the crop (I), the four varieties of lavender obtained an average yield of fresh flowers of 563.3 kg/ha (Table 4) for the control treatment ($N_0P_0K_0$). The use of the mineral fertilizer $N_{60}P_{60}K_{60}$ upon crop establishment determined a very significant yield increase of 141.1 kg/ha.

Table 4. Fertilization influence on flower yield (kg/ha) of *Lavandula angustifolia* (Mill.) varieties (Moara Domnească, 2017-2019)

Lavender variety	Fertilization	2017 (I)		2018 (II)		2019 (III)		Avg*	
		Yield (kg/ha)	Diff. (kg/ha)	Yield (kg/ha)	Diff. (kg/ha)	Yield (kg/ha)	Diff. (kg/ha)	Yield (kg/ha)	Diff. (kg/ha)
Sevstopolis	**$N_0P_0K_0$ (Ct)**	**602.4**	**Ct**	**2,316.2**	**Ct**	**3,239.0**	**Ct**	**2,052.5**	**Ct**
	$N_{60}P_{60}K_{60}$	751.1	148.7***	2,727.3	411.1ns	3,827.9	588.9 ns	2,435.4	382.9 ns
	CAN	683.8	81.4**	3,261.7	945.5***	4,277.9	1,038.9**	2,741.1	688.6***
	$N_{30}P_{30}K_{30+}$M15	708.9	106.5***	3,513.4	1,197.2***	4,750.1	1,511.1	2,990.8	938.3***
	Manure30	667.7	65.3*	4,157.3	1,841.1***	6,194.6	2,955.6	3,673.2	1,620.7***
Vera	**$N_0P_0K_0$ (Ct)**	**638.6**	**Ct**	**2,808.4**	**Ct**	**3,339.0**	**Ct**	**2,262.0**	**Ct**
	$N_{60}P_{60}K_{60}$	798.3	159.7***	3,240.1	431.7 ns	3,950.1	611.1 ns	2,662.8	400.8*
	CAN	715.2	76.6**	3,790.1	981.7***	4,575.1	1,236.1***	3,026.8	764.8***
	$N_{30}P_{30}K_{30+}$M15	740.0	101.4***	4,043.4	1,235.0***	5,027.9	1,688.9***	3,270.4	1,008.5***
	Manure30	702.7	64.1*	4,877.3	2,068.9***	6,411.2	3,072.3***	3,997.1	1,735.1***
Hidcote	**$N_0P_0K_0$ (Ct)**	**551.4**	**Ct**	**2,065.7**	**Ct**	**2,838.9**	**Ct**	**1,818.7**	**Ct**
	$N_{60}P_{60}K_{60}$	673.9	122.5***	2,477.8	412.1 ns	3,455.6	616.7 ns	2,202.4	383.8 ns
	CAN	623.9	72.5**	2,778.9	713.2**	3,922.3	1,083.4**	2,441.7	623.0**
	$N_{30}P_{30}K_{30+}$M15	651.7	100.3***	3,440.1	1,374.4***	4,233.4	1,394.5***	2,775.1	956.4***
	Manure30	615.4	64.0*	3,725.6	1,659.9***	5,794.6	2,955.6***	3,378.5	1,559.9***
Buena Vista	**$N_0P_0K_0$ (Ct)**	**460.6**	**Ct**	**1,916.7**	**Ct**	**2,430.5**	**Ct**	**1,602.6**	**Ct**
	$N_{60}P_{60}K_{60}$	593.9	133.3***	2,344.5	427.8 ns	3,075.4	644.9 ns	2,004.6	402.0*
	CAN	527.8	67.2*	2,627.8	711.1**	3,530.3	1,099.8**	2,228.6	626.0**
	$N_{30}P_{30}K_{30+}$M15	546.6	86.0**	3,016.7	1,100.0***	3,859.9	1,429.4***	2,474.4	871.8***
	Manure30	531.3	70.7**	3,550.1	1,633.4***	5,066.9	2,636.4***	3,049.4	1,446.8***
Avg. varieties	**$N_0P_0K_0$ (Ct)**	**563.3**	**Ct**	**2,276.7**	**Ct**	**2,961.8**	**Ct**	**1,933.9**	**Ct**
	$N_{60}P_{60}K_{60}$	704.3	141.1***	2,697.4	420.7 ns	3,577.2	615.4 ns	2,326.3	392.4*
	CAN	637.7	74.4**	3,114.6	837.9**	4,076.4	1,114.6**	2,609.6	675.6**
	$N_{30}P_{30}K_{30+}$M15	661.8	98.6***	3,503.4	1,226.7***	4,467.8	1,506.0***	2,877.7	943.7***
	Manure30	629.3	66.0*	4,077.6	1,800.8***	5,866.8	2,905.0***	3,524.6	1,590.6***
*Avg. - average of the period 2017-2019		*LSD 5%: 51.5 kg/ha; LSD 1%: 67.8 kg/ha; LSD 0.1%: 88.1 kg/ha*		*LSD 5%: 504.3 kg/ha; LSD 1%: 670.2 kg/ha; LSD 0.1%: 870.7 kg/ha*		*LSD 5%: 649.9 kg/ha; LSD 1%: 863.7 kg/ha; LSD 0.1%:1,122.1 kg/ha*		*LSD 5%: 392.4 kg/ha; LSD 1%: 521.5 kg/ha; LSD 0.1%: 677.5 kg/ha*	

For the second (II) and third (III) of the crop, this treatment generated yield growths compared to the control of 420.7 kg/ha and 615.4 kg/ha respectively, but the differences were not statistically assured.

Compared to control, a very significant yield increase, of 98.6 kg/ha ($p<0.001$), was generated in the first year of the crop (I) by the organo-mineral treatment $N_{30}P_{30}K_{30}$ + M15. For this treatment, in the following years (II,

III), yield growths were also significant, with values of 1,226.7 kg/ha for the IInd and 1,506.0 kg/ha for the IIIrd. Calcium nitrate treatment (CAN) has statistically ensured increases of the average yield obtained by the four lavender varieties, in each of the three years of the crop (Table 4).

Organic fertilization with manure 30 t/ha determined a significant increase (66.0 kg/ha) of the average yield, compared to the unfertilized variant. Longer availability of soil nutrients was provided when applying manure that has generated the growth of the average yield obtained in the IInd and IIIrd year of the crop, with very significant values, of 1,800.8 kg/ha, and 2,905.0 kg/ha, respectively.

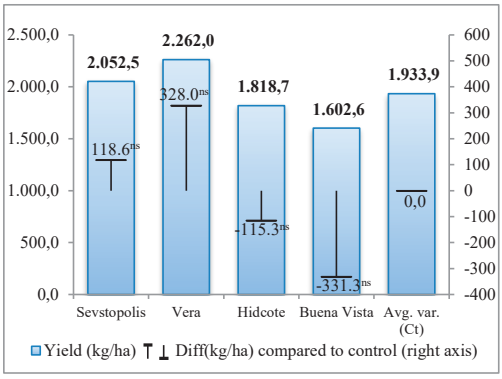
The highest fresh flowers yield growth, due to fertilization, was obtained by Vera variety for the treatment Manure30. This yield increase, of 3,072.3 kg/ha, was obtained in the IIIrd year of the crop and was very significant in statistical terms. The smallest yield increase was generated by the same treatment but in the Ist year of the crop, for the variety Hidcote, with a statistically ensured value of 64.0 kg/ha.

Yield analysis as influenced by variety

Lavender variety influenced the average flowers yield for each of the five fertilization treatments. For the control treatment (Figure 1), compared to the average yield of the four varieties, yield growths of 118.6 kg/ha and 328.0 kg/ha were obtained for the varieties Sevstopolis and Vera.

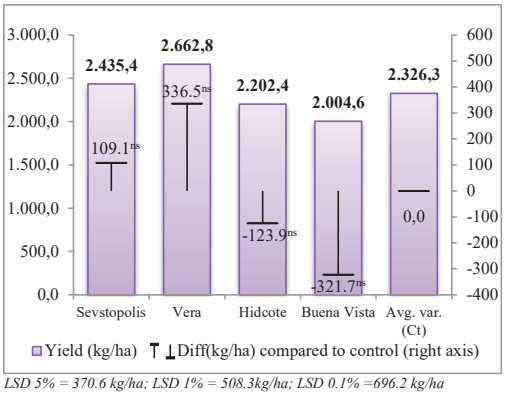
Buena Vista recorded a smaller yield (-331.3 kg/ha) compared to the average yield of the four varieties, and Hidcote also had a smaller yield (-115.3 kg/ha). Both differences were not statistically ensured (Figure 1). When mineral fertilization $N_{60}P_{60}K_{60}$ was applied, Vera obtained a significant yield increase of 417.2 kg/ha compared to the average yield of the varieties, while Buena Vista had a significant yield decrease of -380.9 kg/ha (Figure 2). For Sevstopolis and Hidcote, yield differences were not statistically ensured.

For calcium nitrate (CAN) treatment, Sevtopolis and Vera varieties obtained yield growths of 131.6 kg/ha, and 417.2 kg/ha respectively, only the latter being statistically ensured (Figure 3). Hidcote and Buena Vista varieties had smaller flower yields compared to the average of the four varieties, but only for Buena Vista the difference (-380.9 kg/ha) was significant ($p<0.05$).



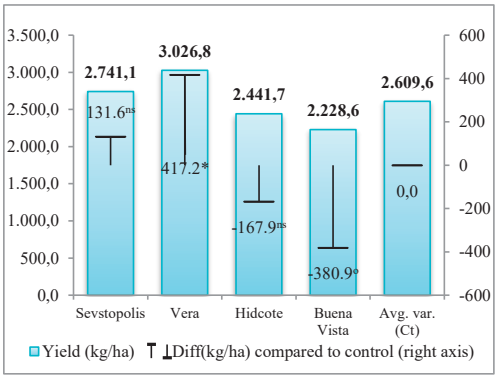
LSD 5% = 370.6 kg/ha; LSD 1% = 508.3kg/ha; LSD 0.1% =696.2 kg/ha

Figure 1. Variety influence on lavender flower yield (kg/ha) for **$N_0P_0K_0$** fertilization, average 2017-2019



LSD 5% = 370.6 kg/ha; LSD 1% = 508.3kg/ha; LSD 0.1% =696.2 kg/ha

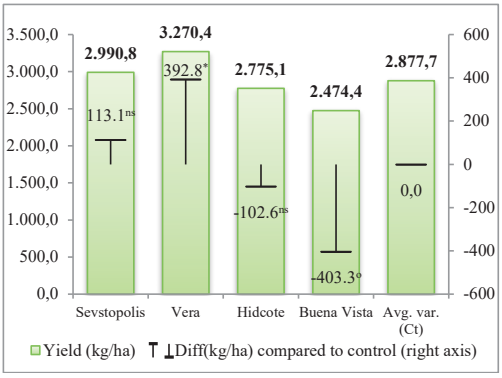
Figure 2. Variety influence on lavender flower yield (kg/ha) for **$N_{60}P_{60}K_{60}$** fertilization, average 2017-2019



LSD 5% = 370.6 kg/ha; LSD 1% = 508.3kg/ha; LSD 0.1% =696.2 kg/ha

Figure 3. Variety influence on lavender flower yield (kg/ha) for **CAN** fertilization, average 2017-2019

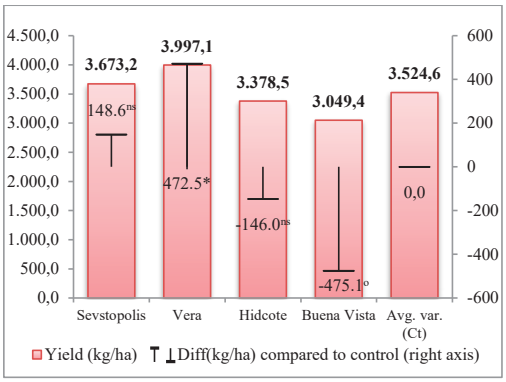
When organo-mineral fertilizer $N_{30}P_{30}K_{60+}M15$ was applied (Figure 4), yield differences were statistically visible for Vera (with a yield increase of 392.8 kg/ha) and Buena Vista (with a yield decrease of -403.3 kg/ha) varieties. For Sevtopolis and Hidcote, yield varied compared to the average of the four varieties, but differences were not significant (p>0.05).



LSD 5% = 370.6 kg/ha; LSD 1% = 508.3kg/ha; LSD 0.1% =696.2 kg/ha

Figure 4. Variety influence on lavender flower yield (kg/ha) for **$N_{30}P_{30}K_{60+}$M15** fertilization, average 2017-2019

For the organic treatment Manure30 (Figure 5), both Sevstopolis and Hidcote obtained yield differences compared to the average yield of the varieties, which were insignificant in statistical terms. On the other hand, Vera and Buena Vista have statistically ensured differences of 472.5 kg/ha and -475.0 kg/ha.



LSD 5% = 370.6 kg/ha; LSD 1% = 508.3kg/ha; LSD 0.1% =696.2 kg/ha

Figure 5. Variety influence on lavender flower yield (kg/ha) for **Manure30** fertilization, average 2017-2019

Analyzing differences among varieties (Table 5) it is observed that, for each fertilization treatment, Vera variety (a_2) had higher fresh flower yields compared to Sevstopolis (a_2), but the differences were not significant (p>0.05). Compared to Hidcote (a_3) and Buena Vista (a_4), Vera also obtained higher yields, and the differences were distinctly and significant.

The same trend was observed for Sevtopolis when compared to Hidcote and Buena Vista. The yield differences between Sevtopolis and Hidcote were not significant, while the increases compared to Buena Vista were statistically ensured.

Hidcote variety obtained higher yields compared to Buena Vista, but the increases were not statistically significant.

Table 5. Yield differences (kg/ha) between *Lavandula angustifolia* (Mill.) varieties (Moara Domnească, average 2017-2019)

Fertilization	**a_2-a_1**	**a_1-a_3**	**a_1-a_4**	**a_2-a_3**	**a_2-a_4**	**a_3-a_4**
$N_0P_0K_0$ (Ct))	209.5^{ns}	233.8 ^{ns}	449.9**	443.3**	659.4**	216.1 ^{ns}
$N_{60}P_{60}K_{60}$	227.4 ^{ns}	233.0 ^{ns}	430.8**	460.4**	658.2**	197.9 ^{ns}
CAN	285.7 ^{ns}	299.4 ^{ns}	512.5**	585.1**	798.1***	213.1 ^{ns}
$N_{30}P_{30}K_{30+}M15$	279.6 ^{ns}	215.7 ^{ns}	516.4**	495.4**	796.0***	300.6 ^{ns}
Manure30	323.9^{ns}	247.7^{ns}	623.8**	618.6**	947.7***	329.1 ^{ns}

LSD 5% = 370.6 kg/ha; LSD 1% = 508.3kg/ha; LSD 0.1% =696.2 kg/ha

Yield correlation to plants development

The average fresh flowers yield of the four lavender varieties was closely related to plants' vegetative development.

The strongest correlation, with a coefficient $r = 0.9390$, was observed between the fresh flower yield and the number of floral stems (Figure 6), and the regression coefficient $R^2 = 0.8817$ highlights that 88% of the yield depends on the number of floral stems.

Analyzing yield as a dependent variable in relation to plants' height reflects a close dependence, supported by a correlation coefficient $r = 0.8594$ (Figure 7).

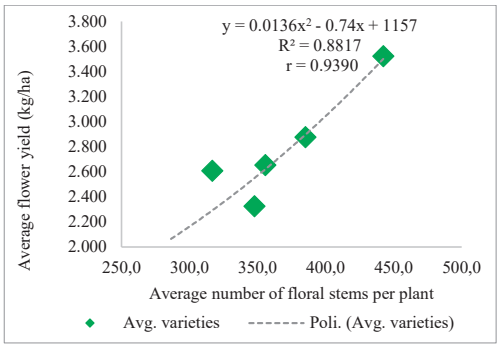


Figure 6. Correlation of lavender flower yield to the number of floral stems, average 2017-2019

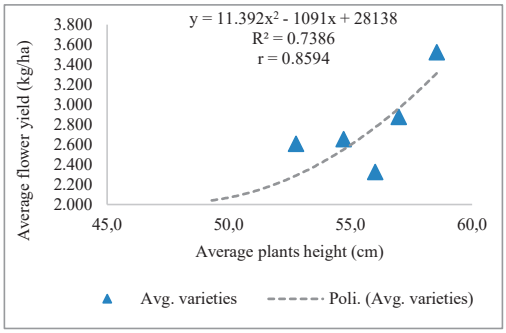


Figure 7. Correlation of lavender flower yield to the height of plants, average 2017-2019

CONCLUSIONS

The results of the research on *Lavandula angustifolia* grown in South East Romania show that both, plants development and fresh flowers yield were influenced by fertilizer's type and amount, but also by the lavender variety.

While in the first year of the crop (I), mineral fertilization generated higher values in terms of plants growth, crop development and yield, for the IInd and IIIrd years, the highest results were obtained when organic fertilization was applied. On crop establishment (I), flower yields were increased due to mineral fertilization by 13.2% (CAN) and 25.7% ($N_{60}P_{60}K_{60}$). Organo-mineral fertilization $N_{30}P_{30}K_{30}$+M15 generated a yield increase of 17.5%, while organic treatment (Manure30) ensured a yield growth of 11.7%. In the following years of crop development, the yield obtained for organic treatment were 4,077.6 kg/ha (II) and 5,866.0 (III), higher by 79.1% and 98.1%, respectively compared to the unfertilized.

Vera was the variety with the most developed plants. This variety also obtained the highest flower yields, in each of the three years of crop development, with values of 798.3 kg/ha (Ist).

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DEFENSIVE ROLE OF EXTRAFLORAL NECTARIES

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Abstract

Plants have to deal with a diverse assemblage of herbivores, which can consume significant amounts of biomass and reduce plant reproductive success. Consequently, plants have developed a diversity of structures and evolutionary strategies to provide protection against herbivory. One of these strategies is represented by extrafloral nectaries, which are nectar secretory structures involved in the indirect defense of plants. Extrafloral nectaries attract adult and predatory parasitoids, leading to a significant reduction in the number of pests that attack plants. Plant-insect interactions are old, and formed the structural basis of many of the terrestrial environments. These relationships directly involve a wide variety of consumption-resource networks, placing plants under enormous pressure of evolution caused by higher trophic levels, especially by herbivores. This paper aims to present a short review about the properties and role in defense of extrafloral nectaries.

Key words*: extrafloral nectaries, defense mechanism, indirect defense.*

INTRODUCTION

Nectar glands produce carbohydrate exudates and can be found on any vegetative or reproductive plant structure (Aguirre et al., 2013). These are specialized structures present on the plant components and are called floral (located on the flower organs) and extrafloral (located on vegetative organs of the plant) nectaries (Garcia de Almeida et al., 2012; Coutinho et al., 2012). It is known that floral nectar glands play a direct role in pollination providing nectar to visitors. In contrast, extrafloral nectaries are not directly involved in pollination, they play a vital role in maintaining a beneficial relationship of mutualism between plant and insects (Garcia de Almeida et al., 2012). Extrafloral nectar contains mainly sugars, so their secretion can be influenced by photosynthesis (Fang-Fang & Jin, 2015). Generally, the extrafloral nectar is known to be an indirect defense mechanism. Along with ants, other insects such as wasps and mosquitoes use this feed source, thus providing different degrees of pest protection. For example, it has been repeatedly demonstrated that the presence of ants increases the protection of the whole plant, while other visitors can act as commensals or even plant pests (Kost & Heil, 2005).

Plants secrete nectar to attract animals that function predominantly as pollinators as in the case of floral nectar, or pest control as in the case of extrafloral nectar. Because nectars are usually aqueous solutions containing mainly sugars and aminoacids, but also other nutrients (Jamont et al., 2013), they are susceptible to infestation with microorganisms that can use nectar-like tissues as entry doors to infect the plant. Nectar secreting tissues therefore require an effective protection shield against pathogenic lesions (Escalante-Péreza et al., 2012). The aim of this study is to present a short review about the properties and role in defense of extrafloral nectaries.

GENERAL ASPECTS CONCERNING EXTRAFLORAL NECTARIES AND EXTRAFLORAL NECTAR

Knowledge of plant defense systems against pests is crucial to understanding trophic relationships in terrestrial ecosystems. Defense systems can act alone or combined during foliar

development (Calixto et al., 2015). In most food chains, herbivore insects are one of the main energy flow pipes between autotrophic plants and the rest of the food chain. Thus, it is not surprising that the pests have led to the evolution of a wide range of effective plant defense mechanisms (Agrawal, 2007). Lack of mobility restricts plants' ability to disperse pollen and signs and protect themselves from pests but is compensated by the presence of floral and extrafloral nectaries glands. Extrafloral nectar is secreted by specialized organs that can appear on all plant structures, but are not involved in pollination. These have been described for about a thousand plant species, which are part of more than 93 plant families of flowers and ferns, but are absent in gymnosperm.

Extrafloral nectar is distributed across plant structures (e.g. spikes, pedicels, buds, calyx, leaves, petioles, bractes or stems) and occurs in over 108 families and 745 genera of ferns and angiosperms (Kost & Heil, 2005; Dattilo et al., 2015). When on the leaves, extrafloral nectar secreting glands develop at the beginning of the budding period, and their activity (time and productivity) is variable among species, depending on the phenological development of the plants (Calixto et al., 2015). Extrafloral nectar glands often secrete large volumes of nectar over a much longer period than floral nectar glands (Géneau et al., 2013).

Extrafloral nectaries are aqueous solutions that mainly contain sucrose, glucose and fructose, but other sugars, amino acids and other organic compounds may also be present in certain species. The secreted sugars are mainly derived from the phloem or are synthesized in the nectar region. Extrafloral nectaries secretes small amounts of nectar throughout the day. Secretion of nectar can follow circadian cycles or can be relatively constant day and night. The nectar secretion mechanisms are still poorly understood: some researchers have described secretion as a passive process, while others have described it as an elimination of excess sugars. However, there is evidence that nectar secretion is an active secretory process: requires metabolic energy consumption, and extrafloral nectaries have secretory cells that contain a large number of mitochondria and have dense protoplasts and large nucleus. Originally discovered in *Macaranga tanarius*, it is suggested that nectar secretion is induced by leaf damage. Inhibitors that suppress the release of linolenic acid or interfere with the production of linolenic acid hydroperoxides completely suppress the EN flow induced by lesions and therefore clearly demonstrate the involvement of oxilipine signaling in EN induction. Interestingly, the attack of some *Agriotes lineatus* under the earth on cotton plants (*Gossypium herbaceum*) induces extrafloral nectar production on the aerial side of the plant. Most studies have focused on the protective effect of ants on whole plants or individual parts of plants, demonstrating a beneficial function of these insects. However, there are conflicting observations that did not detect a measurable preventive effect of the ants attracted to the EN. In these cases, the lack of plant protection can be explained by (1) differences between the degree of aggression of the attracted species of ants, (2) differences between the drilling behavior of the ant species in different habitats and (3) a variable susceptibility of the pests to the predatory ant. In addition to ants, extrafloral nectar attracts a wide spectrum of other arthropods including *Araneae*, *Diptera*, *Coleoptera* and *Hymenoptera*. Due to their predatory or parasitoid life forms, many of these non-ants, such as different species of wasps, jumping spiders (*Salticidae*), mites or flies, can also reduce the number of pests. Both ants and wasps exert beneficial effects on plants that secrete extrafloral nectar. The emission of floral aromas by plants is very important for attracting pollinators to their floral nectar. Beyond these odors, which communicate the location, abundance and quality of these nectars at higher trophic levels, other mechanisms may help guide the arthropods feeding on EN to other sources of nectar further away. First, some extrafloral nectar secretory glands are colored, providing visual indications for arthropods. In addition, increased amounts of both HIPV and EN may allow arthropods to utilize volatile organic compounds emitted as a clue to detecting nectar sources at longer distances (Arimura et al., 2005).

Understanding the role of structure, function, ecology, and evolution of plant secreting structures provides meaningful information to

understand the different types of insect-plant interactions and floral anatomy in relation to reproductive biology (Garcia de Almeida et al., 2012). Because nectar secretion is directly related to the protection of ants against pests, various factors can influence the phenotypic plasticity of a plant species to optimize the compromise between nectar secretion and defensive benefits. For example, in arid and semi-arid mediums with high day driven temperature and low humidity, many insects such as ants and pests are more active at night, mainly due to their eco-physiological limitations. Moreover, some studies have shown that pests have a greater preference for younger and tastier leaves.

It is therefore possible to expect that in these seasonal environments, plants will secrete larger amounts of nectar at night when the pest pressure is higher in more vulnerable tissues (e.g. apical branches) (Dattilo et al., 2015). Many plants interact with carnivores as an "indirect defense" against pests. For example, it is well established that plants attract, feed or host other organisms and this fact can reduce the pressure of the enemy. Plant features involved in this context may be volatile organic compounds, extrafloral nectar, feed organisms and structures used as shelters or nesting spaces (Marques Fortuna, 2013).

Nectar is an important food source for adult parasitoids and can increase longevity and fecundity and thus increase the parasitic rate. Both floral and extrafloral nectar are food sources for parasitoids. The extrafloral nectar of *Centaurea cyanus* L. (Asteraceae) is produced by sepals. The production of nectar begins two weeks before the flowering of the plants and is maintained (in parallel with the production of floral nectar) during the flowering period. It has been observed that parasitoids feed on the extrafloral nectar of *C. cyanus*, and it has been shown that this type of feeding leads to increased longevity and fertility of *Microplitis mediator*. Parasitoids must find hosts (insects) for breeding and a source of food for adult parasitoid nutrition. In the field, these two resources are often separated by space, and parasitoids have been shown to alternate the search for the host and the search for food depending on their physiological state. Parasitoids use both olfactory and visual indicators to locate hosts and food sources (Géneau et al., 2013).

EXTRAFLORAL NECTARIES - WANTED AND UNWANTED VISITORS

Ants are omnipresent terrestrial organisms, especially abundant in shrubs and trees of tropical forests and savannah. Many ant species use plant surfaces as feed substrates to look for both live prey and dead prey as well as for different types of plant-derived food. The ants that feed on extrafloral nectar increase individual survival, and increase their colony growth rate and reproduction. The main advantage that ants provide to plants is effective protection against natural enemies, thus improving the condition of the plant (Assuncão et al., 2014).

Among plant-insect interactions, the relationship between ants and plants is a partnership where the ants are attracted to the extrafloral nectar rich in sugars, while providing protection against pests (Garcia de Almeida et al., 2012; Dattilo et al., 2015). Many resistance-related links are inducible or can be initiated for faster and stronger induction once the lesions appear. This phenotypic plasticity helps balance the costs and benefits of the defense as it ensures that costly protections are only expressed when they are effectively needed. Among the inducible traits, it appears that all plants respond to the damage caused by pests with increased emissions of volatile organic compounds (VOCs), and the plants in many taxa are also responsible for the secretion of extrafloral nectar (EN). Both VOCs and ENs attract adult and predatory parasitoids, leading to a significant reduction in the number of pests that attack plants. Plants have numerous features that offer resistance to most potential pests and pathogens. Many features act directly against these enemies through their toxic, removal or antimicrobial effects, or act as mechanical barriers (Stenberg et al., 2015).

Plant-insect interactions are old, and formed the structural basis of many of the terrestrial environments. These relationships directly involve a wide variety of consumption-resource networks, placing plants under enormous pressure of evolution caused by higher trophic

levels, especially by herbivores. In this respect, researchers are unanimous in highlighting the importance of the chemical and physical characteristics of plants that represent the first line of pest defense. Chemical defense is made up of secondary compounds, such as alkaloids and tannins. Physical defense is mainly morphological or mechanical, such as the presence of tenacity of leaves, trichomes, spinners or latex. Trichomes were considered an effective defense system against herbivorous insects. Resistance can be improved during foliage development, and fully extended leaves have a foliar hardness higher than young ones. Foliar tenacity can act as a powerful defense system that affects morphology, food behavior, and ultimately spatial and temporal patterns of pests (Calixto et al., 2015). The plants also possess other defensive strategies such as biotic defense, or association with a predator. In this situation, plants provide food and / or shelter for predators in exchange for protection. Extrafloral nectar represents the classic example for this type of defense system. Extrafloral nectaries produce a carbohydrate-rich liquid with diluted compounds of amino acids, lipids, phenols, alkaloids and volatile organic compounds, attracting a wide variety of predatory arthropods including ants. Several studies have demonstrated the effectiveness of ants in protecting plants against foliar pests. However, others have failed or have shown only a partially effective defense provided by ants at vegetative parts of plants. Indeed, Assuncão et al. (2014) has shown that ants visiting extrafloral nectar secretory plants can remove pollinators and cause indirect losses. In addition to ants, spiders visiting extrafloral nectar secretory plants can also provide protection.

Interactions between plant and herbivorous insects are among the most striking challenges for ecologists around the world and have been shown to play central roles in the evolution of plant pest defenses. An important and well-known defense mechanism against pests is the indirect defense generated by a third trophic level: the enemies of the pests. An example that occurs mainly in the tropics is mediated by the supply of extrafloral nectar for ants, which protects host plants against pests. In systems involving three trophic levels, such as plants, pests and predators, a trophic cascade describes the top-down positive effects of the third trophic level on the producing species. Associating with animal species can provide plants with protection against pests, diseases and, occasionally, competitors (do Nascimento & Del-Claro, 2010).

Mutualism between ants and plants is an interaction system where plants can provide habitats and/or food for ants, which in turn protects plants against pests that they hunt or remove. The most common nutritional reward that plants give to ants is extrafloral nectar. Several authors have shown that associations between ants and extrafloral nectar secretory plants can reduce foliar pests and/or increase the number of fruits. However, organisms interact not only directly but also indirectly, and indirect effects are important forces that lead ecological communities that can also cause variations in interspecific relationship outcomes. In fact, some studies have shown that the ants' positive effects on ant-plant associations are not universal (do Nascimento & Del-Claro, 2010). Plants and ants have widespread relationships that are mediated by the supply of extrafloral nectar to ants that protect plants against pests. However, these interactions between plants and ants are optional and vary in time and space, mainly depending on the characteristics of ants' species such as density and aggression. Generally, the results of these relationships are positive, but in isolated cases the presence of ants is neutral or negative for plants. Some studies suggest that aggressive attacks or simply the presence of ants could reduce the rate of sighting of pollinating insects, such as bees (Assuncão et al., 2014).

Plants colonized and damaged by herbivorous insects produce a group of volatile organic compounds often called herbivore-induced vegetal volatile compounds (HIPVs), which may include chemical substances that act as repellents for herbivorous pests and as attracting agents for the antagonist organisms of these pests, such as predators and parasites. On the one hand, these signals indicate that the plant is already infested and therefore less suitable as a host, but on the other hand it may increase the search for food by predators and parasitoids. It is widely accepted that plants

respond to the attack of specific herbivore insect species through direct and indirect induced defenses. In direct defense, chemicals target the pest, resulting in delayed development or death (e.g. HIPV), while in the case of indirect defense, chemicals (e.g. HIPV) lead to increased mortality by recruiting predators and parasitoids. Studies on mechanisms leading to HIPV production have revealed the role of their specific elicitors. These elicitors can activate different signaling pathways in the plant, resulting in the accumulation or release of defensive chemicals. In addition, it has been observed that there are also intact plants that naturally produce similar VOCs without any damage from herbivores. Biological control agents (natural enemies) use a wide range of such VOCs to locate their prey (Khan et al., 2008). Activating specific responses requires recognition and adequate response to the attacking enemy, and most events that eventually lead to gene activation (the signaling pathway) occur within minutes. Among the many signaling molecules involved, reactive oxygen species (ROS) and intracellular calcium signatures belong to early events that are responsible for most chemical and molecular reaction cascades (Maffei et al., 2007).

With the coexistence of at least 100 million years, plants and insects evolved a variety of beneficial and harmful interactions. To avoid overloading, the plants have developed a chemical defense capable of producing an effective and often drastic reduction in insect feeding. These defense systems have to be orchestrated both in time and space through extremely complex regulatory networks, which are themselves further modulated by interactions with other signaling paths. Integrated responses ultimately lead to a characteristic pattern of gene expression resulting, among many other activities, in the production of phytochemicals directed against the invading or feeding organisms. This has been achieved during the evolution of constituent components (e.g. primary and secondary metabolites, but also thorns, trichomes, etc.) and induced defense (e.g. secondary metabolites, cell wall strengthening, production of ROS etc.). Direct defense is represented by plant features which by themselves affect the performance of insects and are generally classified by their way of action. Among the secondary metabolites, many phytochemicals function as toxins by poisoning unadapted pests and by forcing adapted insects to invest scarce resources in detoxification. In addition to direct defense, plants express features that facilitate top-down control of pest populations by attracting predators and parasitoids of the herbivorous insects. Thus, indirect protection mediated by VOC release caused by insect attack has received the greatest attention, but the production of nectar-induced pests by extrafloral glands works similarly (Maffei et al., 2007).

Insects can locate their hosts, even if host plants are often hidden in a number of other plants, and volatile plant compounds play an important role in this hosting process. Furthermore, these VOC-mediated interactions of plants with organisms with higher trophic levels suggest that they communicate similarly with each other. Exposure to VOCs alone, without effective pest attack, can directly lead to increased defense. Alternatively, VOC exposure can allow nearby plants to prepare their defense for immediate use once the pest moves from the neighboring plant to attack the receiver. Moreover, volatile compounds in primary host plants can also attract other insects, such as male aphids. Interestingly, parasitoids also use herbivorous insects' responses to assess habitat returns and adapt the residence time of patches. Besides, volatile plant emissions are inducible by other biotrophic as well as abiotic agents. After their release from leaves, flowers and fruits in the atmosphere and from the roots into the soil, the phytochemical compounds of plants protect the plants against pests and pathogens or provide reproductive advantages by attracting pollinators. Furthermore, certain volatile compounds can act as air signals that amplify direct and indirect defense in the distant parts of the same plants (Maffei et al., 2007).

In addition to the use of direct phytochemicals, several stages are involved, starting with the detection of insect feeding to the indirect plant responses. These include leaf tissue disruption, elicitor release, signal cascades, and activation of transcription factors that ultimately lead to

cellular response of plants. Current research into the interaction between plants and insects focuses primarily on transcriptomics, genomics, proteomics and metabolomics, which are late events induced by biotic stress. In contrast, events from the first seconds to minutes that are involved in signal recognition and transduction are still poorly understood (Maffei et al., 2007).

Understanding insect-plant interaction is of interest not only from an ecological and evolutionary perspective, but also for the development of new crop protection strategies. Due to the massive damage caused by herbivores to valuable crops, the deciphering of early plant signals is one of the most interesting areas of research to defend it. ROS and calcium signaling appear to be a common event in induced pest control processes (both in chewing and perforation) and against pathogens, but the way the plant distinguishes enemies lies in the speed and intensity of the damage, as well as in the nature of the elicitor specificity delivered to the attacked plant cells. VOC production has been demonstrated for a wide range of biotrophic attacks and even in this case, the plant responds with specific but variable mixtures that can attract predators of the attacking enemies. Despite all the evidence, the link between early aggressor perception, generation of ROS and secondary messengers, and specific VOC emission is still far from clear and much work is still needed to better understand the important link between recognizing a certain biotic stress and appropriate plant responses (Maffei et al., 2007).

Pest attacks cause changes in the herbal bouquet released by plants. These volatile compounds induced by herbivorous insects (HIPVs) have been interpreted as part of the indirect defense (Turlings & Ton, 2006; Dicke & Baldwin, 2010). However, given that no study has yet investigated whether HIPV is in favor of a plant's robustness, its defensive function needs to be established. In addition, pests, pathogens, pollinators and competitors also respond to HIPV and neighboring plants from native populations also emit volatile compounds that provide a background odor. These considerations enrich the evolutionary context of HIPVs and complicate predictions about their adaptive value (Dicke & Baldwin, 2010).

In addition to the release of volatile compounds, plants attract and maintain parasitoids providing shelter (such as cavities or tricks for ants and mites) or food (such as pollen, floral nectar, extrafloral nectar and sap) (Arimura et al., 2005; Stenberg et al., 2015).

Most species of parasitoids require more resources to complete their life cycle and to maximize their health, such as hosts, shelter and food. Adult parasitic females need to look not only for hosts to ensure the development of larvae, but also for food. Adult food is required to perform basic metabolic functions, provide large amounts of energy for flight and to acquire nutrients to be allocated to oovigens in the case of synovigene species (Jamont et al., 2013).

This food contains carbohydrates and amino acids, and is consumed by a wide range of parasitoids, most commonly during the adult stage. In particular, ingestion of nectar enhances the longevity and efficiency of parasitic predation. These types of food affect the performance, behavior and voracity of the predators, this effect can optimize the effectiveness of biological control using genotypes of plants of a certain quality. In particular, the secretion of extrafloral nectar usually reduces the number of herbivorous insects on the respective plants. In the context of horticulture, there are reports saying that plants that secrete extrafloral nectar have better protection from pests, they produce larger amounts of pollen, or provide additional shelter for ants and mites. Carbohydrate availability is a common obstacle for carnivores, while herbivorous insects are usually limited by protein supply. Therefore, carbohydrate-based rewards can shift the balance in favor of the third trophic level, even when a specific reward is also used by herbivorous insects (Stenberg et al., 2015). While the exploitation of floral nectar by parasitoids has been extensively studied, little is known about how parasitoids locate extrafloral nectar and whether the availability of extrafloral nectar increases the rate of pest parasitization in the field (Géneau et al., 2013). The presence of sugar can play a major role in the performance of parasitoids in a biological control context (Jamont et al.,

2013). Carbohydrate sources provide the parasitoids with the essential energy and nutrients needed to meet their nutritional needs and thus can play a major role in the success of parasitoid reproduction. The availability of adequate carbohydrate sources for increasing survival rate and fertility of adult parasitoids has been demonstrated. If the crop does not provide sources of nectar, sugars can be obtained from vegetation that is not part of the crop or from honey produced by insects in *Sternorrhyncha* that feed on the plant. Floral nectar may be abundant, but is limited to the, often short, flowering period and may not be accessible to parasitoids in deep corolla flowers due to their short mouths. Parasitoids can also have strong competition for floral nectar with other nectar-feeding insects such as lepidopterans, bees and *Syrphydae* family (Géneau et al., 2013).

Many aphids are major agricultural pests due to their unparalleled reproductive ability and ability to manipulate the physiology of host plants. The growth of aphid population and its impact on plant health are strongly influenced by interactions with other organisms, including plant pathogens, endophytes, aphids endosymbionts, predators, parasites, ants and other herbivorous insects. Numerous molecular and genomic resources have recently been developed to identify the sources of aphids resistance in plants, as well as potential innovative targets for aphids control. Furthermore, the same model systems that are used to explore direct molecular interactions between plants and aphids can be used to study the ecological context in which they occur. The Aphididae family comprises more than 4300 species, all of them specialized in feeding on the sap of the plants. Aphids can have a negative impact on the host plants largely due to their ability to quickly populate the surrounding space. Unlike most insects, they can reproduce clonally and give birth to young life, and the aphid's embryonic development begins before the birth of its mother. These features allow for short-term generations; the nymphs of certain species of aphids can reach maturity in just five days. Depending on the densities of the population, the aphid colonies invest in the production of morphs without wings with high fertility or less prolific offspring with wings that can be dispersed in new host plants. This wing dimorphism allows aphids to utilize ephemeral herbaceous hosts in summer and migrate to perennial super-terrestrial hosts in the fall. Such host alternation is associated with cyclic parthenogenesis, in which aphids reproduce cloned on summer hosts and produce an egg-over-egg stage by sexual reproduction in autumn (Goggin, 2007).

Floral nectar and pollen or extrafloral nectar can also enhance predation by attracting natural enemies, supporting the life stages of non-carnivorous parasites and providing alternative food sources for predators when prey abundance is low. Although these alternative food sources are preferred, they may interfere with biological control for plant protection. In some cases, the direct defense of plants against pests can be counterintuitive to indirect defense. Aphid population growth is also influenced by mutual or antagonistic relationships with other insects (Goggin, 2007).

CONCLUSIONS

Nectariferous glands are glands that produce carbohydrate exudates and can be located on any vegetative or reproductive structure of the plant. These are specialized structures, present on the plant components and are called floral (located on the flower organs) and extrafloral (located on the vegetative organs of the plant). The position and type of secreted nectar are often correlated with the efficiency of reproduction.

Extrafloral nectar contains mainly sugars, so their secretion can be influenced by photosynthesis. Extrafloral nectar is used as a food source by several groups of insects. EN is produced for a longer period of time compared to floral nectar, appearing earlier and continuing to be produced after flowering. The composition of EN differs depending on the species. The production of EN depends on the photosynthetic activity, the nutrients available in the soil, the health of the plant, the air temperature and its humidity.

Plant defense mechanisms against pests are not limited to physical and chemical barriers that directly aim to affect attackers, thus becoming increasingly obvious that plants also use indirect defense strategies. One form of indirect

defense of plants is to attract predators and parasitoids by signaling the presence of a potential prey or host with the help of the extrafloral nectaries. This attraction of the third trophic level is one of the supposed functions of the plant volatile compounds induced by herbivorous insects (HIPV), which are released more or less specifically in response to the attack of pests.

The survival strategies of plants are associated with their secretory tissues in different environments, which probably results from the two evolutionary tendencies: one aimed at protecting against pests and the other related to attracting pollinators.

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THE USE OF ARTIFICIAL SEED TECHNOLOGY IN THE PRODUCTION OF HORTICULTURAL PLANTS (REVIEW)

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Abstract

Artificial seeds technology allows to produce in large quantities, in vitro somatic embryos for seedless plant that share some similar properties of natural embryos, and can be used for in field propagation of selected plant species that have commercial value. Somatic embryos are the primary plant material for the production of synthetic seeds. This technology contributes to supply genetically homogeneous seeds, unlike traditional hybrid seeds, obtained from a gametic process which are known to produce plants that are different from the characteristics of the parent plants. They simply encapsulate the somatic embryos with a mixture of nutrient gel that contains carbon, organic and inorganic salts, vitamins, hormones, and antimicrobials to protect embryos from damage. It also allows the growth and germination to happen without unwanted differences. This study inquires the artificial seeds technology and their importance in facing the actual challenges of plant production. Previous studies have been reviewed for a better understanding of the field of artificial seed production.

Key words*: in vitro, sodium alginate, somatic embryos, tissue culture.*

INTRODUCTION

Three horticultural plant species: potato (*Solanum tuberosum* L.), fig (*Ficus carica* L.) and Chinese jujube (*Ziziphus jujuba* Mill.) have been nominated for this study due to their economic importance and high requirement by many countries.

One of the main objectives of the study is the use of somatic embryos by applying and adopting the method and mechanisms of accurate propagation, as well the analyse and assessment of the production of synthetic seeds - artificial seeds.

Schleiden (1838) and Schwann (1839) have proven that the basic building unit of living organisms is the cell, which has the ability to grow if the right environment is in contact with it so that a complete plant can form from it.

The process of tissue culture isolated single differentiated cells and planted them on a saline solution recommended by Knop (1865) and supported by glucose sugar. This process led to growth cells size and the accumulation of starch in them, but it failed to motivate them to divide. However, it set the ideas for the emergence of tissue culture science. Murashige and Skoog (1962) were able to prepare an integrated nutritional medium of salts, whose concentrations increased about 25 times the nutritional medium prepared by Knop.

This medium was called MS and it is still largely used to this day for the *in vitro* cultivation of plant tissues. Tissue culture is the basis for the development of somatic embryos and the production of artificial seeds.

Various plant techniques, have been used for the production of somatic embryos *in vitro*, which have shared some similar properties to natural zygotic embryos. Non-seed producing plants or propagation of elite plant species have commercial value (Saiprasad, 2001).

For imitating the natural seeds, somatic embryos are covered with nutrient gel consisted of basic organic or inorganic salts, compounds containing carbon, plant growth regulators and antimicrobials to protect the embryos from lesion while handling as well as allowing the growth and germination to take place. Some agents already been prepared for encapsulation such as SA complexed by $CaCl_2$ have been noticed and found to be the most convenient.

The production of artificial seeds depends on several factors such as selection of virus free seeds among other various diseases, in addition of seeds viability, high germination and moisture content.

Synthetic seeds have potential for a considerable level of cost lowering (Kok-Siong *et al.*, 2012; Roy and Mandal, 2008) with rapid multiplication of plant with genetic uniformity (Saiprasad, 2001). Artificial seeds are described as artificially encapsulated somatic embryos, which represent any vegetative part of plants (Rihan *et al.*, 2017). Because they are obtained from somatic cells, it is possible to be used for clonal propagation. Artificial seeds have different implementation in plant biotechnology - for instance in clonal propagation, germplasm conservation, plant breeding where propagation via normal seeds is not probable, easy storage, genetic uniformity etc. For some ornamental plants, propagation through somatic embryogenesis and synthetic seeds have been regarded as the only way out. Encapsulation technology is a fast-growing research domain in biotechnology and broadly studied. Artificial seeds are convenient for conservation and delivery of tissue-cultured plants, which is why many types of plants, fruits or cereal have been grown from artificial seeds.

The artificial or synthetic seeds are obtained mainly by deriving somatic embryos from plant tissue cultures and encapsulating them with the help of a hydrogel. Production technology of synthetic seeds in horticultural plants offered new techniques for preparing seed analogues from the micropropagules, like protocorm-like body (PLB) formation and plant regeneration (Bapat and Rao, 1988). Micropropagules are encased in productive coatings of gelling agents such as alginate, agar, carrageenan, gellan gum (gelrite), sodium pectate, and carboxyl methyl cellulose (Kok-Siong *et al.*, 2012).

Micro shoots encapsulation and somatic embryos as well as retrieval of plantlets have been reported in a number of plant species, such as: cauliflower (Kok-Siong *et al.*, 2012), sandalwood (Bapat and Rao,1988), rosemary (Al Masoody and Stănică, 2015), apple (Piccioni *et al.*, 1996) (Micheli *et al.*, 2008), mulberry (Micheli *et al.*, 2017), banana (Ganapathi *et al.*, 1992) cardamom, sugar beet (Tsai and Saunders, 1999) rice and peer (Mower *et al.*, 2007) and other plants (Falcinelli *et al.*, 1997). Seeds production by utilizing somatic embryos and other kinds of explants is possibly fruitful for the huge scale propagation of superior hybrids of important species. The synthetic seed technology may only be successful with competent. Different micropropagules have been regarded for purpose of producing artificial seeds (Stănică, 1999); consequently, somatic embryos and axillary shoot buds have been favoured.

MATERIALS AND METHODS

As method the literature review as a critical look at the existing research that is significant to the field, was used.

RESULTS AND DISCUSSIONS

1.1. Importance of Artificial Seeds

The significance is based on the advantages that could be gained by producing artificial seeds which can bring good and higher percentage of outputs, specifically when seeds are implemented in shorter time, labour and lower cost as well as the possibility to be stored for longer time, since "seeds are desiccation tolerant, durable and quiescent due to protective coat. Such properties of seeds are also used for germplasm preservation in seed repositories" (Patricia *et al.*, 2004). "Many strategies can be used to evaluate plant genetic structure from *in vitro* derived plant clones, but most of them have limitations" (Patricia *et al.*, 2004). The researcher pointed out that such a topic may add a value to previous literature as well as contributing in bridging the gap in literature concerning production of synthetic seeds in horticultural plants using somatic embryos and other types of explants obtained *in vitro*. True seeds can be produced at the end of reproductive stage by the process of reproduction. A regular plant might take a long time to achieve reproduction stage, this matter implies that we are to wait to the end of reproduction stage of a plant to get seeds. But, artificial seeds can be available within one month or less. The majority of regular plants bear flower and produce their seeds at a

specified season. But, producing artificial seeds is not time dependent. It can be at any. The work on some kinds of plants is delayed because of the presence of long periods of dormancy, whereas growing artificial seeds, this period may be reduced where the life cycle of a plant can be shortened. It is probable to produce artificial seeds for any desired crops. Artificial seeds are possible to be applicable at large scale monocultures in addition to mixed genotype plantations. Artificial seed coating has the ability to deliver beneficial adjuvant like growth promoting plant nutrients as well as growth control agents Artificial seeds assist in studying the role of endosperm in addition to seed coat formation.

1.2. Is there a need for artificial seeds?

Many species are now considered to be on the verge of extension. Growing desertification and disappearing forests raises the chances of extinction for many plant native species, most of which cannot be propagated and which produce low quantities of seed. This is where synthetic seed play an important part as a substitutes (Kumar, 1998). By using synthetic seeds, the risks emerging from adjusted plants in reproduction might be prevented. Such risks include the possibility of gene introduction from different species during multiplication, which can become unstable. Artificial seeds can be used trough somatic hybrid propagation (Hwang *et al.*, 2005). Seeds are considered zygotic embryos with supported and improved nutritive tissues that provide protection. This layer makes them desiccation tolerant and durable, making the seeds useful for germplasm preserving in seed repositories. Zygotic embryos include progeny from two parents, which means that the growing or breeding process includes inbred lines that produce hybrid progeny when crossed. However, at crossing, genetic barriers occur for fruits and ornamental plants. Forest trees on the other hand have a too long generation time. In this cases propagation should be achieved in a vegetative manner, by using open pollinated seeds. Zygotic seeds have been replaced following the discovery of somatic embryogenesis, respectively in the 50s when they were created from somatic cells (Mohan, 2000; Jaiswal *et al.*, 2001). The somatic embryos emerging from shoots, roots, cotyledon leaves, epicotyls, hypocotyls, embryogenic calli, as well as protocorm or protocorm-like bodies, which can be perceived as clones originating from a single parent. They never become quiescent in the processes of in vitro culture. The synthetic seeds do not always need a synthetic protective layer. The use of somatic embryos as seeds with functional capacity is studies in the field of technology of artificial or synthetic seed. Their economic importance relies greatly in germplasm preservation and plant production for commercial purposes.

1.3. Types of artificial seeds

Synthetic seeds can be uncoated quiescent – somatic- embryos or uncoated non - q. s.e. The non-q. ones are used for crop production micro propagated trough tissue cultures, whereas the quiescent ones are useful for germplasm storage. The non-q. s.e. in a hydrated encapsulation provide a cost-effective seed. The dehydrated ones encapsulated in protective artificial layers are useful due to their handling qualities. The somatic embryos become quiescent and the protective layer hardens, making them be more resistant for a longer period of time: "the seed coat softens, allowing the somatic embryo to resume growth, enlarging and emerging from the encapsulation" (Grey, 2003).

1.4. Advantages of artificial seeds

Throughout this study, the work will refer to three plants, namely, potato, figs and Chinese jujube and the explants will be chosen from these three plants are tubers, and buds respectively. The artificial seeds are somatic embryos and each plant uses a specific part for micropropagation and packaging to reach artificial seed production. "The propagation of plants by artificial seeds widens the horizon of plant biotechnology and agriculture" (Kinoshita, 1992), "the technology provides methods for preparation of seed analogues from the micropropagules such as axillary shoots, apical shoot tips, embryogenic calli, somatic embryos as well as protocorm or protocorm-like bodies" (Jaiswal *et al.*, 2001). Sodium is a chemical element of the Na symbol and its atomic number 11. It is a white coloured

substance, characterized by its great chemical activity. It reacts in the air and burns with yellow flame. In addition, it is famous for being highly reactive with water and air humidity, so it is stored in oils or oil derivatives, there is no free sodium in nature. There is a relatively large abundance of sodium, it is the sixth most abundant chemical element in the earth's crust, and it is found in many minerals such as Feldspar, Sudalite and Halalite. Sodium salts are highly soluble in water, most notably sodium chloride, which is the main cause of saline water. Sodium has one stable which is 23Na. Sodium has an important vital role; it is classified as a basic nutrient for humans, animals and some plants. Na + Na are the essential cations in extracellular fluid, and have a key role in controlling blood pressure and osmotic pressure in the body, in addition to the role in the transmission of nerve signals. Sodium is more chemically active than lithium, but less than potassium. Sodium can be dissolved in a completely oxygen-free atmosphere without any reaction. The interaction of sodium with oxygen is a special case, since the interaction between them depends on the presence of moisture, in the absence of water does not interact with sodium with oxygen. Alginic acid is also called alginate. It is a polycyclic polychromide widely spread in cellular walls of cellular algae. When mixed with water, it binds together and forms glue, Sodium alginate is a chemical compound with the formula NaC6H7O6. It is the sodium salt of alginic acid (algal acid), and it is in the form of gum extracted from the walls of algae cells. Sodium alginate is a good candidate for the removal of radioactive substances from the body, such as iodine-131 and strontium-90, which are produced by non-radioactive isotopes. The delivery of encapsulated material will save several subcultures for obtaining plants as well as eliminating the stage of acclimatization of in vitro plants. The uniform and simultaneous production of encapsulated propagules followed by uniform germination may remove many drawbacks connected to natural seeds. Many plant systems have been recognised to come up with a huge number of embryos in culture sharing many features similar to natural embryos consisting of germination, which lead to production. For imitating the natural seeds, embryos produced from cultures, are encapsulated in a nutrient gel consisting of basic salts either organic or inorganic, as well as carbon source, plant hormones and antimicrobial agents and coated totally for protecting the embryos from damages such as mechanical during handling, also for allowing developing and germination to take place without any undesirable variations. Several agents for encapsulation and sodium alginate complexing with calcium chloride have proved to be the most convenient. Through this method various types of synthetic seeds have been prepared: hydrated and desiccated. Hydrated synthetic seeds consist of embryos encapsulated individually in a hydrogel, while in desiccated kind, the coating mixture has been dried for several hours inside a sterile hood. The propagules (embryos/axillary bud's/shoot tips) are accurately separated from aseptic cultures and blot dried on filter paper, and are then mixed in sodium alginate prepared in nutrient medium. Then, and manually the propagules are picked up by forceps, and then dropped into a solution of calcium chloride for a period of 40 minutes. After the period of incubation, the artificial seeds were recovered by calcium chloride solution decanting then washing them in sterile water for 4 times before being cultured on nutrient medium or on a filter paper, cotton or soil for purpose of growth and plants conversion. There may be some possible artificial seed systems, relying on the type of artificial seed which is produced, the requirements for artificial seeds, the economic feasibility "will vary greatly among species" (Pond and Cameron, 2003). Production of artificial seeds depend on several steps, which can be summarized as follows:

- **First** comes crop selection, which relies upon commercial and tech potential, then the assembly of a somatic embryo system.
- **The second step** consists of clonal production, system optimization and embryo production and automation of embryo production;
- **The third step** consists of treatment of mature embryos that triggers quiescence;
- **The forth is** encapsulation development and coating system, optimization and automation.

- **The fifth step** consists of determination of economic practicableness of adopting the artificial seed delivery system for a specific crop compared with alternative propagation techniques (cost–benefit analysis regarding encapsulation). In general, some procedures apply to quite one species whereas alternative steps could also be species-specific (Pond and Cameron, 2003).

1.4.1. Encapsulation of somatic embryos

Isolated s. embryos were placed in a Na alginate-based solution. Based on the encapsulation adopted, and suctioned through a micropipette to supply protection. so as to seal the capsules, they're submerged in a $CaCl_2$ solution for a while then in sterile water for forty min. This method is enforced beneath antiseptic conditions. Then, the artificial seeds of potatoes, figs, Chinese jujube are cultivated through a germination medium in Petri dishes by the employment of macro and micro nutrients from the MS medium. It is further supplemented with thirty g/l of disaccharide and seven g/l of agar, then left within the culture chamber at 25ºC with no light.

1.4.2 Applications of Artificial seeds

Synthetic seeds are used in biotechnology for cultivating various plant species. They are also used due to the potential for storing heterozygous plants genetically or with a single outstanding combination that could not be kept in regular methods of seed production "due to genetic recombination exists in every generation for seed multiplication" (Gray, 1997). The applications of artificial seeds is debated in various academic fields. It is important to note that somatic embryogenesis can be an alternative for the sterile species which produce no seeds. Tropical species also produce seeds that cannot be dired and long time storage in gene banks is not likely. "The artificial seeds can be an alternative as more is learned about the mechanism by which this type of seed has no tolerance to desiccation" (Leprince *et al.*, 1993).One limitation of micropropagation is the similarity of the physical tissue culture site, laboratories and greenhouses in order to synchronize the peak of demanded propagules period in the market (Gray and Purohit, 1991).The ornamental plants market is rising annually. The huge cost of production of such species is given by the efforts of the micro propagation and required manpower in the later propagation stages and production. "The use of somatic embryogenesis system in these species would significantly reduce labor costs" (Chee and Cantliffe, 1992) Coniferous forest species may be propagated cheaply, the regular breeding programs in such species are considered very time consuming because its life cycle of conifers is long. Coniferous forests are regarded as too heterogeneous. "Artificial seeds have the ability to clone those overhanging trees at reasonable cost and in minimum time" (Desai *et al.*, 1997). In the commercial field, it is not seen easy to come up with low-cost hybrid seed species like cotton and soybean because they contain cleistogamous flowers and abscission problems because the seed comes from self-pollinating species. Hybrid seed has been made and produced in small quantities in a very laborious by hand pollination. Such small volume of hybrid seed can be largely going up through synthetic seed technology. "the hybrid force would be used commercially to originate a significant reduction in costs" (Tian and Brown, 2000) . In some vegetable species, hybrid seeds are considered expensive, so the plant value is too high. For instance, tomatoes and seedless watermelon hybrid seeds are utilized in very high cost. The reason is that pollination is implemented manually, a matter that requires intensive labour. Whereas, in other species, vegetative reproduction is utilized, it takes much time, space and labor. "The use of artificial seed technology can significantly reduce costs by reducing the labor required, time and space in case of these plants"(Chee and Cantliffe, 1992).The majority of fruit species are propagated by vegetative means due to the fact that the presence of self-incompatibility and breeding cycles very long. "The use of synthetic seed facilitates its spread" (Towill, 1998).The most fruitful synthetic seed would be in the conservation of germplasm of the mentioned species. At the moment, seed banks have been dealt with as live plants in the field. Such method of conservation is too expensive and exposed to danger, as it is exposed to natural risks and disasters. The utility of artificial seeds would keep these

clones in a narrow space, under controlled conditions and without the risks or danger of natural disasters. Moreover, this system of germplasm conservation can be fruitful in tropical species where conservation methods are insufficient. (http://www.fao.org/3/v1430e/V1430E06.htm)

1.5. Previous studies

Literature review is important to access to the most accurate details and consequences. Another important thing to use the previous research is to give the researcher knowledge of the history of the evolution of the subject, and opens his eyes on points not to pay attention to and may be key to the solution. According to Ghanbarali *et al.* (2016), "Optimization of the conditions for production of synthetic seeds by encapsulation of axillary buds derived from minituber sprouts in potato (*Solanum tuberosum)*". The function of alginate encapsulation of axillary buds originated from potato minituber sprouts (PMSs) have been studied and optimized. It has been concluded that synthetic seeds have been obtained in potato using protocols on basis of other explants, normally nodal segments and single-node cuttings, as sources of encapsulable material. In terms of alginate encapsulation, the protocol described in this work is the same as these protocols. It has to be noted that these explants are obtained from in vitro propagated plantlets, a matter that implicates that the in vitro plantlets ought to be obtained. It is more time, labour and cost consuming than previous methods, "where the explants (PMSs) are obtained under *in vivo* conditions through minituber sprouting during 2 months (Ghanbarali *et al.*, 2016). Rosna *et al. (*2013), in their study “Synthetic Seeds Production and Regeneration of Oxalis triangularis for Mass Propagation and Conservation”, the potential of tissue culture technique has been discussed as an alternative method for mass propagation and conservation of this ornamental plant for future uses and exploitation. The key results were Synthetic seeds created by encapsulation of micro shoots of *Oxalis triangularis* in sodium alginate solution. The micro shoots have originated from stem explants of this species after being cultured for a period of 30 days on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BAP. After thirty days of storage, the ability of artificial seeds has been retained for germinating and provide high regeneration rate (90%) and the percentage of survival rate has also been high (77-86%) as compared to control. After 7 days of storage, the mean number of shoots formed was the highest in all treatments root formation was observed. It has been concluded that production of synthetic seeds was attempted from this species and the synthetic seeds survive after seven and thirty days after being stored at 4°C.The synthetic seeds conversion rate to complete plants after seven days was 96.67% with 4.57 mean shoots, whereas after thirty days of storage, the conversion rate goes down slightly to 90% with 3.97 shoots formation per bead (Rosna *et al.*, 2013). Shengrui (2013) has published an important study titled *Past, Present, and Future of Jujubes - Chinese Dates in the United States*, this study summarizes jujube importation, culture history and current jujube cultivars in the USA. It handles current issues with jujubes and probable solutions to them. Jujube adapts and grows well, in addition, it could become a valuable industry in the United States within 15 to 20 years. According to (Remya *et al.*, 2013), storage at 4°C is effective for long term preservation of artificial seeds. Determining the optimal temperature for the long-term storage of artificial seeds is imperative for the conservation and transport of useful germplasm resources; the basic part of biodiversity conservation. Maximum number of multiple shoots have been produced in MS medium supported by thidiazuron (TDZ) (3.0 µM) + naphthalene acetic acid (NAA) (1.0 µM). Thidiazuron has been reported as effective when combined with auxin (NAA) and cytokinin (BAP) in arousing morphogenic reactions. Moreover, it has been concluded that half strength liquid MS medium supplemented with 5 µM IBA was effective for root induction from the microshoots of *Solanum nigrum* with auxin. IBA has been reported to be beneficial for root induction during *in vitro* propagation of *S. nigrum* using nodal explants (Remya *et al.*, 2013).

Kok-Siong *et al.*, 2012, in their study "Production of Artificial seeds derived from encapsulated *in vitro* microshoots of cauliflower, *Brassica oleracea* var. *Botrytis*", a

high number of micro shoots (21 ± 2.31) of cauliflower was obtained. The aim of this work has been to assess the effects of various plant growth regulators (PGRs) on the multiple shoots induction adopting hypocotyls as explants, *in vitro* micro shoots produced have been harvested and encapsulated in sodium alginate for creating artificial seeds. The ability of storage and *in vitro* germination rates of artificial seeds have also been assessed.

The artificial seeds lasted for twelve days (after seven days storage) and fourteen days (after thirty days storage) to germinate on MS basal medium. It has been observed through this work that PGR or hormones supplemented to MS medium. In the current study, 0.1 mg/L NAA and 5.0 mg/L BAP effectively induced a large number of double shoots on cauliflower hypocotyl explants for production of downstream artificial seeds. The germination percentage of encapsulated micro shoots has been influenced by encapsulation matrix composition and pre-germination storage duration. Isolated micro shoots encapsulated in MS supplemented with 0.3 mg/L NAA and 3.0 mg/L BAP presented high germination percentage for seven and thirty days of pre-germination storage period. (Kok-Siong *et al.*, 2012). Dhabhai and Prakash (2012), in their study "Production and Applications of Artificial seeds: A Review", the types, production methods, benefits and different applications of synthetic seeds have been reviewed. Shoot tips, axillary buds have been utilized in the preparation process of artificial seeds. Artificial seeds possess a variety of applications in plant biotechnology. For some plants, as for instance ornamental plants, propagation through s. e. and synthetic seeds have been the only way out. It has been seen that artificial seeds have a broad extended applicability in a huge scale plant propagation. In the case of ornamental and extinct plant species, it is the sole means of propagation. Except this, artificial seeds have been directed and adopted for commercial purpose production of autogamous plant species, genetically adjusted plants, conifers, algae etc. To conclude, "the technology of artificial seed has affected almost every aspect of plant biotechnology and has the potential to become the most promising and viable technology for large scale production of plants (Dhabhai and Prakash, 2012).

Hwang *et al.* (2011) studied the propagation of perennial brown alga *Sargassum fulvellum* by somatic embryogenesis and synthetic seeds production. *Sargassum fulvellum* is a brown alga introduced to the seaweed cultivation industry. Such species presents acceptable potential for diversifying seaweed cultivation. Hwang *et al.* (2011) investigated growth and maturation of this alga by somatic embryogenesis and artificial seeds**.**

Bradford and Still (2004), in their study "Applications of Hydro time Analysis in Seed Testing", aimed to show the hydro time in the process of describing the connection between water potential and rates of artificial seed germination.. The model depends on data derived from germination time courses, therefore it needs data for several time points at the time of germination at some water potentials. Experience with a broad array of priming and pelleting methods implicate that, relying on the cultivar, seed lot, and specific treatments adopted, influences on all three parameters of the hydro time model could be noticed. It has been concluded by this study that analysis of hydro time could offer several indices regarding seed quality in connection with stress tolerance, speed and uniformity of germination; in addition, the development of automated imaging for scoring germination can facilitate hydro time analysis applications (Bradford and Still, 2004).

Sarkar (1998), in his study "Synseeds in potato: An investigation using nutrient-encapsulated *in vitro* nodal segments", investigated the production of synthetic seeds for potato propagation by the use of alginate-mediated, nutrient-encapsulated, *in vitro* nodal shoot segments. Micropropagated single node cuttings 5 mm long were encapsulated in calcium-free 3% sodium alginate–MS solution and they have been incubated under light (ca. 60 μE m^{-2} s^{-1} light intensity) at 24 ± 1°C for 3, 6 or 9 days. The encapsulated segments have been treated with rooting hormone powder before and after light incubation, then they have been planted by using plastic trays, they consist of 1:1 mixture of soil and FYM. A decline in survival of alginate capsules took place when they have been incubated under

light for 3 days or more. Rooting hormone powder application at the time of planting has been effective for establishment of soil for alginate capsules. 57% encapsulated segments have survived in the soil when they have been incubated under light for three days and, they have been treated with rooting hormone powder during the period of planting. The study has shown that micropropagated nodal segments encapsulated in alginate–MS solution may be utilized for purpose of producing potato propagule (Sarkar, 1998).

Simmonds (1997), in the study "A review of potato propagation by means of seed, as distinct from clonal propagation by tubers" has concentrated on the majority of cultivated potatoes are vegetative propagated, outbred auto tetraploids. Disease problems have been shown and the maintenance of vegetative stocks. There have been suggestions for propagating the crop by sexual seed so as to evade disease problems. A success has been fulfilled but control is necessary for avoiding inbreeding depression as well as avoiding seed propagation which is not as cheap or simple as hoped. The idea has raised a broad interest in the tropics and has growing practical impact on China, India and Vietnam. There has been a considerable tendency for using tuberlets borne on crowded nursery plants. There has been emerging recognition that seed propagation are complementary rather than competitive and that good breeding programmes will serve both (Simmonds, 1997)

Kariuki (1991), in his research paper "Production of synthetic seeds from nodal segments of *Solanum nigrum*", presented an efficient protocol for the production of synthetic seeds in *Solanum nigrum*, a medicinal plant. Artificial seeds were made by encapsulating nodal segments of *S. nigrum* in calcium alginate gel. 3% (w/v) sodium alginate and 100 mM CaCl $2H_2O$ were seen most convenient for encapsulation of nodal segments. 22 maximum number of multiple shoots have been made in Murashige and Skoog medium supplemented with thidiazuron (3.0 μM) + naphthalene acetic acid (1.0 μM). The encapsulated nodal segments could regenerate after sixty days when they are stored at 4°C. The shoots have been routed by the use of a half strength liquid MS medium supplemented with 5 μM Indole butyric acid and have been acclimated to greenhouse circumstances. This work shows and describes an efficient protocol for the producing synthetic seeds. The protocol can be adopted as an alternative technique for germplasm conservation of this valuable medicinal plant (Kariuki, 1991). By adopting artificial seeds process, the tissue culture raised plants may be regenerated on a simplified medium eliminating subcultures, it may reduce the cost of process and functions. Developing protocols for recovery of plants from artificial seeds under non-sterile circumstances might be of a larger influence. Despite the fact that a large number of plants may be made in tissue cultures through multiple shoot cultures, their delivery is cumbersome. Embryos or shoots are to be isolated and transferred for rooting to conduct root shoot balance, and the plants ought to be hardened in the green house before field planting. Direct sowing regarding artificial seeds in the soil does not require acclimatization usually needed for the tissue cultured plants. It offers an ideal delivery system enabling an easy flexibility in handling as compared to big parcels of plants. in synthetic seeds technology, for big scale commercialization, improved product of propagules is important. Present tissue culture techniques have not generated sufficient propagules and are not enough to meet the requirements of commercial use of artificial seeds technology methods. Standardization for synchronization of developed propagules followed by automation of all operation of sorting, encapsulation and germination of the coated propagules may improve the pace in producing synthetic seeds.

1.6. Summary on previous studies

According to the literature review, the benefits of this study can be summarized as follows:

- The problem of the research and the determination of its dimensions and areas: by looking at what has been written studies and research on the problem
- To enrich the problem of this study with knowledge, studies, hypotheses, and the results reached by others

The study included the identification of the tools used in this study as well as the ideas and procedures that have been used.

Previous studies on the topics of production and applications of artificial seeds in horticultural plants using somatic embryos and other types of explants as well as the studies regarding producing artificial seeds of the three plants namely, potato, fig and Chinese jujube and the explants will be chosen from these three plants are tubers, and buds respectively. At the conclusion of research, the researcher is going to link the results obtained throughout this study with those mentioned in previous studies so that he could make a comparison and contrast process for showing aspects of similarity, and contrast points. So, such previous studies and the present research show that the scientific gap in research is incomplete and more new research study shall be required.

Conclusions of such mentioned previous studies have been as follows:

- synthetic seeds have been obtained in potato using protocols on grounds of other explants, normally nodal segments and single-node cuttings, like sources of encapsulable material
- the synthetic seeds survive after seven and thirty days after being stored at 4°C
- jujube taxonomy, biology, adaptation, propagation, and research conducted have been described.
- storage at 4°C is effective for long term preservation of artificial seeds. Determining the optimal temperature for the long-term storage of artificial seeds is imperative for the conservation and transport of useful germplasm resources
- assessment of the effects of various plant growing regulators on the multiple shoots induction following hypocotyls as explants, *in vitro* micro shoots produced have been encapsulated in sodium alginate for making artificial seeds.
- artificial seeds have a broad extended applicability in a huge scale plant propagation.
- some studies present the properties and features regarding different bioactive substances in this different plants and seeds.
- applying artificial seeds process, the tissue culture raised plants may be regenerated on a simplified medium eliminating subcultures.

CONCLUSIONS

- The tissue culture raised plants and artificial seeds process can be regenerated on a simplified medium eliminating subcultures, such processes may lower the cost of process and functions.
- Synthetic seeds were made by encapsulating the micro shoots derived from multiple shoot induction, and for the aim of creating artificial seeds, an effective technique of multiple shoots induction ought to be developed so that a large number of micro shoots for encapsulation could be provided.
- Several agents have been made for encapsulation and sodium alginate complexing with calcium chloride has been discovered to be the most convenient.
- Applying artificial seeds production process, may be regenerated on a simplified medium eliminating subcultures, it may reduce the cost, time, limitations as well as some functions.
- Artificial seeds provide applications in the field of plant biotech
- Furthermore, Clinical and Pathological studies should be conducted to isolate and characterize the bioactive components present in the selected plants.
- The technology of artificial seed influences plant biotechnology and is considered in important technique for big scale plant production.
- Artificial seeds offer an ideal delivery system enabling easy flexibility in handling and transport as compared to large parcels of seedlings or plants.
- Development of protocols for direct recovery of plants from synthetic seeds under non sterile conditions may have a greater impact
- There is emerging recognition that vegetative and seed propagation are complementary rather than competitive and that good breeding programmes will therefore serve both.
- It may be probable to produce artificial seeds in any desired crops.

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OBSERVATIONS ON THE STRUCTURE AND DYNAMICS OF CARBID SPECIES IN SOME APPLE TREE ORCHARDS ACCORDING TO THE GROWING AREA AND IN THE CONTEXT OF CLIMATE CHANGE

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Abstract

Investigations into the entomofauna of carabides study in apple tree orchards were carried out using the soil traps type Barber. The collection of the material was done in both sampling during May-August, at intervals of 7 to 15 days, for each of the two stages 2010-2011 and 2018 to 2019. At each harvest, the biological material has been taken from traps and at the same time, the salt solution 25 % concentration has been completed or replaced. It would be ideal for certain species (predators and deleterious) to be in balance so that harmful species do not cause damage. In this paper it was initiate a comparative study of the carabids found in apple tree plantations in Iasi and Vaslui counties who was carried out in the context of the evolution of the climatic conditions. From the collected biological material, only carabids species were selected, which were then determined to the species level. Of these most commonly collected carabid species were: Pseudophonus rufipes Mull., Harpalus distinguendus Duft., Ophonus azureus F., Harpalus aeneus F., etc

Key words*: soil traps, Barber type, climatic values.*

INTRODUCTION

Carabidae is an exceedingly diverse family, to telong the Coleopera order of class Insecta. They are distributed worldwide and are adapted for every possible habitat on our planet (except marine and polar regions) (Talmaciu M. et al., 1998).

Habitat and food specificity make them ecologically and economically significant as indicator species and pests respectively. The World record of identified species is the most species have been reported from Europa.

The beetle diversity is enormous; they display a great deal of ecological importance. Some of them are specialized feeder of animal and plant debris, while some are not. (Varvara M. et al., 1995). Many of species are destructive; by feeding on vital plant parts like flowers, fruits and seeds, which ultimately damage our economy. (Varvara M. et al., 1996)

The numbers of the predatory species are biological control agents of agricultural pests beetles of the family Carabidae (ground bugs) feeds on insect pest like aphids and another larvae stage that damages crops. (Paulian F., 1974).

The present study helps in understanding the seasonal variation in the diversity of beetles from Iasi and Vaslui country which have been identified using the determinant books (Du Chatenet G., 1990; Grozea I., 2006; Panin I., 1951; Reitter E., 1908; Rogojanu V., 1979).

MATERIALS AND METHODS

The soil traps type Barber by which we collected the carabids from the fruit orchards ecosystems, it they consisted of the introduction into the soil of plastic pots of 500 ml volume into which 25% sodium chloride solution is poured as a fixative liquid (Herea M. et al., 2011; Talmaciu M. et al., 2017)

Six traps, on two rows, placed from the edge inward, were placed in the studied biotope, at 10 m row spacing and 6 m for the traps on row spacing.

Samples were collected every 3 years of observation (2011 and 2018-2019) between May and September at approximately 10-15 day intervals.

The setting up of the experimental stationary was implemented in one of the plots of the apple tree plantation at the Didactic Station of

the Faculty of Horticulture from Iasi, and in one of the plots belonging to the SC Lot Service SRL Farm, Vaslui County.

RESULTS AND DISCUSSIONS

Environmental or ecological factors exercise complex and continuous action on the population of the species and all organisms in the biocenosis. Ecological factors influence the number of insect populations due to their organic or inorganic nature and the consistency of their actions over time and space. Due to these nature factors, imbalances occur which lead to acceleration or delay in the development of certain organisms, to the reduction or numerical increase of the population of species and even the change in the structure and dynamic change of ecosystems.

Some of the most important anorganic factors influencing geographical distribution, growth and activity of insects are climatic factors.

The monthly temperatures recorded between 2011 and 2018-2019 in the Iasi area are presented in Table 1.

Table 1. The thermal regime in, 2011 and 2018-2019 (source: www.tutiempo.net)

Iasi Monthly average temperature (°C)			
Month / Year	2011	2018	2019
January	1,1	3,2	2
February	3,8	5,7	2,6
March	4,9	9,2	7
April	12,4	12,6	10,9
May	17,2	16	16,8
June	19,9	20	20,5
July	22,2	21,6	24,2
August	23,4	21	24,5
September	15,1	17,5	20
October	13	12,4	6,5
November	8,7	8	2,6
December	1,3	3,6	-1,5

In 2011, the average annual temperature was 12°C and the average maximum and minimum temperatures were 17.7°C and 6.4°C, respectively.

In 2018, the annual average temperature was 12.6°C and the average maximum and minimum temperatures were 18.4°C and 7.3°C respectively.

The year 2019 was until September a year with high summer temperatures and a small amount of precipitation.

The monthly temperatures recorded between 2011 and 2018-2019 in Vaslui County are shown in Table 2.

In 2011 the annual average temperature was 11.1°C and the maximum and minimum average temperatures were 18.2°C, 5.8°C, respectively.

In 2018 the average annual temperature was 11.7°C and the average maximum and minimum temperatures were 18°C and 6.4°C, respectively.

As in Iasi county, in 2019, during the entire growing plants season of climate regime it was characterized that one year with high summer temperatures and a small amount of rainfall.

Table 2. The thermal regime in 2011 and 2018-2019 (source: www.tutiempo.net)

Vaslui Monthly average temperature (°C)			
Month / Year	2011	2018	2019
January	-0,4	1,7	0,3
February	3,6	5,1	1,3
March	4,9	9	6,5
April	12,1	11,9	10,1
May	17,3	15,1	16,5
June	19,2	18,4	19,3
July	21	20,4	23,2
August	22,3	20,7	22,9
September	14,1	16,7	16,6
October	11,2	11,5	8,8
November	7,9	6,3	3,2
December	-1,1	2,5	-0,1

As climatic conditions influence very much the entire activity in agrobiocenosis, data on the rainfall regime of the two areas considered in the study were also recorded.

The monthly rainfall between 2011 and 2018-2019 in Iasi County is shown in Table 3 and the relative humidity in Table 4

In 2011 the total rainfall was 676.58 mm and the average humidity for the 365 days was 70.5%.

In 2018 the total rainfall was 314.6 mm and the average humidity for the 365 days was 72.8%.

The year 2019 was until the end of the research on September was shown as a year with a small amount of rainfall, which was not a great advantage for plant cultivation.

Monthly rainfall between 2011 and 2018-2019 in the Vaslui area is shown in Table 5 and relative air humidity in Table 6.

Table 3. The fainfall regime in 2011 and 2018-2019 (source: www.tutiempo.net)

Iasi Monthly average rainfall (mm)			
Month / Year	2011	2018	2019
January	56,38	29,8	38,61
February	19,4	16,4	31,24
March	28,6	11,4	22,36
April	24,8	19,9	26,41
May	30,2	19,2	70,62
June	83,6	62,1	27,18
July	26,2	38,7	61,22
August	100,6	38,6	30,47
September	30,73	16,4	13,3
October	43,17	18,1	44,25
November	42,16	30,3	43,1
December	36,0	13,7	31,9

Table 4. Relative humidity in Iasi, 2011 and, 2018-2019 (source: www.tutiempo.net)

Iasi Average monthly of relative humidity%			
Month / Year	2011	2018	2019
January	93	81,2	86,9
February	88,9	79,0	49,8
March	84,3	72,1	65,2
April	73	77,53	64,9
May	74,7	62,9	74,2
June	80,6	73,2	78,3
July	63	69,5	65,9
August	58,1	65,5	69,2
September	77,4	56,1	17,6
October	81,1	73,0	34,6
November	89	78,0	87,4
December	90,5	82,12	80,1

Table 5. The fainfall regime in, 2011 and 2018-2019 (source: www.tutiempo.net)

Vaslui Monthly average rainfall (mm)			
Month / Year	2011	2018	2019
January	24,88	18,8	32,78
February	17,53	19,05	19,82
March	74,92	13,47	9,14
April	47,24	46,74	42,67
May	65,53	70,09	31,51
June	142,76	38,34	51,82
July	35,81	57,4	4,83
August	40,38	34,29	21,84
September	61,95	34,78	26,6
October	90,17	50,29	64,2
November	28,43	34,54	37,0
December	2,28	63,47	47,2

Table 6. Relative humidity in, 2011and 2018-2019 (source: www.tutiempo.net)

Vaslui Average monthly of relative humidity %			
Month / Year	2011	2018	2019
January	83,4	88,7	83,6
February	75,2	73,4	73,7
March	70,2	61,2	62,8
April	64,1	69,5	56,7
May	63,1	72,2	68,2
June	76,8	69,5	68,7
July	64,1	74,5	57,1
August	63	68,6	58,9
September	76,6	70,8	62,3
October	79,1	76	68,5
November	83,4	82,1	83
December	87,4	87,5	82,9

In Vaslui County, in 2011 the total rainfall was 631.88 mm and the average humidity over the 365 days was 73.8%.

In 2018 the total rainfall was 481.26 mm and the average humidity for the 365 days was 74,5%.

Just like in Iasi County, and in Vaslui County in 2019 until September there was a small amount of precipitation.

Moisture and precipitation also play an important role in the life and dynamics of the insect population, as water is indispensable for the vital processes of the organism, thus the moisture of the food substrate acting as a limiting factor in the dynamics of the beetle population.

Results on the status of the collections in the two stages (2011 and 2018-2019) in the two experimental stationary

We have achieved the following results following biological material collections during the research period at the Iasi stationary.

The *Carabidae* family has totaled the highest number of specimens collected with genus, determined/identified by means of determiners (Du Chatenet G., 1990 ; Grozea I., 2006; Panin I., 1951; Reitter E., 1908; Rogojanu V., 1979): *Amara, Bembidion, Brachinus, Calathus, Carabus, Cicindela, Cymindis, Harpalus, Microroles, Notiophilus, Pseudophonus, Ophonus* and *Pterostichus* totalized 1103 specimens collected (Table 7).

In 2011, were collected 224 specimens from biological material collections and the species with the highest abundance were represended by: *Harpalus distinguendus*, *Amara eurynota, Calathus fuscipes*, *Anisodactylus signatus*, *Brachinus crepitans*, *Pterostichus niger* and *Notiophilus palustris.*

In 2018, the number of insect species collected totaled 416 and the species with the the most abundant of specimens collected was collected: *Harpalus distinguendus, Pseudophonus rufipes Amara eurynota*, *Amara familiaris*, *Pseudophonus griseus*, *Brachinus crepitans* and *Carabus coriaceus.*

In 2019, the number of insects collected totaled 463 and the species were found to have the the most abundant of specimens collected: *Calathus fuscipes*, *Pseudophonus rufipes, Carabus coriaceus, Harpalus distinguendus* and *Pterostichus cylindrichus.*

Table 7. The family, genera and species belonging to *Carabidae* collected from apple orchards to stationary from Iasi

Family	Genus	Name of species	Year			Total
			2011	2018	2019	
Carabidae	Abax	Abax carinatus Duft	-	**3**	**4**	**7**
	Amara	Amara eurynota Panz.	21	60	0	81
		Amara aenea Dejean	1	0	8	9
		Amara crenata Dejean	3	2	1	5
		Amara familiaris Duft.	12	35	7	54
		Amara similata Gyll	3	-	-	3
	Anisodactylus	Anisodactylus signatus Paz	15	17	15	47
	Attagemus	Attagemus unicolor	5	-	-	5
	Brachinus	Brachinus crepitans L.	11	25	18	54
		Brachysomus hirtus	19	-	-	19
	Calathus	Calathus ambiguus Payk	-	2	13	15
		Calathus fuscipes Goeze	45	1	96	142
	Carabus	Carabus besseri Fischer	2	7	1	10
		Carabus coriaceus L	5	20	48	73
		Carabus cancellatus Illyg	3	4	-	7
		Carabus scabrisculus Ol	-	-	21	21
	Harpalus	Harpalus distinguendus Duft.	53	110	35	198
		Harpalus tardus Panz.	2	3	2	7
		Harpalus calceatus Duft	-	-	2	2
	Leistus	Leistus ferrugineus L	-	-	1	1
	Microlestes	Microlestes maurus Strm.	3	-	-	3
	Nebria	Nebria picicornis F	-	9	2	11
	Notiophilus	Notiophilus palustris Duft.	10	-	-	10
	Pterostichus	Pterostichus niger Schaller	11	2	11	24
		Pterostichus cylindrichus Hr.	-	5	20	25
	Pseudophonus	Pseudophonus griseus Panz.	-	35	66	101
		Pseudophonus rufipes Müll	-	72	83	155
	Poecilus	Poecilus cupreus		2	3	5
	Zabrus	Zabrus tenebrioides Goeze.		2	6	8
16 genus		**29 species**	**224**	**416**	**463**	**1103**

From biological material collections during research periods at Vaslui stationary we achieved the following results.

The Carabidae family whose representatives we selected from the total number of species collected totaled the highest number of specimens collected 293 are represented by the genres: *Brachysomus, Amara, Carabus, Calathus, Harpalus, Licinus, Leystus and Pterostichus* (Table 8).

In 2011, 89 specimens were collected from biological material collections and the species with the highest abundance were: *Amara aenea , Amara crenata, Attagemus unicolor, Calathus fuscipes, Harpalus calceatus, Harpalus tardus, Leistus ferrugineus, Licinus cassideus and Pterostichus vulgaris*

In 2018, the number of insect species collected totaled 104specimens and the species with the highest number of specimens collected were: *Amara aenea, Attagemus unicolor, Brachysomus hirtus, Harpalus aeneus, Harpalus tardus, Licinus cassideus* and *Pterostichus vulgaris* .

In 2019, the species of insects collected totaled 10 specimens , and the species were the highest collected were: *Attagemus unicolor, Calathus fuscipes, Carabus violaceus, Harpalus aeneus, Harpalus calceatus, Harpalus tardus, Leistus ferrugineus, Licinus cassideus* and *Pterostichus vulgaris.*

Table 8. The family, genera and species belonging to *Carabidae* collected from apple orchards to stationary from Vaslui

	Genus	Name of species	Year			Total
			2011	2018	2019	
Carabidae	Amara	Amara aenea Dejean	10	24	1	35
		Amara crenata Dejean	4	2	1	7
		Amara eurynota Panz.	2	1	-	3
	Attagemus	Attagemus unicolor	12	26	15	53
	Brachysomus	Brachysomus hirtus	2	9	-	11
	Calathus	Calathus fuscipes Goeze	21	0	18	39
	Carabus	Carabus violaceus L.	2	4	22	28
	Harpalus	Harpalus aeneus F.	-	12	7	19
		Harpalus calceatus Duft.	3	2	5	10
		Harpalus distinguendus Duft.	2	1	12	15
		Harpalus pubescens	1	-	1	2
		Harpalus tardus Panz.	12	13	11	36
	Leistus	Leistus ferrugineus	4	2	-	6
	Licinus	Licinus cassideus L.	5	3	3	11
	Pterostichus	Pterostichus vulgaris L.	9	5	4	18
10 genus		**20 species**	**89**	**104**	**100**	**293**

Following the centralization of the data obtained in the three years of research from the two areas located in the counties of Iasi and Vaslui in two apple tree plantations, we recorded (Table 9) a total of 34 species of carabidae with a total of 1395 specimens, of which 329 specimens were collected in 2011, in 2018 we recorded 520 specimens and 2019 recorded the highest number of carabids equal to 563.

Table 9. The family, genera and species belonging to *Carabidae* collected from apple orchards

Family	Genus	Name of species	Total Iasi	Total Vaslui	Total samples
Carabidae -	Abax	Abax carinatus Duft	7	-	7
	Amara	Amara eurynota Panz.	81	3	84
		Amara aenea Dejean	9	35	44
		Amara crenata Dejean	5	7	12
		Amara familiaris Duft.	54	-	54
		Amara similata Gyll	3	-	3
	Anisodactylus	Anisodactylus signatus Paz	47	-	47
	Attagemus	Attagemus unicolor	5	53	58
	Brachinus	Brachinus crepitans L.	54	-	54
		Brachysomus hirtus	19	11	30
	Calathus	Calathus ambiguus Payk	15	-	15
		Calathus fuscipes Goeze	142	39	181

	Carabus	Carabus besseri Fischer	10	-	10
		Carabus coriaceus L	73	-	73
		Carabus cancellatus Illyg	7	-	7
		Carabus scabrisculus Ol	21	-	21
		Carabus violaceus L	-	28	28
	Harpalus	Harpalus distinguendus Duft.	198	15	213
		Harpalus aeneus F.	-	19	19
		Harpalus tardus Panz.	7	36	43
		Harpalus calceatus Duft	2	10	12
		Harpalus pubescensL.	-	2	2
	Leistus	Leistus ferrugineus L	1	6	7
	Licinus	Licinus cassideus L.	-	11	11
	Microlestes	Microlestes maurus Strm.	3	-	3
	Nebria	Nebria picicornis F	11	-	11
	Notiophilus	Notiophilus palustris Duft.	10	-	10
	Pterostichus	Pterostichus niger Schaller	24	-	24
		Pterostichus cylindrichus Hr.	25	-	25
		Pterostichus vulgaris H.	-	18	18
	Pseudophonus	Pseudophonus griseus Panz.	101	-	101
		Pseudophonus rufipes Müll	155	-	155
	Poecilus	Poecilus cupreus L	5	-	5
	Zabrus	Zabrus tenebrioides Goeze.	8	-	8
16 genus		**29 species**	**1102**	**293**	**1395**

CONCLUSIONS

In 2011 and 2018-2019, in the apple fruit-growing plantations belonging to the Iasi and Vaslui stationary, Barber-type traps were installed for collecting species of epigeous carabids. These traps functioned from May until August.

The collected saples of carabids (1395 specimens) belong to 17 genus and 34 species.

The species with the highest number of collected samples were: *Harpalus distinguendus* (213 specimens), *Calathus fuscipes* (181 specimens), *Pseudophonus rufipes* (155 specimens), Pseudophonus griseus (101 specimens), *Amara eurynota* (84 specimens), *Carabus oriaceus* (73 specimens), *Attagemus unicolor* (58 specimens), *Amara familiaris* (54 specimens), *Brachinus crepitans* (54 specimens), *Anisodactylus signatus* (47 specimens), *Amara aenea* (44 specimens), *Harpalus tardus* (43 specimens), *Carabus violaceus* (28 specimens), *Pterostichus cylindrichus* (25 specimens), *Pterostichus niger* (24 specimens) and *Carabus scabrisculus* (21 specimens).

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AVANTGUARD OF ROMANIAN RESEARCH: *MURRAYA KOENIGII* L. - AN AMAZING FLOWER AND MEDICINAL PLANT

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Abstract

Murraya koenigii L. (Rutaceae), known as the curry leaf tree is native to the Indian subcontinent and cultivated in tropical and subtropical regions. The fresh and roasted leaves of the curry tree are widely used in the Asiatic cuisine for seasoning different dishes. The essential oil can be extracted from its flowers and leaves and used in the cosmetic industry. The Ayurvedic medicine uses the leaves of Murraya koenigii, due to their high therapeutic properties. The curry leaf tree can grow very well in our climate but must be protected from freezing. The research pointed out that the bioactive principles of analyzed curry tree plants are highly valuable for medicinal purposes. The morphological analyses showed that the leaves are pinnate, with 11-25 leaflets, each leaflet 2-4 cm long and 1-2 cm broad. The anatomical analyses of the leaf showed a cuticularized upper & lower epidermis and a biseriate palisade & spongy parenchyma.

Key words*: curry tree, therapeutic proprieties, plant morphology, leaf anatomy.*

INTRODUCTION

Murraya koenigii belongs to family *Rutaceae* which has 150 genera and over 1600 species. It is a tropical to sub-tropical evergreen shrub or small tree native of India where is called "sweet neem" or "kadi patta" and Sri Lanka and widely distributed in South-East Asia, Australia and the Pacific Islands (Jain et al., 2012). It can grow up to 4-6 m in height and diameter is about 15-40 cm with short trunk. The foliage is the real standout and is arranged alternately on the stem. The mature leaves are pinnate and 15-30 cm long, with 11-25 leaflets, each leaflet length is about 2-4 cm and breadth is of 1-2 cm; margins irregularly create, petioles 2-3 mm long (Chauhan, 1999). Curry leaf plant has an upright, open growth appearance, aromatic and pungent leaves. Its flowers are bisexual, complete, sweetly scented, white, stalked, regular with the diameter of fully opened flower being in average of 10-12 cm inflorescence, terminal cymes each bearing 60-90 flowers (Saini & Reddy, 2013). The flowers are followed by green pea sized berry-like fruits which turn into black shiny when ripe. The fruit is edible, but the seed is poisonous and should be removed prior to use. *Murraya koenigii* leaves are highly aromatic and very valued for seasoning various dishes. Curry tree has been used in cooking for hundreds of years. Curry tree leaves are a section of the Indian seasoning called curry which is a compilation of many herbs and spices. In India, Thailand and other Asian countries, curry leaves are an essential ingredient of sauces, soups, stews, fish curries (Malaysia, Indonesia), rice, potatoes, etc., but they play a more delicate, aromatic role in the background of the flavor profile. Curry leaves have the strongest aroma and most pronounced flavor when used freshly picked, but they retain their flavor even after drying. In their fresh form, they have a short shelf life and they do not keep well in the refrigerator (Hema et al., 2011). Relating the curry leaf tree uses, its flowers are used in the parfum & cosmetics industry and to religious ceremonies. The wood of curry tree is highly valued to handcraft and tools making. *Murraya koenigii* is also called "the magic plant of the Indian Sub-Continent" thanks to its culinary and medicinal properties. In the Ayurvedic medicine, the root, bark and

leaves of *Murraya koenigii* are used from centuries to treat and prevent different diseases and body disorders (Muthulinggam & Subramanian, 2015; Parrota, 2001). The bioactive compounds of curry tree are used in many tribal and villages communities by traditional medical practi-tioners, but nowadays these compounds are widely used in pharmaceutic industry, as well (Shyamapada, 2016; Harish et al., 2012). The leaves of *Murraya koenigii* have amazing medicinal properties, such as: anti-diabetic (Arunselvan et al., 2006; Achyut et al., 2005; Grover et al., 2003; Yadav et al., 2002; Khan et al., 1995), antioxidant (Arunselvan et al., 2007; Baliga et al., 2003; Deshmukh et al., 1986; Singh & Sharma, 1978), antimicrobial (Dheeraj et al., 2014; Vinuthan et al., 2004; Goutam & Purohit, 1974;), anti-inflammatory (Mathur et al., 2011; Muthumani et al., 2009), analgesic (Das & Biswas, 2012; Gupta et al., 2011), hepato-protective (Roy et al., 2014; Pande et al., 2009), anti-hypercholesterolemia (Khan et al., 1996) and anti-atherosclerotic (Vinuthan et al., 2007), anti-mutagenic (Zahin et al., 2013), anti-anxiety and anti-depressant (Sharma et al., 2017). *Murraya koenigii* leaves have cancer fighting properties (Ghasemzadeh et al., 2014; Iyer et al., 1990). They have the ability to control diarrhea, dysentery, indigestion (Adebajo et al., 2004). The fresh juice of curry leaves is also used as eye treatment for certain eye disorders, especially in arresting the development of cataract (Surbhi & Meenakshi, 2016). Using curry leaves for hair problems, such as dandruff, hair fall and greying hair can be extremely beneficial (Saini, 2013). The stems are used as anthelmintic, febrifuge, foul ulcer (Sharma et al., 2011), in treatment of vomiting and flatulence (Kumar et al., 1999; Nadkarni, 1995; Parmar & Kaushal, 1982); they are very popular for strengthening the gums and teeth (Sivakumar & Meera, 2013). The bark and the roots are used as a stimulant, cure eruptions and against fungi (Das et al., 1965) and bites of poisonous animals (Rao et al., 2013; Shivkanya et al., 2009). Leaves are rich in many bioactive compounds like, alkaloids (Chakrabarty et al., 1997), volatile oils (Chowdhury et al., 2008), furocoumarins, terpenoids, tannins, glycosides, polyphenols and flavonoids (Adebajo & Reisch, 2000). In addition, leaves are also rich in fibers, minerals and vitamins, such as calcium, carotene, vitamin A, phosphorous, calcium, iron, vitamin B2, niacin and vitamin C (Narendhirakannan et al., 2005). The two carbazole alkaloids namely mahanimbine and koenigine found in these leaves showed higher antioxidant activities (Singh, 2014; Kale & More, 2014). Mahenine, a carbazole alkaloid isolated from curry leaf, has been reported to induce apoptosis in human myeloid HL-60 cancer cells by down regulating cell survival factors and disrupting the cell cycle progression (Bhattacharya et al., 2010; Parmar et al., 2010; Roy et al., 2004). Anti-tumorigenic activity of a curry leaf extract against MCF-7 breast cancer cells has been reported by Handral et al. (2012) and Kok et al. (2012). Antioxidant effect of curry leaf powder in chicken and goat meat products has also been reported (Devatkal et al., 2012; Biswas et al., 2006). *Murraya koenigii* bio-active compounds showed a real inhibition of *Staphylococcus epidermidis*, *Streptococcus uberis*, *Pseudomonas aeruginosa*, *Escherichia coli, Corynebacterium gravis* and *Bacillus cereus* on different studies applied to human-beings and animals, as well (Mathur et al., 2010).

MATERIALS AND METHODS

Curry leaf plants (*Murraya koenigii* L.) originated from Hyderabad, India were taken into cultivation last 3 years in our country. They grew as potted plants outdoor between May and October and indoor in the cold season. Fresh leaves & leaflets, rachis and stem fragments were anatomically studied. The material was sectioned manually using razor blades in order to obtain semi-permanent and permanent slides for microscopic study. The sections were clarified with chloral hydrate for 24 hours, then washed and stained with carmine alaunate and green iodine (Luchian & Teodosiu, 2019; Georgescu et al., 2015; Savulescu & Hoza, 2010). The anatomical analyses were performed at the University of Agronomical Sciences and Veterinary Medicine of Bucharest and the bio-chemical analyses were performed at "Chem-Analyst" Laboratory, district 6, Bucharest. The pictures and measurements were made using Leica

DM1000 LED, Leica DFC295 Video Camera, Leica S8 APO Stereo Microscope, Optika Microscope and Sony photo camera. The pictures were taken using light microscope with different magnifications. *Murraya koenigii* leaves have been studied by various authors (Mohammad et al., 2013; Syed et al., 2015).

RESULTS AND DISCUSSIONS

There are three varieties of *Murraya koenigii*: regular, dwarf and gamthi. The regular type has a fast growing; it is tall and the most culinary used. The dwarf variety is 25-30 cm in height and it's not typically grown to use culinary, but as a decorative plant or ground cover. The gamthi curry plant is only 15-20 cm in size, being the most fragrant and flavorful and grows the slowest of the three. As concern the growing conditions, all three varieties of *Murraya koenigii* are frost tender. In the experiment were used plants of *M. koenigii* belonging to regular variety (Figures 1-3).

Figure 1. *Murraya koenigii* seedlings - year 2018

Figure 2. Plants of *Murraya koenigii* - year 2019

Figure 3. Regular variety of *M. koenigii* - year 2020

In cold countries, tropical species need protection from freezing. As an indoor plant in temperate areas, curry leaf develops and flowers from spring till autumn. During the resting period in the winter months, the curry tree leaves might turn yellow and drop off; this generally means that the plant is about to go dormant (Figure 4). In the cold season, *M. koenigii* needs full sunlight or partial shade and a bright windowsill away from any draughts or radiators where the temperature range stays above at least 12°C. The plants can tolerate a temperature range up to 37°C, but below 16°C their growth is affected.

Figure. 4. Mature plants of *Murraya koenigii* affected by winter rest (downward foliage dropping) - year 2020

A real problem with indoor cultivation of *Murraya koenigii* is represented by the air dryness mixed with a warm temperature which encourage the attack of red spider mite and specific pathogens, such as mildew (Figures 5 and 6).

Figure 5. Plants of *M. koenigii* slightly attackedby mildew and red spider mite - year 2020

Murraya koenigii needs and a rich, well-draining soil. Curry leaf tree can be cultivated in a wide range of soils. Red sandy loam soil is ideal for curry leaf cultivation. *Murraya koenigii* plants don't need a particular soil pH level to survive. It doesn't require much in terms of water and it is semi - drought tolerant.

Figure 6. *Murraya koenigii* plants at the end of the winter - year 2020

Three years after sowing, *Murraya koenigii* started to bloom (Figure 7). Daily 1-2 flowers of its inflorescence were fully opened and their fragrance could be smelled from few metres distance. The flowers of *Murraya koenigii* don't produce any allergic reaction. For this reason, curry leaf tree doesn't represent any risk to children or animals, but a sharp attention must be paid to its seeds which are poisonous.

Figure 7. Inflorescences of *Murraya koenigii* - springtime of the year 2020

Staking could be necessary if planted in a windy location. The trees sucker vigorously, so a limited space is a good option. They grow well in a pot and they are very attractive plants for verandas, balconies, terraces or backyards.

M. koenigii propagation is mainly through cuttings or seeds. For raising seedlings, well-ripe fruits are collected from high-yielding curry leaf plants. Within three to four days of fruits collection, the seeds should be pulped and sown in nursery beds or poly bags filled with a mixture of 1:1:1 sand, soil and farmyard manure. Seeds germinate in three weeks. One-year-old seedlings are planted in a pot. Growing *M. koenigii* from seed is not an easy task because the germination process is fickle. For propagating curry tree through cuttings, will be selected a branch or a stem which is neither flexible or nor stiff, means semi-hard. The cutting must be of 5 mm in diameter and about 8-10 cm long with 4 to 5 leaflets. In about ten days, the cutting will produce new buds. Curry leaf can also be propagated by root suckers process. It is very important to start pruning curry leaf tree early in its growth stage. In order to keep plant tight, compact and producing the greatest amount of tasty foliage, is recommended pruning it annually, removing near 1/3 from the top/branches. Within a few weeks, the plant will generate side shoots and fill out into a mini-grove of numerous bushy stalks with pointed leaves. That is a sign that curry leaf plants are healthy and thriving. Curry leaves are picked or harvested 15 months after planting. Commercial harvest can be started from three-year-old plants.

Macroscopic determinations

Murraya koenigii is semi deciduous, unarmed aromatic shrub. Curry leaves are shiny and smooth with paler undersides, having characteristic aroma. Mature leaves are pinnate, estipulate, having reticulate venation and having ovate lanceolate with an oblique base with 11-21 leaflets which are short stalked, alternate, gland dotted and having 0,5 cm long petiole. The young stems are green in color with sweet aromatic odor and characteristic taste. The outer surface is smooth, soft and glabrous.

The mature stems of *M. koenigii* are dark brown (unpeeled) and yellow-brown (peeled) in color with slight aromatic odor and specific taste. The outer surface is smooth and hard. The fracture of bark is splintery.

During three years (2018-2020) was analyzed the growth of *Murraya koenigii* plants, by measuring yearly their height, number of

pinnate leaves and leaflets. As regard the number of leaves and leaflets, in the Table 1, is observed that every year their number is increased averagely with near 50%, to all variants of the experience. Following this rate, in 2020, the number of leaves and leaflets has become double than in 2018. Relating the height of *Murraya koenigii* plants, their size increased yearly with 20-25 %, as is showed in Figures 8 and 9. *Murraya koenigii* grows slowly at seedling stage and 2-3 years after sowing and is a sensitive plant to the attack of different pathogens. Starting with 2019, the curry leaf plants were fertilized twice a month with *Cropmax* and *Vitaflora* (alternatively) - both fertilizers containing macro and micronutrients. This slow growth rate of curry leaf in our country is due to the metabolic adjustments to stressors, mainly the weather conditions (temperature and humidity fluctuations, light intensity). Due to these aspects, *Murraya koenigii* requires a professional care and a strict pest control.

Microscopic determinations

Sections of leaf, midrib, rachis and stem of *Murraya koenigii* L. were analyzed using optic and electronic microscopes.

Leaf anatomy

The presence of the following was observed: epidermis (upper and lower), mesophyll with palisade & spongy parenchyma, epidermal cells, stomata, vascular bundle and epidermal trichomes (Figures 10, 11, 12 and 13).

Table 1. Pinnate leaves and leaflets number of *Murraya koenigii* L. between the years 2018-2020

Variant / Average	Number of pinnate leaves 2018	Number of pinnate leaves 2019	Number of pinnate leaves 2020	Number of leaflets/plant 2018	Number of leaflets/plant 2019	Number of leaflets/plant 2020
V1	4	7	11	25	58	103
V2	5	9	14	32	81	119
V3	4	8	13	29	76	110
V4	6	11	16	38	97	152
V5	13	18	22	107	145	188
V6	14	18	23	116	141	199
V7	14	19	25	113	148	215
V8	15	22	27	127	185	232
V9	14	21	28	119	167	239
V10	16	23	31	144	196	263
Average	**10.5**	**15.6**	**21.0**	**85.0**	**129.4**	**182.0**

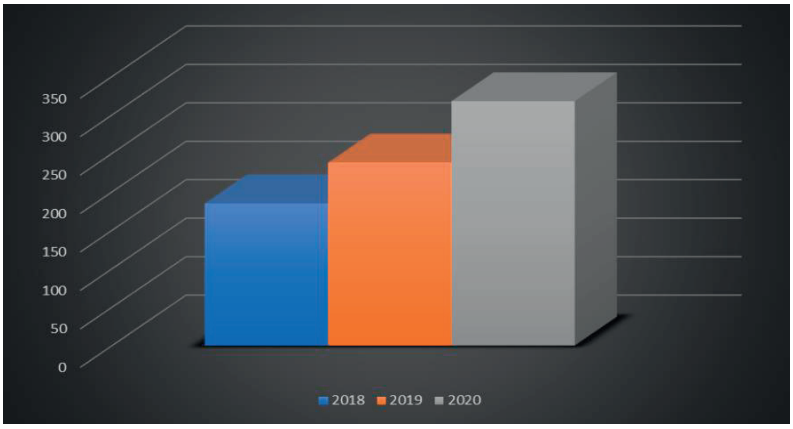

Figure 8. Average yearly increase of *Murraya koenigii* plants

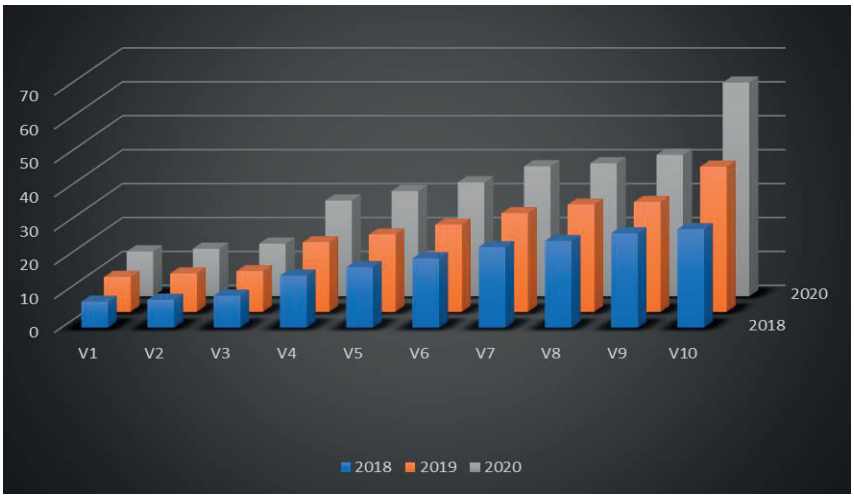

Figure 9. Growth dynamics of *Murraya koenigii* plants

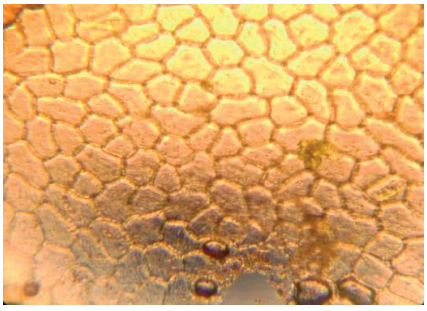

Figure 10. Upper epidermis

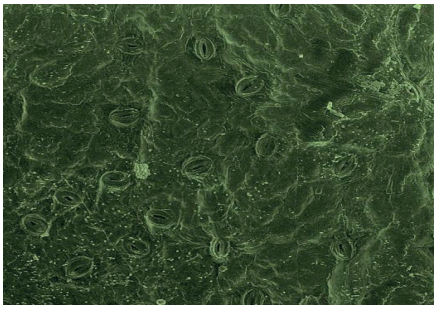

Figure 12. Lower epidermis viewed at SEM

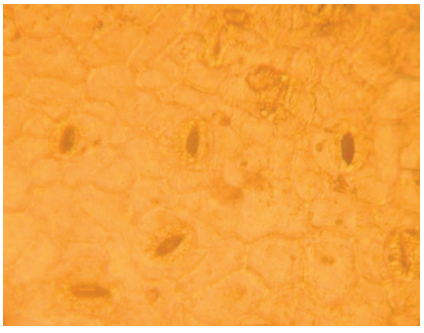

Figure 11. Lower epidermis with stomata

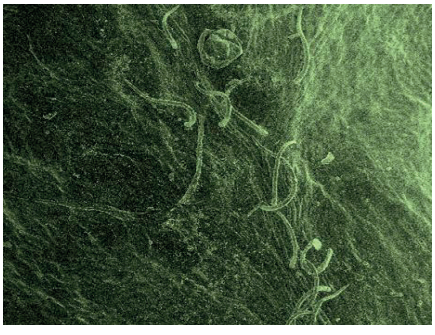

Figure 13. Lower epidermis with trichomes (SEM)

The epidermis presents a layer of rectangular cells. The upper epidermis is covered with cuticle. The lower epidermis presents stomata. The mesophyll presents a palisade tissue with 2 layers of cells (Figure 14).

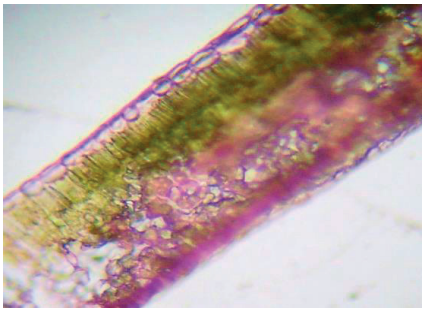

Figure 14. Cross section of *Murraya koenigii* leaf

The ground tissue is oval to polygonal. The parenchyma cells present chlorophyll contents and vascular bundle. Towards the vascular bundles, a bundle of fibers is present on the upper side. The xylem and phloem portions of vascular bundle consist of their basic elements. The leaf shows the presence of non-glandular trichomes (with higher density on the mid - vein of the abaxial leaf surface), parenchymatous pith in petiole, long pericyclic fibers in the midrib (Jain et al., 2017). Trichomes distribution on the leaf was restricted predominantly to the mid-vein and edges, only one type of long, slender non-glandular trichomes was observed on the leaf and stem. The trichomes are vital to the survival of the plant and collectively provide an adaptive edge to the plant by regulating evapotranspiration, shielding from harmful rays and deterring insects and predators. The trichomes play a key role in plant defense, especially with regard to phytophagous insects, avoiding insect feeding & oviposition responses and the nutrition of larvae (Fahn, 2000; Duke, 1994; Thomas, 1991).

Midrib anatomy

The leaf through midrib region flattens towards upper and lower epidermis; unicellular, non-glandular trichomes arise from the abaxial epidermis; adaxial and abaxial hypodermis bi or tri seriate, composed of isodiametric collenchy-matous cells with calcium oxalate crystals; vascular bundle forms an arch with the adaxial xylem and the abaxial phloem (Figure 15).

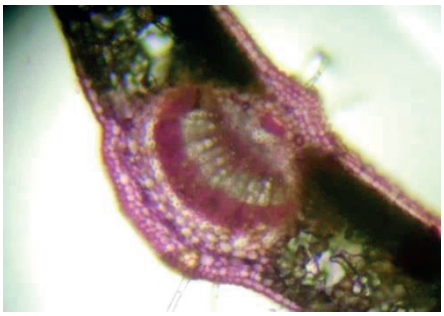

Figure 15. Midrib of curry tree leaf - cross section

Rachis anatomy

The epidermis of curry tree rachis has a single layer of isodiametric cells which are covered with a thick cuticle; unicellular, non-glandular, curved, cortex many-layered, parenchymatous, hypodermal cortical cells are smaller, isodiametric, intercellular spaces; abundant pyramidal calcium oxalate crystals, present in cortical cells; cortex in the hypodermal region is traversed by lysigenous cavities; vascular bundle is encircled by a ring of 2 or 3 layered sclerenchymatous pericycle, xylem parenchyma and xylem fibers with thick walls; phloem is situated towards the periphery of xylem ring and contains sieve tubes, companion cells, phloem parenchyma and phloem fibers; medullary rays are uniseriate; the cells contain calcium oxalate crystals; the pith is large containing parenchymatous cells (Duartea & Deburb, 2005), as are showed in the Figures 16 and 17.

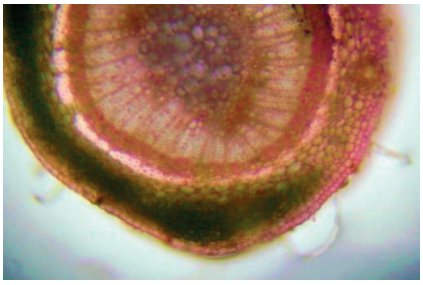

Figure 16. Rachis of curry tree

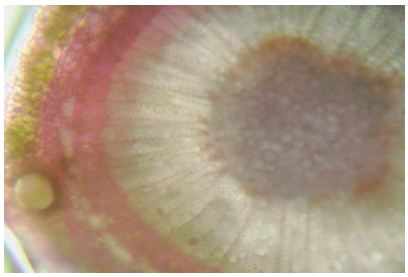

Figure 17. Rachis of *M. koenigii* with oil gland

Stem anatomy

The cross section of *Murraya koenigii* stem (Figure 18) presents: uniseriate epidermis covered with a thick cuticle; it presents trichomes. The cortex is composed by 3-6 layers of cells. It presents oil glands (Figure 19), positioned just below the epidermis, a vascular. Bundle and pith in the center. In the cortex was observed a sclerenchymatic ring composed by 2-3 layers of cells (Jarald et al., 2008).

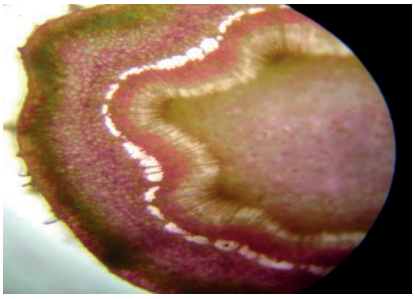

Figure 18. *Murraya koenigii* stem - cross section

The sclerenchymatic cells concentration is effective in withstanding environmental pressures, such as damage by wind and to fend off herbivores (Metcalfe & Chalk, 1988).

The pith consists of walled polygonal, parenchy-matous cells bearing starch grains - clearly showed at the transverse section of *M. koenigii* pith. The pith serves also to produce crystals and other ergastic substances (Dutta, 2001). The vascular system consist of a cylinder of xylem produced towards the inside and a cylinder of phloem outward. Vascular bundles are of collateral, conjoint and open type.

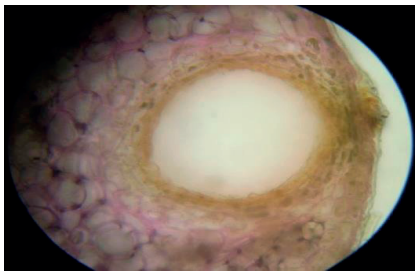

Figure 19. *Murraya koenigii* stem with oil gland

Biochemical compounds analyses

Fresh leaves of Murraya *koenigii* L. were collected in March 2020 from the plants taken into the experience. The biochemical analyses were done at "Chem-Analyst" private laboratory, 101 L Timisoara Avenue, district 6, Bucharest. Total polyphenol (TPP) content was determined using Folin - Ciocalteu method (Johansen, 1940), as modified by Yi and Wetzstein (2010), Vaidya et al. (2013) and further modified by Meena et al. (2017). The analyses showed that, at the humidity of 11.24%, the total content of polyphenols, expressed in gallic acid was of 1259.61 mg %; the flavones content, expressed in rutin was of 0.30 mg %; caffeic acid 1.97 g %; carotene 0.043 g %; calcium 42.03 mg/kg; iron 129.43 mg/kg; magnesium 23.89 mg/kg; zinc 55.33 mg/kg.

The antioxidants reduce the risk of cancer and heart diseases (Lafuente et al., 2009) and enhance immunity; therefore, it is imperative that they should be supplied to body through external sources (Ganesan et al., 2013). Plant-based antioxidants block free radicals produced through oxidation, thus inhibiting chain reactions that could lead to degradation and death of cells in human body. Antioxidants are marketed as beneficial to us by protecting the skin, supporting digestion and the immune system (Das et al., 2011).

The total phenols of *Murraya koenigii* leaves showed an amount with ~ one quarter higher than the polyphenols amount contained in fresh fruits of *Aronia melanocarpa* L. (black chokeberries) - recognized to be very rich in polyphenols, as is shown in the study of Toma (Singh) et al. (2019).

The flavonoids (including the flavones) are precious for their antioxidative, antimicrobial, anticancer and cardio protective effects. Rutin

also strengthens the capillaries, and, therefore, can reduce the symptoms of hemophilia. It also may help to prevent venous edema of the legs. Rutin, as ferulic acid, can reduce the cytotoxicity of oxidized LDL cholesterol and lower the risk of heart disease. The amount of rutin found in *M. koenigii* leaves was near similar to that existing in the fruits of *Aronia melanocarpa*, well known for its high number of flavonoids, as is shown in the study of Atanassova & Bagdassarian (2009).

The amount of caffeic acid (a very important antioxidant) found *in Murraya koenigii* leaves was ~ four times larger, in comparison with the amount of caffeic acid found in the leaves of *Psidium guajava* (guava), a tropical plant very rich in antioxidants which was studied in Romania mainly for its pharmacological potential, as is mentioned in the scientific paper of Toma (Singh) and Luchian (2019). Caffeic acid is considered to have many health benefits, including anti-inflammatory, anticancer and antiviral properties. It may help boost the performance of athletes and improves significantly the hair health & growth (In resources for authors: Caffeic acid. (n.d.) Healthline. Retrieved from: https://www.health-line.com/health/caffeic-acid). Curry tree leaves contained high values of carotene, calcium, magnesium, iron and zinc, with a real contribution to the health. Thus, the beta- carotene is an antioxidant that converts to vitamin A and plays a very important role to improve cognitive function, promote good skin health, reduce macular degeneration, contribute to lungs health, prevent cancer, etc. (In resources for authors: Benefits of Beta Carotene and How to Get It. (n.d.) Healthline. Retrieved from: https://www.healthline.com/health/betacaroten-benefits). Calcium is important for overall health. Almost every cell in our body uses calcium in some way. Some areas where our bodies use calcium is in our nervous system, muscles, heart and bones (In resources for authors: Why is Calcium Important. (n.d). Medicine.wisc.edu. Retrieved from: https://www.medicine.wisc.edu/rheumatology/why - calcium - important). Magnesium helps to maintain normal nerves and muscles function, supports a healthy immune system, keeps the heartbeat steady and helps bones remain strong. It also helps adjust blood glucose levels. Magnesium aids in the production of energy and protein. Diets high in protein, calcium or vitamin D will increase the need for magnesium (In resources for authors: Magnesium in diet. (n.d.). Medline Plus. Retrieved from: https://medlineplus.gov/ency/article/002423/magne -sium-in-diet). Iron is the mineral responsible for the production of hemoglobin, the substance in red blood cells that helps blood carry oxygen throughout the body. If the amount of iron is not enough, then is installed the anemia which determines the following effects: tiredness, lack of concentration, breathing problem, dizziness, headache, feeling cold (In resources for authors: Why is Iron Important in My Diet? (n.d.). Med.umich.edu. Retrieved from: http://www.med.umich.edu/cancer/files/why-is-iron-important.pdf). Zinc is needed for the body's defensive (immune) system to properly work.

It plays a role in cell division, cell growth, wound healing and the breakdown of carbohydrates. Zinc is also needed for the senses of smell and taste (In resources for authors: Zinc in diet. (n.d.). Medline Plus. Retrieved from: https://medline-plus.gov/ency/article/002416/zinc-in-diet.) It is essential for bones, skin, nails and hair beauty, as well. Polyphenols & flavonoids activity at *Murraya koenigii* was studied by many researchers, such as Ghasemzadek et al. (2014), Rani et al. (2012), Ningappa et al. (2008), Tachibana et al. (2003), Middleton et al. (2000). The bio-compounds analyses of potted curry tree plants grown in our country revealed quite similar values of them cultivated or growing wildly in their origin places. It points out that the pharmacologic potential of *Murraya koenigii* is very precious, making this plant a possible solution for preventing and treating a large range of diseases and body disorders.

CONCLUSIONS

In Romania, *Murraya koenigii* L. cultivation represents an avantguard research.

Curry leaf tree has showed a good adaptability in our country.

In the climate of Romania, *Murraya koenigii* needs protection from freezing.

The curry tree grew very well as potted plant outdoor between May and October and indoor in the cold season.

Murraya koenigii started to bloom three years after sowing.

The plants can tolerate a temperature range up to 37°C, but below 16°C their growth is affected.

During three years (2018-2020) was analyzed the growth of *Murraya koenigii* plants, by measuring yearly their height, number of pinnate leaves and leaflets.

Every year the number of leaves and leaflets have increased averagely with near 50%, to all variants of the experience.

The height of *Murraya koenigii* plants has increased yearly with 20-25%.

Murraya koenigii grows slowly at seedling stage and 2-3 years after sowing.

The slow growth rate of curry leaf in our country is due to the metabolic adjustments to stressors, mainly the weather conditions, such as temperature & humidity fluctuations and light intensity.

A special attention to *Murraya koenigii* care and pest control must be paid.

Curry leaf plants cultivated indoor in the cold period have faced a medium attack of red spider mite and a slight attack of mildew.

Sections of leaf, midrib, rachis and stem of *Murraya koenigii* L. were anatomically studied. The microscopic analyses showed that the growth and development of potted curry tree plants follows the same dimensions and characteristics as those of their origin places.

The bio-compounds analyses of potted curry tree plants grown in our country revealed quite similar values of them cultivated or growing wildly in their native areas.

The study points out that the pharmacologic potential of *Murraya koenigii* L. is very precious, making this plant a possible solution for preventing and treating a large range of diseases and body disorders.

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