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CONTENT

CUPRINS

STUDY ON THE CHEMICAL COMPOSITION OF SOME BABY-FOOD AND JUICE PRODUCTS FOR CHILDREN

STUDIU ASUPRA COMPOZIȚIEI CHIMICE A UNOR PRODUSE DE TIP BABY-FOOD ŞI SUCURI DESTINATE COPIILOR

EPERJESSY Diana Beatrice[1], TROFIN Alina[2]*, TRINCĂ Lucia Carmen[2], UNGUREANU Elena[2], SANDU Tatiana[2]

*Corresponding author e-mail: alina.trofin@gmail.com

Abstract. *Blended food or baby-food products marketed for children contain a source of protein, often meat, or can be made entirely from cereals, fruit blends and vegetables. These products must be appropriate from the point of view of both nutritional and safety. Fruit or vegetable juices are recommended as a good source of vitamins and as an additional source of water for healthy infants and young children. With all their benefits, the addition of sugar from the marketed products should be carefully controlled so as not to lead to obesity or metabolic disorder. This paper includes the determination of the content of ions present in three commercially available juices and three types of baby-food from fruits, vegetables and meat.*

Key words: baby-food, juice, fruits, vegetables

Rezumat. *Hrana pasată sau produsele de tip baby-food comercializate pentru copii, conțin în compoziția lor o sursă de proteine, de multe ori carnea, sau pot fi în totalitate din cereale, amestecuri de fructe dar şi legume. Aceste produse trebuie să corespundă din punct de vedere al siguranței dar şi din cel nutritiv. Sucurile de fructe sau legume sunt recomandate ca fiind o bună sursă de vitamine şi o sursă suplimentară de apă pentru sugarii sănătoşi şi copiii mici. Cu toate beneficiile acestora, adausul de zahăr din produsele comercializate trebuie atent controlat pentru a nu conduce la obezitate sau dereglări metabolice. Lucrarea de față include determinarea conținutului unor ioni prezenți în trei sucuri comercializate şi în trei tipuri de hrană pasată din fructe, legume şi carne.*

Cuvinte cheie: hrană pasată, sucuri, fructe, legume

INTRODUCTION

In the first two years of life of the child, optimal nutrition promotes healthy growth and improves cognitive development. It also reduces the risk of becoming overweight or obese and offers the possibility of developing later in life.

For blended food products intended for infants and young children, the following must be taken into account when meat (beef, poultry, fish and organs) is used as a source of protein:

[1]"Saint Mary" Children's Clinical Emergency Hospital Iaşi, Romania
[2]University of Agricultural Sciences and Veterinary Medicine from Iaşi, Romania

- each source of meat protein (beef, poultry, fish, organs) must be greater than 25% of the total protein content;

- the protein source must not represent less than 40% of the weight of the product;

- the protein level in these sources must be greater than or equal to 1,7 g / 100 kJ (7 g / 100 kcal). (Banu, 2010)

Fruit-based baby-food is an important source of energy, basic nutrients, fiber, vitamins, minerals, polyphenols and other sources of antioxidants provided by the fruit in the composition. Along with fruit or vegetable pure, cereals of different type may be included in the mix for their high fiber content. (Mongeau *et.al*, 2001)

Fruit juices are accounted for about 50% of all fruits consumed by children aged between 2 and 18 and one third of all fruits and vegetables consumed by preschool children. Soft drinks are those products which are made from flavored concentrates, fruit or vegetable juices, fruit syrups, flavoring substances (natural or synthetic), flat or mineral water, vitamins and other substances, with or without added carbon dioxide and sweeteners (sugar, saccharin, glucose or other).

The carbohydrate concentration ranges from 11 grams (0.4 kcal / mL) to > 16 grams% (0.64 kcal / mL). The juice contains a small amount of protein and minerals. Some of the juices naturally contain a certain amount of potassium, vitamin A and vitamin C. (Dias, 2012)

Fruits and vegetables are often featured in names of commercial baby foods. Fruits are more common than vegetables in names of the 329 baby food taken into a study made in Spain.

The six most common fruits and vegetables in the names were all relatively sweet: apple, banana, tomato, mango, carrot and sweet potato. Their percentage in the foods ranged from a median of 94% for sweet-spoonable to 13% for dry-savory products. Fruit juice was added to 18% of products. (Garcia *et al*, 2016)

This paper aims to analyze the acidity and some of the main ions content in three commercial baby food products made from mixed fruits, mixed vegetables and chicken and three soft drinks based on concentrated juice (oranges; raspberry and apple; apple, carrot and orange).

MATERIAL AND METHOD

The following soft drink brands were analyzed, with the characteristics offered by the producers:

P 1 – Orange soft drink from Tymbark;

P 2 - Raspberry and apple soft drink from Figo Kids;

P 3 – Carrot and fruit (apple and orange) juice with honey from Tedi;

P 4 – Fruit blended baby food (orange, peach, pineapple) from Hipp;

P 5 – Vegetables mixed baby food (carrot, potato, peas-cauliflower) from Hipp;

P 6 – Baby food mixture with chicken meat from Hipp.

Fig. 1 Samples of juices and baby-food products taken into analysis

The following physical and chemical parameters were tested: moisture and dry matter (by oven drying method at 105^{0}C), titratable acidity (volumetric method), nitrites content (Griess reagent colorimetric method and Spekol 1100), chloride content (Mohr method with silver nitrate reagent), sulphite content (iodometry titration method) and phosphate content (sulphuric molybdenum reagent colorimetric method and Spekol 1100). In order to determine the considered parameters, especially the ions content, the juices were filtered and the baby-food samples were extracted with distilled water and also filtered.

RESULTS AND DISCUSSIONS

Moisture and dry matter were initially determined in order to asses the water / mineral and nutrients ratio intake of the considered products (fig. 2).

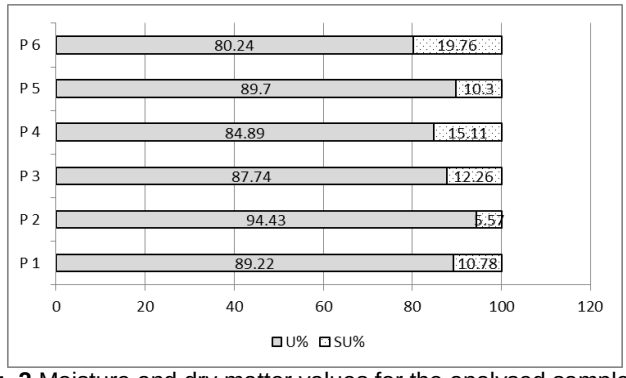
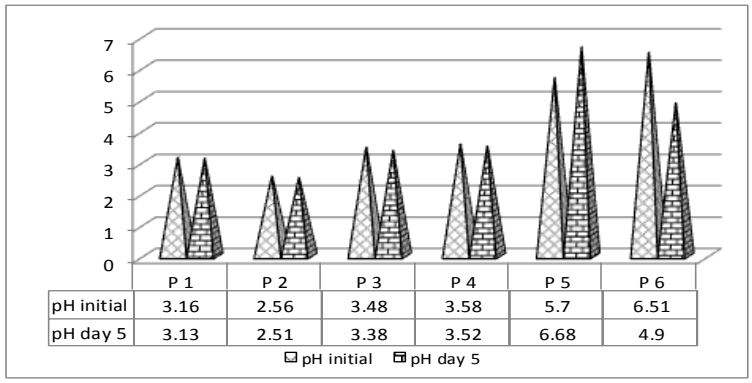


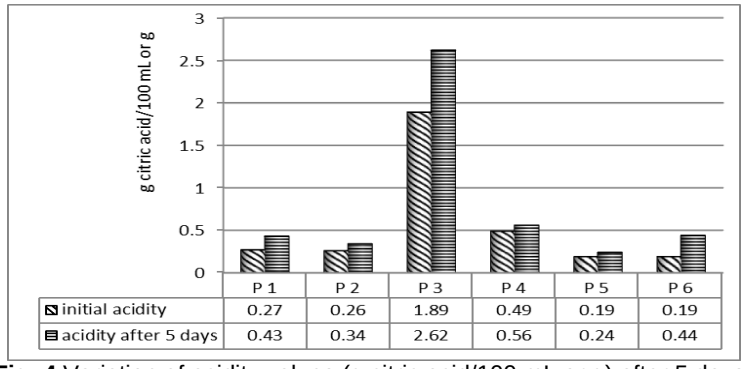
Fig. 2 Moisture and dry matter values for the analysed samples

The pH values were determined when the jars and packeges were open and again after five days in the refrigerator. In correlation to the determined pH values, the acidity was masured for each sample, initially and after five days, expressed in g citric acid/100 mL or g of sample. Recorded data for these two parameters are shown in figures 3 and 4.



	P 1	P 2	P 3	P 4	P 5	P 6
pH initial	3.16	2.56	3.48	3.58	5.7	6.51
pH day 5	3.13	2.51	3.38	3.52	6.68	4.9

Fig. 3 Variation of pH-values during 5 days storage in refrigeration conditions



	P 1	P 2	P 3	P 4	P 5	P 6
initial acidity	0.27	0.26	1.89	0.49	0.19	0.19
acidity after 5 days	0.43	0.34	2.62	0.56	0.24	0.44

Fig. 4 Variation of acidity values (g citric acid/100 mL or g) after 5 days

Chloride ions come from the raw material and from the addition of salt or other preservatives. From the analysis of the obtained results it was found that the sample P3 (carrot juice and fruit - apple + orange - with added honey) recorded the highest value for chloride ion content of 0.3748 g Cl - per 100 mL of sample (fig. 5).

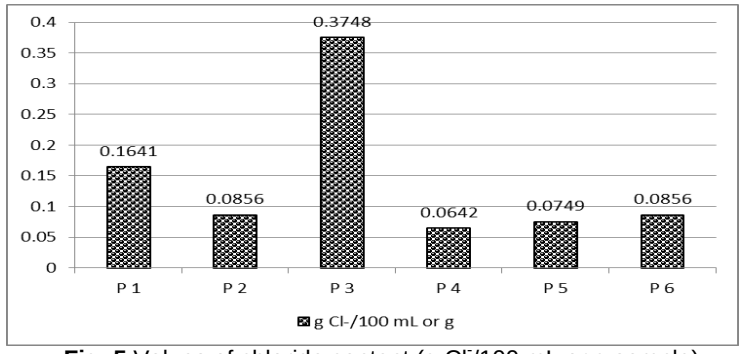


Fig. 5 Values of chloride content (g Cl^-/100 mL or g sample)

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) approved in 2002 an acceptable daily intake (ADI) of 0-3,7 mg / kg bodyweight for nitrates and of 0-0,07 mg / kg for nitrites. JECFA also states that ADI does not apply to infants under the age of 3 months (EFSA, 2008). The obtained values are presented in figure 6.

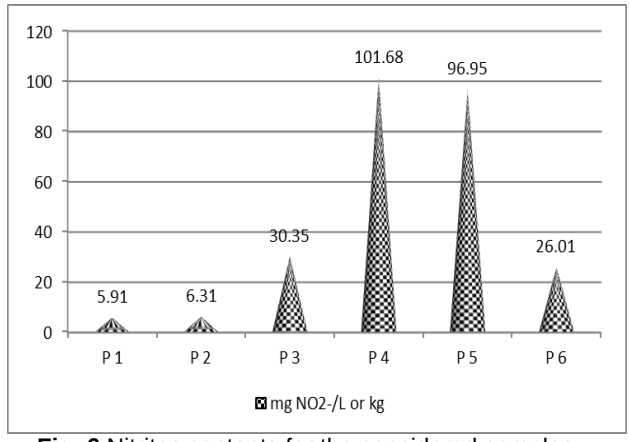


Fig. 6 Nitrites contents for the considered samples

Usually, sulphites are used as preservatives for beverages, but in the case of chicken based baby-food, the sulphite content was higher, with a value of 116.88 mg in 100 grams sample (fig. 7).

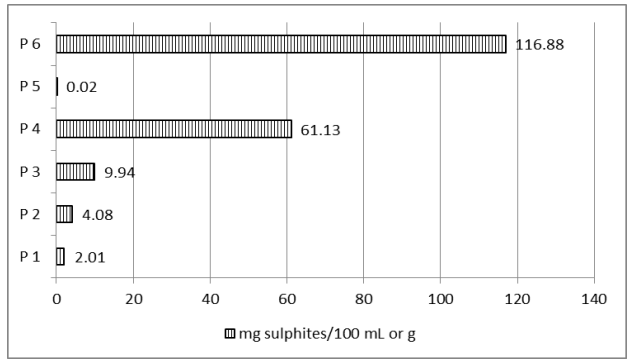


Fig. 7 Sulphites content expressed as mg/100 mL or g sample

For chicken based baby-food, with 40% meat, only phosphate free ions in the deproteinized extract were dosed, therefore the P4 and P5 samples from vegetables and fruit have higher values. Knowing that the maximum allowed level for phosphate ion in food for children between 1 and 13 years is 3000 - 4000 mg per day, it is observed that the samples fall within the tolerable limits, considering only the free phosphate ions in the extract for sample P6 (fig. 8).

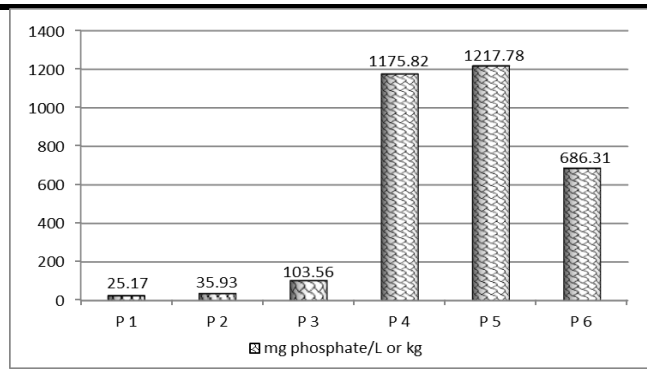


Fig. 8 Phosphate content expressed as g phosphate ion/kg sample

CONCLUSIONS

1. In terms of pH values and variation, the vegetables based and the chicken-based blended baby-food registered the highest values and a modification of 1, respectively 1.4 pH units after five days;

2. From the analysed juices, the highest free acids content, both initial and after 5 days, registered for carrot, apple and orange juice with honey from Tedi;

3. The same product showed highest values for chloride ion, as well, but a daily portion could not exceed the limit, considering a total allowed intake of 2 g of salt (1.21 g Cl^-) a day for children aged between 1 - 3 years;

4. The fruit and vegetable blended baby-food (P4 and P5) presented higher amounts of nitrites, most likely from the processed raw material and the fertilizers used in their culture;

6. The vegetable-based baby-food P5 presented a very low content in sulphite ions, but the highest amount of phosphate; in rest, the blended food products presented higher values for these ions than the juices, as normal.

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ANALYSIS OF CHEMICAL PARAMETERS FOR THREE TYPES OF RECONSTITUTED MILK POWDER

ANALIZA UNOR PARAMETRI CHIMICI PENTRU TREI TIPURI DE LAPTE PRAF RECONSTITUIT

EPERJESSY Diana Beatrice[2], TROFIN Alina[1], UNGUREANU Elena[1], TRINCĂ Lucia Carmen[1], SANDU Tatiana[1]

[*]Corresponding author e-mail: alina.trofin@gmail.com

Abstract. *The infant formula means a breast milk substitute specifically made to meet the nutritional requirements of infants during the first few months of life until appropriate complementary feed is introduced. Milk intake in the baby's diet is recommended for at least the first two years of life, maternal - at least six months, the animal or the formula of reconstituted milk powder - further, due to the essential nutritional principles in growth and physical and cognitive development. The chemical parameters analyzed in the present paper follow the quality of three milk powders (infant formula, next step formula and lactose-free formula) and three types of water (marketed, tapped and filtered) used for reconstitution with regard to acidity and content in nitrite, chloride, sulphite and phosphate ions.*

Key words: milk powder, nitrite, chloride, sulphite, phosphate

Rezumat. *Formula pentru sugari înseamnă un înlocuitor de lapte matern fabricat special pentru a satisface, prin el însuşi, cerinţele nutriţionale ale sugarilor în primele luni de viaţă până la introducerea hranei complementare adecvate. Aportul de lapte în alimentaţia copilului este recomandat cel puţin în primii doi ani de viaţă, matern – minim şase luni, animal sau formula de lapte praf reconstituit – în continuare, datorită principiilor nutritive esenţiale în creşterea ;i dezvoltarea fizică şi cognitivă.*

Parametrii chimici analizaţi în prezenta lucrare urmăresc calitatea a trei formule de lapte praf (de început, de continuare şi delactozat) şi a trei tipuri de apă (comercializată, de robinet şi filtrată) folosite pentru reconstituire, în privinţa aciditătii şi a conţinutului în ioni azotit, clor, sulfit şi fosfat.

Cuvinte cheie: lapte praf, azotit, clor, sulfit, fosfat

INTRODUCTION

Some categories of infant and young children baby-food, especially powdered milk formulas with different peculiarities, are frequently recommended by pediatricians, by age group and according to the nutritional needs of each case.

"Infant formulas" means those foods which are intended for the particular nutritional use of infants during the first 4 to 6 months of life and which may satisfy themselves the nutritional requirements of this category of persons.

[1]University of Agricultural Sciences and Veterinary Medicine Iasi, Romania
[2] Saint Mary Emergency Children Hospital Iasi, Romania

"Follow-on formulas" means foods for particular nutritional uses in children over 4 months of age and which constitute the main liquid component of the progressively diversified diet of this category of persons (Usturoi, 2008)

Ingredients as well as new techniques in the dairy industry minimize the difference between formulas and maternal milk through molecular biology, allowing for the widespread production of recombinant proteins in human milk and bioactive substances with proven effects on nutrient use and other health benefits.

When developing a new or modified formula for infants, the goal is to reproduce the benefits of breast milk (Vivatvakin, 2010).

The water used for the preparation of milk formulas is very important and therefore requires increased attention. On the milk cans there are instructions indicating the optimal temperature of the water used to prepare the formula, but the water source is not specified, only that it must be safe to consume. But it is recommended to use for this purpose microbiologically tested tap water or bottled flat water with a low degree of hardness.

Also, the level of nitrites in water must be below 10 mg/L as a maximum allowed limit for the water used for reconstitute powder milk formulas. Therefore, water from untested wells, especially from rural areas, is inappropriate for use in small children's diet.

Cold storage also influences the physical-chemical parameters of the milk powder, with very significant effects on titratable acidity, humidity and dry matter content. (Semeniuc *et al*, 2012)

This paper aims to analyze changes in physicochemical parameters for three types of formulas (infant, follow-on, lactose free) combined with three types of water (tap, filtered and bottled) during storage at 4-6^{0}C.

MATERIAL AND METHOD

The following samples were analyzed, with the characteristics offered by the producers in case of the formulas:

P 1 – Bottled Baby spring flat water;
P 2 – Filtered tap water;
P 3 – Tap water;
P 4 – Bebelac 1 infant formula 0 – 4 months;
P 5 – Bebelac 2 follow-on formula 6 – 12 months;
P 6 – Topfer lactose free formula.

The following physical and chemical parameters were tested: moisture and dry matter (by oven drying method at 105^{0}C) for the milk formulas, pH (potentiometric method), titratable acidity (volumetric method and expression in mg lactic acid/100 mL), hardness (volumetric complexometry method and expression in German degrees) for the analyzed water types, nitrites content (Griess reagent colorimetric method and Spekol 1100), chloride content (Mohr method with silver nitrate reagent), sulphite content (iodometry titration method) and phosphate content (sulphuric molybdenum reagent colorimetric method and Spekol 1100). In order to determine the considered parameters, especially the ones using colorimetric methods, the reconstituted milk samples were deproteinized and filtered.

The considered samples were numbered according to the original samples, therefore resulted nine reconstituted milk samples (for example P4-1 = Bebelac 1 formula reconstituted with bottled Baby spring flat water).

RESULTS AND DISCUSSIONS

As regards the moisture and dry matter of the analyzed powder milk formulas, the values are shown in table 1.

Table 1

Humidity and dry matter values for the analysed milk formulas

Sample	Humidity (%)	Dry matter (%)
P 4	2.4339	97.5661
P 5	2.0283	97.9716
P 6	1.2469	98.7530

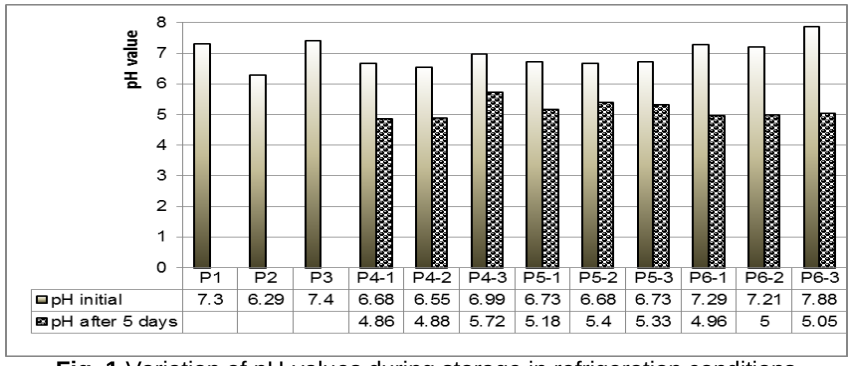
Hardness values for the types of water used in combination with the formulas were tested through complexometry method using a standard 0.001 M solution of Na_2EDTA and eriochrome black T as indicator. The lowest value was registered for P 1, the commercial Baby spring flat water (tab. 2).

Table 2

Hardness value in German degrees for tested water samples

Sample	Humidity (%)
P 1	7.1496
P 2	8.1629
P 3	10.9215

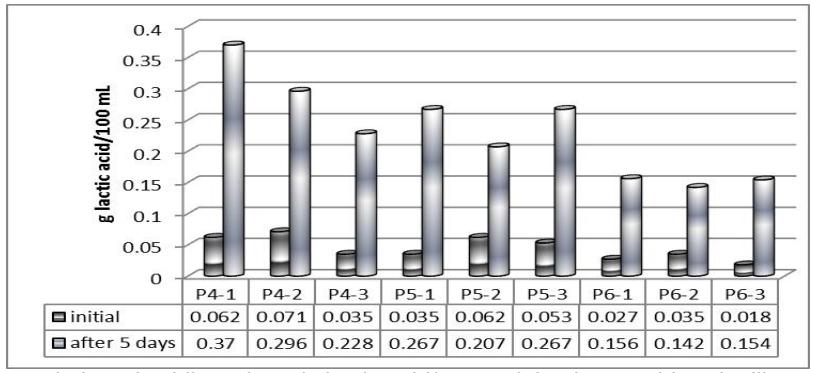
The pH values were determined initially when the formulas were mixed with heated water and again after five days, the samples of reconstituted milk being maintained at 4-6°C. Recorded data are shown in figure 1, observing that the biggest drop in pH value was registered for the P6-3 combination.



	P1	P2	P3	P4-1	P4-2	P4-3	P5-1	P5-2	P5-3	P6-1	P6-2	P6-3
pH initial	7.3	6.29	7.4	6.68	6.55	6.99	6.73	6.68	6.73	7.29	7.21	7.88
pH after 5 days				4.86	4.88	5.72	5.18	5.4	5.33	4.96	5	5.05

Fig. 1 Variation of pH-values during storage in refrigeration conditions

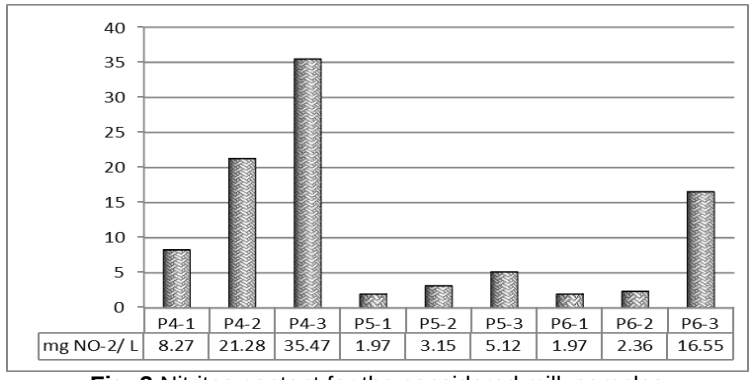
Acidity was expressed in g lactic acid/100 mL and the determinations were also made initially and after five days from the reconstitution of the milk samples. The obtained values are presented in figure 2.



	P4-1	P4-2	P4-3	P5-1	P5-2	P5-3	P6-1	P6-2	P6-3
initial	0.062	0.071	0.035	0.035	0.062	0.053	0.027	0.035	0.018
after 5 days	0.37	0.296	0.228	0.267	0.207	0.267	0.156	0.142	0.154

Fig. 2 Variation of acidity values (g lactic acid/100 mL) for the considered milk samples

The acceptable daily intake established by World Health Organization for nitrates is 0-3,7 mg / kg bodyweight and for nitrites is 0-0,07 mg / kg. It is also stated that these values do not apply to infants under the age of 3 months (EFSA, 2008; Yeh *et. al*, 2013), therefore the nitrite content (adding the reduced nitrates) for the milk samples was determined and presented in figure 3.



	P4-1	P4-2	P4-3	P5-1	P5-2	P5-3	P6-1	P6-2	P6-3
mg NO-2/ L	8.27	21.28	35.47	1.97	3.15	5.12	1.97	2.36	16.55

Fig. 3 Nitrites content for the considered milk samples

From the analysis of the chloride ion in the reconstituted milk types it was found that in general, the sample P4 and P5 (Bebelac 2 and Topfer) recorded higher values for chloride content, combined with all three types of water (fig. 4).

The sulphite content was higher also for the reconstituted P4 and P5 formulas, varying slightly with the water used; the only sample sulphite free was P4-1, the mixture of Bebelac 1 and bottled Baby spring flat water (fig. 5).

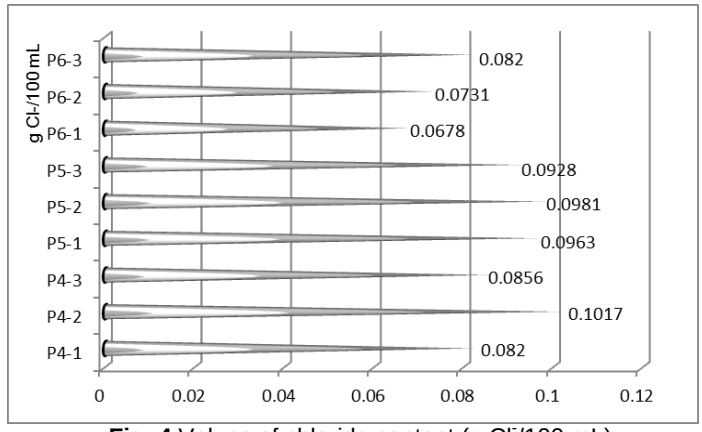


Fig. 4 Values of chloride content (g Cl^-/100 mL)

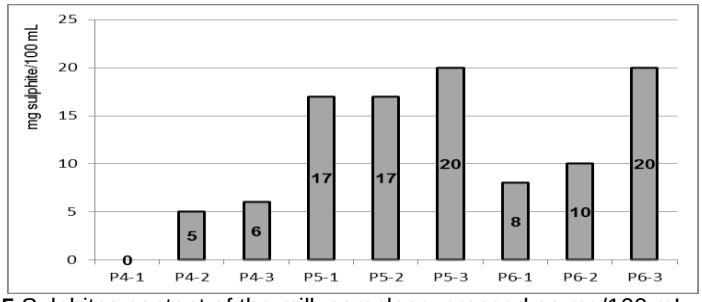


Fig. 5 Sulphites content of the milk samplesexpressed as mg/100 mL

The maximum allowed intake for phosphate ion in food for children 1 to 3 years old is 3000 mg per day; considering that milk is the main source of phosphate for infants, we tested these reconstituted formulas, observing that the highest values were obtained for P6 (Topfer lactose free formula), followed by P4 (Bebelac 1) (fig. 6).

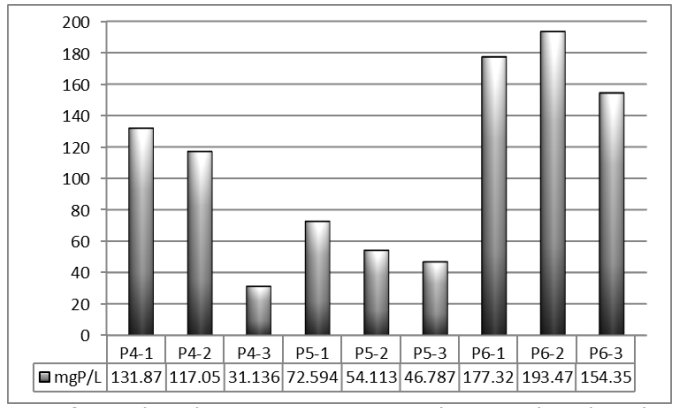


Fig. 8 Phosphate content expressed as mg phosphate ion/L

CONCLUSIONS

1. Dry matter content of the considered three brands of formula varied between 98.75 – 95.56%;

2. Most of the reconstituted milk samples dropped 1 or 2 units of pH in five days during refrigeration, except P6-3, where pH decreased with 2.83 units;

3. Free acids content increased in five days of storage for all the milk samples three times or more; the sample with the biggest difference was P4-1;

4. The chloride content varied from 0.0678 to 0.1017 g Cl^-/100 ml, with small differences between samples;

5. The lowest nitrites content was found for P1 formula, in general, no matter the added water type considered;

6. Sulphite content showed smaller values also for Bebelac 1 infant formula, while Topfer lactose free formula provided the highest intake of phosphate ions.

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GRAVITATIONAL SEDOMENTOMETRICAL ANALYSES APPLIED TO THE SARKANDA GRASS LIGNIN

ANALIZE SEDIMENTOMETRICE GRAVITAȚIONALE APLICATE LIGNINEI DIN IARBĂ

UNGUREANU Elena[1*], UNGUREANU O.C.[2], TROFIN Alina[1]
ARITON Adina-Mirela[3], JITĂREANU Carmenica Doina[1], POPA V.I.[4]
[*]Corresponding author e-mail: eungureanu@uaiasi.ro

Abstract. *The lignin stands out by a very large range of applications in extremely various domains. The adsorption-desorption capacity, ion exchange capacity and its catalytic properties are just a few specific characteristics which are emphasizing the importance of harnessing the lignins. In this paper is shown by sedimentometrical analyses that lignin can be used in agriculture and zootech. The sarkanda grass lignin (L_2) offered by the Granit Recherche Developement S.A. company, Lausanne-Schwitzerland was synthesized from annual plants.*
Key words: sedomentometrical analyses, sarkanda grass lignin, density, pycnometer, sedomentometrical curves

Rezumat. *Lignina se remarcă printr-o gamă foarte largă de aplicații în domenii extrem de diverse. Capacitatea de absorbție-desorbție, capacitatea de schimb ionic, proprietățile catalitice sunt doar câteva repere specifice care recomandă şi evidențiază importanța valorificării ligninelor. În această lucrare, se arată prin sedimentarea gravitațională, că lignina se poate utiliza în agricultură şi zootehnie, Lignina sarkanda grass lignin (L_2) oferită de firma Granit Recherche Developement S.A. Lausanne-Elveția a fost sintetizată din plante anuale.*
Cuvinte cheie: analize sedimentometrice, lignină din iarbă, densitate, picnometru, curbe sedimentometrice

INTRODUCTION

Lignin comprises as much as 30 percent of wood's mass and therefore the development of novel technologies for the use of lignin in composites is an attractive, environmentally intelligent goal (Area *et al,* 2014).

Until now, the attempts to develop an industry, which would put in good use residual lignin, had only partial success. This situation is due especially with a lot of difficulties met in usage and processing of the lignin (Popa, 2015, 2016).

These difficulties are connected to its complex chemical structure, different chemical units, chemical bonds with other components and a great capacity of this

[1]University of Agricultural Sciences and Veterinary Medicine Iasi, Romania
[2] "V.Goldiş" West University of Arad, Romania
[3]Station for Cattle Breedind, Dancu, Iasi, Romania
[4] "Gh. Asachi" Technical University of Iasi, Romania.

natural aromatic polymer to be modified irreversibly during chemical reactions or under the action of physical agents. It is known that lignin has a very complex structure, which varies depending on the plant species, separation method and modification reactions that may induce particular characteristics (Ungureanu, 2011).

One of the pursued directions refers to ameliorating the properties of lignin resulted from processes of chemical wood and annual plants processing through reactions that may lead to increased functionality and diversification of the fields of use for lignin. Due to its regeneration, capacity through photosynthesis, vegetal biomass and its components (including lignin) will become in the future sources of raw material with a high degree of capitalization (Ungureanu *et al,* 2006, Căpraru *et al*, 2009).

In this paper is shown by sedimentometrical analyses that lignin can be used in agriculture and zootechnics, cellulose and paper industry, constructions, metallurgy or as catalysts.

MATERIAL AND METHOD

The following materials have been used:

- Sarkanda grass lignin (L_2), offered by Granit Recherché Dévelopement, having the characteristics described in table 1.

Table 1

Characteristics of sarkanda grass lignin L_2

Characteristics	L_2
Relative humidity, %	5.20
Ash, %	2.40
pH in suspension	3.20
Manganese, %	0.69
Nitrogen, %	1.2
Uronic acid, %	0
Solubility in acids, %	2
Insolubility in acids, %	87

- RS-71 Tensio-tixometer gravimetric sedimentation balance;
- Steel ball crusher;
- Toluene;
- Distilled water.

Methods

- picnometer method;
- gravitational sedimentometrical method.

Work procedure*:* 45 g of sarkanda grass lignin were weighed, crushed for 30 min. and dissolved in 1L of distilled water.

The electrostatic forces of attraction between the hydroxylic groups of the lignin and the dipoles of the dissociated water are so powerful that a colloidal-hydric aggregate is formed and its volume is smaller that the sum of volumes that interact (water-lignin). Experimental data were statistically processed with the aid of the *Unscrambler* application.

RESULTS AND DISCUSSIONS

Based on the standard curve (fig.1) 10 sedimentation curves were plotted according to the dependence q(g) = f(t, s), and the experimental data are also listed in tables.

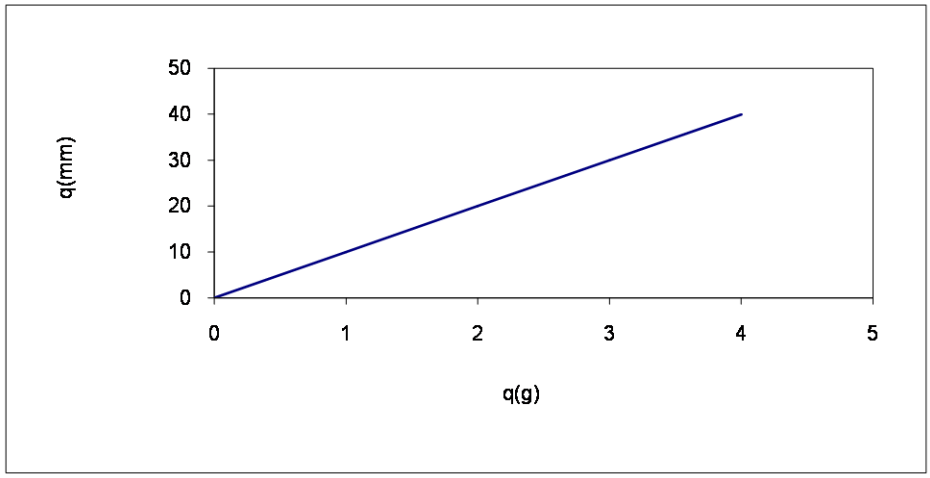


Fig. 1 The standard curve

10 sedimentation curves in q(mm) = f(t, s) coordinates were obtained using RS-71 Tensio-tixometer under constant conditions (mass lignin = 45 g/L water).

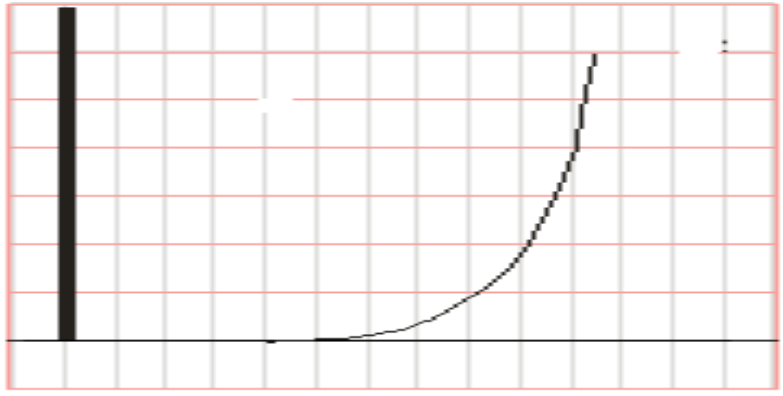


Fig. 2 Exemple of sedimentation curve

These sedimentation curves were also listed in table 2.

Table 2

Parameter values of the sedimentation curves

No det.	t (mm)	t (sec)	q (mm)	q (g)
1	3	7.85	3.51	0.25
2	6	15.7	5.1	0.41
3	9	23.49	7.0	0.59
4	12	31.40	8.0	0.60
5	15	39.25	9.0	0.73
6	18	47.10	10.6	0.82
7	21	54.95	11.0	0.86
8	24	62.80	11.4	0.85
9	27	70.65	12.0	0.90
10	30	78.50	12.6	0.98
11	33	86.35	12.7	0.98
12	36	94.20	13.0	1.02
13	39	102.05	13.6	1.03
14	42	109.90	13.6	1.04
15	45	117.75	14.0	1.11
16	48	125.60	14.0	1.11
17	51	133.45	14.6	1.18
18	54	141.30	14.6	1.18
19	57	149.15	14.7	1.18
20	60	157.00	14.8	1.18

Further on it was obtained the most expected sedimentation curve plotting the values of sediment quantity, q(g) and time t(s), for the reproductible measurements (2, 3, 4, 5, 9) (fig. 3).

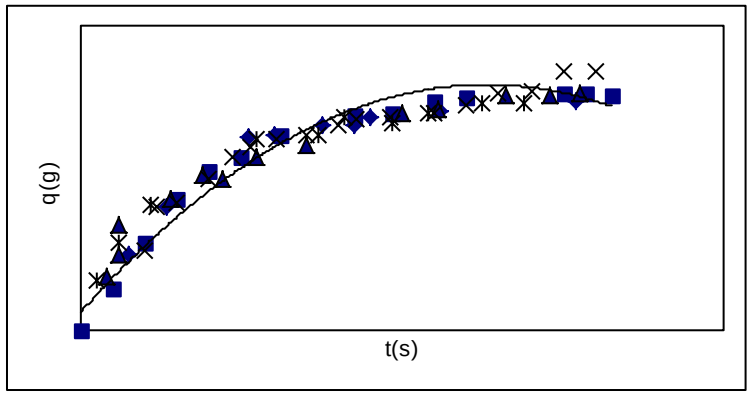
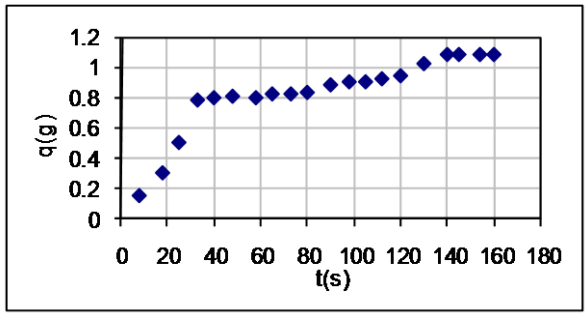


Fig. 3 General sedimentation curve

Measurements 1, 6, 7 and 10 are not reproductible due to a non-uniform distribution of the scattered particles obtained before the recordings (fig. 4a. and b.).

Based on the general theory of sedimentation in gravimetric field of micro-heterogeneous systems, the radius boundaries of the scattered particles in ground lignin were evaluated. According to the determinations performed the amount of

deposited lignin was Q = 1.18 g. In order to determine the density of lignin, the picnometer method was employed. In table 3 are presented the values obtained experimentally by weighing or theoretically determined.



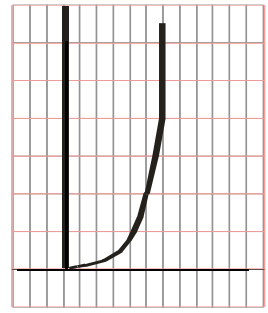


Fig. 4 Sedimentation curves using:
a. q(g) = f(t, s) dependence
b. tensio-tixometer q(mm) = f(t, min.) dependence

Table 3

Density of lignin measured by picnometer method

m_1 (g)	m_2 (g)	m_3 (g)	m_4 (g)
13.6512	14.6515	23.0630	22.4048
13.6512	14.6508	23.0622	22.4048
13.6512	14.6486	23.0642	22.4048

m_1 – empty pycnometer mass; m_2 – pycnometer mass + solid; m_3 – pycnometer mass + solid + liquid; m_4 – pycnometer mass + liquid (toluene); ρ_s - solid density

By graphical derivation of the sedimentation curve (fig.2), the sedimentation rates were obtained at certain periods of time, t = 0, 14, 30, 70, 80, 90, 100, 105 s. These rates were used to determine various fractions radii of the disperse system (tab. 4).

Table 4

Sedimentation rates corresponding to the most expected sedimentation curve for the reproductible results

Time (s)	Sediment quantity (g)	Sedimentation rates (mm/s)
0	0	0.037
14	0.21	0.0165
30	0.63	0.0142
70	0.92	0.0034
80	0.97	0.0028
90	1.01	0.0018
100	1.02	0.0011
105	1.03	0.0140

Based on the resulted sedimentation rates, the particle radii of lignin were obtained (tab. 5).

Table 5

Values of disperse particle radii of lignin obtained by sedimentation in gravitational field.

Fractions number	Dimension of superior and inferior sieve mesh (mm)	Diameter a_i (mm)	Beam r_i (mm)	
			By rieving	By sedimentation gravitational feils
1	1.25 – 1.10	1.125	0.560	-
2	1.0 – 0.05	0.815	0.402	0.33 (0)
3	0.63 – 0.25	0.440	0.210	0.1572 (14)
4	0.25 – 0.20	0.225	0.1125	0.140 (30)
5	0.20 – 0.125	0.1625	0.0811	0.0741 (70)
6	0.16 – 0.10	0.130	0.0649	0.0628 (80)
7	0.10 – 0.09	0.095	0.0474	0.0523 (90)
8	0.09 – 0.08	0.085	0.0424	0.044 (100)
9	0.08 – 0.07	0.075	0.0369	0.038 (105)

CONCLUSIONS

1.The sedimentometrical method applied in gravitational field confirms that the reproducibility of the experimental data depends on the uniform distribution degree of the analyzed disperse particle.

2.In order to determine the lignin density, the picnometer method was success fully employed.

3.The variation range of the disperse particle radius in ground lignin can be determined either by sieving or by sedimentation in gravitational field.

4.The analysis of the values obtained for particle dimensions of wheat straw lignin using both methods leads to a general conclusion that the obtained data are reproductible.

5.Using the gravitational sedimentation, it can be determined in a shorter period of time the particle dimensions comparing with the sieving method, which is a much more complex one.

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SENSORIAL AND PHYSICAL-CHEMICAL ANALYSIS OF A "000" WHEAT FLOUR TYPE OBTAINED INTO A MID CAPACITY MILL

ANALIZA SENZORIALĂ ŞI FIZICO-CHIMICĂ A FĂINII DE GRÂU TIP "000" PRODUSĂ ÎNTR-O MOARĂ DE CAPACITATE MEDIE

AVARVAREI B.V.[1], NISTOR C.E.[1*], USTUROI AL.[1]
[*]Corresponding author e-mail: is_cata@yahoo.com

Abstract. *The aim of the paper was to effectuate a sensorial and physical-chemical analyse for a "000" wheat flour type which was obtained into a mid capacity mill. Sensorial analyse of wheat flour targeted on appreciation of colour, taste, smell as well as on flour infestation degree. From physical-chemical analysis view point, the aims of the current paper were focused on the following parameters: moisture, wet gluten, ash, acidity, granulosity and falling index.*
Key words: "000" wheat flour type, sensorial characteristics, physical-chemical properties.

Rezumat. *Scopul prezentei lucrări este de a efectua o analiză senzorială şi fizico-chimică pentru făina de grâu de tip "000" care este produsă într-o moară cu o capacitate medie. Analizele senzoriale au vizat aprecierea culorii, gustului, mirosului precum şi gradul de infestare a făinii. Din punct de vedere a analizelor fizico-chimice lucrarea de faţă s-a axat pe studierea următorilor parametri: umiditate, conţinutul de gluten umed, conţinutul de cenuşă, aciditate, granulozitate şi indicele de cădere.*
Cuvinte cheie: făină de grâu tip "000", caracteristici senzoriale, proprietăți fizico-chimice

INTRODUCTION

From the ancient times cereals represented one of the main food source of mankind, fact which lead to interweaving of their processing history with mankind history and thus with material cultural history of humanity (Zaharia, 2011).

During development of civilisation and human society, cereal production as well as the specific technologies for their processing known a permanent improvement and were always adapted to food demands and needs of mankind (Avarvarei, 1999).

Cereals belong to category of agricultural plants which are cultivated for seed obtaining. In Romania, the main cultivated cereal plants are: wheat, corn, rye, barley, oat, millet, sorghum.

Due to the fact that cereal seeds have a high content in starch are also called

[1]University of Agricultural Sciences and Veterinary Medicine from Iaşi, Romania

starchy agricultural products. Cereal seeds have in their composition sugars, proteins, vitamins, mineral salts and fats, substances which are very necessary both for human nourishment as well as for animal's one (Mogârzan and Robu, 2005; Roman *et al.,* 2011).

Having in view the role and importance of wheat in human nourishment we could affirm that in parallel with evolution of human civilisation took place an evolution, respectively an improvement of its processing technologies.

The obtaining of wheat flour was at beginning a household occupation, in the way that each family had the necessary tools for its grinding. Some of the most primitive tools for flour obtaining were grindstones (fig. 1) (Alban and Chester, 2005).

Grinding of cereals using grindstones was realized by their rubbing between a larger stone, which have a plain surface and a smaller stone which was hand moved.

Fig. 1 Ancient grindstones (Alban and Chester, 2005)

a b

Fig. 2 Roman grindstone a) manual drive; b) animal drive

In ancient Egypt were founded hand carved stones which were formed from a stone provided with a central hollow, where cereals were introduced and grinded using an elongated stone, with repeated moves of arms.

Grinding of cereals in ancient Rome, was effectuated with stone mills (fig. 2), where milling process was realized by their friction between a mobile stone and a fixed one (Alban and Chester, 2005).

Initially stone drive was manually realized, using arms' force through some handles.

Then it was a gradually passed to driving of those mills by using animal power, water power as well as by using wind power (Alban and Chester, 2005).

In milling industry the technological flow for wheat flour have the following stages: reception (quantitative, qualitative) – storage – cleaning of impurities – conditioning – peeling – grinding – sifting – storage for maturation – packing – storage – delivery.

The aim of the paper was to effectuate a sensorial and physical-chemical analyse for a "000" wheat flour type which was obtained into a mid capacity mill.

Sensorial analyse of wheat flour targeted on appreciation of colour, taste, smell as well as on flour infestation degree.

From physical-chemical analysis view point, the aims of the current paper were focused on the following parameters: moisture, wet gluten, ash, acidity, granulosity and falling index.

MATERIAL AND METHOD

Sensorial characteristics of wheat flour were determined in according with Romanian national standard SR EN ISO 90:2007 (Sensorial characteristics of wheat flour).

Determination of flours' colour is based on comparison of analysed sample's colour with colour of some well established flour benchmarks. It is a quality index which is often used in practice for establishing, with some relativity, of extraction degree for certain flour. Particles which come from endosperm have a white-yellowish colour, due to yellow pigments (xanthophylls and its esters, carotene and oxidation products resulted due to chlorophyll cleaving). Parts which came from coating have a dark colour, due to flavonic pigments. By grinding a part of yellow pigments are eliminated together with bran and the most of it remain in endosperm particles which form the flour, thus determining its colour (Bordei, 2004).

To establish the colour of flour will be weighted around 50 grams of flour, which will be stretched into a rectangular layer with dimensions of 4 x 5 cm, on a wooden shovel; the layer's thickness must be around 0.5 cm. Near this layer will be stretched an equal quantity of benchmark flour, this layer must have the same dimensions. Flour will be pressed using a glass plate, and after pressing bran particles as well as other particles which could be contained by flour, will be obviously observed at surface. After that the shovel with samples, will be introduced in water into a slightly oblique position. Flour layers will be compared in dry state as well as in wet state. Moistening is realised in the following way: shovel with pressed flour sample is introduced slightly oblique into a recipient with cold water, maintaining duration is around 1 minute. The shovel with wet flour it will come out of the water and will be placed from drying, at room temperature for 5-10 minutes. After that will be examined the analysed samples in comparison with standard sample, both in diffuse light and also in direct light. During observations shovel must be placed in such a way in which the light fascicle to be perpendicular on its surface. Analyse is realised in wet state because by wetting colours became more evident and the presence of bran could be better observed.

For *determination of flavour* into a Berzelius glass will be introduced a quantity of 5 grams of flour sample and after that will be added 25 cm^3 of distillate water which was previously heated at 60-65°C. Content will be homogenized for around 1 minute, and after that glass will be covered with a laboratory watch glass and will be placed for resting for about 4-5 minutes. After that the watch glass is removed and the formed suspension will be smelled.

Flour must have a pleasant smell specific to wheat. Flours which are not suitable will present unpleasant smells. If flour presents a honey flavour this means that flour was contaminated by mites, and the presence of altered fish odour is the proof that during milling process were also grinded seeds which were attacked by blight (Mogârzan and Robu, 2005). Another way for determination of flour flavour is to take in hand a quantity at around 5 grams from flour sample and after was slightly rubbed with the other hand, it is smelled.

For *taste determination* will be taken 1 gram from analyzed flour sample, will be chewed, appreciating in this way the taste and the possible presence of some mineral impurities (soil, sand) due to characteristic noise which those one produce during mastication process. Normally flour has a sweetish taste, pleasant, characteristic to wheat. The presence of strange tastes could be due to unsuitable storage conditions or flour's infestation. Altered flour, due to rancid fats, has a bitter taste (Banu *et al.*, 1998).

Determination of infestation is based on sifting of a flour sample (0.5 kg) using a silk sieve or a number 4XX synthetic fibre fabric sieve; the remained residuum will be examined with a 5x magnifying glass, for observing the possible presence of insects or mites (live, dead or part of them). This determination is realised in according with Romanian standard demands SR ISO 6639-1/2/3/4 (Cereals and leguminous. Determination of hidden infestation with insects).

Infestation with mites could be also checked by:

- strong honey flavour of flour;
- crumbling after around one hour of a cone realised from 100 flour grams (built through a conic funnel);
- presence of some characteristic tracks on plain surface of flour (Mogârzan and Robu, 2005).

The realised physical-chemical analyses were: moisture, wet gluten, ash, acidity, granulosity and falling index.

Determination of moisture (M%) was realised by drying at oven, in according with the demands impose by standard SR EN ISO 712:2010 (Cereals and products from cereals. Moisture determination. Reference method).

Method is based on determination of mass loosening due to heating into an oven at a temperature of 130±2°C, for 60 minutes. Into a weighting ampoule, with low form and lid, which was previously heated at 130±2°C, will weighted a quantity of 5 flour grams, with a 0.001 g precision. Ampoule with flour will be placed (with the lid near it) into an oven which was previously heated at 130±2°C. It will be kept into oven at 130±2°C, for 60 minutes. Ampoule will be extracted from oven, will be covered with the lid and will be placed, for chilling till temperature of environment, into desiccators with anhydride $CaCl_2$. After chilling (30-60 minutes), ampoule will be weighted with the same 0.001 precision. It will be realised two subjected to analysis determinations for the same sample.

Moisture (%) will be calculated using formula [1] and the result will be expressed with one decimal:

$$M\ (\%) = [(m_1-m_2)/(m_1-m)] \times 100 \quad [1]$$

in which:

m is mass of empty ampoule (g);

m_1 is mass of ampoule with analyzed flour sample, before drying (g);

m_2 is mass of ampoule with analyzed flour sample, after drying (g).

As final result, will be used the arithmetic mean of those two determinations, if repeatability conditions are fulfilled. That means that the differences between two determinations effectuated in parallel, in the same laboratory, by the same operator and from the same sample aren't allowed to exceed 0.3%.

Determination of wet gluten was realized in according with Romanian standard SR EN ISO 21415-1/2/3/4/2007 (Wheat and wheat flour. Gluten content).

Protein substances under the form of gluten are separated, due to washing of batter prepared from flour sample with a NaCl solution and airing of obtained gluten.

Into porcelain mortar will be placed 25 sample grams, which were weighted with a precision of 0.01 g. It will be added 12.5 cm^3 of NaCl solution and after that, by using a pestle, will be kneaded for 3-4 minutes, till will be obtained homogenous dough. The obtained dough will be washed just after knead, manually or mechanically, with a NaCl solution, above a silk sieve.

Wet gluten content (%) will be calculated using formula [2] and the result will be expressed with one decimal.

$$\text{Wet gluten}\ (\%) = (m_1/m) \times 100 \quad [2]$$

in which:
m is mass of analyzed flour sample (g);
m_1 is mass of gluten which remained after drying (g).

As final result, will be used the arithmetic mean of those two determinations, if repeatability conditions are fulfilled. That means that the differences between two determinations effectuated in parallel, in the same laboratory, by the same operator and from the same sample aren't allowed to exceed 2% (which means 2 grams of wet gluten for 100 grams of sample).

Determination of ash was effectuated in conformity with Romanian standard SR EN ISO 2171:2010 (Determination of ash from cereals and from products obtained from it).

The standard stipulates that total mineral substances represent the residuum which is obtained after sample calcinations at a temperature of 525±25°C till a constant weight is obtained. So, into clean and dried porcelain melting pot which was previously calibrated will be weighted, using an analytical balance, 5 grams from analyzed product. The moisture from oven will be eliminated by its adjustment at a temperature of 125°C, after that being subjected to burning till carbonization at the flame of a gas burner for 10-15 minutes. At the end of carbonization operation, melting pots will be introduced, using some pliers with long arms, into calcinations oven which was adjusted at a temperature of 525±25°C where will be kept uninterrupted for 16-18 hours. Calcinations operation will be repeated by 1-2 exposures in oven for around 1 hour, till a constant weight if reached.

Ash content (%) will be calculated in according with formula [3].

$$\text{Ash (\%)} = (m_1/m) \times 100 \quad [3]$$

where:
m is quantity of analyzed product, calculated as difference between mass of melting pot with sample before drying and its calibration (g);
m_1 is ash quantity, (g), which is calculated as being the difference between mass of melting pot with ash and mass of empty pot.

Determination of falling index was realized in conformity with standard SR EN ISO 3093:2010 (Wheat, rye and flours, durum wheat and durum wheat flour. Determination of Hagberg–Perten falling index).

This index measures indirectly the amylase activity, due to fast gelling of an aqueous suspension of integral grist from wheat of flour, into a boiling water bath as well as by measurement of liquefaction produced by α-amylase to starch gel which contained into analyzed sample. Hagberg–Perten falling index is expressed in seconds and optimal values are in interval 220-280 seconds. Values over 280 seconds indicate that flours have a low amylase activity and the ones under 220 seconds show that flours have an intense amylase activity.

Determination of flour's acidity was realized in concordance with the demands imposed by standard SR 877-1996 (Wheat flour. Determination of flour's acidity).

Was utilized the method of suspension in water, so into an Erlenmeyer balloon are placed 5 grams of flour weighted with a precision of 0.01 g. Is added 50 cm^3 distillate water and the content is homogenized for 5-10 minutes, avoiding formation of lumps. At the end of homogenization will be added 3 drops of phenolphthalein and will be titrated with NaOH 0.1 n till apparition of a pink colour, which must persist for one minute. Will be realized two determinations for the same sample which was subjected to analysis.

Commonly acidity is expressed in acidity degrees, 1 acidity degree representing acidity for 100 g product, which will be neutralized by 1 cm^3 NaOH 0.1 n solution.

Acidity (degrees) will be calculated by using formula [4] and the result will be expressed with one decimal.

$$\text{Acidity (degrees)} = [(V \times 0.1)/m] \times 100 \quad [4]$$

where:
V is volume of NaOH 0.1 n solution used at titration (cm^3);
0.1 is normality of sodium hydroxide solution;
m is mass of analyzed sample (g).

As final result, will be utilised the arithmetic mean of those two determinations, if repeatability conditions are fulfilled. This refers that difference between two determinations effectuated in parallel, in the same laboratory, by the same operator and from the same sample aren't allowed to exceed 0.2 acidity degrees.

Determination of flour's granulosity, have as principle the sifting of flour through a specific sieve for the type which will be analyzed whereupon is weighted the residuum on the rarer sieve and what's going on the dense sieve.

It will be weighted with a precision of 0.01 g, 100 grams from analyzed sample that will sift through sieve, manually or mechanically. If it is used a manually sifting its duration will be 6 minutes, with application of 80-100 movements come and go/minute. For realizing an intensification of sifting, together with flour sample, on sieve will be placed rubber balls or rings, which will be removed at sifting finalization. In case in which the analyzed sample present a moisture above 16% these one will be placed on a sheet of paper into a thin layer whereupon will be let for drying for 2-3 hours, at environment temperature, till moisture will decrease under the value of 15%, after which will proceed to sifting. Separate will be weighted, with a precision of 0.01 g, residuum remained on rarer sieve, obtaining directly the final result.

RESULTS AND DISCUSSIONS

Sensorial appreciation of wheat flour

Sensorial appreciation of wheat flour presumed effectuation of the following analysis: determination of flour's colour, taste, smell and its infestation degree.

It was utilised method of sensorial qualities appreciation through score method. Analyze was effectuated by a team of 6 specialists whom at the end of tasting give ratings for each of those 4 analyzed properties (colour, taste, smell, infestation degree).

Scoring scale was from 0 to 4; in case of flour's colour, taste and smell mark 0 was minimum and mark 4 was represented the maximum value which a sensorial feature was able to obtain. Regarding infestation degree, mark 4 was attributed to the flour which wasn't infested while mark 0 is given to a very infested flour by mites and which is unsuitable in the process of obtaining bakery, pastry and confectionery products.

In table 1 are presented the results of sensorial analysis at the end of examination of "000" wheat flour type which was obtained into a mid capacity mill.

The maximum score which could be obtained by "000" wheat flour type for each of those four analyzed features was 24 points and the minimum score which could be achieved was 0 points (tab. 1).

Table 1

Sensorial analysis of "000" wheat flour type

No.	Colour (points)	Taste (points)	Smell (points)	Infestation degree (points)	TOTAL (points)
Specialist 1	4	3	3	4	14
Specialist 2	4	3	3	4	14
Specialist 3	4	3	4	4	15
Specialist 4	4	4	4	4	16
Specialist 5	4	4	4	4	16
Specialist 6	4	4	4	4	16
TOTAL	24	21	22	24	

As we can observe from table 1 regarding colour, assortment "000" wheat flour recorded a total score of 24 points which means that specialists gave maximum marks for this characteristic.

Referring to the taste of "000" wheat flour type (tab. 1) we remark that the total score was 21, three from specialists noting this characteristic with 3 points while 3 of them gave the maximum score of 4 points.

Analyzing the obtained score by "000" wheat flour type regarding smell (tab. 1) could be observed that two specialists gave the mark 3, while the other four members of tasting team gave the maximum score of 4 points. Totally for smell feature "000" wheat flour type achieved a number of 22 points.

Regarding infestation degree of "000" wheat flour type (tab. 1) could be observed that this one had a score of 24 points, which means that none of those specialists didn't determine that analyzed product was infested by mites, all of them giving the maximum rating.

From analyse of data presented in table 1 could be observed that the total score given by those six specialists was situated in interval 14 points – 16 points (which is the maxim value which could be reached by "000" wheat flour type). Two of the team members evaluated globally the sensorial characteristics of studied product with a number of 14 points, one gave 15 points for the studied product, and three specialists evaluated the sensorial characteristics with maximum total score of 16 points.

A possible explication of those small fluctuations in appreciation of sensorial features of studied product could be that each specialist has its own sensations regarding taste and smell.

Physical-chemical appreciation of wheat flour

For a physical-chemical appreciation of "000" wheat flour type was analyzed the following parameters: moisture, wet gluten content, ash content, falling index (Hagberg-Perten), acidity, granulosity. The obtained results at the end of laboratory determinations were compared with the values imposed by national standard (tab. 2).

Determination of moisture

Regarding moisture of "000" wheat flour type was obtained a mean value,

for this feature, of 14.29±0.028%. For moisture the minimum obtained value was 14.2% while the maximum founded value was 14.4%. Studied parameter had slightly low values than value of 14.5% which is imposed by national standard (tab. 2). Variation coefficient which was calculated had a value of 0.613% which shown a very good homogeneity for the studied character (tab. 2).

Table 2

Physical-chemical appreciation of wheat flour

Physical-chemical characteristic	Standard	Results (n=10)			
		Minimum	Maximum	$\overline{X} \pm s_{\overline{X}}$	V (%)
Moisture (%)	14.5	14.2	14.4	14.29±0.028	0.613
Wet gluten (%)	min. 30	30.1	30.5	30.29±0.038	0.395
Ash (%)	max. 0.48	0.45	0.47	0.46±0.003	1.775
Falling index (sec.)	220-280	256	262	259.2±0.573	0.700
Acidity (grade)	2.2	2.0	2.1	2.09±0.010	1.513
Granulosity (%)	8	7.6	7.9	7.78±0.33	1.328

Determination of wet gluten content

Regarding content of wet gluten from "000" wheat flour type, could be observed that obtained values oscillated between a minimum value of 30.1% and a maximum value of 30.5%. Mean value obtained for wet gluten content was 30.29±0.038%, in conditions in which national standard impose a minimum value of 30% for wet gluten (tab. 2). Variation coefficient, for this feature, had the value of 0.395% fact which shown that also this studied character had a very good homogeneity inside lot (tab. 2).

Determination of ash content

"000" wheat flour type had a mean ash content of 0.46±0.003%, in conditions in which minimum was situated at a value of 0.45% and maximum was 0.47% (tab. 2). For ash content national standard impose a maximum value of 0.48%, so we could affirm that studied product "000" wheat flour type meets the imposed requirements. For this feature, variation coefficient, recorded the value of 1.775% (tab. 2) which allow us to affirm that studied parameter presented a very good homogeneity inside lot.

Determination of Hagberg-Perten falling index

Analyzing Hagberg-Perten falling index for "000" wheat flour type, could be observe that obtained values oscillated in interval 256 seconds (minimum value) and 262 seconds (maximum value). National standard impose that this parameter to fit into interval 220 seconds and 280 seconds, from which result that mean value (259.2±0.573 seconds) obtained by us is in according with those demands (tab. 2). Variation coefficient (0.700%) obtained for Hagberg-Perten falling index shows the fact that inside lot homogeneity was very good (tab. 2).

Determination of acidity

Regarding acidity of "000" wheat flour type could be observed that was obtained a mean value of 2.09±0.010 acidity degrees in conditions in which

standard provides a value of maximum 2.2 acidity degrees (tab. 2). The obtained values at the end of effectuated experiments were situated into interval between 2.0 acidity degrees (minimum value) and 2.1 acidity degrees (maximum value). Variability coefficient obtained a value of 1.513%, so this fact allows us to affirm that inside studied lot homogeneity for this parameter was very good (tab. 2).

Determination of granulosity

Granulosity or finesse of "000" wheat flour type, recorded a minimum value of 7.6% while maximum value was 7.9%. Mean value obtained for this parameter was 7.78±0.33% being slightly inferior to standard which impose a value of 8%. For variability coefficient was obtained a value of 1.328%. This fact allows us to say that inside studied lot also the homogeneity for this parameter was a very good one (tab. 2).

CONCLUSIONS

Based on sensorial and physical-chemical analysis effectuated on "000" wheat flour type a series of conclusions could be drawn.

1. Regarding colour "000" wheat flour type assortment recorded a total score of 24 points which means that specialist gave maximum marks for this feature.

2. Regarding taste of "000" wheat flour type it is observed that total score was 21 points, three from specialists noting this characteristic with 3 points, while other 3 of them gave the maximum score of 4 points.

3. Regarding smell of "000" wheat flour type, this parameter accumulated a number of 22 points. Two specialists according mark 3, while the other four members of tasting team accorded maxim score of 4 points.

4. Regarding infestation degree of "000" wheat flour type it could be observed that this one had a score of 24 points, which means that all specialists gave the maximum mark.

5. Total score given by those six specialists was situated in interval 14 points – 16 points (which is the maxim value which could be reached by "000" wheat flour type). Two of the team members evaluated globally the sensorial characteristics of studied product with a number of 14 points, one gave 15 points for the studied product, and three specialists evaluated the sensorial characteristics with maximum total score of 16 points.

6. So we could affirm that from sensorial point of view "000" wheat flour type is in according with the demands imposed by the nowadays legislation.

7. Moisture of "000" wheat flour type had a mean value of 14.29±0.028%. Minimum obtained value was 14.2% while founded maximum value was 14.4%. Variation coefficient had a value of 0.613% which shown a very good homogeneity for the studied character.

8. Wet gluten content from "000" wheat flour type recorded values which oscillated between a minimum value of 30.1% and a maximum value of 30.5%. Mean value obtained for wet gluten content was 30.29±0.038%; variation

coefficient, for this feature, had the value of 0.395% fact which shown that also this studied character had a very good homogeneity inside lot.

9. "000" wheat flour type, had a mean ash content of 0.46±0.003%, in conditions in which minimum was situated at a value of 0.45% and maximum was 0.47%. Variation coefficient, recorded the value of 1.775%, so studied parameter presented a very good homogeneity inside lot.

10. Hagberg-Perten falling index for "000" wheat flour type, had values which oscillated into interval 256 seconds (minimum value) and 262 seconds (maximum value). Variation coefficient (0.700%) obtained for Hagberg-Perten falling index shows the fact that inside lot homogeneity was very good.

11. Acidity of "000" wheat flour type recorded a mean value of 2.09±0.010 acidity degrees in conditions in which standard provides a value of maximum 2.2 acidity degrees. The obtained values were situated into interval between 2.0 acidity degrees (minimum value) and 2.1 acidity degrees (maximum value). For variability coefficient was obtained a value of 1.513%, so homogeneity for this parameter was a very good one.

12. Granulosity of "000" wheat flour type, recorded a minimum value of 7.6% while maximum value was 7.9%; mean value obtained for this parameter was 7.78±0.33%. For variability coefficient was obtained a value of 1.328% fact which show us that inside studied lot homogeneity for this parameter was a very good one.

13. We consider that physical-chemical speaking "000" wheat flour type fulfils all the demands imposed by nowadays legislation.

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APPRECIATION OF THE FACTORS' INFLUENCE ON THE VIGOR - I INDEX, TREATMENT x GENOTYPE x STORAGE DURATION OF CORN SEEDS (*ZEA MAYS* L.)

APRECIEREA INFLUENȚEI FACTORILOR ASUPRA INDEXULUI VIGORII-I, TRATAMENT x GENOTIP x DURATA PĂSTRĂRII LA SĂMÂNȚA DE PORUMB (*ZEA MAYS* L.)

GOCAN Tincuța Marta[1], ANDREICA Ileana[1], RÓZSA S.[1], LAZĂR V.[1], BĂRBOS A.[1]*

*Corresponding author e-mail: adrianbarbos@gmail.com

Abstract. *The size of the values of the parameters that characterize the quality of the seed from the aspect of the physiological and physical manifestation, has a very important role in the measures to improve the cultivation technologies for obtaining safe and superior harvests qualitatively and quantitatively, therefore it is necessary to promote a seed with high biological value, high production capacity, resistance to diseases, pests and stress conditions. The particularity of the seeds destined for sowing is that they can be kept in different forms for a longer period of time, offering the safety of production. Analysing the data by the size of the range and the size of the coefficient of variation (Cv) it can be seen that there are obvious differences between the variants, regarding the value of these indicators. There were large decreases in the values of the index the force registered in this stage "after 12 months", in the variant treated with fungicide + insecticide, except for Turda 200 and Turda Star hybrids.*
Key words: hybrid, quality, analysis, corn, batch

Rezumat. *Mărimea valorilor parametrilor ce caracterizează calitatea seminței sub aspectul manifestării fiziologice şi fizice are un rol foarte important în cadrul măsurilor de îmbunătățire a tehnologiilor de cultură pentru obținerea unor recolte sigure şi superioare din punct de vedere calitativ şi cantitativ, de aceea este necesar a promova o sămânță cu o valoare biologică ridicată, capacitate de producție mare, rezistență la boli, dăunători şi condiții de stres. Particularitatea semințelor destinate însămânțării este că acestea pot fi păstrate sub diferite forme pe o perioadă mai lungă de timp, oferind siguranța producției. Analizând datele după mărimea intervalului şi mărimea coeficientului de variație (Cv) se poate constata, că există diferențe evidente între variante, în ce priveşte valoarea acestor indicatori. S-au constat scăderi mari ale valorilor indexului vigorii înregistrați în această etapă „după 12 luni", la varianta tratată cu fungicid+insecticid, exceptând hibrizii Turda 200 şi Turda Star.*
Cuvinte cheie: hibrid, calitate, analize, porumb, lot

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania

INTRODUCTION

The evaluation and identification of high-performance seed lots is an important initiative, which contributes to the success of the production even from the sowing phase, by using for the purpose proposed seeds with an active state of health, natural resistance, able to develop normal germs and to ensures a uniform sunrise.

It is necessary to know the real status of a lot of seeds in terms of quality, to provide the necessary information to the user, helping him to decide the way, the conditions and the moment of use of the lot. Different batches of seeds, even if they have the same (sometimes almost identical) germination, does not mean that they have the same physiological potential, that is to say, when sowing in the field, they do not have the same germination and germination.

In order to maintain seed viability at a high-level during storage, it is compulsory to select for seed, seeds that have high levels of viability before storage (Modi, 2004). According to Burris (2000), the quality of the seeds influences the production (Burris, 2000) in the vast majority of the cultivated species, but especially in the case of maize, and Hermanus-Maree (2008) in the work "Prediction of Field Emergence of maize hybrids exposed to cold and wet conditions", specifies that the physiological potential of the seed, respectively its quality, can be evidenced by two fundamental components of it, namely, the viability and vigour of the growths (Hermanus-Maree, 2008). Initially, these two terms were used confusingly to define seed performance.

If things are simple in the case of viability - rendered by the percentage of seeds with live embryos, regarding the term of force, things are much more complex. The International Congress of the "Seed Testing Association" (ISTA), held in Washington (1950), on the topic of seed quality, identifies the force as a component of the physiological potential of the seed, independent of the germination indicator, and expresses its confidence that it is a separate and essential component of seed quality (Marcos-Filho, 2015).

Milosevic and Cirovic (1994) state that in order to obtain accurate information about the quality of a lot of seeds, different vigour tests must be used (Milosevici and Cirovic, 1994). The germination reflects the maximum germination potential of the seeds under optimal conditions, correlating with the emergence in the field only if, there are almost optimal conditions, so it is not a good indicator, to correlate with the emergence in the field, instead the size of the growth and the dry weight of these increases and a series of combined indicators, besides the fact that they are good indicators, correlated with the emergence in the field, also helps to differentiate the lots of seeds regarding the expression of the physiological potential (Divsalar *et al.,* 2013).

Measurements on growth and then the indicators calculated as a combination of germination and growth size play a very important role in predicting good seed behaviour in the field (Copeland and Mcdonald, 1995).

MATERIAL AND METHOD

The hybrids taken in the study were produced at the Agricultural Research and Development Station Turda is located in the north western part of the municipality of Turda, Cluj county. Their cultivation was on a soil with a clay-clay texture, with a neutral pH on 0-20 cm and weak acid on 20-40 cm depth, good and very good supply with NPK and microelements the soil content in humus being medium, according to the analyses made by the specialized laboratory.

Studied hybrids are:

- ✓ TURDA 200, double hybrid, early, FAO group 290, serrated grain, yellow-gold color, MMB 260-330g, grain / chick percentage 76-79%, fat content of coil 4.0-5.4; average production potential 8,100-10,600 kg / ha; It is noted by its adaptability to a wide range of ecological and technological conditions, through the possibilities of capitalizing on the intensive technological conditions, as well as through the good and stable production results during the almost 40 years of use in culture (1976-2015);
- ✓ TURDA 165, trilinear hybrid, early FAO group 280, serrated bob, medium high waist; the earliest Romanian hybrid in culture, good resistance to fall of plants; MMB 300-330 g, percentage of grains / stalks 80-83%, content of starch rich 70-72%, production potential 10.700-13.700 kg / ha;
- ✓ TURDA 201, trilinear hybrid, semi-impure, FAO 340 group, toothed grain, good resistance to cryptogamic and harmful diseases and plant fall; MMB 315-335g, percentage of grains / stalks 80-83%, starch rich content 70-74%; production potential of 9.000-13.600 kg / ha;
- ✓ TURDA FAVORIT, simple hybrid, semi-impure, FAO 380 group, very good resistance to falling and breaking the stems, good resistance to diseases and pests; semi-serrated grain, yellow-gold color, MMB 280-310g, percentage of grains / stalks 78-82%, starch rich content 70-73%; production potential of 10,000-13.500 kg / ha, at a density of 60-65000 plants / ha;
- ✓ TURDA STAR, trilinear hybrid, semi-impure, FAO 370 group, good resistance to low temperatures from the first part of the vegetation period, to fall and break, drought, burns, bean disease, diseases and pests; serrated grain, yellow color, MMB 310g, grain / grain percentage 79-82%, starch rich content 70-71%; production potential of 10,000-12.800 kg / ha the pleasant appearance (stay green) of the plants at physiological maturity, the saps are easily harvested.

The inbred lines taken in the study: LC 223 Nrf.T; LC 223 Nrf.C; LC 363; LC 763, are parental forms of simple commercial hybrids or pollinating (paternal) forms of trilinear hybrids.

At the end of the germination test (after 7 days), 5 seedlings from normal germs were selected randomly, from each repetition, retaining only the seedlings from 3 repetitions.

RESULTS AND DISCUSSIONS

Considering the importance of this index in the appreciation of the vigour of the seeds, respectively their performances during the germination and emergence in the field, as well as a good indicator, used in the differentiation of the lots in terms of quality (Gupta, 1993), it was considered to be necessary that besides the average the result of each experimental variant to present the main indicators of the dispersion: the variance, the amplitude of the variation and the coefficient of variability (tab. 1).

Analysing the data by the size of the range and the size of the coefficient of variation (Cv) it can be seen that there are obvious differences between the variants, regarding the value of these indicators. The close values of the interval indicate a grouping of the observations of the repetitions around the mean, of course this case also corresponds to small values of the coefficient of variability (tab. 1).

Table 1

Variability of the distribution of results in the vigour – I index analysis

TREATMENT	HYBRID	BEFORE SOWING		AFTER 12 MONTHS		AFTER 24 MONTHS		AFTER 36 MONTHS	
		$\bar{I}_{index} \pm t_{5\%} * s_{index}$	Cv[%]	$\bar{I}_{index} \pm t_{5\%} * s_{index}$	Cv[%]	$\bar{I}_{index} \pm t_{5\%} * s_{index}$	Cv[%]	$\bar{I}_{index} \pm t_{5\%} * s_{index}$	Cv[%]
UNTREATED	Turda 200	3425.3-3810.7	2.1	3312.2-3711.2	2.3	3132.5-3567.5	2.6	3019.2-3447.4	2.6
	Turda 165	3165.2-3521.4	2.1	3091.3-3518.7	2.5	2742-3258	3.4	2392-2908	3.9
	Turda 201	4788.6-5137.8	1.9	3333.5-3735.1	2.2	2942-3458	3.2	2523-3110.4	4.2
	Turda Star	3368.3-3724.3	2	3518.5-3791.5	2.6	3079.9-3653.5	3.4	2928.5-3504.9	3.6
	Turda Favorit	3201.8-3580.2	2.2	3039.2-3627.4	3.5	2802-3398	3.8	2680.8-3225.2	3.7
CONTROLLED ENVIRONMENT	Turda 200	3424.3-3811.7	2.1	2769.2-3246.8	3.1	1909.2-2455.4	5	1456.2-2051.4	6.8
	Turda 165	3165.4-3521.4	2.1	2304.5-3012.3	5.3	954.9-1724.5	**11.5**	830.2-1623.2	**13**
	Turda 201	3491.7-3841.7	1.9	2380.9-3075.7	5.1	1129.1-1898.9	**10.2**	639.1-1408.9	**15.1**
	Turda Star	3368.3-3724.3	2	2882.9-3356.1	3.05	1726.6-2406.8	6.6	1263.7-1936.3	8.4
	Turda Favorit	3201.8-3580.2	2.2	2207.7-2925.7	5.6	1009.3-1768.7	**11.04**	852.7-1647.3	**12.8**
FUNGICID	Turda 200	3505.2-3864.8	2.02	3577.2-4017.6	2.3	3448.6-3885.4	2.3	3126.6-3573.4	2.6
	Turda 165	3376.8-3769.8	2.2	3447.6-3884.4	2.4	2853.7-3337.1	3.1	2225.4-2746.6	4.2
	Turda 201	3717.4-4104.6	1.9	3676.6-4130	2.3	3067.5-3638.5	3.4	2600.2-3171.2	3.9
	Turda Star	3638.2-3985.8	1.8	3572-3994	2.2	3298-3720	2.4	2797.9-3244.7	2.9
	Turda Favorit	3509.9-3946.7	2.3	3418.5-3989.5	3.1	2810.6-3366.6	3.6	2718.1-3313.9	3.9
FUNGICID + INSECTICID	Turda 200	3663.5-3936.5	1.4	3544-3966	2.2	2041.8-2631.6	5.1	1781.9-2387.5	5.8
	Turda 165	3437.2-3834.4	2.2	2634.2-3239.8	4.1	996.1-1740.9	**10.9**	665.8-1460.2	**15.4**
	Turda 201	3764.2-4235.8	2.3	3099.7-3720.3	3.6	1448.5-2218.1	8.4	1043.4-1862.6	**11.3**
	Turda Star	3646.8-4019.2	1.9	3459.9-3906.7	2.4	2127.1-2722.9	4.9	1789.7-2410.3	5.9
	Turda Favorit	3542-3964	2.2	2607.5-3178.5	3.9	1222.7-1977.3	**9.5**	860.8-1672.6	**12.9**

It is found that as the storage time increases, at each treatment level and in the case of each hybrid, the values of the coefficient of variability increase. Also, these values are much higher in the variants in which the seed is subjected to stress reaching in some cases at levels above the average variability. This fact explains the different reaction of hybrids to the force in such environments. However, most of the values of the coefficients of variation have values below 10%, which proves that the experimentation was done correctly, and the results have scientific value.

It has been shown previously that the values of the recorded increases, as well as the values of the vigour-I index, are dependent on the duration of storage, treatment and genotype.

Taking into account the structural circle in which the hierarchy of these factors regarding the contribution to the total variability is shown, as well as the conclusions regarding the hard-to-describe reactions regarding the behaviour of these hybrids under different experimentation conditions, it was sought to establish by the known mathematical procedures, the laws of variation of these structures as well as of the index of vigour, to describe as accurately as possible the reactions of the studied hybrids, to the irreversible process of aging and the storage media.

The suitability of these dependencies was assessed by the values of the coefficient of determination R^2 (tab. 2) and the recording of the smallest values for the residual variance.

As can be seen the form of these laws chosen as appropriate is very varied, from those in linear form to those of the exponential form.

Table 2

Functional dependences of vigour-I index

Treatment	Average of vigour-I index				The law of appropriate variation	The regression coefficients	Correlation (R^2)
	Before	12 months	24 months	36 months			
Untreated	3513.08	3447.86	3203.34	2973.94	$y=a+b*x$	a= 3563.846 b= -15.516	0.95
Controlled environment	3513.08	2816.38	1698.34	1370.90	$y= a*b^x$	a= 3609.603 b= 0.972	0.97
Fungicide	3740.98	3770.74	3342.60	2951.80	$y=a*x^2 + b*x+c$	a= -0.730; b= 2.987 c=3765.742	0.93
Fungicide + insecticide	3804.36	3335.66	1912.10	1588.08	$y=a*x^2 + b*x+c$	a= 0.251; b= -76.312 c=3907	0.94

This shows that the aging process of the seeds belonging to the studied hybrids is complex, the phenomena and the biochemical and physiological processes that occur inside the seeds as they grow older, are influenced by the genetic particularities of the genotype but also by the storage conditions.

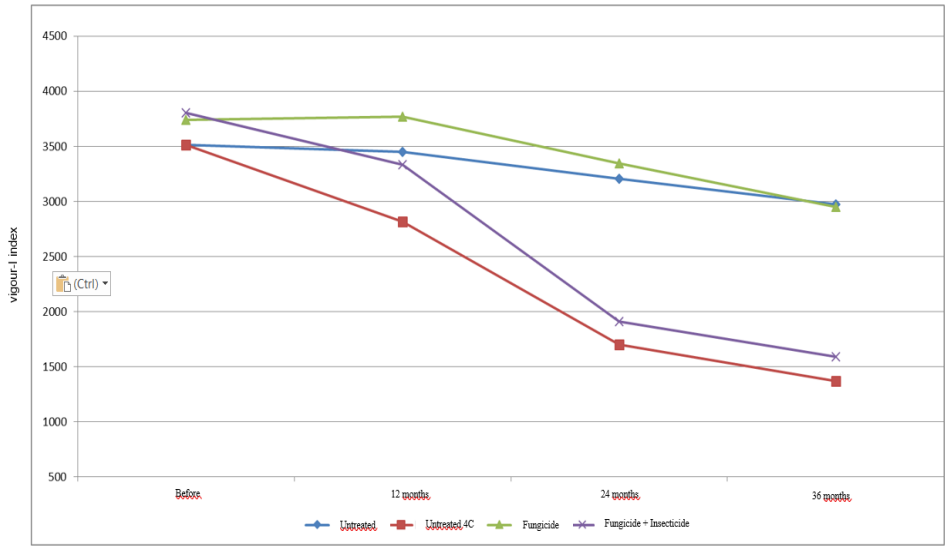

Fig. 1 Variation of hybrids vigour-I index during storage duration

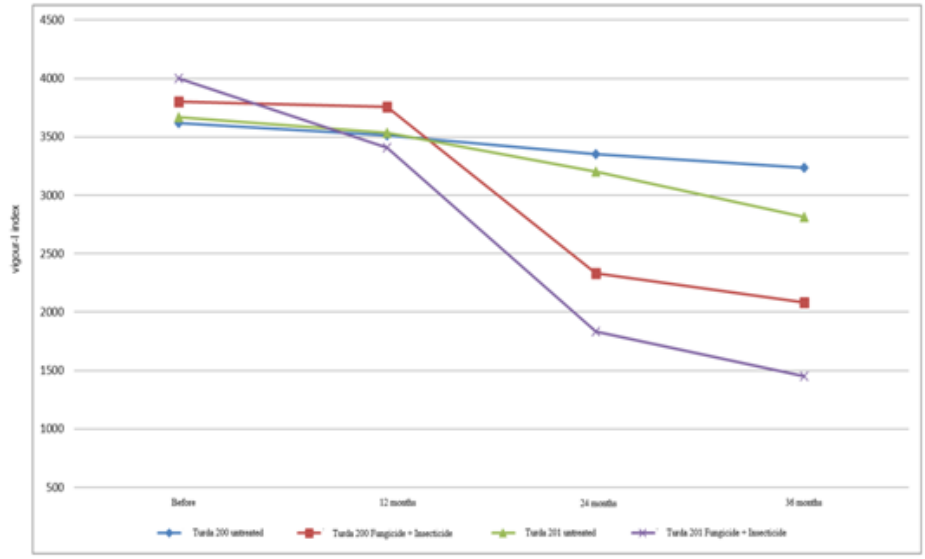

Fig. 2 The comparative study of vigour-I index for hybrids Turda 200 and Turda 201 in environment: untreated and treated with fungicide + insecticide

For a more obvious presentation of the variation of the values describing the index of force using our average values, its evolution during the retention period was graphically represented (fig. 1) and a comparative graphical representation for two Turda 200 and Turda 201 hybrids in two distinct environments: untreated and treated with fungicide + insecticide (fig. 2).

In both graphs, in the "untreated environment" you can see a natural evolution of this indicator, but in the other environments the evolution is difficult to describe. There is a “break” in the values recorded for this index between the stages “after 12 months” and “after 24 months”.

Thus, the rate of growth that directly influences the size of the index of vigour I, slows down in the variants in which the seed is subjected to stress, seems from a certain moment. It can be seen in figure 2 the good resistance with the shelf life and under stress conditions of the Turda 200 hybrid.

CONCLUSIONS

1. In the variant in which the seed was subjected to stress, for example the Turda 165 hybrid, it also has in the "after 12 months" stage the same germination of 92% as the one from the initial moment. The same is true of the Turda 201 hybrid, but after this retention period, in this environment, the force index records, for both hybrids, drastic decreases.

2. There were large decreases in the values of the index of force registered in this stage "after 12 months", in the variant treated with fungicide + insecticide, except for Turda 200 and Turda Star hybrids.

3. With the increase of the retention period (after 36 months) pronounced decreases of the value of the index of force were registered in all hybrids.

4. If obvious decreases of germination were recorded "after 24 months" of storage, in the case of the recorded values and the graphs plotted, for the index of force, a break in the values between the stages "after 12 months" and "after 24 months" is observed. This observation has great practical utility.

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STUDIES ON THE MORPHOBIOLOGICAL CHARACTERISTICS, PRODUCTIVITY AND RESISTANCE TO HIGH TEMPERATURES AT TOMATOES

STUDII PRIVIND CARACTERELE MORFOBIOLOGICE, PRODUCTIVITATEA ŞI REZISTENŢA LA TEMPERATURI ÎNALTE LA TOMATE

MIHNEA Nadejda[1*], LUPAŞCU Galina[1], VÎNATORU C.[2*], CRISTEA N.[1]
*Corresponding author e-mail: mihneanadea@yahoo.com, costel_vinatoru@yahoo.com

Abstract. *The paper presents the results of the appreciation of varieties and lines from the collection of tomatoes of the Institute of Genetics, Plant Physiology and Plant Protection, Republic of Moldova based on productivity, resistance to stressing temperatures and some fruit characters. The analysis of the useful characters of the studied forms from the collection revealed a wide variability in the fruit characters, the overall productivity, the share of the fruits, which allows selection and recommendation of the most valuable forms for hybridization and obtaining new varieties and hybrids of different destination. Cluster analysis (k-means method) demonstrated that the 38°C temperature level manifested a higher discriminative capacity of tomato clusters based on root and strain length (controlled conditions), which revealed the more pronounced interaction specificity with this temperature level. Were identified clusters of tomato genotypes with diminished reaction at stressful temperatures, which is important for the involvement in programs to improve genotypes with increased resistance to heat.*
Key words: tomato, variability, fruit characters, productivity, strength, stressful temperatures

Rezumat. *În lucrare sunt prezentate rezultatele aprecierii unor soiuri şi linii din colecţia tomatelor de cultură ale Institutului de Genetică, Fiziologie şi Protecţie a Plantelor, Republica Moldova în baza productivităţii, rezistenţei la temperaturi stresante şi unor caractere ale fructului. Analiza caracterelor utile ale formelor studiate din colecţie a evidenţiat o variabilitate largă privind caracterele fructului, productivitatea generală, cota fructelor marfă, ceea ce permite selectarea şi recomandarea celor mai valoroase forme pentru hibridare şi obţinere a soiurilor şi hibrizilor noi cu diferită destinaţie. Analiza clusteriană (metoda k-mediilor) a demonstrat că nivelul de temperatură de 38^0C, a manifestat o capacitate discriminantă mai înaltă a clusterelor de tomate în baza caracterelor lungimea rădăciniţei şi tulpiniţei (condiţii controlate), ceea ce relevă specificitatea mai pronunţată de interacţiune cu acest nivel de temperatură. Au fost identificate clustere de genotipuri de tomate cu reacţie diminuată la temperaturi stresante, ceea ce are importanţă pentru implicarea în programele de ameliorare a genotipurilor cu rezistenţă sporită la arşiţă.*
Cuvinte-cheie: tomate, variabilitate, caractere ale fructului, productivitate, rezistenţă, temperaturi stresante

[1]Institute of Genetics, Physiology and Plant Protection, Republic of Moldova
[2]Vegetable Research and Development Station Buzău, Romania

INTRODUCTION

The morphobiological characteristics of tomato fruit are determined by several genes that interacting with environmental factors at different stages of plant growth, provide a strong variability of character (Lippman and Tanksley, 2001). Consumers' predilections for size, aesthetic aspect of the fruit, and the need for certain pericarpic particularities related to transportability, make the actual interest of the breeders more attractive for their variability for practical use.

More and more frequent environmental imbalances in recent decades lead to considerable decreases in productivity and quality of agricultural production including tomatoes. In this connection, research has been intensified in the field of plant resistance to unfavorable biotic and abiotic factors (Hazra *et al.,* 2007; Mihnea *et al*., 2013; Mihnea *et al*., 2015; Mihnea, 2016; Lupascu *et al.,* 2017).

Higher than optimal temperatures can cause physiological disturbances in tomato plants, leading to a decrease in the vegetation period, accelerating the flowering and baking of fruits (Ansary, 2006), or to the nonuniform passage of these processes (Adams and Valdes, 2002; Mulholland *et al.,* 2003).

At temperatures higher than 35°C, germination, flowering, meiosis development, large fruit formation, egg development and viability, as well as embryo development (Wahid *et al.,* 2007) are affected by most varieties of tomatoes. Under stressful conditions, resistant tomato genotypes have the ability to form a much larger number of fruits than the sensitive ones (Comlekcioglu *et al.*, 2010). This situation requires the creation of tomato genotypes with resistance to extreme environmental factors (Venema *et al*., 2005; Hazra *et al.,* 2007; Mihnea *et al.*, 2016).

An important role has the tomato fruit properties. The main characteristics of the tomato fruit are: the mass, the fruit index (the ratio of the length to the fruit diameter), the thickness of the pericarp, the thickness of the mesocarp, the locule number. Knowing the degree of their variability allows for more efficient use of the original material in breeding research (Mihnea, 2012).

The purpose of the research was to study the phenotypic variability of some important quantitative characters in tomatoes and the lability of some perspective genotypes at stressful temperatures for their inclusion in the breeding process.

MATERIAL AND METHODS

As research material, they served 12 varieties and 6 lines of different origins. The lines and varieties Kristina, Florina, Măriuca, Darsirius and Buzău 47 are of Romanian origin and were kindly offered by Dr. Costel Vinatoru from the Buzau Research and Development Station for Vegetables. The experiments were performed in laboratory and field conditions on the experimental field of the Institute of Genetics, Plant Physiology and Plant Protection, Republic of Moldova.

Testing of the high temperature genotype reaction was performed according to the method proposed by Ivakin (1979), based on the growth capacity of the plants after keeping them at high temperatures for 6 hours. High temperature temperatures of 35ºC, 38ºC and 42ºC were used to analyze the influence of high temperatures on the embryonic root and stem.

Tomatoes were grown by seedling culture in three repetitions by standard method (Ersova, 1978). Sowing in the greenhouse took place in the third decade of March, planting in the field in the second decade of May. Under field conditions the morphological description was performed according to the UPOV descriptor (2011). The data obtained were statistically processed in the STATISTICA 7 software package.

RESULTS AND DISCUSSIONS

The data obtained showed essential differences of genotypes according to the average fruit weight. There were 7 large fruit shapes: Santa Maria, Pontina, Kristina, Florina, L 11, L 66, L 713. The character variation range was 10.2-35.5 g, and the coefficient of variation in the analyzed tomato group was 22.0%, indicating the rather pronounced variability of character. Forms evaluated based on the thickness of pericarp were very different, the index ranging from 3.3 ... 9.5 mm (fig. 1). The calculation of the coefficient of variation showed a significant variability of the assessed character – 23.8%.

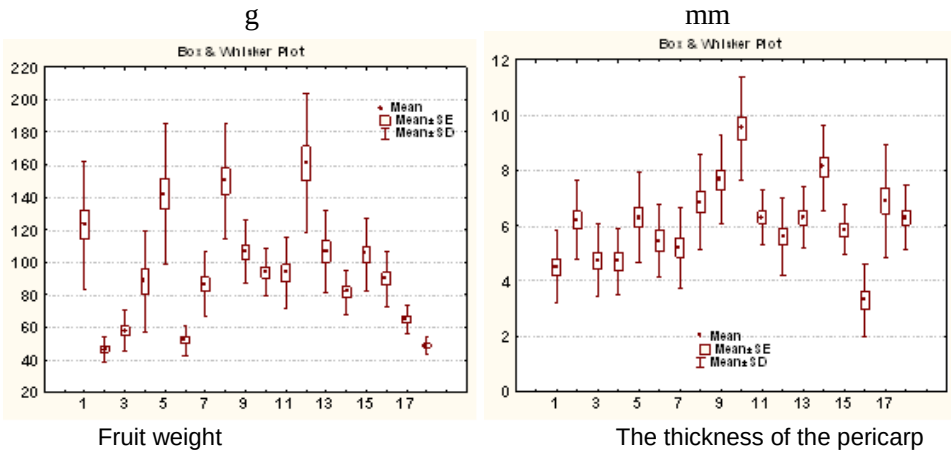


Fig. 1 Morphobiological characters of tomato fruit
1 – Santa Maria, 2 – Roma, 3 – Luci, 4 – Alex, 5 - Pontina, 6 – Flacăra, 7 – Kristina, 8 – Florina, 9 – Măriuca, 10 – Darsirius, 11 – L 10, 12 – 11, 13 – L 66, 14 – L 71(România), 15 – L 713, 16 – L 714, 17 – Buzău 47, 18 – Marglobe

In our researches, there was an insignificant variability of the length and width of the fruit to the studied forms (4.0 ... 10.8% and 3.8 ... 14.3%, respectively). Most forms had a coefficient of variation not exceeding 10% (tab. 1). The average variability of the samples tested was 7.4% for the fruit length and 9.4% for the fruit width, which allows the characters to be characterized as having low heterogeneity.

The data showed a wide range of mesocarp thickness variability in the analyzed samples: V = 9.5-24.3%, the average of the parameter constituting 15.3%, which shows an average variability of the study group according to the analyzed character.

The study of the locule number in the fruit showed that for most varieties it was 2-3, 5-8, with regular settlement, and the average coefficient of variation was 19.2%.

Table 1

Phenotypic variability of some tomato fruit characters

Variety, lines	Length of fruit mm		Fruit diameter mm		Mesocarp thickness mm		Number of locule number	
	$x \pm m_x$, g	V,%	$x \pm m_x$, mm	V,%	$x \pm m_x$, mm	V,%	$x \pm m_x$, mm	V,%
Santa Maria, control	50.1±4.2	8.4	67.4±2.2	14.3	50.1±1.9	16.7	5.6±0.3	22.0
Roma	45.5±0.6	6.3	43.1±0.8	7.9	27.2±1.1	18.5	2.1±0.1	14.7
Luci	66.6±1.6	10.8	39.2±0,6	7.0	25.5±0.8	13.5	2.9±0.1	17.2
Alex	69.1±1.5	8.6	48.5±2.1	17.3	36.0±2.4	24.3	3.2±0.2	17.6
Pontin	58.9±1.0	7.7	65.6±1.7	11.6	55.5±3.7	22.1	4.2±0.2	21.3
Flacăra	44.4±0.7	6.9	44.7±0.7	7.0	63.1±1.5	10.9	2.6±0.1	23.0
Kristina	50.9±1.0	7.5	56.5±1.2	9.3	41.4±1.0	10.4	3.0±0.1	13.4
Florina	62.6±1.2	8.6	69.7±1.4	8.8	53.0±1.5	12.3	4.4±0.2	20.1
Măriuca	65.0±1.2	8.2	56.9±1.0	8.2	36.9±1.3	16.0	3.1±0.1	16.7
Darsirius	68.9±1.4	9.0	52.1±0.9	7.3	27.5±1.1	18.1	2.3±0.1	24.8
L 10B	51.8±1.1	9.3	55.6±1.5	12.1	41.2±1.5	16.0	2.8±0.1	18.7
L 11	59.5±0.9	6.6	70.4±1.9	12.2	52.2±1.8	15.4	4.9±0.2	18.0
L 66	54.2±0.8	5.8	60.7±1.6	10.9	47.2±1.1	9.5	3.1±0.2	22.3
L 71	56.7±1.0	7.6	51.8±0.6	5.4	32.8±0.9	12.8	2.1±0.1	14.7
L 713	45.0±0.6	6.3	62.8±1.3	9.3	50.7±1.8	12.4	4.3±0.2	17.4
L 714	46.4±0.7	6.1	61.3±1.5	10.1	50.9±1.6	12.4	6.4±0.3	21,2
Buzău 47	53.2±0.6	5.1	48.5±0.8	7.3	29.7±1.0	14.4	2.3±0.1	24.8
Marglobe	44.4±0.4	4.0	43.9±0.4	3.8	27.9±1.2	19.9	2.2±0.1	17.0
Mean:		7.4		9.4		15.3		19.2

Testing of tomato varieties has made it possible to elucidate a significant productivity variability that depends both on genotype and climatic conditions. The productivity of varieties studied in 2018 varied between 22.0 t / ha (Luci) and 78.6 t / ha (Măriuca) (fig. 2). An increased productivity compared to the control variety, the total harvest of which was 32.9 t /ha, was recorded in the genotypes Măriuca (78.6 t / ha), Flacăra (63.4 t / ha), L 66 (61 , 4 t / ha), Florina (59.7 t / ha), L 11 (59.4 t / ha), Darsirius (56.0 t / ha) L 71 (50.6 t / ha). The harvest of varieties in the studied varieties proved to be quite high and ranged from 34.3% (Marglobe) to 90.4% (Darsirius).

Subsequently, varieties and lines that exhibited a complex of valuable characters were tested under laboratory conditions to determine the reaction of juvenile plants to stressful temperatures.

As a result of the appreciation of tomato genotypes based on the resistance of the embryonic root and the stem, the resistance to high temperatures of the plantlets varies widely and depends both on the genotype and on the temperature level (tab. 2).

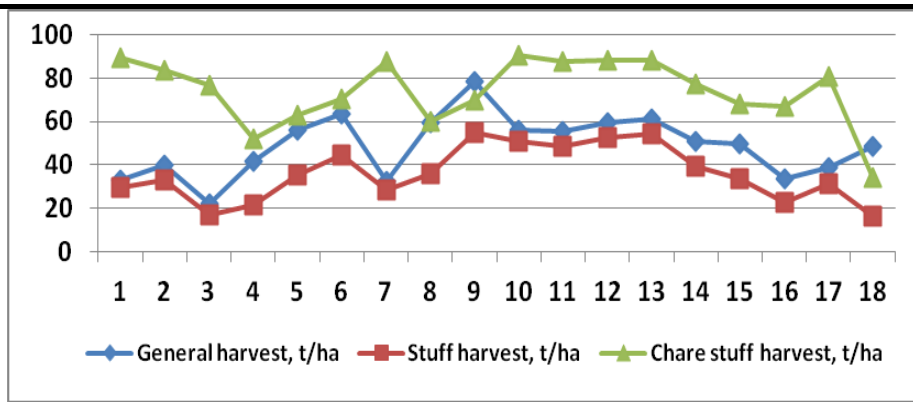


Fig. 2 The characteristic of growing tomato forms based on productivity
1– Santa Maria, 2 – Roma,3 – Luci, 4 – Alex, 5 – Pontina (Dacia), 6 – Flacăra, 7 – Kristina, 8 – Florina, 9 – Măriuca, 10 – Darsirius, 11 – L 10B, 12 – L11, 13 – L 66, 14 – L 71,15 – L 15, 16 – L 714, 17 – Buzău 47, 18 – Marglobe

Table 2

Variability of resistance to high temperatures in tomatoes

Variety, line	Resistance,%					
	Based on the embryonic root length			Based on stem length		
	35^0C	38^0C	42^0C	35^0C	38^0C	42^0C
Roma	80.0	108.6	71.8	75.9	62.7	64.1
Pontina	101.9	71.7	54.2	119.7	74.5	82.9
Flacara	90.7	85.8	48.1	101.1	83.5	92.2
Florina	81.8	64.5	48.8	57.6	70.7	52.3
Mariuca	85.7	89.3	44.6	67.7	89.0	45.4
L 10B	102.2	107.7	81.5	74.0	54.4	64.1
L 11	79.4	70.0	57.2	67.5	37.8	93.0
L 66	89.9	96.6	52.8	55.8	57.0	44.5
L 71	103.4	103.4	76.5	89.6	92.5	103.5
Mary Gratefully	81.9	77.2	63.9	90.0	72.2	68.0

The genotypes showed increased resistance of the embryonic root at 35 and 38ºC except Florina, with an average resistance (64.5%).

Increased resistance to 42ºC was manifested by Mary Gratefully (63.9%), Rome (71.8%) and L 71 (76.5%), L 10B (81.5%). The strain resistance variability was higher and ranged from 37.8 to 119.7%. Of particular interest for the further improvement are the genotypes Mary Gratefully and L 71 which show resistance of both characters.

As can be seen from the data presented (Figure 3A), the influence of stressful temperatures on the growth of the embryonic root in the tomato genotypes included in the study is different. In the case of the thermal regime of 35 ° C the values of character in optimal conditions ranged from 37.3 ... 62.2 mm,

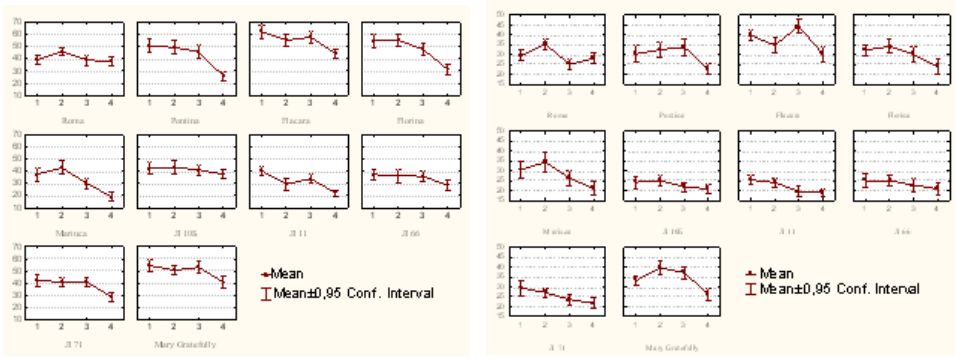
and in stressful conditions – 29.4 ... 51.3 mm. In Pontina, Mary Gratefully, Flame, L 71, L 66 and L 11, the diminution of character showed values of -3.1; -6.8; -11.7; -1.9; -3.2; -27.1% - compared with the witness.

In the case of the thermal regime of 38°C, the decrease of the length of the root has registered values of 2.6 ... 19.3%. There was 13.9% stimulation with Măriuca variety and insignificant inhibition – Mary Gratefully and L 10B, L 66, L 71 lines. Under the action of 42ºC temperature, embryonic root growth was suppressed in most of the analyzed forms. The level of diminution of character showed values of -0.5 (Rome); -12.8 (L 10B); -24.1 (L 66); -25.3 (Mary Gratefully); -29.2 (Flame); -31.1 (L 71); -42.8 (Florina); -45.2 (L 11); -48.9 (Măriuca); -49.3% (Pontina). As a result of the genotype appreciation according to the resistance of the root, Roma variety and L 10B high resistance line were selected, and Mary Gratefully varieties, flame and L 66, L 71 resistant lines.

The stem length of the analyzed genotypes ranged from 24.3 ... 39.7 mm (fig. 3B). Under the influence of high temperatures, there was a quite different reaction and variability: in 19 cases inhibition occurred (-5.5%, ... -31.1%) and in 11 – stimulation of the stem (+ 3.3%. .. + 32.2%). The strong inhibition under the influence of the temperature of 38ºC was attested in Marici (-28.4%) and L 11 (-22.8%); (-25.0%), Florina (-25.8%), Mariuka (-31.1%), L 11 (-25.6), L 71 (-26.5%); growth stimulation was recorded at 7 genotypes at 35ºC, three at 38ºC and at a genotype at 42ºC. It is worth mentioning that the varieties Rome, Mary Gratefully and the L 10B, L 66 lines showed resistance of both characters, so they are of interest for improvement as possible donors of resistance to heat.

The processing of the experimental data by bifactorial analysis of the variance allowed the appreciation of variability and degree of influence of temperature, genotype and their interaction on the variability of the evaluated characters.

mm



A B

Fig. 3 Influence of stressful temperatures on the growth of tomato root embryo (A) and on the growth of tomato strain (B);
Vertically: the length, mm; Horizontally: 1- 25^{0}C; 2 – 35^{0}C; 3 – 38^{0}C; 42^{0}C.

It was found that the contribution of genotype, temperature, and genotype x temperature interaction to the growth of tomato embryonic roots was 33.7; 63.1; 2.5%, and for stem growth – 44.8; 50.2; 4.0% respectively (tab. 3). So the temperature (63.1 and 50.2%) has a major influence on the growth of the embryonic root and the stem. It is worth mentioning that in the case of the strain the role of the genotype increased by 11.1%.

Table 3

Factorial analysis of tomato *genotype x high temperature* relations

Source of variation	Freedom degree	Average square sum	Contribution to source of variation,%
Root length			
Genotype	9	12182*	33.7
Temperature	3	22806*	63.1
Genotype x temperature interaction	27	892*	2.5
Random effects		266	0.7
Stem length			
Genotype	9	5363*	44.8
Temperature	3	6011*	50.2
Genotype x temperature interaction	27	483*	4.0
Random effects	579	126	1.0

*- p≤0.05.

CONCLUSIONS

1. The analysis of the useful characters of the studied forms in the collection revealed a wide variability in the fruit characters, the overall productivity, the share of the f*ruit, etc., which allows selection and recommendation of the most valuable forms for hybridization and obtaining* of new varieties and hybrids with different destination.

2. As a result of the analysis of the reaction of varieties and tomato lines at different stress levels (35°C, 38°C, 42°C) based on the stem and embryonic root lengths it was found that temperatures of 35ºC and 38ºC in most cases did not significantly influence the growth of the roots. Significant repression was recorded only at the line L 11 (-27.1) under the influence of the temperature of 35ºC and at L 310 (-15.0%) under the influence of the temperature of 38ºC. Growth of the root at the evaluated lines was most strongly influenced by temperature 42ºC, mean values relative to the control ranging from -0.5 ... -45.2%.

3. By bifactorial analysis of variance, it was found that for both the embryonic root and the tomato strain, the major contribution to the character variation source had the temperature (63.1 and 50.2%, respectively). It should be noted that an imported role he had the genotype, its factor weight constituted 33.7% for the embryonic root and 44.8% for the stem, which shows the high

opportunity for improvement of the character by identifying genotypes that do not show a pronounced sensitivity at high temperature (35 ... 42°C).

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INTERACTIVE EFFECTS OF RHIZOBACTERIA INOCULATION AND PHOSPHORUS ON PROLINE AND NITROGEN CONTENTS IN ROOTS AND NODULES OF SOYBEAN GROWN UNDER CONSTANT P INSUFFICIENCY AND WATER DEFICIT

EFECTELE INTERACTIVE ALE INOCULĂRII RIZOBACTERIEI ŞI FOSFORULUI ASUPRA CONŢINUTULUI DE PROLINĂ ŞI AZOT ÎN RĂDĂCINILE ŞI NODULII DE SOIA CRESCUŢI ÎN CONDIŢII DE INSUFICIENŢĂ CONSTANTĂ DE P ŞI DEFICIT DE APĂ

***ROTARU V.*[1]**

[*]Corresponding author e-mail: rotaruvlad@yahoo.com

Abstract. *Plant osmolytes play an important role in plant tolerance to abiotic stress factors. A greenhouse experiment with soybean was carried out with N-fixer rhizobacteria Bradyrhizobium japonicum applied singly or in combination with P (20 and 100 mg kg^{-1} dry soil) and two soil moisture regimes: 70% of whole holding capacity (WHC) as control and water stress 35% WHC. All plants were grown under same moisture conditions until the flowering stage. Water deficit conditions were imposed at flowering stage of plants for 12 days. Plants cultivated under the combined effects of P deficiency and drought exhibited the highest proline accumulation. Soybean inoculated with B. japonicum showed increased root proline concentrations compared to uninoculated plants subjected to temporary water deficit. Integrated use of inoculation and a moderate dose of P had a synergic effect on free proline accumulation in roots. The results revealed that combined influence of drought and P insufficiency significantly increased proline concentrations in nodules rather than in roots. Integrated use of rhizobacteria strain and a moderate dose of P decreased free amino acid accumulation in nodules under drought. There was a synergic interaction between the rhizobacteria strain and P in terms of leaves and stems nitrogen contents of soybean. In conclusion, soybean inoculation with B. japonicum in conjunction with P fertilization partially attenuates the adverse effects of constant low P availability and temporary drought on plants.*

Keywords: *Bradyrhizobium japonicum,* soybean, phosphorus, proline, nitrogen, soil moisture

Rezumat. *Compuşii din plante cu acţiune osmotică au rol important în formarea tolerantei plantelor la factorii de stres abiotic. A fost organizat un experiment cu plantele de soia cu aplicarea rizobacteriilor Bradyrhizobium japonicum atât separată cât şi in combinare cu fosforul (20 si 100 mg P/kg sol) şi cultivate la două regimuri de umiditate a solului - 70% din capacitatea totală*

[1]Institute of Genetics, Physiology and Plant Protection, Republic of Moldova

de apă (CTA) a solului şi 35% CTA. Toate plantele au fost crescute la umiditatea optimă până la faza înfloritului. Deficitul de apă din sol a fost declanşat în faza înfloritului deplin pe o perioadă de 12 zile. Cea mai mare acumulare de prolină s-a înregistrat la plantele cultivate la insuficiența de fosfor şi supuse deficitului de umiditate. Plantele de soia inoculate cu tulpina de B. japonicum au arătat un conținut sporit de prolină în rădăcini comparativ cu plantele din varianta control. Aplicarea integrată a rizobacteriilor şi P a manifestat acțiune sinergică la nivel de conținut de prolina în rădăcini. Rezultatele experimentale au demonstrat că seceta şi insuficiența de fosfor au majorat mai pronunțat concentrația de prolină în nodozități decât în rădăcini. Utilizarea combinata a rizobacteriilor şi P în doză moderată a micşorat acest indice în nodozități la plantele supuse secetei temporare. S-a stabilit un efect sinergic la administrarea combinată a rizobacteriilor B. japonicum şi P asupra conținutului de azot în frunze şi tulpini. In concluzie, inocularea semințelor cu tulpina B. japonicum in combinare cu fertilizarea cu P contribuie la atenuarea parțială a efectelor secetei şi deficitului de P asupra plantelor de soia.

Cuvinte cheie: Insuficiență de fosfor, prolină, *Bradyrhizobium japonicum,* soia, umiditate

INTRODCUTION

Among the environmental stresses, drought stress (Khan *et al.*, 2018; Reddy *et al.,* 2004) along with phosphorus deficiency (Lynch, 2007) are the most adverse factors for legumes growth and productivity. It is estimated that drought stress may cause a 50% loss in crop plants (Kasim *et al.,* 2013). Investigations demonstrated water deficiency affects plants at physiological, biochemical and metabolic levels such as osmoprotectants (Glick, 2014). Likewise, the P insuffiency induces damage in crops physiology diminishing their tolerance to unfavorable environmental conditions (Jin *et al.,* 2006). Among legumes, soybean (*Glycine max* L.) is a crop that is one of the major plant protein and vegetable oil resources and plays an important role in agricultural sustainability. This species is very sensitive to water deficit, especially during flowering and onset of pods (R3) (Liu *et al*., 2017). In addition, Sa-T and Israel (1995) reported that P deficiency diminished some physiological parameters: especially nitrogen assimilation in soybean plants under normal irrigation regime.

Therefore, these two adverse environmental conditions could seriously affect soybean production in many countries. There are studies, which demonstrated that the P supplementation leads to improvement of nitrogen assimilation and drought resistance of soybean (Jin *et al.,* 2006). Plant adaptation to environmental stresses is associated with metabolic adjustments that lead to the accumulation of several compatible organic solutes like sugars, polyamines, betaine, quaternary ammonium compounds, carbohydrates, proline and other amino acids (Hashem *et al.,* 2015). It was well documented that proline is accumulated in many plant species under environmental stress conditions as an osmoregulator (Alexieva *et al*., 2001; Ashraf, 2010). The accumulation of this

metabolite in plant tissues contributes to water homeostasis and improvement of plant growth under unfavorable conditions.

Nowadays large varieties of microorganisms' strains are used worldwide with the aim to enhance crop productivity and their resistance to abiotic stresses (Bhattacharya *et al.,* 2012; Khan *et al.,* 2018). It is documented that under normal ecological conditions soybean obtains nitrogen through biological nitrogen fixation by establishing association with rhizobia (Bulgarelli *et al.,* 2017). However, several studies demonstrated that N-fixer bacteria increased plant growth and N uptake under deficit irrigation (Liu *et al*., 2017). Rhizobacteria symbiosis could improve the legume plant nitrogen status by increasing the nutrient uptake and N-fixation to satisfy plants and nodules development (Bulgarelli *et al*., 2017). Low P availability persists in many soils, which together with inadequate compatible rhizobacteria strain to a particular legume result into low tolerance to abiotic factors and poor productivity of plants. To our knowledge, however, proline accumulation and nitrogen contents in soybean plants as influenced by rhizobacteria alone or in combination with P fertilization under water stress have not yet been well documented. Therefore, this study was to investigate the interactive effects of rhizobacteria *Bradyrhizobium japonicum* and P on proline contents in roots and nodules as well as nitrogen concentrations of soybean cultivated on soil of low P availability and temporary drought.

MATERIAL AND METHODS

Soybean (*Glycine max* L. Merrill, cv Horboveanca) plants inoculated with *Bradyrhizobium japonicum* 646 (Rh denoted in the text) alone or in conjunction with P application were grown at two soil moisture levels: normal as 70% WHC (water holding capacity) and temporary drought - 35% WHC. The soil used for the experiment was chernoziom carbonated with low P availability (18 mg P/kg soil) and mixed with sand in order to create P insufficiency conditions. Uninoculated plants served as control treatment. Before planting, the P was applied in soil at rate 0; 20 mg P/kg soil and 100 mg P/kg soil. Before sowing, the soybean seeds were thoroughly mixed with *B. japonicum* inoculants (10^8 cfu/mL). All plants from each treatment were grown under normal soil moisture until the beginning of flowering (R1) stage. After that ~~a~~ half of plants were subjected to temporary water deficit for 12 days. Moisture content of each pot was weighed every 3 days during the whole processing procedure, and was maintained at 70% of the maximum water-holding capacity. The experiment was in a completely randomized design and four replications were performed for flowering- stage and four for maturity grain stage, for each of the treatments. Free proline in roots and nodules was quantified spectrophotometrically according to Bates *et al* (1973). The Kjeldahl method was used to measure nitrogen content of plant samples. The obtained data were analyzed by using Statistic 7 program. Values were presented as means with standard errors (SE) from three independent treatments. The differences in the means were determined by the least significant difference (LSD) (P=0.05) test.

RESULTS AND DISCUSSIONS

In this study, an effort was made to assess the influence of inoculation of soybean with *B. japonicum* alone and in combination with phosphorus on proline accumulation in roots and nodules and nitrogen content in soybean plants grown under constant low P availability and temporary drought.

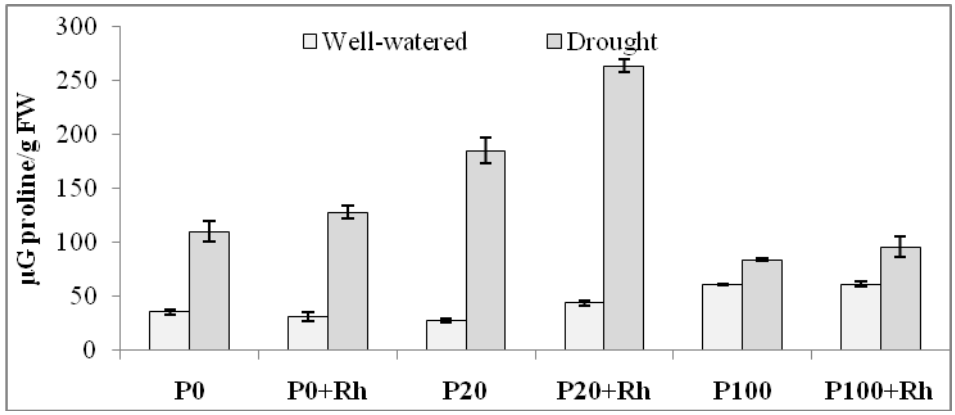

Fig. 1 The effect of *Bradyrhizobium japonicum* (Rh) applied alone or in combination with phosphorus (P) on proline content in roots under moderate drought. Columns are means ± SE.

Experimental results revealed that rhizobacteria and soil humidity had an impact on the pattern of proline concentrations in soybean roots. The contents of proline in soybean roots in relation to treatments and soil moisture level are shown in figure 1. It was noticed that imposition of drought stress caused an accumulation of proline in roots. Plants exposure to drought resulted in a multifold increase in proline concentration in roots tissues of uninoculated (3,1 fold) and inoculated (up to 6 fold) treatments indicating that proline was produced by the plants as response to the stress and inoculation with rhizobacteria further improved stress response of the plants in terms of proline content in roots. The soybean inoculation with *B. japonicum* alone under low P availability but well watered conditions did not change significantly this parameter in roots compared to uninoculated treatment. Similarly, the inoculation with *B. japonicum* in conjunction with P supplementation at a higher rate (100 mg/kg soil) did not influence the proline accumulation in roots for well-watered soybean plants. However, the seed inoculation with *B. japonicum* increased the proline concentration by 16,4% in comparison to uninoculated plants under drought conditions. Under water deficit conditions it was registered a significant increase of proline concentration in roots in treatment with a moderate dose of P in conjunction with seeds bacteria inoculation where this parameter increased by 42.2% compared to reference treatment (P20). Plants accumulating higher contents of free proline show increased stress tolerance (Ashraf, 2010). The

increase of proline in drought stressed soybean plants corroborate with the findings of other studies for wheat (Jatav *et al.,* 2012) and for lupine (Egamberdieva *et al*., 2017). Shukla *et al* (2015) have reported that priming *Triticum aestivum* L. with *T. harzianum* improved drought stress tolerance by mediating enhanced synthesis and accumulation of proline, thereby conferring tolerance to drought stress. Our study's results reflect increased accumulation of proline in inoculated soybean plants ~~as~~ compared to uninoculated plants. A similar observation was reported by Vardharajula *et al* (2011) for maize, where *Bacillus* spp. improved plant growth and tolerance to drought stress via enhanced production of proline, amino acids and soluble sugars. It seems that bacteria strain application could attenuate the drought effects and improve water status in plants. We assume that integrated application of P and rhizobacteria provided better conditions for proline synthesis which in turn induced plant tolerance to drought. Plants subjected to stressed conditions show higher activity of proline synthesizing enzymes while ~~as~~ the activity of catabolizing enzymes is down regulated (Iqbal *et al*., 2015; Khan *et al.,* 2015). The increase in proline accumulation due to rhizobacteria inoculation may occur because of its direct impact on the metabolizing machinery. In addition, the obtained results could be explained due to the fact that nitrogen-fixer bacteria improve nitrogen (N) nutrition in crops which was associated with more synthesis of amino acids, particularly proline which is an amino acid rich in nitrogen.

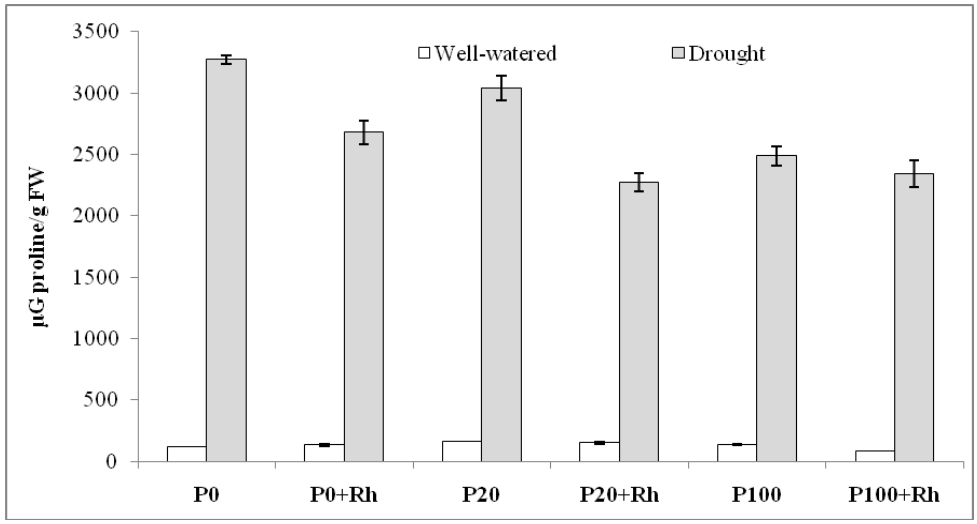

Fig. 2 The effect of *Bradyrhizobium japonicum* (Rh) applied alone or in combination with phosphorus (P) on proline content in nodules under moderate drought. Columns are means ± SE.

In our previous study (Rotaru, 2018) we did not evaluate the changes of proline in nodules of soybean in relation to rhizobacteria inoculation. It is necessary to emphasize that nodules are more sensitive to water deficit and P

deficiency than other soybean organs (Getachew, 2014). The contents of proline in nodules in relation to treatments and soil moisture levels are shown in Figure 2. Experimental results of this research revealed that proline accumulation in nodules was higher than in roots. A significant increase was observed in the proline contents of nodules of soybean plants growing under water stress in contrast to nodules of plants grown under normal soil moisture conditions. We suggest that a higher increase of proline in nodules rather than ~~in~~ roots demonstrated that nodules are more susceptible to water deficit condition. Generally, the interactive effect of P insufficiency and water deficit leads to the highest level of proline concentration in nodules. The application of *B. japonicum* in conjunction with P decreased the proline concentrations by 33.8% and 6.1% in treatments with moderate and high dose of P, respectively (fig. 2). The highest accumulation of this metabolite in nodules under P insufficiency might be due to the fact that nodules are the primary sinks of P and the nodules tissues are abundant in nitrogen source which maintained the proline synthesis at a higher level. Moreover, the increased accumulation of proline showed the efficacy of inoculation treatments in maintaining osmotic potential, which may contribute to cellular adaptation to abiotic stress. In this respect, our findings are corroborated ~~to~~ with those related in studies performed by Sharifi *et al* (2011). Similar results were reported by Egamberdieva *et al* (2017), who observed an improving lupine drought tolerance following treatment with *Bradyrhizobium* species. In this research, it was observed that application of rhizobacteria isolate decreased the proline content in nodules of plants subjected to temporary water deficit and low P supply. However, plants fertilization with a moderate rate of P (20 mg/kg soil) increased proline accumulation in nodules by 34.9% ~~of~~ for well-watered plants compared to unfertilized ones. The application of *B. japonicum* together with a moderate dose of P fertilizer did not significantly change this parameter. The fertilizer application alone at high dose (100 mg P/kg soil) less increased this parameter to a smaller extent in nodules, by only 13.4%. In contrast, the integrated use of rhizobacteria and higher dose of P diminished the concentration of proline in nodules by 56.2%. Perhaps, plants in this treatment were not in stressed state and thereby the proline accumulation decreased. In addition, this result could be explained partially by the biological dilution factor because nodules were better developed compared to those of plants grown under suboptimal P nutrition conditions.

Normal plant growth and the potential to attenuate the adverse effects of unfavorable environments correlated with nitrogen status of crops. It is well known that nitrogen is a major component of the proline molecules and plays an important role in photosynthesis and tolerance of crops. Additionally, certain investigations showed that proline plays an important role of improving energy generation and storage by influencing the nitrogen metabolism (Hashem *et al*., 2015).

Table 1

Interactive effect of *B. japonicum* (Rh*) and* P supplementation on nitrogen contents in soybean plant parts in relation to soil moisture. Data presented are the means ± SE (n = 3)

Treatments	Leaves	Stems	Roots	Nodules
	Normal irrigation, 70% WHC			
P0	4.64 ± 0.26	1.82 ± 0.17	2.74 ± 0.10	4.67 ± 0.09
P0+Rh	4.88 ± 0.17	1.93 ± 0.06	2.72 ± 0.07	5.30 ± 0.13
P20	4.07 ± 0.18	1.45 ± 0.09	2.36 ± 0.11	5.12 ± 0.11
P20+Rh	4.41 ± 0.25	1.54 ± 0.05	2.24 ± 0.11	4.78 ± 0.07
P100	3.37 ± 0.14	1.10 ± 0.05	2.11 ± 0.04	4.65 ± 0.02
P100+Rh	3.96 ± 0.28	1.38 ± 0.06	2.16 ±0.10	4.98 ± 0.05
Water deficit, 35% WHC				
P0	4.45 ± 0.22	1.97 ± 0.07	2.52 ± 0.01	*
P0+Rh	4.23 ± 0.10	2.0 ± 0.09	2.59 ± 0.02	*
P20	4.12 ± 0.17	2.01 ± 0.16	2.38 ± 0.010	5.36 ± 0.03
P20+Rh	3.99 ± 0.10	2.0 ± 0.10	2.31 ± 0.03	5.39 ± 0.14
P100	3.28 ± 0.12	1.24 ± 0.13	2.09 ± 0.01	5.69 ± 0.12
P100+Rh	3.63 ± 0.13	1.39 ± 0.10	2.12 ± 0.04	5.91 ± 0.09

**Insufficiency of nodules mass*

It was well known that seed inoculation with *Bradyrhizobium japonicum* species contributes to improving nitrogen nutrition of soybean plants. However, its magnitude effect depends on environmental conditions, in particular by the P availability for plants and soil moisture regime. The experimental results regarding the effect of this bacterium applied alone or in conjunction with P on nitrogen contents in different parts of plants under normal soil moisture (70% WHC) and temporary water deficit (35% WHC) are presented in table 1. The inoculation increased this parameter by 5.2% in leaves of plants cultivated under P insufficiency and well-watered conditions compared to uninoculated ones. However, the application of *B. japonicum* in conjunction with P at rate 100 mg/kg soil considerably increased the nitrogen content in leaves by 17.5% compared to reference plants. Likewise, the highest increase of this physiological trait by 25.4% was observed in stems. Evidently, these results demonstrate that soybean displays a positive response to P fertilization. Hence, study's data are in concordance with results of other research (Getachew, 2014). There were not significant changes in nitrogen contents in roots due to rhizobacteria strain application irrespective of P

treatments under normal soil moisture level. Regarding nodules, under normal irrigation regime the application of *B. japonicum* increased N content in nodules of plants cultivated in P-deficit soil. The same trend was observed in experiments with soybean by Sa-T and Israel (1995). The application of P at rate 20 mg/kg soil increased nutrient concentration in the symbiotic apparatus by 9.6% compared to unfertilized plants.

Basically, the rhizobacteria inoculation of plants subjected to temporary drought did not have a remarkable impact on nitrogen status of soybean plants. Although, it is necessary to note that there was a slight improvement of nitrogen status in leaves and stems by 10.7% and 12.1% under low soil moisture due to combined use of rhizobacteria and P supplementation (tab. 1). Concerning the nitrogen contents in roots and nodules, there were not registered changes in any studied treatments under water limited conditions. Probably, the water deficit in soil restricted the positive influence of rhizobacteria and the symbiotic system activity was reduced along with decreasing the mineral nitrogen uptake by roots.

CONCLUSIONS

On basis of experimental data we suggested that inoculation with *B. japonicum* could partially compensate the drought effects and improve soybean plant development through enhanced production of proline, probably, other osmolytes too and result in better absorption of water and nutrients from soil. Improved osmolyte proline concentration in roots by integrated use of rhizobacteria *B. japonicum* and P fertilization could have a beneficial impact on root physiological activity by enhancing root system access to more nutrients and water from soil.

Thus, experimental data of this study revealed that rhizobacteria application in conjunction with adequate phosphorus nutrition improved nitrogen content in soybean under not water stress plants, but *B. japonicum* strain did not significantly change nitrogen status in roots and nodules under drought conditions.

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THE SATISFACTION DEGREE OF FOOD PROTECTED REFLECTED PATIENTS ON FRUIT AND VEGETABLES CONSUMPTION

GRADUL DE SATISFACȚIE AL PACIENȚILOR PROTEZAȚI AMOVIBIL REFLECTAT ASUPRA CONSUMULUI DE FRUCTE ŞI LEGUME

VASILIU Mihela Păpuşa[1], TOMIȚĂ Daniela Ivona[1*], SACHELARIE Liliana[1], FUIOAGĂ P.C.[1], POPOVICI Diana[2], STADOLEANU Carmen[1]

*Corresponding author e-mail: daniela.tomita@yahoo.com

Abstract. *A healthy diet of the elderly is important in improving oral health. But the emergence of editorialism prevents the consumption of diversified foods. A nourishing and varied diet, including vegetables, fruits, cereals, dairy products and protein, is of great value especially at the elderly. Many patients regard the mobilizable dentures as a sign of aging and therefore accept them hard, and the accommodation process is difficult. After a rather long period of non-protection, when inserting a prosthesis, it is very difficult to chew or speak with it. This is less common in prosthetic patients. Therefore, restoring the integrity of dental arches by applying mobilizable prostheses increases the satisfaction of edent patients during mastication.*

Key words: oral health, vegetables, fruits, minerals, vitamins, removable dentures, satisfaction

Rezumat. *O alimentație sănătoasă, a persoanelor în vârstă, este importantă în ameliorarea stării de sănătate orală. Dar apariția stării de edentație împiedică consumul alimentelor diversificate. O dietă hrănitoare şi variată, în care sunt incluse legumele, fructele, cerealele, produse lactate şi proteinele, au o importanță beneficămai ales pentru persoanele mai vârstnice. Mulți pacienți privesc protezele dentare mobilizabile ca şi un semn al îmbătrânirii şi de aceea le acceptă greu, iar procesul de acomodare este îngreunat. După o perioadă destul de lungă de neprotezare, în momentul inserării unei proteze se obişnuiesc foarte greu să mestece sau să vorbească cu aceasta. Acest lucru este mai puțin întâlnit la pacienții purtători de proteze. De aceea refacerea integrității arcadelor dentare prin aplicarea de proteze mobilizabile, creşte gradul de satisfacție a pacienților edentați .*

Cuvinte cheie: sănătate orală, legume, fructe, minerale, vitamine, proteze dentare mobilizabile, satisfacție

INTRODUCTION

Problems with mobilizable prostheses can affect patient satisfaction and lifetime of the prosthesis. Hummel *et al.* (2002) reported that 65% of patients

[1]„Apollonia“ University, Faculty of Dental Medicine, Iaşi, România
[2]„Gr. T. Popa“ University of Medicine and Pharmacy, Iaşi, România

carrying mobilizable prostheses have at least one problem, the lack of stability being the most common. Other authors have shown that loss of retention, aspects related to the vertical dimension of occlusion, trauma and ulceration play a significant role in patient satisfaction (Bilhan, 2012). Koyama *et al.* found a significant relationship between age, edentar ridge, number of occlusal stops, pain and discomfort, the shape of artificial teeth, and the patient's desire to use these types of prostheses (Koyama, 2010). It was found that pain and discomfort are the most important causes of interruption of the mobilization of mobile prostheses (Akeel, 2010).

Lack of communication with the treating physician makes it possible to discontinue the mobilization of the mobilizable prostheses, but the patient is unaware that the dentures help mastication properly, thus obtaining all the nutrients necessary for the body.

Dental prostheses improve overall physical condition, provide control over the diet, and without their consumption of raw fruits and vegetables is difficult.

It can be assumed that the quality of prosthetic treatment can also affect oral health, so oral health is related to quality of life because prosthetic and surgical treatment is performed to improve patient satisfaction (Douglass, 2002; Inoue, 2011).

MATERIAL AND METHOD

The study was conducted over a period of 2 years, attended by 37 patients (23 women and 14 men) with a large partial protuberance, prosthetic prostheses 13 and classic removable prostheses - acrylic and modern - skeletonized and total - 24. This study is based on the completion of questionnaires for patients who have addressed the Clinic of Dental Prosthetics, Apollonia University in Iasi, in the urban area, to improve the quality of mastication with dental prostheses. The age of selected patients is between 40 and 75 years of age, with an average age of 57.5 years and the absence of any debilitating systemic illness. Prior to conducting the study, the research protocol was approved by the Institutional Ethics Commission.

For the patient satisfaction form, we used a questionnaire developed by Siqueira *et al* (de Siqueira GP, 2013) in this study. This questionnaire evaluates patient satisfaction in four categories: (1) mastication, (2) aspect, (3), (4) comfort level. In each part, patient satisfaction was assessed using the Likert analog visual scale ranging from 0 to 10, with zero representing the lowest satisfaction and 10 representing the highest satisfaction in mobilizable prosthesis carriers. Average satisfaction was calculated in all four categories (Zlataric, 2008).

RESULTS AND DISCUSSIONS

The lack of stability and retention of mandibular prostheses that increase over time with continuous residual resorption, impaired masticability are the major complaints of total prosthesis carriers (Van Waas, 1990). These handicaps combined with the resulting pain and impairment of patients' ability to communicate can cause dissatisfaction.

The use of mobilizable dentures is strongly influenced by patient satisfaction. Factors such as patient's attitude and personality, the quality of the mobilizable prostheses, the oral hygiene of patients, the tolerance and the level of comfort can influence the satisfaction of prosthetically treated patients with mobilizable prostheses (Akeel, 2010).

In our study too, all evaluated cases had moderate to high (6.2 out of 10) levels of satisfaction, the result of the questionnaire is shown in table 1.

Table 1

Distribution of average and standard deviation of four demographic variables

	CHEWING		FACIAL ESTHETICS		SPEECH		COMFORT	
	Mediate ± SD	**p**	**Mediate ±SD**	**p**	**Mediate ± SD**	**p**	**Mediate ± SD**	**p**
Age								
Less than 50 years old	4.4±4.0	0.004	4.9±3.9	0.01	4.7±3.9	0.01	4.4±4.0	0.004
Over 50 years	7.3±2.4		7.3±2.4		7.3±2.4		7.3±2.4	
Sex								
Men	5.1±3.8	0.07	5.6±3.7	0.13	5.4±3.7	0.1	5.1±3.8	0.06
Ladies	7.1±2.7		7.1±2.8		7.06±2.8		7.1±2.8	
Kennedy Classification								
I	6.5±3.5		6.8±3.4		6.7±3.2		6.5±3.6	
II	6.0±3.2	0.41	6.3±3.2	0.32	6.1±3.2	0.35	6.0±3.2	0.37
III	5.6±3.8		5.8±3.6		5.6±3.6		5.5±3.8	
IV	9.0±		9.0±		9.0±		9.0±	
Spring								
Maxillary	7.4±3.0	0.004	7.5±2.9	0.005	7.4±3.0	0.004	7.4±3.0	0.006
Mandibulary	5.1±3.5		5.3±3.5		5.1±3.5		5.1±3.6	
The type of doctor								
Student	5.6±3.4		5.8±3.4		7.5±3.0		7.5±2.9	
Prosthetic specialist	8.1±2.8	0.009	8.1±1.5	0.003	5.5±3.4	0.005	5.3±3.5	0.004
Removable partial prosthesis								
Chromium-cobalt	6.01±3.4		6.15±3.4		6.15±3.4		6.01±3.5	
Acrylic	9.0±1.4	0.22	9.0±1.4	0.22	9.0±1.4	0.22	9.0±1.4	0.24
The total	6.1±3.4		6.25±3.3		6.25±3.3		6.1±3.5	

Patient satisfaction in patients aged 50 years or older was significantly higher in all four categories compared to patients aged less than 50 years ($P < 0.05$).

Our study shows that patients aged 50 years or older were more satisfied with the mobilization of prostheses compared to those younger than 50 years, and patients aged 45 to 65 were much more satisfied than the 65-year-old.

Also in our study, patient satisfaction is not gender-related, but women tended to be more satisfied than men.

There was no difference in patient satisfaction and the type of Kennedy classification of the crest edited in this study.

In all four categories, there were no significant differences between men and women. The Kennedy classification had no influence on patient satisfaction (P> 0.05). When all four categories were analyzed, patients with jaw mobilizable prostheses were much more satisfied than those with mandibular mobilizable prostheses. Patients who wore jaw mobilizable prostheses were more satisfied than patients who had mandibular partial prosthesis (P <0.05). Patients treated by specialist doctors were more satisfied than those treated by students (P <0.05). There was no significant difference between patients who had acrylic-based prostheses or chromium-cobalt metal base (P> 0.05).

In the questions in the questionnaire regarding the lack of retention during speech, one of the frequent prosthetic wearers' complications, 36%, was dissatisfied with this inconvenience (fig. 1). Consumption of soft foods in 54% caused inconvenience, while in 28%, the inconvenience was caused by hard foods (fig. 2).

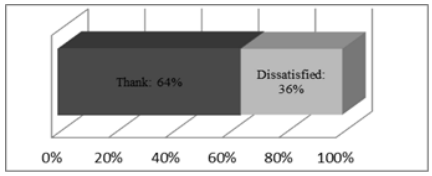

Fig. 1 Lack of speech retention

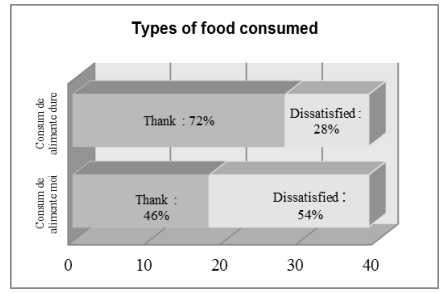

Fig. 2 Satisfaction of patients with food consumption

The health of teeth and dental tissues is determined by the consistency of foods. Consumption of hard, crunchy foods like raw vegetables and fruits (carrot, sweet pepper, turnips, radishes, apples, peaches, pears, quinces) contributes to the cleaning and self-cleaning of dental surfaces as well as to the massage of surrounding soft tissues with stimulating action on gingival circulation.

Therefore, daily consumption of hard fruits (apples, pears) and a series of vegetables (cucumbers, tomatoes, salad, spinach, cauliflower, broccoli,celery) are recommended because have a high water content that reduces the effect of sugars saliva. But for the consumption of these foods, edentuous patients should use as much stable prostheses as possible in the prosthetic field.

Also, 33% of patients reported having difficulties in the tolerance of the prosthesis (fig. 3). Thanks to the insertion / disinfection capacity of the prosthesis in the oral cavity, 74% was declared (fig. 4), and with regard to the aesthetic aspect of the prosthesis, 82% were satisfied (fig. 5).

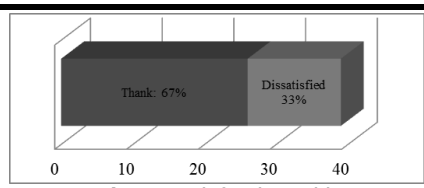

Fig. 3 Satisfaction with prosthetic tolerance

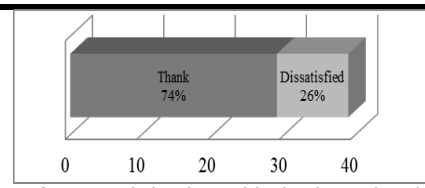

Fig. 4 Satisfaction with the insertion / disassembly capability of the prosthesis in the oral cavity

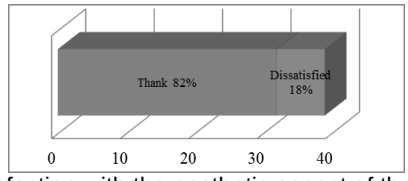

Fig. 5 Satisfaction with the aesthetic aspect of the prosthesis

Removable prosthetic therapy is still widely used due to an economical procedure and easy to apply to patients in need of partial mobilization of prosthesis. However, this prosthesis may be associated with several complications. It is known that the most common complication of removable prostheses is loss of retention, denture destabilization and ulceration, patient discontent with mastectomy, noise, comfort of prostheses and aesthetics, due to an incorrect vertical dimension and, of the tall vertical dimension. The cause of the failures is related to the destructive action of the design and production of inadequate prostheses. Some patients in this study complained about poor adaptation and improper prosthesis manufacture. This complication is an essential reason to replace them. In addition, loss of retention has caused dissatisfaction with patients associated with the function. Loss of prosthesis retention may affect the ability of patients to chew.

As a result of the study, the rate of complications of mobile prostheses decreases, if they are correctly made and inserted in the prosthetic field, and the degree of patient satisfaction associated with mobilizable prosthesis, odontal health and periodontal position of the remaining teeth, the masticatory stability increases significantly. The effect of various types of prostheses on satisfaction are aspects that increase the quality of life of patients.

CONCLUSIONS

1. The types of complications of patients with partial dysfunctions occurring during the mobilization of mobile dentures may affect the acceptance and the degree of satisfaction

2. The instability of prostheses in the prosthetic field leads to loss of retention, which causes discontent among patients related to the ability to chew especially hard foods.

3. Ulcerations that result from poor insertion of mobilizable prostheses or fracture of prostheses negatively affect the ability of mastication and phonation.

4. From the above results, it is concluded that patients with correctly made mobilizable dentures have a high level of satisfaction both aesthetically and functionally, along with a positive impact on good nutrition, by increasing the consumption of fruits and vegetables raw, required oral health of patients.

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INFLUENCE OF DIFFERENT TYPES OF GRANULATED SUBSTRATE ON *CORDYCEPS MILITARIS* MUSHROOM MYCELIUM GROWTH

INFLUENŢA DIFERITELOR TIPURI DE SUBSTRAT GRANULAT ASUPRA CREŞTERII MICELIULUI CIUPERCII *CORDYCEPS MILITARIS*

RÓZSA Melinda[1], APAHIDEAN Maria[1]*
*Corresponding author e-mail: apahidean_maria@yahoo.com

Abstract. *Mushroom mycelium is a biological preparate that is obtained under sterile laboratory conditions and which, in an optimal microclimate, can reproduce the mushrooms from which it originates. Mushroom mycelium is used to inoculate the prepared substrates. This inoculum consists of a supportive material completely colonized by fungal mycelium. The type of support material varies depending on the cultivated mushroom species, although rye is the choice of most mycelium producers. In our experience, we have been looking at how to grow Cordyceps militaris mushroom mycelium on various substrates of cereal grains. The growth was followed for 10 days under laboratory conditions, and the most intense increase was recorded on millet grains with an average increase of 1.65 mm/day and the worst growth was recorded in the case of rye with an increase average of 1.05 mm/day.*
Key words: Cordyceps, mycelium, protein addition

Rezumat. *Miceliul de ciuperci este un preparat biologic ce se obţine în condiţii sterile de laborator şi care, plasat într-un microclimat optim, poate reproduce ciuperca din care provine. Miceliul de ciuperci este utilizat pentru a inocula substraturile preparate. Acest inocul este format dintr-un material de suport complet colonizat de miceliul ciupercii. Tipul de material de suport, variază în funcţie de speciile de ciuperci cultivate, cu toate că boabele de secară sunt alegerea majorităţii producătorilor de miceliu. În experienţa derulată am urmărit modul de creştere a miceliului ciupercii Cordyceps militaris pe diferite substraturi formate din boabe de cereale. Creşterea s-a urmărit timp de 10 zile în condiţii de laborator, iar creşterea cea mai intensă s-a înregistrat pe boabele de mei cu o creştere medie de 1,65 mm/zi, iar creşterea cea mai slabă s-a înregistrat în cazul boabelor de secară cu o creştere medie de 1,05 mm/zi.*
Cuvinte cheie: Cordyceps, miceliu, adaos proteic

INTRODUCTION

Cordyceps militaris (L.) Link is an entomopathogenic fungus, belonging to the Ascomycetes class, is one of the most important medicinal mushrooms which has been used popularly as a folk tonic food and a crude drug in East Asia (Ying *et*

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania

al., 1987). This medicinal mushroom, contains many kinds of active components, such as polysaccharides, cordycepin, mannitol and ergosterol, and due to its several physiological activities, it is currently used in medicine (Mizuno, 1999; Song *et al.,* 1998; Nag and Wang 2005). In the nature, is widely distributed, from sub-tropical to temperate regions around the world (Mains, 1958).

Until now, more than 375 species of Cordyceps were discovered all around the world, of which about 135 species have been originated in China (Jiang, 2004).

Cordyceps militaris (L.) Link is the type of Cordyceps species, which naturally parasitizes larvae or pupae of lepidopteran insects, and has a worldwide distribution (Kryukov *et al.,* 2011; Shrestha *et al.,* 2012).

All Cordyceps mushrooms are endoparasitoid species, mainly on insects and other arthropods, just a few species are parasitic other fungi (Shrestha *et al.* 2004).

Brownbridge *et al*. (1993) have studied common entomopathogenic fungi in forest soils and Hajek et al. (2000), have been isolated entomopathogenic fungi from the interface between leaf litter and the organic layer of soil.

In natural condition, Cordyceps militaris mycelium colonizes the body of an insect, the spores of the fungus germinate and produce a germ tube that penetrates the cuticle, allowing the mycelia to grow inside the host body (Tanada and Kaya 1993, Inglis *et al.,* 2001).

In last year's Cordyceps militaris is cultivated in liquid or in solid media (Das *et al.* 2010). In solid media different supplemented grain types and seeds are used (Chen *et al.* 2011, Shrestha *et al.* 2012, Wen *et al.* 2014, Yi *et al*. 2014, Rozsa *et al*. 2016 a, b, c, d).

Mushroom mycelium is used to inoculate prepared substrates. This inoculum consists of a support material completely colonized by the mushroom mycelium. The type of support material varies depending on the cultivated mushroom species, although rye beans are the choice of most mycelium producers (Rozsa *et al.,* 2016 a, b).

In 1932, Dr. James Sinden patented a new process of mycelium production, using cereal grains, as a support material for mycelium. Since then, rye has been the most commonly used cereal, although both millet and wheat have been used. The new approach of Sinden, has set a new standard for inoculation and is the basis for most modern inoculum productions. The distinct advantage of inoculation on cereal grains is the increase in the number of inoculation places. Each coarse grain thus becomes a point through which the mycelium can spread. Thus, one liter of the inoculum on rye grains contains about 25,000 grains, representing a vast improvement of the inoculum, compared to the coarse materials that have been used before (Rozsa *et al.,* 2016 a, b, c, d).

MATERIAL AND METHOD

To produce mycelium on cereal grains, these are moistened at 48-52% humidity, then sterilized in the autoclave or in a pressure cooker for 1 hour at 121 °C, in the containers to be inoculated (fig. 1).

After the cereal jars were autoclaved, they were placed in the sterile room and allowed to cool.

After removing the hot jars from the pressure cooker or autoclave, they were stirred to break up the grains caught on top and evenly distribute the wet and dry cores, according to the method described by (Rozsa *et al.*, 2016 a, b, c, d). Shaking prevents the beans from sticking and hardening on the bottom of the jar.

Fig. 1 Preparation of jars with cereals for sterilization (original).

The biological material used to inoculate cereal grains was a pure mycelium culture on agar substrate (fig. 2).

Fig. 2 Pure culture of *Cordyceps militaris* mycelium (original).

Bottle inoculation (fig. 3), was carried out at the flame of a gas bulb according to the method described by (Rozsa *et al*., 2016 a, b, c, d).

Fig. 3 Bottle inoculation (original)

In this experience we used as a protein additive dried and ground silkworm (*Bombyx mori*) chrysalis, in concentration of 10 grams per litre of cereals.

RESULTS AND DISCUSSIONS

The evolution of mycelium growth on the substrates used in this experiment with protein addition is shown in figure 4. The evolution of mycelium growth on the substrates used in this experiment without protein addition is shown in figure 5.

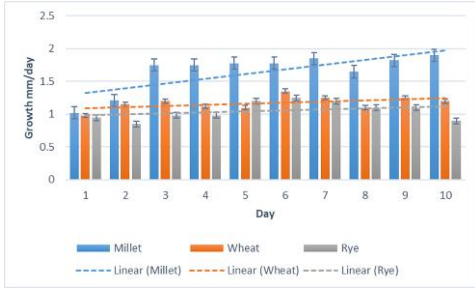

Fig. 4 The evolution of mycelium growth substrates with protein addition.

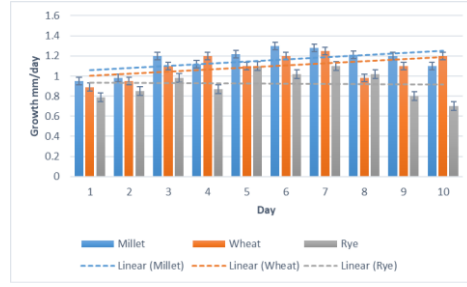

Fig. 5 The evolution of mycelium growth substrates without protein addition.

Following the unilateral influence of the substrate on mycelium growth, considering the substrate with rye, considering that most of the industrial producers of mycelium use as rye substrate, the millet substrate recorded very significant differences of growth 0.43 mm compared to the control considered, being followed by the wheat substrate with distinctly significant differences 0.15 mm.

Following the unilateral influence of protein addition on mycelium growth, there was a significant difference of 0.11 mm on substrates with additional protein addition and a significant negative difference on those without additional protein addition.

Following the combined influence of the factor's additional protein addition and substrate, at the substrate with millet and additional protein addition, there was a significant difference of 0.24 mm compared to the considered control.

The combined influence of substrate factors and additional protein addition once again revealed thousands based on or without additional protein addition, which in the case of additional protein addition recorded very significant differences, 0.60 mm compared to the average of the experience, and in the absence of additional protein addition, positive distinct significantly differences 0.26 mm from the control of experience.

The results obtained are comparable to those found in the literature, thus Wen *et al.* 2014 mentions an average increase of 0.3 mm / day, but without mentioning whether or not additional protein was used.

According to Yi et al. 2014 mentions an increase of 0.25 mm / day, with the addition of peptone.

CONCLUSIONS

1. The fastest growth of the mycelium of the *Cordyceps militaris* mushroom was recorded on millet grains, followed by wheat.

2. Adding additional protein powder represented by cocoons of silkworm (*Bombyx mori*) has positively influenced the growth and development of the mycelium fungus Cordyceps Militaris.

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CULTIVATION AND EXTRACTION OF VOLATILE OILS OBTAINED FROM NEW VARIETIES OF BASIL (*OCIMUM BASILICUM L., FAM. LAMIACEAE*)

CULTIVAREA ŞI EXTRACŢIA ULEIURILOR VOLATILE OBŢINUTE DIN SOIURI NOI DE BUSUIOC (*OCIMUM BASILICUM L., FAM. LAMIACEAE*)

STAN (TUDORA) Cătălina[1,2*], BURNICHI F.[3], MUSCALU Adriana[2], VINTILĂ M.[4], ISRAEL-ROMING Florentina[1]

*Corresponding author e-mail:cmc_tudora@yahoo.com

Abstract. *The paper presents the experimental research carried out within INMA Bucharest regarding the cultivation of two new varieties, 'Aromat de Buzau' and 'Serafim', created by SCDL Buzau. These belong to the two varieties of basil (Ocimum basilicum L., Lamiaceae) yellow and purple, which were grown in the agricultural years 2017 and 2018, years that were different in terms of agrometeorological conditions. It also presents the method for obtaining vegetal extracts (volatile oil and floral water), obtained by processing the vegetal raw material while applying a process based on water vapour pressure distillation. Extraction yields obtained are presented comparatively. The results are the premises for obtaining new products with a high market value, which can be applied in the future for vegetable crops protection in greenhouses and solariums.*

Key words: basil, Aromat de Buzau, Serafim, extraction, volatile oils, floral waters

Rezumat. *Lucrarea prezintă cercetările experimentale realizate în cadrul INMA Bucureşti, referitoare la cultivarea a două soiuri noi, 'Aromat de Buzau' şi 'Serafim', create de SCDL Buzău. Acestea aparţin celor două varietăţi de busuioc (Ocimum basilicum L., fam. Lamiaceae) galben şi violaceu, ce au fost cultivate în anii agricoli 2017 şi 2018, ani diferiţi din punct de vedere al condiţiilor agrometeorologice. De asemenea, este prezentată metoda de obţinere a unor extracte vegetale (ulei volatil şi apă florală), obţinute prin prelucrarea materiei prime vegetale, printr-un procedeu ce are la bază distilarea cu vapori de apă sub presiune. Se prezintă comparativ randamentele de extracţie obţinute. Rezultatele constituie premisele obţinerii unor produse noi cu valoare de piaţă ridicată, care se vor putea aplica pe viitor în protecţia culturilor legumicole din sere şi solarii.*

Cuvinte cheie: busuioc, Aromat de Buzău, Serafim, extracţie, uleiuri volatile, ape florale

INTRODUCTION

Culture of basil is known and appreciated in Romania from ancient times,

[1]University of Agronomical Sciences and Veterinary Medicine Bucharest, Romania
[2]National Institute of Research-Development for Machines and Installations Designed to Agriculture and Food Industry (INMA) Bucharest, Romania
[3]Vegetable Research and Development Station Buzau, Romania
[4]IICDIMPH-HORTING, Bucharest, Romania

both because of the medicinal, culinary properties, but also of the spiritual symbolism that this plant has. Basil is grown today in many other parts of the world (France, Egypt, Hungary, Morocco, USA, Greece, Israel).

Currently, at least 150 varieties are grown, each with its own type of volatile oil, characterized by its distinctive flavor. Depending on the chemical composition of the oil, basil has three biochemical profiles:

- Basil grown in Europe (french basil, sweet, garden) and America have the major components of volatile oil represented by: 4-5% eugenol, 2-3% of methylchavicol (estragol), very good quality linalool at least 50%, with a fine aroma and without camphor;
- Common African basil, indian basil - with major methylchavicol (estragol) 50-70%, linalool 8-30% and camphor content;
- Basil with high content of eugenol (Viturro *et al.*, 1999).

Oil obtained by water vapor entrainment from leaves and floral peaks differs depending on the chemotip from which the raw material originates. More than 200 chemicals have been reported in basil oil, including monoterpenes, triterpenes, sesquiterpene, flavonoids and aromatic compounds. Major components of basil oil include: linalool, estragol (methyl chavicol), anethole, eugenol and methyl eugenol (Qing *et al.*, 2016).

MATERIAL AND METHOD

The plant material used for the establishment of the crop was represented by seedlings belonging to new varieties (creations of SCDL Buzau) from two varieties of basil:

- ***'Aromat de Buzau'*** variety, that belongs to Yellow Basil variety (Homologation Certificate no. 1367 / 22.03.2006)
- ***'Serafim'*** variety, that belongs to Violaceous Basil variety **(**Homologation Certificate No. 4432 / 22.05.2017)

The main characteristics of the ***'Aromat de Buzau' variety*** (Burnichi *et al.,* 2014) (fig. 1.a): Semi-varieties, with are well defined genetic constitution, very well adapted to the environmental conditions existing in our country. The variety is easily recognizable due to its specific flavor, but also by distinct characters: the color of the leaf, the length of the shoots, the ability to preserve its properties during the preservation period. The mature plant is in the form of a strongly branched, slightly flowing bush with a height of 40-60 cm; The leaves are oval, slightly pointed at the top, light green; Flowers are white, somewhat rarer than those of common basil, and the fruits (seeds) are very small (600-800 seeds/g), black and with a high storage capacity without greatly diminishing germinative ability (4-6 years). From the point of view of the relationship with the environmental factors there are no special requirements, preferring the fertile and heavily sunny soils. Cultivation technology supports great flexibility depending on the purpose of the crop and can be cultivated both by direct sowing and seedling. The variety can be grown in an ecological system, and due to the flavor that the plant releases through all its organs there are no losses caused by diseases or pests. Average shoots production is around of 10 - 12 t / ha. The main characteristics of the ***'Serafim' variety*** (Burnichi *et al.,* 2014), (fig. 1.b): Semi-sweet variety, a variety of red-violet basil with an unknown cross,

blooming from summer to autumn. Plant width: 60-70 cm, diameter of the bushes: 40-45 cm, richly branched, with slightly jagged leaves. Unusual, strong, purple-purple color is due to the high content of anthocyanins. The flower has pink-violet corolla, seeds are elliptic 2/1 mm, black color - slightly brown, mate, MMB = 1.3 g, at 1 g = 750 seeds with high storage capacity (4-6 years) without diminishing their germination. It can be used for culinary, ornamental and medicinal purposes, and it is also possible to use it in the perfume industry due to the very pleasant flavor of cloves. It can be cultivated successfully in protected areas, in the open field, but also in the pots. An average yield is about 9 - 10 t ha.

Fig.1.a Yellow Basil *'Aromat de Buzau'* variety

Fig.1.b Violaceum Basil *'Serafim'* variety

In order to obtain the volatile oils and the flood waters of basil, the Hydraulic distillation (pressure water vapor distillation from a separate generator) was used as a method, using a french-based installation, AURA DISTILLATEUR, with a main tank capacity of 130 liters.The extraction yield was calculated using the formula:

Extraction yield (ml/kg) = V/M,

where: V = the volume of essential oil obtained from the plant sample (mL);
M = sample mass of herbs (kg).

The institution where the research was conducted was HORTING Bucharest, and the research method used to obtain the volatile oils was Hydrodistillation (distillation with water vapor under pressure).

RESULTS AND DISCUSSIONS

On the existing land at INMA Bucharest (Baneasa area), under the climatic conditions of the years 2017 and 2018, on a brown-red soil of the forest, the crops with the two basil varieties were established by planting seedlings procured from SCDL Buzau. From an agro-climatic point of view, 2017 was characterized on average by an increase in temperature by 0.7°C above normal climatic (1981-2010). The amount of precipitation accumulated in 2017 was 6% higher than normal climatological (1981-2010). Decreases in rainfall were positive in four of the 12 months of the year under review, ranging from 2% in February to 73% in October, while negative deviations were recorded over the next four months, January, March, June and August, ranging from 12% in March to 37% in January (2017 Report-NMA). For 2018, the average air temperature exceeded by 1.35°C the climatological norm in force (multi-annual average 1981-2010). In 2018 there were 9 months with positive monthly thermal

deviations between 0.3 and 4.7°C, compared to the period 1981- 2010, important in terms of the plant vegetation period, being the moons (+2, 2°C), June (+ 0.9°C), August (+ 1.8°C), September (+ 1.1°C), October (+ 2.0°C). The amount of rainfall for this year was 698.8 mm, 10% higher than normal climatological (1981-2010). Monthly precipitation declines were negative, oscillating between 6% in November and 64% in April (Communique 2018-ANM).

The crop technology applied to the two new varieties of basil included the following technological links:

• *The basic fertilization* was done with the autumn spring, when organic fertilizers (40 t/ha of manure) and mineral fertilizers (phosphorus 40-50 kg / ha and 30-40 kg / ha sa).

• *Soil works* began in the spring before planting, when the land was worked with the grower and the harrow, to maintain moisture in the soil and to remove ephemeral weeds. Even though basil is not too pretentious to the type of soil, however, it needs to be loosened and well mobilized.

• *Establishment of the basil culture* (in both cases) was done by seedlings. For the production of seedlings it sowed in the second decade of March in multiplier greenhouses, using approx. 6 g seeds / m^2. The care work on seedlings was the usual. The seedlings needed for the area of 200 square meters (area of the experimental lots) were produced by sowing directly in the alveoli in greenhouses, by SCDL Buzau.

• *Seedling planting* was done in the field early in May using a planting scheme: 1.00 m/between the lines (for mechanization of maintenance) and 35-40 cm between plants in the row, so that the areas of 2 × 200 m about 1,200 pieces were planted. seedlings of Yellow Basil, variety *'Aromat de Buzau'* and Violaceous Basil, *'Serafim'*, variety.

• *Maintenance work* consisted of manual and mechanical drills. The first hoeing manual has been executed after the operation of planting the seedlings, and the following hoeing were executed whenever needed, both for combating weeds between the rows of plants/from plants in the row, but also for loosening the soil and combating crust formed around the plants. The main weeds found in culture were the ephemeral: like *Veronica sp*., Buckwheat climbing - *Polygonum convolvulus*, then weeds summer: Gloomy - *Setaria sp.*, Pigweed - *Amarantuhus retoflexus*; Bindweed - *Convolvulus arvensis*, Greasy grass - *Portulaca oleracea.* They were the main problem and were eliminated by manual and mechanical works, the culture is kept "clean" without the application of synthetic chemicals.

•*Watering* was done whenever it was needed (especially in August that was a water-scarce month), with watering norms=300-400 m^3 of water, using sprinkling. Noteworthy, that in the climatic conditions of the year 2018, July was a very rainy month with norms above average, which negatively influenced vegetative growth and development (implicitly the accumulation of volatile oils) for both varieties of basil.

• *Harvesting* was done following the degree of accumulation of volatile oil in

plants, when on the central inflorescence, the verticals at the base have brownish-red fruits. This work was staggered, obtaining 2 crops (in August and early October). In bothcases, the work was done mechanically, in sunny weather, using a Herbal Harvesting Equipment, an experimental model, the creation of the institute. After the production of the vegetable raw material, the following step was carried out: the production of volatile oil and floral water from the two basil varieties, 'Aromat de Buzau' and 'Serafim' using french-derived plant (Aura Distilllateur). For this, the plant material must meet technical reception conditions: no organic foreign bodies are allowed, the maximum permissible admissible level of impurities must be max. 5% (floral stems, brunched flowers), mineral alloys max. 0.5%, max. 11% (Verzea, 2002). At the start of the drive operation, when the plant product is rich in volatile oils, steam production was moderate, cooling the strong refrigerant and condensing rapidly to avoid losses. When distillation is done too quickly, water vapor drives less volatile oil. The first portions of distillate contain the largest amount of hydrophilic principles: aldehydes, alcohols, acids, etc. and have a pleasant flavor. The following portions are opalescent, have a less pleasant aroma and contain terpenic hydrocarbons, which are hardly soluble in water. The end of hydro destillation is marked by the lack of odor of the distillate. The collected distillate mixes well (by stirring) to make a saturated solution, and the excess volatile oil is collected at the surface of the Florentine vessel (oil separator). By the hydrodynamic procedure of the two basil varieties, a distillate, consisting of a volatile / essential oil and floral water, called the hydrolyte (a mixture of volatile/essential oil and floral water) was obtained, which was then separated. It is worth anyting that both the quantity and especially the quality of the volatile/essential oil and the floral water obtained differ according to the type of soil and especially the agro-climatic conditions of the respective production year. Once obtained, they were stored in dark glass containers, clean and sterilized at low temperature. Table 1 shows the field culture technology of basil (both varieties).

Table 1

Technological data sheet of basil culture

Technological sequence	Yellow Basil variety ***'Aromat de Buzau'***	Violaceous Basil variety ***'Serafim'***
Destruction of plant remains	tractor of 55–75 CP in aggregate with the disk harrow and adjustable harrows	
Fertilization	in order to ensure permanently the nutritive elements which are necessary for plants. Basic fertilisation with organic/chemical composts is made with a 55-75 CP tractor in aggregate with the machine which furnishes organic/chemical composts	
Ploughing	depth 23-25 cm if the soil is humid enough, with a tractor of 55–75 CP in aggregate with plough and star-toothed horrow or disk harrow and adjustable harrows	
Maintenance ploughing	multiple disking in order to maintain the field without weeds	

Preparing germination bed	is made with a tractor of 55–75 CP in aggregate with combinator	
Planting	at the beginning of May, daily temperatures reaches values over 17ºC. Is made when the seedling have 6-7 cm hight.	
Culture irrigation	are made in order to ensure the water necessary during the growth an flowering periods	
Harvest	when 25-30% of plants had flowered, by cutting the airy part at 8-12 cm up from the soil. Can be realised 2-3 harvests/an	
Extracţ ion yield	5 mL of volatile oil/1 kg of vegetable material	1 mL of volatile oil/1 kg of vegetable material

CONCLUSIONS

1. The two varieties taken in crop are valuable varieties, and crop technology does not involve high cost to the surface unit. The yields obtained are large at the surface unit (10 - 12 t / ha).

2. The yield of extraction depends on the type of plant and the distillation time, and 1 kg of the plant produces 1 liter of hydrolysate. The extraction yield obtained for the two basil was 1 mL of volatile oil/1 kg of vegetable material for *Violaceum Basil* - 'Serafim' variety and 5 mL of volatile oil/1 kg of vegetable material for *Yellow Basil* - *'Aromat de Buzau'* variety.

3. The volatile oils and floral waters that are obtained are of superior quality with quality chemical compounds (linalol, estragol, eugenol), search and use in the perfume and cosmetics industry.

4. It is also possible to create the premises for obtaining new products with high market value, which can be applied in the future in the protection of greenhouse crops in greenhouses and solariums.

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BIOCHEMICAL PARAMETERS OF TOMATO UNDER CHEMICAL FERTILIZERS

INFLUENȚA FERTILIZĂRII CHIMICE LA TOMATE ASUPRA UNOR INDICATORI BIOCHIMICI

STOLERU V. [1*]***, MIHALACHE G.*** [1, 2]***, PERES C.*** [1]***, TELIBAN G.*** [1]***, COJOCARU A.*** [1]***, MURARU V.M.*** [3]

*Corresponding author e-mail: vstoleru@uaiasi.ro

Abstract. *Fertilizers play an important role in providing nutrients to plants and in sustaining an optimal crop yield. In general, plants need three major elements for their optimal growth and development: nitrogen (N), phosphorus (P) and potassium (K). Most of the modern chemical fertilizers contain one or all of these nutrients. Other important elements are sulphur, magnesium and calcium. Micronutrients such as iron, chlorine, copper, manganese, zinc, molybdenum and boron are needed just in small amounts, but are equally important for the plants. In this context, at the UASVM Iasi was organized an experiment to obtain important information regarding the effects of chemical nutrients on tomato biochemical indicators.The water content, the minerals and the tannin content from fruits varied under different chemical fertilizers.*
Key words: chemical fertilizers, tomato, biochemical parameters

Rezumat. *Fertilizatorii joacă un rol esențial în furnizarea de nutrienți pentru plante şi în obținerea unei producții optime. În general, plantele au nevoie de trei elemente principale pentru creştere şi dezvoltare: azot (N), fosfor (P) şi potasiu (K). Majoritatea îngrăşămintelor chimice moderne conțin unul sau toți aceşti nutrienți. Alte elemente importante sunt sulful, magneziul şi calciul. Micronutrienții precum fierul, clorul, cuprul, manganul, zincul, molibdenul şi borul sunt necesari doar în cantități mici. În acest context, au fost testate efectele fertilizării chimice asupra unor indicatori biochimici la plantele de tomate. Rezultatele au variat în funcție de tratamentul chimic aplicat.*
Cuvinte cheie: fertilizare chimică, tomate, indicatori biochimici

INTRODUCTION

Vegetables in general and tomatoes in particular, have specific nutrient consumption, which differs depending on the planned commercial production or the biological production according to the growth and development phenophases. During the vegetation period, the highest consumption of macro- and

[1]University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania
[2] Integrated Center of Environmental Science Studies in the North East Region (CERNESIM), The "Alexandru Ioan Cuza" University of Iasi, Romania
[3] National Institute of Research – Development for Machines and Installations Designed to Agriculture and Food Industry – INMA Bucharest, Romania

microelements occur in the fruiting stage, when the need for nutrients is double or even triple. For an optimal growth and development, tomatoes take from soil or from different substrates high amounts of macroelements (N, P, K, Ca, Mg and S) and microelements (Fe, Zn, Mn, Cu, B and Mo). The nutrient content in the leaves of tomato plants is closely related to the process of photosynthesis and the production of edible parts.

MATERIAL AND METHOD

Tomato seeds were initially germinated in a growth chamber, under controlled conditions (22°C, 75% relative humidity, 10 h - light, 10.000 Lux). At the appearance of the first two leaves, the tomato seedlings were transferred into 400 cm^3 plastic pots, using Kekkila peat as substrate. Once the plants were 21 days old, they were moved to the experimental greenhouse of "Ion Ionescu de la Brad" University of Agronomy and Veterinary Medicine, Iaşi, under similar conditions to those in the growth chamber. At 42 days, the plants were transplanted into 12 L plastic pots, using the same substrate and 10 different fertilization treatments consisting of solutions with macroelements ($MgSO_4$, KNO_3, K_2SO_4, Ca $(NO_3)_2$, KH_2PO_4, KCl) and a treatment with microelements (Na_2MoO_4, $Na_2[B_4O_5(OH)_4]$]•$8H_2O$, Cu, Mn, Zn, Fe) were applied. The treatments with macroelements used in the experiment were the following: (1) $MgSO_4$, (2) KNO_3, (3) K_2SO_4, (4) Ca $(NO_3)_2$,(5) KH_2PO_4, (6) KCl, (7) $MgSO_4$ + KNO_3 + KH_2PO_4, (8) K_2SO_4 + Ca $(NO_3)_2$ + KCl, (9) the mixture between the 6 macro-elements and (10) one macro-element/day. The proportion of the elements used in the mixture was 1:1. For each plant an amount of 30 ml/solution/day was applied for 21 days along with 0.5 - 1 L of water/day, depending on the phenological phase. The microelements were applied foliarly to all the fertilized plants, in a concentration of 0.02 g/plant/week. For control plants (V11), the macroelement solution was replaced with water, and the microelements were not applied.

Water content

The water content of the samples was determined according to AOAC, 2000, at 105 °C.

Mineral content

The content of iron, calcium, magnesium and zinc was determined by atomic absorption spectrometry according to the methods described in AOAC, 2003.

Tannins content

An amount of 2 g of sample was transferred into 50 ml of distilled water. The resulting mixture was heated to 60°C and then filtered. A volume of 10 ml of 4% copper acetate solution was added to the hot filtrate, which was then boiled for 10 minutes. The resulted precipitate was filtered, dried with filter paper and transferred to a previously weighed crucible. The precipitate was then weighed, incinerated in an oven at 550°C, cooled in the desiccators and then weighted again. The difference between the weight of the sample before and after incineration represents the content of tannins.

RESULTS AND DISCUSSIONS

The water content of the tomato fruits varied along with the fertilization treatment. Therefore, it was observed that the highest water content was recorded for the tomato fruits fertilized with $MgSO_4$ + KNO_3 + KH_2PO_4 + K_2SO_4 + Ca $(NO_3)_2$ + KCl (V9) - 93.62%, followed by those treated with KNO_3 (V2) - 93.58% and by those whose treatment was done with KH_2PO_4 (V5) - 93.51% (fig.1). The smallest water content was registered for the tomato fruits fertilized with K_2SO_4 (V3) - 92.33%. The water content of tomato fruits treated with: the mix consisting of $MgSO_4$, KNO_3, KH_2PO_4, K_2SO_4, $Ca(NO_3)_2$ and KCl (V9), KNO_3 (V2), KH_2PO_4 (V5), $MgSO_4$ + KNO_3 + KH_2PO_4 (V7), the daily cyclic treatment with each macroelement (V10) and $Ca(NO_3)_2$ (V4) was significantly higher than the water content of the fruits of the untreated plants (V11) (fig.1). For the rest of the treatments (V1, V3, V6, V8), the water content of the fruits was significantly lower compared to that of the fruits of the control plants (V11) (fig.1).

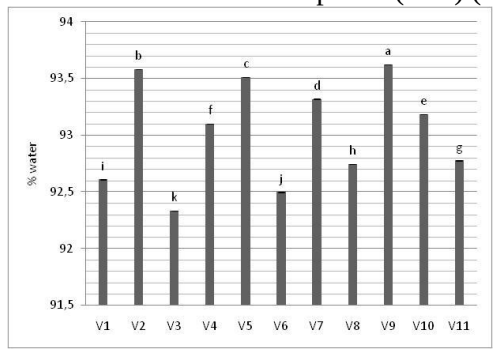

Fig.1 Water content in tomato fruits of cv Brillant F1 under the influence of different fertilization treatments. Different letters mean significant differences between variants according to the Tukey test ($p \leq 0.5$).

The mineral content of the tomato fruits was influenced by the type of fertilization used in the experiment. The highest iron content was recorded for the unfertilized tomato fruits (V11) and those fertilized daily with one macroelement, in a cyclical treatment (V10). The amount of iron registered was 0.051 mg/100 g dry matter and 0.048 mg/100 g respectively. Significantly smaller amounts of iron were recorded for the rest of the treated tomato fruits as compared to those mentioned above. The minimum iron amount was recorded for the fruits of the tomato plants fertilized with $MgSO_4$ (V1), namely 0.01 mg/100 g dry matter. No significant differences among the iron content were observed between the tomato fruits of V5, V6, V7,V8 and V9; V3 – V7; V2, V3 and V4, also between V1 and V2 treatments (fig.2).

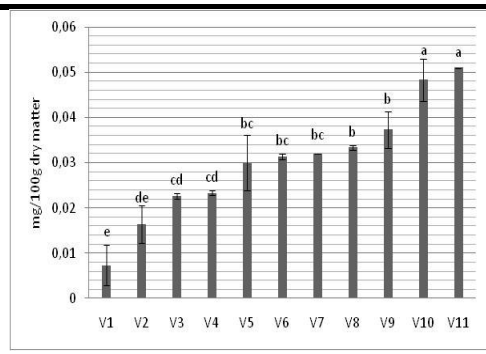

Fig.2 Iron content in tomato fruits of cv Brillant F1 under the influence of different fertilization treatments. Different letters mean significant differences between variants according to the Tukey test ($p \leq 0.5$).

In terms of calcium content, it varied between 8.18 mg/100 g dry matter and 8.15 mg/100 g dry matter. The maximum amount of calcium was recorded for the tomato fruits of the plants fertilized with $MgSO_4$ (V1), while the minimum content was for those of the plants which were included in a daily cyclic treatment with each macroelement (V10) and the tomato fruits of the untreated plants (V11). The amount of the calcium in the tomato fruits of V1 was significant bigger as compared with that registered for the rest of the treatments. Significant differences were also observed between V2 or V3 and V4-V11.The calcium content of the tomato fruits fertilized with the mix of $MgSO_4$ + KNO_3 + KH_2PO_4 + K_2SO_4 + $Ca(NO_3)_2$ + KCl (V9), K_2SO_4 + $Ca(NO_3)_2$ + KCl (V8) and $MgSO_4$ + KNO_3 + KH_2PO_4 (V7) did not differ in significantly way as compared with V6, V10 and V11 (Fig.3). Also, no significant differences were seen between the calcium content of the tomato fruits of V2 and V3, V4 and V5.

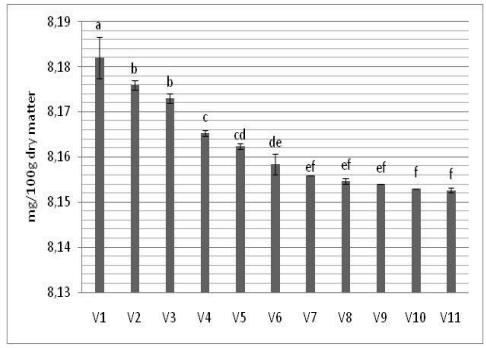

Fig.3 Calcium content in tomato fruits of cv Brillant F1 under the influence of different fertilization treatments. Different letters mean significant differences between variants according to the Tukey test ($p \leq 0.5$).

The magnesium content of the tomato fruits, as in the case of iron, was maximum for the unfertilized plants - V11, but also for those daily fertilized with each macroelement - V10 (2.95 mg/100g dry matter). The lowest magnesium

content was of 2.91 mg/100g dry matter registered for the tomatoes treated with $MgSO_4$ (V1). The content obtained for V10 and V11 were significantly higher than that recorded for the rest of the treatments. No significant differences were observed between V2, V3 and V4; V6, V7 and V8, respectively V5, V6 and V7 (fig.4).

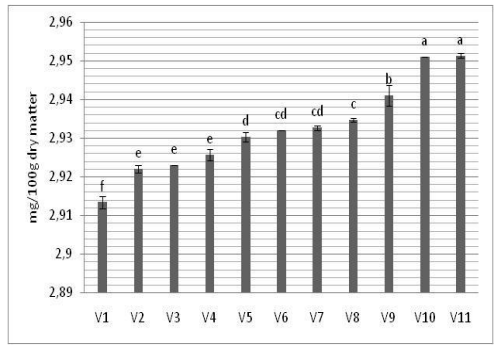

Fig.4 Magnesium content in tomato fruits of cv Brillant F1 under the influence of different fertilization treatments. Different letters mean significant differences between variants according to the Tukey test ($p \leq 0.5$).

Finally, the zinc content of tomatoes fruits fertilized with KCl (V6), the mixtures consisting of: $MgSO_4$ + KNO_3 + KH_2PO_4 (V7); K_2SO_4 + $Ca(NO_3)_2$ + KCl (V8); $MgSO_4$ + KNO_3 + KH_2PO_4 + K_2SO_4 + $Ca(NO_3)_2$+ KCl), the daily cyclic treatment with each macroelement (V11) and the unfertilized plants (V11) was significantly higher as compared to V1-V4 treatments (fig.5).

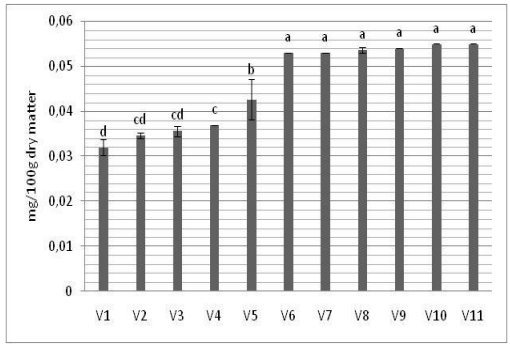

Fig.5 Zinc content in tomato fruits of cv Brillant F1 under the influence of different fertilization treatments. Different letters mean significant differences between variants according to the Tukey test ($p \leq 0.5$).

As in the case of water and minerals content, the tannins content varied depending on the fertilization treatment. The highest amount of tannins was recorded in the fruit of untreated tomatoes (V11), but also in those treated with K_2SO_4 (V3), between which no significant differences were registered (0.34 g/100g fresh matter), followed by those treated with KCl (V6) - 0.33 g/100g fresh matter). The minimum content recorded was for the plants treated with KNO_3

(V2) - 0.28 g/100g fresh matter, followed by those fertilized with KH_2PO_4 (V5) and those with the mix $MgSO_4$ + KNO_3 + KH_2PO_4 + K_2SO_4 + $Ca(NO_3)_2$ + KCl (V9) - 0.29 g/100g fresh matter (fig.6).

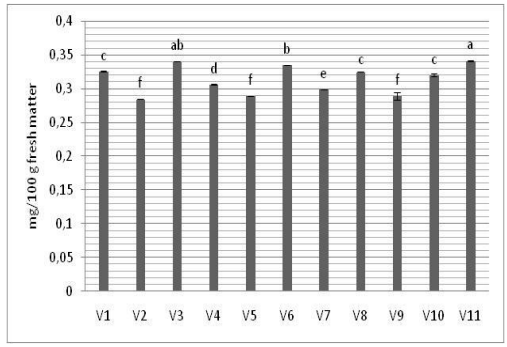

Fig.6 Tannin content in tomato fruits of cv Brillant F1 under the influence of different fertilization treatments. Different letters mean significant differences between variants according to the Tukey test ($p \leq 0.5$).

CONCLUSIONS

1. The tested biochemical parameters of the tomato fruits from cv Brillant F1 plants are highly influenced by the chemical fertilization treatments.

2. The mineral content of the tomato fruits varied according to the analyzed mineral and the fertilization treatment.

Acknowledgments*: This work was supported by the CNCS-UEFISCDI, PN III PCCDI 41/2018 project.*

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BIOSTIMULANT EFFECTS OF REGLALG ON BIOCHEMICAL PARAMETERS OF HORTICULTURE PLANTS

EFECTELE BIOSTIMULANTE ALE REGLALGULUI ASUPRA PARAMETRILOR BIOCHIMICI AI PLANTELOR HORTICOLE

CAUŞ Maria[1]*, DASCALIUC Al.[1]
*Corresponding author e-mail: mcausmcv@yahoo.com

Abstract. *In this study, data are reported on the biostimulant pre-germination treatment effects of plant growth regulator (PGR) Reglalg on cucumber seeds germination, plant growth parameters, and biochemical indices, including catalase and peroxidase activity, on the total polyphenols content and total antioxidant activity in the roots. Spraying with Reglalg tomato plants under field conditions during the season also demonstrated a beneficial effect on plant growth and development, leaf chitinase activity, for both early and late repining cultivars. Soaking cucumber seeds in Reglalg and spraying with it tomato plants during vegetation represents a promising method of applying Reglalg to horticulture crops.*
Key words: PGR *Reglalg*, *Cucumis sativus* L., *Solanum lycopersicum* L., biochemical parameters

Rezumat. *În acest studiu se aduc date despre efectele biostimulante ale regulatorului de creştere a plantelor (RCP) Reglalg, utilizat la tratarea seminţelor de castravete înainte de germinare, asupra germinării seminţelor, parametrilor de creştere a plantulelor, precum şi asupra indicilor biochimici, inclusiv activitatea catalazei, peroxidazei, conţinutului total de polifenoli şi a activităţii antioxidante totale din rădăcini. De asemenea, este demonstrat efectul benefic al RCP Reglalg, utilizat la stropirea plantelor de roşii în condiţii de câmp pe parcursul vegetaţiei, asupra creşterii şi dezvoltării plantelor, activităţii chitinazelor din frunze, atât soiului mediu-timpurii, cât şi tardiv. Îmbibarea seminţelor de castravete în soluţiile RCP Reglalg şi stropirea plantelor de tomate în timpul vegetaţiei cu acest preparat reprezintă o metodă promiţătoare de aplicare a Reglalgului pentru culturile horticole.*
Cuvinte cheie: RCP Reglalg, *Cucumis sativus* L., *Solanum lycopersicum* L., parametrii biochimici

INTRODUCTION

In recent years, due to the global warming, agricultural crops, including horticultures, are exposed to abiotic and biotic stress factors (Bedsworth and Hanak, 2010). In response to stress factors, excessive production of reactive oxygen species (ROS) are formed in plant tissues, which affect the most physiological processes, including plant growth and yield (Gill and Tuteja, 2010). Plants possess

[1]Institute of Genetics, Physiology and Plant Protection, Chisinau, Republic of Moldova

enzymatic and non enzymatic mechanisms to annihilate the excess of the ROS, produced under unfavorable conditions (Gill and Tuteja, 2010). An important role in providing resistance of plants to stresses factors plays the enzymatic activity of peroxidase, catalase, chitinases, and a number of metabolic compounds, including polyphenols compounds (Takenaka *et al.,* 2009; Gomes and Garcia, 2013).

In agricultural technologies for cultivation of vegetable crops, including cucumber and tomato, plant growth regulators (PGR) are widely applied for seed treatments before sowing, as well as for plant treatments during vegetation to increase plant resistance to unfavorable growth conditions, ensuring the quality and productivity of plants (Cauş *et al*., 2016; Dascaliuc *et al.,* 2018).

The purpose of this study was to investigate the effects of PGR *Reglalg*, *when* applied for the treatment of cucumber seeds and for spraying of field grown tomato plants during the season, on the plant growth parameters and some antioxidant indices.

MATERIAL AND METHODS

Seeds of *Cucumis sativus* L. cv. Concurent, used in the study, were immersed during of 24 hours in the dark at 6ºC in water (control) or in water solutions containing $5 \cdot 10^{-4}$, $2{,}5 \cdot 10^{-4}$ and $1{,}25 \cdot 10^{-4}$ % active substance of *Reglalg*. Then treated seeds were transferred for germinations in the dark at 25ºC and 80 % relative humidity of the air. Seedlings of 3 days old were collected and used to assess their growth parameters, the enzymatic activity of peroxidase and catalase, the total polyphenols content and total antioxidant capacity in their roots.

Plants of tomato *Solanum lycopersicum* L., cultivars Julihirsutian (early ripening) and Anatolie (late ripening) grown under field conditions were sprayed during the vegetation period with water (control) or an aqueous solution containing 2.5 • 10-4 % active substance *Reglalg*.

The activities of peroxidase (PO) and *catalase* (Cat) in soluble protein fractions of cucumber seedling roots, the *total polyphenols content* (TPC) and *total antioxidant capacity* (TAC) in hydro ethanol extracts of cucumber seedling roots were determined by the methods described earlier (Caus *et al.,*2016).*Chitinase activity in* tomato leaves extracts was determined according to (Neale *et al.,* 1990).Each experiment was performed three times in triplicate. The arithmetic means and their standard deviations (SD) were calculated.

RESULTS AND DISCUSSIONS

The results showed that soaking cucumber seeds in *Reglalg* influenced germination and seedling growth parameters. As can be seen from table 1, the stimulation of biomass accumulation and stem height depended on the *Regalg*'s active substance concentrations in the aqueous solutions used for seed treatments. And also that *Reglalg* concentrations of $2.5 \cdot 10^{-4}$ and $1.25 \cdot 10^{-4}$ % had stimulatory effects both on the increase of the root and air biomass and on the growth of stems in height.

Table 1

The influence of various concentrations of the *Regalg*'s active substance in the aqueous solutions, applied to seeds prior to germination on the height of stems, fresh weight of roots and aerial part of 3 days old cucumber seedlings

Treatments applied to seeds prior to germination	Concentration of the Regalg's active substance in the aqueous solution, %	Biomass accumulation, % against control		Height of stems, % against control
		Roots	The aerial part of the plants	
Control (H2O)	--------	100	100	100
Reglalg (R)	$5 \cdot 10^{-4}$	100.2 ± 1.24	110.0 ± 1.76	122.6 ± 4.92
	$2.5 \cdot 10^{-4}$	**123.3 ± 1.70**	**119.0 ± 0.82**	**125.2 ± 5.66**
	$1.25 \cdot 10^{-4}$	122.0 ± 1.49	113.2 ± 0.36	121.0 ± 9.05
*At the 3rd day the control biomass of roots and aerial parts reached 0.05 g/plant and respectively 0.09 g/plant, and the height of stems – 2.7 cm.				

Utilization of aqueous solution containing $2.5 \cdot 10^{-4}$ % *Regalg*'s active substance showed the tendency to more strongly influence the growth of plant biomass and the height of the plantlets (tab. 1). The literature data demonstrates that germination processes and subsequent growth of plants are accompanied by the formation of ROS in plant tissues and the activation of the antioxidant system, including antioxidant enzymes, involved in the detoxification of free radicals excess (Gomes and Garcia, 2013). In this context we intended to determine whether the application of aqueous solution containing different concentrations of the *Reglalg* active substance for seeds treatments subsequently can modify the activity of peroxidase (PO) and catalase (Cat), involved in the annihilation of excessive H_2O_2, which is one of the more stable and harmful ROS in plant cells.

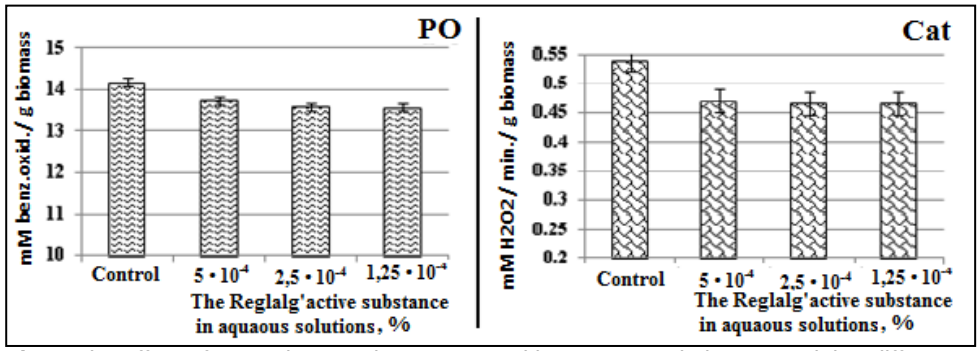

Fig. 1 The effect of cucumber seeds treatment with aqueous solutions containing different concentrations of the Reglalg's active substance on the activity of peroxidase (PO) and catalase (Cat) of soluble protein fractions separated from roots of 3-days plantlets

Figure 1 presents the data on the changes of PO and Cat activity in cucumber seedling roots under the influence of different concentrations of *Reglalg* active substance in aqueous solutions used for seed treatments. It can be

observed, that compared to the control, PO and Cat activity decreased significantly in roots of cucumber seedling, grown from seeds treated with different concentrations of *Reglalg* (fig. 1). This suggests that during the growth and formation of cucumber root system there is no excess production of H_2O_2 in root seedlings grown from seeds treated with *Reglalg*.

Considering that the application of different concentrations of *Reglalg* has influenced the PO and Cat activities, we have proposed to determine whether seed treatment with solutions of *Reglalg* caused induction of changes in the total polyphenols content and total antioxidant capacity, which together with the enzymes system, play an important role in determining the processes of SRO cleavage in plants cells. The analysis of total polyphenols contents (TPC) and their total antioxidant capacity (TAC) in the extracts from cucumber root seedlings, grown from seeds treated with *Reglalg* demonstrated an increase of the TPC as well as their TAC at all concentrations of *Reglalg* (fig. 2). The highest level of TPC and TAC was observed in cucumber roots of seedlings grown from seeds treated with aqueous solution containing $2.5 \cdot 10^{-4}$% *Regalg*'s active substance (fig. 2*)*. So, these results support our hypothesis that the *Reglalg*, applied to the seeds in various concentrations, especially of $2.5 \cdot 10^{-4}$ % , favours the growth and development of cucumber plants as well as the TPC and TAC increase of seedling roots.

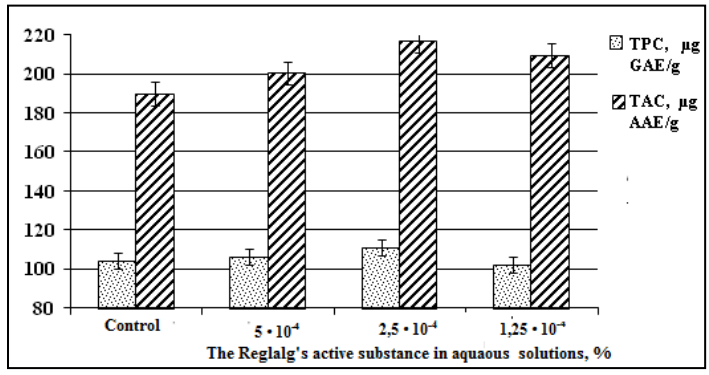

Fig. 2 The effect of cucumber seeds treatment with aqueous solutions containing different concentrations of the Reglalg's active substance on the total polyphenol content (TPC) and total antioxidant capacity (TAC) of 3 day old cucumber plant root extracts

Another purpose of this study was to investigate the effect of applying the aqueous solution containing $2.5 \cdot 10^{-4}$ % *Regalg*'s active substance by spraying field grown tomato plants during their vegetation season. The research focussed on establishing the influence of *Reglalg* on plant growth and development as well as on the possible changes of chitinase activity in leaves of early and late ripening tomato cultivars.

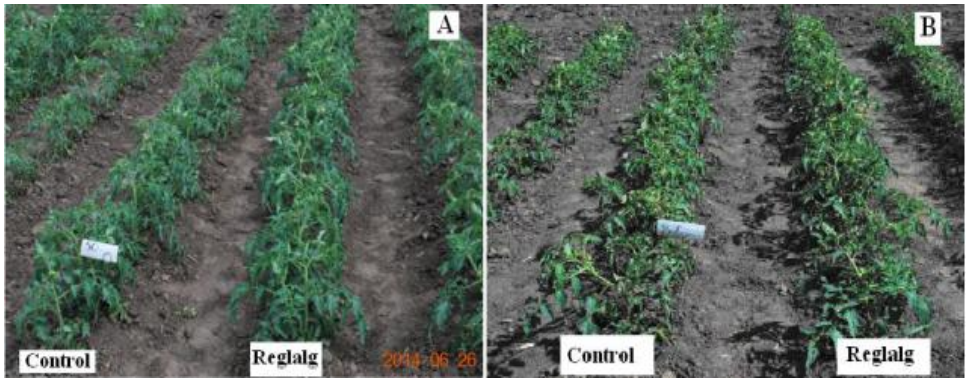

Fig. 3 Foto of tomato plants *Solanum lycopersicum* L., early (A) and late (B) ripening cultivars under field conditions treated with water (control) and an aqueous solution containing 2.5 • 10-4 % active substance of the Reglalg during the vegetation period.

The field grown tomato plants presented in figure 3A and 3B demonstrate the beneficial effect of *Reglalg* on the growth of both early and late ripening cultivars of tomato, which is already manifest before the plants flowering period (June, 10). It should be noted that the treatment of tomato plants with *Reglalg* induced an enhancement of the vegetative growth, flowering and percentage of fruit binding, stimulated fructification and increased plant productivity (data not shown).

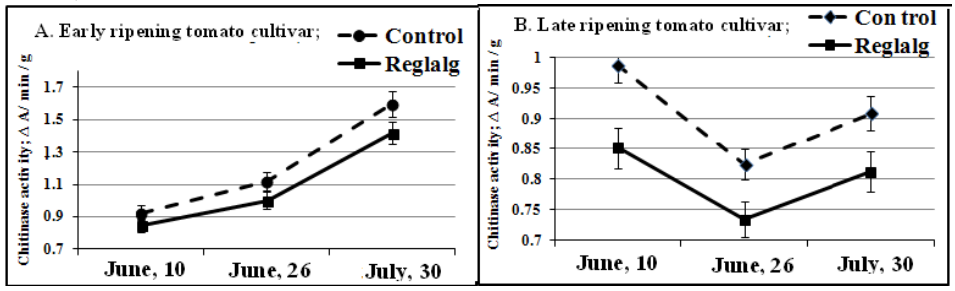

Fig. 4 Effect of foliar application of an aqueous solution containing 2.5 • 10-4 % active substance of the *Reglalg* during the vegetation period, every 10 days, on the leaf chitinase activity of early (A) and late (B) ripening tomato cultivars.

The effects of *Reglalg* application on the enzyme activity of chitinase, extracted from the leaves of tomatoes plants are shown in figure 4. Analyzing the data (fig. 4), we can see that the chitinase activity in leaf extracts of both cultivars treated with *Reglalg* during their vegetation was at the lower level then in leaves of control plants, treated with water. But, the extracts from early ripening cv. Julihirsutian tomatoes leaves (fig. 4A) were characterized by a higher level of chitinase activity, compared to the enzyme activity of late ripening Anatoli cultivar (fig. 2B). Aging of plants is associated with increased activity of chitinases in both cultivars, but in the late ripening cultivar it occurs later, after

the initial decrease in chitinase activity towards the end of June (fig. 4B). Chitinases play an important role in the defensive response of plants to the action of biotic factors (Takenaka *et al.,* 2009). In accordance with the reaction of plants to the respective factors, an induction of the genes responsible for the biosynthesis and activation of the chitinases takes place. On the other hand, in addition to the apparent role of chitinases in the defensive reaction of plants to biotic agents, it is assumed that these enzymes are involved in plant development processes, including floral apparatus formation, leaf senescence and embryogenesis (Neale *et al*., 1990; Zhi-Ping Xie *et al.,* 1996). Our results demonstrated that the aging of plants is associated with increased chitinase activity. They also support the vision of the "rejuvenation" effect of *Reglalg* on tomato plants. Decreasing level of chitinases activity in plants treated with *Reglalg* supports this view.

CONCLUSION

The results demonstrated that PGR *Reglalg* can be effectively used in agriculture practice for pre-germination treatments of cucumber seeds and for foliar application in spraying tomato plants during vegetation.

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PRODUCTIVITY OF APRICOR OCHARD BY THE METHOD OF CONDUCTING THE CROWN IN THE PERIOD OF GROWING AND FRUCTIFICATION OF THE TREES

PRODUCTIVITATEA PLANTAȚIEI DE CAIS ÎN FUNCȚIE DE MODUL DE CONDUCERE A COROANEI ÎN PERIOADA DE CREŞTERE ŞI FRUCTIFICARE A POMILOR

NEGRU I. [1*]***, PESTEANU A.*** [1]
[*]Corresponding author e-mail: a.pesteanu@uasm.md

Abstract. *The experimental plot is placed in the orchard "Agroparc Management" Ltd. founded in 2015 year. The study subject of the experience was Spring Blush and Pinkcot apricot varieties grafted on Mirobalan 29C rootstock, conducted by 6 forms of crowns. The distance of plantation is 5.0 x 3.0 m. The research was conducted during the period of 2018 year. During the research, it was studied, amount of flowers and degree of setting, number of fruits, mean fruit weight and yield. It was established that, the formation of crowns of the apricot trees influence on amount of flowers, degree of setting, number of fruits, mean fruit weight and yield of studied varieties.*
Key words: Apricot, varieties, crowns, setting, yield

Rezumat. *Lotul experimental a fost amplasat în livada întreprinderii SRL „Agroparc Management", fondată în anul 2015. Ca obiect de studiu au servit pomii de cais de soiurile Spring Blush şi Pinkcot, altoite pe portaltoiul Mirobalan 29C, conduse după 6 forme de coroană. Distanța de plantare 5,0x3,0 m. Cercetările au fost efectuate pe parcursul anului 2018. Pe parcursul cercetărilor sa studiat ponderea ramurilor de diferită vârstă, cantitatea de flori şi gradul de legare, cantitatea de fructe, greutatea medie şi recolta. Sa stabilit că forma de conducere a coroanei la cais a influențat asupra ponderii ramurilor de diferită vârstă, cantității de flori şi gradului de legare, cantității şi greutății medii a unui fruct, producției obținute.*
Cuvinte cheie: Cais, soiuri, coroane, grad legare, producție

INTRODUCTION

The culture of the apricot, whose fruits - apricots - are highly sought after, both for fresh consumption and in the food industry, has long been viewed with distrust, and considered risky, due to the traits related to low resistance to frost, sensitivity at specific diseases, premature loss of trees from plantations and short duration of fruit storage (Balan *et al.,* 2008; Cimpoieş, 2018; Cociu *et al.,* 1993).

Nowadays, in the Republic of Moldova, for the establishment of apricot plantations are used vigorous varieties / rootstocks, for which crowns with large gauges are recommended, where the vegetal macrostructure prevails over the

[1]State Agricultural University of Moldova, Chişinău, Republic of Moldova

rodent microstructure(Babuc, 2012; Cimpoieş, 2018; Pesteanu *et al.,* 2018).

At the European level, the culture of the apricot in the last two decades has undergone great transformations regarding the assortment of varieties and rootstocks, the crown form, reaching today remarkable performances in the technology of cultivation of the mentioned species by means of harvests of 25-30 t/ha of fruits of superior quality (Cociu *et al.,* 1993; Hoza, 2005; Pîntea, 2019).

The optimization of the structure of the apricot plantation can be achieved only through the implementation in production of new crown shapes suitable for the species concerned in the intensive system. This would allow for higher, constant and competitive quality productions (Negru, 2018; Pesteanu *et al*., 2018).

The aim of this study was to evaluate the behavior of six crown shapes on the precocity and yield of two new apricot varieties grafted on Mirobalan 29C rootstock in the climatic conditions of the Republic of Moldova.

MATERIAL AND METHOD

The researches carried out during the year 2018 in the intensive apricot orchard of the company "Agroparc Management". The plantation was founded in the spring of 2015, with one-year-old trees from the Spring Blush and Pinkcot varieties, grafted onto the Mirobalan 29C rootstock. Planting distance 5.0x3.0m.

In order to solve the suitability of apricot varieties to different forms of crown management, the following variants have been studied: 1 – Non-layered (control); 2 - Mixed pyramid; 3 – Slender Spindle; 4 – Open vase; 5 – Free palmetto; 6 - Pal - spindel.

Plots were placed in blocks, each variant having 4 repetitions. Each rehearsal consisted of 8 trees. The researches were carried out under field and laboratory conditions according to accepted methods of carrying out the experiments for studying apple orchards.

Statistical processing of the data was performed by the method of dispersion analysis.

RESULTS AND DISCUSSIONS

The apricot is a species that bearies both on anticipated shoots and annual branches of different growth waves, as well as on fruit formations that have differentiated on branches with the age of 2 years.

Studying the weight of the anticipated branches (tab. 1) we record that the Pinkcot variety recorded higher values compared to the Spring Blush variety. If, in the Spring Blush variety, the weight of the anticipated shoots on the variants in the study constituted from 6.0 to 21.5%, then in the Pinkcot variety it ranged from 34.3 to 50.6%. These anticipated branches will bloom later and to a certain extent will prevent the affection of the low temperatures during the late spring.

In contrast, in the Spring Blush variety, the percentage of annual branches predominates compared to the Pinkcot variety, where the index in the study constituted, respectively 73.3-77.2% and 36.3-52.1%.

In the case of two-year-old branches, a higher weight was registered in the

Spring Blush variety (3.6-15.4%) compared to the Pinkcot variety (6.3-13.3%).

In the case of three-year old branches, higher values were registered for the Pinkcot variety (0.8-4.1%) compared to the Spring Blush variety (0.4-1.8%).

Studying the influence of the formation of the crown on the weight of the branches of different ages within the crown, we note that in the Spring Blush variety a higher share of the anticipated branches was recorded in the control variant (21.5%) and slender spindle (21.2%) and mixed pyramid (20.0%). At the formation of the trees after the open vase was registered the lowest values (6.0%). The other variants recorded average values. A higher weight of the branches with the age of 2 years was obtained in the case of the open vase.

Studying how the vegetative and rodent macrostructure developed within the Pinkcot variety, we record that the proportion of the anticipated branches and of the one year ones was approximately the same, constituting respectively 34.3-50.6% and 36.3-52.1%.

Table 1

The structure of the crown in apricot trees according to the biological particularities of the variety and the way of its management, a. 2018,%

Variety	The crown type	Branches lenght			
		anticipated	annual	two years old	three years old
Spring Blush	Non-layered (control)	21.5	73.3	3.9	1.3
	Mixed pyramid	20.0	74.3	4.0	1.7
	Slender Spindle	21.2	73.6	3.6	1.6
	Open vase	6.0	77.2	16.4	0.4
	Free palmetto	15.6	75.6	7.0	1.8
	Pal-spindel	17.4	77.7	3.9	0.9
Pinkcot	Non-layered (control)	50.6	38.7	8.1	2.6
	Mixed pyramid	34.3	52.1	9.5	4.1
	Slender Spindle	53.8	36.3	6.3	3.5
	Open vase	41.4	44.5	13.3	0.8
	Free palmetto	48.5	42.9	6.6	2.0
	Pal-spindel	48.8	40.2	9.9	1.1

Within the anticipated branches higher values were obtained in the variants formed as slender spindle (53.8%) and non-layered pyramid (50.6%). Mean values were recorded in the variants conducted in the form of free palmetto (48.5%) and pal-spindle (48.8%), and lower values when crown formation after the open vase (41.4%), mixed pyramid (34.3%).

Higher values of the quantity of flowers in the period of 2018 at the Spring Blush variety was recorded in the crown shape as open vase 1440 pcs/tree. Further down, the mixed pyramid is placed - 934 pcs/tree, slender spindle - 888 pcs/tree, pal-spindle - 873 pcs/tree, free palmetto - 838 pcs/tree and control variant - 813 pcs/tree (tab. 2).

As a result of pollination, the fertilization of the reproductive organs the

greatest amount of fruits was registered at the formation of the open vase crown - 255 pcs/tree. In the case of the formation of apricot trees after the mixed pyramid crown, the amount of fruits constituted - 168 pcs/tree, free palmetto - 149 pcs/tree and of the Non-layered (control) - 144 pcs/tree. The smallest amount of fruits was registered in the pal-spindel crown - 110 pcs/tree and slender spindle - 119 pcs/tree.

For the apricot trees of the Spring Blush variety the degree of setting was influenced by the way the crowns are run. A higher degree of setting was recorded in the variants where a shorter cut was used in the formation. At the formation of the crowns after the mixed pyramid system the degree of setting of the productive organs constituted 18.0%. In the case of open vase, Non-layered (control) crown and free palmetto, the index in the study ranged from 17.7 to 17.8%.

Table 2

The quantity of flowers and the degree of binding of the reproductive organs to the apricot depending on the biological particularities of the variety and the way of conducting the crown, a. 2018

variety	The crown type	The quantity of flowers, pcs/tree	The quantity of fruits, pcs/tree	The setting degree, %
Spring Blush	Non-layered (control)	813	144	17.7
	Mixed pyramid	934	168	18.0
	Slender Spindle	888	119	13.4
	Open vase	1440	255	17.7
	Free palmetto	838	149	17.8
	Pal-spindel	873	110	12.6
Pinkcot	Non-layered (control)	971	201	20.7
	Mixed pyramid	1098	235	21.4
	Slender Spindle	1074	187	17.4
	Open vase	1943	375	19.3
	Free palmetto	919	193	21.0
	Pal-spindel	962	153	15.9
LSD 5%		17.2	3.9	-

The lowest values of the degree of setting of the ovaries were recorded in pal-spindel variant - 12.6% and slender spindle - 13.4%. This decrease in the degree of setting can be explained by the fact that a higher degree of crown cutting was used for these crowns.

The legacy shown for the Spring Blush variety is valid and for the Pinkcot variety, but with higher values. The amount of fruit in the case of variants while be conducting by different training mode ranged from 187 pcs/tree to 375 pcs/tree. Higher values than in the previous variety were obtained within the open vase. Further downward is the variant founded after the mixed pyramid crown - 235 pcs/tree, control variant - 201 pcs/tree, free palmetto 193 pcs/tree, slender spindle - 187 pcs/tree and pal-spindle - 153 pcs/tree.

A larger number of flowers within the crowns studied were registered within the crown of the open vase - 1943 pcs/tree, the amount of fruits constituting 375 pcs/tree, and the degree of setting of the reproductive organs was19.3%.

Productivity is the final index after which it is possible to appreciate how all the agrotechnical works were carried out in the respective plantation and which from the links of the technological chain deserves improvement.

The investigations show that fruit production at a tree is closely correlated with the amount of fruits and their average weight (tab. 3).

The biological characteristics of the variety influence the index in question. Lower values of the average weight of a fruit were recorded in the Spring Blush variety (42.3-53.8 g) compared to the Pinkcot variety (50.1-63.1 g).

Table 3

Productivity of the apricot plantation according to the biological particularities of the variety and the way of conducting the crown, a. 2018

Variety	The crown type	The average weight, g	Production		In % compared to witness
			kg/tree	t/ha	
Spring Blush	Non-layered (control)	47.3	6.81	4.54	100.0
	Mixed pyramid	44.7	7.51	5.01	110.3
	Slender Spindle	51.3	6.10	4.07	89.6
	Open vase	42.3	10.78	7.19	158.3
	Free palmetto	46.0	6.85	4.57	100.7
	Pal-spindel	53.8	5.91	3.94	86.7
Pinkcot	Non-layered (control)	56.7	11.40	7.60	100.0
	Mixed pyramid	54.1	12.71	8.48	111.6
	Slender Spindle	58.9	11.01	7.34	96.5
	Open vase	50.1	18.78	12.52	164.4
	Free palmetto	57.1	11.02	7.35	96.7
	Pal-spindel	63.1	9.65	6.44	84.8
LSD 5%		0.76	0.11	-	-

In the case of the Spring Blush variety, lower values of the average weight of the fruit were recorded in the case of the trees formed by open vase crown type (42.3 g). With the increase of the degree of cutting, the average weight of a fruit is increased, registering higher values when the trees was formed after the crowns slender spindle - 51.3 g and pal-spindle - 53.8 g.

The study of apricot production in a tree ranged from 5.91 kg in the case of crowns following the pal spindle system to 10.78 kg/tree when they were conducted after open vase system. Mean values were recorded in the other crowns.

The harvest at a surface unit correlated directly with the production obtained within a tree. Lower values are obtained when trees were formed after the pal-spindel system - 3.94 t/ha, and the highest values at the crown open vase -

7.19 t/ha. The other variants recorded average values, from 4.07 to 5.01 t/ha.

In Pinkcot variety, the lower average apricot weight was recorded in the case of the open vase variant - 50.1 g. With the decrease of the amount of fruits in the crown of the trees, the average weight of the apricot fruits increases, constituting in the trees formed by the pal-spindel system - 63.1 g.

The productivity of the trees on the variants in the study ranged from 9.65 to 18.78 kg. Lower values of fruit production were recorded at crown formation after the pal-spindel system - 9.65 kg/tree, and higher in the open vase variant - 18.78 kg/tree, where the degree of cutting at crown formation was more limited.

The production of apricots per unit area is directly correlated with the productivity of a tree. Higher values for both varieties studied or recorded when the trees were conducted after open vase system. The other variants recorded average or insignificantly lower values.

CONCLUSIONS

1. The quantity of flowers in the studied varieties was closely correlated with the method of crown formation. In the crowns with a larger number of basic branches within the tree there is an increase in the amount of flowers, compared to those where the degree of cutting was higher.

2. Fruit production is correlated by the method of crown conduce, and to obtain early yield to apricot it is necessary to minimize the cuts during the formation period, to increase the weight of the fruit buds in the shortest period of time.

3. Higher yields in the fourth year after planting were recorded in the variant where the trees were conducted by the open vase system, which constituted an increase with 53.8% et the Spring Blush variety and 64.4% et the Pinkcot variety compared to the control variant.

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THE EFFECT OF BIOREGULATOR GERBA 4LG TREARMENT ON LATERAL SHOOT FORMATION IN MAIDEN APPLE TREE

EFECTUL TRATĂRII CU REGULATORUL DE CREŞTERE GERBA 4LGLA OBȚINEREA LĂSTARILOR LATERALI ÎN PEPINIERĂ LA POMII DE MĂR

PESTEANU A.[1]*, BOSTAN M.[1]
Corresponding author e-mail: a.pesteanu@uasm.md

Abstract. *The object of the research was apple varieties Golden Delicious Reinders, Red Velox, Gala Buckeye and Red Jonaprince, grafted on M 9. The grafting method was chip budding. Planting distance was 80x35 cm. In order to intensify the formation of the anticipated shoots in the area of the crown formation, various technological processes were used: 1. Free eyelid growth (control); 2. Topping of apical leaves was combined with two treatments with Gerba 4LG at a dose of 25 ml/liter of water. It was established that the most reasonable garnishing of the crown formation with anticipated shoots at all studied varieties was obtained by topping the apical leaves in the apex area once when the graft reaches 65-70 cm height combined with twice the sprinkling Gerba 4LG at 25 ml/liter of water. The first treatment was done after breaking the apical leaves and the next at 5-7 days.*
Key words: Apple, lateral shoots, growth regulator, quality.

Rezumat. *Obiectul cercetărilor a fost soiurile de măr Golden Delicious Reinders, Red Velox, Gala Buckeye şi Red Jonaprince, altoite pe M 9. Metoda de altoire folosită ocularea în placaj. Distanța de plantare – 80x35 cm. Pentru intensificarea gradului de formare a lăstarilor anticipați s-au utilizat diverse procedee tehnologice: 1. Creştere liberă a oculantului (martor); 2. Ruperea frunzelor apicale din zona apexului combinată cu două tratamente cu regulatorul de creştere Gerba 4LG în doza de 25 ml/litri de apă. S-a stabilit, că cea mai rațională garnisire a zonei de formare a coroanei cu lăstari anticipați la toate soiurile luate în studiu sa obținut la ruperea frunzelor apicale din zona apexului o singură dată când oculantul atinge 65 - 70 cm înălțime combinată cu stropirea de două ori cu regulatorul de creştere Gerba 4LG în doza de 25 ml/litri de apă. Primul tratament de efectuat după ruperea frunzelor apicale, iar următorul la interval de 5-7 zile.*
Cuvinte cheie: Măr, lăstari laterali, regulator de creştere, calitate.

INTRODUCTION

The formation of the crown of trees in the nursery from normal and anticipated shoots is the technological operation by which the height of the trunk is defined and the shape after which the tree will be conducted in the orchard

[1]State Agricultural University of Moldova, Chişinău, Republic of Moldova

(Babuc, 2012; Babuc *et al*., 2013; Cimpoieş, 2012).

If it is planned to form the crown from anticipated shoots to apple trees in field II of the nursery, a decisive role plays the hereditary capacity of the varieties to issue such shoots (Basak and Sozcek, 1986; Gudumac, 2008).

Many fruit growers in crown formation in apple trees also use other preparations like: Paturyl 10 WSC, Arbolin 36 SL, Gerba 4LG etc. (Caglar and Ilgin, 2009; Gastol *et al*., 2012; Hrotkó *et al.,* 1996; Wertheim and Estabrooks, 1994).

In order to increase the degree of emission of the anticipated shoots at the base of the apple trees crown in the second field of the nursery and to provide branched trees it was proposed to study how it will influence on the mentioned index the topping of the apical leaves combined with the treatment with the Gerba 4LG product whose active ingredient is a cytokinin.

MATERIAL AND METHOD

The research was carried out in the fruit nursery of the company "Vagadis" LTD. The trees of the Golden Delicious Reinders, Red Velox, Gala Buckeye and Red Jonaprince were grafted onto the M 9 rootstock.

The planting of the rootstocks in field I was carried out in spring 2015. The grafting method used in field one of the apple nursery was the chip budding. Planting distance - 80x35 cm.

In order to determine the influence of different intervention techniques on the degree of issuance of the anticipated shoots, two variants have been established: V_1 - by free growth of the eyepiece (control); V_2 – topping one-time of the apical leaves in the apex area when the eyepiece was 65 - 70 cm high combined with the application of two treatments with Gerba 4LG in the dose of 25 ml/liters of water on the top of the plant. The first application was made immediately after the apical leaves were broken, and the next one at 5-7 days.

The researches were carried out according to recommended methods for conducting field experiments in the nursery.

The main results obtained were statistically processed by the method of dispersion analysis.

RESULTS AND DISCUSSIONS

Lower diameter values in the rootstock area were recorded in trees of the Red Velox variety - 14.4 mm, while in the Golden Reinders, Gala Buckeye and Red Jonaprince varieties, the index in question was higher and varied from 16, 0 mm to 16.6 mm (tab. 1).

In case of formation of the crown by topping the apical leaves and treating with the growth regulator Gerba 4LG in the dose of 25 ml/liter of water, the index in the case increased by 5.4 - 15.9%. Higher values of the index were recorded at the Golden Reinders (18.6 mm) and Buckeye Gala (18.0 mm).

Higher values of the average diameter below the first branch of the crown were recorded in the variant when the apical leaf was broken and treated with the Gerba 4LG growth regulator in the dose 25 ml/liter of water, where it constituted from 13.1 mm up to 17.0 mm.

Table 1

Diameter in different areas of the tree depending on the biological particularities of the variety and the method of crown formation, mm

The crown formation	In the rootstock area	Under the crown	Above the last branch of the crown
Red Velox variety			
V_1	14.4	-	-
V_2	16.7	13.1	9.1
Golden Reinders variety			
V_1	16.2	14.7	12.6
V_2	18.6	17.0	10.8
Gala Buckeye variety			
V_1	16.0	13.1	11.8
V_2	18.0	15.7	10.9
Red Jonaprince variety			
V_1	16.6	12.6	11.4
V_2	17.5	15.0	8.7

Lower values of the index in the study on different areas of the tree were recorded above the last branch in the crown, which was in direct correlation with the biological particularities of the variety. Lower values on the studied variants were recorded in the Red Jonaprince variety 8.7 - 11.4 mm, in the Gala Buckeye variety constituted 10.9 - 11.8 mm, and in the Golden Reinders variety 10.8 - 12.9 mm.

Table 2

The height of crown structure according to the method used in crown formation, cm

The crown formation	The tree height	The height of the trunk	The length of the crown formation area	The length of the arrow
Red Velox variety				
V_1	149	-	-	149
V_2	135	55	13	67
LSD 0.05	5.7	-	-	3.1
Golden Reinders variety				
V_1	169	59	6	104
V_2	150	55	26	69
LSD 0.05	6.9	2.6	0.43	2.8
Gala Buckeye variety				
V_1	184	58	10	116
V_2	150	55	29	75
LSD 0.05	7.2	2.7	0,46	3.7
Red Jonaprince variety				
V_1	172	59	13	100
V_2	151	60	37	54
LSD 0.05	6.5	2.9	0.56	2.9

The difference between the lower and upper diameter of the crown area in

the Golden Reinders variety was 6.2 mm, in the Gala Buckeye variety 4.8 mm, in the Red Jonaprince 6.3 mm variety, and in the Red Velox variety 4.0 mm.

The investigations carried out on the height of the trees according to the biological particularities of the variety, show us that, on the index in the study, the biological peculiarities of the variety influence. A lower value in the control variant of the height of the trees was recorded in the Red Velox variety - 149 cm. Further growing is the Golden Reinders variety - 169 cm, the Red Jonaprince variety - 172 cm and the Gala Buckeye variety - 184 cm (tab. 2).

In variant V2, where the apical leaf break was performed plus the treatment with the growth regulator Gerba 4LG in the dose 25 ml/liter of water, there was a decrease of the index in the study by 10.4 - 22.6% compared to the control variant. The largest difference was recorded in the Gala Buckeye variety, which is characterized by a greater growth force.

Higher values of the extension pod were recorded in the Red Velox variety 149 cm, and in the Gala Buckeye variety 116 cm. In the Red Jonaprince and Golden Reinders varieties, the index was 100 cm and 104 cm respectively.

The lowest values for the varieties studied were recorded in variant V_2, where the apical leaf was topping and treated with the Gerba 4LG growth regulator. In the case of the Red Jonaprince variety, the index was 54 cm, the Red Velox variety - 67 cm, the Golden Reinders variety - 69 cm, and the Gala Buckeye variety - 76 cm.

Table 3

Number of anticipated branches, average and summed length within the apple trees crown in field II of the apple nursery, depending on the method of crown formation

The crown formation	Number of anticipated branches, pcs/tree	The length of anticipated branches	
		Average, cm	Summed, cm/tree
Red Velox variety			
V_1	-	-	-
V_2	5.0	52.4	262
LSD 0.05	-	-	-
Golden Reinders variety			
V_1	1.8	56.0	101
V_2	8.0	46.9	375
LSD 0.05	0.28	2.6	12.4
Gala Buckeye variety			
V_1	2.8	45.1	126
V_2	10.0	42.5	425
LSD 0.05	0.34	2.1	13.7
Red Jonaprince variety			
V_1	3.3	49.4	153
V_2	12.0	45.3	544
LSD 0.05	0.45	2.6	17.9

The number of anticipated branches, their average and summed length, is influenced by the biological particularities of the variety and the method of crown

formation. In the control version, in the Red Velox variety, no lateral shoots were obtained in the crowning area (tab. 3). In the case of variant V_2, when apart from a single break of the apical leaves there were also two treatments with the growth regulator Gerba 4LG, the number of branches in the area of crown formation constituted 5.0 pcs/tree.

In the trees of the Golden Reinders variety, the number of branches obtained was increasing, constituting on the study variants 1.8-8.0 pcs/tree. At the variety Gala Buckeye and Red Jonaprince, the number of anticipatory branches constituted respectively 2.8 - 10.0 and 3.3 - 12.0 pcs/tree.

The smaller average length of the anticipated branches was obtained at Gala Buckeye variety (42.5-45.1 cm). Further, the Red Jonaprince variety (44.0 - 49.4 cm), the Red Velox variety (52.4 cm) and the Golden Reinders variety (46.9 - 56.0 cm) are growing.

The topping of the apical leaves plus the treatment with the growth regulator Gerba 4LG in the dose 25 ml/liter of water increased the total length of the annual branches depending on the variety from 262 cm to 544 cm in the tree.

Analyzing the yield of trees in terms of the biological particularities of the variety we recorded, that during the researches mentioned index ranged from 32.4 thousand pcs/ha to 33.1 thousand pcs/ha. This insignificant difference is also argued by statistical values (tab. 4).

Table 4

The yield and quality of apple trees obtained by various methods of crown formation

The crown formation	Yield, thousands pcs/ha	Product quality, %	
		I	II
Red Velox variety			
V_1	32.8	-	100.0
V_2	32.6	89.7	10.3
LSD 0.05	1.51	-	-
Golden Reinders variety			
V_1	32.4	8.7	91.7
V_2	32.5	91.3	8.7
LSD 0.05	1.49	-	-
Gala Buckeye variety			
V_1	33.0	16.4	83.6
V_2	33.1	90.7	9.3
LSD 0.05	1.54	-	-
Red Jonaprince variety			
V_1	32.5	18.7	81.3
V_2	32.6	93.7	6.3
LSD 0.05	1.52	-	-

The lowest share of quality seeding material was registered in the Red Velox variety, and then the Golden Reinders variety, Gala Buckeye variety and Red Jonaprince variety are placed. Respectively, at the varieties under study in the

control variant or registered values of 0; 8.6; 16.4 and 18.7%. Following the application of augmentation techniques such as the degree of formation of the anticipated shoots to the varieties in question, the share of quality category I trees increases, to the disadvantage of quality category II trees.

The breaking of the apical leaves plus the treatment with the growth regulator Gerba 4LG in the dose 25 ml/liter of water increased the degree of branching, because in the case of growth it inhibits the formation of auxin and increases the degree of emission of the anticipated shoots. Basically in the respective variant at Gala Buckeye and Red Jonaprince varieties, the share of quality category I trees, constituted correspondingly 90.7% and 93.7%, and those of category II quality 9.3% and 6.3% . Within the varieties Red Velox and Golden Reinders there were no major deviations in the quality of the trees.

The biological parculiarities of the variety and the way the crown is formed has a direct tangency with the processes of plant development, which ultimately affects the quality of the trees obtained in field II of the fruit nursery.

CONCLUSIONS

1. The height of the graft growing in field II of the apple nursery is influenced by the biological particularities of the varieties and the way the crown is formed.

2. For a more uniform trimming in the crown formation area, the apical leaf break should be combined with two treatments with the Gerba 4LG growth regulator with a dose of 25 ml/liter of water 2 times.

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INFLUENCE OF THE CULTURE AREA ON TWO SEABUCKTHORN (*HIPPOPHAE RHAMNOIDES*) CULTIVARS

INFLUENTA AREALULUI DE CULTURA ASUPRA A DOUA CULTIVARE DE CATINA (HIPPOPHAE RHAMNOIDES)

GOCAN Tincuța Marta[1], ANDREICA Ileana[1], BĂRBOS A.[1], RÓZSA S.[1]*, POŞTA Daniela Sabina[2]
*Corresponding author e-mail: drd.rozsa.sandor@gmail.com

Abstract. *Attention to the significance of conventional medicine in the field of health and herbal research are valuable. Hippophae rhamnoides has been used in Chinese and Russian medicine for several decades. The research of medicinal plants has grown more and more, Hippophae rhamnoides being a very important plant, as it contains a biodiversity of both nutritional and medical constituents. Bioactive fruits are one of the most important sources of bioelements constituents, and are used as a nutritional and medicinal alternative. The chemical components give value to the products obtained from this miraculous plant. Sea buckthorn is a pure store of natural antioxidants, rich in flavonoids, glucosides, phenols, terpenes, vitamins E, A, C, B-carotene, and trace elements, including iron, zinc, manganese, very low molecular weight antioxidants, which neutralizes free radicals. The results regarding the soluble dry matter from fresh fruits are between 7.6-12.64%. Total sugar content between 5.57-10.61 mg/100g fresh substance.*
Key words: area, seabuckthorn, determinations, fruits

Rezumat. *Atenția cu privire la semnificația medicinii convenționale în domeniul sănătății şi cercetările privind plantele medicinale sunt valoroase. Hippophae rhamnoides a fost utilizată în medicina chineză şi rusă timp de câteva decenii. Cercetarea plantelor medicinale a crescut din ce în ce mai mult, Hippophae rhamnoides fiind plantă deosebit de importantă, deoarece conține o biodiversitate atât nutrițională, cât şi medicală de constituenți. Fructul bioactiv reprezintă una dintre cele mai importante surse de bioelemente constitutive, şi este folosit ca alternativă nutrițională şi medicinală. Componentele chimice dau valoare produselor obținute din această miraculoasă plantă. Cătina este un magazin pur de antioxidanți naturali, conținutul său bogat de flavonoizi, glucozide, fenoli, terpene, vitaminele E, A, C, B-caroten, şi oligoelemente, inclusiv fier, zinc, mangan, antioxidanți cu greutate moleculară foarte mică, care neutralizează radicalii liberi. Rezultatele în ceea ce priveşte substanța uscată solubilă din fructele proaspete se încadrează între 7.6-12.64%. Cantitatea de zahăr total între 5.57-10.61 mg/100g substanță proaspătă.*
Cuvinte cheie: areal, catină, determinări, fructe

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania
[2]Banat University of Agricultural Sciences and Veterinary Medicine, Timişoara, România

INTRODUCTION

Medicinal plant research remains a topical issue. *Hippophae rhamnoides* L. is a valuable plant, because it contains a biodiversity of both nutritional and medical constituents. Seabuckthorn (*Hippophae rhamnoides* L.) from *Elaeagnaceae* family, is a valuable plant, which has recently gained worldwide attention. It is currently domesticated in many parts of the world, due to its nutrients and medicinal properties (Rousi, 1971; Li, 2003). It is a hardy plant, resistant to drought and cold, useful for soil recovery and farm protection through vigorous vegetative and strong reproduction, the root has nitrogen fixing nodules (Rongsen, 1992).

All component parts of this plant are considered to be a good source of bioactive substances such as vitamins (A, C, E, K, riboflavin, folic acid); carotenoids (carotene, lycopene), phytosterols (ergosterol, stigmasterol, lansterol, amirine), organic acids (malic acid, oxalic acid), polyunsaturated fatty acids and certain essential amino acids (Beveridge *et al.,* 1999; Yang and Kallio, 2002; Pintea *et al.,* 2005).

This plant has been used extensively in the traditional eastern system for medicines in the treatment of asthma, skin diseases, gastric ulcers and lung disorders. A wide range of pharmacological effects have been reported in the papers and treated, being an antioxidant, immunomodulatory, anti-atherogenic, anti-stress, hepatoprotective, radioprotective tissue repair. Currently, the seabuckthorn has gained the status of one of the most sought after in the pharmaceutical and cosmetic industry, in the food processing industries of the world. Several countries exploit commercially and ecologically for improving livelihoods and the conservation environment. The amount of experimental data that prove important bioactive properties and substances is vast and continues to grow rapidly. The presence of valuable chemicals and nutritionally important constituents is still a challenge for scientific papers, their knowledge and importance are clear.

In Romania, the seabuckthorn grows spontaneously in the sub-Carpathian area of Moldova and Muntenia, starting from the upper Siret basin to the Olt river. In the sub-Carpathians of Moldova, it is found on the valleys of the rivers Bistriţa, Trotuş, Putna and Milcov. In the sub-Carpathian area of Buzău county, seabuckthorn has a higher frequency than in other areas of Romania. It is also found on the valleys of the Teleajen and Dâmboviţa rivers, as well as in the Danube delta. New plantations have begun to be established for protection.

Due to its high scavenging capacity, the seabuckthorn is used to consolidate the lands on the slope, but also in the form of a live fence, given the strong thorns that prevent the animals from entering the orchards instead of the fences (Qinxiao and Zhao 2003).

Used in agriculture, it increases the winter resistance of bees. Seabuckthorn biofertilizes the soil with natural nitrogen (300 kg/ha) formed in the root nodules

following the symbiotic activity of the *Actinomices eleagni* fungus (Bălan *et al*., 2001). Seabuckthorn has been introduced to culture since 1980, in order to establish and consolidate degraded lands. The seabuckthorn bloom in April-May, when the average daytime temperature is 12-15 °C and takes place over a period of 15 days. The male plants bloom earlier, while the female plants bloom with the bud (Prokkola, 2003).

MATERIAL AND METHOD

The studied plants were harvested from the following areas:

- ✓ From Orăştie where was established as a protective fence the basic culture being the blueberries.
- ✓ From Banatul Montan, Cornereva commune, Caraş-Severin county.
- ✓ From Cluj were seabuckthorn are cultivated at the Didactic Station for protective fence.

For determinations, branches were collected from five specimens from each location.

Biometric measurements were performed with the ruler and the sander. Weight of fruits and seeds by weighing with analytical balance. Determination of soluble dry matter was determined with refractometer (Bota, 2013). Brix grades (symbol °Bx) are a representative unit for the sugar content of an aqueous solution. A Brix grade corresponds to one gram of sucrose dissolved in 100 grams of solution and represents the concentration of the solution as a percentage by weight (% w / w) (directly, by mass). If the solution contains solids other than pure sucrose, such as other sugars, minerals, etc., then the brix degrees (°Bx) only approximate the content of the dissolved solid.

RESULTS AND DISCUSSIONS

Characteristics of the plants according to the culture area

In Orăştie, the strong variability of seabuckthorn is characterized by predominantly orange fruits, elongated in shape, medium to large in size.

As for the spines, they had a number between 3-11 spines / 10 cm sprout, and their maximum length is 2-8 cm.

The leaves of the female specimens in this area are relatively small, unfit for harvesting leaves for medicinal purposes, with a length between 2.5 - 5.4 cm.

In Caraş-Severin county, the strong variability of seabuckthorn is characterized by predominantly open orange fruits with specimens showing yellow fruits. We can deduct from this, according to the literature, that the percentage of oil in seabuckthorn is higher, which is indicated by their light color. Fruit shape is predominantly spherical, smaller to medium. As for the spines, they had a number between 3-12 spines / 10 cm shoots, and their maximum length is 2-9 cm. The leaves of the female specimens in this area are relatively small, unfit for medicinal leaves, with a length between 3 - 6.2 cm.

In Cluj, the strong variability o seabuckthorn is characterized by predominantly orange fruits with specimens showing yellow fruits. The fruit shape is predominantly spherical, with medium to medium size. As for the

thorns, they had a number between 3-13 thorns / 10 cm sprout, and their maximum length is 2-8.5 cm. The leaves of the female specimens in this area are relatively small, unfit for harvesting leaves for medicinal purposes, with a length between 3 - 6.1 cm.

As for the male specimens, in Orăştie we found in the field very attractive specimens that can be used as paternal parents in the plantations, considering the amount of pollen they can produce. Thus, the length of the threats varies from a minimum of 5 mm to a maximum of 15 mm. Their predominant form is elongated. The thorns found on 10 cm shoots have a minimum of 3 and a maximum of 13 and their length is between 2 and 9 cm.

However, what differs from the female plants is the length of the leaves which is between 6 and 8.6 cm and the width between 4.3 and 6.5 mm. This represents a great quality in terms of the possibility of harvesting the leaves with a high yield for medicinal purposes.

In Caraş-Severin county, in the studied genotypes, the male flowering buds have a variation between 4- 4 cm. The predominant shape is spherical. The number of spines on a portion of 10 cm is between 3-13 and their length between 2 and 8 cm. We observe here a reduction in the size of spines in relation to the populations from Orăştie.

The length of the leaves is between 4.5 - 8.5 cm, therefore larger than the populations from Orăştie. The width of the leaves is between 4-7,6 mmm. The length of the threats to the genotypes in the Cluj area is between 5-16 mm. The thorns are present in a number of 3-14 on a length of 10 cm. The leaf length has a minimum of 4.2 cm and a maximum of 7.1 cm, and the width is between 4 and 7 mm.

Characteristics of the fruits obtained according to the culture area

The size of the fruits was weighed 100 fruits for each variant according to the area of cultivation and according to the shape of the beans (fig. 1). Regardless of the culture locality, fruits with spherical grains have higher values than elongated ones. Analyzing the average of the weight of the fruits in all the origins of the three studied areas, it was found that the average value of the character for the spherical fruits of 100 / fruits registered the value of 38.15g, and for the elongated fruits of 33.82g. These values were in line with the values described by Felicia Elena Andronic, 2017.

The weight of the seeds was measured from 100 fruits from each variant according to the crop area and according to the shape of the grains (fig. 2). Analysing the average seed weight for all the origins of the three studied areas, it was found that the average value of the character for the spherical fruits of 100/fruits registered the value of 1.37g, and for the elongated fruits of 1.43g.

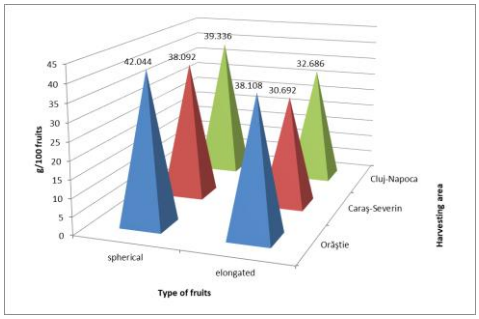

Fig. 1 The influence of the harvesting area on the cultivars, the type of fruits and the weight of the fruits

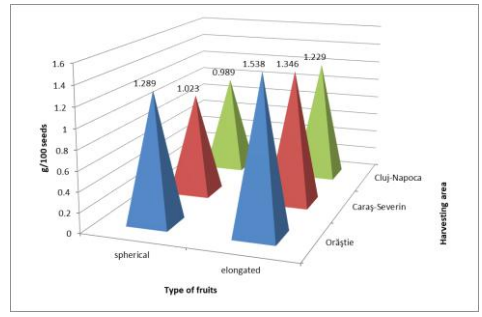

Fig. 2 The influence of the harvesting area on the cultivars, the type of fruits and the weight of the seeds

The results obtained regarding the soluble dry matter are presented in table 1. The highest values were registered in Caraş-Severin county (12.64% soluble dry matter) the data obtained were higher than those obtained by Antoneli *et al*. (2005), for the Carpathian subspecies. The analysis of the variance shows that the Caraş-Severin area fruits have distinctly positive values regarding the dry substance content, while the Cluj area presents significantly negative values compared to the control value considered the average of the experience. The Orăştie area has no statistically assured differences.

Table 1

Soluble solids content in the seabuckthorn from the studied areas

No.	Studied areas	Number of plants	(◦ Brix) Soluble solids			
			(◦ Brix) Mean ± sx	"t"	Signifiance	CV%
1	Orăştie	18	11.73±0,31	1.34	-	14.48
2	Caraş-Severin	20	12.63±0,33	3.51	**	14.51
3	Cluj	19	9.28±0,23	-6.06	*ooo*	13.6
	Mean		**11.21**±0,23			

, **, */ o, oo, ooo Significant at P<0.05; 0.01 and 0.001 (*, **, *** positive,* o, oo, ooo *negative)*

CONCLUSIONS

1. Unlike the Caraş-Severin area, where the plants grow spontaneously, the Orăştie area and the Cluj area present compact bushes, very difficult to penetrate, with strong draft, which have a radius of spreading sometimes to 10 m.

2. The fruiting of these plants is present mostly on the main axis and less on the branches of the second order. It produces fruit that is found in the middle of the bushes, which makes harvesting fruit more difficult.

3. The highest percentage of soluble dry matter is presented by cultivars from Caras-Severin area 12.64%.

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EVALUATION OF DIVERSITY OF SEGMENT OF GRAPEVINE GENETIC RESOURCES IN CONTEXT OF CLIMATE CHALLENGES

EVALUAREA DIVERSITĂȚII UNUI SEGMENT AL RESURSELOR GENETICE ALE VIȚEI DE VIE ÎN CONTEXTUL PROVOCĂRILOR CLIMATICE

SAVIN GH.[1], CORNEA V.[1] , BACA I.[1]
[*]Corresponding author e-mail: ghsavin@yahoo.com

Abstract*. In the paper is presented a segment from the diversity of the grapevine genetic resources accumulated in the Genofond of the Research and Practical Institute for Horticulture and Food Technologies: varieties for table grapes with early maturation, seedlessness. About 30 varieties were highlighted, including with increased or advanced resistance to abiotic and biotic unfavorable factors. Described genotypes represent a wide diversity by genetic origin, the color of the berry, the agrobiological properties. The highlighted sources of useful features are proposed for harness in grapevine breeding, modernization of assortment, inclusivelly in the context of climate challenges.*
Key words*:* grapevine, genofond, genetic resources, assortment, resistance

Rezumat. *În lucrare este prezentat un segment din diversitatea resurselor genetice ale viței de vie acumulate în Genofondul Institutului ştiinţifico-Practic de Horticultură şi Tehnologii Alimentare: genotipuri cu struguri pentru masă cu maturare timpurie, apirenie. Au fost evidențiate circa 30 soiuri, inclusiv cu rezistență sporită sau avansată la factorii abiotici şi biotici nefavorabili. Genotipurile descrise reprezintă o diversitate largă privind originea genetică, culoarea bobului, însuşirile agrobiolgice. Sursele de caracrtere utile evidențiate sunt propuse pentru valorificare în ameliorarea viței de vie, modernizarea sortimentului, inclusiv în contextul provocărilor climatice.*
Cuvinte cheie*:* vița de vie, genofond, resurse genetice, sortiment, rezistență

INTRODUCTION

In the context of Climate Change (CC), defined by the UN by the Rio Convention in 1992 (1992 United Nations Framework Convention on Climate Changes, 1992) and the challenges that follow for humanity, is intensifyed the influence of the main factors in the center of which the breeder operates: the environment, increasingly changing, unpredictable; the dynamic demands of the market and society; the diversity of plant genetic resources, in general, and those of grapevines in particular, which require new strategies and methods in their conservation, evaluation and use. For the Carpathian-Danubian-Pontic region, as a cradle of autochthonous vitiviniculture, the role of genetic resources is primordial

[1] Research and Practical Institute for Horticulture and Food Technologies, Chişinău, Republic of Moldova

for the further progress of the branch, their importance deriving also from the complexity of the CC phenomenon.

Synthesis works of the publications on the impact of CC on the vitiviniculture sector (Mozell *et al.,* 2014; Sacchelli *et al.,* 2016) highlights the multifaceted aspects related to this phenomenon: global warming, as a result of increasing the concentration of greenhouse gases; changing the conditions in the cultivation areas of varieties destined to produce qualitive wines; modification of the chemical composition and quality of the wines, including as a result of the modification of the phenological parameters; raising the sea level, which will affect the areas occupied by the vineyards, firstly in the recognized wine regions; increasing the number of diseases caused by pathogens and pests, their degree of virulence; the implicit socio-ecological and socio-economic effects; the procedures proposed to mitigate these effects.

In order to increase the adaptability of the varieties to the climatic challenges, the breeding of grapevine is of major importance (Savin, 2014; Audeguin *et al.,* 2015), and the breeder's task is to broaden and harness the genetic basis of resistance using the diversity of genetic resources. Lately, plant breeding has advanced by the development and use of molecular marker systems, which aim to accelerate and streamline the obtainment with high accuracy of plant resistance traits and their practical achievements in grapevine breeding (Laucou *et. al.,* 2018; INTEGRAPE, 2019).

The diversity of grapevine Genepool has been and continues to be a precondition for the long-term development of the vitiviniculture sector of the Republic of Moldova (Savin, 2014). In this context is presented a segment of the diversity of grapevine genetic resources accumulated in the Genofond of the National Institute of Vine and Wine, currently the Scientific and Practical Institute of Horticulture and Food Technologies (ISPHTA).

MATERIAL AND METHOD

The studies have been carried out during the two last decades within the grapevine Genofond of ISPHTA, located in the southern part of Chisinau city (46°58′39.65″ N şi 28°46′21.68″ E, altitude 201 m). The weather conditions of the experimental sectors correspond to the conditions of the wine region Codru of the Republic of Moldova and represent the average values for the republic.

The study included genotypes accumulated in Genofond from various vitiviniculture centers of the Earth, varied by genetic origin, the agrobiological properties. Planting scheme 3.0 x 1.25 m, the training system is bilateral cord on high trunk (60 cm). The ampelographic description was made according to the OIV Descriptor (2009).

RESULTS AND DISCUSSIONS

The completion of the current Genofond, composed by the Ampelographic Collection (founded in 1982) and the adjacent sectors, was made, partly, by the transferring the resources from the previous Ampelographic Collection. From the

literature were located the genetic sources with precocity (early and very early), resistance to frost, diseases and pests, seedlessness and, eventually, combinations of these traits. As a result of the expeditions, the exchange of biological material and introduction has been accumulated a valuable Genofond, which at the present stage includes genotypes from 67 vitiviniculture centers of the world (Savin, 2012).

The objectives of researches were the evaluation and documentation of the diversity of accumulated genotypes according the criteria: time of maturity; grape and berry size and attractiveness; quality, including seedlessness; resistance to diseases and pests; increased fertility.

In the group of genotypes with very early time of maturity of the berry, approx. 30 genotypes were highlighted, which can be proposed as genetic sources of earliness (and quality) (tab. 1). Some of them showed an advanced resistance to low temperatures, powdery and downy mildew. It is noted by the increased productivity potential and the advanced percentage of fertile shoots varieties Aromat de Iaşi, Belgradkii rannii, Elegia, Muscat timpuriu de Bucureşti. Genotypes with traits necessary to improve the assortment of grape varieties for table are also present in other ripening times, in particular - late ripening, large berry, presence of aroma, relative or increased resistance to wintering and pathogens: Antigona (YUG), Doci Nimranga (RUS), Vostorg idealinâi (RUS), Xenia (ROM) et al.

The grapevine genofond has an essential role in the creation of seedless varieties. In the context of completing the quantitative and qualitative traits of the assortment, the diversity of the seedless genotypes existing in the Genofond was supplemented with new sources of seedlessness (tab. 2). The varieties Kiş-mis AZOS, Rusbol, Kiş-miş unicalinâi are characterized by complex resistance to unfavorable conditions. The wide diversity of seedless genotypes, introduced in Genofond, seedless varieties and elites created within the institute (Savin, 2012; Savin, 2018) allow to select, evaluate them in order to identify the premises for diversifying the existing assortment: extending the consumption period of the fresh grapes with very early and early varieties, but also late; multiple biological resistance to unfavorable abiotic and biotic factors, reduction of chemical pressure on the environment; potential for obtaining natural, organic products.

The grapes are highlighted by the ability to accumulate, mainly, glucose and fructose, and the seedless varieties tend to accumulate more fructose than glucose, so they are beneficial for sufferers of gastrointestinal diseases and diabetes. In this sense the biological material has a high healing potential, unexplored. As a separate position we highlight the seedless varieties as raw material for industrialization, which are at the beginning of their wide use, offering, in this regard, wide opportunities for the diversification of grape products (including the production of jam, juice, must, marinated, raisins, homogenized, wines and distillates, etc.), as well as job creation.

Table 1

Genotypes with very early – early time of berry maturity (fragment, grapevine Genofond of ISPHTA)

Name of genotype	Contry of origin	Genetic origin	Berry color	Direction of use	Specific particularities
Alma-Atinskii ranii	KAZ	Madelein Angevine n.p.	green yellow	table grapes	very early maturity; muscat flavor; medium resistance
Aromat de Iasi	ROM	Tămâioasă Românească n.p.	green yellow	mixed	medium maturity; muscat flavor; rezistance to frosts, powdery mildew
Arkadia	UKR	Moldova x Cardinal	green yellow	table grapes	early maturity; slightly muscat flavor; high productivity; resistance to downy mildew
Avgalia	RUS	Madelein Angevine x Galan	green yellow	table grapes	very early maturity; relatively resistance to pathogens
Azur	ROM	Coarnă neagră x Cardinal	blue black	table grapes	early maturity; slightly flavor; rezistance to frosts, drought
Belgradskii ranii	YUG	Afuz Ali x Bouvier	green yellow	table grapes	very early maturity; icreased productivity; medium resistance
Elegia	UKR	Shasselas severnaia x Fioletovâi ranni	rose	mixed	very early maturity; muscat flavor; advanced resistance
Kardisah	UKR	Cardinal x Chasselas severnaia	rose	table grapes	very early maturity; muscat flavor; sensitive to the frosts and diseases
Kievskii zolotistâi	UKR	Irsai Oliver x (Malengre ranni + Lignan)	green yellow	table grapes	very early maturity; muscat flavor; medium resistance
Kirghizkii ranii	KGZ	Madelein Angevine x Muscat Vengherschii	green yellow	table grapes	very early maturity; muscat flavor; medium resistance
Muscat Kubanskii	RUS	Malengre ranii x Muscat de Hamburg	green yellow	mixed	early maturity; muscat flavor; increased resistance
Muscat jemciujnâi	UKR	Ceaus rose x Perla de Csaba	green yellow	table grapes	very early maturity; muscat flavor; medium resistance
Muscat timpuriu de Bucuresti	ROM	Coarnă albă x (Regina viilor x Perla de Csaba)	green yellow	table grapes	very early maturity; muscat flavor; medium resistance

Table 2

Seedless genotypes (fragment, grapevine Genofond of ISPHTA)

Name of genotype	Contry of origin	Genetic origin	Berry color	Direction of use	Specific particularities
Călina	ROM	Braghină x Sultanină	rose	table grapes, industr.	medium maturity; good productivity and fertility; medium resistance
Centennial seedless	SUA	Gold x (Emperor x Pirovano 75)	green yellow	table grapes	early maturity; muscat flavor
Himrood	USA	Ontario x Kiş-miş alb	green yellow	table grapes, industr.	early –medium maturity; specific flavor; increased resistance
Interlaken	USA	Ontario x Kiş-miş alb	green yellow	table grapes, industr.	early maturity; increased resistance
Iubilei VIR-a	UZB	Pobeda x Kiş-miş negru	negru	table grapes, industr.	medium-late maturity; medium resistance
Kiş-miş AZOS	RUS		dark red violet	table grapes	medium maturity; advanced resistance
Kiş-miş Hişrau	UZB	Nimrang x Kiş-miş negru	green yellow	table grapes, industr.	medium maturity; medium resistance; transportability
Kiş-miş Novocerkaskii	RUS	Villard blanc x Mecita	green yellow - rose	table grapes	medium-late maturity; high fertility; increased complex resistance
Kiş-miş unicalinăi	RUS	Severnăi x Kiş-miş negru	rose - violet	table grapes, industr.	medium maturity; very productive; resistance to frosts; sensitive to dis.
Mecita	UKR	Ceauş rose x Kiş-miş negru	rose	table grapes	medium maturity; sensitive to the frosts and diseases
Otilia	ROM	Alphonse Lavallee x Perlette	dark red violet	table grapes, industr.	early maturity; good resistance to frosts, drought and diseases
Perlon	ARG	Emperor x Perlette	rose - violet	table grapes	medium-late maturity; sensitive to the frosts and diseases
Rusbol	RUS BGR	Villard blanc x Sverhrannii besemeannăi	green yellow	table grapes, industr.	early maturity; increased resistance
Tarnau	UZB	Nimrang x Kiş-miş negru	green yellow	de vin	medium-late maturity; medium resistance

Considering that some seedless varieties contain by 10-15% more juice, compared to varieties with seeds, it results in benefits (in yield of must) as a plus value. Due to the advanced resistance, the economic effect can be increased by significantly reducing the number of chemical treatments, compative with the sensitive varieties. After all, the use and exploitation of the potential of the latest generation varieties with multiple resistance to the unfavorable abiotic and biotic factors of the environment presents a promising beginning, of perspective through the diversification and sustainability of their productive and qualitative potential, as the basis of eco production.

CONCLUSIONS

In the context of mitigating the challenges of climate change, the studied genotypes represent a segment of the potential for the diversifying the qualitative and quantitative traits favorable for grapevine breeding and amelioration of assortment: very early maturing period - medium-late; the full range of colors of the grain; different degree of seedlessness; specific or muscat aromas; medium, large or very large grapes; increased fertility, diverse use in correlation with biological resistance to abiotic and biotic factors unfavorable to the environment.

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INFLUENCE OF CORBOHYDRATE CONTENT ON GRAFTING IN TABLE GRAPE VARIETIES GELU AND PAULA

INFLUENŢA CONŢINUTULUI DE HIDRAŢI DE CARBON ASUPRA CALUSĂRII LA ALTOIRE LA SOIURILE PENTRU STRUGURI DE MASĂ GELU ŞI PAULA

CIMPOI V.I.[1]*, ROTARU Liliana[1], COLIBABA Lucia Cintia[1], CĂLIN Ioana[1], SCUTĂRAŞU Elena Cristina[1], ALEXANDRU L.C.[2]

*Corresponding author e-mail: cimpoivladut@gmail.com

Abstract. *The factors that determine the formation of the callus at the grafting point, respectively the percentage of vines capable of being planted in the nursery, are of genetic, technological and ecological nature. Thus, the genetic nature of the two symbionts is crucial to the success of grafting. In the present work determinations were made regarding the behavior of table grape varieties Gelu and Paula 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 Paula grape variety (14.32%) and for Gelu variety (13.07%). As a result of the grafting and forcing, the following aspects resulted: the percentage of vines suitable for planting was the highest in the Paula variety grafted on Crăciunel 2, respectively 99%, and in the Gelu variety grafted on Ripariagloire, of 98%. 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 Paula / Crăciunel 2 variant, at 76%. Gelu / Crăciunel 2 variant resulted in obtaining the most vines (7%) that formed root primordia at the intermediate node as well.*

Key words: carbohydrates, callus, primordial roots

Rezumat. *Factorii care condiţionează formarea calusului la punctul de altoire, respectiv procentul de viţe apte de a fi plantate în şcoala de viţe sunt de ordin genetic, tehnologic şi ecologic. Astfel, natura genetică a celor doi simbionţi este hotarâtoare pentru reuşita prinderii la altoire. În lucrarea de faţă s-au făcut determinări privind comportarea soiurilor pentru struguri de masă Gelu şi Paula altoite pe trei portaltoi Riparia Gloire, Berlandieri x Riparia Selectia Oppenheim 4-clona 4 Blaj şi Berlandieri x Riparia Selectia Crăciunel 2. S-a constat că cel mai ridicat conţinut total în hidraţi de carbon l-a avut portaltoiul Riparia gloire (13,49%), urmat de Crăciunel 2 (12,78%) şi SO_{4-4} (12,02%). La soiurile vinifera acelaşi indicator a fost mai mare la soiul Paula (14,32%) şi Gelu (13,07%). În urma altoirii şi forţării au rezultat următoarele aspecte: procentul de viţe apte pentru plantare a fost cel mai mare la soiul Paula altoit*

[1]University of Agricultural Sciences and Veterinary Medicine Iasi, Romania
[2]Vine and Wine Research and Development Station of Iaşi, Romania

pe Crăciunel 2, de 99%, iar la soiul Gelu, altoit pe Riparia gloire, de 98%. Vițele cu sudură complet formată la punctul de altoite, mugurele pornit în vegetație şi primordii de rădăcini a fost în procent maxim, la varianta Paula/Crăciunel 2, în procent de 76%. Varianta Gelu/Crăciunel 2 a dus la obținerea celor mai multe vițe care au format primordii de rădăcini şi la nodul intermediar (7%).

Key words: carbohidrați, calus, rădăcini primordiale

INTRODUCTION

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 ofsubstances 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).

MATERIAL AND METHOD

Research was carried out at the Research and Development Station for Viticulture and Winemaking (SCDVV) in Iasi in 2019. Two varieties of table grapes, Gelu and Paula, obtained at SCDVV Iaşi, were grafted on three rootstocks (Riparia Gloire, Berlandieri x Riparia Selectia Oppenheim 4-clone 4). And Berlandieri x Riparia Selectia 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.

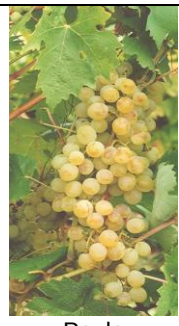
Determinations regarding the total carbohydrate content of the rootstock and scion canes (soluble sugars and starch) by the chemical method with the anthrone reagent and a representation of the starch in the shoots by the colorimetric method based on the staining reaction of the starch with Lugol reagent.

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
 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

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) = $Z \frac{200 \times 4}{m \times 10^6}$ x 100 [%]

% starch (glucose) = $A \frac{500}{m \times 10^6}$ 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 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.

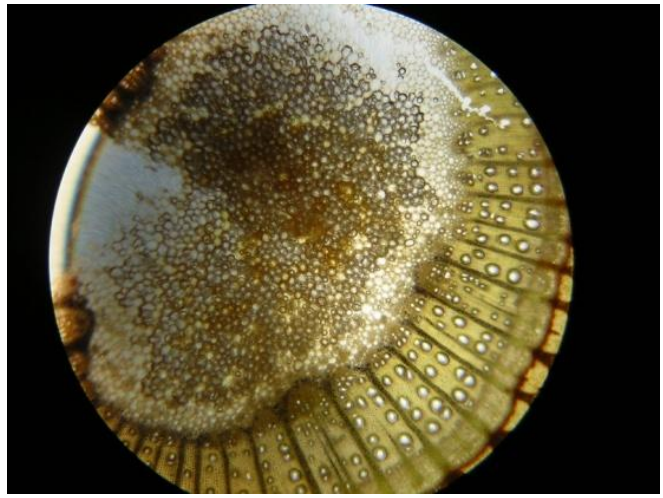


Fig. 1 Aspects of the starch content accumulated in the ropes of rootstock and grape varieties

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
Selecția Oppenheim 4-clona 4 (SO_{4-4})	12.02	9.58	2.44	52.33
Berlandieri x Riparia Selectia Crăciunel 2	12.78	9.79	2.99	52.17

Regarding the results obtained regarding the total carbohydrate content in the string rootstocks (tab. 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 (SO4-4), 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

Rootstock	Total carbohydrate (%)	Soluble sugars (%)	Starch (%)	Rope moisture (%)
Gelu	14.32	8.99	5.33	55.60
Paula	13.07	9.47	3.60	56.04

After analyzing the carbohydrates from the rootstock varieties, the content of the carbohydrates from the rootstock varieties was also analyzed. Of the two varieties taken into consideration, the best result was obtained at the table grape variety Gelu, which recorded a total carbohydrate content of 14.32% (8.99%, representing the percentage of soluble sugars), respectively 5.33% starch). The other variety, Paula, registered a lower result compared to the Gelu variety, having a total carbohydrate content of 13.07% of which 9.47% represents soluble sugars and 3.60% 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% - 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, the Paula / Crăciunel 2 variant recorded the best result (99%), while for the Gelu variety, the best variant was Gelu / Riparia Gloire (98%). From the vines that are suitable for planting, the percentage of grafting was analyzed, here the best results were obtained by the Paula / Crăciunel 2 variant (76%), and Gelu / Riparia Gloire (71%) respectively.

The percentage of vines with the bud that entered vegetation was a good one with values of 71% (Gelu / Riparia Gloire), respectively 76% (Paula / Crăciunel 2). For both varieties taken into consideration, the percentage of vines where the bud did not start vegetation was analyzed, here the variants Gelu / SO4-4 and Gelu / Cracciunel 2 having equal values (30%), while for the other variety, the variant with the highest percentage of buds that did not enter vegetation was Paula / SO4-4 (29%).

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	Callus complete	No callus	Base	Node 2
Gelu	Riparia Gloire	98	71	27	19	45	34	98	2	96	2
	Selecția Oppenheim 4-clona 4 (SO_{4-4})	95	65	30	38	30	27	95	5	92	3
	Berlandieri x Riparia Selectia Crăciunel 2	97	67	30	21	37	39	97	3	90	7
Paula	Riparia Gloire	98	70	28	23	35	40	98	2	95	3
	Selecția Oppenheim 4-clona 4 (SO_{4-4})	97	68	29	35	37	25	97	3	91	6
	Berlandieri x Riparia Selectia Crăciunel 2	99	76	23	28	30	41	99	1	94	5

* 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 grafting (%) - calves with complete callus formed at the grafting point, with buds started in vegetation and with root primordia

The degree of callus forming was another parameter analyzed, here the best results were obtained by the variant Paula / Crăciunel 2 (99%) and Gelu / Riparia Gloire (98%), these having fully formed callus. All the variants were classified according to diameter too, according to specialized literature (7-8.5 mm; 8.6-10 mm; 10.1-12 mm), the results obtained being very varied for each particular variant.

The last parameter analyzed was the one regarding the location of the roots on the grafted vines, following the place where the grafted vines formed root primordia. All the variants taken into account had a high percentage of occurrence of root primordia at the base of grafted vines, compared to their formation at the second node where the highest percentage was obtained in the Gelu / Craciunel 2 (7%) and Paula / SO4- variants. 4 (6%).

CONCLUSIONS

1. 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%).
2. The physiological humidity of the analysed canes was within normal limits, both in the scion and in the rootstocks (52-56%), which showed that the graft material was kept under proper conditions.
3. The callus formation for different diameters shows that the best ratio was at Riparia Gloire and Selecția Crăciunel 2 with the diameters of 8.6-10 mm, respectively 10.1-12 mm, and at the rootstock SO4-4 at the diameters of 7-8.5, respectively 8.6-10 mm.
4. The percentage of vines with the most numerous basal roots was registered at the Riparia Gloire rootstock (95-96%).

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INFLUENCE OF SULPHUR DIOXIDE AND DIMETHYL DICARBONATE ON WHITE WINES QUALITY

INFLUENŢA DIOXIDULUI DE SULF ŞI A DIMETIL DICARBONATULUI ASUPRA CALITĂŢII VINURILOR ALBE

CĂLIN Ioana[1]*, COTEA V. V.[1], LUCHIAN Camelia Elena[1], COLIBABA Lucia Cintia[1], ZAMFIR C.I.[2], SCUTARAŞU Elena Cristina[1], CIMPOI V.I.[1]
*Corresponding author email: ioana.calin16@yahoo.com

Abstract. *Being a complex system in continuous evolution, wine needs different stabilization and conditioning treatments.Sulphur dioxide and dimethyl dicarbonate areone of the most used in winemaking because they have an important role in wine protection and stabilization. For this study, nine wine variants were obtained from a blend of Fetească Regală and Muscat Ottonel varieties. All samples were treated with6 % SO_2 solution and dimethyl dicarbonate liquid solution, in various concentrations. The aim of this experiment was to follow the evolution of physical-chemical and chromatic parameters of wines, depending on treatments used and the analyzes period. The analyzes were repeated and compared at three months difference. Both treatments, SO_2 and dimethyl dicarbonate showed significant influence on the physical-chemical and chromatic characteristics of wines, depending on added substances concentration and the analysis periods, representing a good alternative for modern winemaking.*
Key words: wine stabilization, dimethyl dicarbonate, sulphur dioxide, physical-chemical parameters, color parameters

Rezumat*. Fiind un sistem complex într-o continuă evoluţie, vinul necesită diferite tratamente de stabilizare şi condiţionare. Dioxidul de sulf şi dimetildicarbonatul sunt cele mai utilizate substanţe în ultima perioadă în vinificaţie, deoarece au un rol important în protecţia şi stabilizarea vinurilor. Pentru acest studiu s-a realizat un număr de nouă variante obţinute dintr-un cupaj de Fetească Regală şi Muscat Ottonel. Toate variantele au fost tratate cu o soluţie de dioxid de sulf de concentraţie 6% şi dimetildicarbonat soluţie lichidă, în diferite concentraţii. Scopul acestui studiu a constat în urmărirea evoluţiei parametrilor fizico-chimci şi cromatici ai vinurilor în funcţie de tratamentele administrate şi de perioada de analiză. Analizele au fost efectuate şi comparate, la trei luni diferenţă. Ambele tratamente au avut o influenţă asupra parametrilor fizico-chimici şi de culoare ai vinurilor, în funcţie de concetraţiile administrate şi perioada de analiză, reprezentând o bună alternativă pentru vinificaţia modernă.*
Cuvinte cheie: stabilizarea vinurilor, dimetildicarbonat, dioxid de sulf, parametri fizico-chimici, parametri cromatici

[1] University of Agricultural Sciences and Veterinary Medicine from Iasi, Romania
[2] Romanian Academy, Iasi branch, Romania

INTRODUCTION

Recent studies have focused on the quality of food and beverages, consumers being increasingly concerned about their health. In this context,one of the current main challenges of modern enology is the use of sulphur dioxide (SO_2) in wine-making. Nowadays,attempts are concentrated to replace sulphur dioxide with other substances that play a significant role in wines stabilization. The attention of researchers has focused in particular on wine'scomposition, observing the changes resultedfrom using these stabilization substances.In practice, stability is achieved by subjecting the wine under certain conditions to treatments and operations which as a whole form the conditioning process (Pomohaci, 2001). There area lot of substances that can be used to protect wine'scomposition and itscolor parameters. This group includes those products that protect the must or wine from oxidation and are able to inactivate or kill microorganisms (Cotea, 1985).Sulphur dioxide is used in solution form in various concentrations, have a sickly odor, known to be the most useful andrecommendedpreservative due to its antioxidant, antiseptic and antibacterial actions (Ribéreau-Gayon*et al*., 2006).The origin of sulfur dioxide can be biological and technological. The biological one is produced by yeasts during fermentation and the technological one consists in the introduction inmust and wine of sulfur dioxide in various other forms.Total sulphur dioxide exists as both free sulfur dioxide and bound sulfur dioxide in wine. The active form is represented by free sulfur dioxide that protects thewine against oxidation and microorganisms. When wine pH is low, small amounts of free sulfur dioxide can be effective in microbiological control.Another substance that is increasingly used in enology is dimethyl dicarbonateor DMDC. It's a colorless liquid with a sharpodorbeing ayeastinhibitor and preservative for alcoholic beverages, especially low alcohol wines. It is used as an antimicrobial agent and the efficacy depends on pH values (lower pH requires less DMDC for equivalent antimicrobial action) (Ough *et al*. 1978). Numerous factors such as wine composition, temperature, species, strains, initial contamination are very important to the action of DMDC (Bartowsky, 2009).After its addition in wines, it is immediately decomposedinto alcohol and carbon dioxide, compounds that are always present in wines. DMDC can be used to prevent spoilageyeastgrowth in wines as well as to stop alcoholic fermentation in the production of sweet wines or to disinfect musts by removing native flora present (Costa *et al*., 2008).This compound is in trial to be used instead of SO_2 in vinification (Divol *et al.,* 2005).It wasapproved in the European Union for use in wines at the maximum amount of 200 mg/L at bottling (for wines that contain more than 5 g/L of residual sugar) (Regulation (EC) No 643/2006. However, the inability of DMDC to obstruct numerous bacterialgrowth, using the maximum dose of DMDC legally authorized, and to protect the wine from oxidation makes its use alone in winemaking not sufficient to totally substitute SO_2. A certain synergistic activity exists, increasing the inactivation effect against wine yeast and bacteria between DMDC and sulphur dioxide in both potassium and sodium metabisulphite salt.

The use of DMDC allows asignificant reduction of sulphur content in grape juice or semi-sweet wines (Morata and Loira, 2017).

In this study, a blend of 70 % Muscat Ottonel and 30% FeteascăRegalăgrape varieties from Iasi vineyard was used.

MATERIAL AND METHOD

Muscat Ottonel and FeteascăRegală grapeswere harvested manually in autumn 2018, crushed and destemmed and thenpressed with a hydraulic press and the grape juice resulted was collected in an stainless steel tank to ferment. After fermentation, white wine was divided in 3 aliquot parts in which different amounts of sulfur dioxide have been administered: 40 ppm in the first one, 80 ppm in the second and 160 ppm in the third one.

The aim of this research was to analyze the influence of applied treatments (sulphur dioxide and DMDC) of stabilization onthe physical-chemical parameters of obtained wines samples. Standard analysesaccordingto the International Organization of Vine and Wine methods were repeated at three months differences, first analyses beingrealizedon the 9th of December 2018 and the second one on the 23rd of March 2019 in Laboratory of Oenology from Iasi.Characteristic parameters of color were determined according the Commission Internationaled'Eclairage (CIE, 1976), using characteristics of specific qualities of visual sensation: clarity, tonality, chromatic parameters, saturation, luminosity, hue (OIV-MA-AS2-11). Chromatic characteristics were evaluated using a Specord UV-VIS spectrophotometer. CIELab system characterizes color variations as perceived by the human eye, representing a uniform 3-dimensional space defined by colorimetric coordinates L*, a*, and b*. The vertical axis noted with L* measures from 0 – completely opaque, to 100 - completely transparent, and parameters "+a*" red, "-a*" green, "+b*" yellow, "-b" blue were registered (Main *et. al.,* 2007).

RESULTS AND DISCUSSION

The following physical-chemical parameters have been evaluated in two different period (at three months differences): ethanol content, total acidity, volatile acidity, total sugar,density and pH.Significant changes on white wines composition after the addition of this type of substance can be observed in table 1.

The ethanol concentration represents the result of fermentation sugars from musts (glucose and fructose) and causes some qualitative and quantitative changes in its chemical composition (Moreno and Peinado, 2012). First period of analysis shows an alcoholic strength between 14.3 % vol. (V1, V3)-15.4 % vol. (V7, V9) and second analysis shows values between 14.2 % vol. (V1)-15.3 % vol. (V8).

The total acidity parameter represents the total amount of acids presents in wines. The total acidity of V1, V2, V3 was 5.8 g/L tartaric acid and 6.3g/L at V6.Usually, if a wine has a high acidity level, it will have a low pH. High acid/low pH wines are stable as, in this type of environment, the growth of bacteria and other microorganisms is inhibited.pH is an important parameter in winemaking especially in wine stabilization. Ideal pH levels in wines are between 3.2-3.6 and analyzed samples are within this range.

Table 1

Physical chemical parameters of analyzed samples

Sample	Sulphur dioxide doses(ppm)	DMDC doses (mg/L)	Ethanol % vol. alc.	Total acidity	Volatile acidity	Total sugar g/L	Density δ	pH
9.12.2018								
V1	40	0	14.3	5.8	0.3	17.7	0.9971	3.45
V2	40	100	14.3	5.8	0.27	18.5	0.9969	3.42
V3	40	200	14.4	5.8	0.29	18	0.9968	3.41
V4	80	0	15	6	0.28	18.7	0.9969	3.41
V5	80	100	15.1	6	0.28	18.6	0.9968	3.41
V6	80	200	15	6.3	0.29	17.9	0.9971	3.45
V7	160	0	15.4	6.2	0.26	14.9	0.9953	3.36
V8	160	100	15.3	6.2	0.25	14.7	0.995	3.38
V9	160	200	15.4	6.2	0.27	15.2	0.9949	3.37
23.03.2019								
V1'	40	0	14.2	4.89	0.39	20.7	0.9968	3.42
V2'	40	100	14.4	4.74	0.31	21.1	0.9964	3.41
V3'	40	200	14.3	5.81	0.21	21.6	0.9972	3.26
V4'	80	0	14.9	6.27	0.17	20.7	0.9969	3.25
V5'	80	100	15	6.12	0.18	21.3	0.9969	3.21
V6'	80	200	14.7	6.42	0.2	18.5	0.9963	3.26
V7'	160	0	15.2	6.12	0.17	17.5	0.9954	3.19
V8'	160	100	15.3	6.27	0.16	17.9	0.9953	3.19
V9'	160	200	15.2	6.12	0.16	17.3	0.9954	3.18

The volatile acidity of wines is measured by distillation, a process in which volatile acetic acid (main component= 95-99%) is separated from the other, non-volatile acids present in wines. In this case, insignificantdifferences have been noticed between 0.25 g/L acetic acid (V8)- 0.3 (V1) in first period and 0.16 (V8', V9') - 0.39 g/L acetic acid (V1') during second analysis.

Reductive substances include all the sugars withketonic and aldehydicfunctions and are determined by their reducing action on an alkaline solution of a copper salt (OIV-MA-AS311-01A). Analyzed samples, during the first analysis, present concentrations of 14.7 g/L (V8) and higher concentrations, of 18.7 g/L (V4) and 17.3 g/L (V9') – 21.6 g/L (V3') in the second analysis trial.

Dimetyldicarbonate and sulphur dioxide have influenced the chromatic parameters of analyzed samples to varying degrees observed in table 2. The parameter "a*" presented the highest value at V3 sample (9.00) and the lowest at V9' (0.26). The highest values of "b*" was recorded at the V3' sample with 200 mg/L DMDC and the lowest at V9'. All samples presented positive values with more red and yellow shades.

Table 2

Chromatic parameters of obtained samples

Samples	Luminosity L (0-100)	Colorimetric coordinates		Chroma C	Tone H	Intensity	Tint	Colour simulation
		a red (+) green(-)	b yellow (+) blue(-)					
V1'	89.6	7.16	17.52	18.93	67.76	0.51	1.65	
V2'	84.5	5.55	21.98	22.67	75.84	0.80	191	
V3'	77.8	9.0	23.04	24.73	68.69	1.07	1.58	
V4'	96.9	1.21	6.16	6.27	78.87	0.17	2.26	
V5'	97.4	0.78	6.02	6.07	82.63	0.15	2.53	
V6'	97.2	0.91	6.07	6.14	81.43	0.16	2.42	
V7'	98.5	0.54	5.47	5.50	84.40	0.12	3.99	
V8'	98.2	0.41	5.28	5.30	85.61	0.12	3.57	
V9'	97.92	0.26	5.19	5.20	87.08	0.13	3.15	

The brightness is influenced by luminosity "L*" parameter andidentifiedby high values ranging from V3'-77.8 to V9'-97.72. Chroma values ranged from V9'-5.20 to V3'-24.73.Parameter such as tonality registered positive values for all samples (67.76- 87.08). The tint or hue parameter has been noted with values from V3'-1.58 to V7'-3.99.

CONCLUSIONS

1. Treatments of stabilization play an important role in wine quality, being essentials in modern winemaking. In this experiment, the results show small differences according to the time passed between analyses, which confirmed the synergic action between sulphur dioxide and DMDC.

2. Added substances have a positive effect separately, but, both treatments have a constructive influence to physical-chemical and chromatic parameters of analyzed samples.

3. The results of this study can confirm a good preservation of wine quality and a new alternative to reduce SO_2 concentrations.

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INFLUENCE OF ENZYMES TREATMENT ON THE QUALITY OF SAUVIGNON BLANC WINES

INFLUENȚA TRATAMENTELOR ENZIMATICE ASUPRA CALITĂȚII VINURILOR SAUVIGNON BLANC

SCUTARAŞU Elena Cristina[1*], LUCHIAN Camelia Elena[1], COLIBABA Lucia Cintia[1], COTEA V. V.[1], CĂLIN Ioana[1], ANDRIEŞ M. T.[1], CIMPOI V. I.[1]

*Corresponding author email: cristina_scutarasu@yahoo.com

Abstract. *Enzyme preparations are currently used in modern wine technology for diverse biotransformation reactions from pre-fermentation to fermentation, post-fermentation and wine aging stage. Industrial enzymes gives quantitative (increased juice yields) and qualitative conveniences (improved color extraction and flavor enhancement) and processing benefits (shorter maceration, settling and filtration time). This study investigates the effects of enzyme treatments on physical-chemical, chromatic and organoleptic parameters of white wines obtained in Iasi vineyard.The grapes representing Sauvignon blanc variety were processed in autumn 2018 by the classic method for obtaining white wines. For this study, five commercial enzymes with different activities were used, such as β-glycosidase and pectinases, thus contributing to release aroma compounds, and six variants were obtained. Laboratory analysis of the wine samples was performed according to International Organization of Vine and Wine. Significant influence in the composition of the analyzed samples was observed, depending on the type of enzyme used as pre-treatment. The study results are useful in improving wine-making process and final products quality.*

Key words: glycosidase, pectinases, physical-chemical parameters, Sauvignon blanc

Rezumat. *Preparatele enzimatice sunt frecvent utilizate în vinificația modernă pentru diversele reacții de biotransformare la care participă, încă de la faza de pre-fermentare, fermentare, post-fermentare şi până la stadiul de învechire a vinului. Utilizarea enzimelor la nivel industrial prezintă numeroase avantaje de ordin cantitativ (creşterea randamentului de suc la presare) şi calitativ (îmbunătățirea extracției compuşilor de culoare şi aromă) şi tehnologice (diminuarea timpului de macerare, decantare şi filtrare). Această lucrare are ca scop analiza influenței tratamentelor enzimatice asupra parametrilor fizico-chimici şi cromatici ai unor vinuri albe obținute în podgoria Iaşi. Strugurii din soiul Sauvignon blanc au fost prelucrați în toamna anului 2018 prin metoda clasică de obținerea vinurilor albe. Pentru acest experiment, au fost comparate cinci enzime comerciale de tipul β-glicozidazelor şi pectinazelor, destinate îmbunătățirii extracției compuşilor volatili, rezultând şase variante. Analizarea in laborator a probelor de vin obşinute s-a realizat in conformitate cu reglementările Organizației Internaționale a Viei şi Vinului. S-a observat o influență semnificativă asupra compoziției eşantioanelor analizate, în funcție de tipul enzimei utilizate ca tratament în etapa de pre-fermentație. Rezultatele studiului contribuie laoptimizarea proceselor de vinificație şi a calității vinurilor.*

Cuvinte cheie: glicozidaze, pectinaze, parametri fizico-chimici, Sauvignon blanc

[1] University of Agricultural Sciences and Veterinary Medicine from Iasi, Romania

INTRODUCTION

Enzymes, as biocatalysts, propose a wide diversity of possibilities for improving food and beverage production; their stereo-selectivity is an important advantage. Specific optical conformation may be associated to flavor properties of final products (Christer and López-Munguia, 1994). So, enzymes present significant benefits for industrial application, such as: low temperature condition for activity, which means low energy requirement for the process; less by-products; improvement of product quality; non-toxic when properly used; can be degraded biologically and can also be immobilized to reuse; increase stability and easy separation from the environment (Bayindirli, 2010). In wine-making production, enzyme activity is essential for the development of high-quality wines, principally to ameliorate clarification and filtration of must and wine, improving their stability and enriching the volatile profile or chromatic parameters of wines (Armada *et. al.,* 2010). The actions of the endogenous enzymes are limited to the pH values and SO_2 conditions associated with the wine-making process. Since the grape enzymes are neither efficient nor sufficient under wine-making conditions, commercial enzymes are widely used as additions (Rensburgand Pretorius, 2000). Commercial enzyme products are eco-friendly and present consistent economic benefits (Mojsov, 2013). The use of enzymes in wine production in the European Union is regulated by the International Organisation of Vine and Wine (OIV). Specific resolutions define general aspects of enzymes in wine-making, permitted enzyme activities, mode of application and enzyme activity measurements.

The experiment aimed to analyze the impact of enzymatic preparations on physical-chemical (ethanol content, density, pH, acidity, malic acid, lactic acid, total sugars, free and total SO_2, total dry matter and non-reductive dry extract), chromatic and sensorial parameters of Sauvignon blanc wine samples, obtained from grapescultivated in Iaşi vineyard. The results of this research are usefulin improving the wine-making process and the sensorial quality of final product.

MATERIALS AND METHODS

Grapes samples and winemaking. Sauvignon blanc grapes were harvested in autumn of 2018 at full maturity from Iaşi vineyard and processed by classic white wines technology.*Saccharomyces* yeast (Levuliaesperide®,AEB) at a dose of 20 g/hL and 30 g/hL yeast nutrient (Fermo Plus CH®, AEB) were inoculated and the mixture was separated in six aliquots. Five different commercial enzymes based on pectolytic and β-glycosidase were added to musts before alcoholic fermentation, intended to improve the extraction of volatile components (EndozymThiol®, AEB – V1; Endozym β-Split®, AEB – V2; Zymovarietal aroma G®, SODINAL – V3; Endozym Ice®, AEB – V4; Zimarome®, BSG WINE –V5 and no enzyme– V6), at dose of 3 g/hL (enzymes were diluted with must 1:10) and 3 mL/hL respectively.The doses administered are in correlation with usage specifications of oenological products and with the legislation in

force.The fermentation was carried out at 16-18 °C for three weeks. When the alcoholic fermentation was finished, each variant was filtered through sterile membrane filters followed by sulfur dioxide addition (to preserve wine from microbiological degradation) and bottled. The samples were kept under controlled conditions (constant temperature, zero UV exposure, stable humidity) and analyzed after 5 months.

Physical-chemical parameters were performed accordingto the International Organization of Vine and Wine Compendium methods of analysis: total and volatile acidity, alcoholic strength, real acidity (pH) density, total sugar, free and total sulfur dioxide, malic and lactic acid, total dry extract and non-reducing extract.

Chromatic parameters were studied in accordance with the Commission Internationaled'Eclairage (CIE, 1976), promoted as the OIV standard method for wine color determination. The system uses characteristics of specific qualities of visual sensation: clarity, tonality, chromaticity, saturation, luminosity, tint. Evaluation of chromatic characteristics was made using a Specord UV-VIS spectrophotometer. CIELab system characterizes color variations as perceived by the human eye, representing a uniform 3-dimensional space defined by colorimetric coordinates L*, a*, and b*. The vertical axis noted with L* measures from 0 – completely opaque, to 100 - totally transparent, and parameters "+a*" red, "-a*" green, "+b*" yellow, "-b" blue were registered (OIV, 2019).Distilled water was used as control.

Sensorial characteristics.The panel of tasting was composedof members of the laboratory or external tasting specialists, persons with advanced theory training on the fundamental concepts related to taste. The selected odorsare connected or normally appearing in white wines, such ashoney, citrus, vegetal, ripe fruits, green fruits, hay, roses, etc. The parameters were evaluated with ratings from 0 to 5 and the means were calculated.

Reagents and standard solutions were of analytical grade and were purchased from Merck, Germany.

RESULTS AND DISCUSSIONS

Effects of enzymatic pre-treatment on basic parameters of wine. The analyzed samples were dry wines with 16.2 % vol. The ethanol content in wines was not affected by the type of administrated enzymes as pre-treatment.

Wine's acidity is essential foritspreservation and sensory features. These parameters may be influencedby grape variety, ripeness, climatic conditions, wine-making technology and storage conditions(Samoticha et. al., 2017).The total acidity of the major of variants was 3.3 g of tartaric acid (V1, V3, V5, V6), and 3.2 g of tartaric acid at V2 and V4.

The total dry extract mentions to all non-volatile composites under specified physical conditions (OIV, 2019). The values registered varies between 30 (V2) and 30.7 (V5 and V6) g/L.

The content of the non-reductive extract of wines obtained from Romanian vineyardsfluctuatesamong13 and 35 g/L, according to variety, grapes health situation, wine-making technology and applied treatments (Cotea, 1985). The non-reductive dry extract was 20 g/L for V2, V3, V4, V5 and 20.1 g/L for V1 and V6.

Table 1

Physical-chemical parameters of analyzed wines

Sam-ple	Total Acid. g/L $C_4H_6O_6$	pH	Alc. % vol.	Malic Acid	V.A. g/L $C_2H_4O_2$	Den-sity	Total Sugar g/L	Lac-tic Acid	Free SO_2 mg/L	Total SO_2 mg/L	TDE g/L	NE g/L
V1	3.3	3.4	16.2	0.5	0.29	0.9911	2.2	1.3	15	61	30.2	28
V2	3.2	3.42	16.2	0.3	0.29	0.9910	1.9	1.6	18	61	30	28.1
V3	3.3	3.42	16.2	0.3	0.28	0.9911	2.1	1.6	18	59	30.2	28.1
V4	3.2	3.42	16.2	0.3	0.29	0.9912	2.4	1.5	15	61	30.5	28.1
V5	3.3	3.42	16.2	0.3	0.3	0.9913	2.6	1.4	18	64	30.7	28.1
V6	3.3	3.44	16.2	0.3	0.32	0.9913	2.7	1.6	18	60	30.7	28

Enzymatic treatment influenced the chromatic parameters of analyzed samples to varying degrees. All variants presented a high level of clarity, with more yellow and green shades.The parameter "a*" presented the highest value at V5 sample (-1.02) and the lowest at V1 (0.58). The highest values of "b*" (yellow shade) was recorded at the V1 sample and the lowest at V2.

Table 2

Chromatic parameters of analyzed samples

Sam-ple	Clarity L	Chromaticity		Saturation Chroma C	Tonality H	Lumino-sity	Tint	Colori-metric difference	Tonality difference
		a	b					ΔE	ΔH
V1	98.44	-0.58	6.77	6.8	-85.13	0.12	4.21	3.71	2.38
V2	98.62	-0.7	6.48	6.58	-83.84	0.11	4.55	3.72	2.5
V3	98.63	-0.78	6.65	6.69	-83.32	0.12	4.67	3.87	2.58
V4	98.68	-0.76	6.51	6.55	-83.33	0.11	4.73	3.81	2.56
V5	98.86	-1.02	6.63	6.7	-81.28	0.11	5.59	4.14	2.82
V6	98.54	-0.63	6.5	6.53	-84.46	0.12	4.31	3.64	2.43

Some differences were measured for "L*" parameters, related to brightness. It was manifested by less red and blue color of the wine. The luminous intensity of color - i.e., its degree of lightness - is called its value. Colors can be classified as light or dark when comparing their value. In figure 1, comparative organoleptic graphics of the analyzed samples are represented. Tonality has registered negative values for all samples.

A perceptible color difference between samplestreated with enzymes and control samples can be observed, suggesting that enzyme preparation had an important influence on colorimetric properties. Delta values representthe color difference as compared with the control.Thus, the largest colorimetric difference (ΔE values) was recorded on sample V5, followed by V3, V4, V2, V1 and V6.

Also, ΔH values showthe largest tonality difference in theV5 variant, followed by V3, V4, V2, V6 and V1.

Chroma describes the vividness or dullness of color - in other words, how close the color is to either gray or the pure hue.The tint is how we perceive an object's color (red, orange, green, blue, etc.) and is partially affected by the pH level of the wine.The tint values of analyzed samples ranged from 4.21 (V1) to 5.59 (V5) andchroma values ranged from 6.53 (V6) to 6.77 (V1).

Following the sensory analysis, significant differences can be observed due to the type of enzyme used as pre-treatment.

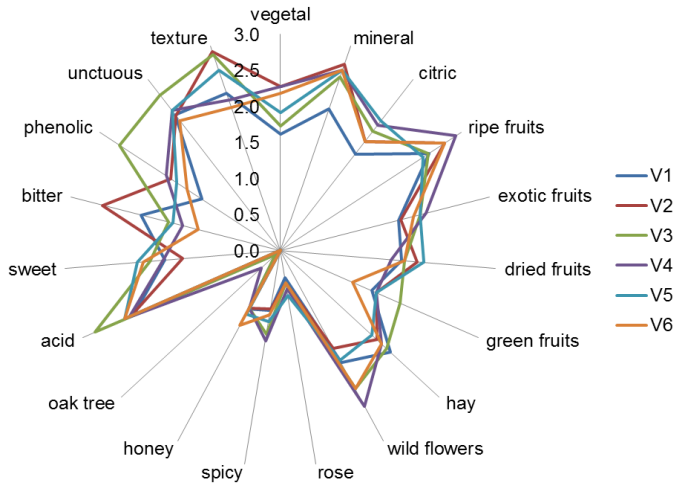

Fig. 1 Comparative organoleptic analysis of wines

This study shows how the general sensory descriptors of the Sauvignon blanc wine can be influenced by enzyme preparations as pre-treatment.Wine texture is counted to be a major product differentiator for wine style and value in the marketplace. Also, clarity and color stability (absence of haze development and the retention of color) are usuallymeasured to be pre-requisites to market success.V1 samplewas noted byintense ripe fruits flavor, hay and mild wildflowers, with a great texture, unctuous and a little bitter. V2 sample showed mild ripe fruits bouquet, vegetal notes and dried fruits taste, with a good texture, and slightly bitter.V3 revealed ripe fruits and wild flowers notes, with a great texture and unctuous. V4sample presented the most intense wild flower touch, fruity notes and medium unctuous degree. V5 stood out by medium fruity notes and less vegetal scent. V6 was noted by ripe fruits fragrance, wild flowers and less green fruits.V1 and V3 variants were appreciated as having the highest acidity.A discreet spicy fragrance was noted especially on V3 and V4 samples. All wines showed a high mineral palate.

CONCLUSIONS

1. The process of winemaking depends on the activity of enzymes. Pre-fermentative treatments applied for this experiment didn't show a relevant effect on the standard physical-chemical parameters of wines.

2. Enzymatic preparations affected chromatic parameters of analyzed samples to varying degrees.

3. By applying enzymatic treatments, an increase in the sensorial complexity and variability of sensorial indices of the samples were obtained.

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RESEARCHES REGARDING THE USE AS A BIOMASS OF VINES RESIDUES RESULTING FROM THE DORMANT PRUNING

CERCETĂRI PRIVIND UTILIZAREA CA BIOMASA A COARDELOR DE VIȚĂ DE VIE REZULTATE DE LA TĂIEREA ÎN USCAT A PLANTAȚIILOR VITICOLE

DUMITRACHI P.E. [1]*, CORDUNEANU Raluca Oana[1], BĂETU M.[1], CÂRLESCU P.[1], ȚENU I.[1]

*Corresponding author e-mail: dumitrachi.emanuel@yahoo.com

Abstract. *At the global level, biomass is considered one of the main forms of renewable energy, as it ensures the conservation of the sun's energy in chemical form, being one of the most popular and universal resources on Earth, used for energy purposes since the discovery of fire by man. Today, biomass can be used for different purposes from room heating to the production of electricity and fuel for cars. Biomass is the biodegradable part of agricultural products, waste and residues, including plant and animal substances, forestry and related industries, as well as the biodegradable part of industrial and urban waste. Considering these elements, we can consider that vine plantations can make a significant contribution to the development of the biomass source, by using the vine ropes resulted from the cuts in the land. The research aimed the determination of the biomass potential of vine plantations, were harvested ropes from wine varieties and at different dates during plant rest to determine the evolution of their humidity. The samples were taken from the didactic resort farm "Vasile Adamachi" plantation of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" of Iasi. The varieties studied were: Busuioaca de Bohotin, Cabernet Sauvignon, Feteasca Alba, Feteasca Neagra, Feteasca Regala, Muscat Ottonel, Pinot Noir and Sauvignon Petit.*
After the research carried out, it was found that the humidity of the strings is different, depending on the variety and the date of harvest.
Key words: vines residues, humidity.

Rezumat. *Pe plan mondial, biomasa este considerată una dintre principalele forme de energie regenerabilă, întrucât asigură păstrarea energiei solare în formă chimică, fiind una dintre cele mai populare şi universale resurse de pe Pământ, utilizată în scopuri energetice din momentul descoperirii focului de către om. Astăzi, biomasa poate fi utilizată în diferite scopuri, de la încălzirea încăperilor până la producerea energiei electrice şi combustibililor pentru automobile. Biomasa este partea biodegradabilă a produselor, deşeurilor şi reziduurilor din agricultură, inclusiv substanţele vegetale şi animale, silvicultură şi industriile conexe, precum şi partea biodegradabilă a deşeurilor industriale şi urbane. Având în vedere aceste elemente, putem considera că plantaţiile de viţă de vie pot contribui semnificativ la dezvoltarea sursei de biomasă prin folosirea cordelor de viţă de vie rezultate de la tăireile în uscat. Cercetările au vizat determinarea potenţialului de biomasă al plantaţiilor de viţă de vie. S-au recoltat coarde de la soiurile pentru vin, şi la diferite date, pe*

[1]University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

durata repausului plantei, pentru a stabili evoluția umidității acestora. Probele au fost preluate din plantația din cadrul Stațiunii Didactice a Universității de Ştiințe Agricole „Ion Ionescu de la Brad" din Iaşi, ferma „Vasile Adamachi". Soiurile luate în studiu au fost: Busuioacă de Bohotin, Cabernet Sauvignon, Fetească Alba, Fetească Neagră, Fetească Regală, Muscat Ottonel, Pinot Noir şi Sauvignon Petit. În urma cercetărilor efectuate s-a constat că umiditatea coardelor este diferită în funcție de soi şi data recoltării.
Cuvinte cheie: coarde de viță de vie, umidități.

INTRODUCTION

Reducing greenhouse gas emissions by 20% (compared to 1990 levels), increasing the use of renewable energy by 20%, improving energy efficiency by 20%, and achieving a share of 10% from renewable sources in the transport sector, represents some of the major objectives of the European Union in terms of energy production and environmental issues by 2020 (European Union. Directive 2009/28 / EC of the European Parliament and of the Council of 23 April 2009 on the Promotion of the Use of Energy from Renewable Sources; European Union: Brussels, Belgium, 2009). In the same idea, in order to stimulate the development of second generation biofuels for transport, and to minimize the impact on the climate, the European Commission has proposed limiting the use of food biofuels from 10 to 5% (European Commission. New Commission Proposal to Minimize the Climate Impacts of Biofuel Production; European Commission: Brussels, Belgium, 2012). This has led to an increase in interest for raw materials from agricultural and industrial processes that do not directly interfere with food production (Psomopoulos *et. al*., 2014).

The use of these agricultural residues (straw, hay, branches from cutting trees and vines, waste eco-friendly green etc. can bring huge benefits, including reducing greenhouse gas emissions, increasing energy efficiency, or lower overall costs of biofuel production (Pari *et al*., 2014; Neri *et. al,* 2016).

To achieve these goals, the use of biomass waste is aimed at. At the global level, biomass is a primary carbon source alongside other renewable energy sources. It can be used as a raw material to produce energy, solid biofuels with high energy value or biochemical fuels, necessary for carrying out economic activities. Thus, at present, biomass contributes about 12% to the primary energy production in the world, and in the developing states it occupies 40-50% of the energy supply needed. Recent statistics have shown that in 2017 global primary energy consumption increased to 13.5 billion tons of oil equivalent, which represents about 565 EJ, with an increase of 1.7% per year (Pradhan *et al.,* 2018).

In this context, the demand for renewable energy becomes unavoidable, biomass representing an inexhaustible reserve and an important energy resource. The vine is a liana that, without the intervention of the horticulturist, has the tendency to climb from year to year to the top, fading to the base, the undesirable consequence being the excessive development to the detriment of the fruit, the

production being thus affected. Cutting the cords keeps the vineyards healthy and productive, this physical process influencing the taste and quality of the wine. Due to the large areas of vines present in Romania, every year a large amount of plant residue is represented by ropes and stubs. They are suitable for burning due to their high cellulose and hemicellulose content. Advanced studies already show that the use of other vegetable residues during the production of wine with high fuel potential is worth considering, and here we refer to the pulp, bark and seeds of pressed fruits. These are characterized by a high concentration of residual oil, an important criterion that can positively influence the calorific value of the fuel produced (Brunerova and Brozek, 2016).

Cutting residues, generally, do not represent a resource for farmers, but on the contrary, an additional cost because the elimination of the residues of vines usually consists of crushing and burying them or burning them (Duca *et al.,* 2016). Regardless of the solution chosen, the implications are negative, burial of the residues affecting a crop of vines already damaged by pathogens in the trunk (aging culture), and the burning of the residues affects the environment through emissions released into the atmosphere or the increased risk of fires. The remains of cuts of the vine ropes can be used in the form of chips or pellets for burning in small, medium and large boilers (Duca *et al.,* 2016).

The term biomass is a generic term attributed to biodegradable and non-fossilized organisms, usually produced directly or indirectly through photosynthesis and used as raw materials for the production of fuels and chemicals.

MATERIAL AND METHOD

In 2018, biomass samples were taken from the ropes obtained from the dry cutting of vines from the old plantation, from the didactic resort of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" of Iasi. Horticultural Farm no. 3 "Vasile Adamachi". The aim was to determine the humidity of the varieties studied.

The vineyard plantation covers an area of 12 ha. The distance between the rows is 2.3 m and between the rows 1.2 m. The plant management system is semi-high, the production elements are located on 60-80 cm high stems, and the cutting system is mixed, the production and replacement elements are being placed on bilateral unilateral cords.

To fulfill the stages necessary for the experiences, vine ropes were cut, depending on the variety (Busuioaca de Bohotin, Cabernet Sauvignon, White Feteasca, Black Feteasca, Royal Feteasca, Muscat Ottonel, Pinot Noir, Sauvignon Petit.

The actual cutting was done with the manual scissors and the folding saw (occasional) removing approximately two strings of wood from each cuttings on each row. After cutting it was done the labeling of the chords according to the varieties and their assembly in turn. After completing the cutting of the strings for each variety, they were transported and stored in the hall within the mechanization department.

The laboratory experiments aimed to determine the percentage of moisture of the chop obtained from the biomass from the vines. Immediately after the ropes of

vines were transported to the protected space within the mechanization chair, they were chopped with the help of a ram chopper, CARAVVAGI BIO 90 (fig. 1). In order to obtain as finely chopped samples as possible, in order to determine the percentage of humidity, a prior screening of the chisel was performed using a screen with small holes (fig. 2).

Fig. 1 CARAVVAGI BIO 90

Fig. 2 Sieve with small holes

After sifting, the fine chop was evenly distributed with the aid of a balance (fig. 3) and inserted into the aluminum capsules that we placed in a forced ventilation oven (fig. 4). The drying was carried out at a temperature of 1050 C, for about 12 hours, without being influenced by factors from the environment (temperature, atmospheric pressure and humidity of the ambient air, etc.). Initially, each capsule was weighed without content (country) and the obtained data recorded in a personal database. The capsule weighing was also performed with a chop, a final weighing being done after drying the sample, in order to determine the humidity. After each weighing, the data obtained were recorded for further processing and determination of humidity. The moisture content in dry basis represents the ratio, expressed as a percentage, between the mass of water present in the solid biofuel and the mass of the same sample in the dry state.

$$U= (m-m_0) /m_0 \times 100 (\%)$$

wherein: m is the mass of the sample before drying (in the natural state), g; m0 - mass of the same sample after drying to a constant value, g.

Fig. 3 Analytical balance Kern ABJ 220-4NM, 220 g

Fig. 4 Samples subjected to drying in the oven FD 53 model with forced ventilation

RESULTS AND DISCUSSIONS

From the obtained data (tab. 1) it follows that the humidity of the strings is different, depending on the variety and the date of harvest. We note that the

lowest percentage of humidity in January was obtained in the Fetească Neagră variety of 43,141%, and the highest in the Fetească Albă variety of 49,272%. In February, the lowest percentage of humidity was recorded in the Cabernet Sauvignon variety of 48,468%, and the highest in the Fetească Albă variety of 51.603%. For March, the lowest percentage of humidity was registered in the Sauvignon Petit variety of 46.928%, and the highest in the Feteasca white variety of 54.198%.

Regarding the determination of the chemical composition, a comparative analysis was followed between the eight varieties of vines (Busuioaca de Bohotin, Black Feteasca, White Feteasca, Royal Feteasca, Savignon Petit, Muscat Ottonel, Pinot Noir and Cabernet Sauvignon) determined in within the specialized laboratory of ICIA Cluj-Napoca.

Table 1

The percentage of moisture of the vine ropes resulting from the cutting in the dry

Varieties	JANUARY (%)	FEBRUARY (%)	MARCH (%)
BUSUIOACĂ DE BOHOTIN	47.810	49.827	53.736
CABERNET SAUVIGNON	45.184	48.468	51.796
FETEASCĂ ALBĂ	49.272	51.603	54.198
FETEASCĂ NEAGRĂ	43.141	49.351	53.199
FETEASCĂ REGALĂ	47.515	49.419	51.990
MUSCAT OTTONEL	47.152	49.587	52.405
PINOT NOIR	48.741	51.019	53.046
SAUVIGNON PETIT	43.217	50.754	46.928

Table 2

Protein content of biomass samples

Biomass samples	N (%)	C (%)	H (%)	O (%)	S (%)	Protein (%)
Sauvignon Petit	1.60	43.1	6.23	45.6	<0.01	10.0
Pinot Noir	0.85	43.9	5.83	46.0	<0.01	5.3
Fetească Regală	0.87	44.0	5.66	46.2	<0.01	5.5
Busuioacă de Bohotin	0.84	43.6	5.89	45.9	<0.01	5.2
Muscat Ottonel	0.90	44.1	6.05	45.6	<0.01	5.6
Cabernet Sauvignon	0.90	43.9	5.98	46.2	<0.01	5.6
Fetească Neagră	1.00	43.8	5.84	45.3	<0.01	6.2
Fetească Albă	1.00	44.6	6.14	45.2	<0.01	6.3

Carbon, hydrogen and sulfur content was determined using a Flash EA 2000 CHNS / O analyzer (Thermo Fisher Scientific, USA) according to ISO 10694: 1995 (Soil quality - Determination of organic and total carbon after dry burning (elemental analysis), ISO 13878: 1998 (Soil quality - Determination of total nitrogen content by dry combustion ("elementary analysis") and ISO 15178: 2000 (Soil quality - Determination of total sulfur by dry combustion) (Tenu *et al.* 2018). The results presented in table 2 show that all samples contained high carbon (C) and oxygen (O), which are in accordance with data reported by El (Achaby *et al.* 2018) for elementary analysis of vines in the SupAgro region of

Montpellier (France). Elemental analysis showed that carbon varied from 43.1% in Savignon Petit to 44.6% in Feteasca Alba, while the hydrogen content ranged from 5.66% in Feteasca Regal, to 6.23% in the sample Sauvignon Petit. Biomass is rich in organic carbon, but low in nitrogen.

All samples contain protein (Sauvignon Blanc has the highest protein content (10%). The S content was below the detection limit in all analyzed samples.

CONCLUSIONS

1. The moisture obtained from dry cutting depends on the variety of vines.
2. Humidity differs depending on the harvesting period.
3. Plant biomass is the most valuable component of renewable energy resources due to the carbon cycle process.

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RESEARCHES REGARDING THE USE AS A BIOMASS OF THE BRANCHES RESULTING FROM ORCHARD PRUNING OF DIFFERENT SPECIES OF TREES

CERCETARI PRIVIND UTILIZAREA CA BIOMASA A RAMURILOR REZULTATE DE LA TĂIERILE DE FRUCTIFICARE DE LA DIFERITE SPECII DE POMI FRUCTIFERI

***DUMITRACHI P.E.* [1]*, *CORDUNEANU Raluca Oana*[1], *BĂETU M.*[1], *CÂRLESCU P.*[1], *ŢENU I.*[1]**

*Corresponding author e-mail: dumitrachi.emanuel@yahoo.com

Abstract. *Biomass is the most abundant renewable resource on the planet. This includes absolutely all the organic material produced by the metabolic processes of living organisms. Biomass can be used in the form of solid or liquid fuels, being used both for direct combustion, for space heating, and for liquid biofuel (bioethanol) for supply the thermal engines. The research carried out had as an objective the collection of the branches resulting from the fruiting cuts from the varieties of apple, hair, cherry and plum, located in the plantations of the Experimental resort from the USAMV Iaşi, Farm "V. Adamachi", and the determination of the calorific power for each category of fruit trees. From the analysis of the results it appears that the calorific power is different from one species to another, as well as depending on the variety of the respective species. Also, the energy value of the harvested branches is high, being close to that of firewood, which allows us to specify that they can be valued as biomass, constituting an important renewable source of solid biofuel, which can be used in the form of pellets and lighters.*

Key words: fruit trees, calorific power.

Rezumat. *Biomasa reprezintă resursa regenerabilă cea mai abundentă de pe planetă. Aceasta include absolut toată materia organică produsă prin procesele metabolice ale organismelor vii. Biomasa poate fi utilizată sub formă de combustibili solizi sau lichizi, fiind folosită atât pentru arderea directă, la încălzirea spaţiilor, cât şi ca biocombustibil lichid (bioetanol) pentru alimentarea motoarelor termice. Cercetările efectuate au avut ca obiectiv colectarea ramurilor rezultate de la tăierile de fructificare de la soiurile de măr, păr, cireş şi prun, aflate în plantaţiile de la Staţiune Experimentală din cadrul USAMV Iaşi, Ferma "V. Adamachi", şi determniarea puterii calorifice pentru fiecare categorie de pomi fructiferi. Din analiza rezultatelor reies că puterea calorifică este diferită de la o specie la alta, precum şi în funcţie de soiul speciei respective. De asemenea, valoarea energetică a ramurilor recoltate este ridicată, fiind apropiată de cea a lemnelor pentru foc, ceea ce ne permite să precizăm că acestea pot fi valoricate ca biomasă, constituind o sursă regenerabilă importantă de*

[1]University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

biocombustibil solid, care poate fi utilizat sub formă de pelete şi brichete.
Cuvinte cheie: pomi fructiferi, putere calorifică

INTRODUCTION

Biomass is the third largest source of primary energy in the world after coal and oil, being the main source of energy for more than half of the world's population and providing approximately 1.25 billion toe (tones of oil equivalent) of primary energy, or about 14% of the world's annual energy consumption (Purohit, 2006; Zeng, 2010).

The main sources of biomass are agricultural and forestry residues, energy crops and organic waste. These sources have been relatively widely used in the EU for a long time, and future growth in biomass production should be linked to more intensive use of energy crops (van Dam *et al.,* 2007). Lately, biomass has been used more and more in energy production, in cogeneration plants, that is in combined heat and energy production. Combined heat and energy generation represents a very valuable potential for significant improvements in overall fuel efficiency. There are different estimates of the potential and role of biomass in the global energy policy of the future, but all current scenarios give biomass an increasing role in energy consumption and predict its significant growth.

MATERIAL AND METHOD

In 2018, biomass samples were taken from the branches obtained from the dry cutting of fruit trees from the didactic resort of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" of Iaşi, the Horticultural Farm "Vasile Adamachi" ". The aim was to determine the humidity of the varieties studied.

The plantation of fruit trees, from which *biomass was harvested, is located on a land that is characterized by the following pedoclimatic* parameters:

• the relief and the micro-relief of the terrain are differentiated by positive and negative forms formed by large plateaus;

• the soil is a slope chernozem, clayey, formed on loessoid clay;

• the texture is different in profile, in the horizon up to 30 cm it is clay or clay-clay, in the horizon 30-50 cm it is only clay and in the depth it is sandy;

• the structure is good due to the high content in humus, being medium glomerular, well developed in the horizon up to 30 and with moderate development in the other horizons not being present specific structural aggregates;

• the climate has a pronounced temperate-continental character, due to the geographical position, the characteristics of the relief and the influences of the Atlantic and Siberian anthropic, which integrate it between the moderate-continental climate of the Central Moldavian Plateau and the excessively-continental one of the Moldavian Plain.

• the distance between the rows is 3.4 m and 1-1.5 m between the trees in a row.

The actual cutting was done with the manual scissors and the folding saw (occasional).

After cutting the branches were labeled (fig. 1) according to the varieties and

their assembly in turn. After completing the cutting of the branches for each variety, they were transported and stored in the hall within the mechanization department.

Fig.1 Labeled branches

Immediately after the ropes of fruit trees were transported to the protected space within the machining department, they were chopped with the help of a ram chopper, CARAVVAGI BIO 90 (fig. 2). In order to take as finely chopped samples as possible, a prior screening of the chisel was performed using a screen with small holes (fig. 3).

Fig.2 CARAVVAGI BIO 90

Fig.3 Sieve with small holes

After sifting, the thin chisel distributed in aluminum capsules inserted into a forced ventilation oven (fig. 4). The drying was carried out at a temperature of 1050 C, for about 12 hours, without being influenced by factors from the environment (temperature, atmospheric pressure and humidity of the ambient air, etc.). Initially, each capsule was weighed without content (country) and the obtained data recorded in a personal database. The capsule weighing (fig. 5) followed with a chop, the last weighing being done after drying the sample, in order to determine the humidity. After each weighing, the data obtained were recorded for further processing and determination of humidity.

Fig. 4 Samples subjected to drying in the oven FD 53 model with forced ventilation

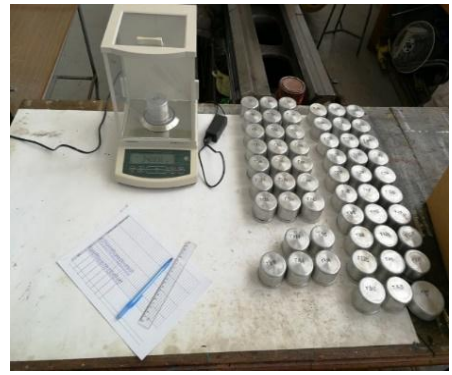

Fig. 5 Analytical balance Kern ABJ 220-4NM, 220 g

The calorific power (the heat of combustion) superior and the inferior of the collected samples was determined in the specialized laboratory of ICIA Cluj-Napoca. Higher calorific power is the amount of heat developed by combustion, when all the water vapor produced in the combustion process is condensed and condensation heat is recovered. The lower calorific value of a solid fuel unit represents the amount of heat developed by combustion, without considering the condensation of water vapor produced in the combustion process. The two components of combustion heat were determined using standard methods.

The gross and net calorific value of the samples were performed using a Parr 6200 Isoperibol calorimeter according to DIN 51900-1: 2000 (Determining the gross calorific value of solid and liquid fuels using the calorimetric pump and calculating the net calorific value) and DIN 51900-2: 2003 (Determination of the gross calorific value of solid and liquid fuels).

For each analysis, 1.0 g pellets were used. A nickel ignition wire was contacted with the pellet. The pump was filled with oxygen at 25 ° C and 1.0 cm3 of distilled water was added. The calorimeter was placed in an isoperibol coating with a distance of 10 mm between all surfaces. The calorimeter pump was immersed in a calorimeter and filled with distilled water. The calorimeter casing was maintained at a constant temperature by circulating water at 27 ° C. The gross calorific value of the samples was calculated from the corrected temperature increase and the actual thermal capacity of the calorimeter.

The net calorific value differs from the gross calorific value by the amount of water in the samples prior to combustion, and that formed during the combustion of hydrogen-containing compounds in the gaseous sample at 25 ° C after combustion.

RESULTS AND DISCUSSIONS

To assess the energy level, by harnessing the biomass resulting from the dry cutting of the trees, the amount of dry matter harvested from the surface of one hectare was calculated and the upper and lower calorific values were determined (tab. 1).

Table 1

Calorific power of samples from dry cutting of payments for fruit trees

Nr. crt.	Variety	**Calorific power** [MJ/g]	
		Upper	Lower
1	Idared 1a	18.10	16.56
2	Idared 2a	17.94	16.44
3	Generos 1a	18.25	16.75
4	Generos 2a	12.34	10.84
5	Jonagold 1a	18.02	16.52
6	Jonagold 2a	18.05	16.61
7	Williams R 1a	16.05	14.62
8	Williams R 2a	17.31	15.86
9	Cure 1a	18.38	16.92
10	Cure 2a	17.05	15.59
11	Rivan 1a	19.01	17.61
12	Rivan 2a	14.05	12.62
13	Van 1a	17.27	15.72
14	Van 2a	18.44	16.94
15	Stanley 1a	15.66	14.16
16	Tuleu 1a	18.71	17.23
17	Centenar 1a	15.12	13.55

The upper and lower heat power of the tree branches depends on the variety, having values between 10.84 MJ / kg for the lower heat and 19.01 MJ / kg for the higher heat.

When comparing the calorific value of the fruit tree branches with that of dry firewood, which is between 12.56 MJ / kg and 16.75 MJ / kg, it is found that the values are very close. This fact determines us to appreciate that the biomass resulting from the dry cutting of the fruit tree plantations is very valuable and can constitute an important energy potential for renewable energy resources.

CONCLUSIONS

If we compare the calorific value of the ropes of fruit trees with that of dry firewood, which is between 12.56 MJ / kg and 16.75 MJ / kg, the values are very close.

From the data obtained we observe that the calorific power is different from one especially the other.

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STUDY REGARDING THE USE OF MULBERRY LEAVES BY *BOMBYX MORI* - TRIUMF HYBRID

STUDIU PRIVIND UTILIZAREA FRUNZEI DE DUD DE CATRE HIBRIDUL DE *BOMBYX MORI* – TRIUMF

***DOLIŞ M.G.*[1], *RAŢU Roxana Nicoleta*[1*], *DINIŢĂ Georgeta*[2]**
[*]Corresponding author e-mail: *roxana.ratu@gmail.com*

Abstract. *In order to assess how efficient is the use of mulberry leaf by the Bombyx mori Triumf larvae hybrid, some determinations were made regarding the nutritional value and digestibility of the worm leaf administered as food (leaves from a Romanian variety, Eforie), during a series of summer growth. The results showed that ongoing vegetation and growth process of this hybrid, the mulberry leaves suffer an aging phenomenon, revealed by diminishing its chemical composition quality. According to this, most of the nutritional substances from mulberry leaves, except cellulose, manifest a continuous decline during the growth period. The digestibility of these nutritional components registered a value of 56.29%, the raw energy value was 4213 kcal/kg dry substance, the digestive energy was 2262 kcal/kg (DS), while the metabolic energy was 2098-2099 kcal/kg (DS). The efficiency of converting ingestion into silk had a value of 9.73% and the digestion was 17.27%.*
Key words: leaves, Mulberry, larvae, energy, use

Rezumat *În scopul aprecierii eficienței utilizării frunzei de dud de catre larvele hibridului de Bombyx mori Triumf, pe parcursul unei serii de creştere pe timp de vară, s-au făcut o serie de determinări legate valoarea nutritivă şi digestibilitatea frunzei de dud administrate ca hrană (frunză de la soiul românesc Eforie). Rezultatele au arătat ca odată cu înaintarea în vegetație şi implicit pe parcursul fiecărei perioade de creştere a larvelor de mătase, frunza de dud suferă un proces de îmbătrânire, tradus prin diminuarea calității acesteia din punct de vedere al compoziției chimice. În concordanță cu acest fapt, la majoritatea substanțelor nutritive din frunza de dud, cu excepția celulozei, s-a observat o scădere continuă a digestibilității de-a lungul perioadei de creştere. Digestibilitatea substanțelor nutritive din frunză, în ansamblu, a înregistrat o valoare de 56,29%. Valoarea energetică a frunzei a fost de 4213 Kcal/kg SU (EB), 2262 Kcal/kg SU (ED), 2098-2099 Kcal/kg SU (EM). Eficiența conversiei ingestei (ECI) în mătase de 9,73%, respectiv a digestei (ECI) de 17,27%.*
Cuvinte cheie: frunză, dud, larve, energie, valorificare

INTRODUCTION

Besides the continuous improvement of the growth technologies, one of the main concerns of the specialists in sericulture is represented by the production of

[1]University of Agricultural Sciences and Veterinary Medicine from Iaşi, Romania
[2]University of Agricultural Sciences and Veterinary Medicine of Bucharest, Romania

biological material of high genetic value as the *Bombyx mori* larvae with an increasing productive potential, more resistant to the environmental factors and to diseases and to use nutrients offered by the Mulberry to the best of their advantage.

Thus, from this point of view, the performances of the used larvae in intensive breeding systems have greatly increased, but at the same time, in order for them to be able to reach their full potential, it is necessary to improve all the factors involved in the breeding process. From the multitude of factors that directly influence the growth process of the larvae and the economic results obtained, it is encountered also nutrition.

The quantity and especially the quality of the worm leaf used in feeding of larvae, directly influence the growth rate, their health and vitality, but also the quantitative and qualitative production of silk. In turn, the quality of the leaf is also influenced by many factors related to the pedoclimatic conditions, season, variety of the mulberry, the way of harvesting and storage etc.

In the specific literature, depending on different factors, the relative humidity values of the Mulberry leaf vary between 65-75% (Doliş, 2008).

Compared with the common Mulberry (69.80-73%), the selected varieties have more water content (Bura *et al.,* 1995). The dried substance from the worm leaf, harvested in the same period, can record, depending on the variety/hybrid, different values, for example, between 23.61% and 27.56% (Matei, 1995).

Also, if the spring moisture of the mulberry leaf is 71.85-77.81%, then it decreases to 68.42-75.64%, in the summer period, respectively to 64.10-73.64%, in the fall (Ifrim, 1998).

Digestibility of the dry substance from the worm leaf decreases from 71.07% in age I, to 39.99% (for male larvae), 48.26% (for female larvae) in age V (Rath et al., 2003). The worm leaf administered to the larvae of the fifth age has an approximate digestibility between 27.99% and 32.44% (Rahmathulla *et al.,* 2002).

The raw leaf protein is estimated to have an average value of 6.16% in the fresh leaf, 20.97% in the dry substance and 24.36% in its organic substance (Doliş, 2008). The raw leaf protein values can vary depending on the season, the time of day, the variety/hybrid of the dude: 32.40% in spring, 28.21% in summer and 24.53% in autumn (Borcescu, 1966), 26.80% in the morning and 29, 10% in the evening (Mărghitaş, 1995), between 22.55% and 25.73% depending on the variety (Matei, 1995). In the specialty literature, for raw leaf protein, the value of digestibility coefficients is between 69.21% and 78.92 (Borcescu, 1966), 60.06% and 74.69% (Petkov, 1980), 71.62% and 93.48% (Matei, 1995).

The limits presented by specific literature regarding the fat content in mulberry leaves are 2.85- 6.07% (Pop, 1967). The values of the digestibility coefficient for raw fat are between 63.28% and 74.19% (Petkov, 1980).

According to the data from the specialized literature, in the common Mulberry the weight of the raw cellulose ranges between 12.33-14.38%, while in the different varieties selected oscillates between 10.43-13.70% (Craiciu, 1966). In

the vegetation period of the mulberry the content in raw cellulose from the leaves increases from 14.47% to 21.16% (Pop, 1967). Increased cellulose content causes aging of the worm leaf, which becomes harder and harsher, therefore harder to consume by, which is why those varieties whose leaves have less cellulose content are considered more valuable. At the beginning of the last century, some authors (Acqua, 1930 – cited by Doliş, 2008) found that the leaf cellulose passes undigested through the digestive tract of the larvae and later it was concluded that this substance has a digestibility of approx. 20% (Legay, 1955 - cited by Doliş, 2008). Recently, some authors state that in the first two ages, raw cellulose would not be digested, but only from the third (8%), its digestibility reaches 21.13% in the third period (Matei, 1995).

The values regarding the mineral substances, offered by the specialized literature, ranges between: 9.13-17.38% (Pop, 1967), 11.52-12.80% (Matei, 1995) and 8.7-13.15% (Bura *et al*., 1995).

At the end of the last century, Romania could be considered an important point on the map of European sericulture. Thus, in her record, Romania can boast in this field with a quite complex literature, as well as with the creation of new varieties and valuable hybrids of worm, as *Bombyx mori*, all being the result of some decade research work of Romanian specialists (Doliş, 2008; Lazăr and Vornicu, 2013; Pătruică, 2013).

For this reason, we consider appropriate to bring a modest contribution to the study of using the mulberry leaf, derived from indigenous varieties, by larvae of breeds or hybrids created in Romania.

MATERIAL AND METHOD

The biological material used in the experiments was represented by a batch of 150 larvae of *Bombyx mori* from Romanian simple hybrid *Triumf*. To be easier to follow, the group was devided into three sub-lots (repetitions) of 50 larvae each, which were raised in paper trays sized according to the age and size of the larvae; in addition, it was also made up a separate lot, with 50 larvae reared separately, but under the same conditions, which served to replace the dead larvae from the experimental group.

The growth of the larvae was in August, in an air-conditioned room, in compliance with all the microclimate factors. Each divided group received the same amount of leaf, from the same variety of worm, *Eforie,* from where samples were previously collected, for chemical analysis.

The Romanian variety of mulberry *Eforie*, which is characterised by a high production capacity, an early budding and a high resistance to freezing and drought. It was selected from a local population from Dobrogea in 1955 and introduced into production in 1970.

Daily and at the same time, from each group were collected, weighed and recorded what was not consumed from the Mulberry leaves and what was excreted by the larvae.

The quantities of residues, respectively of excrements, obtained from each group were summed, the result being divided into three, thus obtaining the average quantity of residues from each 50 larvae. The values obtained were subsequently

used in the calculation relationships to find the digestibility coefficients. Also, from each group were collected samples of excrements, which were mixed in order to obtain medium samples for analyze.

Also, the groups were weighed at the beginning of growth (after hatching) and at the end (before budding), the difference between the two weights, divided by the number of larvae in each group, representing the increase in body mass accumulated by a larva.

From the separated lot were extracted 10 larvae, whose content was determined in dry matter; thus, multiplying the average dry substance content of larvae, calculated from the separated lots, with the increasing body mass of the larvae in the experimental lots, it was determined the average increasing of body mass of a larva.

After gobbling, 15 cocoons were harvested, from which the silk wrapper was separated, weighed and its dry matter content determined, thus obtaining the average dry wool content of the silk wrapper.

The working methods used were mainly the specific ones used to determine the nutritional value of the worm leaf and they were based on the chemical composition (the "proximate analysis" scheme), the digestibility of its components (the "in vivo" method - simple digestibility, with a single control period) and raw energy (use of specific computation equations and regression coefficients recommended by the OKIT system), digestible (calculation equation recommended for monogastric species) and metabolizable (equations recommended for monogastric animals and birds) contained (Halga *et al.,* 2005).

The efficiency of the use of nutrients in the worm leaf by the larvae was expressed by the amount of ingested/digested dry matter required for increasing 1 gram of body mass/weight (silk wrap), respectively by the efficiency of conversion of ingested substances (ECI%) / digested (ECD%) in body mass/weight (Matei, 1995; Rahmathulla *et al.,* 2002; Sarkar, 1993).

RESULTS AND DISCUSSIONS

Table 1 shows the data of the chemical composition evolution of larvae in relation to their age.

The average values obtained for each nutrient separately are set in the limits presented by specific literature, where the data regarding the crude chemical composition of the mulberry leaves varies according to each author, to the research period, to the varieties of mulberry, etc. The average relative humidity of the mulberry leaves during the research was 70.44%, and a decreasing evolution being registered average values between 71.86% (at the first determination corresponding to the first age of the silkworm larvae) and 68.15% (to the last determination when the silkworm larvae are in the age V-th). The dry matter represented 29.56 ± 0.725%.

The crude protein had an average value of 6.18% (20.98±0.670% from DM). It is noticed a progressive decreasing of the protein content throughout the studied period, the content decreasing being with 3.11 percentage points, from 22.14% to 19.03%, respectively (tab. 1).

Table 1

The chemical composition evolution of the Eforie variety mulberry tree leaves during the silkworm larvae growth (%)

Ages	Water	DM	CP		EE		CF		NFE		Ash	
			F*	DM**	F*	DM**	F*	DM**	F*	DM**	F*	DM**
I	71.86	28.14	6.23	22.14	0.85	3.02	4.79	17.02	12.43	44.17	3.84	13.65
II	71.98	28.02	6.21	22.16	0.88	3.14	4.76	16.99	12.24	43.68	3.93	14.03
III	70.68	29.32	6.41	21.86	1.17	3.99	5.26	17.94	12.30	41.95	4.18	14.26
IV	69.53	30.47	6.00	19.69	1.22	4.00	5.58	18.31	13.37	43.89	4.30	14.11
V	68.15	31.85	6.06	19.03	1.38	4.33	6.15	19.31	13.58	42.64	4.68	14.69
$\overline{X}$	70.44	29.56	6.18	20.98	1.10	3.70	5.31	17.88	12.78	43.29	4.19	14.15
$S_{\overline{x}}$	-	0.725	-	0.670	-	0.260	-	0.434	-	0.418	-	0.169
Cv%	-	5.486	-	7.143	-	15.700	-	5.412	-	2.163	-	2.667

* fresh leaves; ** dry matter

The fat content from the mulberry leaves was in average 1.10% in the fresh leaves, and 3.70%±0.260 in DM. It is the only nutrient with a high variability, of 15.70%. The fat content increased uniformly throughout the silkworm larval growth, from 0.85% to 1.38% when it was expressed in fresh leaves, or 3.2% to 4.33% respectively, when it was reported to the dry matter.

The crude cellulose was in average 5.31% in fresh leaves, 17.91±0.434%, respectively when in was reported to DM. Throughout the research, for a month, the crude cellulose increased with 2.29 percentage points, from 17.02% to 19.31%, respectively.

Nitrogen free extract represented in average 43.27±0.418% from the dry matter of the mulberry leaves; the average values decreased from the first determination to the third, from 44.17% to 41.95%, then was an increasing to the fourth determination, being 43.89%, decreasing to the last analyses to 42.64%.

The ash represented in average 4.19% in the fresh leaves and 14.15±0.169% from dry matter. The minerals from the mulberry leaves throughout the research registered a continuous increase from analyse to another. The average values varied from 3.84% to 4.68% to fresh leaves and from 13.65% to 14.69% from dry matter. An exception was registered to the third determination which had a higher value than the fourth one. The increasing in mineral content from mulberry leaves throughout the research was 1.04%.

Knowing the raw chemical composition of the mulberry leaf, using the specific calculation equations, it was possible to assess the nutritional value of the mulberry leaf based on its content of raw energy, which was, on average, over the entire studied period, of 1245 Kcal/kg, in fresh leaf, respectively 4213 Kcal/kg, in the dry matter (tab. 2).

By recording the quantities of the worm leaf administered, the non-consumed and excreted residues and also determining their chemical composition (tab. 3), its digestibility coefficients could subsequently be calculated (tab. 4) and also the content of digestible substances in the leaf (tab. 5).

Table 2

Raw average energy of Mulberry leaf

Specification	%		Caloric equivalent	Kcal/100g		Kcal/100g	
	*	**		*	**	*	**
CP	6.18	20.98	5.72	35.35	120.01	353.5	1200.1
EE	1.10	3.70	9.50	10.45	35.15	104.5	351.5
CF	5.31	17.88	4.79	25.43	85.65	254.3	856.5
NEF	12.78	43.29	4.17	53.29	180.52	532.9	1805.2
						1245	**4213**

Following the complex phenomenon of digestion, nutrients are transformed into simple substances, which can thus be absorbed through the epithelium of the digestive tract, at different levels, thus being retained in the organism of silk larvae, representing practically the difference between the amount of substances ingested through food and the amount of appropriate substances found in droppings. Because not all the substances found in excrement are of dietary origin, some of them are of endogenous origin, which can be obtained by this difference, indicating only apparent digestibility. If you admit the fact that at Bombyx mori excretions are also found in their excrement, which complicates the establishment of the digestibility of nutrients in the wormwood even more accurately, the use of the approximate digestibility term seems to be more correct (Miranda and Takahashi, 1998; Rahmathulla *et al.,* 2004; Rath *et al.,* 2003; Sabhat *et al.,* 2011; Tzenov, 1993).

Table 3

Data needed to calculate digestibility coefficients

The larvae age	Specification	Quantity (g)	Chemical composition (%)					
			DM	CP	EE	CF	NEF	Ash
I	Leaves	15.5	28.14	6.23	0.85	4.79	12.43	3.84
	Leftovers	5.3	63.61	15.01	1.68	13.92	24.01	8.99
	Excreta	0.14	69.82	14.33	15.02	3.35	27.99	9.13
II	Leaves	26	28.02	6.21	0.88	4.76	12.24	3.93
	Leftovers	9.01	59.16	13.01	2.01	13.51	22.61	8.02
	Excreta	0.81	63.22	11.01	4.01	2.43	29.78	15.99
III	Leaves	77	29.32	6.41	1.17	5.26	12.3	4.18
	Leftovers	24.01	58.81	12.33	2.36	15.77	24.02	4.33
	Excreta	3.87	64.53	16.29	2.01	6.09	28.02	12.12
IV	Leaves	242	30.47	6.00	1.22	5.58	13.37	4.30
	Leftovers	65.15	58.06	12.06	1.72	15.71	24.66	3.91
	Excreta	22.02	64.06	11.01	2.66	12.04	27.29	11.06
V	Leaves	1000	31.85	6.06	1.38	6.15	13.58	4.68
	Leftovers	264.12	58.56	11.06	2.61	11.99	24.88	8.02
	Excreta	119.98	62.58	10.38	3.01	16.06	24.02	9.11

During the whole period studied, the digestibility of the dried substance from the worm leaf had a digestibility of 56.29%. The highest digestibility was

recorded in larvae of age I (90.14%), after which, by the end of the larval period, there was a decrease of 35.97 percent.

The raw protein had a digestibility coefficient for the entire studied period of 61.57%. The raw protein digestibility decreased progressively during the studied period, with 27.87%, respectively from 88.19%, in the first larval age, to 60.32%, in the last one. The high digestibility of age I could be explained by the rich content in amides, simple nitrogenous substances, which are found in the young leaf and which are digested much easier than the protein nitrogenous substances, which have the weight in the old leaf.

Table 4

Digestibility coefficients of *Triumf* hybrid

The larvae age	Specification	DM	CP	EE	CF	NEF
I	Leaves	4.3617	0.9657	0.1318	0.7425	1.9267
	Leftovers	3.3713	0.7955	0.089	0.7378	1.2725
	Ingest	0.9904	0.1702	0.0428	0.0047	0.6542
	Excreta	0.0977	0.0201	0.021	0.0047	0.0392
	Digest	0.8927	0.1501	0.0218	0.0000	0.6150
	DC%	90.14	88.19	50.93	0.00	94.01
II	Leaves	7.2852	1.6146	0.2288	1.2376	3.1824
	Leftovers	5.3303	1.1722	0.1811	1.2173	2.0372
	Ingest	1.9549	0.4424	0.0477	0.0203	1.1452
	Excreta	0.5121	0.0892	0.0325	0.0197	0.2412
	Digest	1.4428	0.3532	0.0152	0.0006	0.9040
	DC%	73.80	79.84	31.87	2.96	78.94
III	Leaves	22.5764	4.9357	0.9009	4.0502	9.471
	Leftovers	14.1203	2.9604	0.5666	3.7864	5.7672
	Ingest	8.4561	1.9753	0.3343	0.2638	3.7038
	Excreta	2.4973	0.6304	0.0778	0.2357	1.0844
	Digest	5.9588	1.3449	0.2565	0.0281	2.6194
	DC%	70.47	68.09	76.73	10.65	70.72
IV	Leaves	73.7374	14.52	2.9524	13.5036	32.3554
	Leftovers	37.8261	7.8571	1.1206	10.2351	16.066
	Ingest	35.9113	6.6629	1.8318	3.2685	16.2894
	Excreta	14.106	2.4244	0.5857	2.6512	6.0093
	Digest	21.8053	4.2385	1.2461	0.6173	10.2801
	DC%	60.72	63.61	68.03	18.89	63.11
V	Leaves	318.5000	60.6000	13.8000	61.5000	135.8000
	Leftovers	154.6687	29.2117	6.8935	31.6680	65.7131
	Ingest	163.8313	31.3883	6.9065	29.8320	70.0869
	Excreta	75.0835	12.4539	3.6114	19.2688	28.8192
	Digest	88.7478	18.9344	3.2951	10.5632	41.2677
	DC%	54.17	60.32	47.71	35.41	58.88
I-V	Leaves	426.4607	82.6360	18.0139	81.0339	182.7355
	Leftovers	215.3167	41.9969	8.8508	47.6446	90.8560
	Ingest	211.1440	40.6391	9.1631	33.3893	91.8795
	Excreta	92.2966	15.6180	4.3284	22.1801	36.1933
	Digest	118.8474	25.0211	4.8347	11.2092	55.6862
	DC%	56.29	61.57	52.76	33.57	60.61

The raw fat from the worm leaf had the minimum digestibility value of 31.87%, in the larvae of the second age and maximum of 76.73%, in the larvae of the third age. The results of the digestibility tests regarding the raw fat in the worm leaf are generally inconclusive, as many of these can come from the intestine of the larvae and not from the leaf, which is why, we cannot speak of a determination of the digestibility of the fat itself but of the "ethereal extract", which also contains very large quantities of pigments. Thus, the big differences regarding the evolution of the digestibility of the raw fat during the studied period could be explained.

During the whole larval period, the digestibility of the raw cellulose from the mulberry leaf was 33.57%, being null in age I, after which it increased progressively, reaching the end of the period studied up to the value of 35.41%. This increase in the digestibility of raw cellulose, as the larvae grow older, is in line with the development of the enzymatic equipment in their digestive tract. Thus, if at age I, in the digestive tract of the larvae, the enzymes involved in the process of cellulose digestion are as non-existent, then they gradually increase, reaching the peak at age V, at which point the weight of raw cellulose from the worm leaf it is also bigger. This aspect, however, negatively influences the digestibility of the raw leaf protein, which during the same period, is experiencing a reduction.

Unclaimed extractive substances from the worm leaf had a digestibility over the entire studied period of 60.61%, the digestibility coefficients registering decreasing values, from 94.01%, in the case of the larvae of age I, at 58.88%, in the case of those of fifth age.

Knowing the value of digestibility coefficients, it was possible to calculate the digestible content for each nutrient separately, then the content of digestible substances in the leaf, so when the report was made to the fresh leaf, 146.39 g of Total Digestive Substance/kg were obtained, and when the report was made on the dried substance from the leaf of the mulberry, its nutritional value was 495.50 g TDS/kg (tab. 5).

The determination of the digestible energy content of the worm leaf administered in the feed of silk larvae was made based on the relative digestible content of the nutrients contained in it, using the calorific equivalents recommended for monogastric animal species (tab. 6). In the case of the fresh leaf, the digestible energy content was 668.60 Kcal/kg, and in the case of the dry substance, 2262.43 Kcal/kg.

The calculation of the metabolic energy from the worm leaf administered in the feed of silk larvae was done by multiplying the digestible content of each nutrient with the energy equivalents recommended for monogastric (pig) animal species. Considering, however, the specificity of the silkworm's digestion, respectively the similarity with the digestion of the birds, for the estimation of the metabolic energy from the worm leaf, the energetic equivalents recommended for the birds were used (tab. 7).

Table 5

The nutritional value calculation of the mulberry leaves (g TDN/kg)

Specification	Raw chemical composition %		Digestibility coefficients	Digestive content %		g Total Digestive substance /kg	
	*	**		*	**	*	**
CP	6.18	20.98	61.57	3.81	12.92	38.05	129.17
EE	1.1	3.7	52.76	0.58	1.95	13.06	43.92
CF	5.31	17.88	33.57	1.78	6.00	17.83	60.02
NEF	12.78	43.29	60.61	7.75	26.24	77.46	262.38
Total						**146.39**	**495.50**

* Reported to the fresh leaves; ** reported to DM

Table 6

Digestive energy of Mulberry leaf

Specification	Digestive content %		Caloric equivalent (Kcal/g)	Kcal/kg	
	*	**		*	**
CP	3.81	12.92	5.78	220.22	746.78
EE	0.58	1.95	9.42	54.64	183.69
CF	1.78	6.00	4.40	78.32	264.00
NEF	7.75	26.24	4.07	315.43	1067.97
Total				**668.60**	**2262.43**

* Reported to the fresh leaves; ** reported to DM

Table 7

Metabolic energy of Mulberry leaf

Specification	Digestive content %		Caloric equivalent (Kcal/g)		Kcal/kg			
					*		**	
	*	**	swine	birds	swine	birds	swine	birds
CP	3.81	12.92	5.01	4.26	190.88	162.31	647.29	550.39
EE	0.58	1.95	8.93	9.50	51.79	55.10	174.14	185.25
CF	1.78	6	3.44	4.23	61.23	75.29	206.40	253.80
NEF	7.75	26.24	4.08	4.23	316.20	327.83	1070.59	1109.95
					620.11	**620.53**	**2098.42**	**2099.39**

* Reported to the fresh leaves; ** reported to DM

The average content in metabolic energy from the fresh mulberry leaf was 620.11 Kcal/kg, when the recommended energy ratios for pigs were used, respectively 620.53 Kcal/kg, when the recommended coefficients for birds were used. In relation to the dry matter of the leaf, the content in metabolic energy was on average 2098.42 Kcal/kg, when the recommended energy coefficients for pigs were used, and 2099.39 Kcal/kg, when the recommended coefficients for birds were used.

In order to determine the efficiency of use of the nutrients in worm leaf by the silk larvae, except for the intake and digestion, which were calculated during the course of the digestibility tests, it was necessary to determine the average growth rate of the larvae and the mass of the silk shell. The data necessary for calculating the efficiency of the use of the worm leaf by the larvae, as well as the results obtained in this respect, were centralized in table 8.

Table 8

Efficiency of using Mulberry leaf by *Bombix mori* Triumf larvae hybrid

Average body mass gained during the whole larvae stage (g)	**Living larvae**	5.429
	Dry matter	0.922
Silky shell mass (g Dry Matter)		0.411
Dry Matter of ingested leaf (g)		4.223
Dry Matter of digested leaf (g)		2.380
Ingested Dry Matter/Body mass Dry Matter (g)		4.579
Dry matter ingested/ Body mass Dry Matter (g)		2.580
Dry matter ingested/Silky shell Dry Matter (g)		10.275
Dry matter digested/ Silky shell Dry Matter (g)		5.790
CEI body mass %		21.84
CED body mass %		38.75
CEI silky shell %		9.73
CED silky shell %		17.27

From the data of this table it is observed that in the case of the *Bombyx mori* Triumf larvae hybrid, for every gram of silk wrap is required 10.28 grams of dry matter ingested from the wormwood, respectively 5.79 grams of digested dry matter, resulting in an efficiency of conversion of silk intake (CEI) of 9.73%, respectively of digestion (CED) of 17.27%.

The data obtained from the experience performed, regarding the efficiency of the use of the mulberry leaf by the larvae of Bombyx mori, are comparable with those presented in the literature (Matei, 1995; Rahmathulla *et al*., 2002; Rath *et al.*, 2003; Sarkar., 1993; Tzenov, 1993).

CONCLUSIONS

- Expressed to dry matter from the mulberry leaves, Eforie variety the average values were: CP - 20.98 ± 0.670%, EE - 3.70 ± 0.260%, CF- 17.91 ± 0.4.34%, NEF - 43.27 ± 0.418% and ash - 14.15 ± 0.260%.

- At once with vegetation advancement and implicitly during each growth period of silkworm larvae, the mulberry leaf ages and its quality from the chemical composition point of view is decreasing.

- During the 30 days of the research, was noticed a decreasing of the moisture with 3.71% and of the CP with 3.11% and in the same time an increasing of the CF with 2.29%.

- During the whole period studied, the digestibility of the dried substance from the worm leaf had a digestibility of 56.29%. The dry matter digestibility decreased with 35.97%.

- Digestibility coefficients of the CP (61.57%) and of the NFE (60.61%) from the mulberry leaves decreased during the study with 27.87%, and 35.13%, respectively.

The CF digestibility, null at the beginning, increased progressively till the fifth larval stage when it was 35,41%.

Nutritional value of the mulberry leaves was 496 g TDN/ kg DM.

Throughout the studied period, the gross enrichment of the worm leaf was on average 4213 Kcal/kg, in the dry substance

In the leaf, the content of digestible energy was, in the case of dry matter, 2262 Kcal/kg.

In relation to the dry matter of the leaf, the content in metabolic energy was on average 2098-2099 Kcal/kg.

In the case of the Triumf hybrid, for each gram of silk wrap, 10.28 grams of dry matter ingested from the mulberry tree are required, respectively 5.79 grams of digested dry substance, resulting an efficiency of conversion of ingestion (CEI) into silk of 9.73%, respectively of the digestion (CEI) of 17.27%

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INFLUENCE OF SOME FACTORS ON MULTIPLICATION OF ORNAMENTAL SPECIES *PASSIFLORA COERULEA* AND *PASSIFLORA QUADRANQULARIS*

INFLUENŢA UNOR FACTORI ASUPRA ÎNMULŢIRII SPECIILOR ORNAMENTALE *PASSIFLORA COERULEA* ŞI *PASSIFLORA QUADRANQULARIS*

***CHELARIU Elena Liliana*[1], *DRAGHIA Lucia*[1], *APOSTOL Maria*[1]*, *MANACHE Elena Diana*[1], *AMIŞCULESEI Petronica*[1]**
*Corresponding author e-mail: mariabrinza2007@yahoo.com

Abstract. *In the current paper are presented the results of a research regarding the influence of cuttings' type, substrate and treatments on cuttings rooting at floral species Passiflora coerulea and Passiflora quadranqularis. Research was carried out in the didactical greenhouse of Floriculture discipline from USAMV Iaşi, experiments being organized in 8 variants. During research was observed the influence of cuttings type, substrate and treatments with bio-stimulators on rooting capacity of cuttings. At the end of research was noticed that at those two studied species rooting of cuttings had good results if are treated with a rooting stimulator and placed into a perlite substrate for rooting. The type of manufactured cuttings, it influences to a lesser extent the rooting ability.*
Key words: *Passiflora coerulea, Passiflora quadranqularis*, multiplication by cuttings

Rezumat. *În această lucrare sunt prezentate rezultatele cercetării privind influenţa tipului de butaş, a substratului şi a tratamentelor asupra înrădăcinării butaşilor la speciile floricole Passiflora coerulea şi Passiflora quadranqularis. Cercetările s-au desfăşurat în sera didactică a disciplinei de Floricultură din cadrul USAMV Iaşi, experienţele fiind organizate în 8 variante experimentale. Pe parcursul cercetărilor s-a urmărit influenţa tipului de butaş, a substratului şi a tratamentelor cu biostimulatori asupra capacităţii de înrădăcinare a butaşilor. În urma cercetărilor s-a constat că la cele două specii luate în studiu înrădăcinarea butaşilor se face cu rezultate bune dacă sunt trataţi cu un stimulator de înrădăcinare şi aşezaţi la înrădăcinat în substrat de perlit. Tipul de butaş confecţionat, influenţează într-o mai mică măsură capacitatea de înrădăcinare.*
Cuvinte cheie: *Passiflora coerulea, Passiflora quadranqularis,* înmulţirea prin butaşi

INTRODUCTION

Passiflora genus belongs to Passifloraceae family and include species native from tropical areas (South America, Australia and Asia) (Patil, 2013; Boboc *et al.,* 2017). Ornamentally speaking the most well known are species *Passiflora coerulea* L. and *Passiflora quadranqularis* L.

[1]University of Agricultural Sciences and Veterinary Medicine from Iaşi, Romania

Passiflora coerulea L. is a species of ornamental liana, with climbing port, clinging. Leaves are trefoil, having a dark green colour, with an alternate disposal. In axil, leave present tendrils with the help of which clings on supports. Flowers are big with a diameter of 10-12 cm, having a pink-violet colour. Sepals are, juicy, with a pink-violet colour on internal side, having a length of 3-4 cm. Petals are threadlike and equal as dimensions. It presents numerous and very big stamina. Fruit is fleshy, and seeds are brownish blackish (Draghia and Chelariu, 2011; Chelariu, 2015; Cordoba, 1980; Nagy *et al.,* 1990).

Passiflora quadranqularis L. presents thick, edged and smooth stems, with an intense green colour. Plants have tendrils situated in leaf's axil and have a length of 22.5-35 cm and a thickness of 0.15-0.2 cm. The leave have the limb with an oval or elliptical shape, with entire edge, petiolate, with a length of 10-20 cm and a width of 8-15 cm. Those ones are alternate disposed on edged sprouts. On superior side leaves have a dark green colour, and on inferior side the colour is light green (Draghia and Chelariu, 2011; Geilfus, 1994; Nagy *et al.,* 1990). Flowers have around 12 cm in diameter; the colour could be violet, pink, white or blue. Sepals are ovate and have a length up to 3.5 cm, are fleshy, have a green colour at exterior, and pink or white on the interior side. Petals have an oblong-ovate shape till an oblong-lancelet shape and are equal as dimensions. Stamina is numerous and very big, has the length bigger than petals, have an undulated peak and present alternative transversal stripes, with a violet with white colour. Ovary is elliptical, with a yellowish colour, opaque. Stigmas at the beginning of flowering have a yellow colour and after that became light brown (Avilán *et al*., 1989; Draghia and Chelariu, 2011). Fruit is fleshy, having a length of 10-25 cm and a diameter of 8-10 cm (Cordoba, 1980), weighting 225-450 g or more, mesocarp or pulp have a white colour, reaching 2.5 cm up to 4 cm in diameter, is a juicy fruit with a not so pleasant taste (Nagy *et al.,* 1990).

Multiplication of passiflora species is realised on generative way as well as on vegetative way (Cordoba, 1980; Nagy *et al.,* 1990; Hartmann and Kester, 1997; Draghia and Chelariu, 2011; Chelariu, 2015).

The current paper present the results regarding the influence of some factors on multiplication by cuttings at two ornamental species belonging to genus *Passiflora*, cultivated in pots, in the conditions of didactical greenhouse belonging to Floriculture discipline, from Faculty of Horticulture, Iaşi, Romania.

MATERIAL AND METHOD

Research was carried out into the didactical green house belonging to Floriculture discipline, Faculty of Horticulture, USAMV Iaşi, Romania, during February 2018 – Mai 2019. Study material was represented by two species of ornamental plants, which in conditions of continental temperate climate are cultivated as plants in pots: *Passiflora coerulea* L. 'Amethyst' kind and *Passiflora quadranqularis* L.

For each species was organized three factorial experiences, where the aimed factors were: rooting substrate type, stimulating substance and type of realized cutting.

By combination of factors were obtained, at each species, the following variants: V1 – water + peak cuttings; V2 – water + section cuttings; V3 – perlite + peak cuttings; V4 – perlite + section cuttings; V5 – perlite + peak cuttings + 1 treatment applied at making cuttings; V6 – perlite + section cuttings + 1 treatment applied at making cuttings; V7 – perlite + peak cuttings + 1 treatment applied at making cuttings and another three at a 10 days difference; V8 – perlite + section cuttings + 1 treatment applied at making cuttings and another three at a 10 days difference (tab. 1).

Table 1

Experimental design

Variant	Substrate	Treatment for cuttings	Cuttings type	Cuttings number	Date of realization
V1	water	untreated	sprout peak	10	26.02.2018
V2	water	untreated	sprout section	10	26.02.2018
V3	perlite	untreated	sprout peak	10	26.02.2018
V4	perlite	untreated	sprout section	10	26.02.2018
V5	perlite	1 treatment with Cropmax	sprout peak	10	26.02.2018
V6	perlite	1 treatment with Cropmax	sprout section	10	26.02.2018
V7	perlite	4 treatments with Cropmax	sprout peak	10	26.02.2018
V8	perlite	4 treatments with Cropmax	sprout section	10	26.02.2018

For each experimental variant was utilised biological material (cuttings) as uniform as possible regarding size, respectively development degree of the utilised part of plant. Substrate was utilised water and perlite, and as bio-stimulator Cropmax.

Was aiming to determine the summed influence of substrate, cutting type and bio-stimulator, on cuttings' rooting at *Passiflora coerulea* L. and *Passiflora quadranqularis* L, as well as the determination of unilateral influence of substratem cutting type and bio-stimulator on cuttings' rooting.

The obtained results were centralized in graphs and tables, and statistical interpretation was made by using the limit differences.

RESULTS AND DISCUSSIONS

Analyzing the obtained results we noticed that at first observations, after 10 days from establishing the experience, at *Passiflora coerulea* 'Amethyst' kind rooting started after about 20 days from manufacturing, at cuttings planted in perlite (V3-V8) and after 30 days at variants at which the rooting substrate was water (V1 and V2). At *Passiflora quadranqularis* rooting started after around 10 days at variants with perlite (V3-V8) and after 20 days at the ones with water (V1-V2) (tab. 2).

At the last observations, at *Passiflora coerulea* 'Amethyst' rooting rate varied from 30% to 90%, and at *Passiflora quadranqularis* between 50% and

100%. At the last ones, for variants V7 and V8, rooting finished earlier than at others (tab. 2). At both species the best results were recorded at section cuttings, rooted on perlite substrate and treated with Cropmax (tab. 2, fig. 1).

Table 2

Dynamics of cuttings rooting (%)

Variant	Number of rooted cuttings (after … days)									
	***Passiflora coerulea* 'Amethyst'**					***Passiflora quadrangularis***				
	10	**20**	**30**	**40**	**50**	**10**	**20**	**30**	**40**	**50**
V1	-	-	10	20	30	-	10	30	40	50
V2	-	-	10	30	40	-	20	40	50	60
V3	-	10	20	40	50	20	30	50	70	80
V4	-	10	30	50	60	20	40	60	70	90
V5	-	30	50	60	70	30	50	70	90	100
V6	-	40	50	60	70	40	50	70	90	100
V7	-	30	40	60	80	40	60	80	100	-
V8	-	40	50	70	90	40	60	90	100	-

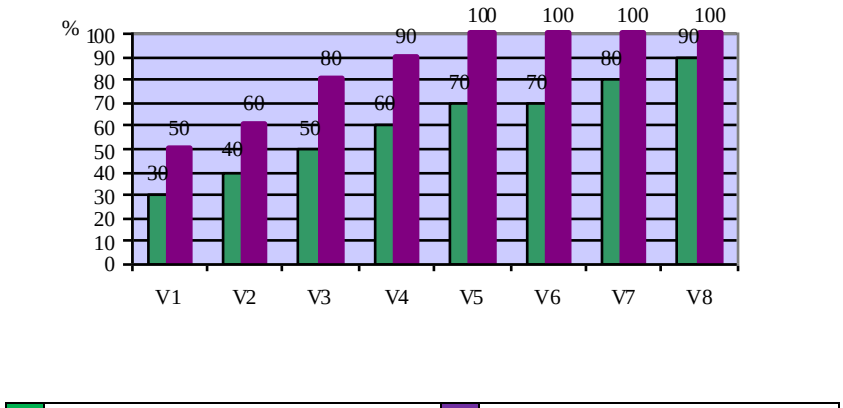

Fig. 1 Rate of cuttings rooting

Statisticallty speaking the influence of interaction, substrate, cutting type and bio-stimulator on rate of rooting for cuttings of *Passiflora* was observed that at *P. coerulea* 'Amethyst' differences face to variants' mean were very significant positive at V8, significant positive at V7. At V1 differences were very significant negative, and at V2 were significant distinct negative. At variants V3-V6, differences face to mean were insignificant (tab. 3). At *Passiflora quadranqularis* differences face to mean were significant positive at variants V5-V8, very significant negative at V1 and V2; at V3 and V4, differences were insignificant (tab. 3).

Table 3

Influence of substrate, cutting type and bio-stimulator influence on cuttings rooting rate

Variant	*Passiflora coerulea* 'Amethyst'			*Passiflora quadranqularis*		
	Rooted cuttings (%)	% face to mean	Difference (±d)	Rooted cuttings (%)	% face to mean	Difference (±d)
V1	30^{000}	48.94	-31.3	50^{000}	58.82	-35.00
V2	40^{00}	65.25	-21.3	60^{000}	70.59	-25.00
V3	50^{ns}	81.57	-11.3	80^{ns}	94.12	-5.00
V4	60^{ns}	97.88	-1.3	90^{ns}	105.88	+5.00
V5	70^{ns}	114.19	+8.7	100*	117.65	+15.00
V6	70^{ns}	114.19	+8.7	100*	117.65	+15.00
V7	80*	130.51	+18.7	100*	117.65	+15.00
V8	90***	146.82	+28.7	100*	117.65	+15.00
Mean	61.3	100.00	0.00	85	100.00	0.00
	LD 5% = 14.3 LD 1% = 19.8 LD 0.1% = 27.5			LD 5% = 11.3 LD 1% = 15.6 LD 0.1% = 21.7		

Statiscally analysing the unilateral influence of substrate on cuttings rooting was observed that perlite determine, face to variants mean, distinct positive significant differences at *P. coerulea* 'Amethyst' and very significant positive differences at *Passiflora quadranqularis*, while substrate represented by water determined distinct significant negative differences at *P. coerulea* 'Amethyst' and very significant negative differences at *Passiflora quadranqularis* (tab. 4).

Table 4

Influence of substrate on cuttings rooting rate

Variant	*Passiflora coerulea* 'Amethyst'			*Passiflora quadranqularis*		
	Rooted cuttings (%)	% face to mean	Difference (±d)	Rooted cuttings (%)	% face to mean	Difference (±d)
water	35.0^{00}	66.67	-17.5	55.0^{000}	73.33	-20.00
perlite	70.0**	133.33	+17.5	95.0***	126.67	+20.00
Mean	52.5	100.00	0.00	75	100.00	0.00
	LD 5% = 15.9 LD 1% = 25.0 LD 0.1% = 42.5			LD 5% = 6.6 LD 1% = 10.4 LD 0.1% = 17.7		

From statistical analysis of unilateral influence of bio-stimulator on cuttings rooting rate was noticed that at both *Passiflora* species, differences face to variants mean were distinct significant positive at treated variants, and distinct significant negative at untreated variants (tab. 5).

Table 5

Influence of bio-stimulator on cuttings rooting rate

Variant	*Passiflora coerulea* 'Amethyst'			*Passiflora quadranqularis*		
	Rooted cuttings (%)	% face to mean	Difference (±d)	Rooted cuttings (%)	% face to mean	Difference (±d)
untreated	45.0^{00}	73.41	-16,3	70.0^{00}	82.35	-15.00
treated	77.5**	126.43	+16,2	100**	117.65	+15.00
Mean	61.3	100.00	0,00	85.0	100.00	0.00
	LD 5% = 8.0 LD 1% = 14.6 LD 0.1% = 32.3			LD 5% = 29.0 LD 1% = 53.3 LD 0.1% = 118.1		

Regarding the unilateral influence of cutting type on its rooting was observed that the differences face to variants mean was insignificant at both species of *Passiflora* (tab. 6).

Table 6

Influence of cutting type of rooting rate of cuttings

Variant	*Passiflora coerulea* 'Amethyst'			*Passiflora quadranqularis*		
	Rooted cuttings (%)	% face to mean	Difference (±d)	Rooted cuttings (%)	% face to mean	Difference (±d)
Peak cuttings	57.5^{ns}	93.80	-3.8	82.5^{ns}	95.06	-2.50
Section cuttings	65.0^{ns}	106.04	+3.7	87.5^{ns}	102.94	+2.50
Mean	61.3	100.00	0.00	85	100.00	0.00
	LD 5% = 8.0 LD 1% = 14.6 LD 0.1% = 32.3			LD 5% = 9.2 LD 1% = 16.9 LD 0.1% = 37.4		

At species *Passiflora coerulea* 'Amethyst' kind, analysing the influence of those three studied factors, on mean length of roots and mean number of roots/cutting was observed that differences face to variants mean is very significant positive at variants V5-V8 and very significant negative at variants V1-V5 (tab. 7). Those results show that treatments with bio-stimulators determine formation of a rich and compact radicular system.

At *Passiflora quadranqularis*, influence of those three factors on mean length of roots was manifested by determination of some very significant positive differences at variants V6-V8 and significant positive at V5; very significant negative differences at V1-V3 and significant negative at V4. Regarding mean number of roots formed on cutting, differences face to variants mean were very significant positive at variants V5-V8, very significant negative differences at V3 and insignificant differences at V4 (tab. 8).

Table 7

Combined influence of substrate, cutting type and bio-stimulator on features of *Passiflora coerulea* 'Amethyst' cuttings

Variant	Mean length of roots (cm)		Mean number of roots (pieces)	
	main	secondary	main	secondary
V1	1.05^{000}	0.45	2.45^{000}	2.10
V2	1.42^{000}	0.56	2.65^{000}	2.34
V3	2.48^{000}	0.68	3.45^{000}	5.85
V4	2.78^{000}	0.85	3.85^{000}	5.97
V5	3.75***	0.98	5.15***	7.15
V6	4.12***	1.12	5.65***	7.85
V7	5.50***	1.24	6.55***	10.20
V8	5.75***	1.37	6.86***	10.85
Mean	3.36	0.91	4.58	6.54
	LD 5% = 0.1 LD 1% = 0.2 LD 0.1% = 0.2		LD 5% = 0.1 LD 1% = 0.2 LD 0.1% =0.3	

Table 8

Combined influence of substrate, cutting type and bio-stimulator on features of *Passiflora quadranqularis* cuttings

Variant	Mean length of roots (cm)		Mean number of roots (pieces)	
	main	secondary		main
V1	2.00^{000}	1.32	3.00^{000}	3.00
V2	2.74^{000}	1.40	3.25^{000}	5.14
V3	2.98^{000}	0.82	5.25^{00}	14.00
V4	3.82^{0}	0.95	5.80^{ns}	14.83
V5	4.92*	0.98	6.70***	15.35
V6	5.60***	1.24	6.90***	15.60
V7	5.96***	1.35	7.35***	16.40
V8	6.10***	1.45	7.64***	16.80
Media	4.27	1.89	5.74	12.64
	LD 5% = 0.4 LD 1% = 0.5 LD 0.1% = 0.7		LD 5% = 0.2 LD 1% = 0.2 LD 0.1% =0.3	

CONCLUSIONS

At *Passiflora coerulea* 'Amethyst' rooting rate varied from 30% to 90%, and at *Passiflora quadranqularis* between 50% and 100%. At both species better results were recorded at section cuttings, rooted on perlite substrate and treated with bio-stimulator.

At *Passiflora quadranqularis,* treatments with bio-stimulator determined a 100% rooting, and application of other four treatments determined a shortage of

cuttings rooting period.

At both studied species, cuttings formed a mass of well developed roots at variants treated with bio-stimulators. Rooting of cuttings is realised with good results if they are treated with a rooting bio-stimulator and is utilised perlite as rooting substrate. The manufactured cutting type, influence in a slightly manner the rooting.

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INFLUENCE OF SOME BIO-STIMULATORS ON OBTAINING OF SEEDLINGS AT *LAVANDULA ANGUSTIFOLIA*

INFLUENȚA UNOR BIOSTIMULATORI ASUPRA PRODUCERII RĂSADURILOR LA *LAVANDULA ANGUSTIFOLIA*

NEAGU Cristina Florina[1], CHELARIU Elena Liliana[1]*, APOSTOL Maria, BERNARDIS R., DRAGHIA Lucia
*Corresponding author e-mail: julia@uaiasi.ro

Abstract. *In the current paper are presented the results regarding influence of treatments with gibberellins GA3 and Razormin on germination of seeds at species Lavandula angustifolia. Research took place in glasshouse conditions in year 2018. Were made observations regarding dynamics of rising and were determined germination velocity and germination rate. The obtained results prove the fact that Lavandula angustifolia seeds have a favourable response to treatment with bio-stimulators.*
Key words: *Lavandula angustifolia*, seedlings, bio-stimulators

Rezumat. *În această lucrare sunt prezentate rezultatele privind influența tratamentelor cu giberelină GA3 şi Razormin asupra germinației semințelor la specia Lavandula angustifolia. Cercetările s-au făcut în condiții de seră în anul 2018. S-au făcut observații asupra dinamicii răsăririi şi s-a determinat velocitatea germinației şi procentul de germinație. Rezultatele obținute demonstrează faptul că semințele de Lavandula angustifolia răspund favorabil la tratamentul cu biostimulatori.*
Cuvinte cheie: *Lavandula angustifolia*, răsaduri, biostimulatori

INTRODUCTION

Lavandula angustifolia is a perennial ornamental species, sempervirens with a sub-shrub aspect, belonging to Lamiaceae family (Cantor, 2009; Draghia and Chelariu, 2015; Butnăraş, 2016; Lelescu, 2013). Lavender present a thin and woody stem, with a height of around 50 cm, leave are small having a grey-green colour, flowers could be white, blue with various nuances till dark-violet. Lavender plants, the "mauve gold" of agriculture, but especially flowers, have a specific, floral, pleasant pungent smell, with a fresh nuance of mint and lemon. Lavender's aroma is considered to be the fragrance of remembrance, the fragrance of romantics (Butnăraş, 2016; Lelescu, 2013).

Lavender's seeds germinate hardly because have a tough tegument and weak permeable. Even if germinate in conditions of a temperature of 12-15^{0}C and constant moisture, germination rate is low (Şerban, 2011).

Due to the fact that in the last years, in Romania, the interest for utilization

[1]University of Agricultural Sciences and Veterinary Medicine from Iaşi, Romania

of lavender increase constantly, as ornamental plant (in landscape designs, as cut flower in fresh state or dried) (Cantor, 2009; Buta and Cantor, 2015; Draghia and Chelariu, 2015), but especially for the obtained oils (Butnăraş, 2016; Lelescu, 2013), we aimed to study the influence of some bio-stimulators on germination of seeds.

MATERIAL AND METHOD

Research was carried out at Farm Neagu Cristina-Florina I.I. from Ţifeşti commune, Vrancea County, Romania, in year 2018.

Research material was represented by *Lavandula angustifolia* 'Sevtopolis' sort (fig. 1). This sort is well known for its adaptation capacity at Romanian climate and for its productive characteristics regarding flowers and essential oil.

Fig. 1 *Lavandula angustifolia* 'Sevtopolis' (original photo)

Were organised three experimental variants: untreated control (V1), seeds treated with gibberellins GA3 0.75g/L (V2) and seeds treated with bio-stimulator Razormin 2mL/L (V3). For each variant were used 100 seeds. At treated variants, before sowing, seeds were kept for 24 hours into a solution of GA3 0.75g/L or in Razormin 2mL/L. Sowing was realised in solarium, into a substrate formed by peat and garden soil, in equal rates**.**

During research were made observations regarding germination dynamics, germination rate and on seedlings morphology. Determinations were realised from 7 to 7 days, from sowing till the moment in which seeds ceased to germinate, after around 28-30 days. The obtained results were synthesised in tables and graphs and were statistically interpreted, using limit differences.

RESULTS AND DISCUSSIONS

Analysing germination dynamics could be observed that treatments with GA3 and Razormin triggered seeds germination with about two weeks earlier that at control variant, so at the first observations, after 7 days from sowing, germination rate was of 15 % at V2 and 8% at V3, and after 21 days, germination rate was between 54 and 66% at treated variants face to 29% at control variant. After 30 days from sowing, germination rate was 82% at variant with GA3 (V2),

76% at variant with Razormin (V3), and at control variant was 57% (tab. 1).

Table 1

Dynamics of emergence (%)

Variant	After … days			
	7 days	14 days	21 days	30 days
V1	-	-	29	57
V2	15	24	54	82
V3	8	31	66	76

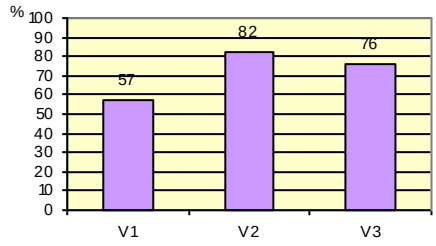

Fig. 2 Germination rate

Analysing from statistically point of view seeds germination rate was observed that treatments with bio-stimulators determined very significant positive differences face to untreated control (tab. 2).

Table 2

Influence of treatments with bio-stimulators on seeds germination

Variants	Nr. of emergent plants -pieces-	% face to mean	Difference	Signification
V1	57	100.0	0.0	control
V2	82	143.86	6.0	***
V3	76	133.33	19.0	***
LD 5%=5.7 pieces; LD 1%= 8.2 pieces; LD 0.1%=14.0 pieces				

Table 3

Growing dynamics of seedlings

Variant	After….days from emergence					
	14 days		21 days		30 days	
	Height -cm-	Nr. of leave -pieces-	Height -cm-	Nr. of leave -pieces-	Height -cm-	Nr. of leave -pieces-
V1	-	-	1.0	2.0	1.64	3.20
V2	1.8	2.60	3.26	5,07	6.17	6.7
V3	1.79	2.83	3.33	5.24	6.52	7.05

After 30 days from sowing seedlings were characterized by a height between 6.7 cm and 7.05 cm at treated variants, face to 3.20 cm at control variant (tab. 3).

From statistical analysis of the results regarding plants' height, as well as the number of leave/seedling was observed that differences face to control are very significant positive at variants with treated seeds (tab. 4, tab. 5).

Table 4

Results regarding height of seedlings

Variants	Height -cm-	% face to control	Difference	Signification
V1	1.64	100.0	0.0	control
V2	6.17	387.5	4.6	***
V3	6.52	406.25	4.9	***
LD 5%=0.1 cm; LD 1%=0.2 cm; LD 0.1%=0.3 cm				

Table 5

Results regarding number of leave at obtained seedlings

Variants	Nr. of leave -pieces-	% face to control	Difference	Signification
V1	3.20	100.0	0.0	control
V2	6.17	193.75	3.0	***
V3	7.05	221.88	3.9	***
LD 5%=0.1 pieces; LD 1%=0.2 pieces; LD 0.1%=0.4 pieces				

CONCLUSIONS

Treatments with GA3 and with Razormin, applied to seeds before sowing, determined the shortage of period till germination started.

Germination rate was 82% at seeds treated with GA3, 76 % at the ones treated with Razormin, face to 57% at control variant.

At variants with treated seeds were obtained net qualitative seedlings, face to control variant (well developed sprouts and rich foliage).

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CONSIDERATIONS ON THE DESIGN OF MICRO-IRRIGATION SYSTEMS IN HORTICULTURAL PLANTS

CONSIDERAȚII PRIVIND PROIECTAREA SISTEMELOR DE MICROIRIGARE LA PLANTAȚII HORTICOLE

LUCA M.[1]*, MARCOIE N.,[1] LUCA AL. L.[2], TĂMĂŞANU F.[2]
*Corresponding author e-mail: mluca2015@yahoo.com

Abstract*. The paper presents an analysis of how to make drip irrigation systems in the plantations of shrubs and fruit trees located in the Plain and the Plateau of Moldavia. Over the last 20 years, drip irrigation systems have been designed for horticultural plantings on relatively small (4-8 ha) areas of a farm. The research shows some shortcomings in the design concept of the irrigation system, starting with the layout scheme, the selection of the watering equipment and the calculation of the operating parameters. The analysis shows that some of the irrigation systems are not carried out according to the characteristics of the wetted horticulture, the pedoclimatic parameters of the soil, the volume of water available at source, etc.*
Key words: shrubs, fruit trees, watering equipment, technological scheme

Rezumat. *Lucrarea prezintă o analiză a modului de realizare a sistemelor de irigaţie prin picurare la plantaţiile de arbuşti şi pomi fructiferi amplasate în Câmpia şi Podişului Moldovei. În ultimii 20 de ani s-au proiectat sisteme de irigaţie prin picurare pentru plantaţii horticole pe suprafeţe relativ mici (4-8 ha) ce fac parte dintr-o fermă. Cercetarea efectuată evidenţiază unele lipsuri în conceptul de proiectare a sistemului de irigaţie, începând de la schema de amenajare, alegerea echipamentelor de udare şi calculul parametrilor de exploatare. Analiza efectuată arată că unele din sistemele de irigaţie nu sunt realizate conform caracteristicilor culturii horticole udate, parametrilor pedoclimatici ai solului, volumului de apă disponibil la sursă etc.*
Cuvinte cheie: arbuşti, pomi fructiferi, echipamente de udare, schema tehnologică

INTRODUCTION

Ensuring optimal life parameters of horticultural plantations under conditions of a continental climate with random variation of precipitation requires the filling of water deficit through irrigation. Drip irrigation is part of the localized irrigation group, along with irrigation through perforated tubes and underground irrigation. The three watering methods make the soil soak at a reduced surface / volume, only in the area of the root-absorbing plant system (Blidaru *et al.,* 1981, Luca, 1989).

Drip irrigation systems in Romania have been developed for the planting of shrubs and fruit trees at the level of the 1980s technique (Luca *et al*., 1994). The area of Moldavia owned before 1989 drip irrigation systems with 45-250 hectares

[1]Technical University "Gheorghe Asachi" of Iasi, Doctoral School, Romania
[2] Polias-Instal Company of Iasi, Romania

of area (bushes, raspberries, currants) and fruit trees (apple, plum). The drip irrigation systems have been developed by capitalizing on the research of the teaching staff from the Department of Hydro-Improvements of the Faculty of Hydrotechnics (Luca *et al.,* 1988).

Actual systems are made on small surfaces of about 3-8 ha integrated in horticultural farms. The implementation of drip irrigation systems is now facilitated by the existence of performance equipment and technologies.

The purpose of the paper is to present a critical analysis of the design of the drip irrigation systems that serve the plantations of shrubs and trees made under Moldova's relief, soil and climate conditions.

MATERIAL AND METHOD

Study area and research method

The theoretical and experimental research was carried out in the following areas:

1. Analysis of the design of the technical documentation for the design of the drip irrigation systems for the geo-physical conditions of Moldova.

2. Analysis of the field adaptation of the drip irrigation equipment to the horticultural plantations in the irrigation systems designed under the relief conditions of the Plain and the Moldavian Plateau.

3. Field investigations of the behaviour of drip irrigation systems designed and made in the Moldavian Plain and Plateau.

The research has analyzed a series of technical documentation of drip irrigation systems designed over the last 10 years in the Plains and Plateaus of Moldova. The analysis focused on drip irrigation systems designed for planting shrubs and fruit trees.

The data used in the research comes from the following sources: technical expertise, draft checks for drip irrigation systems, documentation with technical characteristics of drip irrigation equipment, etc.

Characteristics of irrigation systems by shaping

Drip irrigation systems belong to local irrigation facilities. These arrangements are on small areas ranging from a few hectares to hundreds of hectares belonging to a farm or a single beneficiary (Blidaru *et al*., 1981). The purpose of the irrigation system is to take the water from the source and transport it to the plant. The drip irrigation system is designed in accordance with the topographical, geotechnical, hydro geological, hydrological, pedological and hydroponic conditions. The diagram of the drip irrigation system has the following components (Luca, 1989):

- water outlet adapted according to the nature of the source (underground, surface) and mode of take-off (gravity, pumping);
- adduction flow through water flow: gravity flow channel; a pressure-flow conduit fed by a pumping station;
- a water storage and heating pool when the source is underground;
- supply line to connect the pool to the front installation;

- the frontal installation for filtering, fertilizer dosing, water pumping, control and control of the watering process;
- pipeline network for transporting and distributing water to watering plots;
- hydraulic constructions and installations for the operation of the pipeline network: line and bypass storms, anchor massifs, water flow and water pressure derivation and control systems, hydraulic shock absorbers, pressure regulators;
- watering equipment for the distribution of water to plants.

For a drip irrigation system fed from an underground source (wells, drainage, spring catch), the layout is shown in Fig. 1.

Fig. 1 Groundwater source irrigation system design scheme: S / PA / SPB - water source / pump / pumping station; Cad - conduit; BA - storage basin; IF - frontal installation; RC - pipeline network; EU - watering equipment

The underground drilling type (S / PA / SPB) is equipped with a deepwater take-off pump. Underground water is transported through a conduit (CA) to water storage and heating basin (BA). The basin also has the flow compensation function, taking into account the difference in value between the captured flow rate and the one demanded by the front installation.

Frontal installation with filtering, fertilizer dosing, water pumping, control and control of the watering process.

Watering equipment (EU) consists of watering pipes (CU) on which plant water distribution devices (DD-dripping) are mounted. The equipment is customized by crop type, planting distances and flow rate and water emission area. Watering equipment is differentiated as structure and dripping equipment for irrigating the following crops:

- plantations of shrubs (raspberries, currants, aphids, etc.) with reduced distances between plants;
- plantations of fruit trees (preferably in intensive and super intensive planting systems), with tree distances of 1.50 - 3.00 m;
- vegetable plantations with small plant distances (0.30 - 0.50 m).

a b

Fig. 2 How to mount the watering pipes to the fruit plantations: a - mounting on the ground; b - mounting on the first wire of the trellis (Luca, 1988).

The pipeline network of the trickle irrigation system is made of welded PEHD pipe and less PVC, where the working pressures are 3.0-4.0 bars. Throughout the main pipeline and distribution pipeline there are decommissioning booths in which there are hydraulic installations for controlling flows and pressures. Watering pipes are made of PEID with internal diameters of 10 - 25 mm and operating pressures up to 2.0 bars. Watering pipes are connected to the distribution pipes by special derivation nodes.

Drippers used for irrigation of plantations of shrubs and fruit trees are self-regulating the flow over the length of the watering pipe. Between the first and last dripper mounted on the watering pipe must be a relative pressure difference of about 10% (Keller and Karmeli, 1974).

Parameters for the design of drip irrigation systems for shrub and fruit plantations are as follows:

- the norm of water application of trickle irrigation is considered a fraction of the classic sprinkler watering standard norm; the calculation relationships are as follows (Luca et al., 1988):

$$m_p = \alpha \cdot m, \quad (1)$$

or relationship (Blidaru *et al.,* 1981, Keller and Karmeli, 1974):

$$m_p = \frac{100 H G_v (C - c_0) y}{\mu} \frac{P}{100}, \quad (2)$$

where m_p is the drip watering norm; m - classical watering norm; α - reduction coefficient; G_v - the volumetric weight; H - moist soil depth; C - soil water field capacity; c_0 - coefficient of wilting; y - reduction coefficient; P - percentage of soil actually wetted; η - the yield of uniformity on the watering pipe.

Flow rate of drip irrigation plot is calculated with the relationship (Blidaru *et al.*, 1981, Stăncescu *et al.*, 1984):

$$Q_{PI} = Sq_{u,pond} \frac{1}{\eta_c} \frac{1}{\eta_r} \frac{24}{t} \quad \text{(l/s)} \quad (3)$$

where: Q_{TIS} is the sizing rate of the pumping station in the front installation (l/s), S - irrigated area (ha), $q_{u,\,pond}$ - the weighed hydromodule (l/s.ha), η_c - yield of watering in the field, η_r - the network yield downstream of the pumping station, t - the actual hours of operation of the watering equipment (hours / day).

Watering equipment and front installations are prefabricated components of specialized firms. Irrigation equipment is manufactured on irrigated plant types, diameters, standard lengths, drip mode, flow-pressure range, etc. Watering pipes are pre-fabricated for a certain mounting position: above ground, ground and underground.

RESULTS AND DISCUSSIONS

Over the last 20 years, drip irrigation systems have been designed and built for field, greenhouse and solarium planting. The creation of small horticultural

farms with areas of 3.0 - 10 ha in the area of the Plain and the Moldavian Plateau imposed their equipment with drip irrigation systems. The analysis of some design documentation for drip irrigation systems made in the Plain and the Moldavian Plateau allowed the following conclusions to be drawn:

- a very small number of projects meet a minimum level of requirements regarding the technical content, how to compile your pocketbook, the way of drawing up the plans;

- most technical documentation does not present a correct conception of how to design a drip irrigation system as a layout scheme, how to structure the functional components, the sizing of the hydropower - hydromechanical installations and the related constructions;

- a large part of the projects do not contain the necessary studies for the design of the drip irrigation system: geotechnical study, pedological study, hydrogeological study with drilling pumping tests, climate study etc.;

- most of the technical documentation analyzed do not have a brevity for calculation of parameters of the irrigation regime (irrigation norm, watering norm, system feed flow, watering plot flow, water flow rate and pressure);

- most of the technical documentation analyzed does not have a calculation brevity regarding the hydraulic sizing of the water inlet and the pumping station according to the parameters of the duct (flow, pressure, load loss, inside diameter, material, length)

- the technical documentation analyzed does not present a calculation brevity regarding the hydraulic dimensioning of the pipeline network downstream of the front facility serving the watering parks; no flows and pressures in nodes and pipe sections, pipeline diameters, pipe wall thickness, etc. are calculated;

- watering pipes are not checked when operating with self-regulating drippers (compliance with pressure variation tolerance along pipe length);

- the technical documentation analyzed does not present a calculation brevity for the calculation of resistance and static of hydraulic structures within the irrigation system: the drilling booth, the platform of the front installation, the hydraulic installations houses, the anchor massifs, the road crossings, etc .;

The analysis of the technical documentation presented a negative aspect encountered in many projects, namely the wrong choice of irrigation equipment for irrigation of a particular horticultural crop. Frequently a planting of fruit trees with a 4 x 2.0 m planting scheme, vegetable spreading equipment, where drippers were mounted 0.40 m apart, was chosen.

For some projects, where drilling was provided, no water storage and heating pool was provided. The basin also has the flow compensation function requested by the front installation, when the source flow is lower.

The water used for drip irrigation has well-differentiated quality parameters. Water can be taken from surface sources and from underground sources. The water should be filtered and free of pollutants. Watering a limited volume of soil determines the concentration of pollutants and the short-term

degradation of the site of the horticultural plantation. Drip irrigation contributes with significant amounts of fertilizer on a layer of soil reduced in thickness.

The drawing of the technical documentation has not been developed at the level of design details for many analyzed projects. Hydraulic installations in the hostels and on the pipeline network were not designed and drawn.

Many of the irrigation systems analyzed did not foresee the optimization of the exploitation process by using an automatic program on the computer.

The analysis of the technical documentation of execution has in many cases highlighted the lack of professional training of the staff of the design firms in the field of land improvement works and, in particular, of drip irrigation systems. This situation is also being maintained by the current EU funding legislation that does not condition the design of irrigation systems by firms and engineers certified in this field. A negative contribution to this situation is provided by consultancy firms through lack of professional training in land improvement works.

All the aspects highlighted by the analysis of the design documentation influence the way of execution and the operation of the drip irrigation systems that serve the plantations of bushes and fruit trees.

CONCLUSIONS

1. Drip irrigation systems have a complex constructive and functional structure, requiring designing them in accordance with the type of horticultural plant served, soil characteristics, water source volume and climatic parameters of the site.

2. Drip irrigation system irrigation equipment should be customized on the horticultural planting features served on plant density, root system absorbent depth, water flow rates and service pressures required.

3. The design of drip irrigation systems must be carried out by certified companies in this field and the content of technical documentation must be checked by certified technical verifiers in the field of land improvement systems.

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RESEARCHES ON ENVIRONMENT DEGRADATION IN THE NATURAL SITES ON MOLDOVA RIVER LOWER COURSE

CERCETĂRI PRIVIND DEGRADAREA MEDIULUI ÎN ZONA SITURILOR NATURALE DE PE CURSUL INFERIOR AL RÂULUI MOLDOVA

***LUCA M.*[1]*, SION P. V.* [2]*, AVRAM Mihaela*[1]
*Corresponding author e-mail: mluca2015@yahoo.com

Abstract. *The European Ecological Network Natura 2000 in Romania is present on the lower course of the Moldavian River through the "ROSC10364 Community Site of Rila Moldova between Tupilati and Roman". Research over 15 years has shown that part of the natural reserve is affected by degradation phenomena. In the researched river sector (the minor and major riverbed, the riparian area) are located ballasts, bridges, water catchments, constructions, agricultural holdings, trees and bushes areas, etc. All these have influenced the arrangement of the river bed and the riparian area, which led to the degradation of the river and riparian habitat. Natural and anthropic risk factors have contributed to the degradation of the environment in the researched river area. The most important influences were the floods of the last 15 years, the exploitation of the ballast, the shore circulation areas, the absence of maintenance works in the river bed, etc.*
Key words: environmental degradation, flora, fauna, riparian area, floods

Rezumat. *Reţeaua ecologică europeană Natura 2000 în România este prezentă pe cursul inferior al râului Moldova prin „Situl de importanţă comunitară ROSC10364 Râul Moldova între Tupilaţi şi Roman”. Cercetarea efectuată pe parcursul a circa 15 ani a evidenţiat că o parte din rezervaţia naturală este afectată de fenomene de degradare. Pe sectorul de râu cercetat (albia minoră şi majoră, zona riverană) sunt amplasate balastiere, poduri, captări de apă, construcţii, exploataţii agricole, zone de arbori şi tufişuri etc. Toate acestea au influenţat modul de amenajare a albiei râului şi zonei riverane, situaţie ce a determinat degradarea habitatului fluvial şi riveran. Factorii de risc naturali şi antropici au contribuit la degradarea mediului din zona de râu cercetată. Cele mai importante influenţe au fost date de viiturile din ultimii 15 ani, exploatarea balastului, zonele de circulaţie de pe mal, absenţa lucrărilor de întreţinere a construcţiilor din albia râului etc.*
Cuvinte cheie: degradarea mediului, floră, faună, zona riverană, viituri

INTRODUCTION

The protection and preservation of geographic areas with a particular flora and fauna has led to the creation of "natural sites" protected by law. The ecological network "Natura 2000" has been created in Europe. It has been transposed in

[1]Technical University “Gheorghe Asachi” of Iasi Romania
[2]Technical University “Gheorghe Asachi” of Iasi, Doctoral School, Romania

Romania through community sites differentiated as a structure in various geographic areas. Anthropic actions are limited by law in community sites.

In Romania there is a "National Network of Natural Protected Areas" consisting of parks, natural sites, nature reserves and monuments. These are designated by Law 5/2000, Government Decisions of Romania (GD 2151/2004, GD 1143/2007, etc.). At present, Romania has 30 parks, of which 14 are National Parks and 16 are Natural Parks. Romania has 585 "Natura 2000" sites, of which 437 are of Community importance as an integral part of the European ecological network. Romania owns 926 nature reserves and monuments designated by Law 5/2000 (Lengyel *et al.,* 2016).

Part of the riparian area of the Moldavian river falls into the "ROSC10363 Moldovan Ritual site between Oniceni and Mitesti". The action of natural and anthropogenic factors has intensified the phenomena of degradation on the habitat of the river bed of Moldova and its riparian area (Luca *et al.*, 2018). A particular influence is represented by the floods produced on Moldova, which morphologically modify the bed and cause disturbances in the living conditions of the aquatic fauna. Ballast operations and long-distance transport on the river bank disturb the stability of the environment and pollute the riparian habitat (Luca *et al.,* 2018).

The paper aims at presenting the results of the researches regarding the degradation of the environment in the lower course of the Moldova river and especially in the site of the "Tupilați - Roman" natural site.

MATERIAL AND METHOD

The research was carried out in the natural site "Tupilați - Roman" located on the lower course of the Moldova river. Within the natural site he has chosen a research sector on the Moldavian River between the locality of Pildeşti and Cordun, Neamț County. The research was conducted in several directions: theoretical studies, field studies and numerical simulations.

In the study area were included the minor riverbed of the Moldova River and the riparian area on the Tupilati - Cordun sector. A number of areas affected by environmental degradation and pollution phenomena have been analyzed in this sector.

The data used in the research comes from the following sources: technical expertise in the field of regulation of rivers and coastal defence constructions, A.B.A Siret Bacau Synthesis Reports, projects for works in the riverbed of Moldova and the riparian area, etc.

Part of the data comes from field research conducted by authors on the analyzed river sector (Luca, 2016, Sion, 2019). Authors' studies and research are conducted over a period of about 15 years (2004-2019).

The theoretical and experimental research was carried out in the following areas:

1. Studies on the evolution of natural sites in Romania under the conditions of hydro-climatic changes in the last period of time.

2. Studies and experimental researches on the influence of anthropogenic factors on natural sites located on the Moldova river.

3. Researching the natural and anthropic risk parameters influencing the evolution of the natural site located on the lower course of the Moldova river.

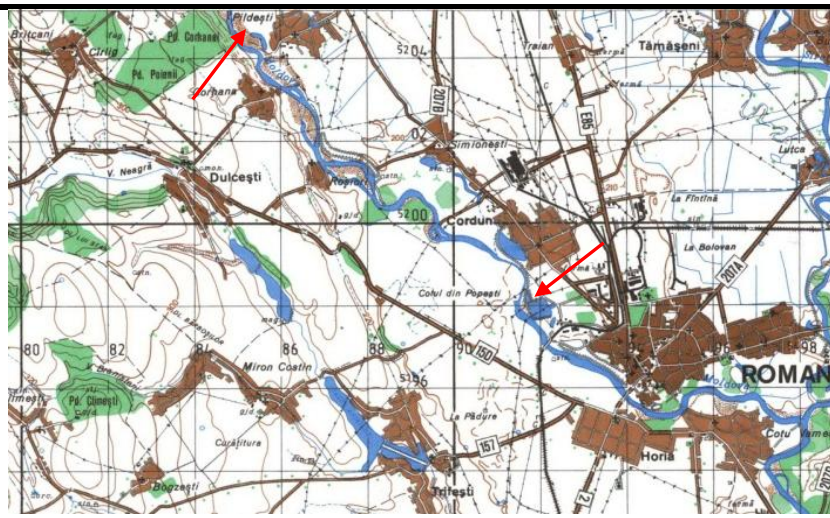


Fig. 1 Localization of the Pildeşti - Cordun Research Area on the Moldovan River From site ROCI0364 Tupilati - Roman on the Moldavian River.

The collection of field data was done by topographic measurements, field analyzes, material samples, photo and video surveys.

Primary data was processed using the statistical, hydrological and hydraulic calculation programs applicable to the case study.

RESULTS AND DISCUSSIONS

The ROSCI0364 - Moldovan River between Tupilați and Roman, as part of the "Natura 2000" site, was established by Government Emergency Ordinance no. 57/2007 on the regime of natural protected areas, conservation of natural habitats, wild flora and fauna, approved with amendments and completions by Law no. 49/2011. The protected area is located on the Moldavian River, including the riparian area, between Tupilați and Roman localities, Neamț County (fig. 1). The natural site has functional relations with four neighboring sites: ROSCI0363 "Moldova River between Oniceni and Miteşti"; ROSCI0365 "Moldova River between Păltinoasa and Ruşi"; ROSCI0378 "River Siret between Paşcani and Roman"; ROSPA0072 "Meadow Middle Meadow". The surface of the natural site is 4,720 ha.

Table 1

Location of ROSCI0364 - Moldova River between Tupilati and Roman

Coordinates		*S*	Alt_{min}	Alt_{med}	Alt_{max}	Biogeographic region
Latitude	Longitude	ha	m	m	m	
N 47^0 2' 13"	E 26^0 45' 16"	4720	175	247	333	Continental
S - surface; Alt - altitude.						

The biogeographic region is of "Continental Steppes Panonic Pontic Alpine". The natural site "Tupilați-Roman" contains habitats such as rivers, lakes, arable land, pastures and deciduous forests. The Moldavian River has the following parameters up to the Tupilați area: S (BH) = 3951 km^2, average altitude = 236 m, length = 176.6 km, slope 1.3 m/Km, multi-annual average flow 32.9 m^3/s suspended solid = 43.2 Kg/s.

The conservation objectives of the site are 10 species of fauna of Community interest, listed in Annex II of Council Directive 92/43 / EEC. The wetland is the specific habitat for 4 species of vertebrates, 4 species of amphibians and 3 species of conservative fish.

Within the natural site, there are a number of mammalian species protected under Annex II: *Lutra lutra, Spermophilius citellus, Myotis myotis şi Myotis blechsteini*. Within the site are species of protected amphibians and reptiles: *Bombina bombina, Bombina Variegata şi Triturus crestatus*. In the river of Moldova and its tributaries there are species of protected fish (*Barbus meridionalis, Cobitis taenia şi Sabanejewia aurata*).

All these species require a conservation status of the aquatic or terrestrial living environment in the coastal area. Thus, *Lutra lutra* is affected by the destruction of the vegetation in the riparian area, the presence of the river bed constructions, the drainage of the wetlands in the meadow, etc. The main threats to *Spermophyllus citellus* are the conversion of pastures into arable land. The use of pesticides in agricultural habitats affects the habitat of *Myotis myotis* and *Myotis flechstein*. Destruction of wetlands, industrial pollution and human activities affect the habitat of *Bombina bombina*. Changing water quality affects habitat at *Triturus cristatus*. *Barbus meridionalis* is influenced by the presence of structures in riverbeds and water pollution.

The research has shown that the site of the natural site is characterized by various human activities (agricultural works, grazing, water abstraction, ballast operations, road transport, etc.). Moldovan and industrial waste is discharged into Moldova, which continuously degrades the living environment of the "Tupilați - Roman" natural site. The Moldovan River has a relatively large capacity to dilute and disperse the pollutants discharged into its bed. But the possibilities of self-restoration of the natural quality of river water are not unlimited. Exceeding some pollution limits can cause major and irreversible changes in aquatic ecosystems (Bica, 2000).

The natural risk factors, especially the hydrological factors and the anthropic factors, influence the morphology of the river bed, the stability of the bed and river construction. But these factors also influence the existing habitat in the minor and major river bed. Disastrous floods produced in recent years have degraded the aquatic and coastal environment.

In the riverside area on the Moldova River, downstream of Pildeşti, there is a Roman water catchments area. Water capture consists of 66 capture wells and seven reinforced concrete boxes located parallel to the river bank. Current maintenance works - repairs and conservation of the sanitary protection area have negatively

influenced the habitat in the meadow area (fig. 2). The sanitary protection zone requires an environment without agricultural crops and the use of fertilizers. This situation does not allow the existence of optimal habitat conditions for some species.

a b

Fig. 2 The riparian area on the left bank of the Moldova River downstream Pildeşti, with the water catchment area: a - general view of the habitat area; b - detail of the sanitary protection area of the capture well, year 2018.

Ensuring the hydraulic parameters at the capture wells required the calibration of the Moldova river bed. Albia has a width of about 400 - 750 m across the catchment section, which causes the migration of the minor bed from the left bank to the right bank to pass through the floods. In order to ensure the water level in the catchment area, regularization works were carried out to move the small bed to the left bank (routing dams, docks, sleepers, bottom sills, shores, etc.) (fig. 3). Multi-annual average flow is 33.0 m^3/s, situation that imposed the presence of the bottom thresholds to ensure a water depth of 0.90 - 1.20 m (fig. 3.a). The works of this type influenced the habitat in a series of species protected in the natural site.

a b

Fig. 3 The state of the natural site in the Pildeşti area on the Moldova river: a - the riverbed branching zone (year 2015), 1 - the new bed in the capture area, 2 - the old riverbed, 3 - digging dam; b - view of calibrated bed and shore defence in capture area (year 2018).

The shore defence is made of geo-bags (fig. 3.b). By way of vertical and horizontal installation of geo-bags, favourable conditions for restoration of the habitat in the minor riverbed of the Moldova river are achieved. Geo-bags reduces water speed, deposition of alluviums and the creation of flora and fauna in the shoreline environment (Luca *et al.,* 2018).

The frequent floods of the years 1992, 2005, 2006, 2008, 2010, 2016, 2018 modified the bed and degraded the river-related settlement work in research. Flows recorded at floods showed high values that exceeded the probability of calculating the adjustment works (Q_{max} = 600 - 1200 m^3/s). The repeated morphological modification of the bedrock has altered the conditions of existence of the aquatic habitat (fig. 2.a and fig. 4.a). The restoration of the bed stability ensures optimal living conditions for the species protected in the natural site.

a b

Fig. 4 The state of the "Tupilati-Roman" natural site on the Moldova River: a - bottom (1) in the calibrated riverbed, Pildeşti area; b - protection of the concrete slab in the Cordun catchments area (year 2018).

A degradation of the natural environment within the site is determined by the intense movement in the riparian area of the ballast transport machines (fig. 5.a). The road is from the ground, with varying widths, with penetrations on the natural ground, which causes a continuous degradation of the riparian area. Noise, dust, and especially pollutant gases dispersed in the air by Diesel engines severely affect the quality of air, water and soil in the natural site area (Sion, 2019).

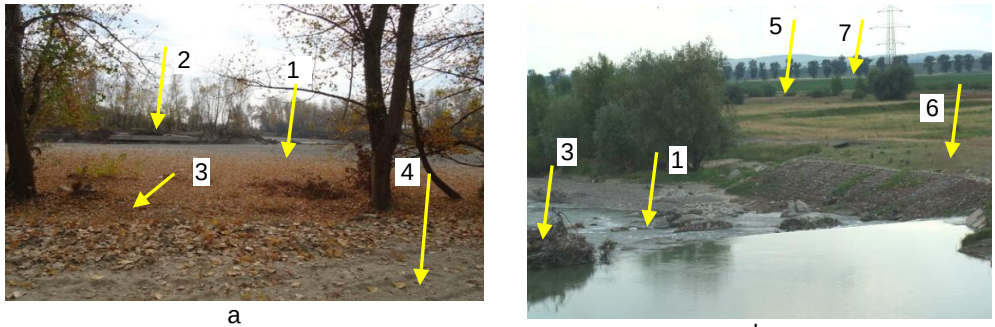
a b

Fig. 5 State of the natural site in the riparian area: a - earth road (1) used for ballast transport, (year 2018); b - the presence of ballasts in the riverbed and the riparian zone.

A degradation factor of the aquatic environment is due to the presence of the ballasts in the locality of Pildeşti and Cordun (fig. 5.b). The research showed the change of the turbidity of the water and the increase of the solid flow

transported on the bedside sector located in the natural site. This situation has greatly disrupted the aquatic habitat of protected species within the natural site.

At the contact between the Pildeşti area and the Cordun area, the Moldova River passes through a vegetation area of shrubs and trees, followed by agricultural crops (fig. 6a). The river has a natural riverbed and a satisfying riparian area for a normal habitat of protected species. Only the presence of traffic on the earth road disrupts the ecological stability of the natural site.



a b

Fig. 6 The state of the ecosystem on the Moldova River in the Cordun area: a - entry into the researched area (year 2018); b - industrial water catchment area Roman, (2011), 1- river riverbed, 2- right river bank, 3-left river bank, 4-way, 5- river right arm, 6-island, 7- had a right arm with farm crops.

In the industrial water catchment area of the Roman town there was a morphological change of the riverbed of the river Moldova (Luca, 2011). The capture of water is "surface", which led to the realization of two arms on the Moldova River (fig. 6.b). The left arm on which the capture is located is regularized (bottom edge, dipstick, shore defence) and rectilinear on a length of about 420 m. The left arm of the river does not provide optimal habitat conditions for the protected species due to high speeds water. The right arm of the river has a curved path with a natural bed at low speeds, favouring a normal habitat. The presence of the island allows the realization of normal living conditions for the protected species.

a b

Fig. 7 Degradation of the aquatic habitat through works in the riverbed of Moldova River: a - transverse works (bottom threshold); b - exploitation of the ballast with morphological degradation of the bed (year 2018).

Hydro-hydraulic work done in the natural site must ensure the living and conservation conditions of mammalian species, which is among the few sites designated for *Spermophilius citellus* and *Lutra lutra*. It is worth mentioning that some constructions and works for regulating the river bed of Moldova exist from a period before the designation of the natural site.

The correct and continuous functioning of natural ecosystems is necessary to ensure living conditions for biological communities. A number of plant and animal species that are integrated into the biotic community depend on certain physical conditions in the site and some ecological processes necessary for their survival (Amoros and Bornette, 2002). Physical conditions include mainly water, temperature, soil type, and organic processes include water circuit, nutrient and nutrition relationships. Changing or losing a certain type of habitat within the natural site determines the loss of species that depend on that type of habitat (Nilsson and Berggren, 2000).

The works included in the building rehabilitation project in the riverbed of Moldova meet the requirements for habitat assurance of the species listed in Annex II of Council Directive 94/43 / EEC.

CONCLUSIONS

1. Natural sites located on the watercourses must effectively collaborate with the riverbed and riparian buildings to ensure the most viable living conditions of the protected species.

2. The research carried out in the area of the "Tupilati - Roman" natural site on a section between Pildeşti and Cordun has revealed a complex of disturbing factors for both the aquatic habitat and the coastal area for the preserved species.

3. The regularization of the river bed of Moldova as well as those executed in the riparian area on the researched section must ensure the living and conservation conditions of the protected species within the natural site in view of the presence of extremely rare species (*Spermophilius citellus* şi *Lutra lutra*).

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ENVIRONMENTAL PROTECTION IN RIVER REGULATORY WORKS USING BIODEGRADABLE MATERIALS

PROTECȚIA MEDIULUI ÎN LUCRĂRILE DE REGULARIZARE A RÂURILOR PRIN UTILIZAREA MATERIALELOR BIODEGRADABILE

SION P.V.[1], LUCA M[2], AVRAM M.[1], TOMA D.[2]
*Corresponding author e-mail: mluca2015@yahoo.com

Abstract*. The paper presents biodegradable methods and materials used to carry out river bed works. River regularization works must help to keep the environment as natural as possible. Biodegradable materials respond to bioengineering methods and concepts for ecological regulation or renaturalisation of river beds. Studies and research have shown the possibility of using biodegradable materials for the execution of shore defense works at the riverbed. The researched biodegradable materials are made of woven sheep wool fabrics and strips. They are used as a support and filter bed at the shoreline contact with the rock in the site. The material has the advantage of natural degradation in about 4-6 years after it has performed its support function. Woollen fabrics are used to make the biological cells used for river bank layout or sloping slopes.*
Key words: shore defenses, wool fabrics, biocells, renaturalisation

Rezumat. *Lucrarea prezintă metode şi materiale biodegradabile utilizate la realizarea lucrărilor din albia râurilor. Lucrările de regularizare a râurilor trebuie să contribuie la păstrarea unui mediu înconjurător cât mai natural. Materialele biodegradabile răspund metodelor şi conceptelor de bioinginerie pentru regularizări ecologice sau renaturalizarea albiilor de râu. Studiile şi cercetarea efectuată au arătat posibilitatea utilizării unor materiale biodegradabile la execuția lucrărilor de apărare de mal la albia râurilor. Materialele de tip biodegradabil cercetate sunt realizate din țesături şi fâşii presate din lână de oaie. Acestea sunt folosite ca strat suport şi filtrant la contactul apărării de mal cu roca din amplasament. Materialul are avantajul unei degradări naturale în circa 4-6 ani, după ce şi-a îndeplinit funcția de suport. Țesăturile de lână sunt folosite la realizarea celulelor biologice utilizate la amenajarea malului râurilor sau la taluzul versanților.*
Cuvinte cheie: apărări de mal, țesături de lână, biocelule, renaturalizare

INTRODUCTION

River regularization works are part of the watercourses in ensuring the stability of hydrological parameters on a determined river sector. Also, the regularization works contribute to achieving favourable environmental conditions

[1]Technical University "Gheorghe Asachi" of Iasi, Doctoral School, Romania
[2] Technical University "Gheorghe Asachi" of Iasi Romania

on a watercourse and its riparian area. The design of shore regulation and defence works is done with hydrological parameters (flows, levels, defences, protected areas, etc.) obtained by statistical processing of data collected over long time periods. Climate changes produced over the last 30 years in Romania have substantially altered the hydrological data, a situation that forced the re-evaluation of some design rules. The European tendency to naturalize river beds which have rigid regularization works and which have negative influences on the environment has led to changes in design concepts (Avram, 2016; Luca, 2018; Bica, 2000).

Naturalization works of river beds have required the use of elastic constructions made of plastics and biodegradable materials. Elastic type works are mainly used in beds located in low cohesive rocks. Materials such as geotextiles have found applications in the structure of river defence works on rivers, but also in the construction of the slopes of various constructions in the earth (Luca *et al.,* 2016).

The purpose of the paper is to present the results of studies and researches on the behaviour of shore defence works, including geodetic and biodegradable materials, with applications on the rivers in Moldova.

MATERIAL AND METHOD

The theoretical and experimental research was carried out in the following areas:

1. Studies on the requirement for the use of biodegradable materials to carry out riverbed regularization works.
2. Studies and researches on types of biodegradable materials with applications for river bed regulating works.
3. On-site research of the behaviour of riverside regulation works made from biodegradable materials.

The research analyzed technical documentation for carrying out river regularization works on the use of biological and natural materials that can be degraded during their exploitation without affecting the aquatic or riparian environment.

The research has analyzed on the ground a series of river regularization works made with biodegradable materials in various river basins on the territory of Romania (B.H. of the Siret River, B.H. of the Olt River).

The data used in the research come from the following sources: technical expertise on the field of river regulation and river defence constructions, water basin management synthesis reports, drafting works, documentation with technical characteristics of biodegradable materials, etc.

The collection of field data was done through specialized analyzes on research fields, material sampling, photo and video surveys.

Primary data has been processed using the statistical, hydrological and hydraulic calculation programs applicable to case studies.

RESULTS AND DISCUSSIONS

Coastal defence works generally have a local character, limited to areas with economic or social objectives located near the watercourse. They must,

however, be treated in such a way as to fit into a water management plan that also protects the environment. The current state of shore defence works requires the use of biodegradable materials for more appropriate environmental protection.

Plastics are widely used to carry out shore defence work due to their high resistance to stretch, flexibility and impermeability, relatively low cost, and ease of operation. Geotextiles, which have large applications for use in a wide range of hydrotechnical construction, are detached from these. But plastics are degradable in a long time or can not be degraded. Biodegradable materials replace some of the plastics, such as geotextiles (Sion, 2019).

The most commonly used biodegradable materials are: earth, stone, wood, textiles, etc.

Stone is the most commonly used material in river regularization. The stone encompasses the works in the form of: massive rockfill, equalizing layers and filters, stone for loading elements made of branches, wicker and wood, stone for the execution of the slopes, defending and consolidating the banks, etc. The stone used is the river stone, rough stone as it results from quarries (natural blocks, stones), crushed stone, carved stone and processed in various ways (moloanes, cubes, bars, tiles et.) (Manoliu, 1973).

Wood is used in the form of raw (round, semi-round wood) and engraved. Woodworks are executed for driven pile, pairs and stakes, wall panels, joining elements etc. Wood is also used in the form of rods, branches, trees, pines, pines, logs, beams, longlines, wale, timber, etc., which are introduced in the river blocks. The branches and threads are used to make fascinations, hedges, and slings used in shore defence (Luca, 2011).

a b

Fig. 1 Biological works in shore defence on Breţcu River, place. Tg. Secuiesc: a - braids/ wattle; b - details on the location of biological protection (Luca, 2011)

An example of biological woodworks has been made by sectors of the Breţcu and Ojdula Rivers, in the area of Tg. Secuiesc, Covasna County. The paper (Luca, 2011) presents the field research on the behaviour of these works. Protective work of the bedside slope at the two biological rivers consists of shafts, waders of willow rods, swamps. The canes and wicker canopies were

placed at the base of the slope. Propagation of floods a year after execution led to the partial degradation of these protection works.

The completed biological defence system takes some time to get into operation and carry out the designed tasks. The high frequency of floods and the low resistance of the bedrock caused a differentiated behaviour on the segments (fig. 1).

At international level, works of biodegradable materials, especially wood, are being carried out constantly in the settlements, in the planning of rivers. These works are required by law to create a natural environment as well as to protect the environment.

a b

Fig. 2 Adjustment works made of wood on rivers: a - the Isar River in the centre of Munich, Germany; b - the Kwaczy River, northern Poland (Sion, 2019).

Biodegradable materials in the form of textile fabrics are planar structures of vegetable materials (eg. jute, coconut) or animal (eg. wool). The fabrics are in the form of fabrics, fusils, pressed bands, etc. The functions performed by them in the shore defence works are;

- separation, when used as a layer of separation between physical properties;
- filtering when it prevents the migration of rock particles, but allows the penetration of water without pressure;
- protection against degradation of slopes or slopes to erosive water, etc.

Biodegradable geotextiles are made of degradable fibers based on natural wool, with or without a jute mesh used to increase mechanical strength. Geotextiles should be placed on smooth surfaces in such a way as to reduce creases or creases from the material. For protection and defence of the slopes, geotextile is attached to the vegetation, in order to avoid soil erosion and slipping through the effects of precipitation and wind. Geotextile is a support for vegetation anchorage, preserves soil moisture even at high temperatures, and by decomposition over time it contributes to feeding the soil with nutrients, thus reintegrating into the natural cycle.

The weaving or nonwoven wool products work together in the structure of the construction elements used to regulate rivers and rocks defence works with rock from the foundation layer, as well as with the building components (Sion, 2019). The textile materials are flexible, which allows for very good molding on the mounting surface (the case of the slopes) (fig.3).

a b

Fig. 3 Biodegradable geotextiles: a - material layout and leaching on the slope; b - tiling of a woollen cloth geotextile (Sion, 2019).

The biodegradable fabric layer made of a woollen fabric used as a support for the location of regularization works and shore defence works has the following characteristics: raw material: natural wool fibbers; standard dimensions: width - 2.0 m, length 25 - 50 m, thickness - 3.0 mm; mechanical parameters: weight - 0.25 kg/m^2, traction resistance - 4 daN/5 cm; MD / CMD prolongation - 125%; water absorption capacity: 140%.

Woollen fabric is a geotextile that degrades biologically in about 2 to 3 years from natural wool fibber without reinforcement of various fibbers. Above the woollen fabric placed on the channel slopes or the bank of the river are placed grass seeds over which is filled a vegetal soil filling. By using this composite it is avoided the formation of sliding plans on the slope and the conditions for the realization of the vegetation layer protection are ensured.

River bank or sloping slope can be protected with biodegradable pre-sown mattresses. They prevent erosion by absorbing the energy resulting from the impact of precipitation, by insulating water and allowing water to pass through material into the rock site. This process cancels both erosion forces. Erosion protection mattresses are made up of coconut or straw trapped together with a lightweight reinforcement mesh of polymers. The polymer grid can be replaced with a jute mesh.The protective mattress is strong and flexible, with a thick texture that creates a barrier to wind and precipitation, which provides total soil and seed protection, contributing to long-term growth and development. The natural components of the mattress degrade biologically without harming the environment. The grid of polymers remains intact for longer and ensures the reinforcement of the grass roots (Sion, 2019).

The use of geotextile from natural or animal fibbers has several advantages:

- the result of the degradation of plant-derived fibbers (eg cotton, jute, hemp, etc.) is carbonated hydrates, which become a natural fertilizer introduced into the soil and helps to grow plants;

- wool geotextile takes over the energy resulting from rainfall and allows water to flow through the material into the soil beneath the wool fabric, helping to reduce the surface erosion phenomenon;

a b

Fig. 4 Mixed riverside protections: a - grilled and sown geo-bags; b - geocells filled with grass roots.

- ensures the development of vegetation on the slope and contributes to the creation of a natural environment;

- geotextiles of vegetal and animal fibers provide protection of the slopes by fixing the vegetation on them, avoiding the erosion of the slope by the action of water and wind.

CONCLUSIONS

1. Ensuring optimal environment protection in the watercourse area requires the use of biodegradable materials for the execution of the regularization works.

2. The biodegradable material most commonly used in the execution of river regularization works is wood but which is limited to use due to low mechanical strengths and rapid degradation over time.

3. Studies and research have shown the use of woollen fabrics and wafers as a supporting layer for the location of river bed settling work on riverbed sites.

4. Biodegradable materials have the advantage of destruction after a period of operation, and degraded components are returned to the soil as nutrients.

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RESEARCH ON THE CONSTRUCTION OF STRUCTURES MICROFERM TYPE ACVAPONICS

CERCETĂRI PRIVIND REALIZAREA STRUCTURILOR ACVAPONICE TIP MICROFERMĂ

URSACHI A.[1], LUCA M.[2]*
*Corresponding author e-mail: mluca2015@yahoo.com

Abstract*. The paper must carry out studies and research on the realization of aquaponic structures under climatic conditions existing in the area of Moldova. The research is focused on designing hydrotechnical care systems to include information: water capture, decanting and disinfection line, water supply line for the planter, wastewater discharge line, air circuit line, filter installation, water treatment plant and disinfection. The hydraulic system deserves the DWC fish and fish basins. The hydrotechnical system deserves to realize an aquaponic structure located in a solarium with an area of 100 m^2.*
Key words: hydraulic system, culture system, fish pools, culture beds

Rezumat. *Lucrarea prezintă studiile şi cercetările privind realizarea structurilor acvaponice în condiţiile climatice existente în zona Moldovei. Cercetarea s-a axat pe conceperea unui sistem hidrotehnic care să cuprindă următoarele componente: captarea apei, linia de alimentare decantată şi dezinfectată, linia de alimentare cu apă a plantelor, linia de evacuare a apei uzate, linia circuitului de aer, instalaţia de filtrare, instalaţia de dezinfecţie. Sistemul hidraulic deserveşte bazinele cu peşti şi bazine cu pat de cultură DWC. Sistemul hidrotehnic deserveşte structura acvaponică amplasată într-un solar cu suprafaţa de 100 m^2.*
Cuvinte cheie: sistem hidraulic, sistem de cultură, bazine de peşti, paturi de cultură

INTRODUCTION

In the next period of time, the human society has to solve two major problems: the water problem and the food problem. The sources of drinking water are constantly decreasing and the phenomenon of pollution is constantly increasing. Finding viable sources of drinking water in Romania has become particularly difficult. Surface water sources are highly polluted. Groundwater sources are becoming more and more polluted. Such a situation is also present in the Moldovan area.

Aquaponics is a biological system for growing plants and fish in a hydraulic system with water recirculation. The two biological components of the system ensure to each other the ideal habitat, with the mutual depollution of the water and the assurance of optimal living conditions (Bernstein, 2011).

[1]Technical University "Gheorghe Asachi" of Iasi, Doctoral School, Romania
[2] Polias-Instal Company of Iasi, Romania

Small volume aquaponics (water volume below 3000 L) are suitable for a family or microfarm (Somerville *et al.,* 2014). A wide range of plants and any species of fish can be introduced into the aquaponic system. In this case, vegetables (spinach, salad, bacon, parsley, etc.) and fruits (strawberries) are suitable. For a number of crops (eg peppers and tomatoes) potassium, calcium and iron should be added. Fish suitable for aquaponics in Romania are carp, rainbow trout. Under the Romanian conditions the Tilapia species can be used. In Romania, aquaponics systems are in the early stages and there is virtually no aquaponics unit on a commercial scale. Most are of small volume and are used as a basis for experimental research.

The purpose of the paper is to present the concept of application of an aquaponic structure with DWC culture technique, small volume for a microfarm, under the climatic conditions of Moldova and for a variation of the type of plants and fish species. The first research phase is focused on designing the hydraulic water supply and drainage circuit.

MATERIAL AND METHOD

The research was carried out in the area of Moldova, respectively in the city of Tomeşti, located on the border of the city of Iasi.

The research area is characterized by the following geophysical characteristics:

- location in the relief area of the Moldavian Plain in contact with the Central Moldavian Plateau area;

- the climate is temperate - continental, in a province sector with aridity; average annual rainfall is 500 - 600 mm; the average annual temperature is 10 ^{0}C: the average annual thermal amplitude is high, with the value 24-26 0C.

- the solar radiation is excessive during the summer and is greatly reduced during the winter; light intensity has a summer value of 30,000 - 100,000 luces in summer, and in winter of 4,000 - 10,000 luces; vegetable plants have a good assimilation to an intensity of 20,000 - 30,000 luces.

For the research, a closed space such as a solarium was used. It was arranged according to the research topic.

Theoretical and experimental research was carried out on the following fields:

1. Design and realization of a hydrotechnical water supply system of a small size aquaponics structure with DWC culture technique in the climatic conditions of the N-E area of Romania.

2. Analysis of how to adapt to the small space of a solarium the hydrotechnical water supply system and the DWC cultivation equipment.

3. Research on the behaviour of small-sized aquaponics structures with DWC culture technique in differentiated exploitation processes by plant and fish species.

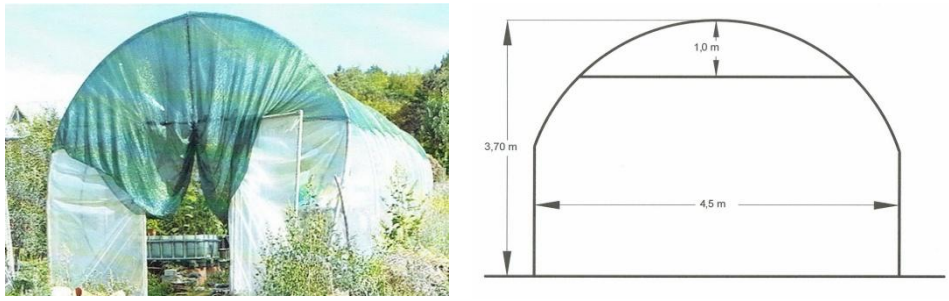
The research analyzed a series of technical documentation of small and medium sized aquaponics structures made internationally and nationally and especially for temperate continental areas with high aridity.

RESULTS AND DISCUSSIONS

An aquaponic system consists of two major components: the fish breeding facility and the plant culture system. Fish are raised in one or more pools, and the

plants are cultivated by several processes that use the waste water from fish. The two major structural components are served by specialized auxiliary installations on the transport of clean and polluted water, on the air supply, on the discharge of waste water and waste, on the lighting and heating of the production space, on the control and monitoring of the exploitation process, etc.

The aquaponic culture system is located in a greenhouse made of a metallic structure covered with HDPE foil with a thickness of 50 microns (single greenhouse, with the shape of a semi-circle roof, high tunnel) (fig. 1). The greenhouse is located on a hill, in an area without trees (shaded area) and with the long side facing the East-West direction. The greenhouse has a length of 20 m and a width of 4.5 m, with an area of 90 m^2. The greenhouse is covered with shading net, where the degree of shading is 75% (Ursachi, 2017).



a b

Fig. 1 Greenhouse for the placement of the aquaponic structure: a - general view of the greenhouse; b - the geometric scheme of the greenhouse (Ursachi, 2017).

The greenhouse is made without a heating system, because the operating time is temporary (only during the growing season). The aquaponic structure was moved during the cold period of the year in a heated space at a temperature of 21 - 22 ^{0}C.

The greenhouse is illuminated naturally and artificially. Artificial lighting is done with growing lamps (fig. 2.a). They operate on 3 light spectra - red, yellow, blue and strictly generate the light spectrum required for plants. Three growth lamps (figs. 2.b and 2.c) have been mounted on the greenhouse skeleton, which provides night illumination with a luminous intensity of 100 luces each. They come into automatic operation, being activated by a photocell sensor (fig. 2.d).

The purpose of the hydrotechnical system is to retrieve water from the source and transport it to the consumer (fish + plant). The layout of the hydrotechnical system is carried out in accordance with the topographic, geotechnical, hydrogeological conditions, the geometrical characteristics of the solarium, the characteristics of the aquaponic installation. The scheme of the hydrotechnical system has the following components:

- water outlet adapted to natural source (underground from the site area, public water supply network); the groundwater is taken after a well equipped with a pump; 25 minutes, Pn 6.0 bar and is metered;

Fig. 2 Greenhouse lighting system: a - service lighting; b - lamp placement scheme; c - lamp; d - lighting trigger sensor (Ursachi, 2017).

- the drilling adduction is made of a PEHD pipe, Dn 63 mm, Pn 4.0 bar; the adduction from the public network is made of galvanized steel pipe with Dn 25 mm, Pn 6.0 bar;

- tank for the storage and heating of water when the source is underground, or for the removal of residual chlorine for water from the public network; the tank has a volume of 250 L and is made of a PVC; the tank is underground and is equipped with an extruded polystyrene thermo-system;

- supply pipe to make the connection between the storage basin and the installation serving the solarium; the pipe is made of HDPE with Dn 63 mm, Pn 4 bar; a water disinfection plant is located on the supply line;

- network of pressure pipes with the role of water transport and distribution at the elements of the aquaponic structure; the pipe network is made of PEHD pipe, Dn 32 mm, Pn 4.0 bar, provided with bypass and control valves, drain valves;

- mechanical filtration and biofiltration systems;

- waste water sewerage network made from a PVC Dn 110 manifold connected to the local sewerage system;

- devices and installations for measuring and controlling the meteorological parameters (temperature and humidity of the air), water (temperature. pH, content of substances), hydraulics (volume of water, flow, pressure).

The fish tanks (fig. 4.a) are made of white plastic, UV resistant and have a volume of 1 m^3 each. Each tank is fed through a PVC pipe Dn 32 with a flow rate of 0.15 L/s. The fish pools were equipped with juvenile Tilapia de Nile and juvenile Tilapia de Mozambique.

The plant cultivation technology is of the "deep water" type (DWC) (fig. 4.b). The technology involves the suspension of plants on a polystyrene plate (fig. 4.b), and the roots are immersed in the water coming from the fish tanks. The water flows gravitationally from fish tanks, through biofilters, to basins with culture beds. The planting scheme in the beds with beds of aquaponic culture was

of the type "companion plants". This concept is found in organic gardens and is based on the idea that plants can benefit from the company of others.

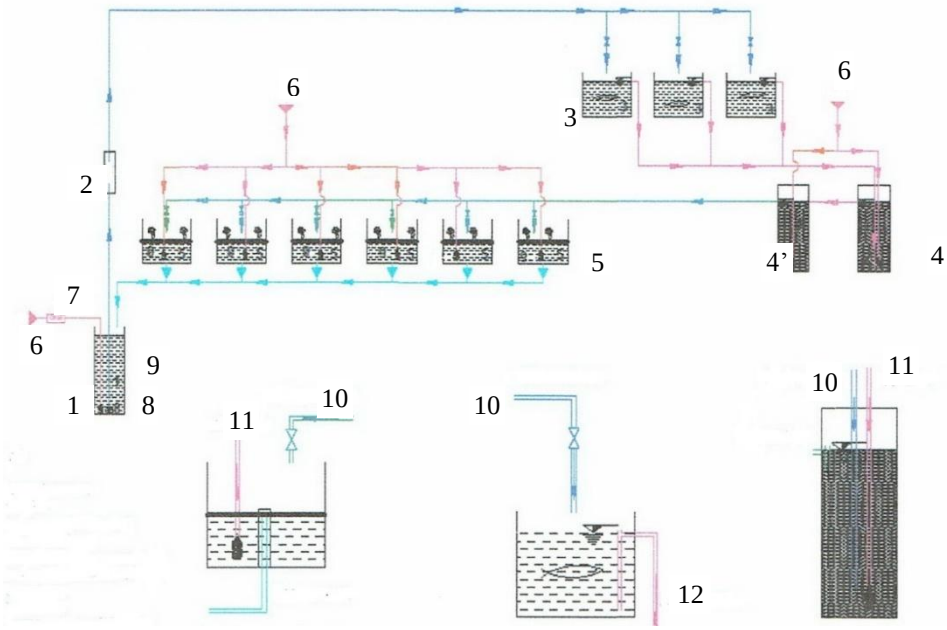


Fig. 3 The functional structure of the aquaponic system: 1 - decanter basin; 2 - UV filter; 3 - fish tanks; 4 - biological biofilter; 4 '- biological + mechanical biofilter; 5 basins with culture bed in DWC system; 6 - air pump; 7 - ozonator; 8 - submersible water supply pump in the aquaponic structure; 9 - aerator with pumice stone; 10 - food; 11 - aeration; 12 - evacuation.

a a b

Fig. 4 The solarium arrangement for the aquaponic structure: a - fish basins; b - pools with culture beds; the suspension plates of the plants (Ursachi, 2017).

The plants used in the aquaponic structure were: *Lactuca sativa* - early green salad, *Lycopersicon esculentum* - tomatoes, *Cucumis sativus* - cucumber, *Capsium annuum* - bell peppers, kapia peppers, peppers, donuts, *Ocimum*

basilicum - basil, *Satureja hortensis* - melo - melon, *Pisum sativum* - peas, *Fragaria sp*. - strawberries, *Citrullus lanatus* - melon, *Solanum melongena* - eggplant.

The process of plant growth is achieved through the participation of nitrifying bacteria (*Nitrosomonas* and *Nitrobacer*), which convert ammonia and urea from fish manure into nitrates, which are consumed by plants.

The basins with culture beds have an area of 1.0 m^2, with a useful volume of 300 l and a water depth of 30 cm. The depth of the water allows the creation of the space for the development of the plant roots. The plant basins are fed in parallel with the flow rate of 0.09 L/s through a PVC pipe Dn 32 connected to the filtration system.

For aerating the culture beds, an air pump with a flow rate of 360 l/h was used, and the bottom of the basins is equipped with pumice stones. The air flow rate for each basin was 1.0 l/min.

The hydraulic system was designed so that after the fish tanks the water flows gravitationally to the drainage tank.

The water disinfection system entered in the hydraulic system is equipped with a UV lamp (fig. 3).

Aquaponic technology can become one of the alternative food production solutions in the future. Aquaponics is a technology that involves low costs and is extremely efficient in environmental protection. This technique can contribute to feeding the population from the disadvantaged areas. Aquaponics is a source of unpolluted food with chemicals (Ursachi and Marcoie, 2017).

CONCLUSIONS

1. Aquaponic crops can be efficiently used in the climatic conditions of Moldova by using small and medium sized systems, with applications in individual households and microfarms.

2. The research carried out under the climatic conditions in the Moldovan area indicates the feasibility of the method in feeding biological food of a microfarm served by a family, but also the existence of a surplus of products that can be used.

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BIODEGRADATION OF SYNTHETIC DYES BY SOME BACTERIAL STRAINS ISOLATED FROM SOIL

BIODEGRADAREA COLORANȚILOR SINTETICI DE CĂTRE TULPINI BACTERIENE IZOLATE DIN SOL

ŞTEDEL Cătălina[1], EFROSE Rodica Cătălina[1], ROŞU Crăița Maria[1 *]
*Corresponding author e-mail: craita2002@yahoo.com

Abstract: *In present study, 19 bacterial strains were isolated from different type of soil (Danube – Delta Biosphere Reserve), molecularly identified (16S rDNA sequencing), and tested for their textile dye biodegradation potential and tolerance level to heavy metals and high salinity. The strains Pseudomonas sp., Bacillus sp. and Thalassospira sp. were found to degrade 72.64% RO16 dye, but only Pseudomonas putida removed (56% decolorization) the RB4 dye. Also, was found a good tolerance to salinity (8% NaCl) in case of Pseudoarthrobacter sp., Enterobacter sp., Thalassospira sp., Bacillus sp., and Pseudomonas sp. strains. Most of the bacterial strains tolerated 70 ppm of cromium (Cr^{6}); only two strains, Cupriavidus respiraculi and Pseudomonas putida showed maximum tolerance to 70 ppm cadmium (Cd^{2+}); all strains of Pseudomonas sp. showed tolerance to 100 ppm lead (Pb^{2+}). The selected strains could be used in bioremediation process of industrial dye waste waters.*

Key words: bacterial strains, Danube – Delta Biosphere Reserve, bioremediation, textile dyes, heavy metals, salinity

Rezumat. *În studiul de față au fost izolate 19 tulpini bacteriene din tipuri variate de sol provenite din Rezervația Biosferei Delta – Dunării, identificate molecular (secvențiere 16S rDNA) și testate din punctul de vedere al capacității de bioremediere a coloranților textili și a nivelului de toleranță la metale grele și salinitate crescută. Tulpinile de Pseudomonas sp., Bacillus sp. și Thalassospira sp. pot degrada 72.64% colorant RO16, iar Pseudomonas putida poate recuperara 56% din colorantul RB4. S-a constatat o bună toleranță la salinitate (8% NaCl) a tulpinilor de Pseudoarthrobacter sp., Enterobacter sp., Thalassospira sp., Bacillus sp., și Pseudomonas sp.. Majoritatea tulpinilor au tolerat cromul in concentrație de 70 ppm (Cr^{6}); tulpinile de Cupriavidus respiraculi și Pseudomonas putida au tolerat 70 ppm cadmiu (Cd^{2+}); toate tulpinile de Pseudomonas sp. au prezentat toleranță la 100 ppm plumb (Pb^{2+}). Tulpinile bacteriene selectate pot fi utilizate in procesul de bioremediere al apelor uzate din industria coloranților.*

Cuvinte cheie: tulpini bacteriene, Rezervația Biosferei Delta – Dunării, bioremediere, coloranți textili, metale grele, salinitate

INTRODUCTION

Bioremediation of contaminated soil and waste waters is a cheap alternative to physico-chemical treatments. Industrial waters are coming from various industries producing or using synthetic pigments and dyes. Due to the complexity

[1]Institute of Biological Research Iasi, Romania

of the composition of these wastewater (heavy metals, high salinity, synthetic dyes recalcitrant to biological degradation, etc.) and for the success of the biotechnological process, some preliminary studies regarding the biodegradation capacity of the dyes and, also for the tolerance of the microorganisms subjected to the new abiotic stress conditions are needed (Ali, 2010; Gadd, 2010). Biological treatments have a high efficiency in detoxifying of diluted industrial effluents and have low operational costs (Allam, 2017).

The present research aim to identify the biodiversity of the microflora isolated from various terrestrial ecosystems (Danube – Delta Biosphere Reserve) in order to select the microorganisms with native bioremediation capacity of the textile dyes and their tolerance to various toxic compounds, especially heavy metals (as components of the dye structure or from various additives used in the dyeing process).

MATERIAL AND METHOD

Sample collection. Bacteria capable of degrading the textile dyes are originated from Danube – Delta Biosphere Reserve (DDBR). Soil samples were collected from various sites and soil type and diluted 1:50 in distilled water. The slurry was plated on yeast – mannitol-agar (YMA) supplemented with Reactive Orange 16 by conventional spread plate method. Plates were incubated at 30°C for 72 hours and colonies with dye adsorbtion capabilities were picked, isolated and purified. Finally, 19 bacterial strains were isolated, identified and maintained at - 80°C in glycerol.

Phylogenetic analysis of 16S rDNA sequences. Bacterial strains were grown in liquid YMB medium and incubated for two days at 28ºC on a rotary shaker. Equal aliquots of bacterial cultures were collected by centrifugation and total genomic DNA was isolated using Bacteria DNA Preparation kit (Jena Bioscience, Germany) according to manufacturer instruction. The conserved region of 16S rDNA was amplify using the fD1 and rD1 universal primers (Weisburg *et al*.,1991) as previously described by Efrose *et al*., 2018. PCR amplification products were purified and directly sequenced on both strands using the same primers as for PCR (CEMIA, Greece). The sequences obtained from the newly isolated strains were corrected and assembled using DNA Baser v. 3.5.4 program and used in the phylogenetic analysis together with the sequences of the reference bacteria retrieved from the NCBI/GenBank database. All sequences were aligned using the CLUSTAL W software and the phylogenetic tree was built with the Neighbor-Joining method based on Kimura's two-parameter model as implemented by *MEGA7* v.*7.0*.26 software package (Kumar *et al*., 2016). Bootstrap confidence levels were calculated for 1000 replicates. The acquired sequences for the selected bacterial strains, were deposited in GenBank/NCBI database using Sequin Application (v. 13.05).

Nucleotide sequence accession numbers. The GenBank accession numbers for the 16S rDNA sequences obtained from the seven selected bacterial strains, which exhibited multiple biotechnological potential are: MH456790 (CR-B4); MH456792 (CR-B16); MH456794 (CR-B20); MH456795 (CR-B421); MH456796 (CR-B32); MH456797 (CR-B33); MH456798 (CR-B34). Accession numbers for the 16S rDNA sequences of the related reference strains are specified in the corresponding phylogenetic tree.

Screening of dye degrading bacteria. The dye biodegradation potential of newly isolated bacteria has been assessed by using various liquid culture media (mineral medium V1 and nutritive medium (TY) V2), as previous described (Stedel *et al*., 2019). Two chemically different synthetic dyes has been tested, as follows: azo-dye Reactive Orange16 (λ_{max}= 495 nm) and antraquinonic dye Reactive Blue 4 (λ_{max}= 595 nm).The stock solutions of dyes (1000 mg L^{-1}) were prepared and filter sterilized (Millipore filter, 0.22µm, Millipore Corp., Bedford, USA). The decolorization assay has been performed in 250-ml flasks contained 100 ml of culture media, supplemented with 20 ppm textile dyes. Bacterial inoculum (2%) was added and the cultures were incubated at 30°C in stationary conditions. The control flasks without inoculums and respectively, dyes were also kept as controls. The spectrophotometric readings (supernatants) were performed after six days of incubation, and the decolorization efficiency was determined as follow:

$$\text{Decolorization (\%)} = \frac{\text{initial absorbance-final absorbance}}{\text{initial absorbance}} x100$$

Stress tolerance. Heavy metal and high salinity tolerance were determined on agarized culture medium (TY) supplemented with heavy metals, at various concentrations: Cr^{6+} ($K_2Cr_2O_7$) and Cd^{2+} ($CdCl_2$) (0.1 - 70 ppm), Pb^{2+} ($Pb(NO_3)_2$) (15 - 600 ppm) and also, NaCl (w/v) (0.1; 0.5; 2.0; 4.0, and 8.0 %). The readings were made after three days of incubation at 30°C. The highest concentration of NaCl and heavy metal salts supporting bacterial growth on agarized plates was defined as the maximum tolerance level.

RESULTS AND DISCUSSIONS

1. Isolation and molecular identification of bacterial strains

A number of seven bacterial strains (CR-B2, CR- B3, CR-B4, CR-B7, CR-B8, CR-B14, CR-B16,) were isolated from agro ecosystems (harvesting sites: Pardina- Chilia Veche and Ostrovu Tataru - cultivated with corn, wheat, barley, autumn barley and rapeseed); also, twelve bacterial strains were isolated from the natural ecosystems (soils from the Murighiol - Dunavățu de Jos area, Sf. Gheorghe, Lake Saraturi, from solonceacs or alluvial soils) (CR-B20, CR-B21, CR-B23, CR-B24, CR-B25, CR-B27, CR-B28, CR-B29, CR-B31, CR-B32, CR-B33, CR-B34).

The isolates relatedness to the previously classified bacterial strains and their taxonomic assignation was assessed by the sequence analysis of 16S rDNA. Nearly complete 16S rDNA sequences were determined by sequencing and used to construct the respective dendrogram (fig. 1). Phylogenetic analysis showed a high diversity of the native bacterial strains and grouped them in ten well supported phyletic groups together with their closely related bacteria. Pair-wise analysis of the acquired sequences revealed that the native bacterial isolates exhibited 98.1 to 100% sequence identity to their closest affiliated bacterial strains comprised in the 16S rDNA phylogeny which belong to various taxonomic groups: *Variovorax* sp., *Cupriavidus* sp., *Pseudomonas* sp.; *Enterobacter* sp., *Thallasopsira* sp., *Starkeya* sp., *Paenibacillus* sp.; *Bacillus* sp.; *Rhodococcus* sp., and *Arthrobacter* sp.

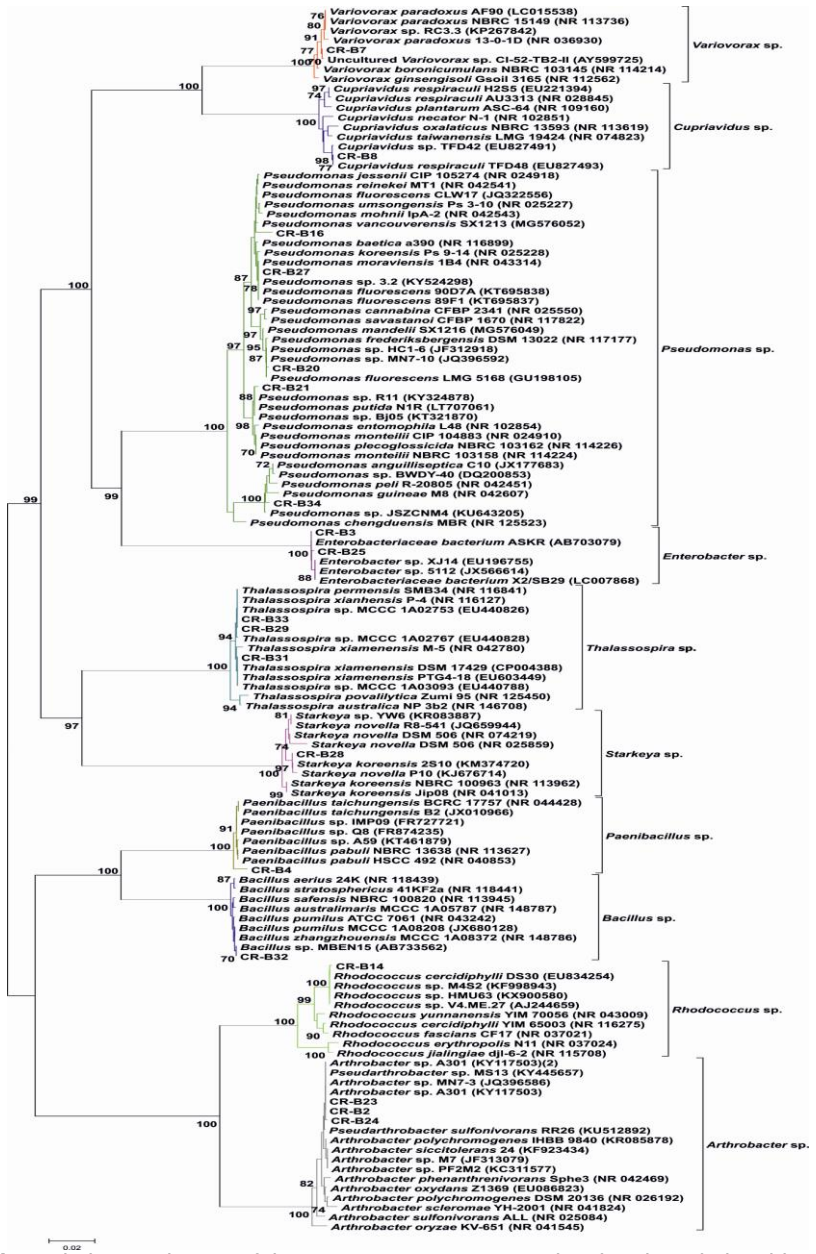

Fig.1 Phylogenetic tree of the 16S rDNA sequences showing the relationships of the newly isolated bacterial strains obtained from various habitats from DDBR with selected reference strains for recognized bacterial species. The Neighbor-Joining dendrogram was constructed using the Kimura 2-parameter model. Bootstrap values (based on 1000 replicates) below 70% are not shown. The scale bar represents 0.02 nucleotide substitutions.

2. Evaluation of the textile dyes biodegradation abilities

The native bioremediation performances of the newly bacterial strains were different. Thus, in the experimental conditions, nine of the newly bacterial strains were able to degrade the Reactive Orange 16 azo dye. Of these, six strains showed a natural decolorization capacity of over 50%, as follows: 96.56% (CR-B34 - *Pseudomonas* sp .); 91.46% - (CR-B20 - *Pseudomonas* sp.); 91% - (CR-B16 - *Pseudomonas* sp.); 91.57% - (CR-B32 - *Bacillus* sp.); 75.23% - (CR-B21- *Pseudomonas putida*) and 72.64% (CR-B33 - *Thalassospira sp.*) in stationary conditions, at 30°C, depending on the establisment of reductive conditions necessary for the degradation of the azo-linkage (after 6 days incubation). Only one strain (CR- B21 - *Pseudomonas putida*) was able to decolorize the antraquinonic RB 4 dye (tab. 1).

Table 1

Screening of soil bacteria isolates for textile dye biodegradation potential

Bacterial strains	Decolorization (%)	
	V1	V2
	Reactive Orange 16	
CR-B2 (*Pseudarthrobacter sp.*)	14.45	16.98
CR-B4 (*Paenibacillus sp.*)	36.42	67.22
CR-B16 (*Pseudomonas sp.*)	89.90	91.05
CR- B20 (*Pseudomonas sp.*)	91.46	37.87
CR- B21 (*Pseudomonas putida*)	47.76	75.23
CR-B24 (*Pseudarthrobacter sp.*)	17.98	19.23
CR-B32 (*Bacillus sp.*)	26.43	91.57
CR-B33 (*Thalassospira sp.*)	16.64	72.64
CR-B34 (*Pseudomonas sp.*)	91.98	96.56
	Reactive Blue 4	
CR- B21 (*Pseudomonas putida*)	56.73	17.46
CR-B34 (*Pseudomonas sp.)*	12.45	5.67

Reactive dyes, such as RB4 and RO16, are synthetic compounds with high water solubility and non-degradable under typical aerobic conditions, in biological treatment systems. Also, the dyes are very poorly adsorbed on the surface of biological solids, which results in a high residual dye load in effluents. The reactive dyes released into the environment represent an important pollution problem due to the coloring of surface waters (rivers), absorbing and reflecting light, which interferes with aquatic ecosystems. They also, have acute and/or chronic toxic effect on living organisms. The bioremediation efficiency of the synthetic dyes by the newly isolated strains can be further increased by optimizing the process parameters (especially carbon and nitrogen sources, pH, aerobic or anaerobic conditions, temperature).

3. Stress tolerance

Related to the high salinity tolerance, many bacterial strains manifested tolerance to high salinity (4% NaCl). The *Pseudoarthrobacter sp*. (CR-B2) and *Enterobacter sp*. (CR-B3) (among isolates from agroecosystems in the Chilia Veche area), as well as the strains of *Thalassospira sp*. (CR-B29, CR-B31, and CR-B33), *Bacillus sp*. (CR-B32)

and *Pseudomonas sp*. (CR-B34) (among isolates from solonceacs type soil, collected from the protected areas in the Murighiol area), showed a good development at the maximum concentration tested of NaCl (8%) (tab. 2).

Table 2

High salinity tolerance of the soil bacteria isolated from RBDD

No.	Geographical origin	Bacterial strains	NaCl (%)				
			0.1	0.5	2.0	4.0	8.0
1.	Chilia Veche – Pardina	CR-B2 (*Pseudarthrobacter sp*.)	+	+	±	±	±
2.	Ostrovu Tataru	CR-B3 (*Enterobacter sp.)*	+	+	+	+	±
3.	Ostrovu Tataru	CR-B4 (*Paenibacillus sp.)*	+	+	+	+	-
4.	Ostrovu Tataru	CR-B7 (*Variovorax paradoxus*)	+	+	-	-	-
5.	Ostrovu Tataru	CR-B8 (*Cupriavidus respiraculi*)	+	+	-	-	-
6.	Stationarul Pardina	CR-B14 (*Rhodococcus sp*.)	+	+	+	+	-
7.	Chilia Veche – Pardina	CR-B16 (*Pseudomonas sp.)*	+	+	+	-	-
8.	Dunavatu de Jos	CR- B20 (*Pseudomonas sp.)*	+	+	+	-	-
9.	Dunavatu de Jos	CR- B21 (*Pseudomonas putida*)	+	+	+	±	-
10.	Dunavatu de Jos	CR-B23 (*Arthrobacter polychromogenes)*	+	+	+	±	-
11	Murighiol – Sf. Gheorghe	CR-B24 (*Pseudarthrobacter sp*.)	+	+	+	±	-
12.	Murighiol – Sf. Gheorghe	CR-B25(*Enterobacter sp*.)	+	+	+	±	-
13.	Murighiol – Sf. Gheorghe	CR-B27 (*Pseudomonas fluorescens*)	+	+	++	-	-
14.	Murighiol – Saraturi Lake	CR-B28 (*Starkeya sp*.)	+	±	±	±	-
15.	Murighiol – Saraturi Lake	CR-B29 (*Thalassospira sp*.)	+	+	+	+	+
16.	Murighiol – Saraturi Lake	CR-B31 (*Thalassospira sp.)*	+	+	+	+	+
17.	Murighiol – Saraturi Lake	CR-B32 (*Bacillus sp*.)	+	+	+	+	+
18.	Murighiol – Saraturi Lake	CR-B33 (*Thalassospira sp*.)	+	+	+	+	+
19.	Murighiol – Saraturi Lake	CR-B34 (*Pseudomonas sp.)*	+	+	+	+	+

+++ very good growth ; ++ week growth; - no growth

Table 3

Heavy metals tolerance of the soil bacteria isolated from RBDD

No.	Bacterial strains	Cd^{2+}					Cr^{6+}	Pb^{2+}			
		5.0	15	30	50	70	70	70	100	200	300
1.	CR-B2 (*Pseudarthrobacter sp*.)	+	-	-	-	-	+	-			
2.	CR-B3 (*Enterobacter sp.)*	+	-	-	-	-	+	+	+		
3.	CR-B4 (*Paenibacillus sp.)*	+	-	-	-	-	+	-			
4.	CR-B7 (*Variovorax paradoxus*)	+	+	+	-	-	+	±			
5.	CR-B8 (*Cupriavidus respiraculi*)	+	+	+	+	+	+	±			
6.	CR-B14 (*Rhodococcus sp*.)	+	-	-	-	-	+	±	-	-	-
7.	CR-B16 (*Pseudomonas sp.)*	+	+	-	-	-	+	+	+	-	-
8.	CR- B20 (*Pseudomonas sp.)*	+	+	+	-	-	+	+	+	+	
9.	CR- B21 (*Pseudomonas putida*)	+	+	+	+	+	+	+	+	+	+
10.	CR-B23 (*Arthrobacter polychromogenes)*	+	-	-	-	-	±	-			
11	CR-B24 (*Pseudarthrobacter sp*.)	+	-	-	-	-	±	-			
12.	CR-B25(*Enterobacter sp*.)	+	+	-	-	-	+	±	±	-	-
13.	CR-B27 (*Pseudomonas fluorescens*)	+	+	-	-	-	+	+	+	-	-
14.	CR-B28 (*Starkeya sp*.)	+	+	-	-	-	+	±			
15.	CR-B29 (*Thalassospira sp*.)	+	+	-	-	-	+	+			
16.	CR-B31 (*Thalassospira sp.)*	+	+	-	-	-	+	+			
17.	CR-B32 (*Bacillus sp*.)	+	+	-	-	-	+	±	-	-	-
18.	CR-B33 (*Thalassospira sp*.)	+	+	-	-	-	+	±			
19.	CR-B34 (*Pseudomonas sp.)*	+	-	-	-	-	+	+	+	+	-

+++ very good growth ; ++ week growth; - no growth

Most of the bacterial strains tolerated concentrations up to 15 ppm Cd^{2+}. Two strains (CR-B7 - *Variovorax sp*. and CR-B20 - *Pseudomonas sp*.) could tolerate up to 30 ppm Cd^{2+}. More, CR-B8 (*Cupriavidus respiraculi*) and CR-B21 (*Pseudomonas putida*) strains tolerated the maximum tested concentration of 70 ppm Cd^{2+}. Related to the tolerance of bacterial isolates to Cr^{6+} additioned to the culture medium, all of them had a good development at the maximum tested concentration (70 ppm). Lower tolerance was observed in the case of *Arthrobacter sp*. (CR-B23) and *Pseudoarthrobacter sp.* (CR-B24) that were sensitive to all tested concentrations. Also, most of the strains manifested a maximum tolerance to 70 ppm Pb^{2+} while *Pseudomonas sp*. (CR-B20; CR-B21, and CR-B34) strains tolerated 100 ppm Pb^{2+}(tab. 3).

CONCLUSIONS

1. In the present work we have reported on the isolation, molecular identification and characterization of the indigenous bacterial strains with textile dye biodegradation potential.

2. The newly isolated bacterial strains obtained from various unfavorable environments from Danube Delta Biosphere Reserve were clustered, based on 16S rDNA phylogeny, in ten distinct lineages, highly affiliated to recognized bacterial species which belong to different taxonomic groups.

3. The GenBank (http://www.ncbi.nlm. nih.gov/GenBank) accession numbers for the sequences acquired from the selected bacterial strains are: MH456790 (CR-B4); MH456792 (CR-B16); MH456794 (CR-B20); MH456795 (CR-B421); MH456796 (CR-B32); MH456797 (CR-B33); MH456798 (CR-B34).

4. A significant result of this study was the isolation of bacterial strains with multiple tolerance to heavy metals, salinity and synthetic dyes, at concentrations ussualy meet in textile dye waste waters.

5. Based on the native potential of bioremediation, the newly isolated bacterial strains can be further exploited to develop a new biotechnology – based bioremediation of industrial waste waters.

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STUDY REGARDING CHEMICAL CHARACTERISTICS OF EGGS GATHERED FROM HENS REARED IN SYSTEMS APPROVED BY EUROPEAN UNION

STUDIU CU PRIVIRE LA CARACTERISTICILE CHIMICE ALE OUĂLOR PROVENITE DE LA GĂINI CRESCUTE ÎN SISTEME AGREATE DE UNIUNEA EUROPEANĂ

USTUROI AL.[1], USTUROI M.G.[1], AVARVAREI B.V.[1*], RAŢU Roxana Nicoleta[1], NISTOR C.E.[1], SIMEANU Cristina[1]
[*]Corresponding author e-mail: bogdan_avarvarei@yahoo.com

Abstract. *Analysis of which results are presented in the current paper are part of an ample study in which we focused on the influence of rearing systems on quality of eggs destined to human consumption.*
Regarding water content in yolk, we mention the fact that the highest value was founded at the eggs gathered from hens reared on ground with access to external paddock (56.12±0.006%) while the lowest value was recorded at the eggs gathered from hens reared in battery, 55.02±0.006%.
For dry matter content the obtained mean values were 43.88±0.005% for yolk of the eggs gathered from hens reared on ground with access to external paddock, 44.06±0.004% for the one gathered from hens reared in loft and 44.98±0.004% at the ones reared in battery.
Protein content from albumen recorded a calculated mean value of 12.17±0.032% for hens reared in free-range system. For hens reared in loft, protein content in mélange was 12.12±0.036% with variation limits which oscillated between 11.93% and 12.22%. For the eggs gathered from hens reared in battery, protein level in mélange was 12.21±0.035.
In the case of fat content the calculated mean value for eggs gathered from hens reared on ground with access to external paddock was 10.64±0.045%; 11.18±0.041% for the ones reared in loft and 11.22±0.049% for the eggs gathered from hens reared in battery.
Key words: consumption eggs, rearing systems, chemical composition

Rezumat. *Analizele ale căror rezultate sunt prezentate în lucrarea de faţă fac parte dintr-un studiu amplu în care s-a urmărit influenţa sistemelor de creştere asupra calităţii ouălor destinate consumului uman.*
Referitor la conţinutul de apă din gălbenuş, menţionăm faptul că valoarea cea mai ridicată a fost regăsită în cazul ouălor provenite de la găinile crescute la sol cu acces la padocul exterior (56.12±0.006%) în timp ce valoarea cea mai scăzută s-a înregistrat la ouăle provenite de la găinile crescute în baterie şi anume 55.02±0.006%.
Pentru conţinutul de substanţă uscată valorile medii obţinute au fost de 43.88±0.005% la gălbenuşul ouălor provenite de la găinile crescute la sol cu acces la padocul exterior, de 44.06±0.004% pentru cel provenit de la găinile crescute în

[1]University of Agricultural Sciences and Veterinary Medicine from Iaşi, Romania

volieră şi de 44.98±0.004% la cele crescute în baterie.
Conţinutul în proteine din albuş a înregistrat o valoare medie calculată pentru găinile crescute în aer liber de 12.17±0.032%. Pentru găinile crescute în volieră, conţinutul proteic din melanj a fost de 12.12±0.036% cu limite de variaţie ce au oscilat între 11.93% şi 12.22%. Pentru ouăle provenite de la păsările crescute în baterie, nivelul proteic din melanj a fost de 12.21±0.035.
În cazul conţinutului în grăsimi valoarea medie calculată pentru ouăle provenite de la găinile crescute la sol cu acces la padocul exterior a fost de 10.64±0.045%, de 11.18±0.041% pentru cele crescute în voliere şi de 11.22±0.049% pentru ouăle provenite de la găinile crescute în baterie.
Cuvinte cheie: ouă de consum, sisteme de creştere, compoziţie chimică

INTRODUCTION

Egg consumption at world level knows an ascendant trend in majority of countries, predictions in this way being of 2-5%/an; in the same context, is estimated an increase with 3-8% of egg production destined for consumption, as well as concentration of birds' flocks into units with a great capacity (Windhorst, 2008).

Phenomenon have at base the role of eggs in human nourishment, those ones being considered food products with a high nutritive value and with a high digestibility degree of its components, as well as stimulating for organism's metabolic functions (Mizumote *et al.*, 2008).

Rearing system influence also the birds' behaviour, being affected both productive level, but mainly the quality of obtained eggs (Petek *et al.*, 2009).

Even if, laying hens were reared into battery cages (in shelters with controlled environment) for a long period of time, this type of exploitation was vehemently challenged, especially in the last period, which is why staring with 1 of January 2012, classical rearing cages were banned in avian practice from Europe, in according with an EU directive which regulates the comfort which must be assured to laying hens, adopted in July 1999 (De Reu *et al.*, 2009).

In the alternative rearing systems, batteries with "furnished cages" (improved) have enjoyed many positive appreciations; this type of cage is equipped with elements which allow exteriorization of some birds' natural instincts (hatch for laying, abrasive stripes for claws, perches for rest, sand bath etc); additionally, the floor area per bird significantly increase in comparison with conventional cages (Ferrante *et al.*, 2009).

Even if offer a new rearing system, modified cage must be tested in practice, to certify the fact that this accommodation variant assure a good welfare state for birds, satisfactory egg productions and decreasing of mortality, provide a base for their future development into another rearing system, superior to the known one (Wang *et al.*, 2009).

Another solution for exploitation of hens which produce eggs destined to consumption is the one on a permanent layer, in shelters with controlled environment. Even if this variant assure a superior comfort to birds (presence of

layer, a greater movement freedom, existence of hatches and even perches for rest), the fact that hens are devoid of beneficial influence of external atmospherically factors lead to a less good heath state (Windhorst, 2008).

Technological variant which attracted many followers is the one of rearing in open shelters with access at external paddocks (free range) which fulfills all the welfare demands for birds (Magdelaine *et al.*, 2010).

However also this system is exposed to sanitary-veterinary risks, with unpredictable consequences on birds; another problem is raised by high contamination degree of shell of the eggs obtained into such a system with implications on eggs' sanity.

MATERIAL AND METHOD

Determination of water content.

It was established through oven drying method. In oven, samples were kept at temperature of +60°C, for 36 hours, for drying.

After this first stage, the analyzed samples were removed from oven and let to cool down for 24 hours.

Dried samples were weighted and the obtained data were introduced into mathematical formula for moisture calculus:

$$U_r\ (\%) = [(m_p - m_{p.usc})/m_p]\ x100$$

in which:

U_r = represent relative water content, (%);

m_p = mass of the sample, in grams;

$m_{p,usc.}$ = mass of dried sample, in grams.

Determination of dry matter content.

It was determined by calculus, using the following formula:

$$SU_r\ (\%) = 100(\%) - U_r\ (\%)$$

where:

U_r = represent relative water content, (%);

SU_r = relative dry matter, (%).

Determination of protein content.

It was determined by Kjeldahl method, using the same named system, which is based on the following principle: nitrogen from organic combinations, by heating with concentrated sulphuric acid, in the presence of a catalyser is transformed into ammonium sulphate.

Samples, weighted at ≈1 g, are quantitative transferred in each of those 6 digestion tubes, adding after that 3–4 g from catalyser mix ($CuSO_4$+K_2SO_4) and 25 ml H_2SO_4 96%.

Samples' digestion took place during 210 minutes, time in which the mix of sample+catalyser+reagent reaches successively three levels of temperature, as follows: 120ºC,, 240ºC, respectively 420ºC.

At the end, tubes are removed for cooling down and, before passing to distillation stage, in each ampoule are introduced 20 ml of distillate water.

For each distillation, UDK7 module consumes 50 ml NaOH 33% and 50 ml distillate water.

After cooling, digestion tubes were taken and attached to distillation port. In the capture glass of nitrogenous solution is added 25 ml H_3BO_3 4% and 5 five drops of Tashiro indicator.

In the next stage, solution for capture glass was subjected to titration with H_2SO_4 0.1N, till colour reversed from green to pale pink.

Volume of H_2SO_4 (0.1N) used for titration, as well as the other quantities of utilised reagents were introduced in the following calculus formula:

$$\text{Protein substances (\%)} = [0.0014(V_1-V_2)x6.25/m] \times 100$$

where:
0.0014 = nitrogen quantity (g) afferent to one ml of sulphuric acid 0.1 n;
V_1 = volume of sulphuric acid 0.1 n (ml) existent in capture glass;
V_2 = volume of sodium hydroxide solution 0.1 n (ml);
m = quantity of product utilised for determination (g);
6.25 = quantity of protein substances (g) afferent to one gram of nitrogen.

Determination of fat content.

It was realized through Soxhlet method, using an extraction device Velp Scientific – SER 148 type.

Analyzed samples, each with a mass into interval 2.5–3 g were packed in filter paper sachets and those ones were placed into device's cartridges, and finally attached at 3 extraction columns.

In the pots in which solvent boils was added petroleum ether at 30–60ºC (80 ml/glass) and chips for boiling uniformity In the moment in which starts the boiling of solvent, cartridges were immersed in pots, being kept in this position for around 30 minute, time in which temperature in solvent bath reached 111ºC (Immersion Phase).

In the next stage of the programme with duration of 120 minutes, cartridges with samples are taken off from solvent pots and took place a continuous washing of samples in ether vapours which flows into a closed circuit (Washing Phase).

In this stage, fats from sample, previously solvated in immersion stage, are leaked into extraction pots together with solvent.

After two hours, programme began the recovering phase (Recover Phase), with duration of 30 minutes in which the last residues of fat substances together with solvent are leaked from cartridges into extraction pots and reagent is recovered into a collecting, into a rate of ≈60% from initial quantity.

Extraction pots are removed from device's columns and are placed in oven for a complete drying and for obtaining a constant weight.

Difference between mass of the pots after extraction and their mass before extraction represent the fat quantity extracted from sample.

This quantity is related to sample mass and is expressed in percents, in according with formula:

$$G\ (\%) = [(m_2 - m_1)/m] \times 100$$

in which:
m_2 = final mass of extraction pot, (g);
m_1 = initial mass of extraction pot, (g);
m = mass of sample, (g DM).

RESULTS AND DISCUSSIONS

Results regarding quality chemical indicators of yolk

Regarding water content in yolk, we mention the fact that the highest value was founded in the case of eggs gathered from hens reared on ground with access to external paddock (56.12±0.006%) while the lowest value was recorded at the eggs gathered from hens gathered in battery, 55.02±0.006% (tab. 1).

For dry matter content the obtained mean values were 43.88±0.005% at yolk of eggs gathered from hens reared on ground with access to external paddock, 44.06±0.004% for the one gathered from hens reared in loft and 44.98±0.004% at the ones reared in battery (tab. 1).

Regarding protein level for yolk from the eggs of the hens reared on ground with access to external paddock the mean value was 13.33±0.006%, variation limits oscillating in interval 13.05% and 13.46% (tab. 1).

Studied character presented a very good homogeneity, value of variation coefficient being 0.067% (tab. 1).

Table 1

Crude chemical composition of yolk

Rearing system	n	Specification	$\overline{X} \pm s_{\overline{X}}$	V%	Min.	Max.
Eggs from hens reared on ground with access to paddock	30	*Water (%)*	56.12±0.006	0.023	57.81	58.22
		D.M. (%)	43.88±0.005	0.031	41.32	42.19
		Proteins (%)	13.33±0.006	0.128	13.04	13.54
		Fats (%)	26.53±0.019	0.463	26.10	26.92
Eggs from hens reared in loft		*Water (%)*	55.94±0.002	0.082	55.11	56.31
		D.M. (%)	44.06±0.004	0.028	43.62	44.68
		Proteins (%)	13.46±0.003	0.067	13.05	13.66
		Fats (%)	26.95±0.022	0.472	26.14	27.41
Eggs from hens reared in battery		*Water (%)*	55.02±0.006	0.031	54.80	55.93
		D.M. (%)	44.98±0.004	0.024	44.28	45.26
		Proteins (%)	13.78±0.005	0.071	13.26	13.94
		Fats (%)	27.04±0.046	0.239	26.67	27.33

At yolk of the eggs gathered from hens reared in loft, variation limits for protein level varied between 13.05% and 13.46% mean value being of 13.66±0.003% (tab. 1).

For the last eggs' category, the ones gathered from hens reared in battery was recorded a mean value for protein content of 13.78±0.005% with a minimum value of 13.26% and a maximum one of 13.94% (tab. 1).

Regarding fat content, were highlighted mean values of 26.53±0.019% for yolk provided by first category of eggs, 26.95±0.022% for the yolk provided from eggs gathered from hens reared in loft and 27.04±0.046% for the one gathred from eggs obtained by hens reared in battery (tab. 1).

Results regarding quality chemical indicators of albumen

Albumen is the egg component with the highest water content, so, for the eggs analyzed by us, the highest level was founded at the eggs gathered from hens reared on ground with access to external paddock, 88.15±0.003%, where variation limits were 88.09% and 88.22% (tab. 2).

For the eggs from hens reared in loft obtained mean of water content was 88.03±0.016% and for the ones from hens reared in battery was obtained a mean value of 87.85±0.012% (tab. 2).

Dry matter content had mean values for those there batches of: 11.85±0.003%; 11.97±0.018 and 12.15±0.023% (tab. 2).

Regarding protein content of the eggs gathered from hens reared on ground was recorded a mean value of 11.12±0.023% with variation limits between 10.94% and 11.18% (tab. 2).

Variation coefficient which was 0.431% allows us to rank the character as being a very homogenous one (tab. 2).

Table 2

Crude chemical composition of albumen

Rearing system	n	Specification	$\overline{X} \pm s_{\overline{X}}$	V%	Min.	Max.
Eggs from hens reared on ground with access to paddock	30	*Water (%)*	88.15±0.003	0.014	88.09	88.22
		D.M. (%)	11.85±0.003	0.045	11.73	11.92
		Proteins (%)	11.12±0.023	0.361	10.94	11.18
		Fats (%)	0.08±0.003	6.302	0.07	0.08
Eggs from hens reared in loft		*Water (%)*	88.03±0.016	0.034	88.01	88.12
		D.M. (%)	11.97±0.018	0.253	11.90	12.03
		Proteins (%)	11.04±0.005	0.168	10.98	11.08
		Fats (%)	0.08±0.004	4.621	0.07	0.08
Eggs from hens reared in battery		*Water (%)*	87.85±0.012	0.027	87.83	88.06
		D.M. (%)	12.15±0.023	0.429	12.01	12.17
		Proteins (%)	10.93±0.022	0.312	10.71	11.05
		Fats (%)	0.09±0.002	5.935	0.08	0.09

For eggs gathered from hens reared in loft the mean value established for protein content was 11.04±0.005% in conditions of a minimum value of 10.98% and a maximum one of 11.08% (tab. 2).

The last category of eggs, the ones gathered from hens reared in battery, recorded a mean value for protein level of 10.93±0.022% with variation limits between 10.71% and 11.05%, and the value of variation coefficient was 0.238% (very homogenous character) (tab. 2).

The last analyzed chemical indicator was represented by fat content in albumen; for the first egg category was determined a mean value of 0.08±0.003% minimum being 0.07% and a maximum value of 0.08% (tab. 2).

Variation coefficient was 7.207%, defining this character as being very homogenous (tab. 2).

For the eggs gathered from hens reared in loft the level of fat from albumen recorded a mean value of 0.08±0.004% with variation limits of 0.07% and 0.08% (tab. 2).

Also, in this case the character was very homogenous, variation coefficient being 5.734% (tab. 2).

For the last category of eggs, the ones gathered from hens reared in battery was obtained a mean value of 0.09±0.002%, minimum value being 0.08% and the maximum value was 0.09%; character being also very homogenous (V% = 5.935) (tab. 2).

Results regarding quality chemical indicator of mélange

Regarding chemical composition of mélange at the level of water content, this one was 75.63±0.021% for the eggs gathered from hens reared on ground in shelters with access to external paddock (tab. 3).

Variation limits were into interval 75.12% and 75.96% (tab. 3), variation coefficient being 0.153%, fact which shown a very good homogeneity of the parameter (tab. 3).

For the eggs gathered from eggs reared in loft the obtained mean value for water content was 75.34±0.063% and for the ones gathered from hens reared in battery was 74.81±0.072% (tab. 3).

Table 3

Crude chemical composition of mélange

Rearing system	n	Specification	$\overline{X} \pm s_{\overline{X}}$	V%	Min.	Max.
Eggs from hens reared on ground with access to paddock	30	*Water (%)*	75.63±0.021	0.172	75.12	75.96
		D.M. (%)	24.37±0.046	0.371	24.02	24.63
		Proteins (%)	12.17±0.032	0.572	12.00	12.28
		Fats (%)	10.64±0.045	1.340	10.46	10.92
Eggs from hens reared in loft		*Water (%)*	75.34±0.063	0.122	75.02	75.37
		D.M. (%)	24.66±0.023	0.298	24.24	24.95
		Proteins (%)	12.12±0.036	0.632	11.93	12.22
		Fats (%)	11.18±0.041	1.139	11.02	11.41
Eggs from hens reared in battery		*Water (%)*	74.81±0.072	0.194	74.32	75.16
		D.M. (%)	25.19±0.083	0.769	24.92	25.41
		Proteins (%)	12.21±0.035	1.718	11.89	12.45
		Fats (%)	11.22±0.025	0.832	11.02	11.46

Regarding dry matter content the mean values were of 24.37±0.046% for the first category of eggs, 24.66±0.023% for the mélange of eggs gathered from hens in loft and 25.19±0.083% at the mélange of eggs gathered from hens reared in battery (tab. 3).

Studied character presented a good homogeneity, value of variation coefficient being into interval 0.298% and 0.769% (tab. 3).

Protein content from albumen recorded a calculated mean value of 12.17±0.032% for hens reared in open air with a minimum of 12.00% and a maximum value of 12.28% (tab. 3).

Regarding studied character this one presented a very good homogeneity, value of variation coefficient being at a level 0.572% (tab. 3).

For hens reared in loft, protein content from mélange was of 12.12±0.036% with variation limits which oscillated between 11.93% and 12.22% (tab. 3).

The studied character presented also in this case a very good homogeneity, value of variation coefficient being of 0.632% (tab. 3).

For eggs gathered from birds reared in battery, protein level in mélange was of 12.21±0.035% minimum being 11.89% and maximum value being 12.45%; value of variation coefficient was 1.718% showing a very good homogeneity of

the character (tab. 3).

For fat content the calculated mean value for eggs gathered from hens reared on ground with access at external paddock was 10.64±0.045%; 11.18±0.041% for the ones reared in loft and 11.22±0.049% for the eggs gathered from hens reared in battery (tab. 3).

In all three cases character was a very homogenous one, variation coefficients being of 1.340%; 1.139%, and respectively 0.832% in case of eggs gathered from hens reared in battery (tab. 3).

CONCLUSIONS

1. Regarding water content in yolk, we mention the fact the highest value was obtained in the case of eggs gathered from hens reared on ground with access of at external paddock (56.12±0.006%) while the lowest value was recorded at the eggs gathered from hens reared in battery, namely 55.02±0.006%.

2. For dry matter content the obtained mean values were 43.88±0.005% for yolk of eggs gathered from hens reared on ground with access to external paddock, 44.06±0.004% for yolk of eggs gathered from hens reared in loft and 44.98±0.004% for yolk of the eggs from hens reared in battery.

3. Regarding protein level for yolk from the eggs of the hens reared on ground with access to external paddock the mean value was 13.33±0.006%, variation limits oscillating in interval 13.05% and 13.46%.

Studied character presented a very good homogeneity, value of variation coefficient being 0.067%.

At yolk of the eggs gathered from hens reared in loft, variation limits for protein level varied between 13.05% and 13.46% mean value being of 13.66±0.003%.

For the last eggs' category, the ones gathered from hens reared in battery was recorded a mean value for protein content of 13.78±0.005% with a minimum value of 13.26% and a maximum one of 13.94%.

4. Regarding fat content, were highlighted mean values of 26.53±0.019% for yolk provided by first category of eggs, 26.95±0.022% for the yolk provided from eggs gathered from hens reared in loft and 27.04±0.046% for the one gathred from eggs obtained by hens reared in battery.

5. Albumen is the egg component with the highest water content, so, for the eggs analyzed by us, the highest level was founded at the eggs gathered from hens reared on ground with access to external paddock, 88.15±0.003%, where variation limits were 88.09% and 88.22%.

For the eggs from hens reared in loft obtained mean of water content was 88.03±0.016% and for the ones from hens reared in battery was obtained a mean value of 87.85±0.012%.

6. Dry matter content had mean values for those there batches of: 11.85±0.003%; 11.97±0.018 and 12.15±0.023%.

7. Regarding protein content of the eggs gathered from hens reared on

ground was recorded a mean value of 11.12±0.023%.

For eggs gathered from hens reared in loft the mean value established for protein content was 11.04±0.005%.

The last category of eggs, the ones gathered from hens reared in battery, recorded a mean value for protein level of 10.93±0.022%.

8. The last analyzed chemical indicator was represented by fat content in albumen; for the first egg category was determined a mean value of 0.08±0.003%.

For the eggs gathered from hens reared in loft the level of fat from albumen recorded a mean value of 0.08±0.004%.

For the last category of eggs, the ones gathered from hens reared in battery were obtained a mean value of 0.09±0.002%.

9. Regarding chemical composition of mélange at the level of water content, this one was 75.63±0.021% for the eggs gathered from hens reared on ground in shelters with access to external paddock.

For the eggs gathered from eggs reared in loft the obtained mean value for water content was 75.34±0.063% and for the ones gathered from hens reared in battery was 74.81±0.072%.

10. Regarding dry matter content the mean values were of 24.37±0.046% for the first category of eggs, 24.66±0.023% for the mélange of eggs gathered from hens in loft and 25.19±0.083% at the mélange of eggs gathered from hens reared in battery.

11. Protein content from albumen recorded a calculated mean value of 12.17±0.032% for hens reared in open air.

For hens reared in loft, protein content from mélange was of 12.12±0.036% with variation limits which oscillated between 11.93% and 12.22%.

For eggs gathered from birds reared in battery, protein level in mélange was of 12.21±0.035%.

12. For fat content the calculated mean value for eggs gathered from hens reared on ground with access at external paddock was 10.64±0.045%; 11.18±0.041% for the ones reared in loft and 11.22±0.049% for the eggs gathered from hens reared in battery.

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OPTIMIZATION OF TOMATO CULTIVATION TECHNOLOGY IN PROTECTED AREAS THROUGH THE USE OF CONTINUOUS ELECTRIC CURRENT

OPTIMIZAREA TEHNOLOGIEI DE CULTIVARE A TOMATELOR ÎN SPAȚII PROTEJATE PRIN UTILIZAREA CURENTULUI ELECTRIC CONTINUU

GHEORGHIȚOAIE M.V.[1], ACHIȚEI V.[1], BODALE I.[1], TELIBAN G.C.[1], STOLERU V.[1*]

[*]Corresponding author, e-mail: vstoleru@uaiasi.ro

Abstract. *Electric and magnetic fields of different intensities can influence the metabolism of plants. The results presented in this paper particularly follow the morphological changes that the electricity may have on tomato plants. The electric and magnetic field has been applied constantly on plants, from the seedling stage, to the moment of reaching the technological maturity of the fruits.*
Key words: tomato, electric field, magnetic field

Rezumat. *Câmpurile electrice şi magnetice de diferite intensităţi pot influenţa metabolismul plantelor. Rezultatele prezentate în această lucrare urmăresc în mod deosebit modificările morfologice pe care curentul electric continuu le poate avea asupra plantelor de tomate. Câmpul electric şi cel magnetic a fost aplicat în mod constant pe plante, de la stadiul de răsad, până la momentul atingerii maturităţii tehnologice a fructelor.*
Cuvinte cheie: tomate, câmp electric, câmp magnetic

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 (Topkins and Bird, 1973; Belyavskaya, 2004). The use of synthetic chemicals has a significant effect of increasing the yield of production, but it has been found that these substances endanger human health (Ahmand and Wani, 2013; Cakmak *et al*., 2010).

Fluctuation of biometric characteristics of the plant is caused by the ion exchange between apoplastic and symplastic space transmitting signals in the phloem wich create a change of electric potential on both sides on membrane cells (Black *et al.,* 1971; Dannehl *et al*., 2011; Rochalska, 2008).

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 (Dayal and Singh, 1986; Blackman, 1924; Collins *et al.,* 1929). For this

[1]University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

purpose, five digital regulated DC power supplies, medical needles, and electrical wires were used (Ward, 1996; Dubinin and Vaulina, 1979; Dannehl *et al.,* 2011).

MATERIAL AND METHOD

Starting from this goal, the aim of the research was to use a continuous electric current in the tomato hybrid Qualitet F1, a hybrid with semi-determined growth that adapts easily to the crops under different technological conditions. In the experiment, six variants of continuous current with an intensity of 0.15 A, 0.30 A and 0.45 A. were used, each variant having a number of 4 repetitions.

For the first three variants, an electric field was used to generate a current that flowed through a spiral wire around plant organs for each repetition. The current intensity were of 0.15 A, 0.30 A, and 0.45 A.

The fourth variant was represented by four plants with two medical needles inserted into each plant. One needle was inserted into the plant stem, and the other into the apical area. Both medical needles were connected to a DC power supply with a current of 1.5 V (the positive pole being connected at the apical area, and the negative one at the base of the plant).

In the case of the fifth variant, two copper electrodes were inserted into the plant substrate. Both electrodes were connected to a DC power supply with a current of 1.5 V.

The sixth variant was for reference. Plants were grown under the same ecological conditions and with the same culture technology.

RESULTS AND DISCUSSIONS

For this experiment, morphological particularities were observed, reaching the following results.

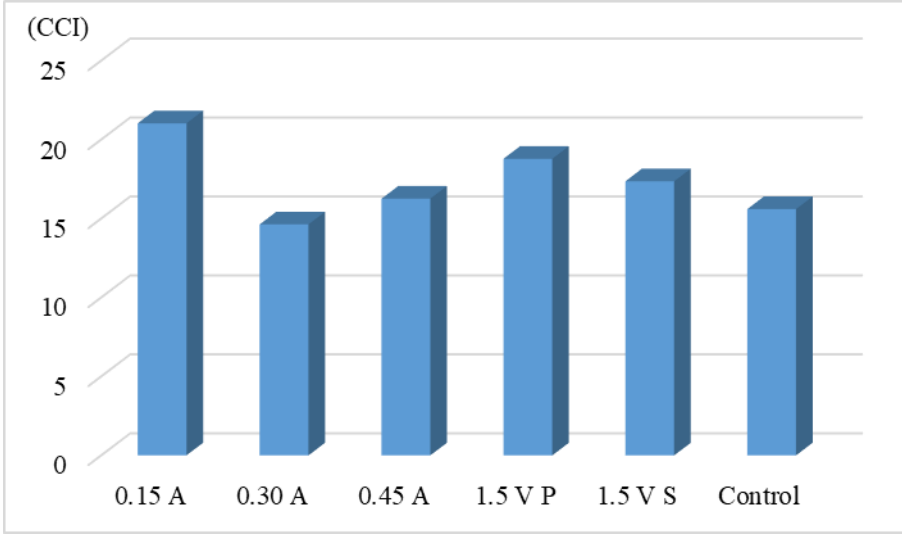

Fig. 1 Chlorophyll content in tomato plant

According to the measurements, the highest chlorophyll content was recorded in the case of the sample where an electric current with a intensity of 0.15 A was used (fig. 1).

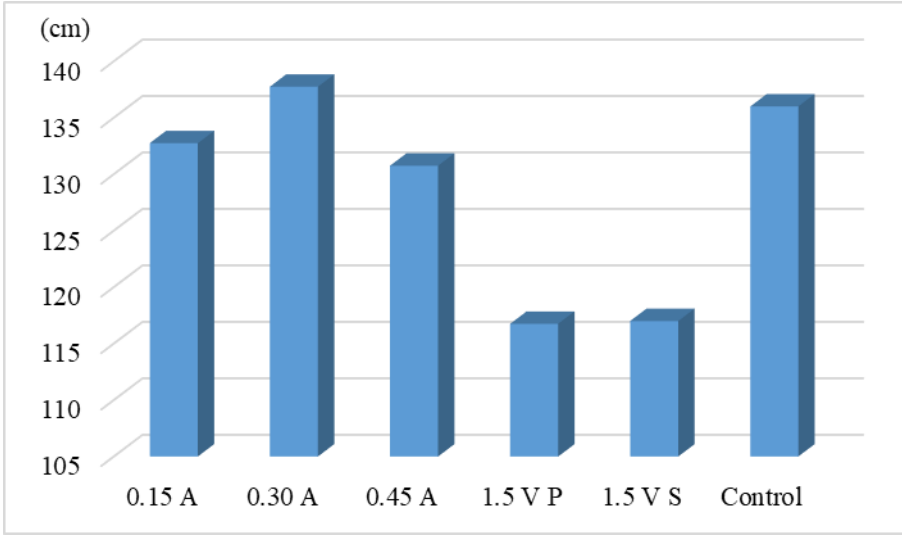

Fig. 2 Plants height of tomato

Another morphological parameter which was measured was the height of plants (fig. 2). The results indicated a wider development in the case of the sample where an electric current intensity of 0.30 A was used.

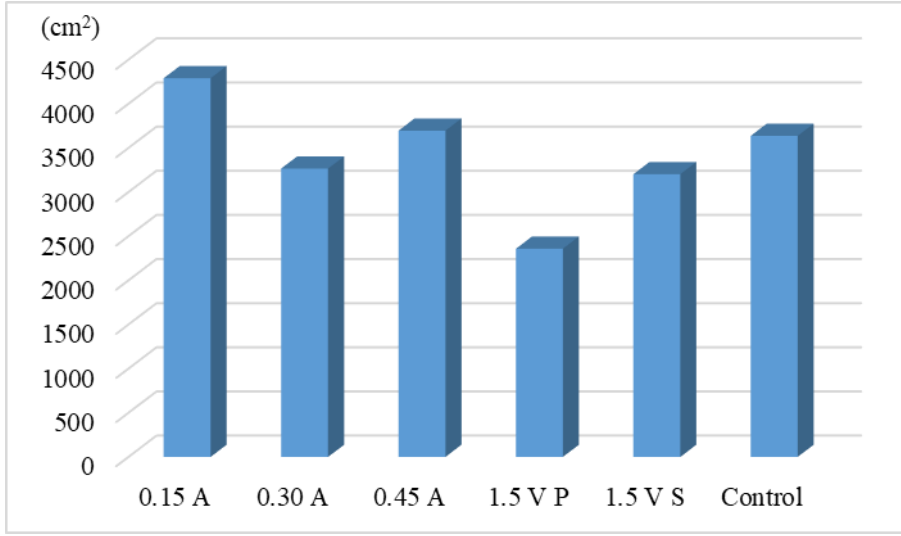

Fig. 3 Tomato area surface

The leaves area was measured using Li-3100 LI-COR. The best results were obtain again for the sample that used an DC current with an intensity of 0.15 A (fig. 3).

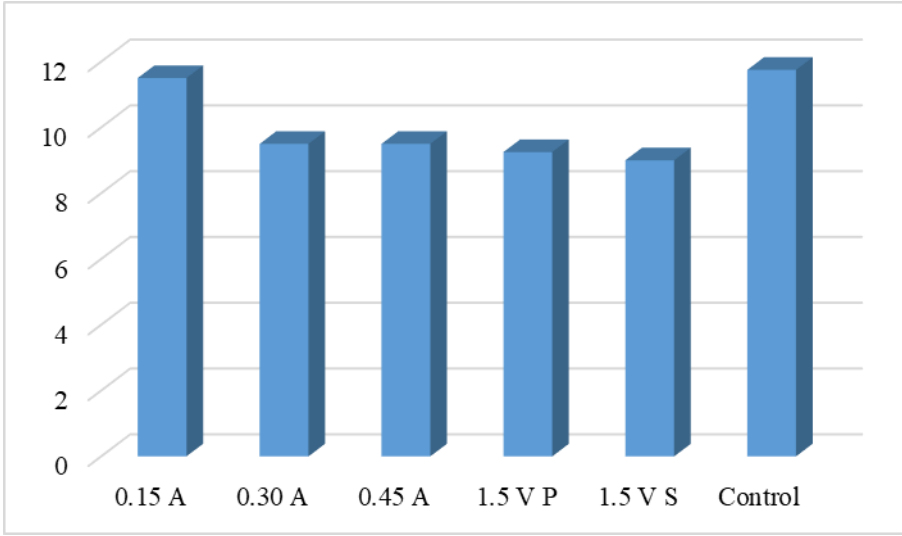

Fig. 4 Tomato leaves number

The average number of leaves (fig. 4) 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.

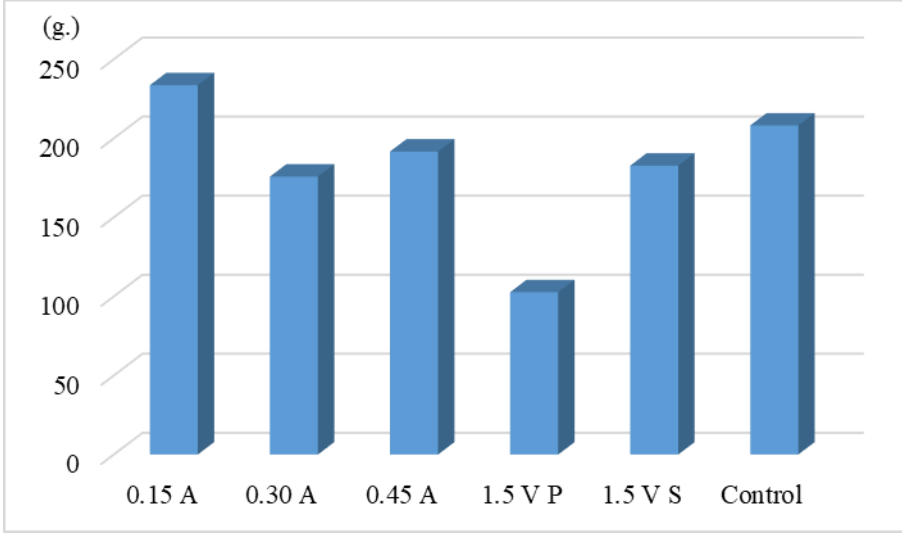

Fig. 5 Tomato plants weight

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 (fig. 5). 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 biological electrodes in the apical area and at the bottom of the stem of tomato plants could be observed a significant lower rate of development.

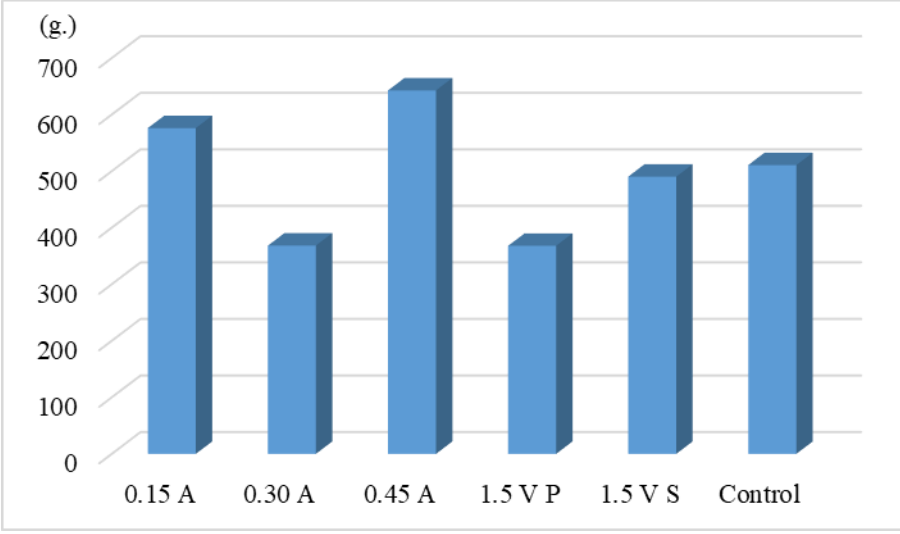

Fig. 6 Average fruits weight of tomato

The best results for the average fruits weight was registred 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 (fig. 6).

CONCLUSIONS

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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THE INFLUENCE OF FERTILIZATION AND MULCHING ON THE EARLY CAULIFLOWER CROP

INFLUENŢA MULCIRII ŞI FERTILIZĂRII ASUPRA CULTURII DE CONOPIDĂ TIMPURIE

TELIBAN G.C.[1], MUNTEANU N.[1], PRECUPEANU Cristina[1], POPA Diana[2], BURDUCEA M.[3], STAN T.[1], ZAICA Ana[4], TALLARITA A.V.[5], STOLERU V.[1*]

[*]Corresponding author, e-mail: vstoleru@uaiasi.ro

Abstract. *The purpose of the present paper is to evaluate the influence of mulching and fertilization on the early cauliflower crop. The research was carried out in the didactic and experimental field of the Vegetable growing discipline, at Agronomy University Iasi. The experiment was of a bifactorial type, testing the influence of mulching with three graduations (unmulched-Ct, mulching with polyethylene film of 15 and 30 µm in thickness) and of fertilization with four graduations (unfertilized-Ct, chemical fertilization, organic fertilization and application of microorganisms), under the conditions of 2018 and 2019. The crop was established by using seedlings of 42 days, in strips of two rows, the distance between strips being of 80 cm and between rows of 60 cm. The distance between the plants in a row was of 25 cm, resulting in a density of about 57 thousand plants per hectare. The results obtained demonstrate the necessity of mulching and fertilizing the early cauliflower crop, the best yields being obtained in the case of the variant mulched with 30 µm foil and fertilized with chemical fertilizers.*

Key words: technological factors, yields, chlorophyll pigments

Rezumat. *Scopul lucrării de faţă este de a evalua influenţa mulcirii şi a fertilizării la cultura de conopidă timpurie. Cercetările au fost efectuate în câmpul didactic şi experimental al disciplinei de Legumicultură, U.S.A.M.V. Iaşi. Experienţa a fost de tip bifactorial, fiind testată influenţa mulcirii cu trei graduări (nemulcit-Mt, mulcire cu folie de polietilenă de 15 şi 30 µm grosime) şi a fertilizării cu patru graduări (nefertilizat-Mt, fertilizare chimică, fertilizare organică şi aplicarea de microorganisme) în condiţiile anilor 2018 şi 2019. Cultura a fost înfiinţată prin răsad de 42 de zile, în benzi de câte două rânduri, distanţa dintre benzi fiind de 80 cm, iar dintre rânduri de 60 cm. Distanţa dintre plante pe rând a fost de 25 cm, rezultând o densitate de aproximativ 57 mii plante pe hectar. Rezultatele obţinute demonstrează necesitatea mulcirii şi fertilizării culturii de conopidă timpurie, producţiile cele mai bune fiind obţinute în cazul variantei mulcite cu folie de 30 µm şi fertilizată cu îngrăşăminte chimice.*

Cuvinte cheie: factori tehnologici, producţii, pigmenţi clorofilieni

[1]University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania
[2]Agricultural Research and Development Station Secuieni-Neamt, Romania
[3]"Alexandru Ioan Cuza" University of Iasi, Romania
[4]INMA Bucharest, Romania
[5]University of Naples Federico II, Italy

INTRODUCTION

Among the technological measures that ensure a high and early yield in the early cauliflower crop, mulching and fertilization play a significant role (Ferdous *et al*., 2017).

Soil mulching is one of the research topics of interest, because it has special influence both on the obtained yield (quantity, quality, precocity) and the soil (the activity of soil microorganisms, its physical and chemical properties). This influence has a beneficial role, which provides mulched lands with this character of sustainability (Stan, 2010; Depalo *et al*., 2016). However, mulching has some disadvantages as well, expressed by the creation of a barrier for oxygen and water, creating differences between the soil temperature and that of the environment (Caruso *et al*., 2019a).

The success of a cauliflower crop also depends on the adequate supply of water and nutrients. The fertilization can be carried out using organic or chemical fertilizers, these being the most commonly used. Lately research is aimed at the use of microorganisms with a role in soil microbiota activity and on plant growth and development (Caruso *et al*., 2019b).

All these aspects lead to the need for in-depth research of these measures. In this context, the purpose of the present research is to evaluate the influence of mulching and fertilization on the early cauliflower crop.

MATERIALS AND METHOD

The research was organized under the experimental conditions of 2018 and 2019 years, at the Didactic Station of Agronomy University Iasi. The experiment was carried out on a soil of the chernozem medium leachate type, with a medium content of nutrients, with 3% organic substance and pH = 6.5. The weather conditions from the experimental period were favorable for this crop.

The experimental protocol dictated the organization of a bifactorial experiment, organized in split plot design, with three repetitions (fig. 1). The influence of two experimental factors was studied in the open field using an early cauliflower hybrid - Vassallo - recommended for an early crop (https://seminte-ingrasaminte-turba.ro/product/conopida-vassallo-f1-crx-13202/):

Factor A – mulching application with three graduations: a_1 = unmulched, mulched with a standard black light density polyethylene film, 15 µm and 30 µm in thickness (a_2 = LDPE 15 µm and a_3 = LDPE 30 µm);

Factor B - four-graduations of fertilization: b_1 = unfertilized variant - as control (Ct), b_2 = chemical fertilization with Nutrispore 30:10:10 (Ch), b_3 = organic fertilization with Orgevit (O), b_4 = application of microorganisms, Micoseeds MB (M).

In both experimental years, the crop was established by using seedlings of 42 days, on April 12th, in strips of two rows each, at distance of 80 cm and between rows of 60 cm. The distance between the plants in a row was of 25 cm, resulting in a density of about 57 thousand plants/hectare (Popescu and Zăvoianu, 2011). The work carried out during the vegetation period was according with specialized literature (Stan and Munteanu, 2001; Zăvoianu, 2010; Ardelean, 2013) consisted of irrigation, (done by dripping), weeding (manually) and two fertilizer application. The first round of fertilization was done before planting - the starter fertilization, with 200 kg Nutrispore,

200 kg Orgevit, and with 60 kg Micoseeds MB, respectively. The second round of fertilization, the root-phase fertilization, was applied out three weeks after planting, using the same fertilizer doses.

Performance of experimental variants was done by evaluation of the yield (kg/ha) and the total chlorophyll pigments (CCI - chlorophyll content index).

The harvest of the yield was done at proper commercial stage of the curd and the chlorophyll contents were achieved before harvesting, by using the CCM-200 plus (Chlorophyll Content Meter).

Fig. 1 Aspects from the cauliflower experiment

The experimental data were processed by appropriate statistical-mathematical methods (Jitareanu, 1999; Leonte and Simioniuc, 2018). The least significant differences (LSD) test was used for the yields, and the Tukey test for chlorophyll content.

RESULTS AND DISCUSSIONS

A. Influences on the yield

The results obtained regarding the influence of mulching on the yield are presented in table 1.

The best results were obtained in the case of mulching with 30 µm foil (22586 kg/ha), with an yield increase of 3976.2 kg/ha (21.37%), compared to the unmulched (control) variant, while the variant mulched with 15 µm foil achieved an yield of 21613 kg/ha, with an yield increase of 3002.8 kg/ha (16.14%), compared to the control variant (18610 kg/ha).

In both cases, the yield differences are positive, statistically covered at a significant level, compared to the unmulched control variant.

Table 1

Results obtained regarding the influence of mulching on the cauliflower yield

No. crt.	Mulch type	Yield			Difference significance
		kg/ha	% compared to the Control	Difference compared to the Control	
1.	Unmulched	18610	100.00	0.0	Ct
2.	LDPE 15 µm	21613	116.14	3002.8	*
3.	LDPE 30 µm	22586	121.37	3976.2	*

LSD 5% = 2537.1 kg/ha; LSD 1% = 4207.2 kg/ha; LSD 0.1% = 7857.8 kg/ha

Regarding the influence of the fertilization on the early cauliflower yield (tab. 2), the highest yield was obtained in the case of the variant fertilized with Nutrispore 30:10:10, which ensured an yield increase of 13852.4 kg/ha, compared to the unfertilized variant.

Table 2

Results obtained regarding the influence of fertilization on the cauliflower yield

No. crt.	Treatment	Yield			Difference significance
		kg/ha	% compared to the Control	Difference compared to the Control	
1.	Unfertilized	15685.7	100.00	0.0	Ct
2.	Ch	29538.1	188.31	13852.4	***
3.	O	20314.3	129.51	4628.6	***
4.	M	18206.9	116.07	2521.2	*

LSD 5% = 2356.9 kg/ha; LSD 1% = 3232.3 kg/ha; LSD 0.1% = 4399.6 kg/ha

From a statistical point of view, the variants treated with different fertilizers achieved yield increases with different degrees of significance, such as: the chemically and organically fertilized variants have presented very significant positive differences, compared to the unfertilized variant (88.31%, and 29.51% respectively), while the variant with microorganisms, registered significant positive differences (16.07%).

Regarding the combined influence of mulching and fertilization (tab. 3), the results highlight the positive influence of the two experimental factors on the early cauliflower yield. The variants in which the mulching and the chemical or organic fertilization were performed simultaneously produced very significant positive differences, compared to the unmulched and unfertilized control variant. Also, the unmulched, but fertilized variant with chemical fertilizers, produced very significant positive results.

The application of microorganisms determined distinctly significant positive differences, in both variants, where soil mulching was performed.

Table 3

Results regarding the influence of the mulching x fertilization combination on the cauliflower yield

No. crt.	Variant	Yield			Difference significance
		kg/ha	% compared to the Ct	Difference compared to the Ct	
1.	Unmulched x unfertilized	13392.1	100.00	0.0	Ct
2.	Unmulched x Ch	28500.0	212.81	15107.9	***
3.	Unmulched x O	18328.6	136.86	4936.5	*
4.	Unmulched x M	14219.0	106.18	827.0	-
5.	LDPE 15 µm x unfertilized	16328.6	121.93	2936.5	-
6.	LDPE 15 µm x Ch	28901.6	215.81	15509.5	***
7.	LDPE 15 µm x O	21257.1	158.73	7865.1	***
8.	LDPE 15 µm x M	19963.5	149.07	6571.4	**
9.	LDPE 30 µm x unfertilized	17336.5	129.45	3944.4	-
10.	LDPE 30 µm x Ch	31212.7	233.07	17820.6	***
11.	LDPE 30 µm x O	21357.1	159.48	7965.1	***
12.	LDPE 30 µm x M	20438.1	152.61	7046.0	**

LSD 5% = 4082,3 kg/ha; LSD 1% = 5598,6 kg/ha; LSD 0.1% = 7620,3 kg/ha

B. Influences on the chlorophyll content

The results regarding the content of chlorophyll pigments, presented in fig. 2, ranges from 30.2 CCI in the unmulched variant fertilized with Micoseeds MB, to 57.2 CCI in the version mulched with 30 µm polyethylene film, which was chemically fertilized.

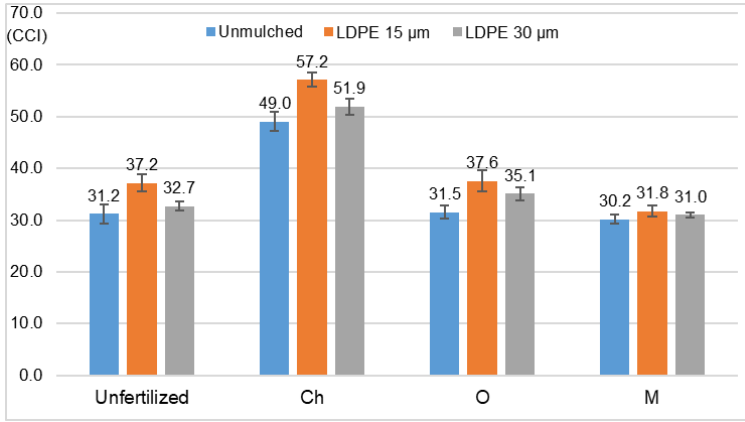

Fig. 2 Results regarding the chlorophyll pigments content in the early cauliflower crop

The chemical fertilization produced the highest values of the CCI, but the lowest values were recorded in the variants fertilized with microorganisms. Regarding mulching, the highest values were recorded in the variants mulched with 15 µm foil, and the lowest values of the CCI coefficient are recorded in the unmulched variants.

CONCLUSIONS

1. The results obtained recommend the use of mulching and fertilization on the early cauliflower crop, fertilization having the primary role in achieving high yields.

2. The best results were obtained in the case of the chemically fertilized variants, which produced the highest yield increase, regardless of the mulching variant used.

3. The highest values of the CCI index are correlated with the highest productions registered in the variants fertilized with chemical fertilizers.

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ECOLOGICAL SOLUTIONS REGARDING THE MONITORIZATION OF THE URBAN ATMOSPHERE POLUTION INVOLVING BIOINDICATING TREES ON THE MAIN STREET ALINEAMENTS IN IAŞI

SOLUȚII ECOLOGICE PRIVIND MONITORIZAREA POLUĂRII ATMOSFERICE URBANE CU AJUTORUL ARBORILOR BIOINDICATORI PE ALINIAMENTELE STRADALE PRINCIPALE DIN MUNICIPIUL IAŞI

SANDU Tatiana[1]*,TROFIN Alina -Elena[1], BERNARDIS R.[1], GRECU Codrina[1]
*Corresponding author email: tatiana_sandu69@yahoo.com

Abstract. *Recent data underline that the evolution of the urban environment in Iaşi was mostly based on developing the street system infrastructure in detriment of the urban green areas, what led inevitably to the intensification of traffic and holding back the interest for developing in a sustainable mode a network of urban green areas. In many European countries a program of monitoring involving bio indicating trees is already implemented, based on the response of these "sentinel trees" to harsher life conditions of the intense traffic and poluted streets compared to the rest of the green areas. The present study aims the importance of dendrological street plantations through their positive impact on the fight against direct pollution toward the pedestrian traffic. The observations were conducted in alignments plantations from three main boulevards in Iaşi, underlining the insufficiency of woody vegetation, the precarious health state of most of the mature specimens, caused by improperly branch cutting, discontinuity of the green vegetation lines or the lack of them. The paper will propose solutions and landscaping measures meant to improve the quality and safety of the pedestrian traffic along the considered boulevards.*
Key words: the urban green areas, bio indicating trees, alignments.

Rezumat. *Ultimele constatări evidențiază că evoluția mediului urban ieşean s-a bazat mai mult pe dezvoltarea infrastructurii rutiere în detrimentul spațiilor verzi urbane, ceea ce a condus în mod inevitabil la intensificarea traficului auto şi la plasarea în plan secundar a interesului pentru conturarea sustenabilă a unei rețele de spații verzi urbane. În multe țări europene este implementat deja un program de monitorizarea a arborilor bioindicatori, pe baza răspunsului acestor "arbori santinelă" la condițiile de viață mai dure ale zonelor stradale aglomerate şi poluate, comparativ cu restul spațiilor verzi. Studiul de față vizează evidențierea importanței plantațiilor dendrologice stra-dale prin impactul pozitiv pe care acestea îl au asupra combaterii poluării directe la nivelul circulațiilor pietonale. Observațiile au fost efectuate în plantațiile de aliniament de la nivelul a trei bulevarde ieşene principale, evidențiind insuficiența vegetației lemnoase, starea fitosanitară precară a majorității exemplarelor mature de arbori, cauzată de aplicarea tăierilor encores-punzătoare în coroană, discontinuitatea fâşiilor verzi sau lipsa acestora. Se vor propune pe vitor soluții şi măsuri peisagere care să amelioreze calitatea şi siguranța traficului pietonal la nivelul bulevardelor analizate.*
Cuvinte cheie: spații verzi urbane, arbori bioindicatori, aliniamente.

[1] University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

INTRODUCTION

Research on polluted air in major cities (Barnes,1972, cited by Peneghi, 2015) has shown that emissions from car transport in the modern urban atmosphere constitute over 90% of the total volume, which is why it is considered that fossil fuel based car transport is the main polluter of urban airspace. .

The European Parliament and the Council of the European Union issued Directive 2008/50 / EC of 21 May 2008 on air quality for the region of Europe, which regulates ambient air quality policies and obliges Member States of the European Union to reduce emissions. of PM2.5 and PM10 in urban areas. In our country, it is required, by Law 104/2011, that the values allowed by PM2.5 and PM10 be maximum 50 μg / m3 for PM10 and 25μg / m3 for PM2.5 in the air breathed (Oros, 2002).

It is known that there is a big difference between the very harsh living conditions (vegetation) of the trees in the road alignments compared to the conditions in the other categories of green spaces in the urban area (Bolea et Chira, 2008). Bio-indicator trees, also called "sentinel trees", can accurately reproduce the level of pollution, they are monitored to determine the degree of pollution and the type of pollutants due to which the woody vegetation is suffering. In other words, bio-indicators integrate the pollution produced over a certain period of time and reflect the effects of this integrated pollution (Oros, 2002).

The current situation of the urban environment of Iasi reveals an un-controlled extension of the urban area, which thus leads to an increase in the traffic of cars, to the detriment of the urban green spaces. This situation is strongly highlighted due to the development of the residential areas, in the absence of a judicious management of the road infrastructure and an increasingly intense traffic. The main link between the north and south of the city is represented by the Nicolina Road - Nicolina Street - Palat Street - Anastasie Panu Street - Elena Doamna Street - Independence Boulevard - Carol I Boulevard - Grigore Ghica Vodă Boulevard, thus becoming the most intensely circulated arteries. So the pedestrian traffic at the level of these boulevards and streets is conducted under unfavorable conditions of pollution with noxes and noise pollution.

The city of Iaşi is included in the management regime I for the polluting particulate matter PM10, based on the results obtained after the evaluation of the air quality, using measurements at fixed points, with the help of the 4 fixed measuring stations that are part of the National Monitoring Network. of Air Quality, as well as results regarding the modeling of the dispersion of pollutants in air, observations made based on local emission inventories.

The studies carried out by the researchers of the Physics laboratory of the University "Alexandru Ioan Cuza" showed that the limits allowed for both types of particles were exceeded, the measurements indicating values that exceed, sometimes, two or even three times the limits imposed by law (tab. 1).

Table 1

Indices of the suspended particles PM2.5 and PM10 obtained by measurements at the level of the boulevards analyzed, in January 2018

Place	$PM_{2,5}$ (µg/m^3)	PM_{10} (µg/m^3)	Admitted value: $PM_{2,5}$ (µg/m^3)	Admitted value: PM_{10} (µg/m^3)
Palatul Culturii	**76**	**174**	**25**	**50**
Palas	56	116		
Bulevardul Independenței	54	143		
Copou (U.A.I.C.)	53	140		

Source:https://www.bzi.ro/exclusiv-harta-explicita-cu-cele-mai-poluate-cartiere-din-orasul-iasi-foto-636358

The possibility of monitoring urban pollution in Iasi by observing the reaction of bio-indicator trees is considered to be about 15 times cheaper than using a network of physico-chemical analyzers (Ruscă and Rusu, 2018). The present paper highlights the need to monitor the highly polluted areas within the city of Iași using the biological response of the bio-indicator trees for the pollution with powders and exhaust gases.

MATERIAL AND METHOD

The observations were made between May 2019 - August 2019, at the level of Carol I, Independence and Strada Palat boulevards in Iasi. To begin with, it was necessary to illustrate the situation of the analyzed areas by highlighting the distances between the built and road areas, marking the situation of the road alignments and the location of the road alignments and highlighting the examples of bio-indicator trees, graphically presented in this paper in the AUTOCAD program. The vegetal component was analyzed by direct visual observation in the field by manually measuring with the roulette wheel, in order to establish the characteristic dendrometric elements for each bioindicator tree, the planting distances between the trees in the alignments, the crown projection on the ground, the trunk diameter, phytosanitary status of specimens determined as bioindicator trees.

A number of 12 alignment trees were monitored belonging to the species *Tilia cordata*, a species mentioned in the literature as the most resistant to air pollution by exhaust gas, this fact highlighting the value of this species as a bioindicator tree. (Tugulea, 2018).

RESULTS AND DISCUSSIONS

Field observations have highlighted the fact that car traffic is the main source of emissions containing harmful particles of small size, known as PM2.5 and PM10. These particles are of the order of microns and have the ability to penetrate the respiratory system to the lungs level, having negative effects on human and animal health, such as eye irritation, coughing, sneezing, allergies, asthma, cardio-vascular problems etc. (Ghidra and Zaharia, 2002). However, in the air polluted by exhaust gases, carbon monoxide, hydrocarbon residues, benzopyrene, aldehydes, nitrogen and sulfur oxides, heavy metals, soot are also encountered etc. (Ruscă and Rusu, 2018).

The analyzed boulevards are characterized by intense pedestrian traffic on both directions of movement, the sidewalks being separated from the road by

continuous and discontinuous green lanes, and on agglomerated segments the existence of a relatively discontinuous protection lane is noted. Road alignments are predominantly composed of mature and young specimens of *Tilia cordata*, *Tilia tomentosa*, *Tilia platy-phyllos*, *Aesculus hippocastanum* and *Fraxinus excelsior*. An analysis of the quality of these alignments shows that the mature trees are approximately 80% debilitated, having an uneven crown, due in large part to the improperly performed cutting actions. Thus, it is noticeable in the level of the crowns of dry branches of the second degree but also numerous "greedy branches" that are unsightly and unevenly distributed in space. The young trees, which are meant to rejuvenate the alignment plantations, have not been properly guarded at the planting, some of them having deviations of the trunk axis, on them no cutting of crown formation was applied according to the corresponding standards. Indeed, specialists recommend that *Tilia* species do not respond well to severe crown cutting (Bolea and Chira, 2008). On the boulevard Carol I, 5 specimens of bio-indicator trees of the species *Tilia cordata* were located and taken into consideration (fig. 1).

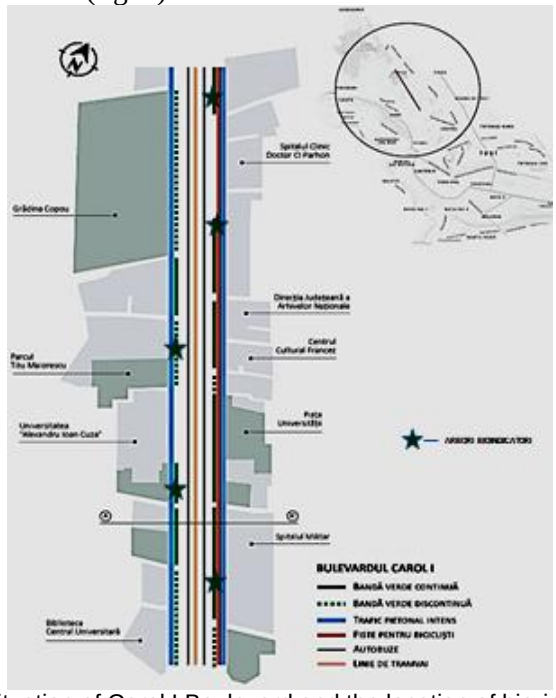

Fig. 1 The situation of Carol I Boulevard and the location of bio-indicator trees

The situation on Independence Boulevard (fig. 2) and Palat Street (fig. 3) has made it clear that for the height interval between 1.50 m and 1.70 m (the breathing interval for the average human height), there is no obstacle to the harmful factors of road traffic. The sidewalks are directly exposed to these harmful elements, since at the level of the separating green strips there are only tree trunks, without a consistent vegetative layer that will filter the emissions of

the road towards the pedestrian traffic. This analysis of the sites under study clearly highlights the need to monitor air pollution using bio-indicator trees. On these two sites, another 6 specimens of *Tilia cordata* (3 for each street alignment - Independence Boulevard and Palat street) were declared as bio-indicator trees, at distances of over 20 m between them for a better coverage of the analyzed site. . The observations will be compared with a control specimen of *Tilia cordata*, of close age with the analyzed ones, but located in the Iasi Botanical Garden, in a noxes pollution-free site due to inexisting car transport.

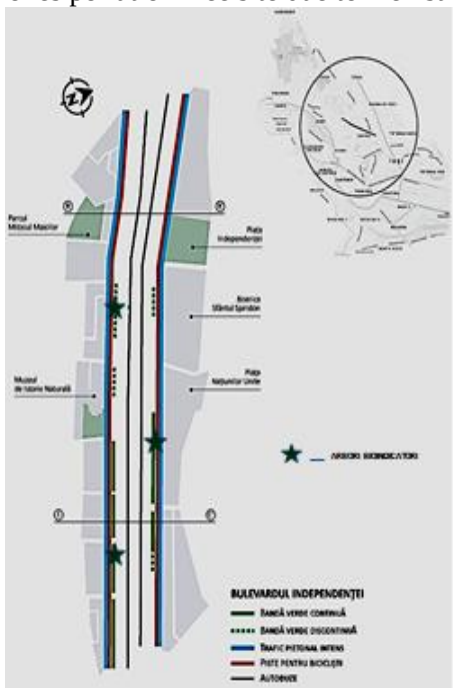

Fig.2 The situation of the Independence Boulevard and the location of the bio-indicator trees

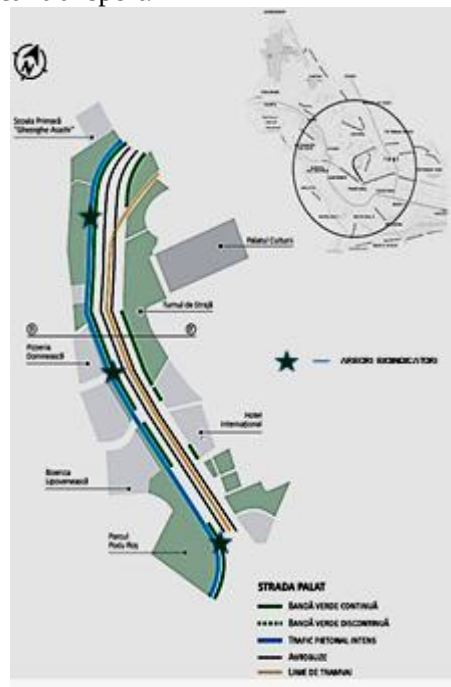

Fig. 3 The situation of the Palace Street and the location of the bio-indicator trees

The results of the field observations are centralized presented in table 2.

Table 2

Centralized situation of the analyzed bio-indicator trees

Bio-indicator trees	The situation of *Tilia cordata* bio-indicator trees (average values)						
	Height (m)	Trunk diameter, etentive bark (cm)	etentive age after dendrometric tables (years)	Crown projection (m)	Diameter/ height ratio	Phytosanitary condition (etentive)	Location
Tc 1	17,5	96	53	4,6	5,48	B	Bv. Carol
Tc 2	16	88	51	4,2	5,50	B	Bv. Carol
Tc 3	15,5	79	50	3,8	5,09	B	Bv. Carol
Tc 4	18	101	54	5,2	5,61	B	Bv. Carol
Tc 5	16	72	51	4,3	4,50	B	Bv. Carol

Tc 6	15	73	51	4,4	4,86	B	Bv. Indep
Tc 7	16,5	78	52	4,8	4,72	B	Bv. Indep
Tc 8	14,5	70	49	4,2	4,82	S	Bv. Indep
Tc 9	16,5	86	52	4,6	5,21	B	Str. Palat
Tc 10	17	92	53	4,8	5,41	B	Str. Palat
Tc 11	17,5	94	54	5,0	5,37	Fb	Str. Palat
Average	16,4	84,5	51,8	4,5	5,14	B	-
Tc control	18	98	52	5,8	5,11	Fb	Gr. Botanica

Legend: Phytosanitary condition: Fb - very good, B - good, S – satisfactory

The choice of the *Tilia cordata* species as a bio-indicator tree is justified by studies that show the high retentive capacity for nitrogen, sulphur and heavy metals, as well as a great capacity for bioaccumulation of silica, aluminum and cadmium in tissues, without significantly affecting biological functions of the species (Untea, 2010 and Peneghi, 2015).

CONCLUSIONS

1. The observations in the 4 established locations, (Carol I Boulevard, Independence Boulevard, Palat Street and Botanical Garden) for establishing a network for monitoring the atmospheric pollution in the road alignment plantations in Iasi, were made on a number of 12 mature specimens of *Tilia cordata* (fluffy linden).

2. The results of the dendrometric observations made during the active vegetation period (May-August 2019) highlighted the following: the phytosanitary state of the majority of the specimens is good, they have heights between 15 ÷ 18 m, the trunk diameter, on average, 91.25 cm, the average age of approx. 52 years, the projection of the crown of approx. 5.15 m and a diameter / height ratio of 5.12.

3. The specimens of the *Tilia cordata* species prove an adequate biological status in order to be included in a network of monitoring bio-indicators of the level of pollution with noxes in the road alignments.

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Consilier editorial: **Vasile VÎNTU**

Tehnoredactori: **Elena Liliana CHELARIU**

Corectori: **Lucia DRAGHIA**
Elena Liliana CHELARIU

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