**ISSN–L=1454-7376**
**(Print)-ISSN 1454-7376**
**(Online)=ISSN 2069-8275**
**(CD-ROM) = ISSN 2069 – 847X**

**UNIVERSITATEA DE ŞTIINȚE AGRICOLE**
**ŞI MEDICINĂ VETERINARĂ**
**“ION IONESCU DE LA BRAD” IAŞI**


# LUCRĂRI ŞTIINȚIFICE

## Vol. 58

## NR. 2

## SERIA HORTICULTURĂ

EDITURA “ION IONESCU DE LA BRAD”

**IAŞI 2015**

ISSN–L=1454-7376
(Print)-ISSN 1454-7376
(Online)=ISSN 2069-8275
(CD-ROM) = ISSN 2069 – 847X

"Ion Ionescu de la Brad" Publishing House Iaşi
ISSN–L=1454-7376
(Print)-ISSN 1454-7376
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(CD-ROM) = ISSN 2069 – 847X

# CONTENT

# CUPRINS

# SYNCHRONIZATION FOR TWO CHAOTIC FINANCE SYSTEMS

## SINCRONIZAREA A DOUĂ SISTEME HAOTICE DIN FINANȚE

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***Abstract***. *Financial systems can exhibit chaotic behaviour and this fact is very important for economic structures. In order to formulate the chaos control in finance, analyzing the dynamics of a chaotic financial system, the synchronization of two systems based on the adaptive feedback method of control is presented in this work. The transient time until synchronization depends on initial conditions of two systems, the strength and number of the controllers.*
***Key words****: finance system, chaos control*

***Rezumat***. *Sistemele economice pot avea comportare haotica si acest fapt este foarte important pentru structurile economice. Pentru a realiza controlul haosului în sistemele financiare, în această lucrare sincronizăm două sisteme haotice, folosind o metodă de feedback. Timpul după care se obține sincronizarea depinde de condițiile initiale ale celor doua sisteme, de intensitatea si numarul functiilor de control.*
***Cuvinte cheie:*** *sistem financiar, controlul haosului*

## INTRODUCTION

Financial systems can exhibit chaotic behaviour and this fact is very important for economic structures. From this point of view the deliberate control of these phenomena have a great practical impact despite the fact that it is very dificult; this is the reason the theoretical models are useful in these situations. Over the last decade, there has been considerable progress in generalizing the concept of synchronization to include the case of coupled chaotic oscillators especially from economical reasons. When the complete synchronization is achieved, the states of both systems become practically identical, while their dynamics in time remains chaotic. Many examples of synchronization have been documented in the literature, but currently theoretical understanding of the phenomena lags behind experimental studies (Grosu I., 1997), (Grosu I., et al, 2008), (Hu M., et al, 2008), (Lerescu A.I., et al, 2004), (Lerescu A.I. et al, 2006), (Oancea S., 2009). The main aim of this paper is to study the synchronization of two chaotic systems based on the adaptive feedback method of control.

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## THEORY

One of the chaotic finance three-dimensional model is given by a nonlinear system of equations (Zhao X.et al., 2011)

$$\dot{x}_1 = x_3 + x_1 (x_2 - a) \quad (1)$$
$$\dot{x}_2 = 1 - bx_2 - x_1^2$$
$$\dot{x}_3 = x_1 - cx_3$$

In this model x denotes the interest rate, y denotes the investment demand and z denotes the price index. The parameter a denotes the savings, b denotes the investment cost and c denotes the commodities demand elasticity.

This system has a chaotic behaviour, for the following constants:

a=0.9, b=0,2, c=1,2 (2)

Figure 1 shows that the attractor projected onto $x_1x_2$ $x_3$ space for the chaotic system (1) with values from (2).

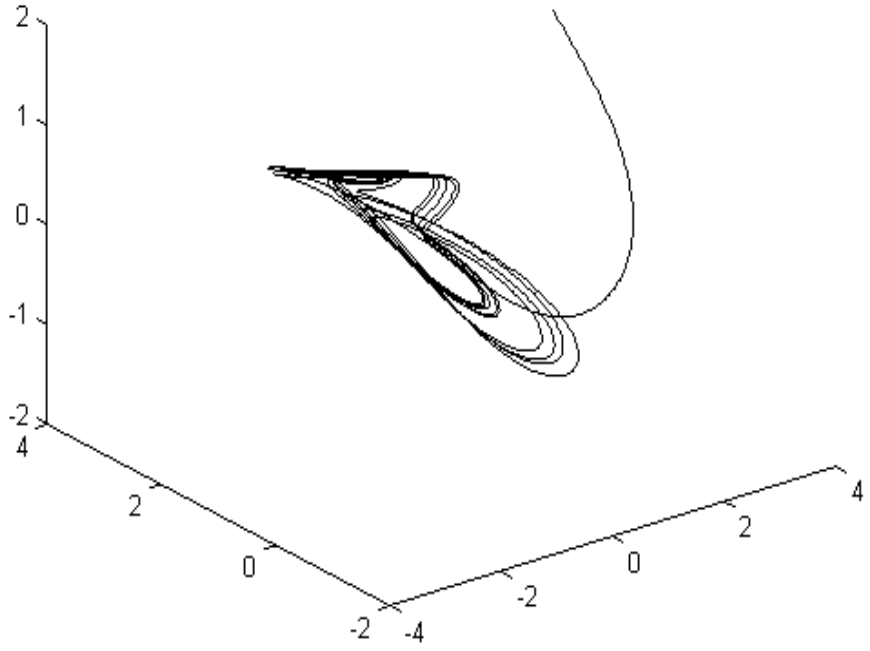


**Fig. 1**– Phase portrait of ( x 1, x2, x3) for system (1)

To synchronize two chaotic systems we used a simple method for chaos synchronization proposed by Guo and coworkers (Guo W., et al, 2009) and used by Oancea (Oancea S., 2009).

If the chaotic system (master) is:

$\dot{x} = f(x)$ where

$x = (x_1, x_2, .....x_n) \in R_n$;

$f(x) = (f_1(x), f_2(x).....f_n(x)) : R^n \rightarrow R^n$

then the slave system is:

$\dot{y} = f(y) + z(y - x)$

where the functions

$\dot{z}_i = -\lambda_i (y_i - x_i)^2$

and $\lambda_i$ are positive constants

## RESULTS AND DISCUSSION

According this method of synchronization, the slave system for the system (1) will be:

$$\dot{y}_1 = y_3 + y_1(y_2 - a) + z_1(y_1 - x_1) \quad (3)$$
$$\dot{y}_2 = 1 - by_2 - y_1^2 + z_2(y_2 - x_2)$$
$$\dot{y}_3 = y_1 - cy_3 + z_3(y_3 - x_3)$$

and for the control strength:

$$\dot{z}_1 = -(y_1 - x_1)^2$$
$$\dot{z}_2 = -(y_2 - x_2)^2 \quad (4)$$
$$\dot{z}_3 = -(y_3 - x_3)^2$$

Figure 2 and 3 demonstrate the syncronization of the two finance systems.

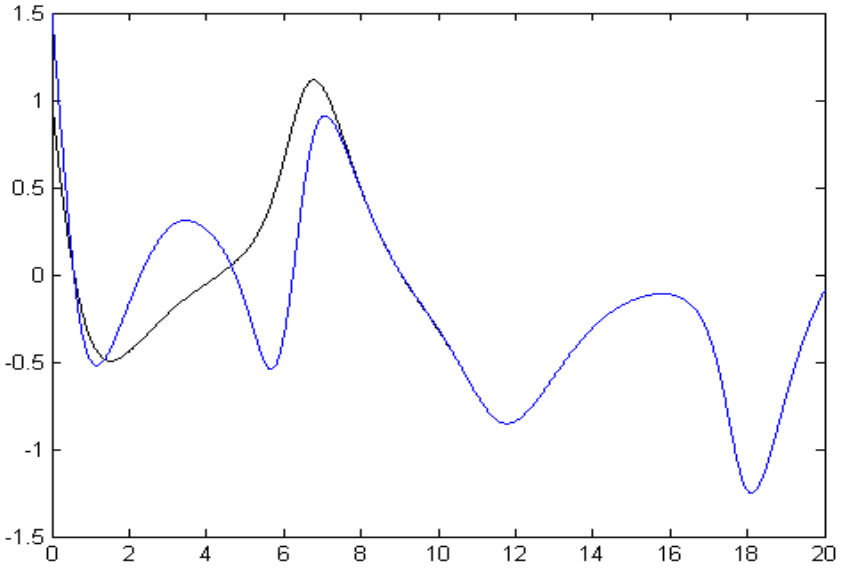


**Fig. 2** – The synchronization of the two chaotic systems

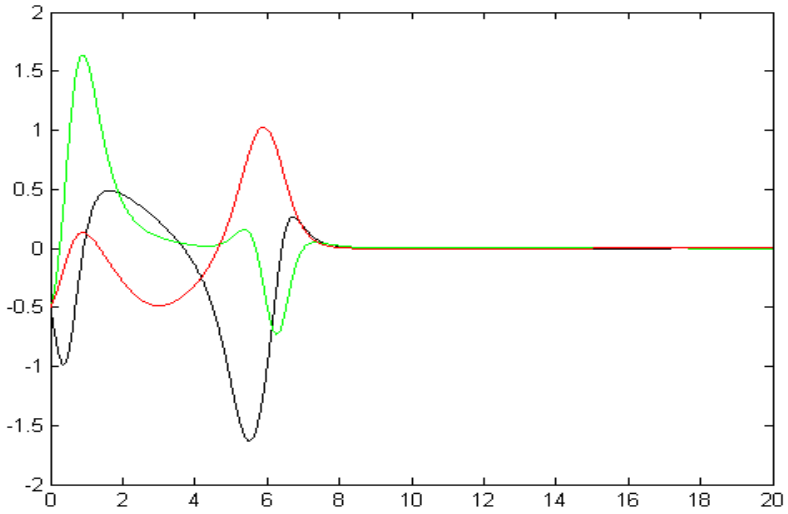


**Fig. 3 –** Synchronization errors between master and slave

Debin Huang (Huang D., 2005), by testing the chaotic systems including the Lorenz system, Rossler system, Chua's circuit, and the Sprott's collection of the simplest chaotic flows found that we can use a single controller to achieve identical synchronization of a three-dimensional system (for Lorenz system this is possible only we add the controller in the second equation). For the two finance systems we achieved the synchronization if one controller is applied only in the first or in the second equation (fig. 3,4 and 5).

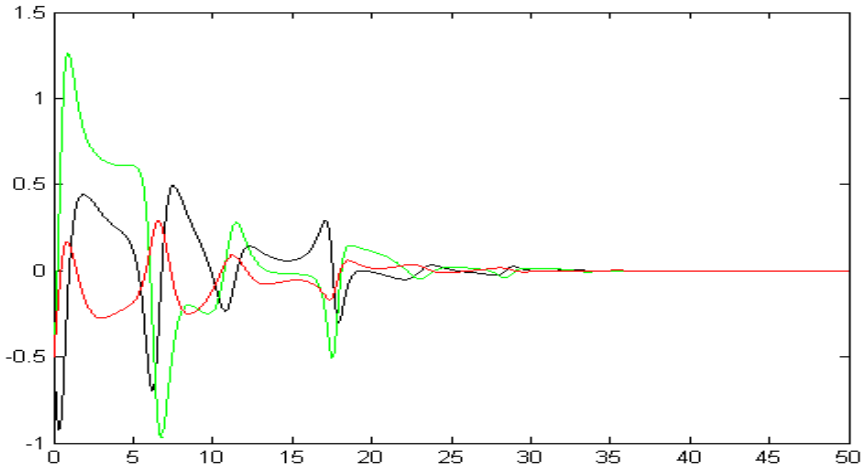


**Fig. 4 –** Synchronization errors between master and slave when the controller is applied in the first equation

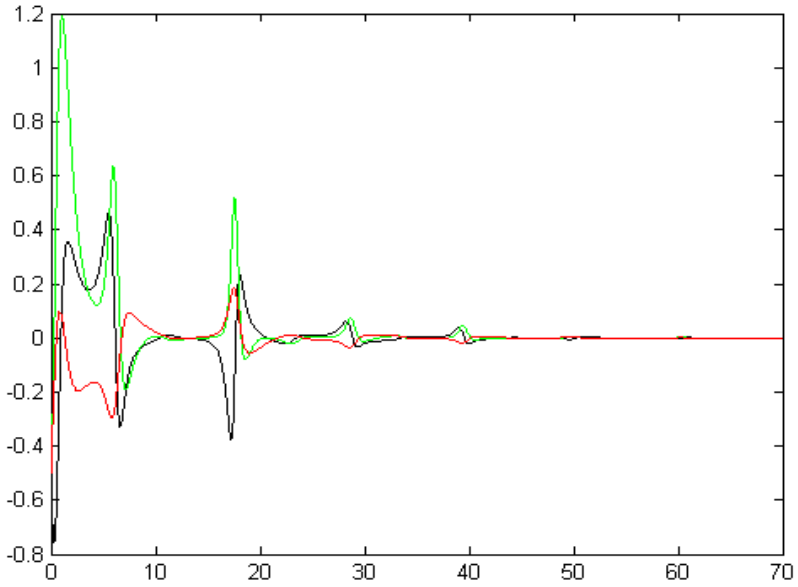


**Fig. 5 –** Synchronization errors between master and slave when the controller is applied in the second equation

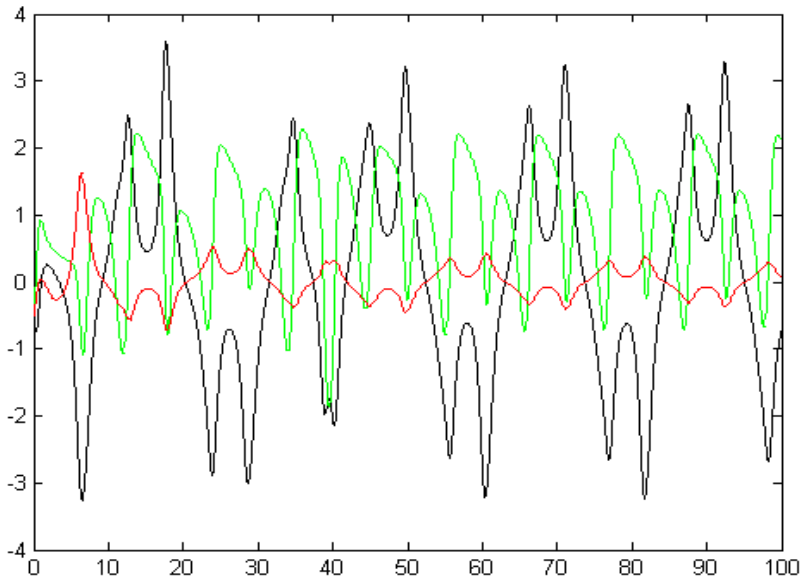


**Fig. 6 –** Synchronization errors between master and slave when the controller is applied in the third equation

## CONCLUSIONS

In this paper we investigated the synchronization of two chaotic finance three-dimensional systems using an adaptive feedback method. The transient time until synchronization depends on initial conditions of two systems, the strength of the controllers and their number. We achieved the synchronization if one controller is applied only in the first or in the second equation, that means in interest rate and investment demand domains. Then we can control this finance system in accordance with recent debates of Wang and Chen (Wang J-W and Chen A-M., 2010) about full global synchronization and partial synchronization in a system of two or three coupled chaotic oscillators. The control method described in this paper is very easy and might be useful in the case of the other chaotic systems.

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# EVALUATION OF AMINOACIDS FORMED IN THE (THERMO) SYNTHESIS OF ACRYLAMIDE DURING THE PROCESSING OF VEGETAL PRODUCTS

## EVALUAREA UNOR AMINOACIZI IN PROCESUL DE (TERMO)SINTEZĂ A ACRILAMIDEI IN PROCESUL DE PRELUCRARE A PRODUSELOR VEGETALE

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***Abstract.*** *Acrylic acid amide, known by its toxic effects exerted upon the biologic systems and by its high incidence in food, justifies its presence through the Maillard reaction, chemical cascade equations that take place during the thermal preparation of vegetals. The present researches are directed towards the gradual evaluation of concentration of certain amino acids in food in order to associate vegetal products that contain amino acids with those containing reducing sugars. In this direction, glutamine, asparagine and arginine were added to semi prepared potatoes before frying. The results can be corroborated with other data from literature that emphasize the role of ammonium carbonate in this synthesis, changing the research towards this aim. Is it possible that the presence of dicarboxylic amino acids favour the acrylamide production versus the basic amino acids?*
***Key words:*** *acrylamide, asparagine, glutamic acid, arginine, olive oil, sunflower oil.*

***Rezumat.*** *Amida acidului acrilic, cunoscută prin efectele sale toxice exercitate asupra sistemelor biologice şi prin incidența crescută în alimente, îşi justifică prezența ca produs al reacției Maillard, cascadă de ecuații chimice, derulate în procesul de prelucrare termică a vegetalelor. Cercetările redate în această lucrare sunt direcționate în sensul evaluării graduale a concentrațiilor anumitor aminoacizi în vederea asocierii potrivite a produselor vegetale ce conțin aminoacizi şi glucide reducătoare. În acest sens, pe şase eşantioane a 5 probe de cartofi semipreparați, s-a adăugat, înainte de prelucrarea termică, asparagină, acid glutamic şi arginină în concentrații constante (1 ppm). Determinarea concentrațiilor de acrilamidă pe probele de cartofi prăjiți au evidențiat concentrații ridicate pe eşantioanele cu adaos de asparagină şi glutamină. Aceste rezultate pot fi coroborate cu date din literatura de specialitate care scot în evidență rolul carbonatului de amoniu în aceasta sinteză, redirecționând cercetarea spre o altă cale de cercetare. Este posibil ca prezența aminoacizilor dicarboxilici să favorizeze producția de acrilamidă comparativ cu prezenta aminoacizilor bazici?*
***Cuvinte cheie:*** *acrilamidă, asparagină, acid glutamic, arginină, ulei de măsline, ulei de floarea soarelui.*

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## INTRODUCTION

Acrylamide (2-propamide) is the amide of an unsaturated acid, the acrylic acid, and it is used in the water potabilization process, the production of paper, cellulose, plastic, food packages, cosmetics etc. (Fig. 1).


**Fig. 1 -** Use of acrylamide (*according to Anca-Irina Burlacu 2009*)

Due to its toxic effects (neurotoxicity, carcinogenesis, genotoxicity, teratogenesis etc.) acrylamide becomes the main concern of toxicologists at the beginning of the 21st century when its adducts to haemoglobin are identified in the blood of some Italian workers (Erikson, 2005, 2006, Burlacu, 2009). The subsequent research conducted by Swedish researchers proved its presence in bread, biscuits, bakery products, snacks, fried potatoes etc. This represents an impulse for the study of its forming mechanism (Mustafa *et al.*, 2008). From the numerous studies conducted worldwide, it results that the thermal processing of food that includes amino acids and glucides is the main way acrylamide is formed (Tayemans, 2004, Vatemm and Shettz, 2003, Braten, 2005) (Fig. 2).

Because the analytical and toxicological studies on the assessment of acrylamide intake through food consumption in Romania are not so numerous, it results necessary to intensify the research on reducing the health risks subsequent to the consumption of qualitatively unsuitable products.

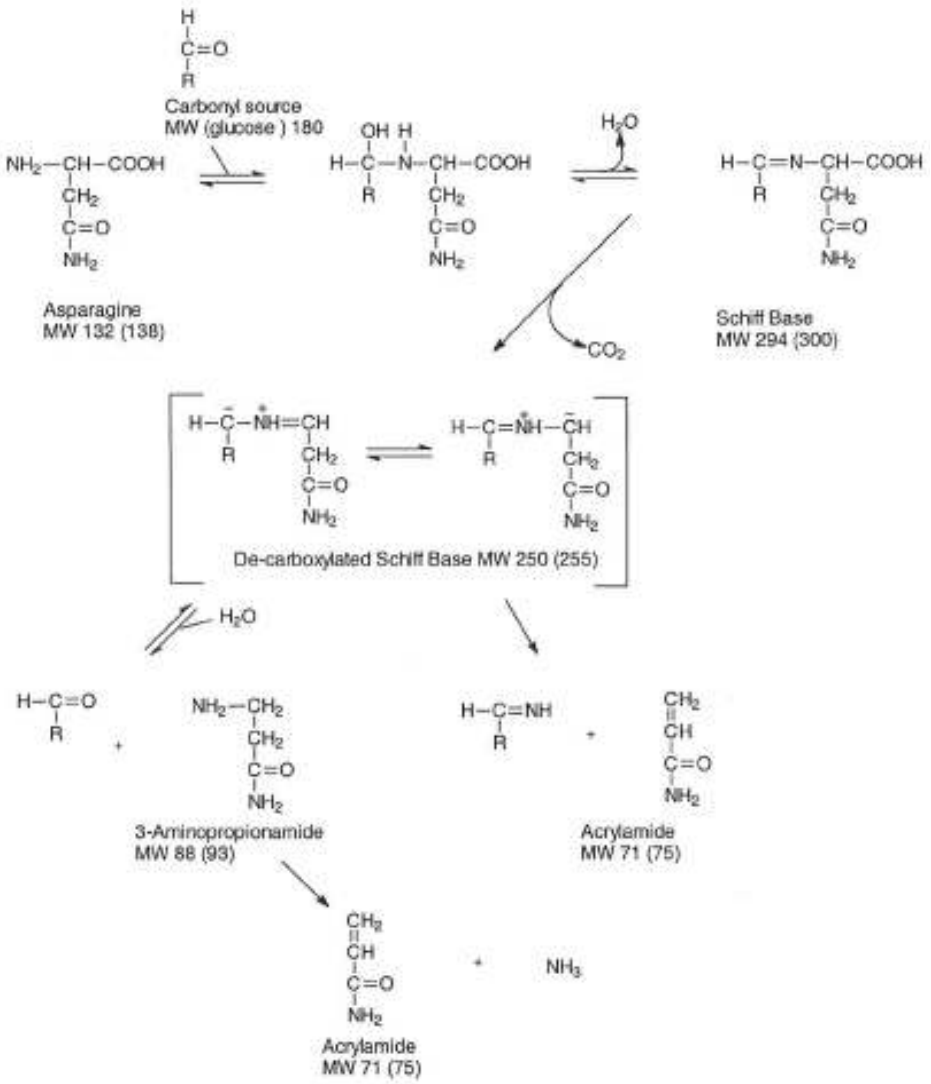


**Fig. 2** - The formation mechanism of acrylamide in Maillard reaction (*according to Tayemans, 2004*)

## MATERIAL AND METHOD

**The experimental model** was designed so to assess the influence of adding three amino acids on acrylamide thermal synthesis: asparagine (aspartic acid amide), glutamic acid (dicarboxylic amino acid) and arginine (basic amino acid). Simultaneously it was attempted to evaluate the influence of the oil used during the thermal processing process. For this there were considered six samples of semi processed frozen potatoes; each sample included three subsamples. The first sample was added asparagine (10 ppm), the second was added the same concentration of

glutamic acid while the third sample was added 10 ppm of arginine (Tab. 1). The first three samples were fried using olive oil and for the last three sunflower oil was used. In all cases the vegetal oil was fresh. At the end of the experiment, it was calculated the acrylamide concentration from each sample. The gas chromatography was used and the results were statistically processed.

*Table 1*

**Experimental model**

| Sample | Olive oil | | | Sample | Sun flower oil | | |
|---|---|---|---|---|---|---|---|
| | Asparagine | Glutamic acid | Arginine | | Asparagine | Glutamic acid | Arginine |
| E1 | 10 ppm | - | - | E4 | 10 ppm | - | - |
| E2 | - | 10 ppm | - | E5 | - | 10 ppm | - |
| E3 | - | - | 10 ppm | E6 | - | - | 10 ppm |
| E0 (etalon) | - | - | - | E0 | - | - | - |

## RESULTS AND DISCUSSIONS

The results were presented in Table 2 and Figures 3 and 4. Comparing the influence of amino acids to the samples studied, it is noticed the high level of acrylamide in all samples enriched by adding amino acids in comparison to the reference sample (2.1 μg).

*Table 2*

**The resulting acrylamide concentrations**

| | **ACRYLAMIDE CONCENTRATION** (μg /10g sample ) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Samples** / **Lipids** | **Asparagine [10 ppm]** | | | **Glutamic acid [10 ppm]** | | | **Arginine [10 ppm]** | | |
| | **min** | **average** | **max** | **min** | **average** | **max** | **min** | **average** | **max** |
| E1 ; E2; E3 / olive oil | 3,00 | 4,26 | 4,9 | 3,9 | 4,31 | 4,85 | 3,8 | 4,03 | 4,8 |
| E4; E5; E6 / sun flower oil | 3,80 | 4,70 | 5,5 | 4,1 | 4,93 | 5,6 | 3,9 | 4,6 | 4,85 |
| E0 (reference) | 2,10 | 2,93 | 3,00 | 2,1 | 2,9 | 3,5 | 2,2 | 2,73 | 3,3 |

The study of the results for the two lipids added during the thermal processing points out that for both olive and sunflower oil, the acrylamide concentration increased proportionally with the amino acids added. Comparing the acrylamide concentration from the two reference samples it is noticed that the

acrylamide value is higher for the sunflower oil (2.73 µg) compared to only 2.1 µg for the olive oil. The study on the evolution of acrylamide concentration in the samples processed with olive oil indicates that the highest acrylamide concentration was recorded at the sample where glutamic acid was added (4.31 µg). The study on acrylamide concentration for the samples processed with sunflower oil indicates the highest value at the sample where glutamic acid was added (4.93 µg) if compared to the concentration obtained when adding asparagine (4,7 µg) and arginine (4,6 µg).

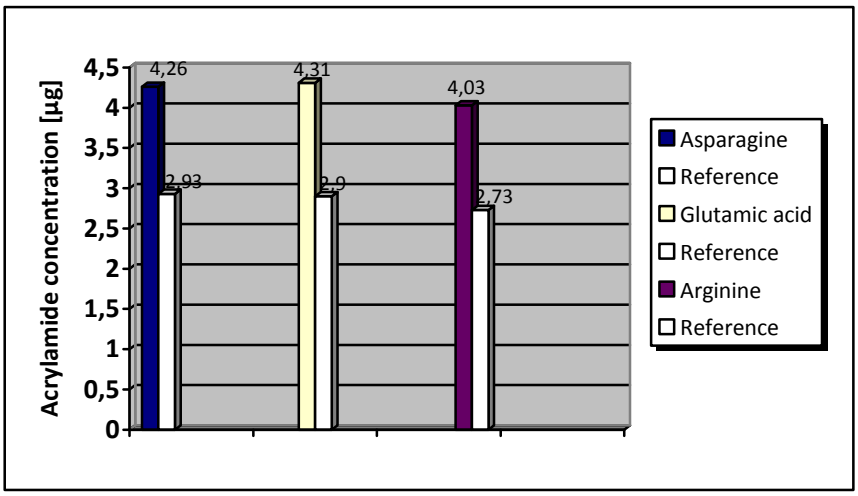


**Fig. 3** - Variation of acrylamide concentration in samples E1, E2, E3 and reference sample (E0) with olive oil

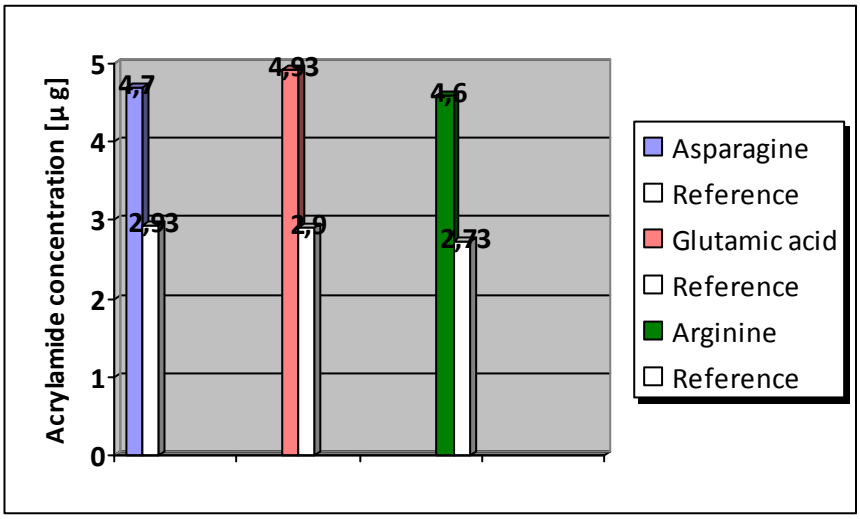


**Fig. 4** - Variation of acrylamide concentration in samples E4, E5, E6 and reference sample (E0) with sunflower oil

## CONCLUSIONS

1. Adding glutamic acid influenced greatly the acrylamide synthesis in the samples studied, its influence being higher than when adding asparagine and arginine.

2. Comparing the oils used for the thermal synthesis of acrylamide in the samples studies it is noticed that the sunflower oil has greater influence than olive oil.

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# DETERMINATION OF RED WINES TOTAL ANTIOXIDANT CAPACITY BY ELECTROMETRIC AND SPECTROPHOTOMETRIC METHODS

## DETERMINAREA CAPACITĂȚII ANTIOXIDANTE TOTALE A UNOR VINURI ROŞII PRIN METODE ELECTROMETRICE ŞI SPECTROFOTOMETRICE

***TRINCĂ Lucia Carmen [1], MARECI D.[2], ARITON Adina Mirela [3], TUCALIUC Roxana Angela[1], CĂLIN M. [1], CHIRUȚĂ C. [1]***
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***Abstract.*** *Phenolic compounds from various food matrices can be investigated with different analytical techniques such as spectrophotometric, cromatographic or electrochemic methods. Cupric Reducing Antioxidant Capacity method (CUPRAC) is a convenient assay for determining total antioxidant capacity in food substrates. In this work, electrochemical and spectrophotometrical approaches were used to estimate the total antioxidant capacity by means of an alternative of the CUPRAC assay, as the concentrations of the participating species ($[Cu(Nc)_2]^{2+}$ and $[Cu(Nc)_2]^{1+}$) can be evaluated by electrochemical and spectrophotometrical methods. The electrometric–CUPRAC methods have been applied to measure total antioxidant capacity of different local wines and validated against the spectrophotometric CUPRAC assay. The electrochemical methods proved to be easy, fast and with low detection limits. A good correlation for the total antioxidant capacity determined by CUPRAC spectrofotometric and electrometric method versus total phenol content (determined by Folin-Ciocalteu Method) was recorded, wich highlights that wines antioxidant activity results mainly from their phenolics content.*
***Key words:*** *wines, antioxidant capacity, electrometric methods, spectrophotometric methods*

***Rezumat.*** *Compuşii fenolici din diverse substraturi alimentare pot fi investigați prin tehnici analitice variate. Metoda CUPRAC reprezintă a tehnică analitică convenabilă pentru determinarea capacității antioxidante totale a substraturilor alimentare. În această lucrare tehnicile electrochimice şi spectrofotometrice utilizate au folosit o variantă a metodei CUPRAC, pentru determinarea concentrației speciilor participante ($[Cu(Nc)_2]^{2+}$ şi $[Cu(Nc)_2]^{1+}$) din vinurile locale. Metodele electrochimice s-au dovedit a fi precise, simple, rapide şi reproductibile. Corelația buna între capacitatea antioxidantă totală (masurată electrochimic şi spectrofotometric) şi conținutul de polifenoli totali (măsurat prin metoda Folin-Ciocalteu) a evidențiat rolul predominant al acestora în determinarea activității antioxidante totale a vinurilor.*
***Cuvinte cheie:*** *vinuri, capacitatea antioxidantă, metode electrometrice, metode spectrofotometrice*

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## INTRODUCTION

Phenolic compounds play one of the most important roles in the quality of wines, as they strongly contribute to the color, mouth feel and palatability of red wines. Phenolic compounds can be classified into phenolic acids (including hydroxybenzoic acids and hydroxycinnamic acids), flavonols, anthocyanins and stilbene derivatives. Phenolic acids are considered to possess the ability to scavenge excess radicals and maintain the balance of reactive oxygen species in the human body (Jiang and Zhang, 2012).

Positive correlations between total phenolics and antioxidant capacity have been reported. According to many authors, antioxidant activity of wines results mainly from their phenolics, whereas the phenolic content and composition depend on the grape variety, vineyard location, cultivation system, climate, soil types, vine cultivation practices, harvesting time, production process and ageing (Lopez-Velez *et. al.,* 2003).

The antioxidant compositions of wine samples are fairly complex, usually involving multiple reaction characteristics and mechanisms, so no single assay will accurately reflect all antioxidants in a complex system, therefore different antioxidant capacity assays may be needed (Badarinath *et. al.,* 2010). The aim of this work was to estimate the total antioxidant capacity by means of an alternative of the CUPRAC assay, as the concentrations of the participating species ($[Cu(Nc)_2]^{2+}$ and $[Cu(Nc)_2]^{1+}$) can be evaluated by both electrochemical and spectrophotometrical methods.

## MATERIAL AND METHOD

**Wine Samples**

Red wine samples (Cabernet Sauvignon, Fetească neagră, Merlot, Pinot Noir) were purchased from local stores. Samples were opened, protected against sunlight and stored at 4˚C. Analyses were carried out within a few days. Each wine was analyzed 5 times.

**Determination of Total Phenolic Content**

Spectrophotometric determination of the TPC was carried out with Folin-Ciocalteu method as adapted for wine analysis using gallic acid as the standard. This method is based on the reduction of a phosphotungsten-phophomolybdate complex by phenolics to blue reaction products. For the preparation of calibration curve, 0.1 mL aliquots of 50, 100, 150, 200, 250 and 300 mg/L aqueous gallic acid solutions or 0.1 mL 20-fold dilution samples of red wines (diluted with 13% (v, v) ethanol) were introduced into a test tube and then 2 mL of 2% sodium carbonate was added. After incubation for 2 min, 0.1 mL of Folin-Ciocalteu's reagent (diluted with water 1:1, v/v) was added. After a further 30 min the absorbance was measured at 750 nm using a spectrophotometer. Results were expressed as mg gallic acid equivalents per litre of wine (mg GAE/L) were estimated.

**Cupric Reducing Antioxidant Capacity (CUPRAC)**

Cupric reducing antioxidant capacity assay was carried out according to Apak et. al., 2004, method:1 ml each of 10 mmM Cu(II), 7.5 mM neocuprine, 1 M ammonium acetate buffer (pH 7) solutions and 0.6 mL water were mixed in a test tube. 0.5 mL of diluted wine or Trolox standard solutions were added to the initial mixture. The absorbance was

measured at 450 nm after 30 minutes. Trolox standard solutions were prepared at a concentration range from 40 to 400 µM.

**Electrochemical investigations:DPV−CUPRAC Assay (Proposed Method)**

Differential pulse voltammetry was performed using a potentiostat model VOLTALAB 40(Potentiostat/Galvanostat/) in a three-electrode configuration, employing two platinum wires as the working/counter electrode, and saturated calomel electrod as the reference electrode. The required volume of antioxidant standards and samples ($20^{th}$ fold wine diluted samples) was added into a mixture solution of the supporting electrolyte and Cu(II)−Nc chelate in the electrochemical cell. The reaction mixture was left to react for 1 min on a stirrer at room temperature. The voltammograms were recorded immediately to minimize adsorption of polyphenols. Work conditions were as follows: scan range, from +0.6 to −0.25 V; pulse size, 50 mV; step size, 4 mV; pulse time, 0.1 s; and sample period, 0.5 s. The cyclic voltammograms were recorded by scanning the potential from +0.8 to −0.2 V at a scanning rate of 40 mV/s. The total antioxidant capacity values of wine samples were expressed as millimoles of trolox equivalents per gram of wine (mmol TE/g wine). The standard calibration curves of trolox and other phenolics were obtained by measuring the currents (Ip) at the cathodic reduction peak potential of the $Cu(Nc)_2^{2+}$ / $Cu(Nc)_2^{+}$ redox couple before and after reaction with antioxidants, taking the difference (ΔIp), and plotting this difference versus concentration of antioxidant standard (Cárdenas et. al. 2014)

**Statistical Analysis**

Statistical analyses were realized using Excel software (Microsoft Office 2007) for calculating the means and the standard errors of the means. Results were expressed within 95% confidence interval as mean ± SD. The results were interpreted by two-way analysis of variance (ANOVA).

## RESULTS AND DISCUSSIONS

Total antioxidant capacity determined values of the tested wine samples measured by the developed voltammetric method (fig. 1) were in good agreement with those of spectrophotometric-CUPRAC method (fig. 2B).

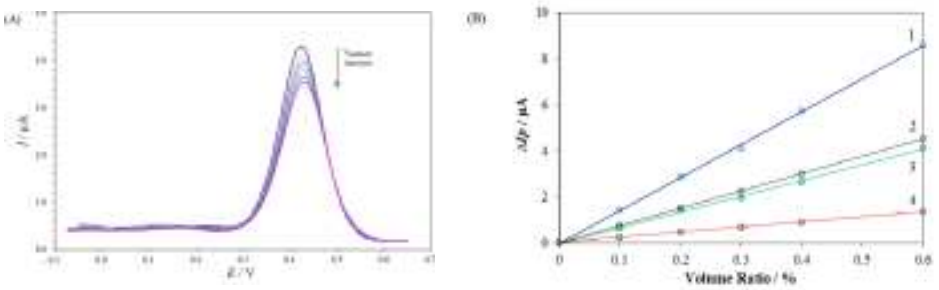


**Fig. 1 -** (A) Differential pulse voltammograms of $Cu(Nc)_2^{2+}$ reagent showing the effect of increasing wine volume parts.(B) Dependence of ΔIp on the volume ratio (V/Vtotal, %) of wines

Total phenols content (fig. 2A) of the antioxidant capacity wines samples were in good agreement with those of spectrophotometric-CUPRAC method (fig. 2B).

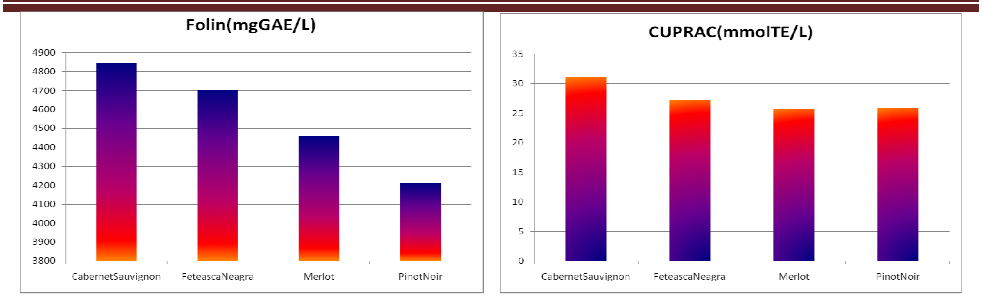


**Fig. 2 -** (A)Total phenols content of red wines measured by FOLIN–CIOCALTEU method. (B) Total antioxidant capacity of red wines measured by CUPRAC spectrophotometric method

## CONCLUSIONS

1. Total antioxidant capacity values of the tested wine samples measured by the developed voltammetric method were in good agreement with those of spectrophotometric-CUPRAC investigation.

2. Electrochemical results showed that total antioxidant capacity of wine samples were in the order: Cabernet Sauvignon > Feteascã neagrã > Merlot > Pinot Noir, in compliance with the results of the spectrophotometric−CUPRAC assay and total phenols content, respectively.

3. A good correlation (0.88) for the total antioxidant capacity determined by CUPRAC spectrofotometric and electrometric method versus total phenols content (determined by Folin-Ciocalteu Method) was recorded.

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# PYRIDAZINE DERIVATIVES WITH ANTIMICROBIAL ACTIVITY

## DERIVAȚI DE PIRIDAZINĂ CU ACTIVITATE ANTIMICROBIANĂ

***TUCALIUC Roxana-Angela [1], TRINCĂ Carmen Lucia[1], MANGALAGIU I.I.[2]***
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***Abstract.*** *Twenty nine pyridazine derivatives (3 salts and 25 pyrrolopyridazine cycloadducts) were prepared and tested in vitro as antimicrobial compounds. Some of them have proved to have a remarkable activity against different microorganisms (germs and fungi).*
***Key words:*** *pyrolopiridazine derivatives, 3+2 dipolar cycloadditions, biological activities.*

***Rezumat.*** *Douăzeci și nouă de derivați de piridazină (trei săruri și douăzeci și cinci de produși de cicloadiție) au fost sintetizați și testați in vitro ca și compuși cu activitate antimicrobiană. O parte din compuși au prezentat o remarcabilă activitate asupra microorganismelor (germeni și fungi).*
***Cuvinte cheie****: derivați pirolopiridazinici, cicloadiții 3+2 dipolare, activitate biologică.*

## INTRODUCTION

Pyridazine derivatives have been extensively investigated because posses different biological activities: anticancer, antituberculosis, antimicrobial, antihypertensive, platelent aggregation inhibitor etc (Mangalagiu, 2011; Rodriguez-Ciria *et al.,* 2003).

In preliminary communications (Zbancioc *et al.,* 2006; Zbancioc *et al.,* 2010; Butnariu *et al.,* 2009; Tucaliuc *et al.,* 2013) is presented the synthesis and spectral analysis of pyrrolopyridazine derivatives. A simple method to obtaine fused pyridazine derivatives, involves 1,3-dipolar cycloaddition of cycloimmonium ylides to variously dipolarophiles (activated alkenes and alkynes).

## MATERIAL AND METHOD

The aim of this work is to perform a thorough study concerning synthesis and activity of some azaheterocycles compounds (pyridazine derivatives).

A facile way to obtain condensed pyridazines is to use ylides as intermediates.

First, by N-alkylation of the pyridazine, we obtained the corresponding cycloimmonium salt (**5-7**) which in alkaline medium generated the ylide (***i***) *in situ*, Fig. 1:

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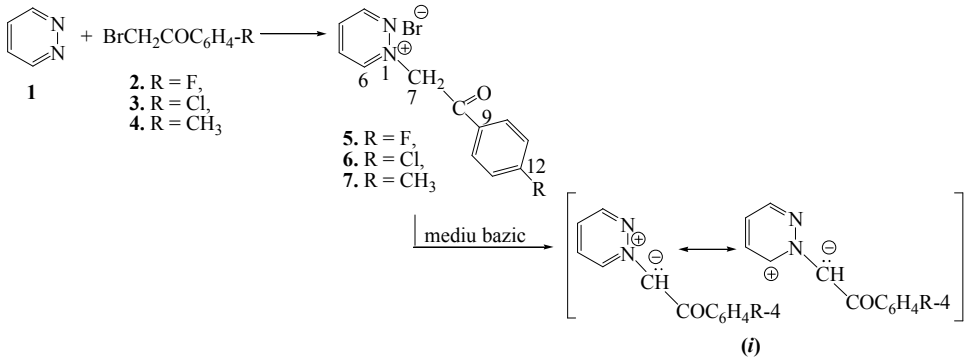



**Fig. 1 -** N-alkylation of the pyridazine.

The N-alkylation of the pyridazine was followed by a 3+2 dipolar cycloaddition of diazinium ylides (***i***) (generated in situ from the corresponding salts) to dipolarophiles (activated alkenes or alkynes), Fig. 2.

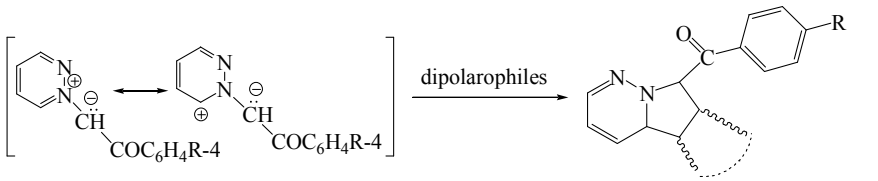
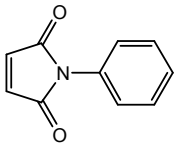
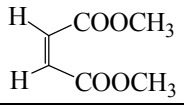
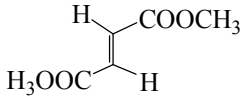



**Fig. 2 -** 3 +2 dipolar cycloaddition of diazinium ylides.

The dipolarophiles used in 1,3-dipolar cycloaddition are activated alkenes and alkynes and are presented in Table 1:

*Table 1*

**Activated alkenes or alkynes (dipolarophiles used in 1,3-dipolar cycloaddition)**

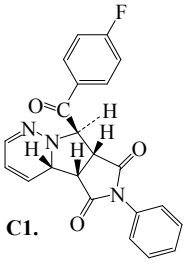
| Dipolarophiles | Structure |
|---|---|
| N-Phenylmaleimide (NPMI) |  |
| maleic ester |  |
| fumaric ester |  |
| acrylonitrile | $H_2C{=}CH{-}CN$ |
| ethyl propiolate | $HC{\equiv}C{-}COOC_2H_5$ |

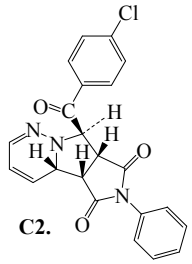
| | |
|---|---|
| dimethyl acetylendicarboxylate (DMAD) | $H_3COOC-C\equiv C-COOCH_3$ |
| 2,2,2-trifluorocrotonate | $F_3C-CH=CH-COOC_2H_5$ |
| 2,2,2-trifluorobutinoate | $F_3C-C\equiv C-COOC_2H_5$ |

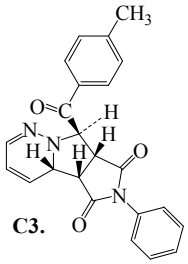
After purification, the structure of the compounds was proved by spectral analysis: the 1H NMR and 13C NMR spectra and two-dimensional experiments 2D-COSY, 2D-HETCOR(HMQC), long range 2D-HETCOR (HMBC) recorded on a Bruker Avance 400 DRX spectrometer at 400/100 MHz. Chemical shifts are given in parts per million (δ-scale), coupling constants (J) in hertz and downfield shift from internal tetramethylsilane (δ 0.00 ppm). The IR spectra were recorded on an FT-IR Shimadzu Prestige 8400s spectrophotometerin KBr. Melting points were determined using an electrothermal apparatus and are uncorrected. Flash chromatography was performed with Aldrich 230e400 mesh silica gel. TLC was carried out on Merck silica gel 60-F-254 plates.

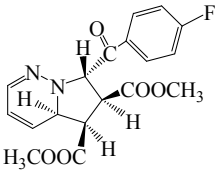
All reagents and solvents employed were of the best grade available and were used without further purification.

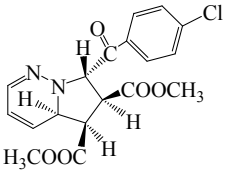
The pyridazine derivatives (**C1-C24**) have the following structures, Fig. 3:

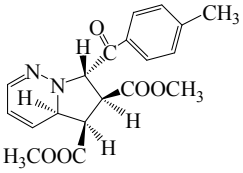

**C1.**

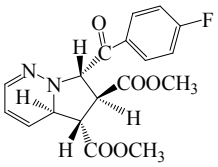

**C2.**

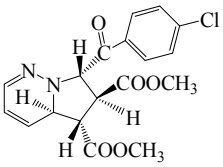

**C3.**

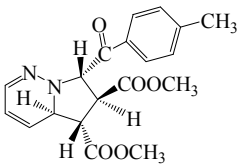

**C4.**


**C5.**


**C6.**


**C7.**


**C8.**


**C9.**

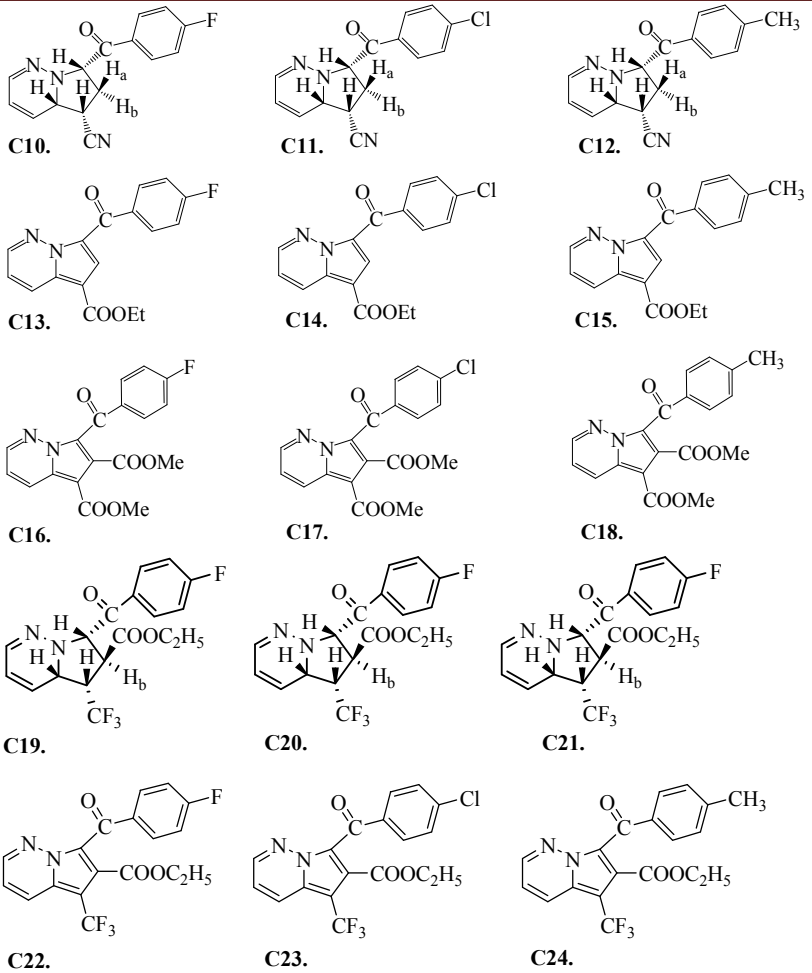



**Fig. 3 -** Pyridazine derivatives obtained by 3 +2 dipolar cycloaddition reaction.

The *in vitro* antibacterial and antifungal activity of the obtained pyridazine compounds was tested having in view that our previous study had proved a certain biological activity in this respect.

The test was performed using the diffusimetric method with rustles steel cylinders based on the diffusion of the tested substances on the gelose surface (for bacteria) and Sabouraud environment (for fungus *Candida albicans*). The cylinders were maintained for 24 h at thermostat, at 34 $^{o}$C for bacteria and at 37 $^{o}$C for *Candida*. The tested substances were previously dissolved in dimethylformamide (DMF) 5% (v/v). A witness solvent sample has been done. The inhibition diameter zone, in mm, of development of microbial strain was measured. A compound is considered active when the difference between the inhibition diameter zone of compound and witness is up to 2 mm (3-4 mm moderate active and up to 5 very active). The results are listed in Table 2.

*Table 2*

**Results of *in vitro* antibacterial and antifungal activities for diazine derivatives described in the text.**

| Product | Strain | | | | | |
|---|---|---|---|---|---|---|
| | ***Staphylococcus aureus Oxford*** | ***Sarciria luteea*** | ***Bacillus subtillis*** | ***Pseudomonas aeruginosa*** | ***Escherichia coli*** | ***Candida albicans*** |
| **C1** | 27 | **63** | 28 | 16 | 25 | 25 |
| **C2** | 28 | **54** | 28 | 18 | 28 | **34** |
| **C3** | 31 | **73** | **37** | 18 | 24 | 29 |
| **C4** | 29 | **60** | **37** | 18 | **35** | 24 |
| **C5** | 33 | **56** | **39** | 18 | **32** | 25 |
| **C6** | **41** | **62** | **35** | 19 | **37** | 30 |
| **C7** | 29 | **51** | **35** | 17 | **35** | 27 |
| **C8** | 23 | **54** | **36** | 15 | **31** | 27 |
| **C9** | **47** | **59** | **38** | 17 | **37** | 29 |
| **C10** | 26 | **50** | 27 | 14 | 20 | 23 |
| **C11** | 30 | **57** | 29 | 14 | 28 | **35** |
| **C12** | 46 | **60** | **39** | 19 | 23 | 30 |
| **C13** | 27 | **48** | 22 | 20 | 23 | 22 |
| **C14** | 25 | **48** | 25 | 20 | **33** | 30 |
| **C15** | **40** | **56** | **37** | 18 | 25 | 28 |
| **C16** | 27 | **52** | 23 | 15 | 21 | 21 |
| **C17** | 30 | **57** | 26 | 18 | **31** | 24 |
| **C18** | **46** | **65** | **36** | 19 | 25 | 29 |
| **C19** | 34 | **54** | 29 | 18 | 26 | 29 |
| **C20** | 36 | **55** | 31 | 16 | 28 | 30 |
| **C21** | 39 | **54** | 31 | 16 | 24 | 28 |
| **C22** | 37 | **62** | 28 | 16 | 23 | 27 |
| **C23** | 37 | **64** | 30 | 18 | 28 | 27 |
| **C24** | 39 | **59** | 29 | 16 | 28 | 29 |

## RESULTS AND DISCUSSIONS

The comparative analysis of the obtained data leads to the conclusions:

- the pyridazine derivatives have an excellent antimicrobial activity (non selective) against *Gram positive* germs, the results on *Sarciria Luteea* being spectacular;

- the cycloadducts have antibacterial activity only against *Escherichia coli* (very active for **C6** and **C9**);

- only two pyridazine derivatives (**C2** and **C11**) have a significant activity against fungus *Candida albicans*.

## CONCLUSIONS

In conclusion, a study concerning synthesis, structure and biological activity of some pyridazine derivatives is reported.

The *in vitro* antibacterial and antifungal activities of the newly obtained diazine compounds were tested, some of the compounds have proved to have a remarkable activity against *Gram positive* germs, the results on *Sarciria luteea*

being spectacular. Against fungus *Candida albicans* pyridazine derivatives have no significant activity.

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# FEAUTURES OF WATER STATUS AND ANTIOXIDANT PROTECTION OF PLANTS WITH DIFFERENT ADAPTATION STRATEGIES TO DROUGHT

## PARTICULARITĂȚILE STATUS-ULUI APEI ŞI PROTECȚIEI ANTIOXIDANTE LA PLANTE CU DIFERITE STRATEGII DE ADAPTARE LA SECETĂ

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***Abstract.*** *There have been registered differences caused by drought in competitive relationships between plant organs of Phaseolus vulgaris L., Phaseolus acutifolius Gray, and Phaseolus lunatus L. The inflorescences and pods of resistant plants possessed a better water homeostasis by account of water reserve from the stems, of root system activity and reactivity of stomata. Reactivity of stomata, hydraulic conductivity adjustment, high ability to restore the water status of the tepari and Lima beans reflected the conservative strategy of tolerant plants. Low tolerance of Phaseolus vulgaris L. plants to drought could be explained as the result of lower stomatal conductance, the occurrence of reactive oxygen species, acceleration of lipid peroxidation, accelerating oxidative destructions.*
***Key words:*** *water status, adaptation, protective antioxidant enzyme, drought*

***Rezumat:*** *S-au înregistrat deosebiri în relațiile competitive dintre organele plantelor de Phaseolus vulgaris L., Phaseolus acutifolius Gray, şi Phaseolus lunatus L., condiționate de secetă. La plantele rezistente, inflorescențele şi păstăile posedă o mai bună homeostatare a apei din contul rezervei de apă din tulpini, activității sistemului radicular şi reactivității stomatelor. Reactivitatea stomatelor, reglarea conductibilității hidraulice, capacitatea înaltă de restabilire a status-ului apei, plantelor de fasolea Phaseolus acutifolius Gray, şi Phaseolus lunatus L., reflectă strategia conservativă a plantelor tolerante. Toleranța scăzută a plantelor de Phaseolus vulgaris L. la secetă este o urmare a diminuării conductibilității stomatelor, apariției speciilor reactive de oxigen, intensificării procesului de oxidare peroxidică a lipidelor, accelerării destrucțiilor oxidative.*
***Cuvinte-cheie:*** *status-ul apei, adaptare, enzimele de protecție antioxidantă, secetă*

## INTRODUCTION

Plant property to minimize the impact of drought and to adapt to sub-optimal soil water content is due to a complex of functional and structural features, which were formed during phylogenesis (Cherry, 1989; Bray, 1993; Smith

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and Griffiths, 1993; Kramer and Boyer, 1995; Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 1999). However, so far it is not clear their role in development of the response reaction to the change of soil water within the meaning of not only the survival of plants, but also in the aspect of "reproductive survival". In this context, it was interesting to clarify the physiological particularities of adaptation to drought for crop plants, belonging to species with different potential of tolerance and productivity.

## MATERIAL AND METHOD

Investigations were carried out on plants of *Phaseolus lunatus* L., cv. *Fetanisa*; *Phaseolus acutifolius* Gray, cv. *Acutifolius* 5; *Phaseolus vulgaris* L., cv. *Nina* - species with different potential for productivity and tolerance to drought, grown in pots Mitcerlih with a capacity of 30 kg oven dry soil under controlled soil water content. The experiments design included treatments: I - control, permanent soil water content 70% of the total water capacity (TWC); II - plants, which have suffered two cycles of drought - first one - phase trifoliate leaves, and second - flowering stage. The study of functional characteristics was performed after 10 days of water stress and after I-st and VII-th day of restoring of optimal hydric regime. Water status parameters were determined by conventional methods. The testing of lipid peroxidation (LPO) was performed by determining the malone dialdehyde (MDA) content; the activity of antioxidant protection enzymes was measured spectrophotemetrically: catalase CAT – using the method of Chance and Machly (1955); ascorbate peroxidase APX – according to Nacano and Asada (1981); guaiacol peroxidase GwPX, glutathione reductase GR and glutathione peroxidase GPX – according to Schadle and Bassham (1977). Statistical analysis of data was performed using set of programs "Statistica 7" - ANOVA for computers.

## RESULTS AND DISCUSSIONS

According to conception of "ecological strategies" (Grime, 1979), the differences of plants with various ecological orientation are determined by physiological nature of adaptation reactions.

*Table 1*

**Peculiarities of the water status of bean plants in drought conditions.**

| Specia/ cultivar | Soil water content % TWC | WC, g·100 $g^{-1}$ f.m. | SD, % of full satura-tion | WHC, % of water lost after 2h of dehydration | HB, g water · $g^{-1}$ dry soil |
|---|---|---|---|---|---|
| ***Ph. lunatus* L., cv. *Fetanisa*** | **70** | 83.2 ± 0,5 | 6.7 ± 0.3 | 7.4 ± 0.2 | 4.7 ± 0.1 |
| | **30** | 80.3 ± 0,1 | 10.1 ± 0.4 | 2.9 ± 0.1 | 3.9 ± 0.02 |
| ***Ph. acutifolius* Gray, cv. *Acutifoliu* 5** | **70** | 83.1± 0,2 | 6.9 ± 0.2 | 11.7 ± 0.4 | 5.5 ± 0.04 |
| | **30** | 83.7 ± 0.2 | 10.5 ± 0.2 | 4.8 ± 0.2 | 4.5 ± 0.1 |
| ***Ph. vulgaris* L., cv. *Nina*** | **70** | 86.5 ± 0.8 | 8.0 ± 0.3 | 14.4 ± 0.5 | 5.6 ± 0.03 |
| | **30** | 81.3 ± 0.5 | 12.8± 0.4 | 6.1 ± 0.2 | 4.1 ± 0.1 |

Note. f.m. - fresh mass

The value of SD to some extent depended on the activity of absorption of root system, on the capacity of protoplasm biopolymers to retain water, as well as, on the intensity of transpiration (Fig. 1).

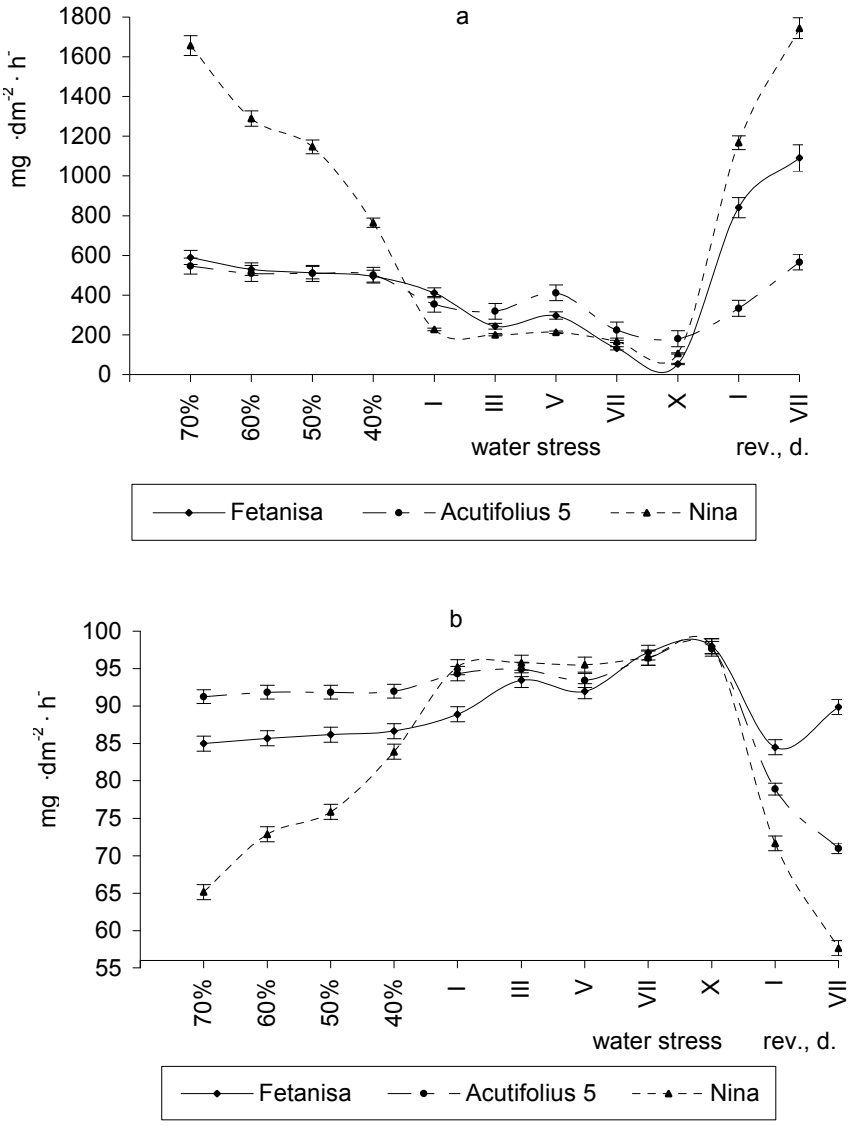


**Fig. 1 -** Modification in the intensity of transpiration (a) and the resistance to diffusion of water through the stomata (b) of *Phaseolus* leaves depending on the gradual change of soil water content.

The data (Table 1) have demonstrated that plants *Phaseolus* of investigated species distinguished by their reactivity to the variation in hydric regime. In drought conditions, a saturation deficiency of (SD) is formed in plant tissue, by which it can be judged on the relationship between the absorption of water from the soil, its transportation in the organs, and consumption during transpiration. After 10 days of drought (30% TWC) the water content (WC) was higher and the DS in leaves was significantly lower for plants with the high potential of tolerance in comparison to ones *Ph. vulgaris* L.

It was stated that the representatives of the species *Ph. acutifolius* Gray at the optimal level of water content lost the water more easily, leading to assumption that their genotypic tolerance was associated not so much with water retention capacity, but with activity of the root system. By the amount of water retained in the leaves under water deficit in the soil, the plants taken in the investigation could be distributed in descending order as follows: Fetanisa > Acutifolius 5 > Nina. Despite the fact that the plants in drought conditions developed a greater capacity to retain water in tissues, the degree of hydration of biocoloizilor (HB) decreased (table 1). The increase of SD in the tissues under the influence of insufficient soil water content resulted in reducing of water consumption in the process of transpiration. The stomata of bean plant responded promptly to changing of soil water content (fig. 1a, b). The transpiration of plants *Ph. vulgaris L.* has been particularly notable. Already at soil water decrease up to 50% TWC, the stomatal resistance for water vapour diffusion increased, and the intensity of transpiration of these plants was reduced by 1.45 times; at 40% TWC the water consumption decreased by 2.16 times. The same for them, a 10-day drought caused the almost complete blockage (93 %) of transpiration. In the early days of water content shortage (30% TWC), the degree of reduction in transpiration of Lima beans was 5 times lower than the usual beans. A 50 percent reduction in water consumption was recorded after 5-7 days of drought.

The impact of drought on water status of vegetative and generative organs was conducted differently (fig. 2).

The maximum quantitative differences were recorded in the net effect of drought on the water status in the roots and inflorescences of plants. Net action of drought on WC in the roots of *Ph. vulgaris* L. cv. Nina was ≈ 3 times higher than the WC in the stems, and 2.3 times higher than in leaves.

It was shown that the inflorescences did not have the capacity to repair the disruption of the water status even after improving the soil water content (Brinza L., 2003). The lower potential of drought resistance *Ph vulgaris* L. plants was due to the occurrence of reactive oxygen species (table 2), which increased oxidative destructions of cellular components. The degree of activation of antioxidant enzymes did not compensate the MDA formation.

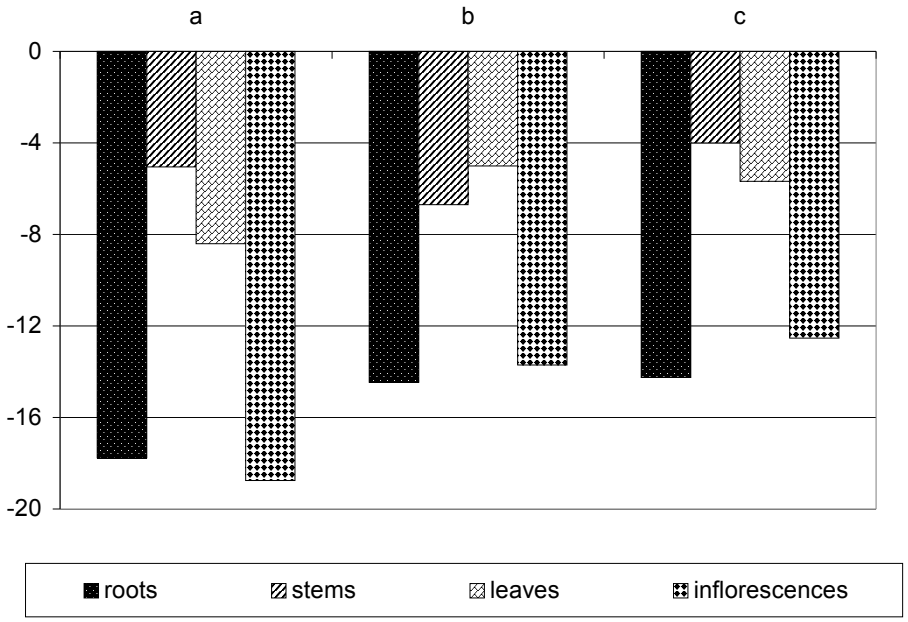


**Fig. 2 -** The effect of drought on water content in different organs of different species of plants *Phaseolus:* a - *Ph. vulgaris* L., cv. Nina; b - *Ph. acutifolius* Gray, cv. Acutifolius 5; c - *Ph. lunatus* L., cv. Fetanisa.

*Table 2*

**Changing in the amount of phospholipids, MDA, and protective antioxidant enzyme activity in the leaves of *Phaseolus* plants during drought conditions.**

| Parameters | Soil water content | | |
|---|---|---|---|
| | 70 % TWC | 30 % TWC | Δ, % control |
| | M ± m | M ± m | |
| **Phospholipids content, mcg.g$^{-1}$ d. s.** | 741.8 ± 4.2 | 320.2 ± 3.8 | -56,8 |
| **MDA content , mcM/g. f. s.** | 7.44 ± 0.2 | 16.2 ± 0.4 | 117,7 |
| **SOD activity, conv. un./ g. f. s.** | 68.3 ±1.1 | 130.0 ± 2.1 | 80,9 |
| **CAT activity, mM/g. f. s.** | 12.0± 0.3 | 21.3 ± 0.2 | 77,5 |
| **APX activity, mM/g. f. s.** | 9.5 ± 0.1 | 23.7 ± 0.2 | 149,5 |
| **GPX activity, mM/g. f. s.** | 126.5 ± 0.9 | 184.0 ± 1.9 | 45,5 |
| **GwPX activity, mM/g. f. s.** | 247.7 ± 2.2 | 392.3 ± 3.3 | 58,4 |
| **GR activity, mM/g. f. s.** | 156.6 ± 1.2 | 264.4 ± 2.2 | 68.4 |

Note. d. s. - dry substance; f. s. - fresh substance; conv. un. - conventional units

The refore, *Phaseolus* plants adapted to suboptimal soil water content due to the self-regulation the water status: by increasing WHC in tissue, by the activity of water absorption in the root system, and by regulation of water consumption in the process of transpiration. The economical water consumption

reflected the conservative strategy of tolerant specia *Ph. lunatus*, L. and *Ph. acutifolius* Gray.

## CONCLUSIONS

1. The conservation strategy of plants spp. *Ph. lunatus* L. and *Ph. acutifolius* Gray was achieved by maintaining a relatively stable and balanced water status, which provided preadaptation to sub-optimal soil water content.

2. The compensatory restoration capacity of physiological processes in stress resistant plants was faster after amelioration of hydric regime.

3. The reduced resistance potential of common bean plants was due to inhibition of physiological processes at higher water potential in cells, to drastic decrease of stomata conductance, reactive oxygen species appearance, to the disorder of coordination degree among the enzyme activities involved in antioxidant protection.

***Acknowledgments**: This investigations were performed with the financial support of young researchers project 15.819.05.08F.*

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# PHYSIOLOGICAL PROCESSES ASSOCIATED WITH THE RESISTANCE OF *GLYCINE MAX* (MERR., L.) PLANTS TO REPEATED WATER STRESS

## PROCESE FIZIOLOGICE, ASOCIATE CU REZISTENȚA PLANTELOR DE *GLYCINE MAX* (MERR., L.) LA STRESUL HIDRIC REPETAT ÎN TIMP

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***Abstract.*** *The experimental data were obtained on the reasoning behind the meaning of water homeostasis significance and antioxidant protection in the event of potential resistance of Glycine max L.(Merr) plants to the hydric and oxidative stresses, conditioned by in time repeated drought. It was established that the primary reaction of soybean plants to drought action is changing the parameters of the water status and tissue dehydration, coupled with the formation of reactive oxygen species (ROS), an increase of malonic dialdehid (DAM) and peroxide oxidation of lipids. Dehydration and ROS formation causes destruction of chloroplasts and diminish fund assimilating pigments in the leaves of both cultivars, especially in sensitive plants.Water stress caused closure of the stomata, the inhibition of carbon dioxide assimilation, with the negative consequences on the of water use efficiency in the production process. The plants, which have suffered a moderate drought at the initial stages of ontogenesis, exhibit a greater tolerance to repeated drought during critical period.*
**Key words**: plants, drought, oxidative stress, photosynthesis, assimilating pigments, tolerance.

***Rezumat:*** *Au fost obținute date experimentale privind argumentarea semnificației capacității de homeostatare a apei și protecției antioxidante în manifestarea potențialului de rezistență a plantelor de Glycine max,L.(Merr) la stresul hidric și oxidativ, condiționat de seceta repetată în timp. S-a stabilit, că reacția primară a plantelor de soia la acțiunea secetei este modificarea parametrilor status-ului apei și deshidratarea țesuturilor, cuplată cu formarea speciilor reactive de oxigen (SRO), majorarea di-aldehidei malonice (DAM) și oxidarea peroxidică a lipidelor. Deshidratarea și generarea SRO, provoacă destrucția cloroplastelor și diminuarea fondului de pigmenți asimilatori în frunzele ambelor cultivare, cu precădere la plantele sensibile. Stresul hidric a condiționat închiderea stomatelor, inhibarea asimilării dioxidului de carbon, cu urmări negative asupra eficienței utilizării apei în procesul de producție. Plantele, care au suportat o secetă moderată la etapele inițiale ale ontogenezei manifestă o toleranță sporită la apariția repetată a secetei în perioada critică.*
**Cuvinte cheie:** plante, secetă, stress oxidative, fotosinteză, asimilare, pigmenți, toleranță.

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## INTRODUCTION

The adverse conditions, repeated over time, can cause the damage cell structures, the discordance of the key physiological processes and morphological changes with severe consequences on productivity. Recent research assume that plants that have suffered a moderate stress at the beginning of vegetation can form so-called "stress memory" - the ability to react adequately to the repeated stress over time (Davies and Zhang , 1991; Kalapa *et al.,* 1996; Beck *et al.,* 2007; Bartol *et al.,* 2013). Recent literature in this field misses the data on the impact of environmental stress repeatedly on the functional processes of the most valuable crop plants. In this context the purpose of the work was to assess the significance homeostation water capacity and antioxidant protection in the event of property plant *Glycine max*, L. (Merr) to react adequately to droughts in time.

## MATERIAL AND METHOD

As subjects of study were used the plants *Glycine max* (Merr., L.) cultivars (cv.) Indra and Enigma grown in containers Mitcerlih with the capacity of 30 kg absolutely dry soil in humidity conditions controlled in the Complex of vegetation IGFPP ASM. The scheme included: - I variant - witness, humidity 70% of the total capacity for soil water (CTA); II variant - 70-35% CTA, stress phase "button formation - beginning of flowering" III variant - 70- 35-70 - 35% CTA, plants exposed to water stress phase "first trifoliate leaf" and repeated in the phase of "button formation - beginning of flowering." The duration of water stress - 7 days. Status of the water parameters were determined by classical methods (Vasseu and Sharkey, 1989), the intensity of CO2 uptake, transpiration, stomatal conductance and water use efficiency were investigated using portable gas analyzer LCA-4. Malone di-aldehyde content DAM -. The activity of key enzymes of antioxidant protection was investigated by the spectrophotometric method: SOD – according; GR - by reducing oxidized glutathione in the presence of NADP · H, λ 340 nm (Schad and Bassham, 1977). The differences between the variants were documented by statistical analysis using the set of programs "Statistica 7" - ANOVA.

## RESULTS AND DISCUSSIONS

The experimental data have shown a reaction and a different degree of parameter change of the status of the water plant *Glycine max* (Merr., L.) to drought conditions (Table. 1). The lack of humidity caused the decrease of the moisture of plant (CA) to both varieties, but especially to the variety Enigma. As a result of dehydration in leaf tissues was created a deficit of saturation (DS) significantly higher compared with control plants, unexposed to drought action: with + 4.47% Indra variety and +16,90% - Enigma plants. To the repeated occurrence of drought conditions in phase of "button formation - beginning of flowering" were found a different reaction to the plant that suffered a moderate water stress in the phase of "first trifoliate leaf" (Tab. 1).

*Table 1*

**The influence of repeated drought in time to "button formation - beginning of flowering" stage on the parameters of water leaves of plants *Glycine max* (Merr., L.)**

| cultivation | Soil mosture, % CTA | CA, g·100g m.p. | | CRA, % water lost from the original content | | DS, % of full saturation | |
|---|---|---|---|---|---|---|---|
| | | M ± m | Δ, % control | M ± m | Δ, % control | M ± m | Δ, % control |
| Indra | Control plants, 70 | 73,8±1,5 | | 18,6±0,8 | | 12,5±0,6 | |
| | Drought , 70-35-70-35 | 73,1±1,5 | -1,03 | 15,6±0,5 | -15,94 | 12,8±0,5 | +2,24 |
| | Drought, 70 -35 | 72,3±1,9 | -2,02 | 15,9±0,3 | -14,44 | 13,1±0,4 | +4,47 |
| Enigma | Control plants, 70 | 77,6±1,4 | | 24,4±0,9 | | 11,5±0,3 | |
| | Drought , 70-35-70-35 | 74,9±1,0 | -3,48 | 16,3±0,6 | -21,81 | 12,5±0,2 | +8,40 |
| | Drought , 70 -35 | 73,7±2,0 | -4,89 | 19,0±0,8 | -25,75 | 13,5±0,2 | +16,90 |

The representatives cv. Enigma, the adaptation effect is less pronounced as a result of the weaker autoregulation property of the status of the water. In plant tissues exposed first to drought stress was formed a deficit of saturation respectively with 4.47 and 16.9% higher compared to control plants and 2.18 and 7.92 higher than plants that they have supported a moderate water stress at the first stage of trifoliate leaves. Water retention capacity values (CRA) in the leaves of plants adapted are higher than the plants exposed for the first time to drought. A hydric suboptional regime in the youthful phase of development makes insufficient the adaptation to the moisture by forming a plant xerophit guy (Brînză, 2005). This phenotype of plants has a better assimilation of homeostatare water in tissues. Therefore, to the molecular level, the stress memory and tolerance to drought is associated with the property's stabilization of domestic water status by increasing its retention capacity by the macromolecules.

One of the first consequences of the stressogenic factors action is intensifying the appearance in the cells of the reactive oxygen species, oxidative stress and the damage cell structures. The data obtained in this paper (Tab. 2) showed that the degree of increase of SRO formation induced by insufficient humidity during the button formation and flowering of plants, is significantly lower in plants that have suffered a moderate water stress in the first trifoliate leaf

stage, compared to plants exposed to the first cycle of drought during the critical period.

*Table 2.*

**The influence of repeated droughts on the activity of antioxidant enzymes and di-malonate aldehyde content in the leaves of soybean plants**

| Cultivation | Soil mosture, % CTA | Content DAM, mkM/g. s. p. | SOD un. conv./ g. s. p. | CAT mM/ g. s.p. | APX mM/ g. s. p. | GlPX mM/ g. s. p. | GlRed., mM/ g. s. p. |
|---|---|---|---|---|---|---|---|
| | | M±m | M±m | M±m | M±m | M±m | M±m |
| Indra | Control plants, 70 | 40,1±0,9 | 26,11±0,9 | 1,2±0,03 | 2,8±0,1 | 77,9±2,1 | 96,8±1,5 |
| | Drought, 70-35-70-35 | 51,0±1,0 | 31,17±0,7 | 1,2±0,02 | 6,9±0,2 | 87,0±2,0 | 150,7±0,9 |
| | Drought, 70 -35 | 56,4±1,2 | 28,12±0,6 | 1,3±0,11 | 3,3±0,3 | 81,6±1,9 | 118,2±2,0 |
| Enigma | Control plants, 70 | 43,0±0,8 | 28,84±0,9 | 1,2±0,20 | 3,0±0,3 | 76,6±2,2 | 111,2±2,5 |
| | Drought, 70-35-70-35 | 55,3±1,3 | 32,30±0,9 | 1,3±0,05 | 5,2±0,5 | 80,4±2,6 | 196,2±2,4 |
| | Drought, 70 -35 | 62,0±1,5 | 30,06±0,8 | 1,1±0,04 | 3,7±0,3 | 78,3±2,4 | 125,6±2,2 |

*Table 3*

**The influence of repeated water stress on the intensity of photosynthesis (IF), transpiration (IT) and stomatal conductance (SC) of plants *Glycine max* (Merr., L.)**

| Cultivation | Soil mosture, % CTA | IF, $mmol \cdot m^{-2} \cdot h^{-1}$ | | IT, $mmol \cdot m^{-2} \cdot h^{-1}$ | | CS, $mmol \cdot m^{-2} \cdot h^{-1}$ | | EUA, $^{*}$ mmol $CO_2$/ mmol $H_2O$ |
|---|---|---|---|---|---|---|---|---|
| | | M±m | Δ, % control | M±m | Δ, % control | M±m | Δ, % control | M±m |
| Indra | Control plants 70 | 9,8±0,3 | | 2,7±0,05 | | 0,14±0,02 | | 3,7±0,2 |
| | Drought, 70-35-70-35 | 6,7±0,5 | -31,5 | 1,6±0,04 | -38,5 | 0,10±0,01 | -28,57 | 4,1±0,2 |
| | Drought, 70 -35 | 4,4±0,4 | -55,7 | 1,3±0,09 | -52,5 | 0,08±0,03 | -42,85 | 3,5±0,1 |
| Enigma | Control plants, 70 | 11,2±1,0 | | 2,4±0,12 | | 0,19±0,05 | | 4,6±0,2 |
| | Drought, 70-35-70-35 | 7,6±1,0 | -32,7 | 1,3±0,08 | -48,7 | 0,13±0,06 | -31,58 | 6,0±0,3 |
| | Drought, 70 -35 | 3,7±0,6 | -67,2 | 1,1±0,07 | -55,3 | 0,08±0,04 | -57,89 | 3,4±0,1 |

It is also remarkable that the same intensity and duration of drought conditions a stronger SO emergence to the plants with less potential for self-regulating status of the water. The degree of increase in content DAM cv. Enigma is higher compared to plants cv. Indra. The action to different types of stress, the tolerance formation is closely linked with the elimination of the SRO by the antioxidant protection systems. SRO cleaving enzyme activity is more pronounced in plants adapted to moderate insufficiency of moisture, especially from representatives cv. Indra. It has been shown that dehydration and SRO eruption had a negative impact on plant photosynthesis (Tab. 3). Water stress conditioned stomatal closure and nearly complete inhibition of uptake of carbon dioxide by leaves, the processes associated with negative consequences on water efficiency in the production process. Stomatal conductance values, carbon assimilation and transpiration plants supposed to repeated water stress is kept to a higher level. So, the data lead to the conclusion that the interrelations of water and SRO form an integrated network which provides under stress conditions the induction of nonspecific protective mechanisms and are part of the plant memory stress.

## CONCLUSIONS

1. The primary reaction of soybean plants to drought action is changing of the water parameters status and tissues dehydrating, coupled with the formation of reactive oxygen species (SRO)

2. Water stress had conditioned stomatal closure, the inhibation of carbon dioxide assimilation, with negative consequences on water use efficiency in the production process.

3. The plants which have suffered a moderate drought in the early stages of ontogenesis show an increased tolerance to drought during a repeated criticism.

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# THE INCREASING OF THE *IN VITRO* MULTIPLICATION RATE FOR GRAPEVINE GENOTYPES BY APPLICATION OF LOW-INTENSITY MILLIMETER WAVES

## SPORIREA COEFICIENTULUI DE MULTIPLICARE *IN VITRO* LA VIȚA DE VIE PRIN UTILIZAREA UNDELOR MILIMETRICE DE INTENSITATE JOASĂ

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***Abstract.*** *The aim of this work involves the application of low-intensity millimeter waves on the meristematic apex for increasing of the in vitro multiplication rate for six grapevine genotypes. After the first 25-35 days of inoculation it was attested the proliferation and development of regenerants, which were subsequently transferred to nutritive media for micropropagation. The rate of explant with shoots varied between 8.39 % and 46.04 % for evaluated genotypes. The millimeter waves conducted to the increasing of this parameter. The major growth was established for Apiren extratimpuriu (by 2.24 times) and the smaller for Prezentabil (by 1.1 times). The number of shoots per explant had the minimal and maximal values certified between 1.0 ÷ 5.98 for control and 2.75 ÷ 27.83 for experimental traits. The growth capacity of this index ranged from 1.48 for Gen Moldova to 4.91 for 1-5-71 genotype.*
***Key words:*** *multiplication rate, in vitro culture, low-intensity millimeter waves, grapevine*

***Rezumat.*** *Scopul cercetărilor constă în sporirea coeficientului de multiplicare in vitro prin iradierea meristemelor cu unde milimetrice de intensitate joasă. În studiu au fost incluse 6 genotipuri de viță de vie. La primele 25-35 zile de la inoculare s-a atestat proliferarea și dezvoltarea regeneranților, care ulterior au fost transferați pe medii nutritive pentru micropropagare. La genotipurile cercetate, cota explantelor cu lăstărași a variat la martor între 8,39 % și 46,04 %, iar iradierea apexurilor a condus la sporirea acesteia. Cea mai majoră creștere (de 2,24 ori), a fost atestată la Apiren extratimpuriu, iar cea mai lejeră (de 1,1 ori) a fost înregistrată la Prezentabil. Numărul de lăstărași per explant a atins valori min/max de 1 și 5,98 pentru varianta martor și respectiv 2,75 și 27,83 pentru varianta experimentală. Potența creșterii acestui indice a fost cuprinsă între 1,48 pentru genotipul Gen Moldova și 4,91 la genotipul 1-5-71.*
***Cuvinte cheie****: rata de multiplicare, cultura in vitro, unde milimetrice de intensitate joasă, vița de vie*

## INTRODUCTION

The microclonal multiplication is applied for rapid propagation of the virus-free material (Grout and Brian, 1999) or for the production, conservation and

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improvement of plant resources (Bairu *et al.,* 2011, Ngezahayo and Liu, 2014), enabling establishment in a short period of time for obtaining of sufficient seedlings. Technical date is justified by efficiency and economic benefit of this procedure.

According to the certification requirements of grapevine planting material the microclonal multiplication methods gets applicability in practice. It is known more techniques for *in vitro* vegetative multiplication or micropropagation. The classic procedure (Chee *et al.,* 1984) involves selection of the shoot, *in vitro* inoculation of apex, shoot production and obtaining of regenerants, shoot multiplication from the subcultured shoots, rooting, *ex vitro* adaptation and transfer of the plantlets in soil substrate. The authors reported the significant increasing of multiplication rate after the first 3-4 passages. The potential for vegetative micropropagation was found after the passage 6, which corresponds to the duration of approximately 330 days of *in vitro* cultivation, which is followed by major spending. Also, prolonged subcultivation in *in vitro* conditions may conduct to the inducing variations somaclonal.

Millimeter waves therapy was found to be highly effective in biology and medicine being demonstrated positive impact on cell proliferation (Betskii *et al.,* 1998).

The aim of the present research is to study the impact of low-intensity millimeter wave irradiation on micropropagation of grapevine meristems with *in vitro* positive response in order to increase the multiplication rate.

## MATERIAL AND METHOD

As biological material for *in vitro* micropropagation were selected 6 grapevine genotypes from the collection of Research and Practical Institute for Horticulture and Food Technologies, Chisinau: 1-15-15, 1-5-71, Apiren roz, Apiren extratimpuriu, Gen Moldova and Prezentabil.

Selected shoots for *in vitro* culture (6-10 cm long) were striped of leaves and rinsed in water with three drops of Tween-20 (0.1%) and under running tap water for 15 min, after that the terminal shoot tips (5-10 mm) containing the apical meristem were excised by a sharp blade.

The explants were sterilized for about 2-3 seconds with 70% ethylic alcohol and 5.2 % calcium hypochlorite (dilution with distillate water 1:1), following by rinsing in three baths of sterile water, of 5 minutes each, to remove the total chlorine.

All sterilization operations, apex excising and their inoculation on culture media were conducted under aseptic conditions. The explants were inoculated in tubes containing 2 ml of Murashige & Skoog nutrient medium (Murashige and Skoog, 1962) supplemented with 6-benzilaminopurine (BAP) (5 μM), 1-naphthylacetic acid (NAA) (0.5 μM), 2% sucrose and 0,7% agar, which proved optimal for all analyzed genotypes. The pH of the nutrient medium was adjusted to 5.7 before autoclaving. To induce regeneration, the tubes were incubated under controlled conditions with a 16 h-light photoperiodicity temperature of $25\pm2^0$C and light - 2000lx. At 2-3 weeks after the inoculation, the explants which established a positive response were irradiated for 20-25 minutes with the low-intensity millimeter wave (λ = 5.6 mm, 53.8 GHz), using the device "IAVI-1" (flux density 1mW/cm$^2$÷10 mW/cm$^2$). After treatment the apices

were restored in growth chamber under the same controlled conditions. Untreated explants were used as control.

The obtained regenerants in the first 25-35 days after inoculation were transferred in Magenta glass jars (100/150 ml) containing 10-12 ml the media for multiplication. Every 4-5 weeks the shoots with 3 - 4 nodes were cutting and inoculated on fresh nutrient media. This procedure has being made up to 6-7 cycles of subculturing. From each cycle of subculturing the plantlets with 3-4 internodes were transferred to medium for risogenesis (Murashige & Skoog, 1962) supplemented with NAA (0.4 µM).

At 6-7 days after passage on this substrate, the regenerants presenting a rooting system with well developed were transferred to 500ml plastic pots with soil mix: peat (1:3). The pots were covered with polyethylene film and transferred to the culture room at 25±2$^{o}$C with a 16 h-light photoperiodicity. After the occurrence of 2-3 new leaves the plantlets were transferred to soil substrate according to the standard techniques.

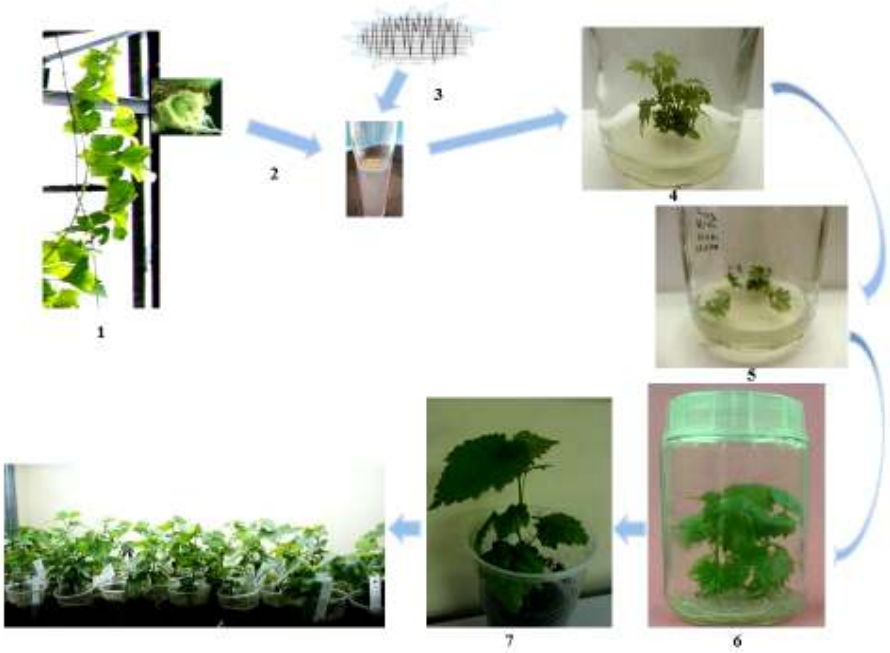


**Fig.1 - Scheme** of microclonal multiplication of the grapevine apex
(1) the selection of the shoot, (2) apex inoculation, (3) irradiation with low-intensity millimeter wave, (4) obtaining regenerants (5) subcultivation and in vitro multiplication (6) rooting and (7) *ex vitro* adaptation of plantlets to soil substrate.

## RESULTS AND DISCUSSIONS

According to the obtained results, the number of survival explants was slight influenced by the applying scheme of multiplication based on the using of millimeter waves (Table 1). For analyzed genotypes, the percentage of explants

that formed shoots had ben ranged from 8.39 for Apiren extratimpuriu to 46.04 for 1-1-15. According to the literature data (Chee *et al.,* 1984) the response of the explants to *in vitro* culture conditions and the number of formed shoots priority are dependent on the genotype. Similar reaction was established and by our results.

The irradiation of apices led to a significant increase on the rate of explants with shoots. The major growth was attested for cultivar Apiren extratimpuriu being noticed an increase of 2.24 times, while the lowest influence was registered for cv. Prezentabil (up 10%).

*Table 1*

**Effect of low-intensity millimeter waves on regeneration of grapevine meristems**

| Genotype | Treatment | Inoculated apex (number) | Survival explant (number) | Explants with shoots (%) | Plantlets per explant (number) |
|---|---|---|---|---|---|
| 1-15-15 | control | 21 | 19 | 46.04 | 5.98 |
| | mmW | 18 | 17 | 75.00 | 9.88 |
| 1-5-71 | control | 16 | 14 | 38.89 | 5.67 |
| | mmW | 12 | 11 | 52.78 | 27.83 |
| Gen Moldova | control | 40 | 37 | 23.99 | 2.64 |
| | mmW | 31 | 29 | 55.83 | 3.92 |
| Apiren Roz | control | 13 | 12 | 16.67 | 1.00 |
| | mmW | 13 | 12 | 25.00 | 2.75 |
| Apiren extratimpuriu | control | 25 | 24 | 8.39 | 2.00 |
| | mmW | 19 | 18 | 18.83 | 9.75 |
| Prezentabil | control | 9 | 8 | 25.00 | 1.00 |
| | mmW | 14 | 14 | 27.50 | 3.50 |

Also for all six investigated genotypes was found a significant increase of the number of shoots derived from an explant. The potency of growth of this index was between 1.48 and 4.91 for the varieties Gen Moldova and 1-5-71 respectively. Based on ANOVA test was found that the number of shoots per explant is determined primarily by genotype. The application of low-intensity millimeter waves had an effect 11.4% (Tab. 2).

*Table 2*

**Analysis of variance of shoots frequency per explant for different grapevine cultivars**

| Source of variance | Sum of Squares | Degree of freedom | Dispersia | Test F | Contribution of the source of variance (%) |
|---|---|---|---|---|---|
| Genotip (A) | 930,348 | 5 | 186.07 | 3.90** | 30.87 |
| Millimeter waves (B) | 343,904 | 1 | 343.904 | 7.20* | 11.41 |
| Interaction AB | 501,2 | 5 | 100.24 | 2.10 | 16.63 |
| Rezidual | 1242,03 | 26 | 47.7704 | | |
| Total | 3014,04 | 37 | | | |

*; ** - significant difference from the control at P ≤ 0.05; 0.01

Significant differences between control and irradiated apices were found for the number of shoots per explant according to the subcultivation cycle (Tab.3). If for control the main multiplication potency is observed after the third cycle of *in vitro* subcultured, while the irradiation conducted to an increase of the number of shoots after the first subculturing. The efficiency of multiplication had the major impact yet 2-3st subculturing cycles.

The accelerating of multiplication was also accompanied by a major augmentation of microclonale rate, and as a result by increasing number of obtained shoots in a short period of time.

The reduction of duration of *in vitro* subcultivation conducted to significant diminution of cost related to the nutrient medium components, growth conditions, number of working days and prevent the risk of inducing somaclonal variations and/or culture vitrification, which appeared in cultures corresponding to the multiplication stage (Bairu *et al.,* 2006).

*Table 3*

**Proliferation of shoots with 3 - 4 nodes in dependence of *in vitro* subculture cycle**

| Subcultivation cycle | Number of shoots per explant | | | | | |
|---|---|---|---|---|---|---|
| | Gen Moldova | | 1-5-71 | | 1-15-15 | |
| | control | mmW | control | mmW | control | mmW |
| First multiplication | - | 1 | - | - | 0.67 | 1 |
| 1st subculture | 0 | 4 | 0 | 12.33 | 0 | 8 |
| 2st subculture | 0 | 4.33 | 0 | 19.67 | 0 | 14.75 |
| 3st subculture | 1.33 | 4.33 | 1 | 12.33 | 1.33 | 10.5 |
| 4st subculture | 2.33 | - | 4.33 | 15 | 4.25 | 4 |
| 5st subculture | 3 | - | 5.5 | - | 2.5 | - |
| 6st subculture | 7 | - | 2 | - | 2 | - |
| 7st subculture | 3 | - | - | - | 1 | - |

According to the obtained results the effect of waves is distinguished by accelerating of multiplication, and also by increasing of the microclonal reproduction rate. Thus, if in the control treatment the value ranged between 2.38 and 3.63 for Gen Moldova and 1-5-71, then as a result of irradiation with millimeter waves this index increase to 4.2 and 14.8 respectively from the same genotypes (Tab. 4).

*Table 4*

**Theoretical potential of micropropagation via *in vitro* multiplication for some grapevine genotypes**

| Subcultivation cycle | Days of culture | Number of shoots with 3 and more nodes | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gen Moldova | | 1-5-71 | | 1-15-15 | |
| | | control | mmW | control | mmW | control | mmW |
| Cultivated explants | | 13 | 15 | 8 | 10 | 9 | 12 |
| Rate of multiplication | | 2.38 | 4.2 | 3.63 | 14.8 | 2.77 | 10.75 |
| Multiplicated explants* | | 18 | 28 | 45 | 15 | 31 | 90 |
| 1st subculture* | 60 | 32.9 | 78.4 | 204.3 | 222 | 95.5 | 806.4 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2nd subculture* | 90 | 60.3 | 219.5 | 927.5 | 3285.6 | 294.1 | 7225.3 |
| 3st subculture* | 120 | 110.3 | 614.7 | 4210.9 | 48626.9 | 905.8 | 64739.1 |
| 4st subculture* | 150 | 201.9 | 1721 | 19117.7 | 719677.8 | 2789.8 | 580062.2 |
| 5st subculture * | 180 | 369.4 | 4818.9 | 86794.4 | 10651231.8 | 8592.4 | 5197357.1 |
| 6st subculture * | 210 | 676.1 | 13492.9 | 394046.7 | 15763830.6 | 26464.6 | 46568319.7 |

*** -** theoretical potency of multiplication reported to ten subcultured regenerants

Based on the data related to this study it is presented that the microclonal multiplication potency in case of application of low-intensity millimeter waves could conduct during 6 subcultivations to an increasing of tens or to thousands of times (Gen Moldova - 19.9 times, 1-5-71 - by 400 and for 1-15-15 - more than 1750 times).

## CONCLUSIONS

Apex irradiation by low-intensity millimeter waves has a positive effect on microclonal propagation leading to an increase in the rate of 1.53 to 3.26 for different analyzed genotypes of *Vitis vinifera*.

As practical perspective the results are more efficient in order to speed multiplication during 3-4 subcultivation, which provide a sufficient number of materials for foundation of grapevine nurseries or other plant species of interest.

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# INVOLVEMENT OF WATER IN COORDINATION AND INTEGRATION OF PLANTS´ FUNCTIONS IN DROUGHT CONDITIONS

## IMPLICAREA APEI ÎN COORDONAREA ŞI INTEGRAREA FUNCȚIILOR PLANTELOR ÎN CONDIȚII DE SECETĂ

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***Abstract.*** *It was established that water in unfavorable conditions is one of the main factors of integration and coordination of physiological processes at the cellular, organ and organism level. The water stress caused disturbances in the functional interrelationships between organs as a result of differences in the degree of dehydration, the occurrence of reactive oxygen species and the antioxidant enzyme activities, especially in roots and inflorescences. The reason of the functional imbalance in drought conditions was the reduction of water and phytohormones transport, disturbed water homeostasis, and decreased enzymatic antioxidant protection. It was confirmed the hypothesis that water is the factor involved in regulating the functional activity of the plant, helps maintain coordinated relations between organs and performs, along with other factors, function integration at the organism level.*
***Key words:*** *plants, drought, coordination, integration, physiological processes, water, phytohormones, reactive oxygen species, antioxidant enzyme.*

***Rezumat.*** *S–a stabilit, că în condiții nefavorabile de umiditate apa reprezintă unul din factorii principali de integrare şi coordonare a proceselor fiziologice la nivel celular, de organ şi organism. Stresul hidric cauzează perturbări în interrelațiile funcționale dintre organe în rezultatul deosebirilor principiale ale gradului de deshidratare, apariției speciilor reactive de oxigen şi schimbării activității enzimelor antioxidante, cu precădere în rădăcini şi inflorescențe. Cauza dezechilibrului funcțional în condiții de secetă este diminuarea/inhibarea transportului apei şi fitohormonilor, dereglarea homeostazei apei, diminuarea protecției enzimatice antioxidante. S-a confirmat ipoteza conform căreia apa este factorul antrenat în reglarea activității funcționale a plantei, contribuie la menținerea relațiilor coordonate dintre organe şi îndeplineşte, de rând cu alți factori, funcția de integrare la nivel nu numai celular, dar şi de organism.*
***Cuvinte cheie:*** *plante, secetă, coordonare, integrare, procese fiziologice, apă, fitohormoni, specii reactive de oxigen, enzime antioxidante.*

## INTRODUCTION

A plant perceives environmental changes as a whole organism, and its resistance to stressogenic action of any unfavorable factor is manifested through a capacity of physiological processes coordination and by maintaining a dynamic

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balance of functions depending on oscillations of ambient conditions (Shinozaki and Yamaguchi-Shinozaki. 1999; Maurel *et al.,* 2010; Aroca *et al.,* 2012). The mechanisms of acclimation to different factors are partly identical, and non-specific primary reactions include the change of the water status and the accelerated generation of reactive oxygen species (ROS), which serve as signaling molecules of activation of protection systems (Boursiac. *et al.,* 2008; Bartolia *et al.,* 2013; Kramer and Boyer,1995). Based on the reported the objective of our study was to elucidate the role of water in the coordination and integration of crop functions in drought conditions.

## MATERIAL AND METHOD

As subjects served plants of *Zea mays* L., cultivar P458, and *Phaseolus vulgaris* L., cultivar Porumbita, representing the genotypes with different resistance potential and different morphological adaptation strategies to insufficient soil water content. The experiments were conducted in the greenhouse complex under controlled hydric regime with plants grown in containers Mitcerlih with 30 kg dry soil. The hydric stress was created by the gradual decline in soil water content range 70 - 60 - 50 to 40 - 30% of total water capacity (TWC) of the soil.

The parameters of the water status have been determined by conventional methods (Vasseu and Sharkey, 1989); transpiration intensity, $CO_2$ assimilation, stomatal conductance - using portable gas analyzer LC*i*. The testing of lipid peroxidation (LPO) intensity was performed by determining the final product - malone dialdehyde (MDA) content using thiobarbituric acid; superoxide dismutase activity SOD – by the method (Чевари *и др.,* 1985); catalase CAT – using the method of Chance B. and Machly A. (1955); ascorbate peroxidase APX – according to Nacano and Asada (1981); glutathione reductase GR and glutathione peroxidase GPX – according to Schadle and Bassham (1977). Differences between variants were documented by ANOVA analysis ("Statistica 7").

## RESULTS AND DISCUSSIONS

It was established that disturbance of internal aqueous medium and disruption of interactive relations between root – leaves - inflorescence occur under conditions of acute lack of soil water content due to different reactions of organs to hydric stress (Table 1).

*Table 1*

**Characteristic parameters of the water status of plant Zea mays L. cultivar P458 exposed to the drought**

| Treatments, soil water content, % TWC | Organ | WC, g·100 $g^{-1}$ f. m. | SD,% of full saturation | WRC, g·100 $g^{-1}$ f. m. |
|---|---|---|---|---|
| **Optimum, 70** | leafs | 80,80 ± 0,44 | 1,68 ± 0,11 | 76,63 ± 0,20 |
| | stems | 89,93 ± 0,54 | 2,56 ± 0,22 | 88,53 ± 0,12 |
| | roots | 89,00 ± 0,31 | 12,47 ± 0,17 | 70,18 ± 0,34 |
| **Drought, 30** | leafs | 78,35 ± 0,33 | 19,81 ± 0,38 | 76,29 ± 0,11 |
| | stems | 89,11 ± 0,31 | 7,29 ± 0,13 | 88,00 ± 0,10 |
| | roots | 73,60 ± 0,22 | 19,44 ± 0,14 | 50,17 ± 0,59 |

Note. WC - water content; f.m. - fresh mass; SD - saturation deficiency; WRC -water retention capacity

The drawback of soil water resulted in more prompt water content reductions in the roots, conditioning an imbalance in water supply of plant aboveground organs. Maximum quantitative differences were recorded in the net effect of the drought, also in the SD and turgidity in plant roots and inflorescences. The same profound changes were established for plants *Ph. vulgaris* L. not only in the water status of the root system, exposed to direct collisions, as well as in the leaves and, especially, in inflorescences (Fig.1).

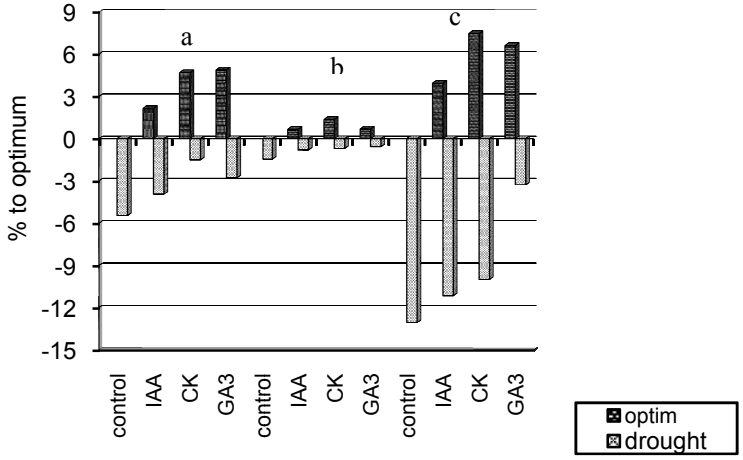


**Fig.1 -** Change of water content in: roots (a), leaves (b) and inflorescences (c) of plant *Ph.vulgaris* L. in drought conditions. Legend: IAA - indolyl acetic acid; CK - cytokinin; $GA_3$ - gibberellic acid.

It is known that the water in the organism fulfills a function of transmitting information: a decrease of water potential in root system induces a closure of stomata faster than the water content decreases in the leaves. There were significant differences in the ability to control the homeostasis of water at plants *Ph. vulgaris* L. and *Zea mays* L. under drought conditions (Table 2).

*Table 2*

**Effect of drought on the intensity of photosynthesis, stomatal conductance and transpiration for maize and beans plants**

| Treatment, soil water content,% TWC | Stomatal conductance, $Mm/m^2/h$ | Intensity of transpiration, $Mm/m^2/h$ | Intensity of photosynthesis, $Mm/m^2/h$ | WUE* Mm $CO_2$/ Mm water |
|---|---|---|---|---|
| ***Ph. vulgaris,* L.** | | | | |
| **70** | 0,04±0,01 | 1,67±0,02 | 4,36±0,08 | 2,61±0,02 |
| **30** | 0,01±0,001 | 0,55±0,01 | 0,06±0,00 | 0,11±0,00 |
| ***Zea mays,* L.** | | | | |
| **70** | 0,10±0,002 | 2,64±0,07 | 9,86±0,16 | 3,73±0,05 |
| **30** | 0,04±0,000 | 0,77±0,01 | 1,95±0,04 | 2,53±0,03 |

* WUE - efficiency of water use

Maize plants in contrast to common bean plants under the same conditions of insufficient soil water content were able to maintain the activity of the physiological processes at a less water potential in the cells - the property

appreciated in the scientific literature as *the protoplast resistance.* Disturbance of functional interrelationships in drought conditions for plants *Ph. vulgaris* L. occured at a significantly higher water potential compared to plants of *Zea mays* L. The dehydration of tissue caused enhanced formation of ROS and organ damage by oxidative destruction of cellular components. The results of the evaluation of the intensity of lipid peroxide oxidation (LPO) in the cell membrane manifested, as shown in Table 3 and Figure 2, that already at the decrease of soil water content up to 40% TWC in the leaves of the plants took place an intensified ROS formation. Compared with the control plants for those exposed to the drought, the malone dialdehyde (MDA) content, considered the marker of oxidative stress and LPO, in leaves of bean plants was increased on average by 25.8 percent and 17.08 percent - in the leaves of maize plants. The evolution of drought in time resulted in intensifying of MDA synthesis and after 3 days of stress their contents prevailed over control plants with 58.5% and 38.6% respectively. Hence, under conditions of equal intensity and duration of drought in the leaves of plants *Ph. vulgaris* L. and *Zea mays* L. developed the oxidative stress, more pronounced in the leaves of sugar beans (Table 3).

*Table 3*

**Dynamics of change of MDA content and protective antioxidant enzyme activities in leaves of plants *Zea mays* L and *Phaseolus vulgaris* L at soil water content fluctuation and evolution in time of drought**

| Soil water content, % TWC | MDA content, mkM /g f. s. | SOD, conv. un. /g f. s. | CAT, mM /g f. s. | APX, mM /g f. s. | GR, mM /g f. s. | GPX, mM /g f. s. |
|---|---|---|---|---|---|---|
| ***Zea mays*, L.** | | | | | | |
| **70** | 8,7±0,2 | 167,4±2,1 | 13,7±0,3 | 8,0±0,1 | 292,5±4,1 | 172,7±2,0 |
| **60** | 8,8±0,2 | 177,8±3,0 | 13,9±0,2 | 8,1±0,2 | 301,8±3,9 | 182,8±2,9 |
| **50** | 8,9±0,2 | 188,2±2,8 | 14,2±0,3 | 8,2±0,1 | 311,0±2,7 | 192,8±2,1 |
| **40** | 10,2±0,3 | 222,5±3,4 | 16,5±0,4 | 10,3±0,2 | 341,8±3,2 | 271,5±3,1 |
| **30, I day** | 11,2±0,2 | 239,5±3,4 | 17,8±0,5 | 15,5±0,2 | 406,0±3,8 | 283,4±2,2 |
| **30, III days** | 12,1±0,4 | 246,4±3,8 | 17,8±0,5 | 16,0±0,3 | 410,2±4,1 | 298,7±3,0 |
| **30, V days** | 21,4±0,3 | 248,4±3,1 | 18,9±0,5 | 16,9±0,3 | 417,1±4,0 | 300,9±2,1 |
| **30, VII days** | 21,7±0,2 | 243,6±2,8 | 17,8±0,4 | 17,6±0,4 | 436,5±3,5 | 320,6±3,2 |
| **30, X days** | 22,0±0,5 | 245,1±2,3 | 16,6±0,5 | 18,5±0,3 | 451,7±3,9 | 356,9±1,0 |
| ***Ph. vulgaris*, L.** | | | | | | |
| **70** | 7,4±0,2 | 68,2±1,0 | 12,0±0,2 | 9,5±0,1 | 156,5±1,2 | 126,4±0,9 |
| **60** | 8,1±0,1 | 74,5±1,1 | 14,9±0,1 | 9,9±0,1 | 163,8±1,1 | 130,2±1,1 |
| **50** | 8,9±0,2 | 80,8±1,0 | 17,8±0,2 | 10,7±0,2 | 169,8±1,0 | 134,0±1,1 |
| **40** | 9,4±0,2 | 84,0±0,8 | 18,2±0,1 | 12,8±0,4 | 178,5±0,9 | 135,4±1,2 |
| **30, I day** | 10,9±0,3 | 94,0±0,7 | 18,9±0,1 | 14,1±0,2 | 189,4±1,6 | 137,9±1,5 |
| **30, III days** | 11,8±0,4 | 100,8±1,8 | 20,8±0,2 | 14,5±0,3 | 205,9±1,4 | 147,4±2,0 |
| **30, V days** | 12,4±0,2 | 103,6±1,6 | 22,0±0,4 | 18,2±0,2 | 209,9±2,1 | 149,7±2,1 |
| **30, VII days** | 16,0±0,5 | 129,3±1,3 | 22,0±0,4 | 22,4±0,1 | 244,7±3,1 | 176,8±1,8 |
| **30, X days** | 19,8±0,4 | 130,0±2,1 | 21,3±0,4 | 23,7±0,2 | 264,4±2,1 | 184,0±1,9 |

In determining the net action of drought on the change of MDA content and the activation of superoxide dismutase, the different effects were found depending

upon duration of the stress factor. If for the maize plants the SOD activation recompensed the effect of superoxide radicals, and MDA content maintained at a relatively balanced level until the 5th day of drought, for the bean plants the SOD activation was not sufficient to dismutation of ROS.

Given that, the antioxidant enzymes showed a maximum activity at different degree of hydration, the dehydration tissues at severe drought conditions could induce and a different degree of their activation. In this case there was a disruption of the degree of compliance between peroxide-producing enzyme and those of its neutralization. When the action of the unfavorable factor was directed to the roots, a result of their strong dehydration was an authentic increase of oxidative destructions more pronounced than in the leaves and, in particular, than in stems (Fig. 2).

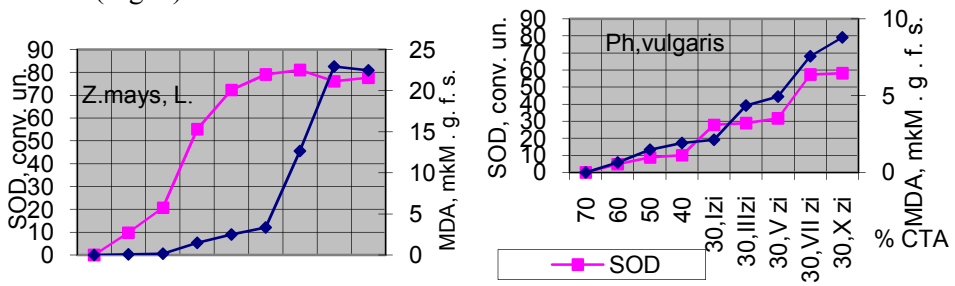


**Fig. 2 -** Net action of drought on MDA content and SOD activity in the leaves of plants of *Zea mays* L. and *Phaseolus vulgaris* L.

A non-significant correlation between catalase activity and parameters of the water status of the leaves for was found for weak drought-resistant plants: r = -0.26 for sensitive plants, while r = -0.84 for tolerant plants. The comparative analysis of the influence of heat accompanied drought on the degree of dehydration and SOD and CAT activity in leaves of weak resistant plants X5P515 allowed to assume that these enzymes had a different sensitivity to the degree of hydration: while SOD activity kept at a relatively high level and after 10 days of water content deficit (30% TWC), the CAT activity decreased after only 3-7 days (Table 3).

The results of the investigations carried out in this study lead to the conclusion that the genetically determined resistance to stress caused by drought was certainly correlated, primarily, with the plant property of self-regulation of the degree of tissue hydration, secondly, with the ability to quickly increase the antioxidant protective system activity.

The cause of functional imbalance in drought conditions was reducing / inhibiting water and plant hormones transportation, impaired water homeostasis, decrease of enzymatic antioxidant protection. The hypothesis has been confirmed that water was the factor involved in regulating the functional activity of the plant, helped maintain coordinated relations between organs and performed, along

with other factors, the function of integration not only at cellular, as well as, at organism level.

## CONCLUSIONS

1. The water stress caused disturbances in the functional interrelationships between organs as a result of principled differences in the degree of dehydration, the occurrence of reactive oxygen species and antioxidant enzyme activities change, especially in roots and inflorescences.

2. The cause of functional imbalance in drought conditions was reducing / inhibiting water and plant hormones, impaired water homeostasis, decrease enzymatic antioxidant protection.

3. The water at unfavorable conditions of soil hydric regime was a factor involved in regulating of functional activity of the plant, which helped to maintain coordinated relations between organs, and performed, along with other factors, the function of integration not only at cellular, but also at organism level.

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# RESEARCHES REGARDING SOME FEATURES OF APPLE POLLEN TUBES

## CERCETĂRI PRIVIND UNELE PARTICULARITĂȚI DE DEZVOLTARE ALE TUBULUI POLINIC LA MĂR

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***Abstract****. The pollen tubes are not always appropriate in pistil penetrating. The object of this work is to present two types of "features" that occur during the pollen germination process in vitro at some apple varieties like Pionier and Generos regarding the development and the pollen tube aspect in experimental conditions on different culture media. The manifestation of these "features" was observed on other additional experiments of apple varieties which suggests that it is an "anomaly germination", especially dependent on the species and it is not just a characteristic of the variety own. The microscopic examination of pollen germination analysis was performed by transmitted light and in phase contrast for highlighting of the pollen tubes and pollen nucleus. This kind of germination was observed in three replicates at the rate of 1 to 5%.*
***Key words****: pollen tubes, anomaly germination.*

***Rezumat****. Tuburile polinice nu sunt totdeauna adecvate străbaterii pistilului. Scopul acestei lucrări este de a prezenta doua tipuri de "particularităţi" care apar în timpul procesului de germinare al polenului in vitro la unele soiuri de măr precum Pionier şi Generos, privind dezvoltarea şi aspectul tubului polinic în condiţii experimentale pe medii diferite de cultură. Manifestarea acestor "particularităţi" a fost observată şi la alte soiuri de măr experimentate suplimentar, ceea ce ne sugerează că este o "anomalie de germinare" dependentă mai ales de specie şi nu este doar o caracteristică proprie a soiului. Examenul microscopic pentru analiza germinării polenului s-a executat în lumină transmisă şi în contrast de fază pentru punerea în evidenţă a tubului polinic cu cei doi nuclei. Acest fel de germinare s-a observat în procent de 1 şi 5%.*
***Cuvinte cheie****: tuburi polinice, anomalie de germinare.*

## INTRODUCTION

In the present research, it was experimented *in vitro,* the capacity of the pollen germination at two apple varieties namely Pionier and Generos that have different repening times. The age of trees varies from 7 to 10 years with medium and late repening period depending on variety (for Pionier, the beginning of September and the end of September and the beginning of Octomber for Generos variety). The natural fertility, vitality and ability of pollen germination *in vivo* (as a biological feature) have major implications in the quality and quantity of fruits

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production. The experiments of laboratory of freshly pollen germination, released from anthers *in vitro* , are a good test for early assessment and forecasting of the flower fertility rates *in vivo* in the fruit trees orechard (Hedhly *et.al,.* 2004). The pollen germination is a different phase preceding of the development phase of the pollen tube in styl. Each of the two stages (germinations and pollen tube growth) are stimulated by other heating conditions such as: (a) moderate temperature which stimulates the secretions of stigma, increases the pollen adhesion and promotes the germination (Hedhly *et.al.,* 2004); (b) the slightly higher temperature that accelerates the growth pollen tube and stimulates the fertilization process (Hedhly *et.al.*, 2004). Other authors (Pădureanu, 2007) have analyzed in other species as *Vitis vinifera* and *Ampelopsis*, some abnormal forms development of pollen tubes during germination, abnormalities correlated with important aspects of genetic and physiological nature of respective taxa during the germination. In another scientific paper, the same author refers to abnormal pollen tubes which may occur in *Lotus corniculatus* during germination (Pădureanu, 2007). The objective of this research is analyzed by microscopic methodes of the pollen tubes that develops during the germination process and by the evidence of some specific defects that may occur during their development/deployment.

## MATERIAL AND METHOD

The samples consisted of open flowers (anthesis) and flowers buds were collected for each variety from 10 trees from both the edge and inside the plot from the orchard. To estimate the germination capacity of pollen the anthers were easily detached from the stamen filament with a sterile needle, there put in a small watch glass with a few drops of distilled water to stimulate the release of the pollen from the anthers, thus making also the pollen hydration. Acording to the experimental results from previuos years we opted for the planting media only with pollen hidrated beforehand. Pollen previously hydrated every watch glass constituted or represented average sample for each variety being assessed. There were used extracted anthers from many flowers, to have a homogeneus sample that represent as closely a biological potential of the pollen at that time. For average sample of each variety were made sawing germination media on liquid medium with distiled water in 2 diferent concentrations of sucrose. The first concentration with sucrose 10%+$H_3BO_3$ 0,01% and the second concentration with sucrose 15%+ 0.01% $H_3BO_3$. To verify the results both their safety and each experiment were made 3 identical repetitions complete sowing and paralel germination, as shown above. For the risk limitation of contamination and to the prevent environmental deterioration of germination, all utensils, filter paper and culture medium were sterilized in advance. The glass blades germination media in small Petri dishes/plates were maintained at 17ºC to 20ºC in a humid atmospheric so as not to evaporate the liquid medium. It was thus avoides the culture medium concentration by the water evaporation and it was maintained the constant concentration of boric acid ($H_3BO_3$) and sucrose. The wet atmosphere was maintained by lining the inside of the Petri dishes with paper filter moistened with sterile distilled water (Andrew and Paraschivoiu, 2003). After seeding the first laboratory tests were made after a test period of 5 hours and verification at 24 hours . For microscopic examination were used the optic microscope type IOR ML4 -M. This was

done by transmitted light and ocular phase contrast 10x and objectives 10x, 20x, 40x. The germination was expressed percentage (G%) by the reference of the germinated pollen to the total pollen to grains germinated existing and counted in the field. As in a current practice it was found to be germinated the grains which had pollen tube length equal to twice the pollen diameter at least. The results obtained from the 3 repetitions / experiment there were expressed as percentage (%) based on the appropriate arithmetic mean.

## RESULTS AND DISCUSSIONS

The germinative capacity (G%) of the apple tree pollen was made evident on sucrose medium 10% in comparision with 15% sucrose medium. It was found that the Generous variety had 60 %, germinative capacity, double than percentage to Pioneer variety as 30% only. However, it is obvious that in general, the germination (G%) of 30 % is considered as acceptable in terms of production. In the specialized literature it is admitted that germination is representative of the fertility degree of flowers and of fruits binding respectively (Ivaşcu, 2001). The pollen tubes that have germinated in the culture media, have varied lengths of approx. 100µ - 200µ. It was observed that pollen tube length (LTP) is dependent on both internal factors (variety) and external factors (in vitro germination conditions). The most well developed (longer) pollen tubes were identified as follows (Table 1, Fig. 1, Fig. 2):

(a) depending on the variety - Generator variety (LTP 270µ max.) compared with Pioneer variety (LTP 150µ max.);

(b) depending on the concentration of sucrose in the germinative medium- the LTP max 15% sucrose are 150µ and 270µ compared with sucrose 10% and 130µ and 200µ respectively.

The pollen tube length of Generous variety faithfully respond to environmental changes and thus the pollen tubes are longer and therefore no uniform trend. The pollen tubes are not always appropriate for pistil pentrating *(in vivo* The apple germination has some "features/particularities" in terms of development and pollen tube aspect , namely:

- **Type “a” peculiarity (features) -** was found in Generous and Pioner apple variety that pollen tubes can sometimes have forked end.

In this case the two branches ending the pollen tube may or may not equal.

In general, the branching pollen tube has a normal growth in the first 1-2 hours, then there is a branch which is usually about equal to the other portion of the free pollen tube and so the final look is the bifurcation /forking. Both varieties have this "peculiarity" in the apical pollen tube and further testing found the same deviation of the tube morphology and other apple varieties as well (Prima and Florina).

The presence/manifestation of the two types of "peculiarit " in pollen tube development and design, has observed in other apple varieties, which suggests

that it is an "anomaly germination" mainly dependent on species and it is not only an own characteristic peculiarity of the variety.

This kind of germination was observed in three replicates at a rate of 1 to 5% (Fig. 3 and Fig. 4).

*Table 1*

**The action of medium composition on (LTP µ) pollen tube development of Romanian apple Pionier and Generos varieties**

| Apple varieties | LTP(µ) Medium with 10% sucrose + 0,01% $H_3BO_3$ | LTP(µ) Medium with 15% sucrose + 0,01%$H_3BO_3$ |
|---|---|---|
| Pionier | 130 | 150 |
| Generos | 200 | 270 |

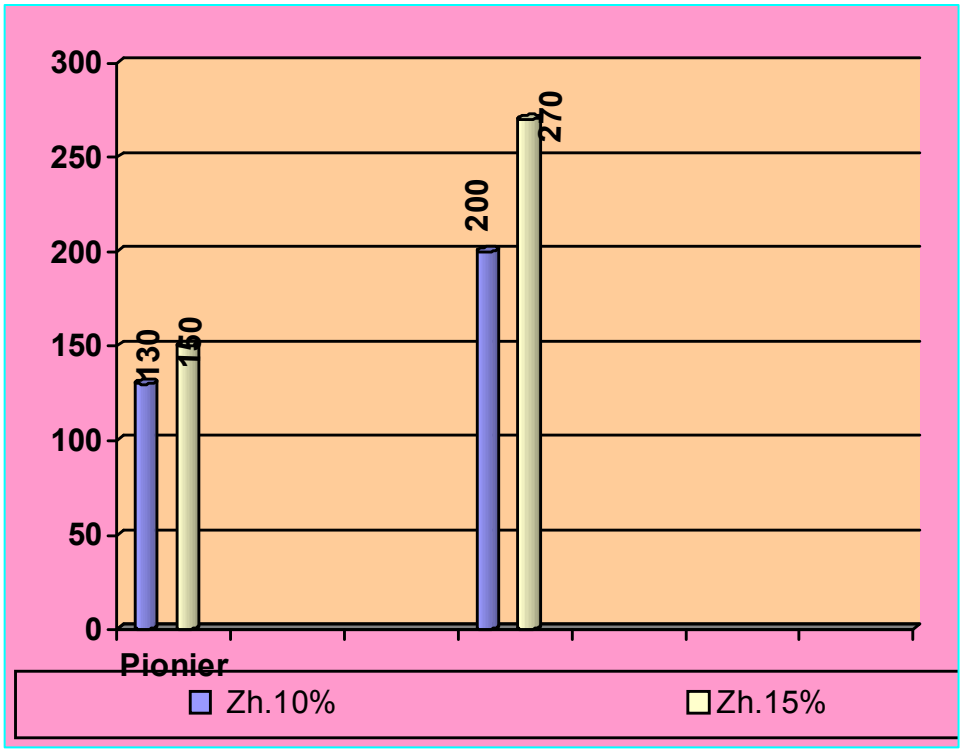


**Fig.1** - Dynamic development of the pollen tube (LTP µ) depending on the composition medium (environment) Romanian apple Pionier and Generous varieties.

**Fig. 2** - Developing Pollen Tube on Pionier variety medium with 10% sucrose.

**Fig. 3 -** The type "a" peculiarity- pollen tube with an apical forking in early stage.

**Fig .4** - The type "a" peculiarity- pollen tube with apical forking in advanced stage.

•**Type "b" peculiarity**- the second feature consist on the spiraling of the free end (extremity), usually, all in the apical zone. These deviation are more rare than branches (forkings); sometimes, the spiraling is reduced to only one or two spirals or even simple folds of the pollen tube. The fold lines are marked because the two bented portions form right angles or sharp. This type of specific

malformation of pollen tube development was observed in both varieties of apple (Fig. 5) .

**Fig. 5 -** Type “b” peculiarity with spiraling pollen tube .

## CONCLUSIONS

1. The fact that the two types of " features " that have appeared in small percentage of 1% to 5% , in pollen tube development and appearance of both varieties and other varieties that was supplementary tasted sugest us that there is an “anomaly of germination”, especially, dependent on variety species and not only a characteristic of the variety own.

2. The champion of germination in both in vitro envionments was the apple variety Generos with G%= 60% to 15% sucrose medium, double values than the Pionier variety.

3. Usually in the fruit tree practice, they consider pollen germination capacity of about 20 % provides binding fruit with a normal fruit production .

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# SOME BIOLOGICAL AND AGRONOMICAL CHARACTERISTICS CONCERNING WALNUT GERMPLASM EVALUATION IN THE CONDITIONS OF REP. MOLDOVA

## UNELE PARTICULARITĂȚI BIOLOGICE ŞI AGRONOMICE PRIVIND EVALUAREA GERMOPLASMEI DE NUC ÎN CONDIȚIILE REP. MOLDOVA

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***Abstract.*** *There are presented some biological and ecological peculiarities of local walnut varieties and new selections in the conditions of Rep. of Moldova. Main evaluated genotypes are characterized by large ecological plasticity and high adaptability to variable local climatic and edaphic conditions. There are demonstrate that formation of lateral pistillate buds there are present within all dichogamous type. But an important percent of fruit (nuts) set in the lateral positions there are rare events. We suppose that type of initiation of pistillate flower buds both in terminal and lateral positions with sustainable fructification of dichogamous of some selected Moldavian walnut selections shows a large biodiversity reserves for the future breeding programs.*
***Key words:*** *walnut, biology, breeding, promotion, Republic of Moldova*

***Rezumat.*** *Sunt prezentate unele caracteristici ale soiurilor şi selecțiilor noi de nuc în condițiile Republicii Moldova. Genotipurile de bază evaluate se caracterizează printr-o largă plasticitate ecologică şi adaptabilitate înaltă la condițiile microclimatice şi edifice variabile locale. S-a demonstrate că la toate tipurile dichogamice are loc formarea mugurilor laterali de rod (femeli). Dar legarea unui procent important al fructelor (nucilor) este un fenomen rar. Tipul de inițiere a mugurilor de rod atât în poziția terminală, cât şi în cea laterală cu producerea sustenabilă de fructe la unele selecții moldoveneşti evaluate ne permite să considerăm că biotipurile locale reprezintă o rezervă de biodiversitate largă, necesară pentru viitoarele programe de ameliorare varietală a nucului.*
***Cuvinte cheie:*** *nucul, biologie, ameliorare, promovare, Republica Moldova*

## INTRODUCTION

In spite of variable pedoclimatical conditions of different agricultural microareals, Republic of Moldova there are favorable region for walnut (*Juglans regia* L.) culture. Therefore for Moldavian peasants nuts always were and will remain a valuable product for food and trade. As a result of realisation of walnut

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breeding programs of the Institute of Horticulture there are evaluated new promising varieties, with late leafing, higher fruit quality and a better shell-meet ratio. Moldavian registered for industrial culture walnut assortment (Catalogul soiurilor de plante al Republicii Moldova, 2015) includes 14 local varieties with high level of resistance to winter and spring temperature stresses and low sensibility to blight); trees productivity increases rapidly and reaches 2-2.5 tones of kernel per hectare in 8-9 years after plantation (Comanici, 1980, Țurcanu and Comanici, 2004). Registered varieties have higher level of resistance to temperature stresses and main diseases of walnut and low sensibility to blight. But the principal trials are: sustainable high productivity and nuts qualities. For temporary testing in orchards there are included also15 foreign and local varieties. Main trials of its promotion are: sustainable productivity and nuts qualities. For new period of walnut culture it is indispensable to promote varieties with high percents of lateral bearing. The new perspectives for walnut culture in the Republic of Moldova was done after adoption of the Walnut Law (Official Monitor of the Rep of Moldova, Nr. 658-XIV, 29.12.1999.), which sets as the goal preserving and development of the existing patrimony, as well as the promotion of industrial "grafted walnut orchards" .

## MATERIAL AND METHOD

As material for researches were used 36 main important varieties and selections from national collections of walnut germplasm, established through complimentary research programmes of the Moldavian Research Institute of Horticulture and Alimentary Technologies, Botanical Garden of ASM etc. As a result of walnut breeding programs (1966-1998 years, 2000-present), based on hybridisation and evaluation of the best genotypes (including selection from local seedling populations) there are good perspectives of improvement of local varieties with new ones, first of all with late leafing (Kishinau variety), higher fruit quality and high percent of fructification from lateral buds (Pescianskii var. and 3 local selections). For investigations there are employed methodical and methodological principles which are approved for breeding and genetics (including embryological ones) of fruit trees species (Cociu and Oprea, 1989, Pîntea, 2004).

## RESULTS AND DISCUSSIONS

According our comparative investigations Moldavian varieties are characterized by high adaptability to diverse local environmental edaphical and microclimatical conditions. In comparative collaborative scientific researches there established good ecological plasticity and adaptability of the main Moldavian varieties also in the neighbor countries Ukraine, Romania, Hungary, Serbia etc.

Type of formation of pistillate flower buds (in terminal and lateral positions) and good fructification of dichogamous of selected Moldavian walnut varieties and selections shows a large biodiversity reserves for the future breeding programs concerning fructification (Tab. 1, Fig. 1, 2, 3). It is evident that formation of lateral

pistillate buds there are present within all type dichogamous type. But fruit set in the lateral positions usually there are rare phenomenon (Tab. 1).

*Table 1*

**Types of pistillate flower buds formation of and fructification of dichogamous main moldavian walnut varieties and selections**

| Nr.crt.. | Variety, type of flowering | Type of formation of pistillate buds (T-terminal, | Type of fructification (T-terminal, L-lateral) | Nr.crt.. | Variety, type of flowering | Type of formation of pistillate buds (T-terminal, | Type of fructification (T-terminal, L-lateral) |
|---|---|---|---|---|---|---|---|
| HOMOGAMOUS | | | | 19. | M-101 | T,T-L | T |
| 1. | I-33 | T,T-L | T, partially | 20. | G-48 | T,T-L | T |
| 2. | D-5, Pescias kii | T,T-L | T-L | 21. | I-69 | T,T-L | T |
| | | | T, partially | 22. | Iv4/5 | T | T |
| | | | T-L | 23. | Is-67 | T,T-L | T |
| | | | | 24. | I-28 | T,T-L | T |
| PROTOGINOUS | | | | PROTANDROUS | | | |
| 3. | Ti-23 | T | T | 25. | T-19 | T | T |
| 4. | B | T,T-L | T | 26. | I-29 | T,T-L | T |
| 5. | I-69 | T | T | 27. | D-10 | T,T-L | T,T-L |
| 6. | I-57 | T | T | 28. | I-24 | T,T-L | T |
| 7. | P-21 | T | T | 29. | Iv.4/7 | T,T-L | T |
| 8. | S-65 | T | T | 30. | I-25 | T | T |
| 9. | Cr-66 | T-L | T | 31. | G-51 | T | T |
| 10. | I-72 | T,T-L | T,T-L | 32. | G-50 | T | T |
| 11. | 2/1 | T,T-L | T | 33. | D-12 | T | T |
| 12. | I-34 | T | T | 34. | I-30 | T | T |
| 13. | P-112 | T | T | 35. | D-I | T | T |
| 14. | I-60 | T | T | 36. | D- | T,T-L | T, partially |
| 15. | I-58 D-17 | T,T-L | T, partially | | | | T-L |
| 16. | | T,T-L | T-L | | | | |
| 17. | G-47 | T,T-L | T | | | | |
| 18. | I-62 | T | T | | | | |
| | D-18 | | T | | | | |

Based on cytoembryological studies of walnut intraspecific hybridization the degree of compatibility and the capacity of compatibility of all dichogamous types was established.

It was also studied the morphohystochemical peculiarities of pistillate flower receptivity to pollination depending on flowering stage. Experimental studied of the terms of more effective pollination of dichogamous varieties in condition of Republic of Moldova shows that walnut embryo sac functional activity in lateral flowers is more long for protoginous early flowering genotypes, and short for early flowering protandrous one (Table 2).

*Table 2*

**Average period of walnut embryo sac functional activity in relation with dichogamous type and flowering period of genotype**

| Dichogamous type and flowering period | Duration of flowering (days) | | Period of active functioning of embryo sac | |
|---|---|---|---|---|
| | Terminal flowers | Lateral flowers | Terminal flowers | Lateral flowers |
| Simultanous | | | | |
| Early | 8 | 8-9 | 5-6 | 4-5 |
| Protoginous | | | | |
| Late | 6 | 6 | 5-6 | 4,5 |
| Early | 6 | 7 | 3-5 | 5-6 |
| Protandrous | | | | |
| Early | 4 | 5 | 2-3 | 2-3 |
| Late | 6 | 6 | 3-4 | 3-5 |

**Fig. 1 -** Simultaneous type of flowering: Homogamous variety Kishinau

**Fig. 2 -** Terminal flowering/ fructification of Skinoskii variety in central pomological zone

**Fig. 3 -** Lateral fructification of introduced French Fernor variety in central pomological zone

From its basic biological and ecological features o Moldavian varieties, it should be noticed the high exigency given the light, which is well seen due to its rare crown. It means the light should get in its crown so high, as well as from the lateral parts for optimization of floral buds induction. It should be noticed also that in inadequate luminosity conditions, the fit of the most dangerous disease which provokes the harvest loss is possible. Thus, in the hillock areas (which is characteristic for Moldova), moist enough, the walnut tree should be planted in southern expositions, south-western or eastern, while in the droughty areas, firstly will be used the following expositions: north-western, north-eastern, and even the northern one, where the soil humidity is better maintained, and the scorching heat is weaker. Walnut, as well as other nut cultures like the heat, producing permanently optimal harvests in the areas with annual medium daily temperature of 8-9 degrees (Germain E., 1999). Even if the walnut trees in the rep. of Moldova during the winter supports normally the temperatures of minus 20-25 degrees, the tardy spring frosts represent a permanent danger for buds and annual shoots. That is why, while choosing the varieties for the orchards setting up, it is necessary to

choose the varieties with a tardy disburgeoning and late flowering of pistillate flowers. This will assure avoidance from the respective frosts. Preliminary agrotechnical experiments show that using biannual pruning systems it is possible to stimulate formation not only of additional annual shoots, but initiation of lateral formation of pistillate buds. In the same time, fruit set in lateral positions depends a lot of optimization of soil water requirements etc.

As a result long time establishment of orchard by seeds, walnut bearing plantations represent a pronounced polymorphism, which does not always correspond to practical, especially marketing requirements. Non-homogeneous production of walnuts is realised in form of walnut kernels remain a problem. From the other hand, preservation of local domestic biotypes represent a sustainable way for long time biodiversity conservation within the species *Juglans regia* L.

## CONCLUSIONS

1. Studies of the initiation and development of pistillate walnut flowers demonstrate that type of formation of pistillate flower buds (in terminal and lateral positions) and sustainable fructification of dichogamous selected Moldavian walnut varieties and selections shows a large diversity reserves for the future breeding programs.

2. Following local breeding program should be axed on creation and promotion of perspective genotypes with high potential of both terminal and lateral types of bearing, especially protandrous ones.

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# RESEARCH ON THE FERTILIZATION AND DRIP IRRIGATION OF TOMATOES UNDER PROTECTED CROP

## CERCETĂRI PRIVIND FERTILIZAREA ȘI IRIGAREA PRIN PICURARE A UNEI CULTURI DE TOMATE ÎN SOLAR

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***Abstract.*** *This article presents the influence of the fertilization methods on the production obtained from a culture of tomatoes located in a protected space. The experiments were organized in a solar belonging to the Vegetable discipline, located in the "V. Adamachi" Didactic Farm, in Iasi County. The aim of the research is to determine the influence of fertilization by irrigation, compared to the classical fertilization and the microorganism one. In this regard, after subjecting the plants to the three types of fertilizer, there were observed differences in the amount of tomatoes per hectare. The highest production, compared to the control one, was obtained by the Minaret cultivar, fertilized dropwise, which is of 111,421 kg / ha.*
***Keywords:*** *fertigation, tomatoes, yield, tunnel.*

***Rezumat.*** *În lucrare se prezintă influența unor metode de fertilizare asupra producției obținute la o cultură de tomate amplasată în spațiu protejat. Experiențele au fost organizate într-un solar aparținând disciplinei de Legumicultură, situat în Ferma Didactică "V. Adamachi" din județul Iaşi. Scopul cercetărilor este de a determina influența fertilizării prin irigare, comparativ cu fertilizarea clasică și cea cu microorganisme. În acest sens, în urma supunerii plantelor celor trei tipuri de fertilizare, s-au constatat diferențe în ceea ce priveşte cantitatea de tomate la hectar. Producția cea mai mare, comparativ cu martorul, a fost obținută de cultivarul Minaret, fertilizat prin picurare, aceasta fiind de 111.421 kg/ha.*
***Cuvinte cheie:*** *fertirigare, tomate, recoltă, solar.*

## INTRODUCTION

Drip irrigation consists of distributing the water slowly, dropwise, to the roots of the plants and it is constant and continuous. This method of irrigation has a number of advantages, such as: enables the accurate dosing of the quantities of water and fertilizer for the plants, allows the automation of the process, reduces water consumption, increases production up to 100% and higher, reduces the occurrence of fungal diseases in the cultures by maintaining them at low

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atmospheric humidity rates, reduces the amount of weeds, thus the number of works in culture, allows performing works in the culture even during irrigation etc (Țenu, 2004, Ceauşescu *et al.,* 1984).

In order for the vegetable plants to absorb the necessary soil minerals, they consume an amount of water about 10,000 times greater than these substances (Grumeza and Drăgănescu, 1983). Fertigation refers to the injection of the fertilizer in the watering system. Through this method of fertilization, plants can receive the appropriate and recommended dosages, depending on the vegetative stage they are in, thus increasing or decreasing the amount of the fertilizer used (Snyder, 2000).

The aim of fertigation is to improve the availability of the nutrients in the wet ground, creating a reserve of nutrients proportional to the degree of culture assimilation. By fertigation, we can determine: the applied amount, the duration of the applications, the proportion of the fertilizer, the start and the end moment of the application. A fertigation system comprises the Venturi injector head control, the metering pumps, the fertilizer tank and the automatic timers.

Another factor for a successful dropwise fertilization is choosing the cultivar, that has to be suitable for the salt stress conditions. (Stoleru *et al.,* 2012; Ciobanu *et al.,* 2009)

## MATERIAL AND METHOD

The research was conducted in a semicircular solar located in the Didactic Farm of "V. Adamachi" within USAMV Iasi, with an area of 270 $m^2$.

The studied plants belong to the Minaret F1 tomato cultivar, being grouped into four work versions (Table 1), in strips, the distance between the plants in a row being 80 cm and between the rows 60 cm, resulting in a density of 31,740 plants/ha. The protection experience strip was founded with tomatoes from the 1600 Buzău variety.

*Table 1*

**Work versions (2015)**

| Current no. | Experimental version | Distance between strips (cm) | Distance between rows (cm) | Distance between plants in a row (cm) |
|---|---|---|---|---|
| **1.** | ***Minaret tomatoes*** x dropwise fertigation | 80 | 60 | 45 |
| **2.** | ***Minaret tomatoes*** x classic fertilization | 80 | 60 | 45 |
| **3.** | ***Minaret tomatoes*** x microorganism fertilization | 80 | 60 | 45 |
| **4.** | ***Minaret tomatoes*** x not fertilized (M) | 80 | 60 | 45 |

In order to irrigate the plants, a dripping line, having the diameter of the strips of 16 mm and the distance between the droppings of 10 cm, was created.

To perform the experiments, a fertigation system was projected (Figure 1) within the disciplines of Agricultural Mechanization, composed of a fertilizer tank, an automatic watering scheduling system and a watering line.

The plants from the 1st version were fertilized dropwise, weekly, every 4 days, the fertigation being carried out in the morning. The fertilizer used was Nutrispore® - NPK (MgO) 10.30.10 (2), Boron (B), Iron (Fe), Manganese (Mn), Zinc (Zn), (PGPR) - 300 kg / ha, Nutrispore ® NPK (MgO) 30.10.15 (2), Boron (B), Iron (Fe), Manganese (Mn), Zinc (Zn), (PGPR) - 425 kg / ha and NPK Nutrispore® 12-48 -8 (2) with Boron (B), Iron (Fe), Manganese (Mn), Zinc (Zn), (PGPR) - 400 kg / ha.

In order to obtain a better fertigation, plant nutrition was conducted in three stages.

1. In the first stage, the irrigation was carried out without fertilizers, which were introduced when the soil was moistened through the fertilizer tank.

2. In the second stage, the fertilizers were introduced into the fertilizer tank, along with 20 L of water. After mixing, the diluted solution has been introduced in the dripping water through the bus, thus showing proper fertigation.

3. In the third stage, the injection was stopped before the watering cycle was completed. The irrigation water allowed the fertilizer to be removed from the system.

The plants from the 2nd version were chemically fertilized with Cristaland® NPK 20-20-20, applied to the basic fertilization of 200 kg/ha, Cristaland® NP 15-50 + 2MgO in an amount of 250 kg/ha, applied in the stage of floral button (first inflorescence) and Cristaland® NPK 9-18-27 + 2 MgO, applied in the first blossom fruit phenophase (3cm), in an amount of 200 kg/ha.

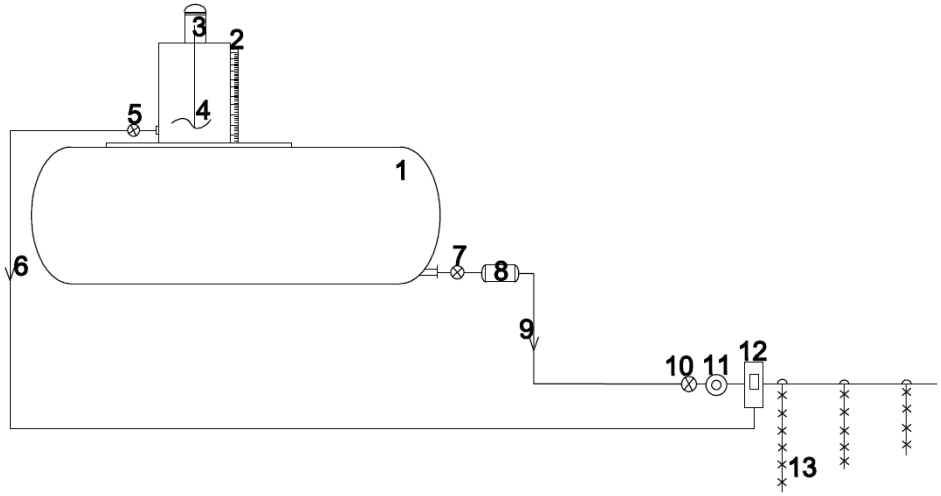


**Fig. 1 -** Fertigation system scheme

1 - water pool; 2- graduated beaker; 3- electric motor; 4 - rotating mixing paddle; 5 - way valve; 6 - water hose with fertilizing solution; 7 - pass valve; 8 - filter; 9 - water hose; 10 - pass valve; 11 - water meter; 12 - developer; 13 - dripper watering band.

The plants from the 3rd version were fertilized with fertilizers based on Micoseed® MB microorganisms, in an amount of 60 kg/ha, applied in order to prepare the ground, 2-3 days before planting the tomatoes. According to the specialty literature, Micoseed MB is a fertilizer based on Glomus sp., Beauveria sp., Metarhizium sp. and Trichoderma sp. (Stoleru *et al.,* 2014).

During the growing season, these plants have been fertilized twice with Nutryaction® at 5 L / ha.

The plants from the 4th version (the control version) were irrigated by dripping, at the same time with the plants from the 1st version, using the same water, in the same amount.

Weekly biometric measurements were performed, which determined the dynamics of the plant growth, depending on the version, aiming the plant's height, the number of inflorescences and the related flowers/ fruits.

The tomato plants were cared for according to the data of the specialty literature (Ciofu *et al.,* 2004; Indrea *and al.,* 2003). Experimental data processing was performed using the analysis of the version (ANOVA), by calculating the limit differences (Săulescu and Săulescu, 1967).

## RESULTS AND DISCUSSION

The dynamics of the tomato plant height in 2015 is shown in Table 2. During the experiments, there were conducted seven biometric measurements every 7 ... 10 days, beginning with the third week after planting. The F1 Minaret tomato cultivar shows a semi-determinate increase, being used for two crop cycles. It presents an average height of 81.2 cm.

*Table 2*

**The dynamics of the tomato plant height (cm)**

| Date / Exp vers. | 13.05. 2015 | 26.05. 2015 | 3.06. 2015 | 10.06. 2015 | 18.06. 2015 | 25.06. 2015 | 2.07. 2015 | Average hight |
|---|---|---|---|---|---|---|---|---|
| V1 (fertig.) | 46,6 | 68,0 | 76,2 | 84,6 | 91,4 | 101,0 | 108,2 | **82,3** |
| V2 (cl. fert.) | 46,0 | 58,2 | 69,2 | 79,6 | 87,2 | 94,0 | 95,8 | **75,7** |
| V3 (m.o.o.) | 44,8 | 59,6 | 73,4 | 80,2 | 87,0 | 95,6 | 95,6 | **76,6** |
| V4 (control) | 71,8 | 79,8 | 86,4 | 92,4 | 96,8 | 100,2 | 104,8 | **90,3** |
| **Mean** | **52,3** | **66,4** | **76,3** | **84,2** | **90,6** | **97,7** | **101,1** | **81,2** |

From Figure 2 we can see that the Minaret hybrid shows an upward increase of the plant height until early July, after which the growth of the plants ceases. At the beginning of the growing season (13.05. and 26. 05.), the highest values of plant height were obtained in the V4 version (the control version).

From the data we find in Table 2 we can say that, in the case of the fertilized versions, the plant height was lower at the beginning of the growing season, as the fertilizers used increased the salt nutrient content in the soil, thus the plants had a lower growth dynamics. In the second half of the growing season, we can see that V1 recorded the highest increase in the height of the tomato plants (108.2 cm).

The results concerning the dynamics of the tomato plants are shown in Table 3. The average number of inflorescences varied between 3.45 (V4) and 4.00 (V1), which shows that the fertigation version ensures the best distribution of the mineral elements in time and space.

The average number of fruits per plant ranged from 11.20, in the classic fertilized version V2, up to 15.34, in the fertigation version, V1. Average values that were close to version 2 have been completed in the control version, V4 (11.78) also.

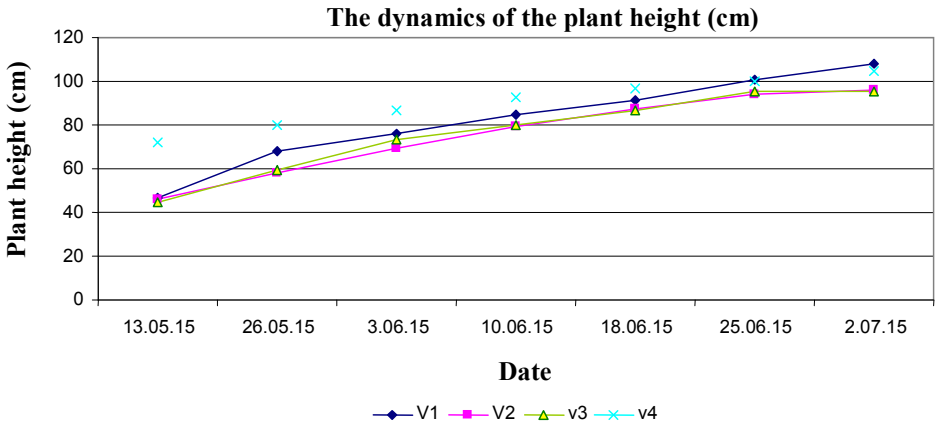


**Fig. 2 -** The plant height dynamics diagram

*Table 3*

**The results concerning the development dynamics of the tomato plants**

| Experimental version | Number of inflorescenses | Number of fruits | Average weight (g) |
|---|---|---|---|
| V1 | 4,00 | 15,34 | 228,80 |
| V2 | 3,68 | 11,20 | 228,20 |
| V3 | 3,91 | 12,54 | 248,00 |
| V4 | 3,45 | 11,78 | 209,00 |

Regarding the average weight of the Minaret tomato fruits, we can say that it ranged from 209 g in V4, up to 248 g in V3.

In the classic fertilized version, the average fruit weight was about 228 g, which indicates that the chemical fertilization favors the earliness of the fruits.

The results concerning the tomato production in 2015 are shown in Table 4. The production of tomatoes in the experiment ranged very widely, from 78,173 kg/ha in the control version, up to 111,422 kg/ha in the fertigation version.

*Table 4*

**The results concerning the tomato production (2015)**

| Experimental version | Total production, kg/ha | Relative production, % | Difference to the control version | Differencial significance |
|---|---|---|---|---|
| V1 | 111422 | 142,5 | 33249 | xxx |
| V2 | 81120 | 103,7 | 2947 | - |
| V3 | 98731 | 126,2 | 20558 | xx |
| V4 | 78173 | 100 | 0 | - |

DL 5 % = 9.136 t/ha; DL 1 % = 14.398 t/ha; DL 0,1 % = 21.438 t/ha

The difference of 33,249 kg/ha, compared to the control version, is considered to be very significant. A significant difference was also achieved in the microorganism fertilized version, the difference being of 20,558 kg/ha.

The classic fertilized version, V2, has made a difference to the control version of 2947 kg/ha, which is considered to be insignificant.

## CONCLUSIONS

1. At the beginning of the growing season, the highest values of plant height were obtained from version V4 (the control version), 79.8 cm, while in the second half of the growing season the heighest increases of the height of tomato plants, of 108.2 cm, were recorded in V1.

2. The average number of fruits per plant varied from 11.20 in the classical fertilized version V2, up to 15.34, in the fertigation version, V1.

3. The average weight of the Minaret tomato fruits ranged from 209 g in V4 (the control version) to 248 g in V3 (microorganism fertilization).

4. The tomato production in the experiment ranged from 78,173 kg/ha in the control version, up to 111422 kg/ha in the fertigation version, which means that the fertigation version provides the best distribution of chemical fertilizers in time and space.

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# PARTIAL RESULTS ON BASIL CROP IN INTERCROPPING SYSTEM

## REZULTATE PARȚIALE ÎNTR-O CULTURĂ DE BUSUIOC ÎN SISTEM DE INTERCROPPING

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***Abstract*** *The paper presents the behavior of basil (Ocimum basilicum L.) crop in two intercropping systems with runner bean (Phaseolus coccineus L.) and cherry tomatoes (Licopersicon esculentum L.). The obtained results were evaluated based on morphological and phenological characteristics of the chosen species, revealing the agrophitotechnological character of alimentary basil and its productivity in the two intercropping systems. Due to the decorative valences that basil presents, which were highlighted in the intercropping systems, this species brings unity and harmony to the specific ornamental vegetable garden of our country.*

***Key words:*** *Alimentary basil, Agrophitotechnological, Decor.*

***Rezumat.*** *Lucrarea prezintă comportamentul specie Ocimum basilicum L. în cazul a două sisteme de cultură, intercropping alături de fasolea mare (Phaseolus coccineus L.)şi tomate cherry (Lycopersicon esculentum L.). Rezultatele obținute au fost evaluate conform caracteristicilor morfologice şi fenologice ale specilor alese, scoțând în evidență caracterul agrofitotehnic al busuiocului alimentar şi productivitatea acestuia încadrul celor două asocieri. Datorită valențelor decorative pe care busuiocul le prezintă, evidențiate în cadrul sistemelor de cultură, această specie contribuie la unitatea şi armonia unei grădini legumicole ornamentale specific țării noastre.*

***Cuvinte cheie:*** *Busuioc alimentar, Agrofitothehnic, Decor.*

## INTRODUCTION

The legend says that basil grew for the first time, in a sacred place, were the Holly Cross was found by Emperors Helen and Constantine. Basil is also known in folk culture as basic, "bosioc" or "mădăcină" and presents numerous religious, medicinal and aesthetic valences. *Ocimum basilicum* L. is considered the king of spices and it is used in kitchens around the world, from spacing of salads to flavoring juices. In Italy basil is the base of the well-known pesto sauce (Fălticeanu and Munteanu, 2003).

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Basil is used in the treatment of medical problems such as infections of the respiratory tract, digestive tract, liver diseases and not least problems of the nervous system. (Fălticeanu and Munteanu, 2003). From a religious point of view basil is used in the sanctifying of holy water and adornment of icons.

From a vegetable point of view *Ocimum basilicum* L. is considered a spice, but in the same time an aromatic plant, which is renowned for its essentials oils, in a percentage of 0,1-0,4 %. Due to these basil is used in cosmetic and perfume industry, giving products a special character. Among its essential oils, basil also has in its composition mineral salts, carotene, vitamin C, phytoncides and many more (Stan *et al.,* 2003).

Aesthetically speaking, basil was used for its numerous ornamental valences, being one of the recommended species using in creating a vegetable kerb (Sima, 2009). This species is mentioned from ancient times in writings due to its habitus, and benefic effects on the human body. In Medieval Europe basil was a part of the medicinal herb garden, among other plants (mint, poppy, fennel) which was situated near the apotheque and medical building (Kluckent, 2007).

Beside the beauty of *Ocimum basilicum*'s L. port, it is also renowned for the repellent effect on pests that can appear in a crop or in a family garden.

Due to the qualities that the basil presents it is a species frequently used in biological agriculture (Fălticeanu and Munteanu, 2003). At the same time, numerous studies present the benefits that this species has on the agro system in crops in intercropping systems. Among these benefits, we can recollect increased soil quality, nutrients and soil activity, a decreased of pest attacks and a better taste of the vegetables that were associated with basil (Wu Hong Ying *et al*., 2010).

This paper presents the behavior of *Ocimum basilicum* L. in two intercropping systems, with runner bean (*Phaseolus coccineus* L.) and cherry tomatoes (*Lycopersicon esculentum* L. var. *cerasiforme*). The necessity of this study is given by the desire to produce healthy vegetables, by eliminating insecticide and chemic fertilizer, which have a toxic afterglow in vegetables or fruits, dangerous for the human body. The objects of this study are to identify the benefits that the basil brings in the two intercropping systems and its productivity.

## MATERIAL AND METHOD

For the fulfillment of the established purpose and objects, an experience was organized in the experimental field of the Department of Vegetable growing, in 2015, in "V. Adamachi" farm of USAMV Iaşi.

The biological material used in the experience was represented by two varieties of basil (*Ocimum basilicum* L.), one with green leafs (f. *bulatum*) and a local population with purple leafs; runner bean (*Phaseolus coccineus* L.) for dried beans; and cherry tomatoes (*Lycopersicon esculentum* L. var. *cerasiforme*).

The experience was conducted between 06.05-11.10. The establishment was realized in intercropping system, using standard seedlings, for basil and cherry tomatoes and seeds for runner bean.

In the first intercropping system, basil with runner bean, the basil seedling were placed at a distance of 30 cm between plants, one purple, one green and 70 cm from the runner bean row. The runner bean crop was realized in the shape of a pyramid, with 10 nests.

In the second intercropping system, basil with cherry tomatoes, the distance between basil seedlings was 25 cm and of 50 cm from the cherry tomatoes row.

The experience was established respecting the crop technology found in the reviewed literature (Stan *et al.,* 2003, Fălticeanu and Munteanu, 2003, Sima, 2009, Ciofu *et al*., 2003). The crops were established on leveled ground, well stocked with nutrients and organic matter.

The applied methods in the study were bibliographic study, experiment and observation, based on the performances of basil the two intercropping systems were evaluated.

## RESULTS AND DISCUSSIONS

Basil is an annual plant and a part of aromatic and spiced vegetables, next to dill, thyme and cicely.

The obtained results from a morphological point of view showed that basil is a well branched species, with an erect port and a taproot, which can be found at the surface of fertile soil. The plants have a strong flavor, with the purple basil having a much more intense perfume. The leaf lamina is an oval-arrow shape, whole for green basil (Fig. 1) and slightly serrate for purple basil (Fig. 2). The flowers are small, zygomorphic, white for green basil and pink for purple basil.

In the first intercropping system, basil with runner bean, based on the conducted research we found that green mass was situated between 2,857 t/ha for purple leafed basil and 4,523 t/ ha for green basil, the average of dried mass was situated between 1,346 t/ha for purple basil and 2,190 t/ha for green leafed basil. The average height was situated between 49-61 cm and the diameter of the plants has varied between 40 cm for the local population of basil and 45 cm for green basil (Tab. 1).

**Fig. 1 -** Green leafed basil

**Fig. 2 -** Purple basil

*Table 1*

**Biometric aspects for first intercropping system with runner bean**

| First establishment | | | Basil Production t/ha | | Height (cm) | Plant diameter (cm) |
|---|---|---|---|---|---|---|
| no | Specifications | | Green sprig | Dried sprig | | |
| 1 | Intercropping with runner bean | V1 | 2,857 | 1,346 | 49 | 40 |
| 2 | | V2 | 4,523 | 2,190 | 61 | 45 |

V1- *O. basilicum*, f. *bulatum*
V2- Local population of purple *O. basilicum*

The second intercropping system, basil with cherry tomatoes, based on the conducted research the green sprig productivity varied between 3,6 t/ha for the local purple population and 8,3 t/ha for the bulatum form. The diameter of the varieties was 27.33 cm for purple basil and 38 cm for green basil, while the plant height was between 41 for purple basil and 62,66 cm for green leafed basil, dimensions that can be seen in Table 2.

*Table 2*

**Biometric aspects for the second intercropping system with cherry tomatoes**

| Second establishment | | | Basil Production t/ha | | Height (cm) | Plant diameter (cm) |
|---|---|---|---|---|---|---|
| no | Specifications | | Green sprig | Dried sprig | | |
| 1 | Intercropping with cherry tomatoes | V1 | 3,600 | 1,492 | 41,00 | 27,33 |
| 2 | | V2 | 8,332 | 3,408 | 62,66 | 38,00 |

V1- *O. basilicum*, f. *bulatum*
V2- Local population of purple *O. basilicum*

**Fig. 3 -** Green basil sprigs

In what concerns the sprigs, the average was 10,66 first degree sprigs, for green basil (Fig. 3), and 9 for purple, in the first intercropping system. In the second intercropping system with cherry tomatoes the average sprig was 7, for purple basil and 8,16 for green basil. The values of the second, third, fourth and fifth sprig average are represented in table 3.

*Table 3*

**Number of sprigs in the two intercropping systems**

| no | Specification | | R I | R II | R III | R IV | R V |
|---|---|---|---|---|---|---|---|
| 1 | Intercropping with runner bean | V1 | 10,66 | 20,66 | 20,66 | 7,00 | 2,66 |
| | | V2 | 9,00 | 18,00 | 18,00 | 3,66 | 0,00 |
| 2 | Intercropping with cherry tomatoes | V1 | 8,16 | 18,33 | 23,66 | 13,83 | 2,16 |
| | | V2 | 7,00 | 17,33 | 8,00 | 1,66 | 0,00 |

V1- *O. basilicum*, f. *bulatum*
V2- Local population of purple *O. Basilicum*

R1- First degree sprig
R2- Second degree sprig
R3- Third degree sprig
R4- Forth degree sprig
R5- Fifth degree sprig

## CONCLUSIONS

1. In both intercropping systems, basil had significant and benefic contributions, regarding pests and soil activity.
2. The quality of taste for the associated species with basil was significant.
3. Regarding green sprig production, the quantity was double in the second intercropping system with cherry tomatoes for both basil varieties.
4. The biometric indicators such as diameter and height presented larger values in basil intercropping with runner bean.
5. The obtain result for first degree sprigs, did not present significant differences between the two intercropping systems.

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# INFLUENCE OF CULTURE SYSTEM ON PRODUCTION AT SOME GRAFTED TOMATO CULTIVARES

## INFLUENȚA SISTEMULUI DE CULTURĂ ASUPRA PRODUCȚIEI LA UNELE CULTIVARE DE TOMATE ALTOITE

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***Abstract.*** *The research has watched the influence of the culture system on the production at some grafted tomato cultivares. The experimental variants were grafted tomatoes: 'Alambra'/'Titron', 'Alambra'/'Beaufort', 'Alambra'/'Suketto', 'Alambra'/'Konkurabe', 'Cypriana'/'Titron', 'Cypriana'/'Beaufort', 'Cypriana'/'Suketto' and 'Cypriana'/'Konkurabe' grown at a density of 12000 and 15000 plants / ha and ungrafted tomatoes (control): 'Alambra' and 'Cypriana', grown at a density 24000 plants / ha. The culture system at grafted plants was with one and two stalks, at ungrafted plants was with one stalk. Were made determinations, observations and interpretations regarding the influence of the culture system on the production (quantity - t/ha, quality - SR 1421/2003 and earliness – days between planting and first harvest). The best results at quantity were obtained from grafted tomatoes with two stalks, then ungrafted tomatoes and grafted tomatoes conducted with a stalks; at commercial quality, grafted tomatoes were superior. At earliness, the grafted plants were late compared with the ungrafted plants. The culture system of the grafted tomatoes influence the production.*
***Key words:*** *grafting, harvest, Lycopersicon esculentum, technology*

***Rezumat.*** *Cercetarea a urmărit influența sistemului de cultură asupra producției la unele cultivare de tomate altoite. Variantele experimentale au fost tomate altoite: 'Alambra'/'Titron', 'Alambra'/'Beaufort', 'Alambra'/'Suketto', 'Alambra'/'Konkurabe', 'Cypriana'/'Titron', 'Cypriana'/'Beaufort', 'Cypriana'/'Suketto' şi 'Cypriana'/'Konkurabe', cultivate la o densitate de 12000 şi 15000 plante/ha şi tomate nealtoite (martor): 'Alambra' şi 'Cypriana', cultivate la o densitate de 24000 plante/ha. Sistemul de conducere la plantele altoite a fost cu unul şi două brațe, la plantele nealtoite a fost cu un braț. S-au realizat determinări, observații şi interpretări privind influența sistemului de cultură asupra producției (cantitate – t/ha, calitate – conform SR 1421/2003 şi timpurietate - număr de zile intre plantare şi prima recoltare). Cele mai bune rezultate la cantitatea de fructe le-au obținut tomatele altoite conduse cu două brațe, apoi tomatele nealtoite şi tomatele altoite conduse cu un braț; la calitatea comercială, tomatele altoite au fost superioare comparativ cu tomatele nealtoite. La timpurietate, plantele altoite au fost mai tardive comparativ cu plantele nealtoite. Sistemul de cultură al tomatelor altoite influențează producția.*
***Cuvinte cheie:*** *altoire, Lycopersicon esculentum, recoltă, tehnologie*

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## INTRODUCTION

The seedlings quality is decisive to ensuring economic efficiency and environmental protection (Dobrin, 2005).The technology for greenhouse tomato production by using of grafted plants is great success all over the world (Japan, Korea, USA, Turkey, Morocco) and in Europe (Italy, Greece etc.) is a tradition in this domain. The modern protected cultures are established by grafted seedlings and its have a higher spread both world and in our country because its have numerous advantages (Iliescu, 2013).

The researches on the vegetable crops grafted have started and in the Researchand Development Institute for Processing and Marketing of Horticultural Products – Horting, Bucharest, Romania in 2002 and continues and today.The researchers showed that grafting improves absorption of water and nutrients (King *et al.,* 2010, Lee 1994). The results on the fruit quality from grafted plants are contradictory (Davis *et al.,* 2008, cited by Doltu. and Bogoescu, 2014).

The researchers from the Institute Horting Bucharest have showed that the grafting influences the production and the earliness of first harvest of eggplants (Doltu and Bogoescu, 2014) studying these aspects at all the vegetables that are grafted.

## MATERIAL AND METHOD

The plants used for grafting have been tomato rootstocks, three $F_1$ hybrids, 'Titron', 'Beaufort', 'Suketto', 'Konkurabe' and scion, two $F_1$ hybrids of tomatoes, 'Alambra' and 'Cypriana'.

The experimental variants were grafted tomatoes, so: 'Alambra'/'Titron', 'Alambra'/'Beaufort', 'Alambra'/'Suketto', 'Alambra'/'Konkurabe', 'Cypriana'/'Titron', 'Cypriana'/'Beaufort', 'Cypriana'/'Suketto' and 'Cypriana'/'Konkurabe' cultured at a density of 12000 and 15000 plants/ha and ungrafted tomatoes (control): 'Alambra' and 'Cypriana', cultured at a densiy of 24000 plants/ha. The directing system of the grafted plants was with one arm and two arms and with one arm at the ungrafted plants.

Were made determinations, observations and interpretations regarding the influence of the culture system on the production (quantity - t/ha, quality - according to SR 1421/2003 and earliness - number of days between planting and first harvest).

Duncan test was used for statistical interpretations of the results and the determination coefficient.

## RESULTS AND DISCUSSIONS

The results on the influence of the culture system on the quantity of tomato fruits/ha is the average from three years of research; the results are presented in Table 1.

From analysis of productions on variants - grafted combinations and variants - ungrafted plants were found higher productions at combinations scion/rootstock: 'Alambra' $F_1$/'Beaufort' (67.00 t/ha), 'Alambra' $F_1$/'Titron' (65.75 t/ha), 'Alambra' $F_1$/'Konkurabe' (64.75 t/ha), 'Alambra' $F_1$/'Suketto' (63.25 t/ha). King *et al.,* 2010 and Lee 1994 confirms higher productions at grafted plants compared with ungrafted plants.

*Table 1*

**The influence of the culture system on tomato production (t/ha)**

| Combination scion x rootstock | Mt. | B1.1 | B1.2 | B2.1 | B2.2 | $\bar{X}$ |
|---|---|---|---|---|---|---|
| 'Alambra'/'Titron' | | 55 | 86 | 51 | 71 | **65.75** |
| 'Cypriana'/'Titron' | | 55 | 84 | 51 | 63 | **63.25** |
| 'Alambra'/'Beaufort' | | 56 | 88 | 52 | 72 | **67.00** |
| 'Cypriana'/'Beaufort' | | 54 | 85 | 50 | 64 | **63.25** |
| 'Alambra'/'Konkurabe' | | 55 | 85 | 49 | 70 | **64.75** |
| 'Cypriana'/'Konkurabe' | | 53 | 83 | 47 | 62 | **61.25** |
| 'Alambra'/'Suketto' | | 52 | 84 | 47 | 70 | **63.25** |
| 'Cypriana'/'Suketto' | | 52 | 81 | 45 | 64 | **60.50** |
| $\bar{x}$ **(grafted)** | | **54.0** | **84.5** | **49.0** | **67.0** | ***63.62*** |
| 'Alambra' | 69 | | | | | **69.0** |
| 'Cypriana' | 62 | | | | | **62.0** |
| $\bar{x}$ **(ungrafted)** | **65.5** | | | | | ***65.5*** |

It is noted that the scion has influenced the production per hectare, 'Alambra' had higher productions, grafted on all rootstocks, compared with the scion 'Cypriana', a maximum production at the combination 'Cypriana' $F_1$/'Titron' (63.25 t/ha), this is the minimum obtained at a combination of the 'Alambra' scion - 'Alambra' $F_1$/'Suketto' (63.25 t/ha). And Palada and Wu, 2005 underlines in his papers the high productions of the grafted plants compared with the plants ungrafted.

Regarding the influence of the conducting system on the production, were evidenced by a higher yield the variants of grafted plants with two stalks at density of 15000 plants/ha (84.5 t/ha) and at density of 12000 plants/ha (67.0 t/ha), compared with control variants – ungrafted plants – which recorded average yield lower (65.5 t/ha). It can be noted higher production at ungrafted variants – 'Alambra' and 'Cypriana' (65.5 t/ha) compared with grafted variants with one stalks (49-54 t/ha). The average productions were interpreted by Duncan test (Fig. 1).

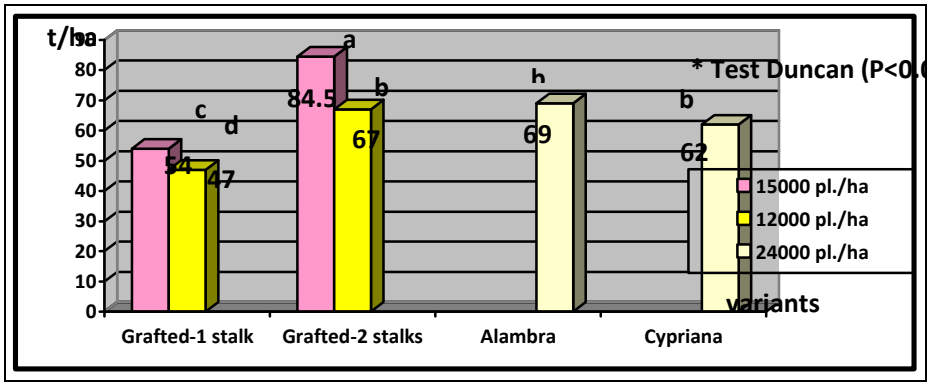


**Fig. 1 -**Statistical interpretation of production (t/ha)

Differences between variants are significant, exception the ungrafted variants and 'Alambra' - grafted, two stalks and 12000 plants/ha. Reducing of the density/ha decreases the tomato quantity/ha.

In order to determine the influence of the culture system on the occurrence of the first crop (degree of earliness) were conducted observations and measurements (date of the first crop, number of days from planting to first harvest) (Table 2).

*Table 2*

**Earliness of tomato culture**
**(date of the first crop, number of days from planting to first harvest)**

| Combination scion /rootstock | Control | | B1.1 | | B1.2 | | B2.1 | | B2.2 | | $\bar{x}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day | No. of days | Day | No. of days | Day | No. of days | Day | No. of days | Day | No. of days | Day |
| 'Alambra' / 'Titron' | | | 01.09 | 88 | 31.08 | 87 | 30.09 | 86 | 31.08 | 87 | 87.00 |
| 'Cypriana' / 'Titron' | | | 02.09 | 89 | 02.09 | 89 | 01.09 | 88 | 31.08 | 87 | 88.25 |
| 'Alambra' / 'Beaufort' | | | 31.08 | 87 | 01.09 | 88 | 31.09 | 87 | 31.08 | 87 | 88.25 |
| 'Cypriana' / 'Beaufort' | | | 02.09 | 89 | 02.09 | 89 | 02.09 | 89 | 01.09 | 88 | 88.75 |
| 'Alambra' / 'Konkurabe' | | | 01.09 | 88 | 31.08 | 87 | 31.08 | 87 | 30.08 | 86 | 87.00 |
| 'Cypriana' / 'Konkurabe' | | | 02.09 | 89 | 02.09 | 89 | 02.09 | 89 | 01.09 | 88 | 88.75 |
| 'Alambra' / 'Suketto' | | | 02.09 | 89 | 31.08 | 87 | 01.09 | 88 | 01.09 | 88 | 88.00 |
| 'Cypriana' / 'Suketto' | | | 03.09 | 90 | 03.09 | 90 | 02.09 | 89 | 02.09 | 89 | 89.50 |
| $\bar{x}$ **grafted** | | | | 88.6 | | 88.3 | | 87.8 | | 87,5 | *88.05* |
| 'Alambra' | 25.08 | 81 | | | | | | | | | |
| 'Cypriana' | 29.08 | 82 | | | | | | | | | |
| $\bar{x}$ **ungrafted** | | **81.5** | | | | | | | | | ***81.50*** |

It is clear that the grafted tomato variants were tardive as 6-7 days compared with the ungrafted tomato variants.

The precocity of the ungrafted tomatoes compared with the grafted tomatoes is signaled and from other specialists (Doltu *et al,* 2012) in scientific papers. The time differences between combinations (rootstock x scion) have varied between 87 days ('Alambra' $F_1$ /'Titron', 'Alambra' $F_1$ /'Konkurabe') and 89.50 days ('Cypriana' $F_1$ /'Suketto'). The conducting system of the strain and density per unit area have influenced little the precocity production, the differences were not significant, the days number from planting to first harvest has varied between 87.5-88.6 days ($\bar{x}$ = 88.05 days) at grafted plants and between 81-82 days ($\bar{x}$ = 81.5 days) at ungrafted plants.

Regarding the influence of the culture system on the percentage of Extra and I$^{st}$ category fruits at the tomato crop, the differences between cultivars or

combinations (scion x rootstock) were not significant (Table 3). The fruit quality was appreciated in accordance with the quality standards for fresh fruits and vegetables SR 1421/2003, for tomatoes.

*Table 3*

**Influence of the culture system on quality, Extra and I$^{st}$ category (%)**

| Variants | Control | B1.1 | B1.2 | B2.1 | B2.2 | $\bar{x}$ |
|---|---|---|---|---|---|---|
| 'Alambra' / 'Titron' | | 85.7 | 84.1 | 85.8 | 84.5 | 85.0 |
| 'Cypriana' / 'Titron' | | 85.3 | 83.8 | 85.1 | 84.1 | 84.6 |
| 'Alambra' / 'Beaufort' | | 86.2 | 84.4 | 86.3 | 84.8 | 85.4 |
| 'Cypriana' / 'Beaufort' | | 85.2 | 84.1 | 85.4 | 84.3 | 84.8 |
| 'Alambra' / 'Konkurabe' | | 84.4 | 83.9 | 84.8 | 84.1 | 84.3 |
| 'Cypriana' / 'Konkurabe' | | 84.1 | 83.8 | 84.5 | 83.8 | 84.0 |
| 'Alambra' / 'Suketto' | | 84.7 | 84.0 | 84.7 | 84.1 | 84.4 |
| 'Cypriana' / 'Suketto' | | 84.2 | 83.7 | 84.5 | 83.6 | 84.0 |
| $\bar{x}$ **grafted plants** | | 85.0 | 84.0 | 85.1 | 84.2 | *84.6* |
| 'Alambra' | 80.9 | | | | | 80.9 |
| 'Cypriana' | 80.4 | | | | | 80.4 |
| $\bar{x}$ **ungrafted plants** | **80.7** | | | | | ***80.7*** |

At density 15000 plants/ha were obtained 85-85.1% of extra and I$^{st}$ category fruits; at density 12000 plants/ha were obtained 84-84.2% of Extra and I$^{st}$ category fruits. Regarding the culture system with one stalk, the Extra and I$^{st}$ category fruits were 84-85% and at culture system with two stalks were between 84.2-85.1%. Analysis of data presented show a high commercial quality of tomato fruits from grafted cultures, 84.6% (Extra and I$^{st}$ fruits) compared with ungrafted cultures, 80.7% (Extra and I$^{st}$) .

**Correlations between the culture system (number of stalks/plant) and production and fruit quality**

In Figure 3 is observed a direct linear correlation between the culture system (number of stalks/plant) and parameters concerning production. The value of the determination coefficient shows that at average production per hectar the correlation significance is strong, very significant ($r^2 = 1$).

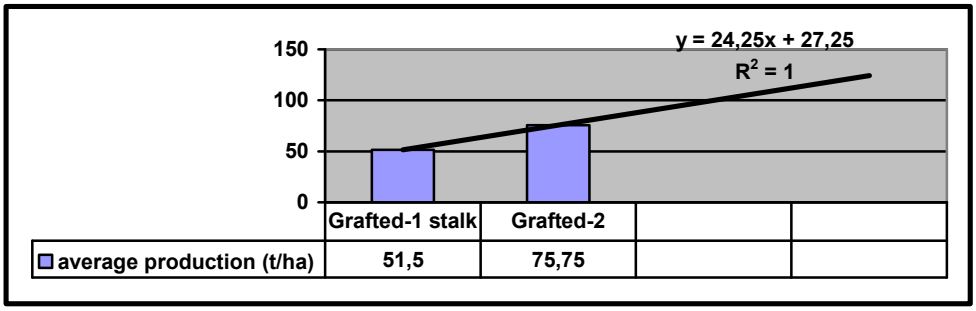


**Fig. 3 -** Influence of culture system (no. of stalks/plants) on production

In Figure 4, it is noted that there is a indirect linear correlation between number of stalks/plant and parameters of fruit quality (Extra and a I-a category).

The value of the determination coefficient shows that at number of stalks/plant, the correlation significance the correlation significance ($r^2$ = 0,9044).

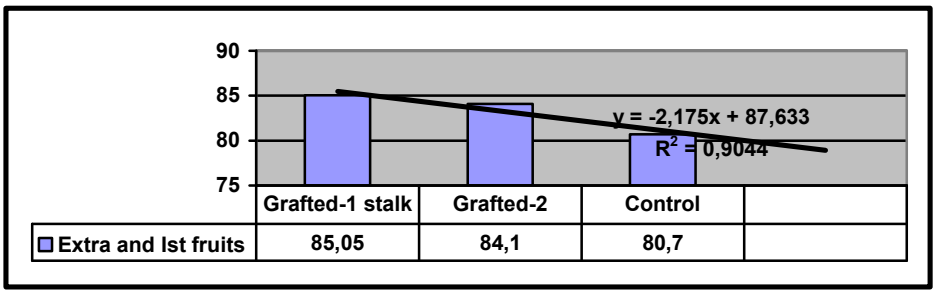


**Fig. 4 -** Influence of culture system (no. of stalks/plants) on tomato quality

## CONCLUSIONS

1. The cultivars scions and rootstocks researched had compatibility by grafting, in order to achieve the desired results (quantity and quality).

2. The grafting induce a slight tardiness.

3. The density per area unit and the culture system influences the production; best results are obtained with a density of plants at half compared with ungrafted cultivars and culture system with two stalks/plant.

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# INFLUENCE OF FERTILIZATION MANAGEMENT ON THE QUANTITY AND QUALITY OF WHITE CABBAGE

## INFLUENȚA FERTILIZĂRII DIFERENȚIATE LA VARZA ALBĂ ASUPRA CANTITĂȚII ȘI CALITĂȚII PRODUCȚIEI

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***Abstract:*** *The cabbage crops is one of the most important spectrum vegetable plants, this is demonstrated by the large area planted and yields obtained. The quality and quantity of white cabbage depend by variety, technology, fertilizer management, and maintenance. The purpose of this work has been to evaluate the influence of management fertilization on early cabbage production and the content of nitrites and nitrates. The favorable effect of fertilization regime on total production at Bourbone cultivar observed by different production from 43.363 t/ha in control to 69.376 t/ha at variant fertilized with Micoseed®. The nitrate content ranged from 0.34 mg/kg in control up to 0.72 mg/kg in the version organic fertilized. The nitrate content ranged from 48.11 mg/kg in control up to 162.14 mg/kg in version fertilized with Nutrifine®.*
***Key words:*** *Brassica oleracea L., var. capitata L., f. alba DC; fertilizers; contaminants; yield*

***Rezumat:*** *Cultura verzei este una dintre cele mai importante în spectrul plantelor legumicole, acest fapt este demonstrat de suprafața mare cultivată dar și de producțiile obținute. Calitatea producției de varză albă precum și cantitatea acesteia, diferă în funcție de soi, tehnologia de cultivare, îngrășăminte administrate, lucrări de întreținere precum și de condițiile meteorologice. Experința de față a avut ca scop studiul influenței fertilizării diferențiate a unei culturi de varză timpurie asupra producției totale si a conținutului de nitriți și nitrați. Efectul favorabil al fertilizării asupra producției totale la cultivarul Bourbone se observă din variația producției de la 43,363 t/ha în varianta martor la 69,376 t/ha varianta fertilizată cu Micoseed®. Conținutul de nitriți a variat de la 0,34 mg/kg în varianta martor până la 0,72 mg/kg în varianta fertilizată organic, iar conținutul de nitrați a variat de la 48,11 mg/kg în varianta martor până la 162,14 mg/kg în varianta fertilizată cu Nutrifine®.*
***Key words:*** *Brassica oleracea L., var. capitata L., f. alba DC; fertilizanți; contaminanți; recoltă.*

## INTRODUCTION

White cabbage is widespread in Romania (15% of the vegetable), being cultivated from early spring until late autumn, using adapted cultivation in this sense (Stan and Munteanu, 2001). White cabbage is resistant specie to low

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temperatures, which gives a good adaptation to climatic conditions and the possibility of extending cultivation conveer.

Early cabbage crop in the NE region of Romania can establish successful during 1-7 April in each year, were obtained production from 29.89 t/ha up to 31.01 t/ha, for a crop density of 47,619 pl./ha (Sabareanu-Stoleru, 2010). According to the weather conditions of every year, planting period can increase or decrease obtained production.

For other areas of Romania such Transylvania, at a density of 71,500 pl./ha, in the conventional system can achieve an average production of between 52-56 t/ha (Csok, 2009).

The cultivar for establishing the crop can also have a major role in achieving high productions (Indrea *et al.,* 2012).

In the cabbage crop system, the cultivar has a decisive influence on production, and the economic efficiency of crop. If in conventional crop be carried out studies on fertilizer influence on production if they are relatively few organic farming and worth studying because it prevents contamination production. The differentiated fertilization in organic agriculture has positive influence on pest control (Stoleru *et al.*, 2012)

## MATERIAL AND METHOD

The experiment with early cabbage crop was carried out in an experimental plot at UASVM Iasi, during 2014.

The experimental crop was established in 7 April 2014, by seedlings of 45 days, with a distance design of 70 x 30 cm (N= 47°11'34,07" E= 27°32'59,63"). Seedlings were been produce in a greenhouse, according with specific literature (Stan and Munteanu, 2001; Indrea *et al.*, 2012).

The soil from the stationary is a mold chambic chernozem, easy antropic, whit the following physicochemical properties, in the substrate of 0-60 cm: clay 32 %, ph=7,11, EC=252.3 µS/cm2, $CaCO_3$=1.03%, OM=28.23 mg/kg, C/N = 5,20, N=4.53 g/kg, P=106.66 ppm, and CEC = 20.9 meq/100g.

The biological material was used the ***Bourbone F1***, recommended for early open field.

To achieve its purpose, treatments were applied fertilizers in organic farming systems organic and conventional as follows: Orgevit® = 1300 kg/ha (applied in five stages, the first stage in 20 April and the following 10 in 10 days), Nutrifine®=1100 kg/ha (like as Orgevit®) and Micoseed®=75 kg/ha (applied in three stages, the first stage in 20 April and the following 10 in 10 days, that the treatment 4 and 5 was used Nutryaction®=5 l/ha)

Micoseed® It is a fertilizer based on microorganisms, particularly based *Glomus sp.* Nutrifine® is a synthetic chemical complex like as NPK 20-20-20. Orgevit® is an organic fertilizer (100%) from chicken manure applied as drops.

The total yield and dynamic of production, according with fertilization schemes, was carried out by weigh the heads, for each harvest.


**Fig.1** - Bourbone cultivar

The principle of humidity determination is based to loss of the drying oven at a temperature of 102°C, until a constant mass. The loss will be calculated as a percentage relative to the initial mass of the sample (Butnariu and Butu, 2014). Determination of heavy metals in vegetables is by dry mineralization. This consists in the destruction by carbonization and incineration (450-500°C) oven sample; ash being thus passed by dissolving in dilute hydrochloric acidan (Butnariu and Butu, 2014). The analyses of the heavy metals in cabbage have been made at the UASVM Iasi and UASVMB Timisoara.

The dates collected have been statistical analyzed by one-way analysis of variance (ANOVA) and least significant differences (LSD) at 5%, 1% and 0.1% confidence levels for production. For the contaminants, the statistical significance were carried, compared with maximum accepted limit (MAL).

## RESULTS AND DISCUSSIONS

### Production results from early white cabbage

In 2014, early white cabbage production ranged from 43.36 t/ha to 69.37 t/ha. In all experimental versions, the production was higher to the control un-fertilized. In the version biological fertilized with microorganisms, total production was the biggest is considered positive very significant.

In the versions organic and chemical fertilized, the total yields obtained were relatively similar, and are superior to control.

*Table 1*

**The influence of fertilization on cabbage yield**

| Experimental version | Average head cabbage (g) | Total yield (t/ha) | Relatively yield *vs.* Control (t/ha) | Differences *vs.* Control (t/ha) |
|---|---|---|---|---|
| Bourbone x Nutrifine | 1276.99 | 53.199 | 122.68 | 9.836** |
| Bourbone x Micoseed | 1665.28 | 69.376 | 159.99 | 26.013*** |
| Bourbone x Orgevit | 1277.69 | 53.228 | 122.75 | 9.865** |
| Martor | 1040.89 | 43.363 | 100.00 | 0 |

DL 5%=4,38t/ha; DL1%=6,63 t/ha; DL 0,01%=10,65 t/ha

**- distinctly significant positive differences; ***- very significant positive differences.

**Results on nitrate and nitrite content on white cabbage**

The nitrites content ranged from 0.34 mg/kg in control up to 0.72 mg/kg in the version organic fertilized. The nitrate content ranged from 48.11 mg/kg in control up to 162.14 mg/kg in version fertilized with Nutrifine®.

From the data obtained on fertilization regime on the content of nitrites and nitrates, we can say that regardless of the amount of fertilizer used and the type of early cabbage does not exceed the maximum acceptable limits, according with scientifically literature (Butnariu and Butu, 2014; Stoleru *et al.*, 2014).

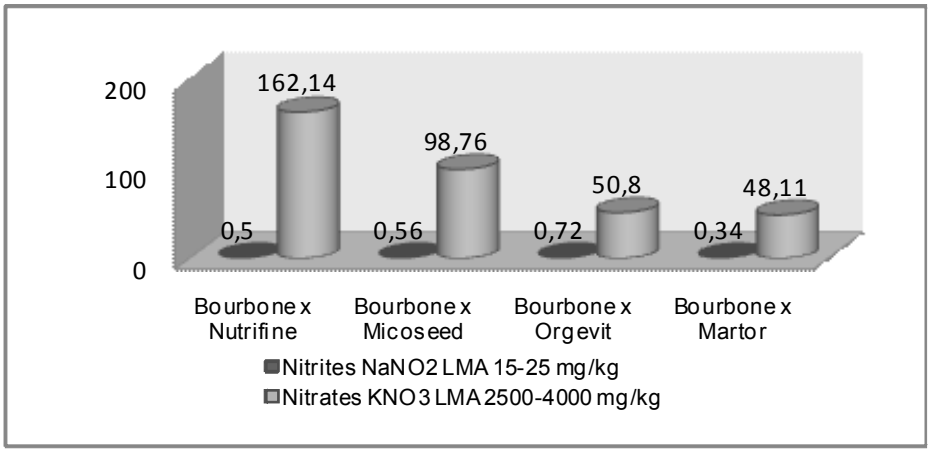


**Fig.2 -** The content of nitrites and nitrates in the white cabbage (mg/kg)

**The influence of fertilization on the content of heavy metals in cabbage**

The results on the influence of fertilization on heavy metal content are presented in Tabel 2. The values obtained for the heavy metals of each variant were compared with the MAL. The content of Cr range from 55 to 67 mg/kg, which shows that each version was exceeded MAL. Cu content varied from 31 to 37 mg/kg, which highlights that regardless of fertilization regime, the content of this element is not influenced.

The Pb element varied in the term of large limits, from 26 mg/kg in the biological version up to 52 mg in Control. In this version has been recorded and who is overcoming MAL who is 50 mg/kg.

Zn content rage from 118 mg/kg in chemical version up to 155 mg/kg.

*Table 2*

**Heavy metal contents on white cabbage**

| Experimental version | Heavy metal content (mg/kg) | | | |
|---|---|---|---|---|
| | Cr | Cu | Pb | Zn |
| Bourbone x Nutrifine | 55 | 37 | 48 | 118 |
| Bourbone x Micoseed | 56 | 33 | 26 | 132 |
| Bourbone x Orgevit | 67 | 35 | 35 | 139 |
| Bourbone x Martor | 58 | 31 | 52 | 155 |
| **MAL** | **50** | **50** | **50** | **150** |

## CONCLUSIONS

The best production result was obtained in the version fertilized with Micoseed® (69,376 t/ha).

Regarding fertilization regime used in experimental version that regardless of the amount used has not influenced the content of nitrates and nitrites.

In most samples, the MAL for heavy metals was not exceeded. Values higher than the MAL were recorded for version control.

The presence of chemical contaminants in most versions, maintaining the support for monitoring investigations in order to protect human health.

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# STUDIES REGARDING THE POSTHARVEST CARE OF CUT FLOWERS FOR SOME ORANAMENTAL *ALLIUMS*

## STUDII PRIVIND PĂSTRAREA FLORILOR TĂIATE LA UNELE SPECII ŞI CULTIVARE ORNAMENTALE DE *ALLIUM*

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***Abstract.*** *This study presents some results regarding the behavior of some Allium species and cultivars cut flowers, which were treated with giberelic acid ($GA_3$) and cycocel (CCC). The floral stems of A. giganteum, A. moly and A.`Purple Rain` were harvested in three different flowering stages (at the opening of 1/4, 1/2 and 3/4 from the total flower number of the inflorescence) and were treated with $GA_3$ and CCC solutions, in 250, 500 and 1000 ppm concentrations. The control was preserved in water. The average of vase resistance is 23,9 days for A. giganteum, 21,3 days for A. 'Purple Rain' and 11,4 days for A. moly. The flower stems which were harvested at the stage of 1/4 and 1/2 open florets had the longest resistance. The most efficient solutions were $GA_3$ 500 ppm and CCC 250 ppm for A. giganteum, $GA_3$ (all concentrations) and CCC 250 ppm for A.'Purple Rain' and only water for A. moly.*
***Key words:*** *Allium, cut flowers, cutting stages, preserving.*

***Rezumat.*** *Lucrarea prezintă rezultate privind comportarea florilor tăiate la specii şi cultivare de Allium păstrate în soluții de acid giberelic ($GA_3$) şi cycocel (CCC). Tijele florale de la A. giganteum, A. moly şi A. 'Purple Rain' au fost recoltate în diferite stadii de deschidere (la 1/4, 1/2 și 3/4 flori deschise în inflorescență) şi au fost păstrate în soluții de la cele două produse, în concentrații de 250, 500 și 1000 ppm. La martor păstrarea s-a făcut în apă. În medie, inflorescențele tăiate la A. giganteum au rezistat 23,9 zile, la A. 'Purple Rain', 21,3 zile, iar la A. moly, 11,4 zile. Inflorescențele recoltate la deschiderea a 1/4 şi 1/2 din numărul de flori au înregistrat cea mai bună rezistență. Soluțiile conservante cele mai eficiente au fost cele de $GA_3$ 500 ppm şi CCC 250 ppm la A. giganteum, cele de $GA_3$ (în toate cele trei concentrații) şi CCC 250 ppm la A. 'Purple Rain' şi numai apa la A. moly.*
***Cuvinte cheie:*** *Allium, flori tăiate, faze de recoltare, păstrare.*

## INTRODUCTION

Known mostly as vegetables, the *Alliums* have also important ornamental traits, which completed by the environmental resistance and the great colour range, make them easy to be used in landscape architecture, floral or interior design (Fritsch and Friesen, 2002). Through the first and the most used ornamental *Alliums* are: *A. giganteum* Regel, *A. moly* L. or *A.*

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*sphaerocephalon* L., plants witch are more and more used today (Davies, 1992; Cottrell, 1999; Kamenetsky and Fritsch, 2002; cited by Harding, 2004).

The interest of the romanian florists for these plants is increasing at the moment and this work is giving some informations about the vase resistance of *Allium* cut flowers, using some different stages and postharvesting treatments with growth regulators (giberelic acid and cycocel). The growth regulators have very large range of using in horticuture, like to reduce the bulb sprouting or to incease the flower number for many ornamental plants, including some Alliums (Pogroszewska *et al.*, 2007; Sardoei, 2014; Toma and Jităreanu, 2007). Krzyminska (2009) highlight that the *Allium* cut flowers postharvest life is different betwen the species: 15-20 days for *A. aflatunense* L., 21-29 days for *A. christophii* L., 28-35 days for *A. giganteum* Regel and 15-19 days for *A. rosenbachianum* L. and that the cutting stage is very important for the postharvest life.

## MATERIAL AND METHOD

The study was realised in may – june, 2015, the material was represented by three *Allium* cultivars *Allium*: *A.* 'Purple Rain'*, A. moly* L. and *A. giganteum* Regel, cultivated in the experimental field of Floriculture, from University of Agricultural Sciences and Veterinary Medicine Iași. The inflorescences were harvested in three flowering stages, as 1/4, 1/2 and 3/4 open florets. There were used different solutions with giberellic acid ($GA_3$) and cycocel (CCC), in 250, 500 and 1000 ppm concentrations, to preserve the cut flowers. The control was kept in distilled water. The preservation solutions were changed at every two days. Every variant had three replications, each one having three flower stems. For every cultivar studied, the experimental factors were represented by the preservation solution (7 graduations) and the cutting stage (three graduations), the experiment accumulating 21 variants (tab. 1).

*Table 1*

**Experimental variants**

| Experimental factors | Specification | Variant/Graduations |
|---|---|---|
| Storage solutions | Distilled water | **$V_1$ (control)** |
| | $GA_3$ | $V_2$ - 250 ppm |
| | | $V_3$ - 500 ppm |
| | | $V_4$ - 1000 ppm |
| | CCC | $V_5$ - 250 ppm |
| | | $V_6$ - 500 ppm |
| | | $V_7$ - 1000 ppm |
| Flowering stage | % open florets | $S_1$ - 1/4 |
| | | $S_2$ - 1/2 |
| | | $S_3$ - 3/4 |
| | | **Average (control)** |

The cutting flower stem length was different, depending on the natural height of the plants: *A.* 'Purple Rain' – 40 cm, *A. moly* – 20 cm, *A. giganteum* – 60 cm. The flowers were preserved in about 22-24$^0$C and about 1000 lx daily illuminance. The obtained data and the results were processed using the variant analysis and the LSD method (Săulescu and Săulescu, 1967).

## RESULTS AND DISCUSSIONS

The cut flowers from the three *Allium* taxa registered a very good vase resistance, especially *A. giganteum* and *A.* 'Purple Rain' (the average longevity - 21.2-23.9 days); at *A. moly* the postharvest longevity was lower with 50% than the other two taxa (11,3 days). The cut flowers resistance was influenced by the both experimental factors (the postharvest treatment and the cutting stage), but it was different betwen the species. From the individul analysis of those two experimental factors, could be observed that each one had an influence over the logevity of the *Allium* cut flowers.

Regarding the *A. giganteum* species, the storage solutions (different like product and concentration) had different influence comparing with the control (water), most of the results being statistically insured, excepting $GA_3$ 250 ppm solution (tab. 2).

*Table 2*

**The influence of the preservation solution over the *A. giganteum* cut flower storage time**

| Variant (preservation solution) | Storage time (days) | Difference toward the control (±) | Significance of difference |
|---|---|---|---|
| **$V_1$ – water (control)** | **24.1** | - | **-** |
| $V_2$ – $GA_3$ 250 ppm | 24.2 | +0.1 | ns |
| $V_3$ - $GA_3$ 500 ppm | 25.8 | +1.7 | *** |
| $V_4$ - $GA_3$ 1000 ppm | 23.7 | -0.4 | o |
| $V_5$ – CCC 250 ppm | 25.0 | +0.9 | *** |
| $V_6$ – CCC 500 ppm | 22.9 | -1.2 | ooo |
| $V_7$ – CCC 1000 ppm | 22.4 | -1.7 | ooo |

LSD 5% = 0,3 LSD 1% = 0,4 LSD 0,1% = 0,6

The $GA_3$ 500 ppm solution had a good influence over the longevity of the cut flowers, extending the vase life with about 2 days, folowed by the $V_5$ variant (CCC 250 ppm) with an extension about 1 day. The CCC solutions with big concentrations (500 and 1000 ppm) have determined the reducing of the flower longevity with 1-2 days, the differences toward the control were very negative significant (tab. 2). Regarding the cutting stage, the results registred differences very significant positives in the stages $S_1$ and $S_2$ (at the stage at 1/4 and 1/2 open florets). The more late harvesting, at 3/4 open florets, shorten the storage time with 1-2 days toward the average of the variants with about 3 days comparing with $S_1$ (tab. 3).

*Table 3*

**The influence of the cutting stage over the *A. giganteum* cut flower storage time**

| Variant (open florets stage) | Storage time (days) | Difference toward the control (±) | Significance of difference |
|---|---|---|---|
| $S_1$ - ¼ open florets | 25.1 | +1.2 | *** |
| $S_2$ - ½ open florets | 24.7 | +0.8 | *** |
| $S_3$ - ¾ open florets | 22.3 | -1.6 | ooo |
| **Average (control)** | **23.9** | - | - |

LSD 5% = 0,3 LSD 1% = 0,5 LSD 0,1% = 0,6

At *A.* 'Purple Rain', the longevity of the cut flowers, even if it outrun 20 days, it is a bit reduced but *A. giganteum*. Between those 7 variants which regards the storage solution, all the $GA_3$ solutions were favorable (with very significant differences) and also the CCC 250 ppm solution (with distinct significant differences). The $V_6$ and $V_7$ variants registered values closed to the control, with positives and negative differences, but without statistically insure (tab. 4).

*Table 4*

**The influence of the preservation solution over the *A.* 'Purple Rain' cut flower storage time**

| Variant (preservation solution) | Storage time (days) | Difference toward the control (±) | Significance of difference |
|---|---|---|---|
| **$V_1$ – water (control)** | **20.4** | **-** | **-** |
| $V_2$ –ppm | 21.3 | +0.9 | *** |
| $V_3$ - $GA_3$ 500 ppm | 22.8 | +2.4 | *** |
| $V_4$ - $GA_3$ 1000 ppm | 22.2 | +1.8 | *** |
| $V_5$ – CCC 250 ppm | 21.0 | +0.6 | ** |
| $V_6$ – CCC 500 ppm | 20.7 | +0.3 | ns |
| $V_7$ – CCC 1000 ppm | 20.2 | -0.2 | ns |

LSD 5% = 0,4 LSD 1% = 0,5 LSD 0,1% = 0,8

Regarding the cutting stage (tab. 5), the best variant is $S_1$, the 1/4 open florets cutting stage, which insure positive differences, very significant toward the average (it extend with 1-2 days the storage time). The harvesting when most of the florets are open, reduce the storage time with almost 2 days toward the average. In $S_2$ stage, the differences are positives but insignificant (tab. 5).

*Table 5*

**The influence of the cutting stage over the *A.* 'Purple Rain' cut flower storage time**

| Variant (open florets stage) | Storage time (days) | Difference toward the control (±) | Significance of difference |
|---|---|---|---|
| $S_1$ - ¼ open florets | 22.8 | +1.6 | *** |
| $S_2$ - ½ open florets | 21.4 | +0.2 | ns |
| $S_3$ - ¾ open florets | 19.5 | -1.7 | ooo |
| **Average (control)** | **21.2** | **-** | **-** |

LSD 5% = 0,3 LSD 1% = 0,5 LSD 0,1% = 0,6

*Table 6*

**The influence of the preservation solution over the *A. moly* cut flower storage time**

| Variant (preservation solution) | Storage time (no. of days) | Difference toward the control (±) | Significance of difference |
|---|---|---|---|
| **$V_1$ – water (control)** | **13** | **-** | **-** |
| $V_2$ – $GA_3$ 250 ppm | 10.1 | -2.9 | ooo |
| $V_3$ - $GA_3$ 500 ppm | 9.9 | -3.1 | ooo |
| $V_4$ - $GA_3$ 1000 ppm | 9.8 | -3.2 | ooo |
| $V_5$ – CCC 250 ppm | 12.7 | -0.3 | ns |
| $V_6$ – CCC 500 ppm | 12.6 | -0.4 | ns |
| $V_7$ – CCC 1000 ppm | 11.2 | -1.8 | o |

LSD 5% = 1.4 LSD 1% = 2.0 LSD 0,1% =2.9

The *A. moly* species has a different behavior toward *A. giganteum* and *A.* 'Purple Rain', especially regarding the storage solution. From the results presented in Table 6, the preservation in water in the most favorable. The $GA_3$ solutions are not suitable, no matter the concentration, they decrease the flower longevity with more than 3 days. The CCC also has negative effect, but the differences toward the control is smaller, insignificant at 250 and 500 ppm or significant at 1000 ppm. The storage time at *A. moly* cut flowers is very less influenced by the cutting stage, is oscillating between 11 and 11.6 days, with the average between the variants by 11.3 days (tab. 7). Between the three variants, the best results registered the cutting stage 1/4 and 1/2 open florets (positive significant differences toward the average).

*Table 7*

**The influence of the cutting stage over the *A. moly* cut flower storage time**

| Variant (open florets stage) | Storage time (days) | Difference toward the control (±) | Significance of difference |
|---|---|---|---|
| $S_1$ - ¼ open florets | 11.6 | +0.3 | * |
| $S_2$ - ½ open florets | 11.4 | +0.1 | * |
| $S_3$ - ¾ open florets | 11.0 | -0.3 | o |
| **Average (control)** | **11.3** | **-** | **-** |

LSD 5% = 0.1 LSD 1% = 0.4 LSD 0,1% = 0.9

*Table 8*

**The combined influence of the experimental factors over the cut flower longevity (days)**

| Experimental factors | | Species (cultivar)/significance of differences | | |
|---|---|---|---|---|
| **Preservation solution** | **Inflorescences cutting stage** | ***A. giganteum*** | ***A.* 'Purple Rain'** | ***A. moly*** |
| $V_1$ water | $S_1$-1/4 open florets | 25.6** | 21.3 | 13.0 $^{ns}$ |
| | $S_2$-1/2 open florets | 25.0 * | 21.3 | 13.0 $^{ns}$ |
| | $S_3$-3/4 open florets | 21.6 $^{ooo}$ | 18.6 $^{ooo}$ | 13.0 $^{ns}$ |
| $V_2$ $GA_3$ 250 ppm | $S_1$-1/4 open florets | 25.0 * | 23.3 *** | 10.0 $^{ns}$ |
| | $S_2$-1/2 open florets | 25.0 * | 21.3 | 10.0 $^{ns}$ |
| | $S_3$-3/4 open florets | 22.6 $^{oo}$ | 19.3 $^{ooo}$ | 10.3 $^{ns}$ |
| $V_3$ $GA_3$ 500 ppm | $S_1$-1/4 open florets | 27.6 *** | 24.6 *** | 10.0 $^{ns}$ |
| | $S_2$-1/2 open florets | 26.6 *** | 23.0 *** | 9.6 $^{ns}$ |
| | $S_3$-3/4 open florets | 23.0 $^{o}$ | 20.6 | 10.0 $^{ns}$ |
| $V_4$ $GA_3$ 1000 ppm | $S_1$-1/4 open florets | 24.6 | 24.6 *** | 10.0 $^{ns}$ |
| | $S_2$-1/2 open florets | 23.6 | 21.3 | 9.6 $^{ns}$ |
| | $S_3$-3/4 open florets | 22.6 $^{oo}$ | 20.6 | 9.6 $^{ns}$ |
| $V_5$ CCC 250 ppm | $S_1$-1/4 open florets | 26.3 *** | 22.3 * | 12.6 $^{ns}$ |
| | $S_2$-1/2 open florets | 26.0 *** | 21.3 | 12.6 $^{ns}$ |
| | $S_3$-3/4 open florets | 22.6 $^{oo}$ | 19.3 $^{ooo}$ | 12.6 $^{ns}$ |
| $V_6$ CCC 500 ppm | $S_1$-1/4 open florets | 23.3 | 22.3 * | 12.6 $^{ns}$ |
| | $S_2$-1/2 open florets | 23.3 | 20.6 | 12.3 $^{ns}$ |
| | $S_3$-3/4 open florets | 22.0 $^{ooo}$ | 19.0 $^{ooo}$ | 12.6 $^{ns}$ |
| $V_7$ CCC 1000 ppm | $S_1$-1/4 open florets | 22.6 $^{oo}$ | 21.0 | 12.6 $^{ns}$ |
| | $S_2$-1/2 open florets | 23.3 | 20.6 | 12.3 $^{ns}$ |
| | $S_3$-3/4 open florets | 21.3 $^{ooo}$ | 19.0 $^{ooo}$ | 12.6 $^{ns}$ |
| **Average** | | **23.9** | **21.1** | **11.4** |
| | | LDS 5% = 0,90<br>LDS 1% = 1,21<br>LDS 0,1% =1,6 | LDS5% =0,96<br>LDS1% =1,23<br>LDS0,1%=1,62 | LDS 5% = 2,8<br>LDS 1% = 3,8<br>LDS0,1%=5,1 |

The interaction of the two experimental factors (tab.8), the best results were obtained at *A. giganteum* by harvesting blossoming in the early stages of opening (S1, S2) and preservation in GA3 500 ppm or CCC 250 ppm; for *A.* 'Purple Rain' is recommended either harvesting in stage 1 and preservation in $GA_3$ solution (250 and 1000 ppm), or harvesting in stage 1 and 2, preservation in $GA_3$ 500 ppm. In all preservative solution and for all harvesting stages, *A. moly* longevity cut flowers presented a non significant differences, but better results in water (tab. 8).

## CONCLUSIONS

1. The cut flower longevity of the three *Allium* (between 9.8 and 25.8 days) justify the possibility to use them in this way.

2. The most valuable as cut flowers (regarding the natural length of the floral stems and longevity) are *A. giganteum* and *A.* 'Purple Rain'.

3. In all cases, the optimum cutting stage of the inflorescences is $S_1$ (1/4 open florets) and $S_2$ (1/2 open florets).

4. As preservation solutions it can be recommended $GA_3$ 500 ppm and CCC 250 ppm at *A. giganteum,* $GA_3$ (all concentrations) and CCC 250 ppm at *A.* 'Purple Rain' and only water for *A. moly.*

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# RESULTS CONCERNING THE CREATION OF CULTIVARS WITH RIPENING AGES AT THE LIMITS OF CHERRIES MATURATION SEASON AND THEIR EVALUATION

## REZULTATE PRIVIND CREAREA DE SOIURI CU EPOCI DE COACERE AFLATE LA EXTREMITĂȚILE SEZONULUI DE MATURARE A CIREŞELOR ŞI EVALUAREA LOR

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***Abstract:*** *The paper's aim is to present the valuable characteristics of a couple cherry cultivars created at SCDP Iaşi, which improve the inland cherry assortment with early and late maturation cultivars, with quality fruits. At the moment the domestic market, but also the external one is unbalanced, favoring the cultivars from the first part of the fruits maturation season (June). The tendency is to create a balance, assured by reducing the weight of the cultivars with average age of fruits maturation and by increasing the weight of the extra early and late cultivars. Analyzing the average productions on three years (2011-2013), from the statistical point of view, it can be seen that the cultivars Marina (31,1 kg/tree) and Margo (30,9 kg/tree) registered significantly positive production differences compared to the witness cultivar Boambe de Cotnari (21,7 kg/tree). In terms of fruits weight (g) and of equatorial diameter (mm), the cultivars Anda (7,6 g and 23,7 mm) and Marina (7,2 g and 22,2 mm) got remarked with significantly positive differences compared to the witness.*
***Key words:*** *objectives, cherry, cultivars, early, late, fruit.*

***Rezumat:*** *Scopul lucrării este de a prezenta caracterele valoroase a unor soiuri de cireş create la SCDP Iaşi, care îmbunătățesc sortimentul de cireş autohton cu soiuri cu maturare timpurie şi târzie, cu fructe de calitate. În acest moment piața internă dar şi cea externă este dezechilibrată în favoarea soiurilor din prima parte a sezonului de maturare a fructelor (luna iunie). Tendința este de realizare a unui echilibru, asigurat prin reducerea ponderii soiurilor cu epocă mijlocie de maturare a fructelor şi a sporirii celor extratimpurii şi târzii. Analizând producțiile medii pe trei ani (2011-2013), din punct de vedere statistic, se constată că soiurile Marina (31,1 kg/pom) şi Margo (30,9 kg/pom) au înregistrat diferențe de producție semnificativ pozitive față de soiul martor Boambe de Cotnari (21,7 kg/pom). Sub aspectul greutății fructelor (g) şi a diametrului ecuatorial (mm), s-au remarcat soiurile Anda (7,6 g şi 23,7 mm) şi Marina (7,2 g şi 22,2 mm) cu diferențe semnificativ pozitive față de martor.*
***Cuvinte cheie:*** *obiective, cireş, soiuri, timpurii, târzii, fruct.*

## INTRODUCTION

Due to the feature of having an earlier ripening age of the fruits compared

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to the other fruit-growing trees species (beginning from May), the cherry tree represents the first ring in the annual chain of fruits production (Budan and Gradinariu, 2000; Grădinariu and Istrate, 2004; Petre, 2006).

The researches concerning the assortment improvement for the cherry species, the cultivars quality and the avoidance of short term congestion on the fresh cherry market are objectives that gain increasingly higher importance.

At the moment, the internal market and also the external one is unbalanced in the favour of the cultivars from the first part of the fruits maturation season (June). The tendency is to create a balance, assured by reducing the share of the cultivars with middle age of fruits maturation and by increasing the share of the extra early and late cultivars (Budan and Grădinariu, 2000).

The aim of the paper is to present the valuable characteristics of some cherry cultivars created at SCDP Iaşi, which improve the inland cherry assortment with cultivars with early and late maturation, with quality fruits.

## MATERIAL AND METHOD

As working material, there were used the cherry genotypes existent in the national collection. The basis of this germplasm background were set in 1981 and it consists at the moment of 555 genotypes from inside the country and from abroad, with old and new cultivars, local cherry biotypes, hybrids and clones.

The method of creating the new cultivars was the classic one and it consisted in making controlled sexed hybridizations, hybrid kernels harvesting, obtaining hybrid saplings, selecting according to the established objectives and testing the hybrids (Cociu & Oprea, 1989).

The time from hybridizations to homologation was long (16-26 years) due to the species biological particularities (low kernel germination, low viability of the saplings in the first years from planting/transplanting, late starting of ripening etc.).

The breeding programme objectives took into account the creation of new cherry cultivars that would be qualitative competitive and with genetic resistance to diseases, pests, stress factors (frost, draught), reduced vigour of trees, flowering lateness and superior quality fruits under commercial, technological aspect and under the aspect of the chemical features, with ripening ages from the extremities of the maturation season of the cherries.

The studies have been taken during 2011 – 2013, having as research material six cherry cultivars (Cătălina, Cetățuia, Marina, Margo, Anda and George). From the six genotypes taken in study, two of them are with early maturation (Cătălina, Cetățuia) and four genotypes are with late maturation (Marina, Margo, Anda and George). The comparison of the cultivars was made against the local area witness Boambe de Cotnari.

For the creation of the new cherry cultivars with early and late maturation of the fruits, there have been used the cultivars Van, Boambe de Cotnari, Bigarreau Drogan, Cilegia di Ottobre, HC. 23/31, Lijana and Fromm.

The selected elites were grafted on mahaleb, after which were planted in competition micro crops at a distance of 4 x 5 m. The trees were led under flattened free pelmet shape, without sustaining system and without irrigation system.

**In experimental planting there were followed**: the trees vigour, (Cociu and Oprea, 1989); the main fructification phenophases (Fleckinger, 1960); physical features

(fruit's and kernel's weight, equatorial diameter of the fruit, the report fruit/kernel, % kernel from the fruit's weight, the epidermis colour); chemical and quality features of the fruits (SUS%, pulp firmness, fruit shape, kernel adherence to pulp, fruits resistance to cracking); the productivity (it was determined according to the fruits production kg/tree and the fertility index that represents % of resulted fruits at 25-30 days after the petals fall and high productivity cultivars are considered to be the ones with values over 30-35%)(Cociu and Oprea, 1989).

The experimental data were statistically interpreted by analyzing the variance and it was calculated the variation coefficient (s%) for which it is arbitrary admitted the following values: 0 -10% - low variation coefficient; 10 – 20% - average variation coefficient; 20 – 30% - high variation coefficient.

## RESULTS AND DISCUSSIONS

Following the notifications and determinations made in the national cherry collection, numerous potential genitors were established for different useful characteristics and features, which have been used in intraspecific controlled hybridizations.

On the basis of these features, there have taken place annual hybridization programmes with the participation of 208 genotypes from which 34 maternal and paternal genitors have been used frequently, being followed 16 features of the trees and of the fruits.

The results after using the germplasm background for creating new cherry cultivars during 1981-2913 can be seen in Table 1. There have been made 774 hybrid combinations with the participation of 208 genitors, pollinating 738,041 flowers from which 135.804 hybrid fruits have been harvested, from which there have been extracted 97.847 kernels good to be sowed.

Out of these kernels, 7.782 hybrids have risen from which 1.720 ripened. Following the selections, 86 hybrids were promoted as elites, 33 being multiplied, planted and studied in competition micro crops (tab. 1).

During 1999-2011, from the elites studied in the competition micro crops, 22 elites have been homologated as new cultivars and in December 2013 four hybrid selections have been registered for homologation at ISTIS (one with early ripening age, two with average ripening age and one selection with late ripening age).

*Table 1*

**The use of germplasm background for creating new cherry cultivars (1981-2013)**

| The hybridization year | Of combinations | Used genitors (nr.) | | Pollinated flowers -nr- | ybrid harvested fruit -nr- | Resulted hybrid kernels -nr- | Arisen saplings -nr- | Hybrids on fruit -nr- | Perspective hybrid selections -nr- | Homologated cultivars -nr- | Hybrid elites suggested for homologation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ♀ | ♂ | | | | | | | | |
| Total 1981-2013 | 774 | 208 | | 738.041 | 135.804 | 97.847 | 7.782 | 1.720 | 86 | 22 | 4 |

The trees vigour for the six cherry cultivars taken in study is average (table 2). The flowering period was between 10th of April and 10th of May, therefore for the cultivars Cetățuia and Cătălina the flowering was early and Anda and Margo cultivars manifested lateness for flowering.

The values registered for natural fertility for the six cultivars were between 9,8% (Cătălina) and 77,8% (Cetățuia), registering a high variation coefficient (59,3%) and they are classified as of high productivity, because the fertility index registered values above 30%, exception being the cultivar Cătălina (tab.2).

The harvesting maturity was registered in the third decade of May (Cătălina and Cetățuia), the end of the third decade of June (Anda, Margo) – the first decade of July (Marina, George) and the number of days from the end of the flowering to maturation was between 27-70 days, registering a high variation coefficient (39 – 32%) (tab. 2).

*Table 2*

**The trees vigour and the main fructification phases for the cherry cultivars with early and late ripening age (2011-2013)**

| Cultivar/ Phenophase | Tree's vigour | Beginning of flowering (phase E) | End of flowering (phase G) | Natural fertility (%) | Fruits maturation date | Nr. Of days from the end of flowering to maturation |
|---|---|---|---|---|---|---|
| **Limit dates (earliest - latest):** | | | | | | |
| Cătălina | middle | 12 - 21.04 | 24.04-1.05 | 9,8 | 22.05-4.06 | 27-35 |
| Cetățuia | middle | 12 - 20.04 | 20 - 27.04 | 77,8 | 22 - 30.05 | 29-37 |
| Marina | middle | 16 - 23.04 | 22.04-01.05 | 64,8 | 18.06-01.07 | 58-62 |
| Anda | middle | 20 - 24.04 | 01 - 05.05 | 45,4 | 18 - 24.06 | 49-55 |
| Margo | middle | 22 - 26.04 | 01 -10.05 | 55,3 | 18 - 24.06 | 44-54 |
| George | middle | 18 - 23.04 | 30.04 -1.05 | 51,2 | 02 -10.07 | 64-70 |
| Variation coefficient (%) | - | 31,9-12,1 | 95,0-166,7 | 59,3 | 57,5 -81,2 | 39,0-32,0 |

No matter the climatic conditions of the year, the order in which the cherry cultivars reach maturity is always kept the same, the only difference being that the time interval between two successive cultivars is longer or shorter (Istrate, 1998).

Analyzing the average productions over three years (2011-2013), from the statistical point of view, it can be noticed that the cultivars Marina (31,1 kg/tree) and Margo (30,9 kg/tree) registered production differences positive significant compared to the witness cultivar Boambe de Cotnari (21,7 kg/tree) (tab. 3).

The weight of the fruit is a dimension that is influenced by the local climatic conditions and by the biological particularities of each cultivar. From the statistical point of view, the cultivars Anda, Marina and Margo registered positive significant differences compared to the witness.

Under the aspect of equatorial diameter (mm), the cultivar Anda got remarked (23,7 mm), with positive significant differences and the cultivars Marina (22,2 mm), Cătălina (21,9 mm) and Margo (21,5 mm) with positive differences compared to the witness (21,2 mm) (tab. 3). As kernel size, the

cultivars registered a weight between 0,14-0,31 g, having a small to middle size according the UPOV questionnaire.

The report fruit/kernel was between 20,3 (Cătălina) and 27,1 (Cetățuia, Anda and Marina) registered very significant positive differences. The percent of the kernel from the fruit's weight registered values between 3,66% (Cetățuia) and 5,0% (George). From the statistical point of view, the cultivars Cetățuia, Anda and Marian registered negative significant differences compared to the witness (tab. 3).

*Table 3*

**Fruits production and physical features of the cherry cultivars (2011-2013)**

| Cultivar/ Biotype | Average production of fruits (kg/tree) | Fruit's average weight (g) | Kernel's average weight (g) | Report fruit/kernel | Kernel from the fruit's weight (%) | Fruit's equatorial diameter (mm) |
|---|---|---|---|---|---|---|
| Cătălina | 19,4 | 6,3 | 0,31 | $20,3^{00}$ | 4,96 | 21,9 |
| Cetățuia | 21,3 | $3,8^{00}$ | $0,14^{0}$ | $27,1^{+++}$ | $3,66^{0}$ | $18,0^{00}$ |
| Boambe de Cotnari (Mt) | **21,7** | **6,7** | **0,33** | **22,1** | **4,92** | **21,2** |
| Marina | $31,1^{+}$ | 7,2 | 0,27 | $26,7^{+++}$ | $3,75^{0}$ | 22,2 |
| Anda | 15,1 | 7,6 | 0,28 | $27,1^{+++}$ | $3,73^{0}$ | $23,7^{++}$ |
| Margo | 30,9+ | 6,8 | 0,30 | 22,7 | 4,43 | 21,5 |
| George | 18,1 | $5,5^{0}$ | 0,27 | $20,4^{00}$ | 5.00 | 19,8 |
| LSD 5% | 8,2 | 1,1 | 0,14 | 0,9 | 1,0 | 1,8 |
| LSD 1% | 11,6 | 1,5 | 0,20 | 1,3 | 1,4 | 2,5 |
| LSD 0,1% | 16,8 | 2,2 | 0,29 | 1,9 | 2,0 | 3,7 |

The fruits colour was from yellow (Anda, Margo), double coloured (Marina) to dark red (Cătălina, Cetățuia, George) (tab. 4).

The pulp firmness is an important quality element, especially for the fruits intended for fresh consumption (Kappel et al., 2000). In our case, the early cultivars (Cătălina, Cetățuia) have semi firm pulp and the late cultivars have firm and very firm pulp.The content in SUS was between 15,8% (Margo) and 18,6% (Anda) (tab. 4).

All the cultivars taken in study manifested a good resistance to the fruits cracking phenomenon, the registered values being under 16%.

*Table 4*

**Physico-chemical and quality features of the fruits (2011-2013)**

| Cultivar | Epidermis colour | Pulp firmness | Fruit shape | SUS -%- | Kernel's adherence to pulp | % of fruits cracked after 6 hours |
|---|---|---|---|---|---|---|
| Cătălina | Dark red | middle | Heart-shaped | 16,7 | Non-adherent | 6,3 |
| Cetățuia | Dark red | middle | Kidney-shaped | 16,9 | Semi-adherent | 15,5 |
| Marina | Double coloured | firm | Heart-shaped | 16,0 | Non-adherent | 10,1 |
| Anda | Yellow | very firm | Kidney-shaped | 18,6 | Non-adherent | 10,0 |
| Margo | Yellow | firm | Heart-shaped | 15,8 | Non-adherent | 1,3 |
| George | Dark red | firm | Heart-shaped | 17,0 | Non-adherent | 4,0 |

## CONCLUSIONS

1. The new created cultivars correspond to the actual goals concerning the trees vigour, flowering lateness, fruits resistance to cracking, productivity, fruits quality and having ripening ages at the extremities of the cherries maturation season.

2. The creation of the new cherry cultivars with early maturation (Cetățuia, Cătălina) and late maturation (Marina, Anda, Margo and George) of the fruits ensures an extending of the season of fresh fruits and fruits for industrialization with 10-15 days.

3. There are perspectives for an increasing consumption of fresh fruits for the cherry species on a period of 42-46 days, with outstanding taste qualities offered also by the unique microclimate which can be found on the axis Cotnari, Iaşi, Răducăneni from the Iaşi County.

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# EVALUATION OF SWEET AND SOUR CHERRY CULTIVARS USEFUL IN GENETIC IMPROVEMENT

## EVALUAREA UNOR SOIURI DE CIREŞ ŞI VIŞIN UTILE ÎN AMELIORAREA GENETICĂ

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***Abstract.*** *During 1999-2014 at RSFG Iasi were approved 28 new sweet cherry cultivars from which 25 with sweet taste and three with bitter taste. The sour cherry have been approved and patented three cultivars in the period 1978-1994. The methods used to obtain new cultivars of cherry were selection of local cultivated flora, artificial hybridization, mass selections obtained by free pollination. As genetic resources were used sweet cherry collection (established and continuous collected during 1980-2006) with 550 genotypes and sour cherry collection (established and collected during 2000-2002) with 130 genotypes. The paper aims to assess six sweet cherry cultivars and four sour cherry cultivars that can be used as genitors in improving these both fruit tree species.*
***Key words:*** *phenologie, trunk section, fruit, diameter, skin color*

***Rezumat.*** *În perioada 1999-2014 la SCDP Iaşi s-au omologat ca soiuri noi de cireş 28 de creaţii din care 25 de cireş dulce şi 3 de cireş amar. La vişin au fost omologate şi brevetate 3 soiuri în perioada 1978-1994. Metodele utilizate pentru obţinerea de noi soiuri de cireş şi vişin au fostselecţia din flora locală cultivată, hibridarea artificială, selecţii în masă obţinute prin polenizarea liberă. Ca resurse genetice au fost folositecolecţia decireş (perioada de infiinţare şi colectare continuă 1980-2006) cu 550 genotipuri şi colecţia devişin (înfiinţare şi colectare 2000-2002) cu 130 genotipuri. Lucrarea are ca scop evaluarea a şase soiuri de cireş şi patru de vişin care pot fi utilizate ca genitori în ameliorarea celor două specii pomicole.*
***Cuvinte cheie:*** *fenologie, secţiunea trunchiului, fruct, diametru, culoare pieliţă.*

## INTRODUCTION

Sweet cherry (*Prunus avium* L.) is a wild, semi-wild or cultivated species while sour cherry (*Prunus cerasus* L.) is just semi-wild or cultivated in Romania (Sîrbu, 2011; Sîrbu and Paraschiv, 2005). In the last thirty years new sweet and sour cherry cultivars with high fruit quality (Sansavini and Lugli, 2005; Kask *et al.,* 2010; Schuster *et. al.,* 2014) was approved. Pérez-Sánchez, 2008 evaluated 25 sweet cherry, four duke and one sour cherry from traditional Spanish cultivars which showed distinctive and interesting agronomical characters such as low

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susceptibility to fruit cracking, high levels of soluble solids, early fruit maturity and great rusticity. The growers from Romania are very interested by newest sweet and sour cherry cultivars due to a program for renewing local fruit growing which at present is ongoing. The paper aims to assess six sweet cherry and four sour cherry cultivars that can be used as genitors in improving these both fruit tree species.

## MATERIAL AND METHOD

In this study during 2013-2014 six Romanian cultivars: 'Cătălina', 'Ludovic', 'Cociu', 'Boambe de Cotnari', 'Galata' and 'Maxut' and four sour cherry cultivars 'Pitic', 'Selecția Cotea', 'De Botoşani' and 'Ilva' were evaluated. All cultivars were grown on *P. mahaleb* L. seedlings rootstock. Three trees presented each cultivar and were planted at spacing of 4 x 5 m, with free palmette crown shape with support system. The orchard was located on a medium sandy clay loam with medium (6%) humus content. Herbicide spraying were mantained along trees rows and grass was cut three times during summer in alleyways. No irrigation, rainfull, frost or birds protection system provided.

Some parameters related to phenological stages, tree and fruit characteristics were determined. Phenological data were determined through the Fleckinger system (Fleckinger, 1960): $B_1$ - the bud swelling: the bud rounds delicate and gains a green light at the top; G- the end of the flowering: the petal of flowers have fallen for 90%. The data of the fruit ripening was established in the time of marketing quality traits (colour, the content of dry matter) specific to each cultivar.

The climatic datawere recorded with the AgroExpert system by the station located on the perimeter of the experimental polygon of the Fruit Growing Research Station, Iaşi - Romania. The active thermal balance (Σt°a) is provided by the sum of average daily temperature grades, which exceeds the biological limit characteristic to the sweet and sour cherry tree, considered to be 5°C (Istrate, 2007).

**Σt°a =ΣT atd – BL**, in which:

**ΣT atd** = sum of average temperature of days between two subsequent phenological stages;

**BL** = the biological limit of fruit tree species (Istrate, 2007).

The statistical interpretation of data was calculated with the variation coeficient (s%) for which are allowed arbitrary the next values: 0 – 10% - low variation coefficient; 10 – 20% - medium variation coefficient; 20 – 30% - great variation coefficient. The statistical analysis was performed with the XLSTAT programme.

## RESULTS AND DISCUSSIONS

During 2013 - 2014 it has been observed a great variability of the number of days and the sum of active degrees of temperature according to the sweet and sour cherry tree cultivars and climatic conditions of the year. Therefore, the period from the swelling of the mixed buds until the fall leaves, the studied cultivars showed large variations invegetation period and the active thermal balance.

*Table 1*

**Phenological data and active thermal balance necessary during the vegetation of sweet and sour cherry cultivars (average 2013-2014)**

| Cultivar | Phenological data | | | | Vegetation period (days) | Active thermal balance (°C) |
|---|---|---|---|---|---|---|
| | Swollen bud ($B_1$) | End of flowering (G) | Fruit ripening | Fall leaves | | |
| **Sweet cherry** | | | | | | |
| Cătălina | 21.03 | 29.04 | 25.05 | 20.10 | 214 | 3.370,2 |
| Ludovic | 31.03 | 29.04 | 11.06 | 21.10 | 205 | 3.352,5 |
| Cociu | 1.04 | 30.04 | 12.06 | 19.10 | 202 | 3.332,7 |
| Boambe de Cotnari | 19.03 | 29.04 | 22.06 | 22.10 | 218 | 3.341,2 |
| Galata | 4.04 | 30.04 | 22.06 | 21.10 | 201 | 3.314,3 |
| Maxut | 1.04 | 29.04 | 14.06 | 22.10 | 205 | 3.356,1 |
| Average | | | | | 207,5 | 3.344,5 |
| STDEV* | | | | | 6,89 | 19,60 |
| COVAR S%** | | | | | 0,0332 | 0,0059 |
| **Sour cherry** | | | | | | |
| Pitic | 30.03 | 2.05 | 25.07 | 24.10 | 209 | 3.346,8 |
| Selecția Cotea | 29.03 | 28.04 | 24.06 | 20.10 | 206 | 3.346,8 |
| De Botoşani | 27.03 | 29.04 | 28.06 | 20.10 | 208 | 3.346,8 |
| Ilva | 29.03 | 2.05 | 1.07 | 22.10 | 208 | 3.371,7 |
| Average | | | | | 207,75 | 3.353,03 |
| STDEV* | | | | | 1,26 | 12,45 |
| COVAR S%** | | | | | 0,0061 | 0,0037 |

*STDEV- standard deviation; **COVAR S% - coefficient of variation %

During the study the values ranged between 201 days with 3.314,3°C as active thermal balance at 'Galata' and 218 days with 3.341,2°C as active thermal balance at 'Boambe de Cotnari' for sweet cherry cultivars. At sour cherry cultivars the values ranged between 206 days with 3.346,8°C as active thermal balance at 'Selecția Cotea' and 209 days with the same active thermal balance at 'Pitic'(table 1). Some tree characteristics are presented in table 2.

Number of young shoots of studied sweet cherry cultivars ranged between 84 at 'Ludovic' and 155 at 'Cătălina' as average for period 2013-2014. Length of young shoots ranged between 25,5 cm at 'Maxut' and 36,9 cm at 'Cociu'. At sour cherry cultivars the number and the length of young shoots ranged between 79 ('Pitic') to 138 ('De Botoşani'), respectively 21 to 37 cm. Trunk cross-sectional area (TCSA) is a parameter which show the vegetative growth of the trees for each cultivar (Radunić *et al.,* 2011; Kurlus, 2004). From this point the view 'Ludovic' for sweet cherry and 'Pitic' for sour cherry cultivars showed smaller vegetative growth (table 2).

*Table 2*

**Tree characteristics of sweet and sour cherry cultivars (average 2013-2014)**

| Cultivar | Young shoots | | TCSA($cm^2$) |
|---|---|---|---|
| | Number | Length (cm) | |
| **Sweet cherry** | | | |
| Cătălina | 155 | 29,8 | 334,5 |
| Ludovic | 84 | 35 | 224,8 |
| Cociu | 123 | 36,9 | 272,6 |
| Boambe de Cotnari | 126 | 32,3 | 299,5 |
| Galata | 120 | 34,6 | 426,5 |
| Maxut | 118 | 25,5 | 497,8 |
| Average | 121 | 32,35 | 342,62 |
| STDEV* | 22,65 | 4,15 | 101,81 |
| COVAR S%** | 0,1871 | 0,1284 | 0,2972 |
| **Sour cherry** | | | |
| Pitic | 79 | 21 | 54 |
| Selecția Cotea | 125 | 36 | 244,6 |
| De Botoşani | 138 | 37 | 232,2 |
| Ilva | 123 | 36 | 101 |
| Average | 116 | 32,5 | 157,95 |
| STDEV* | 22,26 | 6,65 | 82,27 |
| COVAR S%** | 0,1915 | 0,2047 | 0,5208 |

*STDEV- standard deviation; **COVAR S% - coefficient of variation %; *** TCSA - Trunk cross-sectional area

As fruit size 'Ludovic' registered the greatest values that mean 11,4 g as weight with 26,4 mm as fruit diameter (table 3) being a very good size according with other studies about sweet cherry (Sîrbu *et al.,* 2012; Lichev *et al.,* 2004; Girard and Kopp, 1998).

*Table 3*

**Fruit size and skin colour of sweet and sour cherry cultivars (average 2013-2014)**

| Cultivar | Fruit size | | Skin colour |
|---|---|---|---|
| | Weight (g) | Diameter (mm) | |
| **Sweet cherry** | | | |
| Cătălina | 7,8 | 22,5 | Dark red |
| Ludovic | 11,4 | 26,4 | Dark red |
| Cociu | 8,5 | 23,5 | Dark red |
| Boambe de Cotnari | 8 | 22,8 | Bicolour |
| Galata | 4,5 | 18,7 | Bicolour |
| Maxut | 4,3 | 13 | Dark red |
| Average | 5,60 | 18,17 | - |
| STDEV* | 2,08 | 4,92 | - |
| COVAR S%** | 0,3716 | 0,2709 | - |

| Sour cherry | | | |
|---|---|---|---|
| Pitic | 4,2 | 17 | Dark purple |
| Selecţia Cotea | 6,3 | 17,2 | Red purple |
| De Botoşani | 7,7 | 22,1 | Dark red |
| Ilva | 5,2 | 18,3 | Dark red |
| Average | 5,85 | 18,65 | - |
| STDEV* | 1,50 | 2,37 | - |
| COVAR S%** | 0,2568 | 0,1271 | - |

'De Botoşani' registered the greatest values as fruit size with 7,7 g as fruit weight and 22,1 mm as fruit diameter, that being a very good size for sour cherry cultivars according with Grafe and Schuster, 2014.'Maxut' and 'Galata' are cherry cultivars with bitter taste fruits being a row material for processing as traditional products in Romania (Budan, 2014).

## CONCLUSIONS

1. The climate change from recent years have influenced the duration of the phenological phases of different cultivars of sweet and sour cherry.

2. 'Ludovic'showed great fruit size and low vigor of trees being a good genitor for sweet cherry breeding.

3. 'De Botoşani'showed great fruit size while 'Pitic'showed the smaller vegetative growth being a very good genitors for sour cherry breeding.

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# THE ADAPTATION TO CLIMATE CHANGE A GRAPEVINE TECHNOLOGIES

## ADAPTAREA TEHNOLOGIILOR VITICOLE LA SCHIMBARILE CLIMATICE

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***Abstract.*** *Efficiency and stable development of vineyards depends primarily on environmental factors, particularly the deviation from their normal (frost, hail, drought, torrential rains). Natural factors are closely linked to ecological parameters of the territory, landscape, agricultural technique of cultivation. An optimal interaction of all these factors and correct adjustment of agricultural technique leads to an effective Vineyard with a good productivity during the operation. The research was conducted within RDSVW Bujoru, district Galați in climatic conditions of 2012-2014. Experienced technological factors (soil and system maintenance fruit load) differentially influenced grape production. It stands soil maintenance system complete littered with straw mulching, followed by maintenance system of the soil by mulching partial by marc compost.*
***Key words:*** *vines, climate change, the total mulching with straw, composted grape marc partial mulching.*

***Rezumat.*** *Dezvoltarea eficientă şi stabilă a plantațiilor viticole depinde în primul rând de factorii de mediu, îndeosebi de abaterea de la normală a acestora (ger, grindina, secetă, ploi torențiale, etc). Factorii naturali sunt strâns legați de parametrii ecologici ai teritoriului, relief, agrotehnica de cultivare etc. O interacțiune optimă a tuturor acestor factori şi o reglare corectă a agrotehnicii conduce la o viticultură eficientă, cu o productivitate bună pe parcursul perioadei de exploatare. Cercetările s-au efectuat în cadrul SCDVV Bujoru, jud. Galați în condițiile climatice ale anilor 2012-2014. S-a urmărit elaborarea unor soluții tehnologice de diminuare a efectului perturbator al schimbărilor climatice personalizate la nivel de centru viticol, care să asigure productivitatea şi sustenabilitatea pe termen lung a plantațiilor viticole. Factorii tehnologici experimentați (sistem de întreținere al solului) au influențat în mod diferențiat producția de struguri. Se remarcă sistemul de întreținere a solului prin mulcire totală cu paie, urmat de sistemul de întreținere a solului prin mulcire parțială cu tescovină compostată.*
***Cuvinte cheie:*** *viță de vie, schimbări climatice, mulcire totală cu paie, mulcire parțială cu tescovină compostată.*

## INTRODUCTION

Vineyards in southern Moldova are increasingly affected by climate change than occurred in the last period. Reducing the quantity and quality of crops and grape vines in vineyards affected by the occurrence of early autumn frosts,

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excessive negative temperatures, the spring frosts and drought rendered during the growing season, trainer losses for vineyards. We are seeing lately from torrential rainfall, long dry, excessively dry years followed by normal or rainy years. All these things affect the water reserve in the soil which exhibit hydric most times since June. Alternative techniques culture of the vine and in particular the uses of biological methods of crop monoculture contribute to the constraints of time. They tend to establish a new balance in the ecosystem components are considered more natural that respects life and the environment. Vine has developed a vegetative device and therefore is water intensive. It has great possibilities in terms of water supply and rainfall of about 450 mm / year because it has a developed root system with physiological and ecological plasticity large, with a high root pressure and high suction force of the leaves (Dejeu *et al.,* 1997). Climate change is recognized today as one of the most serious environmental challenges, societal and economic issues facing mankind. Climate change affects many sectors. Agriculture is one of the areas most exposed due to its dependence on weather conditions. Negative effects on agricultural yields will be exacerbated by more frequent extreme weather events (floods, heavy rains, heat waves and drought). Most of the effects of climate change on agriculture are resulting in water. The lack of water has a major impact on agricultural production.

## MATERIAL AND METHOD

The experience was made in an experimental polygon in the SCDVV Bujoru planted with Merlot. The research was conducted during 2012-2014 and taking into account the proposed order were studied as follows:

*The soil maintenance,* with the graduations*:*

$V_1$ – *black field* ( plowing autumn, spring plowing, five hoeing mechanical on interval, five manual hoeing on interval, fertilization with N, P, K the optimal dose) (Figure 1);

$V_2$ – *total mulching with plant debris* (plant debris spread along with the range and depth of 10 cm, fertilization with N, P, K the optimal dose) (Figure 2);

$V_3$ – t*he partial mulching mulch marc interval* (spread grape marc compost with layer thickness range of 10 cm, postemergence herbicides on row - two herbicides, fertilizer N, P, K dose reduced by 50%) (Figure 3).

They were made observations and determine the climatic elements and quantity of grapes. Harvesting of grapes and determine the qualitative characteristics made the technological maturity at the same time for all experimental variants.

**Fig. 1 -** Black field

**Fig. 2 -** Total mulching with straw

**Fig. 3 -** Mulching partial with grape marc

## RESULTS AND DISCUSSIONS

Climatic data recorded at the weather station using a system SCDVV Bujoru AGROEXPERT and are shown in Table 1, 2 and fig. 4, 5 and 6 reveal that the amount of viticultural Center Bujoru precipitation and mean air temperatures, the years 2012, 2014 and 2013 are dry a normal year. A big problem is the intensity of extreme values of climate factors, which until now were considered accidental and that in recent years have become common. Of these air temperature and heavy rains are the most (Enache, 2010).

The year 2012 was a very dry year, with a decrease in rainfall regime and an uneven distribution of rainfall. The maximum precipitation was in May (115,8mm) and led to a build script, in fact the whole amount was not recovered, much of it is lost through leakage at ground level. Since June precipitation decreased considerably hovering below multiannuality. Deficit rainfall and maximum temperatures exceeding 30°C have influenced the relative air humidity during certain periods it recorded values below 50%, leading to stressful conditions for growing vines. Compared to the average multi-annual mean air temperature has a relative difference of 103%, 109% amount of active temperatures, humidity 84%, 118% insolation and precipitation 99%.

The growing season of 2013 begins with air temperatures close to normal. Although in June, July and August were recorded maximum air temperatures above 30°C, average air temperature is lower from June to September multiannuality process using lower temperatures during the nights. Rainfall during the growing season of 2013 totaling 516.0 mm. Lack of useful rainfall during July-25 August 2 created stressful conditions for vineyard. The rains that followed the end of August, combined with those of September have largely managed to cover the deficit created in July-August. In September surplus was recorded rainfall of 165,0 mm maximum rainfall recorded but were not fully valorised on 4 consecutive days because of rain amount produced was 187,4 mm. Relative humidity is within the normal range, occurred only sporadically values less than 50% in the first decade of April and in August. Compared to the average multi-annual mean air temperature has a relative difference of 97%, 94% total active temperature, relative humidity of 101%, 108% insolation and precipitation 157%.



Year 2014 was a normal year, with a decrease in rainfall regime and an uneven distribution of rainfall. The maximum rainfall in the months of April was (72,4) and July (84,6mm) and the minimum in May (4.1 mm) and September (3,0 mm). Deficit rainfall and maximum temperatures exceeding 30°C have influenced the relative air humidity during certain periods it recorded values below 50%, leading to stressful conditions for growing vines. Compared to the average multi-annual mean air temperature has a relative difference of 93,9%, 91,6% total active temperature, humidity 104,7%, 88,1% and precipitation sunstroke 99.2 %.

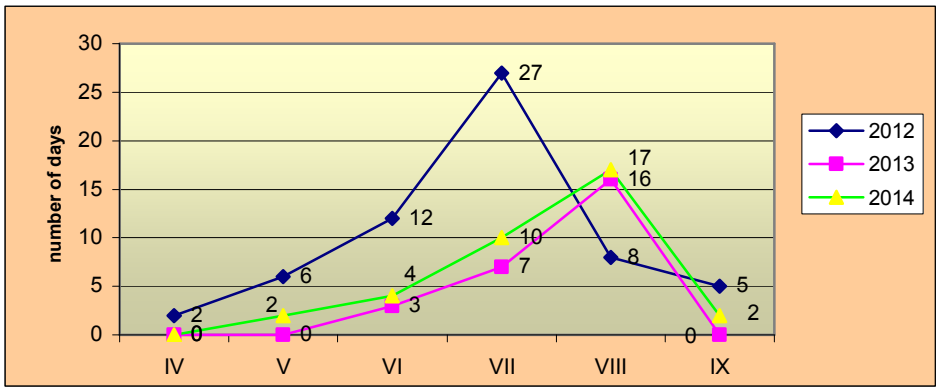


**Fig. 4 -** Number of days with Tmax air above 30ºC

*Table 1*

**Climatic parameters / 2012-2014 relative difference**

| Climatic parameters | The average yearly | 2012 year | Relative difference | 2013 year | Relative difference | 2014 year | Relative difference |
|---|---|---|---|---|---|---|---|
| Tmedie a aerului (ºC) | 11,5 | 11,9 | 103 | 11,1 | 97 | 10,8 | 93,9 |
| Suma T active (ºC) | 3516 | 3838 | 109 | 3295 | 94 | 3220,3 | 91,6 |
| Ur (%) | 73 | 61,6 | 84 | 73,9 | 101 | 76,4 | 104,7 |
| Insolația (ore) | 1796 | 2096 | 118 | 1931 | 108 | 1582,8 | 88,1 |
| Precipitații (mm) | 453,8 | 448,0 | 99 | 713,1 | 157 | 450,4 | 99,2 |

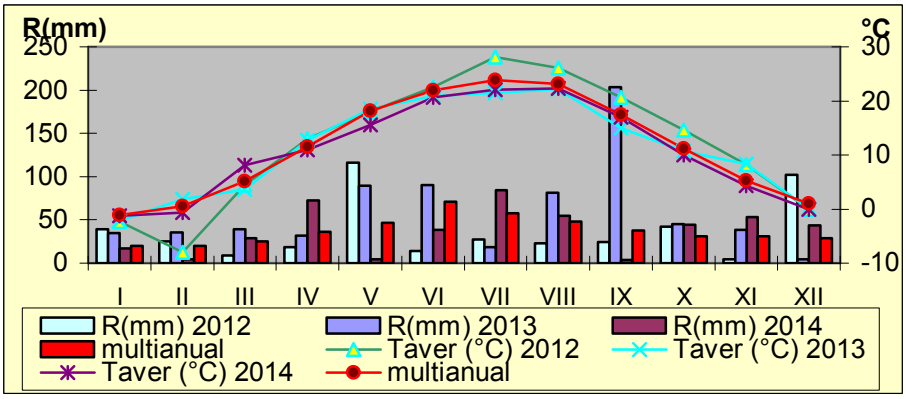


**Fig. 5 -** Monthly distribution of rainfall and average air temperature 2012-2014

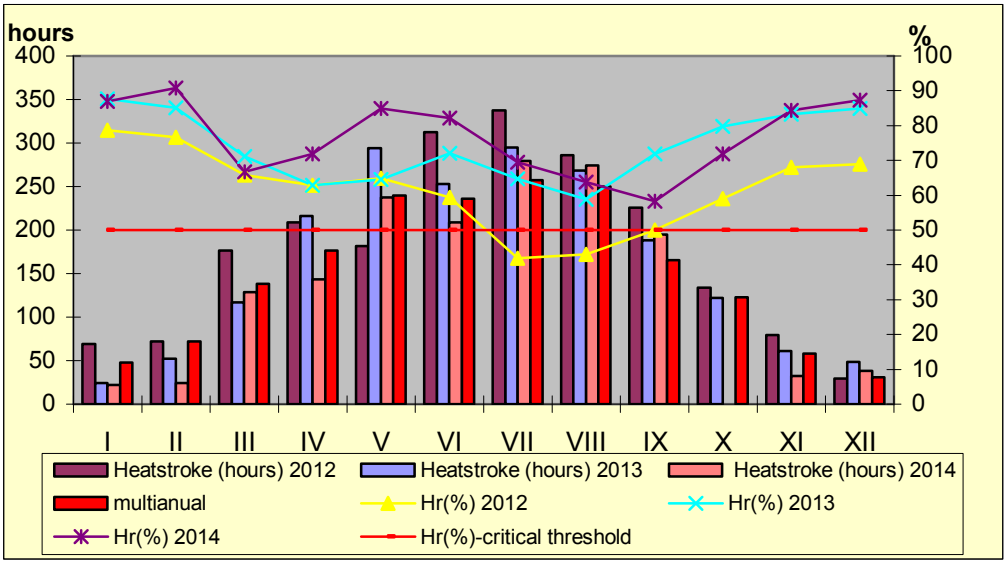


**Fig. 6 -** Heatstroke and relative air humidity 2012-2014

By applying technology to manage vineyards adapted to better conserve water in the soil and water loss is minimized. In terms of productive results show particularly large influence they have on the quantity of grapes experimental variants (fig. 7). The drought of 2012 directly influenced grape production. Analyzing in terms of soil maintenance system influence on the production of grapes, we note that in a dry year as 2012 maximum production was if total mulching with straw, followed by mulching the interval with marc. In a normal year, mulching with grape marc achieved maximum production (18,609 t / ha), followed by mulching with straw total (17,205 t / ha).

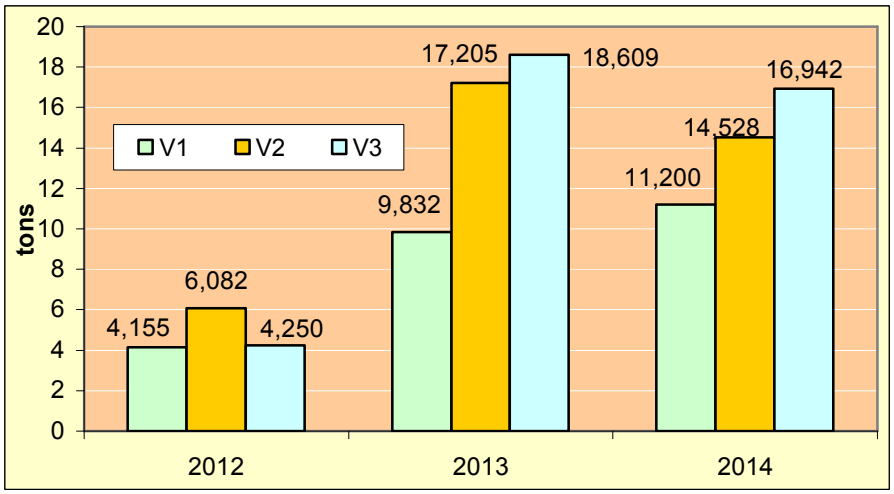


**Fig.7 -** Influence of soil maintenance system on production grape 2012-2014

## CONCLUSIONS

1. Three years taken in observation were different. The year 2012 in terms of climate was unusual, with accentuated water deficit and a surplus thermal regime. The year 2013 presented the favorable climatic conditions for growing grapevine, except the period 2 July to 25 August when rainfall deficit was emphasized. The rains that followed the end of August, combined with those of September have largely managed to cover the deficit created during July- August 25th. Year 2014 was a normal year, with a decrease in rainfall regime and an uneven distribution of rainfall.

2. In a dry year rainfall deficit and higher maximum temperatures of 30°C have influenced the relative humidity, the values recorded during periods of less than 50%. In a normal year relative air humidity is within the normal range, only sporadically recorded values below 50 %.

3. The maintenance of soil influences grape production. In extremely dry years production maximum mulching is recorded in total and in a normal year is highlighted followed by mulching partial mulching total.

4. Mulching is recommended to apply partial and total reducing disruptive effect of climate change in the vineyards, depending on climatic year.

***Acknowledgments**: The study is part of the project undertaken ADER 1.1.6 "Developing technologies to mitigate wine adapted disruptive climate change" conducted in 2011-2014 period.*

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# ECONOMIC AND TECHNOLOGICAL EFFICIENCY OF GIBBERELLIN'S TREATMENT ON SEEDS AND SEEDLESS TABLE GRAPES VARIETIES

## EFICIENTA ECONOMICĂ ŞI TEHNOLOGICĂ A UTILIZĂRII GIBERELINEI LA SOIURILE PENTRU STRUGURI DE MASĂ

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***Abstract***. *Viticulture for the agriculture of Moldova is an important and effective branch, but the table grapes sector is an efficient and more important for small, medium and family business. Increasing the quality of table grapes is a necessity for society and consumers. The purpose of the research from this article is to study the influence of Gobbi Gib 2LG on the quantity, quality and economic efficiency of grapes of Beauty Seedless and Prezentabil varieties. The research was conducted in the vineyards of the „Terra Vitis" LTd, from Southern wine region in Moldova. Research results have shown that the dose of 2.4 l/ha is most useful for conditions by the south part of Moldova, for Beauty seedless variety and the dose of 0,98 l/ha for Prezentabil varietiy.*
***Key words***: *Beauty Seedless, Prezentabil, table grape, growth stimulators.*

***Rezumat***. *Viticultura pentru agricultura Moldovei este o ramură importantă şi eficientă, dar sectorul strugurilor de masă este un sector mai eficient şi mai important pentru businessul mic, mijlociu şi cel de familie. Creşterea calităţii strugurilor de masă este o necesitate pentru societate şi consumatori. Scopul cercetării din acest articol este de a studia influenţa preparatului Gobbi Gib 2LG asupra cantităţii, calităţii şi eficienţei economice a strugurilor de masă la soiurile Beauty Seedless şi Prezentabil. Cercetările a fost efectuate în plantaţiile viticole întreprinderii "Terra Vitis" SRL, din regiunea vitivinicolă sud din Republica Moldova. Rezultatele cercetării au arătat că doza de 2,4 l/ha este cea mai favorabilă pentru condiţiile regiunii de sud a Moldovei, pentru soiul Beauty Seedless şi doza de 0,98 l ha - pentru soiul Prezentabil.*
***Cuvinte cheie***: *Beauty Seedless, Prezentabil, struguri de masă, stimulatori de creştere.*

## INTRODUCTION

Viticulture for the agriculture of Moldova is an important and effective branch, but the table grapes sector is an efficient and more important for small, medium and family business. Increasing the quality of table grapes is a necessity

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for society and consumers. A lot of agricultural branches use different growth regulators or biological active substances, but in Moldova we need to research the influence of these substances on quality of table grapes production, because we do not have registered and approved substances, for use.

The role of growth regulators showed many researches (Wear, 1976; Winkler *et al.*, 1997; Smirnov. *et al.,* 1987) and other. The growth regulators in low concentrations are able to cause essential modifications of the growth and development processes in the plant and their regulation. A specific particularity of the regulators action is their capacity to influence on the processes that are not responding on the influence of normal agricultural practices.

The use of gibberellins within table grape variety technology in most of the countries around the world (Japan, USA, Russia, Italy, Ukraine, Bulgaria etc.) is an obligatory agricultural process. Treating the inflorescences (in the blooming period, in the post fecundation period) leads to considerable modifications of the morphological and mechanical bunch particularities and to productivity increase as well as berries quality modifications.

The researches of Smirnov *et al.,* (1998), Batukaev, (1987), Agafonov *et al.,(*2007), Krasohina, (2008), Derendovskaâ *et al.,* (2009, 2013) it was proved that the application of gibberellins to table grapes varieties lead to increased size and weight of grapes and berries; improve the appearance of grapes; grapes and berries structure modification; improving the processes of accumulation of sugars, formation in some varieties of seedless berries

## MATERIAL AND METHOD

The purpose of the research was to study the influence of the Gobbi Gib 2LG, produced by „L Gobbi" Ltd., Italy on the table grapes varieties productivity.

To achieve the final purpose it was necessary to resolve the following objectives:

- the action of Gobbi Gib 2LG on the table grapes varietes Beauty seedless and Presentabil, on its berry morphological parameters and mechanical properties;
- the action of Gobbi Gib 2LG on the productivity, quality and economic efficiency of the grapes;
- finding out the optimal concentration of the Gobbi Gib 2LG which has a more efficient action within the table grapes seedless varieties;

The research in the field of studying the action of Gobbi Gib 2LG (active substance GA3, the commercial name for L.Gobbi S.R.L. ITALY, this regulator was accepted in Moldova after our research results) as growth regulator needed to increase the productivity and quality of the grapes was effectuated in "Terra-Vitis" Ltd. located in Burlacu village, Cahul district.

The object of study it was the Beauty seedless and Presentabil a table grapes varieties grafted on the Berlandieri x Riparia SO4 rootstock. Density of plants – 2222 plants/ha (3 x 1,5 m). Training system - horizontal cordon. The soil – ordinary black soil.

The experimental variants for Beauty seedless variety – 1. Control; 2. treatment in different phenologycal stages: the technology used in Italy (dose 3.6; 4.6 l/ha) – on 8 cm of shoots length; one week before the blossom; while blossoming 30% of the bloom; while

blossoming 50% of the bloom; while blossoming 80% of the bloom; the treatment of Ø 3-4 mm berries; 8-10 days after the last treating; the technology suggested for Moldova (Mihov, 2010) was the treatment of Ø 3-6 mm berries (2,0 and 2,4 l/ha). In this experiment we use variations and 3 repetitions

The experimental variants for Prezentabil variety – 1. Control; 2. treatment in different phenologycal stages: the technology used in Italy (dose 0,65; 0,82 l/ha) –the treatment of Ø 3-4 mm berries; 8-10 days after the last treating; the technology suggested for Moldova (Mihov, 2010) was the treatment of Ø 3-6 mm berries (0,98 and 1,3 l/ha). In this experiment we use variations and 3 repetitions

## RESULTS AND DISCUSSIONS

**Prezentabil table grape variety.**

*The usage of Gobbi Gib 2LG following the Italian technology.*

*Table 1*

**The reaction of the Prezentabil variety to the Gobbi Gib 2LG treatment in postfecundary period. „Terra vitis" Ltd., 2013, (*Italian technology*)**

| *Index* | *The variant of experience* | | | | | | | deviation of the 2nd variant from the 1st variant, ± | DL 0,95 |
|---|---|---|---|---|---|---|---|---|---|
| | Control-$H_2O$ | *GG2LG-0,65 l/ha* | | | *GG2LG-0,82 l/ha* | | | | |
| | $\bar{x}$ | $\bar{x}$ | deviation from the control, ± | % to the control | $\bar{x}$ | deviation from the control, ± | % to the control | | |
| Bunch weight, g | 503,3 | 586,0 | *+82,7* | *116,4* | 640,0 | *+136,7* | *127,2* | *+54,0* | |
| The quantity of berries per bunch, berries, total | 167,0 | 208,0 | *+41,0* | *124,6* | 214,0 | *+47,0* | *128,1* | *+6,0* | |
| including undeveloped berries | 21,0 | 43,3 | *+22,3* | - | 47,0 | *+26,0* | - | *+3,7* | |
| 100berries' weight,g | 395,8 | 368,4 | *-27,4* | *93,1* | 403,2 | *+7,4* | *101,9* | *+34,8* | |
| Harvest, kg/vine | 3,8 | 4,5 | *+0,7* | *118,4* | 4,9 | *+1,1* | *129,0* | *+0,4* | ***0,49*** |
| The content of:<br>- sugar, %<br>- titratable acidity, g/dm$^3$ | 22,6<br><br>8,9 | 22,0<br><br>8,5 | *-0,6*<br><br>*-0,4* | -<br><br>- | 23,3<br><br>8,3 | *+0,7*<br><br>*-0,6* | -<br><br>- | *+1,3*<br><br>*-0,2* | |
| Price MDL* per kg | 7,00 | 7,50 | *+0,5* | *107,14* | 7,50 | *+0,5* | *107,14* | *,0* | |
| Value, MDL per vine | 26,60 | 33,75 | *+7,2* | *126,88* | 36,75 | *+10,2* | *138,16* | *+3,0* | |
| Total cost, MDL per hectare | 15200,0 | 15400 | *+200,0* | *101,32* | 15500 | *+300,0* | *101,97* | *+100,0* | |
| Sales revenue, MDL per hectare | 59105,2 | 74992,5 | *+15887,3* | *126,88* | 81658,5 | *+22553,3* | *138,16* | *+6666,0* | |
| Profit, MDL per hectare | 43905,2 | 59592,5 | *+15687,3* | *135,73* | 66158,5 | *+22253,3* | *150,68* | *+6566,0* | |
| Profitability, % | 288,85 | 386,96 | *+98,1* | | 426,83 | *+138,0* | | *+39,9* | |

* MDL – Moldova's money, lei (NBM 1 Euro=22,5 MDL)

According to the data reflected in Table 1 it is noted that the grape weight is 503.3 g in the control, but in the 1st variant (0,65 l/ha) - 586.0 g, with 87.2 g or 16.4% more, and in the 2nd (0,82 l/ha) - 640.0 g, with 136.7 g or 27.2% more. The 2nd variant exceeded with 54.0 g the 1st variant. The bunch is big, conically shaped, and dense. The berries have an oblong shape. (Table 1, Figure 1).

The quantity of grapes per vine in the 1st variant was 4.5 kg, with 0.7 kg or 18.4% more in comparison with control variant, but in 2nd variant - 4.9 kg, with 1.1 kg or 29,0% more in comparison with control variant. The 2nd variant exceeded with 0,4 kg the 1st variant. The value of $DL_{0,95}$ – 0,49 kg.

The level of profitability in the 1st variant was 386,96%, with 98,1% more in comparison with control variant, but in 2nd variant - 426,83%, with 138,0% more in comparison with control variant. The 2nd variant exceeded with 39,9% the 1st variant.

*Table 2*

**The reaction of the Prezentabil variety to the Gobbi Gib 2LG treatment in postfecundary period. „Terra vitis" Ltd., 2013 (*Moldova technology*)**

| *Index* | *The variant of experiment* | | | | | | | | DL 0,95 |
|---|---|---|---|---|---|---|---|---|---|
| | Control - $H_2O$ | *GG2LG-0,98l/ha* | | | *GG2LG-1,3l/ha* | | | deviation of the 2nd variant from the 1st variant, ± | |
| | $\bar{x}$ | $\bar{x}$ | deviation from the control, ± | % to the control | $\bar{x}$ | deviation from the control, ± | % to the control | | |
| Bunch weight, g | 503,3 | 649,0 | *+145,7* | *129,0* | 651,5 | *+148,2* | *129,4* | *+2,5* | |
| The quantity of berries per bunch, berries, total | 167,0 | 255,0 | *+88,0* | *152,7* | 196,5 | *+29,5* | *117,7* | *-58,5* | |
| including undeveloped berries | 21,0 | 50,0 | *+29,0* | - | 18,0 | *-3,0* | - | *-32,0* | |
| 100 berries' weight, g | 395,8 | 376,2 | *-19,6* | *95,1* | 404,6 | *+8,8* | *102,2* | *+28,4* | |
| Harvest, kg/vine | 3,8 | 4,9 | *+1,1* | *129,0* | 5,0 | *+1,2* | *131,6* | *+0,1* | *0,49* |
| The content of: - sugar, % | 22,6 | 23,6 | *+1,0* | - | 21,6 | *-1,0* | | *-2,0* | |
| - titratable acidity, g/dm$^3$ | 8,9 | 8,6 | *-0,3* | - | 8,4 | *-0,5* | | *-0,2* | |
| Price MDL* per kg | 7,00 | 7,50 | *+0,5* | *107,14* | 7,50 | *+0,5* | *107,14* | *0* | |
| Value, MDL per vine | 26,60 | 36,75 | *+10,15* | *138,16* | 37,50 | *+10,9* | *140,98* | *+0,75* | |
| Total cost, MDL per hectare | 15200,00 | 15400,00 | *+100,00* | *101,32* | 15500,00 | *+300,0* | *101,97* | *+100,0* | |
| Sales revenue, MDL per hectare | 59105,20 | 81658,50 | *+22553,3* | *138,16* | 83325,00 | *+24219,8* | *140,98* | *+1666,5* | |
| Profit, MDL per hectare | 43905,20 | 66258,50 | *+22353,3* | *150,91* | 67825,00 | *+23919,8* | *154,48* | *+1566,5* | |
| Profitability, % | 288,85 | 430,25 | *+141,4* | | 437,58 | *+148,73* | | *+7,33* | |

* MDL – Moldova's money, lei (NBM 1 Euro=22,5 MDL)

*The usage of Gobbi Gib 2LG following the Moldova technology.*

According to the data reflected in Table 2 it is noted that the grape weight is 503.3 g in the control, but in the 1st variant (0,98 l/ha) - 649.0 g, with 145,7 g or 29.0% more, and in the 2nd (1,3 l/ha) – 651,5 g, with 148,2 g or 29,4% more. The 2nd variant exceeded with 2,5 g the 1st variant. The bunch is big, conically shaped, and dense. The berries have an oblong shape. (Table 2, Figure 1).

The quantity of grapes per vine in the 1st variant was 4.9 kg, with 1,1 kg or

29,0% more in comparison with control variant, but in 2nd variant – 5,0 kg, with 1.2 kg or 31,6% more in comparison with control variant. The 2nd variant exceeded with 0,1 kg the 1st variant. The value of $DL_{0,95}$ – 0,49 kg.

The level of profitability is an important index of economic efficiency. This index in the 1st variant was 430,25%, with 141,4% more in comparison with control variant, but in 2nd variant – 437,58%, with 148,73% more in comparison with control variant. The 2nd variant exceeded with 7,33% the 1st variant.

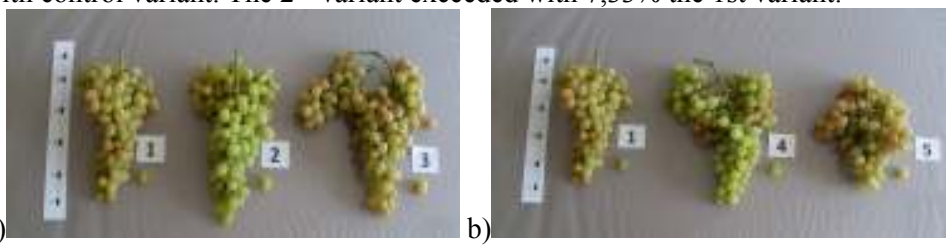


**Fig. 1 -** The Gobbi Gib 2LG influence on the external appearance of the bunch and berries.
a) The Prezentabil variety , "Terra vitis" Ltd., 2013, (Italian technology). The variant of experiment: 1-Control – $H_2O$; 2-GG2LG-0,65 l/ha; 3-GG2LG-0,82 l/ha
b) The Prezentabil variety, "Terra vitis" Ltd., 2013, (Moldova technology). The variant of experiment:1-Control – $H_2O$;2-GG2LG-0,98l/ha;3-GG2LG-1,3l/ha

**Beauty seedless table grape variety.**

*The usage of Gobbi Gib 2LG following the Italian technology.*

*Table 3*

**The reaction of the Beauty seedless variety to the Gobbi Gib 2LG treatment. „Terra vitis" Ltd., (*Italian technology*)**

| *Index* | *The variant of experience* | | | | | | | | DL 0,95 |
|---|---|---|---|---|---|---|---|---|---|
| | Control-$H_2O$ | GG2LG *-3,6 l/ha* | | | GG2LG *-4,6 l/ha* | | | deviation of the 2nd variant from the 1st variant, ± | |
| | $\bar{x}$ | $\bar{x}$ | deviation from the control, ± | % to the control | $\bar{x}$ | deviation from the control, ± | % to the control | | |
| Bunch weight, g | 251,7 | 404,8 | +153,1 | *160,83* | 424,4 | +172,7 | *168,61* | *+19,6* | |
| The quantity of berries per bunch, berries, total | 239 | 245 | +6 | *102,51* | 227,7 | -11,3 | *95,27* | *-17,3* | |
| including undeveloped berries | 24,7 | 5 | -19,7 | *20,24* | 1,7 | -23 | *6,88* | *-3,3* | |
| 100 berries' weight, g | 113,8 | 175,4 | +61,6 | *154,13* | 191,4 | +77,6 | *168,19* | *+16* | |
| Harvest, kg/vine | 3,9 | 6,2 | +2,3 | *158,97* | 6,5 | +2,6 | *166,67* | *+0,3* | ***0,58*** |
| The content of: | | | | | | | | | |
| - sugar, % | 17,3 | 16,6 | -0,7 | *95,95* | 18,2 | +0,9 | *105,20* | *+1,6* | |
| - titratable acidity,g/dm$^3$ | 11,7 | 10,8 | -0,9 | *92,31* | 10,9 | -0,8 | *93,16* | *+0,1* | |
| Price MDL* per kg | 7 | 7,5 | +0,5 | *107,14* | 7,5 | +0,5 | *107,14* | *0* | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Value, MDL per vine | 27,3 | 46,5 | +19,2 | *170,33* | 48,75 | +21,45 | *178,57* | *+2,25* | |
| Total cost, MDL per hectare | 15200 | 15400 | +200 | *101,32* | 15500 | +300 | *101,97* | *+100* | |
| Sales revenue, MDL per hectare | 60660,6 | 103323 | +42662,4 | *170,33* | 108322,5 | +47661,9 | *178,57* | *+4999,5* | |
| Profit, MDL per hectare | 45460,6 | 87923 | +42462,4 | *193,40* | 92822,5 | +47361,9 | *204,18* | *+4899,5* | |
| Profitability, % | 299,08 | 570,93 | +271,85 | | 598,85 | +299,77 | | *+27,92* | |

* MDL – Moldova's money, lei (NBM 1 Euro=22,5 MDL)

According to the data reflected in Table 3 it is noted that the grape weight is 251,7 g in the control, but in the 1st variant (3,6 l/ha) – 404,8 g, with 153,1 g or 60,83% more, and in the 2nd (4,6 l/ha) – 424,4 g, with 172,7 g or 68,61% more. The 2nd variant exceeded with 19,6 g the 1st variant. The bunch is big, conically shaped, and dense. The berries are spherical or slightly oval shape. (Table 3, Figure 2).


**Fig. 2 -** The Gobbi Gib 2LG influence on the external appearance of the bunch and berries. Beauty seedless variety, "Terra vitis" Ltd., 2013. The variants of experience: 1-Control– $H_2O$; 2-GG2LG -3,6 l/ha; 3- GG2LG -4,6 l/ha, (Italian technology); 4-GG2LG -2,0l/ha; 5-GG2LG-2,4l/ha (Moldova technology)

*Table 4*

**The reaction of the Beauty seedless variety to the Gobbi Gib 2LG treatment while the post fecundation period. „Terra vitis" Ltd., 2013, (*Moldova technology*)**

| *Index* | *The variant of experience* | | | | | | | | DL 0,95 |
|---|---|---|---|---|---|---|---|---|---|
| | Control-$H_2O$ | GG2LG *-2,0 l/ha* | | | GG2LG *-2,4 l/ha* | | | deviation of the 2nd variant from the 1st variant, ± | |
| | $\bar{x}$ | $\bar{x}$ | deviation from the control, ± | % to the control | $\bar{x}$ | deviation from the control, ± | % to the control | | |
| Bunch weight, g | 251,7 | 454,8 | +203,1 | *180,69* | 508,3 | +256,6 | *201,95* | *+53,5* | |
| The quantity of berries per bunch, berries, total | 239 | 238 | -1 | *99,58* | 242 | +3 | *101,26* | *+4* | |
| including undeveloped berries | 24,7 | 0 | -24,7 | *0,00* | 1,7 | -23 | *6,88* | *+1,7* | |
| 100 berries' weight, g | 113,8 | 196,3 | +82,5 | *172,50* | 217,6 | +103,8 | *191,21* | *+21,3* | |
| Harvest, kg/vine | 3,9 | 7,0 | +3,1 | *179,49* | 7,8 | +3,9 | *200,00* | *+0,8* | ***1,22*** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| The content of: | | | | | | | | | |
| - sugar, % | 17,3 | 16,1 | -1,2 | *93,06* | 16,1 | -1,2 | *93,06* | *0* | |
| - titratable acidity,g/dm$^3$ | 11,7 | 11,5 | -0,2 | *98,29* | 11,8 | +0,1 | *100,85* | *+0,3* | |
| Price MDL* per kg | 7 | 7,5 | +0,5 | *107,14* | 7,5 | +0,5 | *107,14* | *0* | |
| Value, MDL per vine | 27,3 | 52,5 | +25,2 | *192,31* | 58,5 | +31,2 | *214,29* | *+6* | |
| Total cost, MDL per hectare | 15200 | 15400 | +200 | *101,32* | 15500 | +300 | *101,97* | *+100* | |
| Sales revenue, MDL per hectare | 60660,6 | 116655 | +55994,4 | *192,31* | 129987 | +69326,4 | *214,29* | *+13332* | |
| Profit, MDL per hectare | 45460,6 | 101255 | +55794,4 | *222,73* | 114487 | +69026,4 | *251,84* | *+13232* | |
| Profitability, % | 299,08 | 657,50 | +358,42 | | 738,63 | +439,54 | | *+81,13* | |

***** MDL – Moldova's money, lei (NBM 1 Euro=22,5 MDL)

The quantity of grapes per vine in the 1st variant was 6,2 kg, with 2,3 kg or 58,97% more in comparison with control variant, but in 2nd variant – 6,5 kg, with 2,6 kg or 66,67% more in comparison with control variant. The 2nd variant exceeded with 0,3 kg the 1st variant. The value of $DL_{0,95}$ – 0,58 kg.

The level of profitability in the 1st variant was 570,93%, with 271,85% more in comparison with control variant, but in 2nd variant – 598,85%, with 299,77% more in comparison with control variant. The 2nd variant exceeded with 27,92% the 1st variant.

*The usage of Gobbi Gib 2LG following the Moldova technology.*

According to the data reflected in Table 3 it is noted that the grape weight is 251,7 g in the control, but in the 1st variant (2,0 l/ha) – 454,8 g, with 203,1 g or 80,69% more, and in the 2nd (2,4 l/ha) – 508,3 g, with 256,6 g or 101,95% more. The 2nd variant exceeded with 53,5 g the 1st variant. The bunch is big, conically shaped, and dense. The berries are spherical or slightly oval shape. (Table 4, Figure 2).

The quantity of grapes per vine in the 1st variant was 7,0 kg, with 3,1 kg or 79,49% more in comparison with control variant, but in 2nd variant – 7,8 kg, with 3,9 kg or 100,0% more in comparison with control variant. The 2nd variant exceeded with 0,8 kg the 1st variant. The value of $DL_{0,95}$ – 1,22 kg.

The level of profitability in the 1st variant was 657,5%, with 358,42% more in comparison with control variant, but in 2nd variant – 738,63%, with 439,54% more in comparison with control variant. The 2nd variant exceeded with 81,13% the 1st variant.

Early studies in Moldova with different varieties (Muscat de Hamburg, Loose perlette, Cardinal, Codreanca and others) and there results showed the similar effects of treatments (Nicolaescu *et al,* 2009, 2012, 2015, Mihov, 2010).

## CONCLUSIONS

In finally, for the Prezentabil (seeds variety) table grape variety in the

conditions of southern part of Moldova, high results we obtained in the 2nd variant with the dose of 1.3 l/ha. The level of profitability 437,58%, with 148,73% more in comparison with control variant.

The Beauty seedless (seedless variety) table grape variety in the conditions of southern part of Moldova, high results we obtained in the 2nd variant with the dose of 2,4 l/ha. The level of profitability 738,63%, with 439,54% more in comparison with control variant.

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# STUDIES OF THE MAIN PHYSICAL CHEMICAL CHARACTERISTICS IN SOME GRAPE VARIETIES FROM IAŞI VINEYARD

## STUDIUL PRINCIPALELOR CARACTERE FIZICO-CHIMICE LA UNELE SOIURI DE VIȚĂ DE VIE CULTIVATE ÎN PODGORIA IAȘI

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***Abstract.*** *The article analyses local, newly created grape varieties for table grapes, in order to highlight valuable sources of germplasm as to fulfill the biological and economic potential of Iasi vineyard, area with a low favourability for table grapes cultivation. The main goal is the analysis of structural, physicochemical and biochemical characterization of local varieties for table grapes. Having information regarding the type and concentrations of biochemical compounds available in the grapes, and the relationships between them, technological characteristics, therapeutic value, as well as sources of germoplasm for other grape varieties. The objectives were to determine the structural characteristics of the grapes at full maturity, determination of physico-chemical characteristics of the studied varieties, as well as their qualitative characteristics.*
***Keywords****: new creations, table grapes, table grapes, Iași vineyard, structural analysis*

***Rezumat.*** *Lucrarea constă în analiza unor soiuri autohtone, creații noi românești de viță de vie pentru struguri de masă, în vederea evidențierii surselor de germoplasmă viticolă valoroase, cu scopul de a pune în valoare potențialul biologic și economic al acestora în podgoria Iași, zona cu favorabilitate scăzută culturii de viță de vie pentru soiuri de masă.Scopul principal îl constituie analiza și caracterizarea fizico-structurală și biochimică a unor soiuri autohtone pentru struguri de masă, aparținând speciei Vitis vinifera L., fam.Vitaceae, genul Vitis. Cunoscându-se tipul și concentrațiile de compuși biochimici disponibili în struguri, precum și raportul dintre aceștia, pot fi apreciate însușirile tehnologice, valoarea alimentară și terapeutică, dar și calitățile ameliorative, în perspectiva utilizării acestora ca sursă de germoplasmă viticolă a soiurilor autohtone luate în studiu.Obiectivele urmărite au fost: determinarea caracterelor de structură ale strugurilor la maturitate deplină, determinarea caracterelor fizico-chimice ale soiurilor studiate, caracteristicile de calitate ale soiurilor studiate.*
***Cuvinte cheie****: creații noi, struguri de masă, podgoria Iași, analize structurale*

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## INTRODUCTION

In the north-east area of Romania, the cultivation of table grapes is less favourable, because of limitative climatic conditions (very cold winters and very dry summers). Usually, the table grapes that can be cultivated here are part of Chasselas group. Therefore, the research units are trying to characterize new grape varieties with a higher adaptability degree to the restrictive climatic factors and a shorter vegetation period (Damian *et al*., 2006).

Among Romanian creations that were considered acceptable are: Gelu (SCDVV Iaşi ), Cetățuia, Napoca, Transilvania ( SCH Cluj ), Milcov. This study presents the behavior of each of these grape varieties cultivated in Iasi vineyard. They are characterized by resistance to frost, fertility degree, yield capacity, quantity and quality of grapes (Rotaru *et al*., 2010).

## MATERIAL AND METHOD

The present article studies five grape varieties for table grapes with middle maturation, new Romanian creations: Transilvania, Napoca, Cetățuia, Milcov and Gelu, all cultivated in the Ampelographic Collection of Vasile Adamachi didactic farm Iași. In order to establish the behavior of these grape varieties in Copou-Iași viticultural center, structural and physical-chemical analyses were made on grapes harvested in 2014.

The aims of this article were: analysis of structural characteristics of grapes at full maturity, analysis of physical-chemical characteristics, analysis of quality characteristics (OIV, 2013).

## RESULTS AND DISCUSSIONS

The main physical-chemical results of analysed varieties were centralized as average values, with a standard deviation and are presented in table 1.

After maturation, the weight of a grape ranges between 236,96 g ±3,67 in Milcov variety to 428,30 g ± 8,34 in Gelu variety, values confirmed by specific literature. A tight correlation between weight of grapes, no of berries/grape and weight of 100 berries is registered. A comparison between the 5 grape varieties shows that the highest weight is registered in the case of Gelu variety, of 428,30 g, as well as the highest number of berries (76,33). The highest weight of 100 berries is registered for Transilvania variety, with a minimum of berries /stalk (53,33). The lowest values are registered in the case of Milcov variety, with a weight of 236,96 g, berries/stalk - 69,67, weight of 100 berries 297,7 g.

Skin weight has similar values in all 5 grape varieties, between 0,43 g (Milcov) and 0,61 g (Cetățuia). Pulp weight has a maximum value in the case of Transilvania variety (6,21 g), while the minimum is registered in Milcov (2,45 g).

In general, the weight of the grape stalk is higher than the weight of a grape berry, except in the case of Transilvania variety, where the values are 6,67 g, for the grape stalk and 6,85 g for one berry. The relative humidity coefficient (%) is highest in the case of Milcov grape variety (89,61%), while the lowest total dry substance (%) is the lowest (10,29%).

*Table 1*

**Main structural and physical-chemical characteristics of studied grape varieties**

| Grape varieties / Parameters | Gelu | | Milcov | | Cetățuia | | Transilvania | | Napoca | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Average | ± | Average | ± | Average | ± | Average | ± | Average | ± |
| Grape weight (g) | 428,30 | 5,16 | 236,96 | 3,76 | 352,32 | 8,34 | 371,98 | 31,19 | 282,20 | 33,80 |
| No berries/stalk | 76,33 | 1,53 | 69,67 | 3,79 | 80,00 | 12,29 | 53,33 | 5,51 | 67,67 | 2,52 |
| Berry weight (g) | 5,50 | 0,32 | 3,30 | 0,41 | 4,29 | 0,18 | 6,85 | 0,62 | 3,96 | 0,33 |
| 100 berries weight (g) | 474,23 | 24,31 | 297,70 | 23,38 | 357,24 | 4,89 | 875,17 | 73,96 | 443,32 | 8,38 |
| Stalk weight (g) | 8,49 | 1,03 | 7,05 | 1,11 | 9,12 | 0,52 | 6,67 | 0,79 | 14,23 | 0,74 |
| Seed no/berry | 1,67 | 0,58 | 3,00 | 0,01 | 3,33 | 0,58 | 2,00 | 0,01 | 3,00 | 1,00 |
| Skin weight (g) | 0,59 | 0,07 | 0,43 | 0,07 | 0,61 | 0,02 | 0,51 | 0,09 | 0,54 | 0,12 |
| Pulp weight (g) | 4,32 | 0,21 | 2,45 | 0,13 | 3,54 | 0,33 | 6,21 | 0,97 | 3,79 | 0,56 |
| Seed weight (g) | 0,53 | 0,40 | 0,15 | 0,02 | 0,14 | 0,03 | 0,13 | 0,01 | 0,17 | 0,09 |
| Structural index | 20,89 | 3,44 | 23,12 | 4,37 | 29,46 | 2,70 | 31,09 | 3,15 | 17,85 | 1,65 |
| Berry index | 20,12 | 1,31 | 32,65 | 4,47 | 20,87 | 0,77 | 11,02 | 0,77 | 20,28 | 1,33 |
| Compositional index | 4,28 | 1,88 | 4,27 | 0,45 | 4,72 | 0,53 | 9,81 | 0,06 | 5,48 | 1,29 |

*Table 2*

**Humidity and total dry substance of of studied grape varieties**

| Grape varieties / Parameters | Gelu | Milcov | Cetățuia | Transilvania | Napoca |
|---|---|---|---|---|---|
| Relative humidity (%) | 78,49 | 89,61 | 76,71 | 78,53 | 85,62 |
| Total dry substance (%) | 21,51 | 10,29 | 23,29 | 21,47 | 14,38 |

*Table 3*

**Sugars' concentration of studied grape varieties**

| Grape varieties / Parameters | Gelu | Milcov | Cetățuia | Transilvania | Napoca |
|---|---|---|---|---|---|
| Sugars (g/l) in 2014 | 187,27 | 175,07 | 217,36 | 200,09 | 147,7 |
| Values in literature | 160 | 185 | 175 | 160 | 140 |

The highest concentration of vitamins is found in the case of vitamin C, which, at full maturity, ranges between 3,43±0,03 mg/100 g at Transilvania grape variety and 5,45±0,14 mg/100 g in the case of Milcov grape variety and 5,73±0,43 mg/100 g in Napoca variety.

The pH of the analysed grape varieties is in normal range, between 3,29 in Napoca and 3,53 at Gelu. However, in Gelu, the acidity was found to be at deficit compared to normal values. The majority of samples registered a normal acidity of over 7,1 g/L acid tartaric.

The sugars' concentration in 2014 overpassed in general the specific of the grape varieties, as follows: Cetățuia and Transilvania register 217,36 g/L respectively 200,09 g/L sugars, the only exception being Milcov where only 175,05 g/ L, compared to the specific values, of 180-185 g/L.

The gluco-acidimetric index ranges between 26,53±1,69 (Milcov) – 39,56±2,28 (Cetățuia).

## CONCLUSIONS

The studied grape varieties present values that are proof for their suitability in the viticultural region of North-east of the country, respectively Iasi vineyard.

Gelu, Transilvania and Cetatuia grape varieties have a grape weight of over 350 grams, a good commercial aspect and a better adaptability to Iasi vineyard.

The sugars accumulations have been, in general, higher than the values described in literature.

The values of the glucoacidimetric index are much higher than the inferior limit set by the OIV, 2008, of 20.

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# IMPROVING VINE PERFORMANCE UNDER UNFAVORABLE CONDITIONS OF GROWTH BY USE OF TRACE ELEMENTS AND MICROORGANISMS

## UTILIZAREA COMPOZIȚIILOR DE MICROELEMENTE ȘI MICROORGANISME PENTRU AMELIORAREA FERTILITĂȚII SOLULUI ÎN PLANTAȚIILE VITICOLE

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***Abstract.*** *Long-term plant cultivation imposed by a monoculture such as the vineyards and orchards, leads to exhaustion of the soil, reduction of microbiological activity in rhizosphere, and accumulation of products from pesticides, such as copper and herbicides. The possibility to elaborate a new advanced technology to maintain vineyard health under sustainable conditions and to increase plantation longevity was undertaken in last 6-7 years. The obtained data demonstrate the possibility of developing an effective process for the control of growing conditions and plant nutrition on the soils contaminated with Cu and pesticides, for productivity and resistance to pollution and low temperatures increase, improve product quality, increase the longevity of the vine, reducing chemical pressing on the environment.*
***Key words****: viticulture, trace elements, microorganisms, copper excess, herbicide, plant growth, productivity.*

***Rezumat.*** *Cultivarea pe termen lung a plantelor impusă de către o monocultură precum viile sau livezile, duce la epuizarea solului, reducerea activității microbiologice în rizosferă, și la acumularea produselor din pesticide precum cupru și erbicide. Posibilitatea elaborării unei noi tehnologii avansate de menținere a sănătații podgoriilor în condiții durabile și de creșterea longevității plantațiilor a fost întreprinsă în ultimii 6-7 ani. Datele obținute demonstrează posibilitatea de a dezvolta un proces efectiv pentru controlul condițiilor de creștere și nutriție a plantelor pe soluri contaminate cu pesticide și cupru, pentru creșterea productivității și rezistenței împotriva poluării și temperaturilor scăzute, îmbunătățirea calității produselor, creșterea longevității viei, și reducerea pressingului chimic asupra mediului.*
***Cuvinte cheie:*** *viticultură, microelemente, microorganisme, exces de cupru, erbicid, creșterea plantelor, productivitate.*

## INTRODUCTION

The vineyards and orchards constitute a major factor in agricultural productivity of many countries. However, long-term plant cultivation imposed by a monoculture

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such as in vineyards leads to depletion of essential soil nutrients, reduction of microbiological activity in the rhizosphere and soil pollution due to the accumulation of residues from chemical treatments (Veliksar and Toma, 2012). The biomass of microorganisms in the soils under the vineyard is reduced 3- to 4- fold, because of chemical applications and nutrients available to the plants become exhausted. These adverse cultivation conditions negatively affect plant growth by reducing the capability to face abiotic and biotic stresses such as extreme temperatures and pest attacks, and thus plant production by decreasing fruits' quality and quantity.

A new approach to vineyard cultivation aiming at recovering plant productivity by ameliorating the growing conditions is currently requested to maintain adequate levels of the output of this important fruit crop. The goal is to reduce the chemical load on the ecosystem and to correct plant growth in the soils already suffering from heavy metals and pesticide residues. Because the bioavailability of many microelements to perennial plants is limited, a special complex *Microcom-V* containing 6 essentially for grape trace elements was created to improve metabolism in grapes and studied in different conditions (Veliksar *et al.,* 2011; Veliksar *et al.,* 2008). The complex is applied foliar as a 0,15% water solution during vegetation period. It was proposed that beneficial effects of the nutrients in Microcom -V in 0,5 dose will be supplemented with suspensions of living beneficial microorganisms and their metabolites to create a new complex for plant productivity. Such approach have to possess a wide range of action, be ecological friendly, compatible with the standard technology of grape cultivation and offer cost-saving to be competitive on the world market. Creation of the microbial biotechnology is one of the main areas of modern agriculture (Lütfi and Murat, 2009; Salantur *et al.,* 2006). Using the potential of soil bacteria capable to mobilization of nutrients from the soil and the atmosphere is an important achievement of biotechnology and factor in increasing the productivity of agricultural crops. On the basis of the microbial metabolites the various biological composition are created. They are becoming more widely used in crop production.

## MATERIAL AND METHOD

The researches have been performed in green house and in field conditions (central region of Moldova) on the technical varieties of grape (Codrinschi, Aligote, Traminer, Chardonnay, Presentable) in the years 2010 - 2014. Plants fertilization involves: a) incorporation in soil of suspension of beneficial microorganisms *Azotobacter chroococcum (suspension #1), Pseudomonas fluorescens (suspension #2),* and *Baccilus subtilis* when planting seedlings and b) foliar treatment of plants with metabolites of microorganisms and complex of trace elements Microcom-V. As a subjects of study were used: vine seeds, vine cuttings of two cultivars (Codrinschii and Prezentable, seedlings grown in plastic pots in green house and nursery, fruitful grape plants. The foliar treatment by the micro fertilizers and bacteria metabolites was conducted three terms – before flowering and at the stage of intensive growth with an interval of 12 to 14 days. Water treated plants were used as control. The following analytical methods were used: the content of free amino acids - using an AAA-300 analyzer, the carbohydrate content - according to Bertran;

photosynthetic pigments determination- using ethanol extraction, trace elements content - using an atomic absorption spectrophotometer Perkin Elmer after dry ashing at 480°C.

## RESULTS AND DISCUSSIONS

Effect of microorganisms and trace elements on grape seeds germination and seedlings growth. A promising direction in the improvement of existing technology in viticulture and seedlings production is the application of trace elements and biotechnological products, particularly for vine reproduction from seeds (in the case of breeding new hybrid varieties). Reproduction of vine by seeds is of great importance in the selection too, for new hybrid varieties creation. Vine seeds have low germination energy such as some species of trees and shrubs. Researchers conducted under controlled conditions have shown the possibility to increase seed germination rate of the grape and to improve the quality of planting material by applying trace elements complex Microcom-V and suspension of microorganisms *Ps. fluorescence* - for hybrid seeds obtained by crossing (Tab.1) and *Az. cchroococcum* - for seeds of standard varieties. Recording of seedlings key parameters (height and diameter of shoots) shows that all variants, where seedlings have been treated with suspensions applied separately and in binary combination suspension (1:1), have a better development. These research results show the positive role of bacterial suspension on the growth and development of seedlings.

*Table 1*

**Effect of bacterial suspension on seed germination of hybrid vine obtained by crossing (Madleine Angevine x Moldova)**

| Variants | % of seeds germina-tion | % to control | the length of shoots, cm | % to control | Diameter of main shoot, cm | % to control |
|---|---|---|---|---|---|---|
| Control | 26 | 100 | 33,8 | 100 | 0,10 | 100 |
| Seed treatment with Microcom-V | 68,5 | 263,5 | 35,8 | 105,9 | 0,12 | 120,0 |
| Seed treatment with *Pseudomonas fluorescens* | 74,2 | 285,4 | 72,9 | 215,7 | 0,18 | 180,0 |
| Seed treatment with *Azotobacter chroococcum* | 71,4 | 274,6 | 42,1 | 124,6 | 0,15 | 150,0 |

*Effect of microorganisms and trace elements on growth and development of seedlings produced by vegetative propagation*. It has been determined the effect of trace elements and microorganisms on the growth of vine cuttings, obtained for vegetative propagation under controlled conditions (in green house). A mixture of two suspensions of microorganisms, incorporated into the soil, and their metabolites used for foliar treatment of plants in pots increased the growth of cuttings, in particular root system. Most intense biomass accumulation of seedlings was observed in variants, were extra root triple treatment of plants by metabolites of two strains of microorganisms and half dose of Microcom-V were used (Tab. 2). Intensive growth and increased biomass accumulation can be explained by the fact that PGPR (plant

growth promoting rhizobacteria) produce auxins, citokinine, gibbereline and adjusts the level of endogenous ethylene in plants (Avis *et al.,* 2008; Martinez-Viveros *et al.,* 2010).

Determination of roots and shoots of seedlings length demonstrates that the total biomass increase was largely due to increased of rhizogenesis process - length and quantity of roots, especially small roots - absorbents hairs. Increased absorption surface of roots is very important to improve plant mineral status and quality of planting material. Significant effect of rhizobacteria and trace elements on growth cuttings is associated not only with major content of active substances in the metabolites of microorganisms, but also with increased growth potential of the vine.

*Table 2*

**Biomass accumulation by vine plants under the influence of microorganisms and trace elements. Green house, variety Prezentabil, g/plant.**

| Variants | Roots | Above-ground part of seedlings |
|---|---|---|
| Control | 7.58±2.78 | 16.09±2.32 |
| Suspension of *Pseudomonas fluorescens* + *Azotobacter chroococcum*, în soil | 9.50±1.70 | 30.96±5.91 |
| Suspension of *Ps. fluorescens* + *Az. chroococcum*, în soil + Microcom (0,5) foliar | 9.4±1,89 | 19.41±4.10 |
| Metabolites *of Ps. fluorescens* + *Az. chroococcum*,- foliar | 9.30±1.50 | 22.42±1,95 |
| Metabolites of *Ps. fluorescens* + *Az. Chroococcum* + Microcom (0,5) foliar | 10.68±0.89 | 28.75±2.64 |

*Effect of microorganisms and trace elements on growth and development of plants under the stress imposed by copper and herbicide accumulations in soil.* One of the objectives of the project is highlighting the role of microorganisms and complex of trace elements in reducing the negative effect of herbicides. Currently in viticulture is used most often herbicide Raundap which is quite effective against some weeds, but there is information about the negative effect on grape plants. We included 5 variants in scheme of the experiment: 2 controls - mechanical treatment of soil and without treatment, 1 dose of herbicide Raundap, half dose of herbicide, a consortium of three strains of microorganisms *Pseudomonas putida, Agrobacterium radiobacter, Bacillus subtilis.* Record of the number and type of weeds in mentioned experiment has shown that the number of weeds in variants with microorganisms and herbicide solution sharply drops in comparison to variants with 1 and 0.5 doses of herbicide. This result confirms the information in the literature about the possibility of applying some strains of microorganisms in ecological agriculture.

We studied the nutrient content in the soil under the fruitful grape plants after herbicide application. Over a month after application of Raundap in recommended dose (1 dose) it was observed a decrease in the content of mobile forms of basic nutrients not only from 0-30 cm and 30-60 cm. In the variant with incorporation into the soil of the consortium of microorganisms with the application of herbicide dose of 0.5 it was mentioned a tendency to maintain the contents of the nutrients in the soil at a

stable level. Interesting data was obtained by determining the capacity of a soil nitrification after Raundap application. Herbicide application reduced soil nitrification capacity from 6.8 up to 6.2 mg $N\text{-}NO_3$ / 100g soil, which also indicates about the negative effect of the herbicide on the soil condition and properly - plant productivity. Soil nitrification capacity increased before 8.8 mg $N\text{-}NO_3$ / 100g soil after the common application of herbicide with microorganisms.

It was clearly demonstrated that the use of the recommended dose of herbicide Raundup on the vines violates the nutrient status of plants in the soil (0-60 cm), where are the majority of roots, that adversely affect the productivity and viability of plantations. Adding half of the dose of the suspension of the three strains of microorganisms with halved dose of herbicide is more effective to the weeds than a single dose of a herbicide. More even, they contribute to the maintenance of the mineral status of the soil to the control.

One of the goals of presented study was increase *the vine resistance to excess of one of the heavy metals - Cu***.** Data from the literature and those obtained in our previous experiments show that the accumulation of heavy metals in the surface layers of soil brings unbalance in the soil- plant - atmosphere system, multiple deviations in plant nutrition, worsening of health. The vineyards during the agricultural year are treated repeatedly with different pesticides at the same time and with preparations containing copper and zinc. Cu, which is accumulation in some soils of Moldova exceeds 10-15 times compared to normal soils (background), presents a particular danger. The obtained data demonstrate that addition of suspension of microorganisms decreases obvious negative effect of toxic doses of Cu. It is well pronounced tendency to decrease the toxicity of the metal in variants with suspension of consortium of microorganisms application, especially when adding the complex of micronutrients Microcom-V - foliar treatment (Tab. 3). This confirms our hypothesis about possibility to reduce the toxicity of heavy metals by derivatives of microorganisms applying and foliar fertilization by Microcom-V.

*Table 3*

**The length of shoots and roots of plants in experience with $CuSO_4$, variety "Prezentabil" (10/02/2013), the average of 10 plants**

| Variants | roots length, cm | shoots length, cm | shoots maturati-on cm | % to control | | |
|---|---|---|---|---|---|---|
| | | | | roots length | shoots length | shoots matur. |
| Control | 292.2±28.8 | 26.2±2.6 | 18.8±2.1 | 100 | 100 | 100 |
| Cu – 1200 mg/kg | 229.7±52.4 | 24.4±4.4 | 14.8±4.7 | 178,6 | 193.1 | 178.7 |
| Cu + suspension of microor. #1 în sol | 302.2±55.1 | 25.9±1.9 | 15.33±0.5 | 103.4 | 99.2 | 81.4 |
| Cu + suspension #2 în sol | 301.8±84.4 | 28.8±4.1 | 19.00±2.6 | 103,3 | 110.1 | 100,9 |
| Cu –+ suspens.# 1+2 în sol | 304.3±55.2 | 31.0±3.8 | 21.63±2.4 | 1104.1 | 118.5 | 114.8 |
| Cu + suspen. 1+2 in sol+ Microcom-V- foliar | 359.2±62.9 | 29.6±9.6 | 21.9±8.7 | 122.9 | 113.3 | 116.7 |

*The study of the effect of trace elements and micro-organisms on the fruit-bearing vines.* In the fruit-bearing plant leaves were determined some indices, which are necessary for plant status characterization after fertilization: photosynthetic pigments content, proline, intensity of photosynthesis. The optimization of the metabolic processes in the course of plant vegetation through application of trace elements and metabolites of microorganisms was mentioned, which resulted in shoot growth and maturation, enhancement of plant resistance to frost during the period of dormancy. It contributes to the formation and a fuller manifestation of genetically based potential of frost and winter resistance of vine, increase of plant productivity and longevity.

## CONCLUSION

It was highlighted for the first time the possibility common use of complex of trace elements Microcom-V and saprophytic microorganisms to enhance the growth and development of vine cuttings and fruitful plants. It was established beneficial effect of saprophytic microorganisms on vine growth under unfavorable conditions (copper excess, low temperature, application of herbicides). It was revealed the ability to decrease by 50% the dose of herbicide Raundap adding consortium of 3 microorganisms with the same effect on weed control, maintenance of nitrification activity in soil and plant nutrient status to a higher level. The obtained data demonstrate the possibility of developing an effective process for the control of growing conditions and plant nutrition on the soils contaminated with Cu and pesticides, for productivity and resistance to pollution and low temperatures increase, improve product quality, increase the longevity of the vine, reducing chemical pressing on the environment.

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# *JUNIMEA VINULUI IAȘI* – A LOCAL GROUP OF OENOLOGICAL INITIATIVE AND PROMOTION

## *JUNIMEA VINULUI IAȘI* – UN GRUP LOCAL DE INIȚIATIVĂ ȘI PROMOVARE OENOLOGICĂ

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***Abstract.*** *This paper presents the Junimea Vinului Iaşi group description. This group is made up of wine lovers with different backrounds and hase as a aim to promote the culture of wine quality. The group holds regular meetings were they discussed various oenological topics, also wine tastings and promoting various wine producers in cyberspace and beyond. As a result of a year of activity, the group has developed co-opting various subject areas and develop a series of recommendations that are promoted via internet (social media networking).*
***Key words:*** *wine tasting, oenology, promotion*

***Rezumat.*** *Lucrarea prezintă descrierea grupului Junimea Vinului Iași. Acest grup este constituit din iubitori de vin ce provin din mai multe segmente de activitate și are ca scop să promoveze o cultură a vinurilor de calitate. Grupul organizează întâlniri periodice ce au în discuție diverse teme oenologice, cât și degustări de vinuri și promovarea diferiților producători de vin în spațiul virtual și nu numai. Ca rezultat al unui an de activitate, grupul s-a dezvoltat cooptând diverse arii tematice și elaborează o serie de recomandări care sunt promovate în spațiul virtual (online).*
***Cuvinte cheie:*** *degustare de vin, oenologie, promovare.*

## INTRODUCTION

Wine, in the conception of Louis Pasteur, is the healthiest and more hygienic drink. Beyond the skill of the winemaker, the wine is primarily the expression of the grape variety, the soil in which the vine is planted and climatic conditions of the harvest year. The beneficial effects of wine consumption are well known, they coming from complex combination of multiple compounds, in which phenolic compounds play a very important rol (Mihalca *et al.,* 2010).

In order to achieve end good products that meet the increased demands of internal and external market consumption, Oenology is The science that deals with the study and methods of producing, stabilizing and maturing of wines and other products obtained from grape must and wine.

Color, pleasant smells and taste are resulting from fermentation. They are formed during product development from grapes or formed during maturation and give an ensemble or consonance to the constituents that from wine. When one of those substances are in excess, especially when their are added as various treatments

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or by corrosion effect of the acidity upon containers and mechanical parts, following enzymatic activities and wine lack of basic hygiene, a number of undesirable changes can occur in the product, well known as faults. Besides these, other changes may occur due to the activity of yeast and pathogenic bacteria and they are designated as diseases of wine (Cotea *et al.*, 1982; Cotea *et al.*, 2009).

Quoting Maiorescu from a letter sent to Alecsandri: "*after an enthusiastic moment, but without calculating the full practical means, there comes a time of numbing and also disgust, but finally resumes stamping the old heart with old problems and many lost illusions, but with greater certainty of success into a narrower circle*" (Zub, 1976), we can say that *Junimea Vinului Iași* is a group of wine lovers coming from different backrounds and its aim is to promote quality, especially from Romanian wines varieties.

## MATERIAL AND METHOD

The paper describes *Junimea Vinului Iași* group and its role in the oenological sector. The group, until now, consists of 21 wine lovers, who come from several areas of expertis, namely: restaurant owners, production managers, wine distributors, sommeliers, heads of hall-restaurant, oenologists, doctors and doctoral students etc. Their age is ranging between 20 and 50 years old.

The group holds regular meetings in which they discussed various oenological topics, tasting wines and promoting different wines from almost all producers via social media networking. The data are interpreted by suitable experimental method recommended by the literature (Jităreanu, 1994).

## RESULTS AND DISCUSSIONS

*Junimea Vinului Iași* group was established in the spring of 2014 from the desire to know better the secrets of wine, to socialize and share and to understand what it means: quality of wine. Among other things, the role of the group is to recommend quality wines at an affordable prices to potential consumers, HoReCa (Hotels Restaurants Cafés), specialized wine shops and wine-loving private persons.

To have an objective activity from the start an imported rule was embraced so that this group will not advertise a single producer of wine.

The group holds regular meetings with various oenological issues, while taking place wine tastings (tab. 1; tab. 2; tab. 3; tab. 4; fig. 1; fig. 2).


**Fig. 1 -**The logo and a image from Junimea Vinului Iași meeting

*Table1*

**White wines assessed**

| Winery | Variety | Range | Wine type / production year | Remarks |
|---|---|---|---|---|
| Bucium | Fetească albă | Bucium | medium dry 2014 | harmonious with wild flowers aromas |
| | Fetească albă | Fresca | medium dry 2013 | very expressive nose, pleasant aftertaste |
| Panciu | Fetească albă | Casa Panciu | dry 2014 | impressive, notes of citrus, flower of vine |
| | Chardonnay baricat | Domeniile Panciu | dry 2013 | typicality and pleasant acidity |
| | Muscat Ottonel | Casa Panciu | sweet 2014 | pleasant dessert wine |
| | Cabernet Sauvignon "blanc de noirs" | Domeniile Panciu | dry 2014 | complex with varietal flavor supported by high acidity |
| | Sauvignon blanc | Sagio | dry 2014 | fruitfully, tropical, stout |
| | Aligoté | Domeniile Panciu | dry 2014 | atypical |
| Licorna | Fetească albă | Serafim | dry 2014 | typicality, vine flowers, honey |
| | Chardonnay | Serafim | dry 2014 | balanced sensory |
| | Sauvignon blanc | Serafim | dry 2014 | typicality, stout, citrus |
| | Rhein Riesling | Serafim | dry 2014 | lemon and grapefruit, shock and mint |
| Darie | Chardonnay | Străbun | dry 2013 | baric, aftertaste, butter, peach |
| | Chardonnay | Străbun | dry 2014 | stripling with pleasant aftertaste |
| Familia Hetei | Chardonnay | Familia Hetei | dry 2014 | wine with high potential, butter fresh flavor, round |
| | Pinot Gris | Familia Hetei | dry 2014 | a rustic wine |
| | Tămâioasă românească | Familia Hetei | dry 2014 | imbalance, rose aroma, vapid taste, wine without texture |
| Balla Géza | Pinot Gris | Balla Geza | dry 2013 | bunch of aging |
| Domeniile Tohani | Chardonnay | Domeniile Tohani | dry 2013 | vanilla flavors excess |
| Domeniile Vlădoi | Sauvignon blanc | Ravak | dry 2014 | pleasant notes with fructuos citrus notes |
| | Chardonnay | Ravak | dry 2014 | baric, aftertaste, butter, peach |
| | Sauvignon blanc | Anca Maria | dry 2014 | youthful wine, very pleasant and typical |
| | Riesling Italian | Ravak® | dry 2014 | sprightly, with the nose of hydrocarbons |
| | Muscat Ottonel | Ravak | dry 2014 | intense flavor, pleasant |
| | Fetească regală | Ravak | dry 2014 | pleasant acidity |
| Domeniile | Chardonnay | Hermeziu | dry 2014 | fresh, floral with notes |

| | | | | |
|---|---|---|---|---|
| Lungu Domeniile Lungu | | | | of grapefruit |
| | Sauvignon blanc | Hermeziu | dry 2014 | sprightly, vegetal notes, green fruit |
| | Traminer roz | Hermeziu | dry 2014 | aromas of white flowers |
| Crama Delta Dunarii | Chardonnay | La Sapata | dry 2014 | cork taint |
| | Aligoté | La Sapata | dry 2014 | typically, bio grapes |
| | Aligote/Riesling | La Sapata | dry 2014 | sand after a short summer rain |

Most white wines evaluated had a low content of sugars (dry wines), namely 92%, therefore group recommends consumption as fresh white wines and a low concentration of sugars.

*Table 2*

**Rosé wines assessed**

| Winery | Variety | Range | Wine type / production year | Remarks |
|---|---|---|---|---|
| Lacerta | Blaufrankisch | Lacerta | dry2014 | red fruits and grapefruit |
| Licorna | Burgund mare/Merlot | Serafim | dry 2013 | physalis, cherries, strawberries |
| | Cabernet Sauvignon Shiraz | Serafim | medium dry 2014 | strawberry, pomegranate, sour cherry, raspberry |
| Domeniile Vlădoi | Fetească neagra | Ravak | dry2014 | grapefruit, blood orange |
| | Cabernet Sauvignon | Anca Maria | dry2014 | fresh, harmonious, balanced |
| Familia Hetei | Cabernet franc | Familia Hetei | medium sweet 2014 | cork taint |
| Panciu | Cabernet Sauvignon | Casa Panciu | dry 2014 | is not yet finalized, reductive |
| | Băbească neagră | Casa Panciu | medium dry2014 | very good acidity, floral |
| S.E.R.V.E | Fetească neagră / Merlot | Terra Romana | dry2014 | ripe red berries |
| Bucium | Busuioacă de Bohotin | Fresca | medium dry 2014 | rose petals and basil |
| | Busuioacă de Bohotin | Bucium | dry2014 | rose petals and basil |

Rosé wines, in a proportion of 17% had a higher sugar content, making a positive contribution in terms of their flavor.

*Table 3*

**Red wines assessed**

| Winery | Variety | Range | Wine type /production year | Remarks |
|---|---|---|---|---|
| Lacerta | Cabernet Sauvignon, Merlot, Shiraz | Cuvee IX | dry 2012 | currant, chocolate, vanilla and spices |
| Licorna | Fetească neagră | Serafim | dry 2013 | silky, fruity, harmonious |
| | Cabernet | Serafim | dry 2013 | ripe red fruits, vegetal |

| | | | | |
|---|---|---|---|---|
| | Sauvignon | | | |
| | Merlot | Serafim | dry 2013 | sour cherry, bitter cherry jam |
| Tohani | Cabernet Sauvignon | Princiar | dry 2012 | berries |
| | Pinot noir | Moșia Tohani | dry 2011 | typical |
| Panciu | Fetească neagră | Domeniile Panciu | dry 2012 | black cherry, mellow, spicy |
| | Cabernet Sauvignon Fetească neagră | Sagio | dry 2011 | complex, dried plums, pepper and pimento |
| Domeniile Vlădoi | Fetească neagră | Pivnița Basarabilor | dry 2012 | dried plums |
| | Merlot | Pivnița Basarabilor | dry 2011 | low acidity |
| | Cabernet Sauvignon | Pivnița Basarabilor | dry 2012 | grassy, balanced |
| Zoresti | Cabernet Sauvignon | Zoresti | dry 2012 | with potential |
| Halewood | Fetască neagră | Halewood | dry 2012 | without expressivity |
| Rothenberg | Merlot | Emeritus | dry 2010 | rotten sour cherry |
| Vinicom | Feteasca neagră | Cramele Copoului | dry 2013 | black cherry, plum |
| | Fetească neagră | Cramele Copoului | dry 2013 | black cherry, highly oxidized |
| Familia Hetei | Feteasca neagră | Familia Hetei | dry 2012 | wiped |
| | Merlot | Familia Hetei | dry 2013 | atypical, without body |
| | Shiraz | Familia Hetei | dry 2012 | slightly spicy without body |

Red wines evaluated are more complex, requiring to breath for a time, but not very long period, and so generally no more then one hour.

*Table 4*

**Sparkling wines assessed**

| Wine cellar | Variety | Range | Type | Colour | Remarks |
|---|---|---|---|---|---|
| Cricova | Chardonnay/Pinot | Cuvee Prestige | raw | white | special flavor, finesse |
| Halewood | Chardonnay / Pinot | Rhein | raw | white | intense aroma of yeast |
| Jidvei | Chardonnay / Pinot | Romantine | extra raw | white | intense aroma of yeast |
| Panciu | Fetească albă / Fetească regală | Domeniile Panciu | raw | white | very good pearling, fine flavors |
| Riviera | Fetească regală | Târnave | raw | white | no special flavors |
| Balla Géza | Mustoasă de Măderat | Signum | raw | white | fragrant, pleasant |
| Cricova | Fetească albă | Crisecco | raw | white | special flavor, |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | finesse |
| Bucium | | Grand Casino | raw | white | erased, without intense flavors |
| Villa Yustina | | Villa Yustina | raw | white | poor flavor |
| Halewood | Pinot noir | Rhein rose | raw | rose | fine aromas of yeast |
| Cricova | Pinot noir | Brut rose | raw | rose | too sweet |
| Jidvei | Pinot noir | Romantine | raw | rose | too intense color |

Sparkling wines were mostly made by the traditional method and they could be a good starter product.

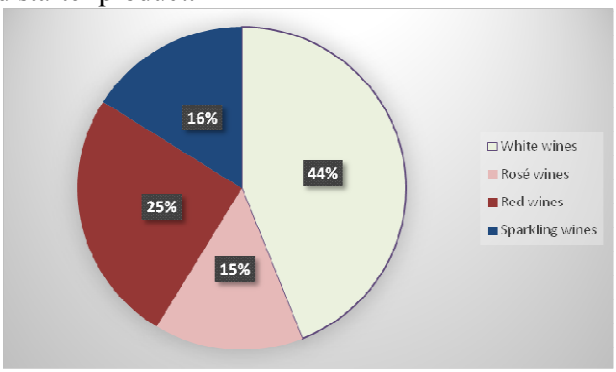


**Fig. 2** - Distribution of evaluated wine types

Following these meetings, a number of beneficial conclusions and recommendations are drawn for both participating producers and intereseted consumers regarding price-quality ratio and special features.

## CONCLUSIONS

1. *Junimea Vinului Iași* group aims to promote quality wines, especially wines from Romanian varieties. Also, the role of the group is to recommend to potential customers quality wines at an affordable price, both for HoReCa locations or specialized wine shops and wine-loving private persons.

2. The local initiative group is established with specialists coming from different various sectors of expertise and want to promote wine, an old product (nourishment) in the culture of the Carpathian-Danubian-Pontic space without being directly involved in commercial aspects.

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# PHYSICO CHEMICAL ANALYSIS OF CYPRIOT AND ROMANIAN RED WINE FOR QUALITY CONFIRMATION

## ANALIZA PARAMETRILOR FIZICO CHIMICI A UNOR VINURI CIPRIOTE ŞI ROMÂNEŞTI PENTRU CONFIRMAREA CALITĂȚII

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***Abstract.*** *The production of winemaking in Cyprus and in Romania dates back to ancient times with years of tradition and had an important role in the everyday life. To ensure the quality of wines produced in the two countries, it is absolutely necessary to characterize and establish the typicity and physical-chemical parameters in order to achieve a database. The aim of this research was the comparative analysis of Cypriot and Romanian wine samples using standard methods of OIV, for determining the physico-chemical parameters of red wine samples (volatile acidity, total acidity, free SO2, total SO2, TDM, Sugars, pH, alcoholic content). Following the results, we observed statistically significant differences between samples. These results, together with the metal and phenolic compounds content analysis will be used to confirm the authenticity of the Romanian and Cypriot wines.*

***Key words:*** *wine, physico-chemical parameters, authenticity*

***Rezumat.*** *Producerea de vinuri în Cipru și România este o tradiție pentru cele două popoare încă din cele mai vechi timpuri. Este absolut necesar, pentru a se asigura calitatea vinurilor produse în cele două țări, caracterizarea și stabilirea caracteristicilor fizico - chimice și a tipicității vinurilor produse, în vederea realizării unei baze de date. Scopul acestei lucrări constă în analiza comparativă a unor probe de vinuri roșii, cipriote și românesti, cu ajutorul metodelor standard OIV pentru stabilirea parametrilor fizico-chimici (aciditate volatilă, aciditate totală, $SO_2$ liber, $SO_2$ total, TDM, conținutul de zaharuri, pH, concentrația alcoolică). In urma analizei rezultatelor obținute s-au observat diferențe semnificative între probele analizate. Aceste rezultate, împreună cu analiza conținutului de metale și de compuși fenolici, vor fi utilizate pentru confirmarea autenticității vinurilor românești și cipriote.*

***Cuvinte cheie:*** *vin, parametri fizico-chimici, autenticitate*

## INTRODUCTION

Studying the authenticity of wine is one of the most important issues in food quality control validating to guarantee the safety and to protect the rights of consumers and producers. A chemical approach to assuming the properties of wine is

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based on analysis of chemical composition as a unique combination of constituents (Arvanitoyannis, 2010).

The authenticity of wine may be a subject of legal interest, especially when it involves economic costs or causes negative health effects (Cozzolino and Heather, 2013).

Practiced since earliest times, wine fake has become more sophisticated in the present day, taking many forms. Consumers and manufacturers are all interested to have consistent analytical tools and information to allow the authentication and detection of wine adulteration or incorrect classification (Schlesier *et al.,* 2009).

The production of winemaking in Cyprus and in Romania dates back to ancient times with 4000 years of tradition and had an important role in the everyday life.

Cyprus is situated in the southeastern Mediterranean, at the crossroads of the three continents of Europe, Asia and Africa. It is the third largest island in the Mediterranean after Sicily and Sardinia. The vineyards of Cyprus are mostly on the southern peaks of the Troodos Mountain, near Limassol. The second area is in the south-western part of the island near Pafos. The long, sunny Mediterranean summers are a great asset; they ripen the fruit and give it its full flavor. The winters are mild and the gentle winds and breezes check the frosts, dispel the fogs and protect the vines and grapes from fungus diseases (Cyprus remains one of the few phylloxera-free wine-producing countries in the world) (Psaras and Zambartas, 1981).

Romania is one of the top ten wine producing countries in the world and one of the top six in Europe, along with France, Spain, Italy, Germany and Portugal. Wine production follows the same trends as grape production (Arvanitoyannis *et al.,* 1999).

The scope of this work consists in the comparative study on the chemical and physical properties of commercial and authentic wine samples from Romania and Cyprus.

## MATERIAL AND METHOD

Were analyzed eleven commercial red wines from Cyprus and eight Romanian red wines: five authentic wines from ampelographic collection, vinified on the Research Center and three commercial wine.

Wine analyzes were performed in the Laboratory of Oenology from Oenology Researche Center of Romanian Academy Iasi Brunch. Physico-chemical analyzes were carried out according to the methods specified in the international standards and studies (Garrido and Borges, 2011; Iland *et al.,* 2004; Ribéreau-Gayon, 1972). The following were registered: total acidity, volatile acidity, total and free $SO_2$, sugars and alcohol of the wine. The methods used are according to the OIV (*** OIV, 2013).

## RESULTS AND DISCUSSIONS

The physico-chemical characteristics for the analysed Cipriot wines are present in table 1 and for Romanian wines in table 2. Analyzed parameter values vary significantly from one country to another as well as from one sample to another.

The higher value of the pH is 3.88 for Romanian wines, in the Cabernet Sauvignon 2012 c sample. The Cypriot wines are characterized by the pH-values exceeding 4, for example Morakanella has a pH of 4.4.

Alcoholic concentration varies in wine samples from Cyprus from very low values - 5.28 (in Merlot ), uncharacteristic for a product called wine, to higher values - 14.65 (in Maratheutiko 3). Romanian wines have an alcohol concentration between 9.72 and 14.49.

The values of total acidity, is between 8.11 (Cabernet Franc) and 3.05 (Morakanella) in samples of wine from Cyprus and between 9.43 and 4.42 in local wine samples.

*Table 1*

**Physical-chemical analysis of Cipriot wines**

| Sample | Free $SO_2$ (mg/L) | Total $SO_2$ (mg/L) | Volatile acidity (g/L acetic acid) | Total acidity (g/L tartaric acid) | □ (g/L) | Alco-holic conc. (%) | Remanent sugars (g/L) | pH |
|---|---|---|---|---|---|---|---|---|
| Merlot | 9.13 | 42 | 0.37 | 5.9 | 1.005 | 5.28 | 4.33 | 3.9 |
| Mara-theutiko1 | 15.07 | 68.71 | 0.23 | 5.16 | 0.997 | 10.64 | 3.56 | 3.8 |
| Giannoudi | 14.61 | 66.66 | 0.21 | 3.64 | 0.995 | 10.94 | 2.8 | 4.1 |
| Mara-theutiko 2 | 15.3 | 72.82 | 0.12 | 5.16 | 0.993 | 12.71 | 3.92 | 3.5 |
| Mara-theutiko 3 | 8.49 | 90.86 | 0.4 | 5.11 | 0.992 | 14.65 | 3.4 | 3.5 |
| Cabernet Franc | 22.37 | 71 | 0.32 | 8.11 | 0.994 | 13.4 | 3.3 | 3.4 |
| Morak-anella | 2.97 | 171.22 | 0.5 | 3.05 | 0.993 | 10.67 | 0.83 | 4.4 |
| Mauro | 31.73 | 66.2 | 0.78 | 3.44 | 1.000 | 6.18 | 2.15 | 4.2 |
| Ofthalmo | 19.18 | 64.83 | 0.45 | 4.81 | 0.991 | 13.04 | 2.09 | 3.56 |
| Mara-theutiko 4 | 31.05 | 70.77 | 0.42 | 6.09 | 0.993 | 11.66 | 1.8 | 3.14 |
| Gian-noudi | 28.54 | 51.59 | 0.4 | 3.34 | 0.999 | 7.04 | 2.05 | 4.3 |

*Table 2*

**Physical-chemical analysis of Romanian wines**

| Sample | Free $SO_2$ (mg/L) | Total $SO_2$ (mg/L) | Volatile acidity (g/L acetic acid) | Total acidity (g/L tartaric acid) | □ (g/L) | Alcoholic conc. (%) | Remanent sugars (g/L) | pH |
|---|---|---|---|---|---|---|---|---|
| Pinot noir 2014 a | 16.2 | 46.79 | 0.23 | 6.77 | 0.991 | 14.26 | 3.55 | 3.41 |
| Feteasca neagra 2014 c | 13.7 | 56.16 | 0.2 | 7.47 | 0.994 | 10.68 | 1.67 | 3.31 |
| Pinot gri 2014 a | 65.52 | 138.57 | 0.33 | 6.68 | 0.989 | 14.49 | 4.81 | 3.24 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Babeasca neagra 2014 a | 10.5 | 40.41 | 0.25 | 9.43 | 0.994 | 9.72 | 1.42 | 3.11 |
| Feteasca neagra 2013 c | 23.74 | 75.79 | 0.63 | 4.42 | 0.994 | 13.72 | 2.59 | 3.78 |
| Babeasca neagra 2013 a | 20.09 | 55.47 | 0.26 | 8.45 | 0.997 | 10.02 | 14.86 | 3.16 |
| Cabernet Sauvignon 2013 a | 6.81 | 38.09 | 0.22 | 6 | 0.992 | 11.58 | 1.44 | 3.42 |
| Cabernet sauvignon 2012 c | 36.54 | 99.72 | 0.14 | 7.52 | 0.994 | 12.21 | 2.84 | 3.88 |

a= authentic; c=comercial

## CONCLUSIONS

1. pH values for samples coming from Cyprus are higher than those of Romanian wine samples and reached more than 4.

2. Low alcoholic concentration in some samples of wine vinified in Cyprus is not correlated with the climatic conditions of the Island; the high temperature should lead to obtain wines with higher alcohol concentrations.

3. Even if the processing of grapes to obtain wine is a preoccupation for hundreds years to the both countries, the results indicates that though, the wine industry has evolved differently, probably under the influence of traditions and local practices, resulting wines with clear defined and authentic characteristics.

***Acknowledgments**: the research was funded by the PN-II-RO-CY-2013-1 project, nr. 764/2014.*

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# COMPARATIVE STUDY OF SOME COMPOSITIONAL CHARACTERISTICS OF WHITE AND RED WINES OBTAINED BY FERMENTATION WITH INDIGENOUS AND SELECTED YEAST

## STUDIUL COMPARATIV AL UNOR CARACTERISTICI COMPOZIȚIONALE LA UNELE VINURI ALBE ȘI ROȘII OBȚINUTE ÎN URMA FERMENTĂRII CU LEVURI INDIGENE ȘI LEVURI SELECȚIONATE

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***Abstract***. *International competition existing in the wine market, consumer demand in terms of adapting to new styles of wines and growing concerns about environmental sustainability in the wine production causes a continuous research of a new technique for modeling the profile of a wine. A new concern for the researchers is the exploitation of indigenous yeasts as a commercial option. This is because the wines made with indigenous yeasts are supposed to have a more complex aromatic level and more specificity. In the case of intensive wine production that already is successfully using selected yeasts the key problem that it comes is the technological efficiency. Thus, if the selected yeast allow a rapid fermentation, has a good tolerance to high levels of ethanol, a reduced production of acetic acid, there remains the question whether native yeast can provide for the technologist the same benefits or even superior results.*

***Key words:*** *fermentation, selected yeast, indigenous yeast, technological efficiency, compositional characteristics*

***Rezumat.*** *Concurența internațională existentă pe piața vitivinicolă, cererea consumatorilor în ceea ce privește adaptarea unor noi stiluri de vinuri, precum și preocupările crescânde cu privire la sustenabilitatea ecologică a producției de vin determină o cercetare continuă a unor noi tehnici de modelare a profilului unui vin. O nouă preocupare a cercetătorilor o reprezintă exploatarea levurilor indigene ca și opțiune comercială. Aceasta se datorează faptului că vinurile realizate cu drojdii indigene sunt percepute ca având o complexitate mai mare la nivel aromatic, dar și o specificitate mai mare. În cazul producției vinicole intensive care deja utilizează cu succes drojdii selecționate se pune problema eficenței tehnologice. Astfel, dacă drojdiile selecționate permit realizarea unei fermentații rapide, prezintă o toleranță bună la nivelurile crescute de etanol, o producție redusă de acid acetic, rămâne deschisă problema dacă drojdiile indigene pot oferi tehnologului aceleași avantaje sau chiar rezultate superioare.*

***Cuvinte cheie:*** *fermentație, levuri indigene, levuri selecționate, eficență tehnologică, caracteristici compoziționale*

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## INTRODUCTION

Wine by definition is a product resulting from biological and biochemical complexes interactions that occur between grapes (grape juice) and various microorganisms (fungi, yeasts, bacteria ) with the participation of mycoviruses and bacteriophages (Fleet, 2003).

In most countries, wine production is based on using selected yeasts able to determine obtaining wines with superior features: high alcohol level, pleasant aromas, reduced production of acetic acid in a relatively short time.

However, using selected yeasts inevitably lead to a standardization of wine styles, so in the end the idea of specificity and biodiversity is much diminished.

The present study aims to identify physico-chemical differences in wines produced by fermentation with indigenous yeasts and selected yeasts.

## MATERIAL AND METHOD

The wines analyzed were obtained both from white grape varieties, namely: Fetească regală, Fetească albă, Tămâioasă românească , Muscat Ottonel and black grape varieties: Fetească neagră, Cabernet Sauvignon, Merlot and Băbească neagră. After the vinification process of each grape variety there were obtained two technological variants, namely: control- variant (M) fermented with indigenous yeasts and V1- variant fermented with selected yeast. It is also necessary to mention that the comparative analysis was performed on wine samples coming from the same varieties.

In a first stage, the wines were subjected to common physico-chemical analyzes, respective: acidity, density, pH, alcohol concentration, etc. The analytical methods used to determine these parameters were in accordance with the European standards and those imposed by the OIV (OIV, 2011). In the second stage the wines were studied in terms of phenolic component and it was imposed to do some photometric analyzes with Shimadzu UV-1800 spectrophotometer.

The reading of D280 index polyphenols was achieved at an 280 nm absorbance and for the Folin-Ciocalteu index it was used the method described by Watherhouse in 2002, so in this way the phenolic compounds are expressed by using a gallic acid standard curve with the following concentrations: 50, 100, 250, 500 mg / L (Watherhouse, 2002).

For an objective evaluation of the characteristic color of the wines the recommended method used was the one described by "Eclairage International Comission", namely: Cie Lab 76 (Cotea *et a*l., 2009). The recording of transmittance spectra was performed using a UV-VIS spectrophotometer Carl Zeiss SPECORD coupled with an IBM-PC computer. In this way was made the digitization and the automatic recording of transmittance spectrum in a file stored on the computer. Digitized spectral data was processed with the program "WINECOLOR" to obtain the chromatic parameters L, a, b, c, and H□.

## RESULTS AND DISCUSSIONS

This article is a comparative study between different samples of wines obtained from different varieties on which was applied a fermentation process using indigenous and selected yeast.

*Table 1*

**Physico-chemical analysis of the wines**

| Wines considered | $SO_2$ mg/L | | Vol. acidity ($g/L C_2H_4O_2$) | Total acidity 8($g/L C_4H_6O_6$) | Relative density | Alch. conc. (% vol.) | Reductive subst. (g/L) | T.D.E (g/L) | N.E. (g/L) | pH |
|---|---|---|---|---|---|---|---|---|---|---|
| | Free | Total | | | | | | | | |
| **Fetească regală- M** | 28.64 | 92.7 | 0.58 | 7.23 | 0.9993 | 11.93 | 2.02 | 29.18 | 22.06 | 3.4 |
| **Fetească regală- $V_1$** | 26.23 | 75.88 | 0.59 | 7.5 | 0.9933 | 12.1 | 1.23 | 26.39 | 25.12 | 3.34 |
| **Fetească albă- M** | 49.13 | 102.89 | 0.48 | 6.89 | 0.9989 | 12.01 | 2.1 | 30.36 | 21.25 | 3.46 |
| **Fetească albă- $V_1$** | 33.71 | 92.08 | 0.46 | 6.95 | 0.9931 | 12.18 | 1.77 | 27.63 | 19.04 | 3.31 |
| **Tâmâioasă românească- M** | 31.27 | 87.36 | 0.44 | 5.13 | 0.9939 | 11.38 | 2.37 | 24.53 | 22.12 | 3.71 |
| **Tâmâioasă românească – $V_1$** | 29.59 | 85.71 | 0.42 | 5.11 | 0.9924 | 11.81 | 2.31 | 22.71 | 20.46 | 3.68 |
| **Muscat Ottonel- M** | 22.89 | 79.13 | 0.46 | 4.92 | 0.9947 | 11.42 | 3.24 | 25.58 | 22.28 | 3.62 |
| **Muscat Ottonel- $V_1$** | 24.03 | 81.53 | 0.43 | 4.87 | 0.9958 | 11.45 | 3.18 | 29.24 | 26.3 | 3.68 |
| **Fetească neagră- M** | 21.3 | 105.24 | 0.33 | 6.58 | 0.9923 | 10.89 | 1.15 | 25.69 | 22.72 | 3.6 |
| **Fetească neagră- $V_1$** | 25.12 | 77.53 | 0.29 | 6.99 | 0.9928 | 11.23 | 1.21 | 25.36 | 24.09 | 3.6 |
| **Cabernet Sauvignon - M** | 25.18 | 71.19 | 0.69 | 6.34 | 0.9912 | 13.18 | 1.14 | 27.68 | 26.71 | 3.71 |
| **Cabernet sauvignon- $V_1$** | 22.34 | 68.12 | 0.58 | 6.49 | 0.9935 | 13.46 | 1.78 | 29.1 | 27.35 | 3.74 |
| **Merlot- M** | 26.52 | 105.78 | 0.49 | 6.28 | 0.9929 | 11.89 | 1.24 | 25.46 | 24.27 | 3.69 |
| **Merlot- $V_1$** | 19.37 | 92.18 | 0.45 | 6.78 | 0.9934 | 12.23 | 1.38 | 26.29 | 25.12 | 3.57 |
| **Băbească neagră- M** | 20.28 | 75.36 | 0.45 | 5.96 | 0.9933 | 11.59 | 1.18 | 22.37 | 21.11 | 3.63 |
| **Băbească neagră- V1** | 11.77 | 37.45 | 0.23 | 6.05 | 0.9938 | 11.97 | 1.59 | 24.52 | 22.96 | 3.66 |

The compositional charactheristics of the analysed wines are presented in the Table 1. Observing two parameters: the alcohol concentration and the reductive substances, the wine samples that were analyzed can be included in the category of dry quality wines.

Following the physico-chemical analyses that were conducted on the wine samples, it can be seen that no major differences were found between the parameters of the control samples and those of the samples treated with selected yeasts.

Though, a slightly difference could be observed in the case of Băbească neagră wine samples concerning the volatile acidity feature. Thereby, the Băbească neagră – control sample fermented with indigenous yeasts had a higher value of volatile acidity, 0.45 g/L acetic acid than the Băbească neagră sample fermented with selected yeasts which has recorded a lower value of 0.23 g/L acetic acid.

Analyzing the alcohol concentration, it can be observed that this parameter presented insignificant variations, except wine samples obtained from Fetească neagră variety. So, the control sample obtained from Fetească neagră grapes recorded a lower value of the alcohol concentration feature of 10.89 %, than the V1 sample obtained from the same variety, respectively 11.23 %.

For each pair of samples, using the spectrophotometry it was observed the variation of two features, namely: the index of total polyphenols (IPT) and the Folin-Ciocâlteu index (IFC).

In the case of Fetească regală, Tămâioasă românească, Merlot and Băbească neagră samples, there were no significant differences recordered for the index of total polyphenols (IPT) and Folin-Ciocâlteu index (figure 1). However, the Fetească albă control sample presented a higher value for the Folin- Ciocâlteu index of 4.46 than the Fetească albă V1- sample which registered a lower value of 3.48.

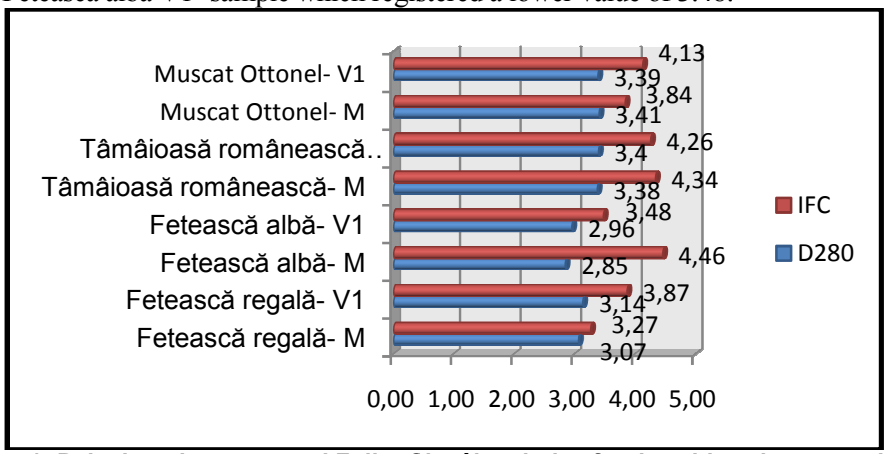


**Fig. 1- Polyphenol content and Folin- Ciocâlteu index for the white wines samples**

Observing the Muscat Ottonel samples, it can be seen that the control sample had a lower value of the polyphenol index with reducing proprieties than the sample V1 treated with selected yeasts.

The Fetească neagră sample treated with selected yeasts presented higher values for both Folin- Ciocâlteu index and total polyphenols index, respectively 32.79 and 25.26 than the control sample.

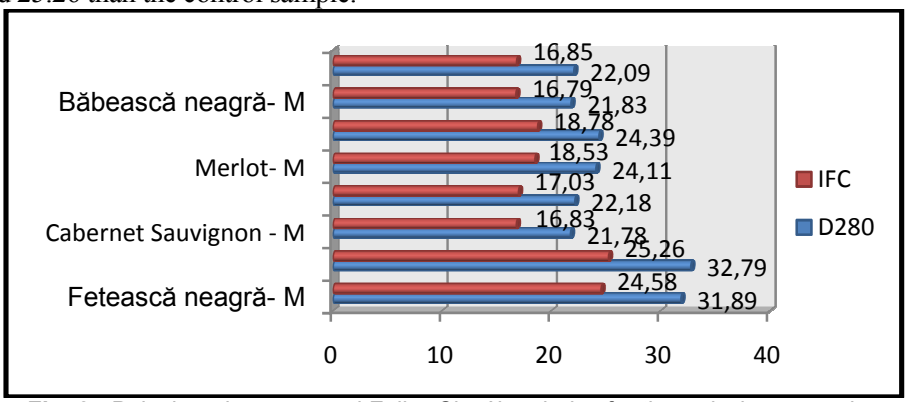


**Fig. 2 -** Polyphenol content and Folin- Ciocâlteu index for the red wines samples

In what concerns the Cabernet Sauvignon samples, from Figure 2, it can be seen the same variations both for the Folin- Ciocâlteu and total polyphenols index that were noted in the case of Fetească neagră samples.

Thereby, the Cabernet Sauvignon samples treated with selected yeasts presented higher values for Folin- Ciocâlteu and total polyphenols index than the control samples.

*Table 2*

**Values of chromatic parameters of the analyzed wines**

| Samples | L | Color coordinates | | C | H | I | N | Color |
|---|---|---|---|---|---|---|---|---|
| | | a | b | | | | | |
| **F. R. - M** | 95.36 | 1.75 | 5.53 | 4.63 | -80.39 | 0.21 | 1.97 | |
| **F. R. - $V_1$** | 99.27 | 1.46 | 3.92 | 1.98 | -76.59 | 0.15 | 0.05 | |
| **F. A. - M** | 98.10 | 1.64 | 7.01 | 7.04 | -84.65 | 0.14 | 3.83 | |
| **F. A. - $V_1$** | 98.22 | 1.69 | 6.98 | 7.00 | -84.24 | 0.13 | 4.00 | |
| **T. R. - M** | 93.66 | 2.05 | 8.56 | 7.28 | -75.61 | 1.02 | 1.37 | |
| **T. R. – $V_1$** | 92.87 | 2.41 | 8.08 | 7.56 | -77.21 | 0.23 | 1.48 | |
| **M. O. - M** | 94.53 | -0.27 | 5.86 | 5.33 | -81.42 | 0.98 | 1.21 | |
| **M. O. - $V_1$** | 95.88 | -0.51 | 5.92 | 6.08 | -80.89 | 0.96 | 0.99 | |
| **F. N. - M** | 37.69 | 49.56 | 28.97 | 57.29 | 30.28 | 3.51 | 0.81 | |
| **F. N. - $V_1$** | 30.98 | 52.11 | 40.89 | 66.25 | 38.08 | 4.79 | 0.85 | |
| **C. S. - M** | 6.79 | 35.92 | 11.48 | 37.64 | 17.77 | 13.43 | 0.59 | |
| **C. S. - $V_1$** | 6.78 | 35.89 | 11.47 | 3761 | 17.75 | 13.47 | 0.59 | |
| **Merlot- M** | 31.88 | 57.94 | 41.21 | 71.09 | 35.32 | 5.11 | 0.69 | |
| **Merlot- $V_1$** | 31.14 | 57.01 | 45.23 | 72.76 | 38.41 | 5.39 | 0.71 | |
| **B. N. - M** | 64.52 | 43.89 | 20.48 | 48.47 | 25.08 | 1.79 | 0.69 | |
| **B. N. - V1** | 55.01 | 52.61 | 15.87 | 54.98 | 16.81 | 2.04 | 0.68 | |

*L- clarity; C- saturation; H- tone; I- intensity of color; N- color tint.

Analyzing data obtained using the spectrophometer, it can be observed (Table 2) that the white wine samples presented specific yellow and light red shades of color, excepting the Muscat Ottonel samples where predominated the green and yellow shades of color. In the case of red wine samples, as expected the red and yellow shades of colour prevailed.

As expected, from the analysis of the L-parameter (brightness), it resulted that the white wine samples presented a high level of clarity. However, the Fetească regală samples and the Fetească albă samples had higher values of L-parameter than the other white wine samples analyzed. In the case of red wine samples, the lowest values of brightness were recorded by the Cabernet Sauvignon samples and the highest values were registered by the Băbească neagră samples. The facts stated above were sustained once again by the simulated colors from Table 2.

In order to emphasis the impact of the yeast on the physico- chemical features of the analyzed samples it was conducted a paired t-test. For each pair of samples, it was considered a general hypothesis: that the fermentation process conducted with indigenous yeasts (control samples) and with selected yeasts (V1-samples) had a significant influence on the physico-chemical features of the analyzed wines.

The statistical results revealed for each pair of samples the value of P (significance) was higher than 0.05. Therefore, it can be stated that the variable didn't had a significant impact on the physico-chemical features of the analyzed pairs.

## CONCLUSIONS

Following the results of the physico- chemical characteristics (specifically the alcohol concentration and the reductive substances) obtained by analyzing the considered pair of samples, these could be included in the category of quality dry wines.

So, in this preliminary study, it can be concluded that the type of yeasts used (indigenous yeasts and selected yeasts) didn`t had a major influence on the physico-chemical features, such as: alcohol, density, volatile acidity, total acidity, T.D.E, N.E., pH, etc. This statement is supported once again even by the statistical analysis performed, for which it was obtained a value higher than 0.05 for the P (significance) feature.

However, it has been observed that using different types of yeasts did had a slightly influence on the phenolic profile of the wines. In this case, remains open the question of the yeast influence on the phenolic and flavor components.

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# PRELIMINARY RESULTS ON ESTABLISMENT THE OPTIMAL EXTRACTION CONDITIONS STAGED PHENOLIC COMPOUNDS IN GRAPE SEED PROANTHOCYANIDINS

## REZULTATE PRELIMINARII PRIVIND STABILIREA CONDIȚIILOR OPTIME DE EXTRACȚIE ETAPIZATĂ A COMPUŞILOR FENOLICI PROANTOCIANIDINICI

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***Abstract.*** *Condensed tannins from grape seed the waste, also known as proanthocyanidins extracted with solvents, are soluble in water and therefore can not be used practically as the bioactive antifungal, antibacterial, cytostatic. In this context imposed, establish the optimal conditions for the method of extraction of phenolic compounds in stages: report solid / solvent extraction time, the number of extraction steps. They were tested five reports of plant material / solvent (1/4, 1/5, 1/6, 1/7, 1/8), following the dynamics of the extraction process of phenolic compounds in two steps, by monitoring the optical density at 280 nm. In the two-stage extraction, the maximum value of the optical density was recorded after eight hours, in the ratio 1/4, and the reports 1/5 - 1/8 after 10 hours. Cumulated extracts presented in terms of variations in the concentration of polyphenol, amounts being comprised between 2.81 to 2.88 g GAE / 100 g of delipidated grist.*
***Key words:*** *seed, extraction, report, time*

***Rezumat.*** *Taninurile condensate din deşeul de semințe de struguri, numite şi proantocianidine, extrase cu solvenți, nu sunt solubile în apă, motiv pentru care nu pot fi utilizate practic ca substanțe bioactive antifungice, antibacteriene, citostatice. În acest context s-a impus, stabilirea condițiilor optime pentru aplicarea metodei de extracție etapizată a compuşilor fenolici: raportul solid/solvent, timpul de extracție, numărul de etape de extracție. Au fost testate cinci rapoarte material vegetal/solvent (1/4, 1/5, 1/6, 1/7, 1/8), urmărindu-se dinamica procesului de extracție a compuşilor fenolici în două etape, prin monitorizarea densității optice la 280 nm. În cele două etape de extracție, valoarea maximă a densității optice s-a înregistrat după opt ore, la raportul 1/4, iar în cazul rapoartelor 1/5 – 1/8 după 10 ore. Extractele cumulate au prezentat variații nesemnificative în ceea ce priveşte concentrația de polifenoli totali, valorile fiind cuprinse între 2,81 – 2,88 g GAE/100 g şrot delipidat.*
***Cuvinte cheie:*** *semințe, extracții, raport, timp*

## INTRODUCTION

Grape seeds represent 38-52% of the dry pomace and are rich in oil, phenolic compounds, organic phosphorus compounds and minerals. Proanthocyanidins from

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the grape seed grits, resulting from extraction of the oil, are not soluble in water and therefore can not be used practically as bioactive antifungal, antibacterial, cytostatic substances . Their hydrosolubility represent the main prerequisite for the practical use.

The extractive quantitative methods used are continuously optimized thanks to deepen, on the one hand of the knowledge concerning the chemical nature of the phenolic compounds and on the other hand, the experience achievedregarding the physical factors involved in these extractive processes, namely: the size of the particles plant materials, the solvents that were used, the temperature of the process, the extraction time with the solvent plant material contact. In the literature there can be find numerous studies on these aspects, the authors using a variety of solvents, different temperature ranges and different reports solid / liquid extraction time (Spigno *et al.,* 2007; Huh *et al.,* 2004; Bensaruk *et al.,* 2012; Chew *et al.,* 2011; Khanal *et al.,* 2009; Amendola *et al., 2010*; Ross *et al.,* 2011; Lapornik *et al.,* 2005).

The study presents partial results obtained in the laboratory concerning the establishment of the optimal plant material or grape seed meal delipidated / solvent volume and the optimum extraction of phenolic compounds.

By applying a discontinuous process in the stationary and in the mining parameters ttaht t were seted, it was intended to achieve the best extraction yields of polyphenols from the seed oilcake delipidated with hexane, in a context of low

## MATERIAL AND METHOD

The determination of the optimal raport solid / solvent (S / L), meal grape seed / alcohol 96 was conducted in 500 ml Erlenmeyer flasks closed with a glass stopper and wrapped in aluminum foil to avoid distortion of the phenolic compounds from the action of the light .

Five reports have been tested: 1/4, 1/5, 1/6, 1/7, and 1/8, while maintaining a constant amount of 25 g of delipidated seed meal to which was added 100 mL, 125 mL, 150 mL, 175 mL and 200 mL of ethyl alcohol 96°C. The bottles that were prepared were placed on the mechanical stirrer programmed to 200 rpm, under a temperature of 25ºC. For each report two extractions were performed.

The extraction process monitoring was performed by determining the optical density (OD) at a 280 nm wavelength every two hours. At the timeframes referred it was also determined the concentration of the polyphenols, the samples being diluted 1/10. The amount of polyphenols was determined with Folin Cicâlteu reactif according to the Singleton and Rossi method, 1965. The concentration of polyphenols was determined on the basis of gallic acid standard curve, the amounts being expressed in g / l eq. gallic acid (EAG).

## RESULTS AND DISCUSSIONS

Monitoring optical density shows a progressive increase during the extractive processes of total polyphenols compounds (Figures 1 and 2). The maximum optical density was recorded after eight hours in the two extractions at the ratio S / L 1/4.

For the reports 1/5 - 1/8 the maximum optical density was recorded after 10

hours but this is much more evident in the second extraction step.

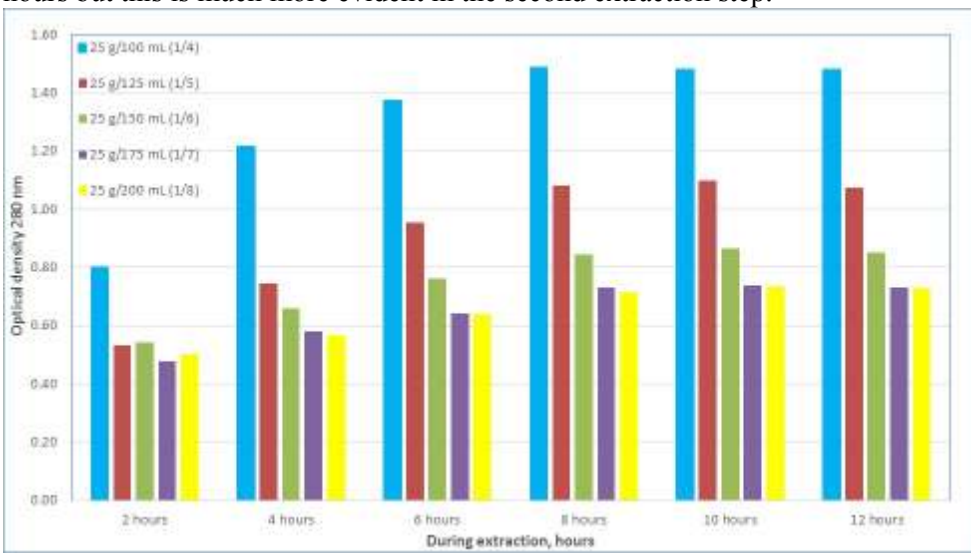


**Fig. 1 –** The dynamics of extraction processes phenolic compounds, during stage I

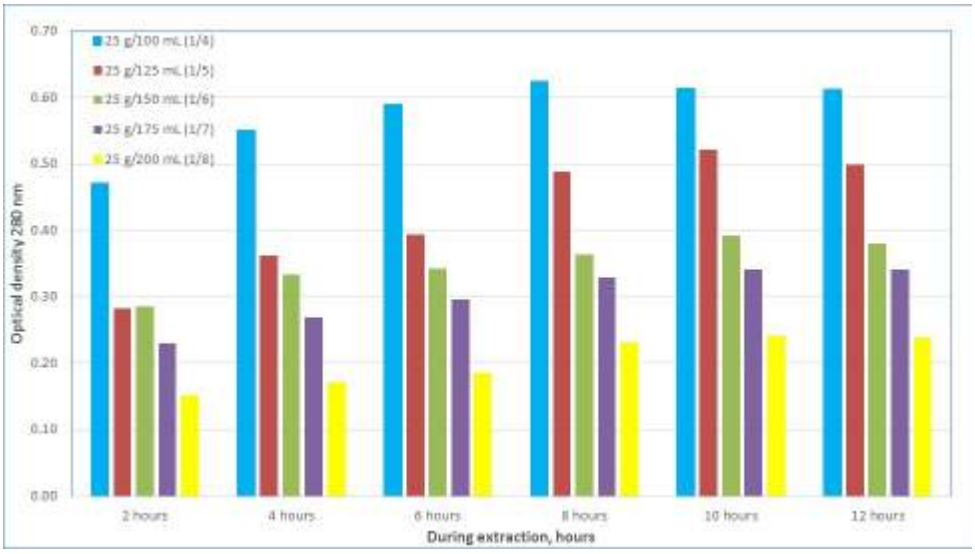


**Fig. 2** – The dynamics of extraction processes phenolic compounds, during stage II

After 8-10 hours of extraction, the values of the optimum density slightly increase, having decreasing trends after 12 hours, so the prolongation of extraction is no longer justified (Figure 3).

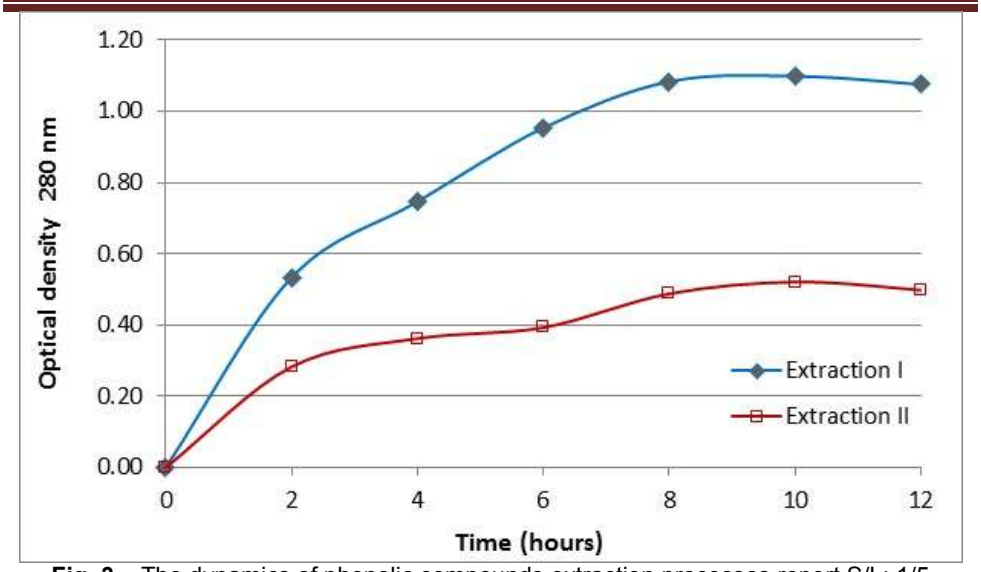


**Fig. 3** – The dynamics of phenolic compounds extraction processes report S/L: 1/5

The results obtained by determining the concentration of total polyphenols compounds in the crude extracts obtained in stages I and II for each report solid / liquid are presented in Tables 1 and 2.

*Table 1*

**The concentration of polyphenol compounds, g GAE/100 g delipidated grist - stage I extraction**

| The extraction time, hours | report solid/liquid, g/mL | | | | |
|---|---|---|---|---|---|
| | 1/4 | 1/5 | 1/6 | 1/7 | 1/8 |
| 2 hours | 1,80 | 1,89 | 1,78 | 2,06 | 1,71 |
| 4 hours | 2,09 | 2,15 | 1,94 | 2,25 | 2,23 |
| 6 hours | 2,19 | 2,18 | 2,04 | 2,30 | 2,29 |
| 8 hours | 2,26 | 2,22 | 2,15 | 2,31 | 2,35 |
| 10 hours | 2,20 | 2,26 | 2,18 | 2,34 | 2,40 |
| 12 hours | 2,19 | 2,26 | 2,17 | 2,33 | 2,34 |

*Table 2*

**The concentration of polyphenol compounds, g GAE/100 g delipidated grist - stage II extraction**

| The extraction time, hours | report solid/liquid, g/mL | | | | |
|---|---|---|---|---|---|
| | 1/4 | 1/5 | 1/6 | 1/7 | 1/8 |
| 2 hours | 0,40 | 0,49 | 0,53 | 0,39 | 0,34 |
| 4 hours | 0,47 | 0,52 | 0,60 | 0,47 | 0,40 |
| 6 hours | 0,54 | 0,53 | 0,61 | 0,47 | 0,42 |
| 8 hours | 0,58 | 0,56 | 0,62 | 0,48 | 0,45 |
| 10 hours | 0,59 | 0,57 | 0,63 | 0,49 | 0,48 |
| 12 hours | 0,56 | 0,56 | 0,62 | 0,48 | 0,47 |

In the first extraction step, it is noted that the higher value for the total

polyphenols compounds was extracted after 10 hours at the report solid / liquid 1/8, namely 2.40 g / 100 g delipidated meal. In the case of the reports extracts 1/4, 1/5, and 1/7 16 similar values of total polyphenols concentrations were obtained, but lower than the extract obtained in 1/8 ratio (Table 1).

In the second stage of extraction close values of total polyphenols concentration were obtained after 10 hours of extraction area for the reports 1/5, 1/8, this value being reached with the report 1/4 after eight hours of extraction (Table 2).

In the two extraction steps five crude polyphenol extracts resulted with insignificant variations in the concentration of total polyphenols, the values measured being situated between 2,05- 2,88 g GAE / 100 g of delipidated meal (Table 3).

*Table 3*

**The total polyphenol compounds (I + II extraction), g GAE/100 g delipidated grist**

| The extraction time, hours | report solid/liquid, g/mL | | | | |
|---|---|---|---|---|---|
| | 1/4 | 1/5 | 1/6 | 1/7 | 1/8 |
| **2 hours** | 2,20 | 2,38 | 2,31 | 2,45 | 2,05 |
| **4 hours** | 2,56 | 2,67 | 2,54 | 2,72 | 2,63 |
| **6 hours** | 2,72 | 2,71 | 2,65 | 2,77 | 2,71 |
| **8 hours** | 2,84 | 2,78 | 2,77 | 2,79 | 2,80 |
| **10 hours** | 2,79 | 2,83 | 2,81 | 2,83 | 2,88 |
| **12 hours** | 2,75 | 2,82 | 2,79 | 2,81 | 2,81 |

In the case of the report 1/4 the highest quantity of phenolic compounds was extracted after eight hours, namely 2.84 g GAE / 100 g, and in the case of other reports (1/5, 1/6, 1/7 and 1/8) after 10 hours of extraction, 2.81 - 2.88 g / GAE / 100 g.

It can be also noted that regardless the solid / liquid and the time of extraction, the highest amount of phenolic compounds, over 80% was extracted in the first stage (Figure 3).

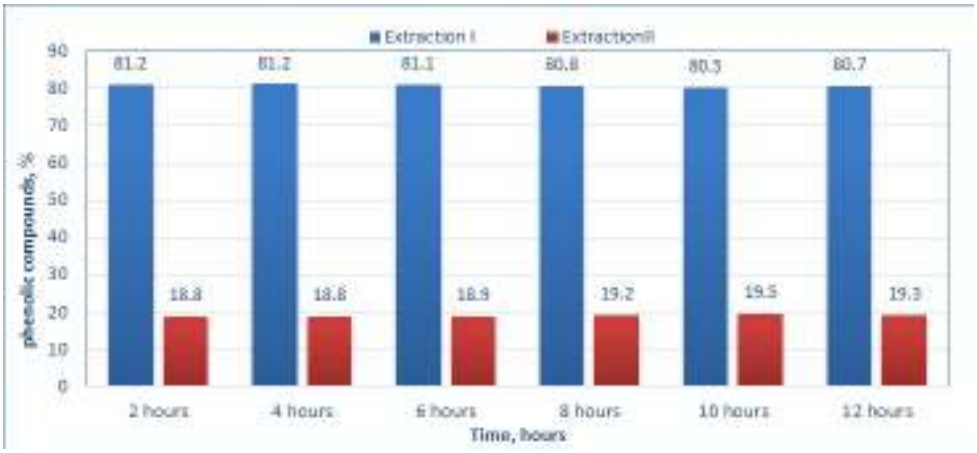


**Fig. 3 -** The percentage concentration of total phenolic compounds, during the two-stage extraction

The results obtained confirm the data reported in the literature and justifies further research or the study of the influence of the type of solvent and the source (variety) of plant material.

## CONCLUSIONS

1. In the two-stage extraction, the maximum value of the optical density was recorded after eight hours at the report solid / liquid 1/4 and in the case of the reports 1/5 - 1/8 after 10 hours. After 10 hours of extraction, the values of the optical density increased insignificantly having decreasing trends after 12 hours.

2. In the first extraction stage, the highest amount of total polyphenolic compounds was extracted after 10 hours at the S / L 1/8, and 2.4 g / 100 g delipidated meal. In the second extraction stage, the data shows close values of total polyphenols concentration after 10 hours of extraction area for the reports 1/5, 1/8, this value being reached also in the case of 1/4 ratio after eight hours of extraction.

3. The crude polyphenol extracts obtained in the two phases presented insignificant variations in terms of the concentration of total polyphenols (2.81 - 2.88 g GAE / 100 g of delipidated meal).

***Acknowledgments**: This work was carried out by the Partnership in priority areas - PN II, developed with the support of MEN -UEFISCDI, project no. 183/2014 (PN-II-PT-PCCA-2013-4-0333) "Technology of capitalization of the bioactive elements from the grape seed waste with usefulness in the food and pharmaceutical industry, plant and environmental protection (Acronym: PROVITIS)"*

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# COMPARATIVE STUDY OF ANTIRADICAL PROPERTIES OF SOME ROMANIAN WINES FROM THE COMMERCIAL PRODUCTS

## STUDIU COMPARATIV AL PROPRIETĂȚILOR ANTIRADICALICE LA UNELE VINURI ROMÂNEȘTI DIN OFERTA COMERCIALĂ

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***Abstract.*** *Assessment of the bioactive properties of productsis important as well as assessing the amount of alcohol from wines. This property aroused the interest of researchers in the last 3 decades because the literature shows awide range of bioactive phenolic compounds and there positive properties. The study presents two methods for evaluating the antiradical potential of wineas a complex product to consumers. By contrasta suite of phenolic substances with beneficial effects of the product are presented. We analyzed four white and one red wine commercially available. Results varywidely but are higherthan some observations from laborator ymicro-samples.*
**Key words:** *commercialwine, DPPH, chemiluminescence, phenolic compounds*

***Rezumat.****Evaluarea proprietăților bioactive ale unor produse este la fel de importantă precum și evaluarea cantității de alcool a vinurilor. Această proprietate a stârnit în ultimele 3 decenii interesul cercetătorilor deoarece datele din literatură prezintă o serie largă de compuși fenolici cu proprietăți bioactive. Studiul de față prezintă două metode de evaluare a efectului antiradicalic pe care vinul ca un complex îl prezintă pentru consumatori. Prin contrast este prezentată suita de substanțe fenolice are formează efectele benefice ale produsului. Sunt analizate 4 vinuri albe și unul roșu din oferta comercială. Rezultatele variază în limite largi dar sunt mai mari decât unele observații de la microproblele de laborator.*
**Cuvinte cheie:** *vin comercial, DPPH, chemiluminiscență, compuși fenolici*

## INTRODUCTION

Radicals are defined as atoms, compounds, or ions that contain an unpaired electron in their atomic or molecular orbital. Free radicals or free species are well recognized for having both negative and beneficial effects on living systems. From those oxygen free radicals, commonly referred to as reactive oxygen species (ROS), but in addition to these are also reactive nitrogen species (RNS) generated at low/moderate concentrations. They are important in numerous physiological functions including: regulation of vascular tone, sensing of oxygen tension, enhancement of

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signal transduction from various membranes, and oxidative stress responses that ensure the maintenance of redox homeostasis (Halliwell and Gutteridge, 1989).

The evolution of normal metabolic processes such as respiration and photosynthesis unavoidably leads to the production of free radicals. In contrast, an overproduction of ROS can cause damage to cellular lipids, proteins, or DNA, inhibiting their normal function. However, organisms have evolved a complex array of defences comprising of both endogenous (enzymatic and non-enzymatic) and exogenous antioxidants to control cellular levels of free radicals (Jaeschke, 2010). These species have been recognised to protect cells by scavenging free radicals from the body (causing a breakdown of radical chain reactions), suppressing free radical formation or by reducing oxidised cellular components (Valko *et al.,* 2007).

The methods develop are to evaluate the antioxidant capacity of whole plants other foods. Traditionally these are often referred to as off-line or batch style approaches as they are generally performed in spectroscopic cuvettes. Two of the more commonly used off-line techniques are based on measuring the ability of exogenous antioxidants to reduce a stable radical (representative of in vivo free radicals). The first approach involves the purple chromophore radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH°) which is reduced by an antioxidant(s) to its corresponding pale yellow hydrazine. This can be spectrophotometrically monitored as a decrease in absorbance at 515-517 nm. After a period of time, the degree of decolourisation and decrease in absorbance is proportional to the number of electrons captured, and therefore to the concentration and antioxidant activity of the species itself. The results of this technique are generally reported as the ED50, defined as the amount of antioxidant necessary to decrease the initial DPPH° concentration by 50% (Brand-Williams *et al.,* 1995).

## MATERIAL AND METHOD

In this study we are examining the possibly to improve radical scavenging determination so the number of sample is reduced at four white wine (Huși Averești Zghihară 2013; Cotnari: Fetească albă 2011, Fetească albă 2013 and Tămâioasă românească 2011) and one red wine, Fetească neagră Dealu Mare year of production 2013, all commerciale available on romanian market. For avoiding any kind of legal problem we are just mention the grape sort, region and year of production.

The platform for the antiradical depletion DPPH is made with a Visible or UV-Vis spectrophotometer,in our case multi-plate reader Tecan M200 Pro (fig. 1) withpolymethyl methacrylate well plates. A series of dilution is needed so it can calculate the final result. This dilution are: Reference solution $D_0$: 9 mL of the DPPH° methanolic solution + 100 µL of methanolMeOH; dilution $D_1$: 1/40 dilution of wine (4 mL of the DPPH + 100 µL wine); dilution $D_2$: 1/80 dilution of wine (4 mL DPPH° + 50 µL wine); $D_3$: 1/160 dilution of wine (4 mL DPPH° + 25 µL wine); $D_4$: 1/320 dilution of wine (4 mL DPPH° + 12.5 µL wine); $D_5$:1/640 dilution of wine (4 mL DPPH° + 6.25 µL wine).

The antioxidant activities is evaluated based on free DPPH° radicals remaining in the medium after the reaction between the methanolic DPPH° solution and the samples to be tested.For each dilution from $D_0$to $D_5$, the reduction in the absorbance is determined at 515 nm at 0 min and every 1 min. for 14 min., and every 10 min. until the reaction reaches

a plateau in about 1 hour. In some cases the percentage of DPPH at the plateau level can be calculated: DPPH° remaining in a stable state (%) = [(Ci - Cf) / Ci] × 100. The antioxidant activity of the wine is thus defined by the dilution of wine required to decrease the initial concentration of DPPH° by 50%: Effective dilution = ED50.

**Fig. 1** - Multi-plate reader used for the determination of the DPPH scavenging activity

Determination of water-soluble and lipid-soluble antioxidative capacity (ACW and ACL) in wine is realised using the methodology put in place by the Analytik Jena company (***, internet). The reaction kits are conserve to freezer until needed. The reaction uses luminoland the activation is done inside the Photochem device by a high intensity UV lamp. The results are in nmol/L of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid for the ACL determination) and nmol/L ascorbic acid for ACW.

We have used as a platform we developed a method presented in Journal of Chromatography A. (Castellari M. et al., 2008). For the analysis of phenolic compounds, samples were processed on a Shimadzu HPLC consisting of: quaternary pump Shimadzu Prominence series LC-20AD with five-channel degasser DGU-20A5 Shimadzu Prominence series, autoinjector SIL-20AC HT Shimadzu Prominence series (injection volume: 10 µL, sample temperature 20 °C), column oven CTO-20AC Shimadzu Prominence series, diode array detector SPD-M30A Shimadzu Prominence series (190-600 nm), chromatographic system controller CBM-20A Shimadzu Prominence series PC connectivity via LAN. We optimized the gradient using trifluoroacetic acid (TFA) as an eluent acidification of 1% MeOH (A channel) and 50% MeOH (B channel) acidified to 2.15 pH with TFA. The column system is composed of a pre-column SecurityGuard ULTRA Cartridges UHPLC C18 for 4.6 mm ID coupletto Kinetex 2.6 µm C18 100Å 150×4.6 mm columns manufactured by Phenomenex. The elution flow is 0.85 mL/min and the column compartment is set at 50 °C so we can make a total run in 60 min.

## RESULTS AND DISCUSSIONS

In the past we implemented the classical methodology in a Uv-Vis spectrometer. The problem with this kind of determination is related to the dilution of the sample. If the radical scavenging activity is greater than the concentration on DPPH is methanolic solution, the reaction is fast under 2-3 minutes and you can miss the initial concentration or the start of the reaction. So from our experience the manipulation and shaking for mixing the reactance is more or less taking 1 maybe 2 minute. In order to avoid this kind of problems we

began to experiment, with multi-plate readers. In this study the adding and mixing is done automatically, but still another problem appeared at low volumes the dominant substance MeOH begins to evaporate during the reaction, so the number of repetition are at more than 9 in order to get reproducible results.

In figure 2 it's presented the effect of concentration of red wine upon different dilution in 5 min from start of the reaction. The other figure presents the instability of this solution in time as result of wine matrix (the lowest dilution).

**Fig. 2 -** Consumption of methanolic solution of DPPH radical by colour change (from purple to yellow) in the five dilution ($D_1$, $D_2$, $D_3$, $D_4$ and $D_5$) compared to the control $D_0$


**Fig. 3 -** Consumption of methanolic solution of DPPH radical, by colour changing of the diluted sample (from purple to yellow) in a single dilution (D5) for 60 minutes in air

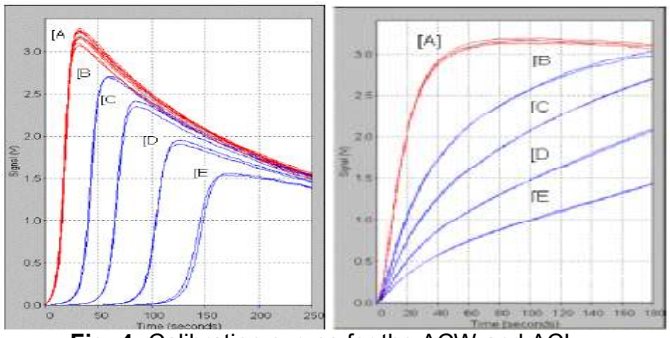


**Fig. 4 -**Calibration curves for the ACW and ACL

The analysis wine with the PhotoChem device is more robust and reproducible (n=3). In the figure 4 are presented the different types of kinetics involved in the foto-induced radical formation.

*Table 1*

**Wine anti-scavenging activity**

| WINES Grape sort Region/Producer Year of production | DPPH ED50 | Ascorbic acid equivalents [mmol/l] | Trolox equivalents [mmol/l] |
|---|---|---|---|
| ZghihărăAverești 2013 | 1/35 | 1.84 | 0.28 |
| FeteascăalbăCotnari 2011 | 1/18 | 1.37 | 0.19 |
| FeteascăalbăCotnari 2013 | 1/23 | 1.38 | 0.22 |
| TămâioasăromâneascăCotnari 2011 | 1/58 | 1.41 | 0.27 |
| FeteascăneagrăDealu Mare 2013 | 1/324 | 3.01 | 0.52 |

*Table 2*

**Phenolic compound quantified in wines**

| mg/L | Zghihară Averești 2013 | Fetească albă Cotnari 2011 | Fetească albă Cotnari 2013 | Tămâioasă românească Cotnari 2011 | Fetească neagră Dealu Mare 2013 |
|---|---|---|---|---|---|
| gallic acid | 272.63 | 439.74 | 173.38 | 675.59 | 6315.54 |
| protocatechuic acid | 33.28 | 28.38 | 22.62 | 59.13 | 79.41 |
| p-hydroxybenzoic acid | 946.15 | 293.65 | 329.32 | 676.87 | 816.66 |
| gentisic acid | 16346.53 | 5070.44 | 5740.75 | 11506.64 | 13686.39 |
| B1 | 11.31 | 42.34 | 64.36 | 49.19 | 123.11 |
| catechin | 25.29 | 28.20 | 32.39 | 69.03 | 75.63 |
| m-hidroxybenzoic acid | 29.27 | 182 | 95.53 | 66.43 | 2307.29 |
| vanillic acid | 36.65 | 48.21 | 59.12 | 40.86 | 594.48 |
| caffeic acid | 115.87 | 491.33 | 66.36 | 264.33 | 562.87 |
| chlorogenic acid | 1.5 | 4.83 | 2.88 | 5.03 | 28.73 |
| B2 | 1.85 | 1.61 | 1.39 | 3.45 | 57.75 |
| syringic acid | 1.63 | 6.24 | 3.93 | 5.32 | 211.1 |
| epicathechin | 0.46 | 36.6 | 1.23 | 33.98 | 132.85 |
| p-coumaric acid | 21.71 | 100.98 | 24.91 | 68.04 | 201.71 |
| ferulic acid | 46.03 | 248.94 | 249.85 | 129.03 | 45.68 |
| salicylic acid | 9.13 | 18.63 | 108.05 | 4.16 | 60.53 |
| polydatine | 0.12 | 0.04 | 0.03 | 0.31 | 7.45 |
| sinapinic acid | 1.54 | 0.45 | 0.62 | 0.29 | 0.71 |
| hyperoside | 0.59 | 0.04 | 0.04 | 0.07 | 0.34 |
| ellagic acid | 0.05 | - | - | 0.06 | 2.89 |
| trans-resveratrol | 12.45 | 10.28 | 7.6 | 11.73 | 471.71 |
| rutin | 11.55 | 7.13 | 13.4 | 9.39 | 32.22 |
| cis-resveratrol | 0.31 | 2.89 | 0.64 | 0.74 | 0.96 |
| morin | 0.12 | 0.49 | 0.35 | 0.45 | 0.08 |
| quercitine | 1.18 | 7.33 | 1.62 | 9.57 | 3.54 |
| ∑ eq./L | 116.45 | 44.6 | 44.76 | 87.81 | 161.97 |

In table 1 the antiradical activity of red wine is overwhelming up to 9 time greater than white wine and also the maceration of Tămâioasa is not a factor of improvement. The age of the wine are diminishing the activity. The acidity of the Zghihara wine could have some effect on the ACW determination. In case of wine the ACL values are not to be correlated with the lipophilic compound's, but mainly with the complexity of water non soluble phenolic substances present in wine.

The phenolic composition in table 2 show haw complex is the composition of different wine. Each winehas a different phenolic composition and this reflects the maceration or the technology specific for different region or people, but the total results concurs with bioactive proprieties determined.

## CONCLUSIONS

1. The improved method for evaluating antiradical capacity enables more stable analysis with DPPH stable radical.

2. The antiradical ability of white wines commercially available is proportional to the amount of phenolic compounds detected whit LC method.

***Acknowledgments**: This research was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

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# ANALYSIS OF ORGANIC ACIDS IN GRAPE AND WINE BY PRIOR FRACTIONATION ON SPE

## EVALUAREA FRACȚIILOR DE SEPARARE PENTRU ANALIZA ACIZILOR ORGANICI DIN STRUGURI ȘI VINURI

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***Abstract.*** *Analysis of organic acids from wines haze some restriction related to the phenolic compound present in the matrix who can be retain very strongly to the stationary phases. The purpose of the study is the development of efficient separation methods that can be used both for the analysis of organic acids in the wine and grapes. The grapes and wines varieties analysed are Zghihară, Fetească regală, Fetească albă, Busuioacă de Bohotin, Fetească neagră, Merlot and Cabernet Sauvignon. The extraction of acids from grapes was made on activated charcoal, C18 and SDVB materials. Acid separation is done in two ways: one is using two columns with C18 stationary phase and the second one is with an ion exchange stationary phase as a pre separation column. In the case of grapes analysis, the methods are limited by the level of solid material used in extraction that can ranged results from 80 to 105% recovery. These methods can be useful for analysing 10 organic acids with little to no sample preparation.*
***Key words:*** *grape, liquid chromatography, organic acids, wine*

***Rezumat.*** *Analiza acizilor organici din vinuri prezintă câteva vicisitudini, în special, din punctul de vedere al compușilor fenolici prezenți în probe, care pot se pot reține puternic pe faza staționară. Scopul acestui studiu/articol este să dezvolte metod eeficiente de separare, care să poată fi utilizate la analiza acizilor organici atât la vinuri cât și la struguri. Soiurile de struguri și vinurile analizate sunt: Zghihară, Fetească regală, Fetească albă, Busuioacă de Bohotin, Fetească neagră, Merlot și Cabernet Sauvignon. Extracția acizilor organici din struguri a realizată cu cărbune activ, C18 și pe SDVB. Strategia de separare aplicată pentru acizi a fost dual: prima cu utilizarea a două coloane cu fază staționară C18 și a doua cu ajutorul unei coloane de schimb ionic pentru pre-separarea amestecului de substanțe. Experimental s-a observat că în cazul strugurilor metoda este limitată de cantitatea de material adsorbant folosită la extracție, astfel nivelul de recuperare a variat între 80 și 105%. Aceste metode pot fi utilizate pentru analiza a 10 acizi organici, fără eliminarea altor interferenți din probele analizate sau clean-up.*
***Cuvintec heie:*** *struguri, cromatografie de lichide, aciziorganici, vin*

## INTRODUCTION

Acid determination has a great importance for the characterisation of wine composition evolution with implication in chemical and biochemical processes.

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The grape contains most of the acids involved in the glycolytic and shikimic acid pathways as well as in the Krebs and glyoxylic acid cycles, the rest remaining unmodified, being transmitted in wine (Ribereau-Gayon *et al.,* 2006).

For the analysis of the acids the are different methods that are all using liquid chromatography, but basically is ion chromatography with litter ionic strength eluents and separation phases, as shown by the methodology of separation know-how at core level (*** OIV, 2015: OIV-MA-AS313-04:R2009; OIV-MA-AS313-17:R2004)

## MATERIAL AND METHOD

The grapes and wines varieties analyzed are noted as follows: 1. Zghihară, 2. Fetească regală, 3.Fetească albă, 4. Busuioacă de Bohotin, 5. Fetească neagră, 6. Merlot and 7. Cabernet Sauvignon from the Ampelographic Collection of U.S.A.M.V. Iaşi, Copou centre, Iaşi. The grapes were processed using the classical fermentation for white grape variety and maceration-fermentation technology for red grape variety, as follows: after crushing and destemming, the marc was homogenised, Saccharomyces cerevisiae selected yeasts (30 g/100 kg), and pectolytic and proteolitic enzymes (1.5 g/100 kg) were added in case of red varieties. The white marc was pressed with a hydraulic press and then the must is put in 25 L glass vessel for alcoholic fermentation. The maceration-fermentation was done in 35 L static plastic vessels, for 72 hours, with pumping over the must six times/ day, for 15 minutes. After the end of maceration-fermentation, the marc was pressed with a hydraulic press, the resulted wine ending in demijohns of 25 L for finishing its alcoholic fermentation. A week after the alcoholic fermentation ended, the wine was racked and fined. Bottling was done after filtering with SA-995 plaques.

The extraction experiments for acids content from grapes was made on following material: activated charcoal, C18 and SDVB materials. The cartridges were activated with specific methodology and the final loading is done with 12% ethanolic solution.

For separation of the acids a Shimadzu Prominence LC20 series was used with the following composition: 2 quaternary pumps LC-20AD with DGU-20A5 degassers; SIL-20AC autosampler (20 °C thermostatic controlled sample temperature); columns oven CTO-20AC (at 20 °C); diode array detector SPD-M20A, FCV 20AH valve system; system controller CBM-20A coupled via LAN to an external PC unit where LabSolution 5.3 is controlling, collecting and process the chromatographic information. Two column are used: Prevail Organic Acid 250-4.6-5 (column 1 – pre-separation column) and-or Prevail Organic Acid 150-4.6-3 (column 2 – separation column). The injecting volume is 2 µL. The flow rate is at 0.9 mL/min with some variation for the washing the column 1 (second pump) for economy of solvents. The program developed is isocratic with two pumps: first pump for eluting the compound of interest on a series the 2 columns (loading) and the second pump is for washing the first column. When the acid are eluted from the first column to the second column the valve is switch so the second pump is washing the first column of phenolic compounds and the first pomp is continuing to separate the acids in the second much faster column.At minute 8.8 the position of the changing valve is changed for washing the column 1 with water then acetonitrile (AcCN)and at minute 25 the changing valve is putting the 2 columns in series so the hall system is re-equilibration until 45 min.

## RESULTS AND DISCUSSIONS

During the first attempts we have used only one column as the OIV methodology sugest (OIV-MA-AS313-04:R 2009). A Supelco 610H S-DVB 300×7.8 mmcolomn, 7 µm, for an injection of 10 µL, in isocratic mode at 0.5 mL/min. with 10 mMphosphoric acid ($H_3PO_4$). The optimal temperature is around 30 °C for the common detection at 210 nm for the: 1 –tartaricacid; 2 – malicacid; 3 – lacticacid; 4 –shikimicacid; 5 –fumaricacid; 6 –succinicacid; 7 –citricacid and 8 – aceticacid. We spotted some problems so the method is good, but only at low temperatures around 15-20 °C, but with peak broadening, works at low concentration of acid so you need to guess the best dilution for musts. For wines this method is not good due to overlapping peaks, so we tried the other version with two columns: a Superspher RP-8 250-4 mm and an Prevail Organic Acid 250-4.6 mm both 5µm. The injection is 10 µL elution isocratic at 0.4 mL/min. (which has generatedat 20 °Ca maximum at ~190 bar backpressure) for the elution system $KH_2PO_4$ 70 g/L, $(NH_4)_2SO_4$ 14 g/L, pH 2,1 adjusted with $H_3PO_4$. The separation is good in this case but the backpressure and the salts are clogging the LC system.

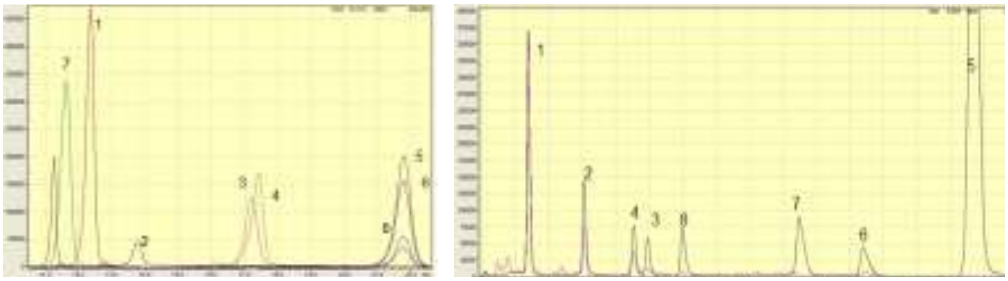

**Fig. 1 -** Separation of organic acids with well-known methods

This method is good but also remain the problem with SPE clean-up and the long time that is taking for the acid to elute.

Taking this in the consideration the experience (figure 2) we improve the method of separation by 2 columns, but in this case the backpressure is reduce and the elution time due to the shorter and more faster columns is greatly improved. Because the results are now better we introduced other 2 acids that can be separated as well. This method is well suited for grapes (must) or for wines (fig. 3) without the usage of the SPE cartridges (fig. 4). The presence of phenolic compound is showed in figure 4 so the washing of the firs column is a must. In the past methods a large peak is appearing in the chromatogram due to the tannins in red wine so the SPE clean-up in mainly important to red wines. But a problem raised from the utilisation of clean-up cartridges: what is the recovery rate and this can influence the natural distribution in must-grapes.

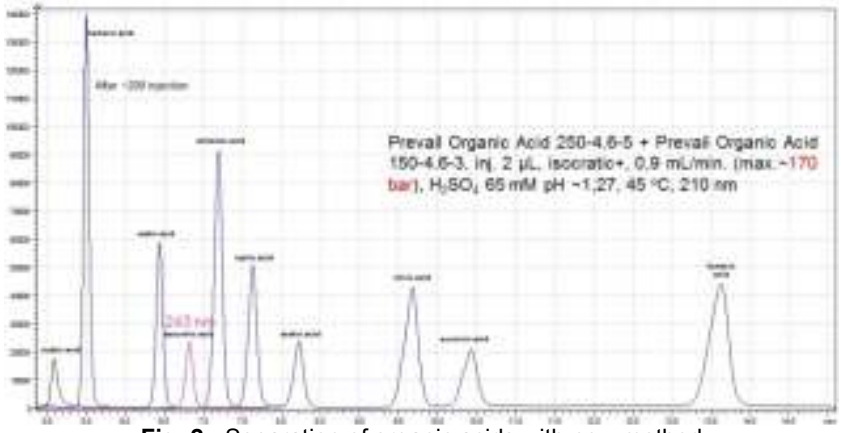


**Fig. 2 -** Separation of organic acids with new method

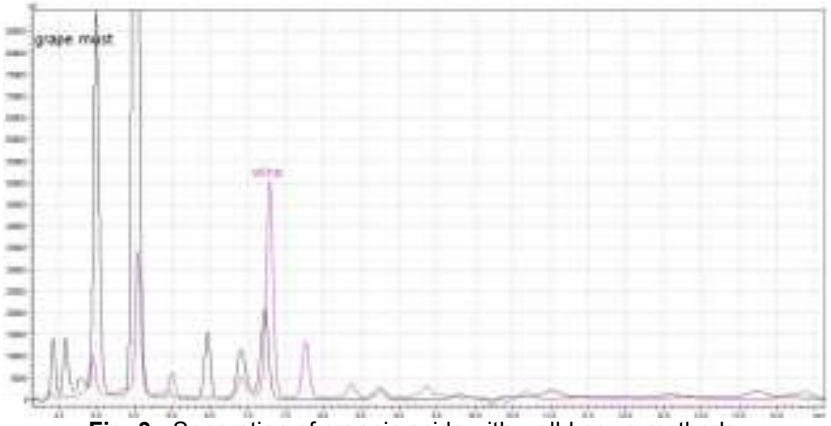


**Fig. 3 -** Separation of organic acids with well-known methods

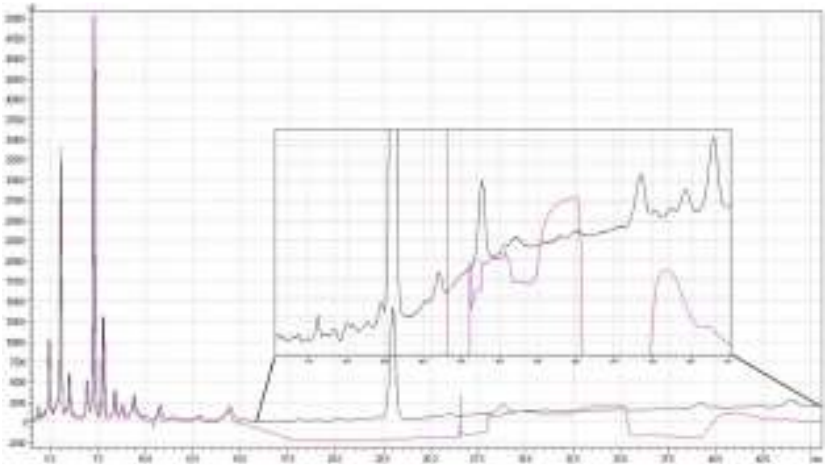

**Fig. 4 -** Effect of column washing and re-equilibration

In table 1 are the results for the recovery rates in the case of main organic acids. The percentage is the result of three repetition on the same cartridge and a three different cartridges, so we have sufficient information. This is the case of Cabernet Sauvignon grapes and the results confirm that the composition is influenced because the standard deviation varies more than 5% and in some cases of acids the recovery with standard deviation do not reach the initial concentration. Some other factor can be responsible for the results but for now this step can by eliminated if is possible.

*Table 1*

**Recovery of main organic acids2014 harvest**

| Extraction material for 10 g red grapes / Recovery (%) | tartaric acid | malic acid | lactic acid | acetic acid | citric acid | succinic acid | fumaric acid |
|---|---|---|---|---|---|---|---|
| activated charcoal 3 g | 85±15 | 87±13 | 88±9 | 90±13 | 95±4 | 96±9 | 94±10 |
| C18 3 g/600 mL | 88±11 | 91±11 | 94±10 | 85±22 | 92±10 | 97±2 | 92±7 |
| Lichrolut EN (40-120 µm) 3g/600 mL, SDVB | 90±17 | 82±16 | 89±15 | 90±15 | 99±11 | 91±4 | 99±8 |

With this method we analysed directly with-out clean-up the content of organic acids in grapes (table 2) and we saw large amount of malic acid greater then tartaric. Interesting is the presence of shikimic acid in the grapes as a stress related factor in rose and grape grapes.

*Table 2*

**Grape/must organic acids content in the 2014 harvest**

| value±SD (g/L) from direct must | tartaric acid | malic acid | shikimic acid | citric acid |
|---|---|---|---|---|
| Fetească regală | 7,40±0,20 | 6,73±0,57 | - | 0,29±0,05 |
| Fetească albă | 6,13±0,17 | 6,62±0,56 | - | 0,30±0,05 |
| Busuioacă de Bohotin | 5,35±0,14 | 6,65±0,57 | 0,026±0,004 | 0,74±0,13 |
| Fetească neagră | 7,31±0,20 | 8,26±0,70 | 0,093±0,013 | 0,31±0,05 |

After a period of 6 mouths the processed wine is opened and the same procedure without clean-up is used to evaluate the acid content of the samples. The Zghihara wine is the most acidic product because the content of tartaric, malic and also succinic acid is high. The succinic acid is the result of fermentation process and in some cases has the influence of the wine taste attribute. Beside Zghihara this compound is in high concentration at red wines. Another factor that is present an these results is evidence that the bacterial fermentation at red wine from Merlot and Cabernet Sauvignon, kwon as malo-lactic fermentation, is finished do to the larger amount of lactic acid and the low amount for malic acid. The citric and acetic acid have high content at the Merlot based wine do to some secondary fermentation mainly acetic during malo-lactic fermentation. Fumaric

acid is in low concentration (mg/L) but he plays an important role in wine protection metallic hazes.

*Table 3*

**Wine organic acidscontent in the2014 harvest, value±SD (g/L)**

| tartaric acid | malic acid | lactic acid | shikimic acid | acetic acid | succinic acid | citric acid | fumaric acid |
|---|---|---|---|---|---|---|---|
| 4,50±0,07 | 3,24±0,28 | 0,14±0,01 | 0,071±0,010 | 1,19±0,13 | 2,29±0,21 | 0,18±0,03 | 0,0001±0,00007 |
| 2,30±0,06 | 0,93±0,08 | 0,12±0,01 | 0,011±0,002 | 1,49±0,16 | 0,79±0,07 | 0,15±0,03 | 0,0007±0,00016 |
| 1,93±0,05 | 0,82±0,07 | 0,71±0,07 | 0,008±0,001 | 2,07±0,23 | 0,80±0,08 | 0,15±0,03 | 0,0047±0,00014 |
| 1,55±0,04 | 1,85±0,16 | 0,23±0,02 | 0,007±0,001 | 1,33±0,15 | 0,62±0,06 | 0,37±0,06 | 0,0012±0,00028 |
| 1,71±0,05 | 1,46±0,12 | 0,17±0,02 | 0,022±0,003 | 1,32±0,15 | 1,26±0,12 | 0,15±0,03 | 0,0051±0,00122 |
| 2,67±0,11 | 0,05±0,01 | 1,80±0,17 | 0,042±0,006 | 3,37±0,31 | 1,37±0,18 | 0,47±0,08 | 0,0004±0,00010 |
| 2,78±0,08 | 0,07±0,02 | 2,40±0,23 | 0,028±0,004 | 2,40±0,27 | 1,12±0,10 | 0,19±0,07 | 0,0003±0,00006 |

1. Zghihară, 2. Fetească regală, 3. Fetească albă, 4. Busuioacă de Bohotin, 5. Fetească neagră, 6. Merlot and 7. Cabernet Sauvignon

## CONCLUSIONS

1. In the present research work, we demonstrated that this methods can be useful for analysing 10 organic acids, directly from wine with little to no sample preparation.

2. Different materials can influence the concentration of organic acids.

3. Characterization of the grapes, must and wine acid composition to make the necessary correction of composition, if they are necessary.

***Acknowledgments***: *This research was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

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# ASPECTS REGARDING THE EVOLUTION OF ACIDITY DURING ALCOHOLIC FERMENTATION OF CERTAIN MUSTS

## ASPECTE REFERITOARE LA EVOLUȚIA ACIDITĂȚII ÎN TIMPUL FERMENTAȚIEI ALCOOLICE A UNOR MUSTURI

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***Abstract:*** *These work presents data regarding the acidity variation during the alcoholic fermentation of the must in comparison with the evolution of the main compositional characteristics, respectively, reducing sugars, alcohol, potassium, calcium, sodium cations, and total polyphenols index (ITP). The experiments were conducted on two musts from white varieties (Muscat Ottonel, Fetească albă) and must obtained from a red variety (Cabernet Sauvignon) from Bujoru wine center. Depending on the main acids (tartaric, malic, lactic, succinic, citric and the volatile acids), in all grape musts, during the metabolism of sugars in the wort, there is, generally, an increase in total acidity (to around 7-8 % vol. alcohol), after which it slowly decreases until the end of alcoholic fermentation.*
***Key words:*** *must, acidity, alcoholic fermentation, acids, total polyphenols index.*

***Rezumat:*** *În lucrare se prezintă date referitoare la evoluția acidității în timpul fermentației alcoolice a unor musturi în comparație cu evoluția principalelor caracteristici de compoziție, respectiv zaharuri reducătoare, alcool, cationii de potasiu, calciu, sodiu şi indicile de polifenoli totali (IPT). Experimentările au fost efectuate pe două musturi provenite din soiuri albe (Muscat Ottonel, Fetească albă) şi pe mustuiala obținută dintr-un soi roşu (Cabernet Sauvignon), provenite din centrului viticol Bujoru. În funcție de principalii acizi (tartric, malic, lactic, succinic, citric şi de cei volatili), la toate soiurile, în timpul metabolizării zaharurilor din must, se observă, în general, o creştere a valorii acidității totale (până în jurul valorii de 7-8 % vol. alcool), după care aceasta scade lent până la sfârşitul fermentației alcoolice.*
***Cuvinte cheie:*** *must, aciditate, fermentație alcoolică, acizi, polifenoli totali.*

## INTRODUCTION

Knowing the wine acidity composition is very important for oenology, because it permits to correlate the real acidity of wine with the intensity of sower taste, sensed at the sensorial analyses. At gustatory appreciation of the wines acidity, it must take into consideration the fact that the sower taste is influenced also by other compounds as alcohol, glycerol, sugars and by the neutralizing

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effect of potassium and calcium ions (Cotea, 1985). Because of this reason it is necessary that during the alcoholic fermentation, in direct relation with the evolution of the compounds responsible for the acidity, to monitor the formation and modification of the aforesaid compounds. This work presents data regarding the acidity variation (volatile, total and real acidity) during the alcoholic fermentation of the grape must in comparison with the evolution of the principal compositional characteristics, as it is the reducing sugars, alcohol, tartaric, malic, lactic, succinic and citric acids, potassium, calcium and sodium cations and total polyphenols index (IPT).

## MATERIAL AND METHOD

The studies regarding the acidity variation during the alcoholic fermentation of grape must were done from September to November 2014, in the wine cellar of SC Eurofruct SRL. The experiments were performed on two grape musts from white varieties (Fetească albă, Muscat Ottonel) and on the marc of one red grape variety (Cabernet Sauvignon), from Bujoru wine center, Dealu Bujorului vineyard. The grapes from the studied varieties were harvested between September 29$^{th}$ and October 17$^{th}$ as follows: Feteasca albă on 29$^{th}$ of September, Muscat Ottonel on 4$^{th}$ of October and Cabernet Sauvignon on 17$^{th}$ of October. After crushing and destemming each white grape varieties, by treating it with pectolytic enzymes, macerating it for 24 hours and pressing the marc, were obtained 800 L of grape must from each variety. The grape must was transferred in 900 L stainless steel containers and inoculated with selected yeast strains. The alcoholic fermentation was conducted at control temperature (between 16 and 20 ºC) and ended after 216 hours in case of Muscat Ottonel grape must and 192 hours in case of Feteasca albă must. At the initial moment and at different time intervals, respectively at 0, 24, 48, 72, 120, 168 and 216 hours, for Muscat Ottonel variety were taken the samples $MO_0$, $MO_1$, $MO_2$, $MO_3$, $MO_4$, $MO_5$ and $MO_6$. For Feteasca albă at the initial moment and at different time intervals, respectively 0, 24, 48, 72, 96, 120, 168 and 216 hours, were taken the samples $FA_0$, $FA_1$, $FA_2$, $FA_3$, $FA_4$, $FA_5$ and $FA_6$. The marc obtained by crushing-destemming 1100 Kg red grapes, after treating it with pectolytic enzymes and selected yeast strains, was transferred to a 1300 L stainless steel tanks, with lid, in a room at 20 ± 3ºC. The maceration-fermentation of Cabernet Sauvignon marc took place for 144 hours, after which the must obtained without and with pressing was deposited into an 900 L stainless steel container with a hinged lid and pressure and temperature (16 – 20 ºC) system control. At the initial moment and at different time intervals, respectively at 0, 48, 96, 144, 192, 240 and 600, were taken the samples $CS_0$, $CS_1$, $CS_2$, $CS_3$, $CS_4$, $CS_5$ and $CS_6$.

Each taken sample, after filtration and decarbonization, was analysed in terms of principal physico chemical analyses. The analyses regarding the principal compositional characteristics (reducing sugars, alcohol, total acidity, volatile acidity, pH, tartric acid, malic acid, lactic acid, succinic acid, citric acid, potassium, calcium, sodium and total polyphenol index – IPT at 280 nm) were done using the methods indicated in national and international standards in force (***2012) or in the scientific literature (Țârdea, 2007; Cotea *et al*. 2009). Together with the absolute values obtained are presented the relative deviations ($\delta$r) in (%), which changed the main components of the sample of must analyzed.

The calculation of the concentration of each acid in the must analysed samples was done according to the scientific literature (Ribereau-Gayon *et. al.* 1972; Würdig and Woller, 1989; Odăgeriu *et. al.,* 2005) based on the calculation methodology presented by Odăgeriu, 2006.

## RESULTS AND DISCUSSIONS

Tables 1, 2 and 3 present data concerning the evolution of the main compositional characteristics of the must-wine mixture during the alcoholic fermentation at the grape varieties studied. They had, for the most part, similar evolution at the must studied samples.

Thus, the transformation of the initial musts ($FA_{0,}$ $MO_0$ $CS_0$) into final wines ($FA_{6,}$ $MO_6$ $CS_6$) is shown by the variation of the flowing parameters: 198÷2 g/L reducing sugars, 0.00÷11.55 % vol. alcohol; 4.77÷5.58 g/L $C_4H_6O_6$ total acidity, 0.00÷0.34 g/L $C_2H_4O_2$ volatile acidity; 3.884÷3.874 pH; 3.80÷1.62 g/L total tartaric acid, 0.00÷0.54 g/L succinic acid; 0.00÷0.38 g/L lactic acid; 0.00÷0.18 g/L citric acid; 0.00÷0.32 g/L other acids; 1160÷640 mg/L potassium; 98÷70 mg/L calcium, 19 mg/L sodium, 6.8÷6.0 IPT for Fetească albă; 210÷2 g/L reducing sugars, 0.00÷12.24 % vol. alcohol; 3.48÷5.02 g/L $C_4H_6O_6$ total acidity; 0.00÷0.40 g/L $C_2H_4O_2$ volatile acidity; 4.005÷3.923 pH; 3.33÷1.52 g/L total tartaric acid; 0.00÷0.57 g/L succinic acid; 0.00÷0.43 g/L lactic acid; 0.00÷0.20 g/L citric acid; 0.00÷0.41 g/L other acids; 1200÷790 mg/L potassium; 106÷77 mg/L calcium, 21 mg/L sodium, 8.9÷8.1 IPT for Muscat Ottonel; 227÷4 g/L reducing sugars, 0.00÷13.10 % vol. alcohol; 4.30÷6.35 g/L $C_4H_6O_6$ total acidity; 0.00÷0.75 g/L $C_2H_4O_2$ volatile acidity; 3.495÷3.725 pH; 3.65÷1.37 g/L total tartaric acid; 2.39÷1.02 g/L malic acid; 0.00÷0.72 g/L succinic acid; 0.00÷1.08 g/L lactic acid; 0.00÷0.29 g/L citric acid; 0.00÷0.51 g/L other acids; 1340÷800 mg/L potassium; 114÷88 mg/L calcium, 23 mg/L sodium, 14.6÷44.6 IPT for Cabernet Sauvignon.

Total acidity, expressed as g/L $C_4H_6O_6$, comparing with the initial values in grape musts, at all the studied varieties, raised in proportion of 17.0 % for Feteasca albă, 44.3 % for Muscat Ottonel and 47.7 for Cabernet Sauvignon.

The volatile acidity, expressed as g/L $C_2H_4O_2$, had un increasing trend at all the studied varieties, reaching at the end the value of 0.34 g/L at Fetească albă, 0,40 at Muscat Ottonel and 0.75 at Cabernet Sauvignon.

Total tartaric acid ($H_2T$) concentration linearly decreases at all the studied samples, respectively in percentages of 57,4 at Fetească albă, 54.4 at Muscat Ottonel and 62.5 at Cabernet Sauvignon. Malic acid ($H_2M$) registered constant values of 2.12 and 1.45 g/L at Fetească albă and Muscat Ottonel varieties and decreases in percentage of 43.9 at Cabernet Sauvignon variety, as a result of partial malolactic fermentation that took place simultaneous with the alcoholic fermentation.

*Table 1*

**Variation of main compositional characteristics of Fetească albă musts during alcohol fermentation**

| Sample | Time (hours) | Total sugars | | Alcohol | | Total acidity | | Volatile acidity | | Real acidity (pH) | | Total tartaric acid ($H_2T$) | | Malic acid ($H_2M$) | $H_2T$/ $H_2M$ ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | g/L | $o_r$ (%) | % vol. | $\delta_r$ (%) | g/L $C_4H_6O_6$ | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | |
| $FA_0$ | 0 | 198 | 0.0 | 0.00 | 0.0 | 4.77 | 0.0 | 0.00 | 0.0 | 3.884 | 0.0 | 3.80 | 0.0 | 2.12 | 1.79 |
| $FA_1$ | 24 | 158 | -20.2 | 2.34 | 20.3 | 5.34 | 11.9 | 0.02 | 5.9 | 3.770 | -2.9 | 3.52 | -7.4 | 2.12 | 1.66 |
| $FA_2$ | 48 | 99 | -50.0 | 5.85 | 50.6 | 6.04 | 26.6 | 0.10 | 29.4 | 3.642 | -6.2 | 3.35 | -1.,8 | 2.12 | 1.58 |
| $FA_3$ | 72 | 65 | -67.2 | 7.84 | 67.9 | 6.36 | 33.3 | 0.16 | 47.1 | 3.511 | -9.6 | 3.07 | -19.2 | 2.12 | 1.45 |
| $FA_4$ | 120 | 32 | -83.8 | 9.76 | 84.5 | 5.75 | 20.5 | 0.22 | 64.7 | 3.662 | -5.7 | 2.22 | -41.6 | 2.12 | 1.05 |
| $FA_5$ | 168 | 13 | -93.4 | 10.88 | 94.2 | 5.68 | 19.1 | 0.27 | 79.4 | 3.755 | -3.3 | 1.95 | -48.7 | 2.12 | 0.92 |
| $FA_6$ | 216 | 2 | -99.0 | 11.55 | 100.0 | 5.58 | 17.0 | 0.34 | 100.0 | 3.874 | -0.3 | 1.62 | -57.4 | 2.12 | 0.76 |

*Table 1 (continued)*

| Sample | Time (hours) | Succinic acid | | Lactic acid | | Citric acid | | Other acids | | Potassium ($K^+$) | | Calcium ($Ca^{2+}$) | | Sodium ($Na^+$) | IPT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | mg/L | $\delta_r$ (%) | mg/L | $\delta_r$ (%) | mg/L | $D_{280}$ |
| $FA_0$ | 0 | 0.00 | 0.0 | 0.00 | 0.0 | 0.00 | 0.0 | 0.00 | 0.00 | 1160 | 0.0 | 98 | 0.0 | 19 | 6.8 |
| $FA_1$ | 24 | 0.17 | 31.5 | 0.05 | 13.2 | 0.03 | 16.7 | 0.07 | 1.19 | 1090 | -6.0 | 97 | -1.0 | 19 | 6.7 |
| $FA_2$ | 48 | 0.31 | 57.4 | 0.13 | 34.2 | 0.06 | 33.3 | 0.19 | 3.22 | 1060 | -8.6 | 89 | -9.2 | 19 | 6.5 |
| $FA_3$ | 72 | 0.40 | 74.1 | 0.20 | 52.6 | 0.10 | 55.6 | 0.24 | 4.07 | 1005 | -13.4 | 82 | -16.3 | 19 | 6.4 |
| $FA_4$ | 120 | 0.46 | 85.2 | 0.27 | 71.1 | 0.12 | 66.7 | 0.26 | 4.40 | 800 | -31.0 | 73 | -25.5 | 19 | 6.3 |
| $FA_5$ | 168 | 0.49 | 90.7 | 0.33 | 86.8 | 0.14 | 77.8 | 0.29 | 4.91 | 730 | -37.1 | 71 | -27.6 | 19 | 6.2 |
| $FA_6$ | 216 | 0.54 | 100.0 | 0.38 | 100.0 | 0.18 | 100.0 | 0.32 | 5.42 | 640 | -44.8 | 70 | -28.6 | 19 | 6.0 |

*Table 2*

**Variation of main compositional characteristics of Muscat Ottonel musts during alcohol fermentation**

| Sample | Time (hours) | Total sugars | | Alcohol | | Total acidity | | Volatile acidity | | Real acidity (pH) | | Total tartaric acid ($H_2T$) | | Malic acid ($H_2M$) | $H_2T/H_2M$ ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | g/L | $\delta_r$ (%) | % vol. | $\delta_r$ (%) | g/L $C_4H_6O_6$ | $\delta_r$ (%) | | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | |
| $MO_0$ | 0 | 210 | 0.0 | 0.00 | 0.0 | 3.48 | 0.0 | 0.00 | 0.0 | 4.005 | 0.0 | 3.33 | 0.0 | 1.45 | 2.30 |
| $MO_1$ | 24 | 160 | -23.8 | 2.92 | 23.9 | 4.10 | 17.8 | 0.05 | 12.5 | 3.893 | -2.8 | 2.98 | -10.5 | 1.45 | 2.06 |
| $MO_2$ | 48 | 104 | -50.5 | 6.24 | 51.0 | 4.78 | 37.4 | 0.14 | 35.0 | 3.842 | -4.1 | 2.56 | -23.1 | 1.45 | 1.77 |
| $MO_3$ | 72 | 73 | -65.2 | 8.05 | 65.8 | 5.00 | 43.7 | 0.22 | 55.0 | 3.812 | -4.8 | 2.38 | -28.5 | 1.45 | 1.64 |
| $MO_4$ | 120 | 33 | -84.3 | 10.43 | 85.2 | 5.09 | 46.3 | 0.27 | 67.5 | 3.835 | -4.2 | 2.17 | -34.8 | 1.45 | 1.50 |
| $MO_5$ | 168 | 12 | -94.3 | 11.64 | 95.1 | 5.05 | 45.1 | 0.33 | 82.5 | 3.886 | -3.0 | 1.87 | -43.8 | 1.45 | 1.29 |
| $MO_6$ | 216 | 2 | -99.0 | 12.24 | 100.0 | 5.02 | 44.3 | 0.40 | 100.0 | 3.923 | -2.0 | 1.52 | -54.4 | 1.45 | 1.05 |

*Table 2 (continued)*

| Sample | Time (hours) | Succinic acid | | Lactic acid | | Citric acid | | Other acids | | Potassium ($K^+$) | | Calcium ($Ca^{2+}$) | | Sodium ($Na^+$) | IPT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | mg/L | $\delta_r$ (%) | mg/L | $\delta_r$ (%) | mg/L | $D_{280}$ |
| $MO_0$ | 0 | 0.00 | 0.0 | 0.00 | 0.0 | 0.00 | 0.0 | 0.00 | 0.00 | 1200 | 0.0 | 106 | 0.0 | 21 | 8.9 |
| $MO_1$ | 24 | 0.20 | 35.1 | 0.10 | 23.3 | 0.05 | 25.0 | 0.04 | 0.68 | 1120 | -6.7 | 102 | -3.8 | 21 | 8.8 |
| $MO_2$ | 48 | 0.34 | 59.6 | 0.18 | 41.9 | 0.08 | 40.0 | 0.28 | 4.74 | 1025 | -14.6 | 95 | -10.4 | 21 | 8.6 |
| $MO_3$ | 72 | 0.43 | 75.4 | 0.25 | 58.1 | 0.12 | 60.0 | 0.33 | 5.59 | 985 | -17.9 | 88 | -17.0 | 21 | 8.5 |
| $MO_4$ | 120 | 0.49 | 86.0 | 0.32 | 74,.4 | 0.14 | 70.0 | 0.35 | 5.93 | 950 | -20.8 | 81 | -23.6 | 21 | 8.4 |
| $MO_5$ | 168 | 0.52 | 91.2 | 0.38 | 88.4 | 0.16 | 80.0 | 0.38 | 6.44 | 870 | -27.5 | 79 | -25.5 | 21 | 8.3 |
| $MO_6$ | 216 | 0.57 | 100.0 | 0.43 | 100.0 | 0.20 | 100.0 | 0.41 | 6.94 | 790 | -34.2 | 77 | -27.4 | 21 | 8.1 |

*Table 3*

**Variation of main compositional characteristics of Cabernet Sauvignon musts during alcohol fermentation**

| Sample | Time (hours) | Total sugars | | Alcohol | | Total acidity | | Volatile acidity | | Real acidity (pH) | | Total tartaric acid ($H_2T$) | | Malic acid ($H_2M$) | | $H_2T/H_2M$ ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | g/L | $\delta_r$ (%) | % vol. | $\delta_r$ (%) | g/L $C_4H_6O_6$ | $\delta_r$ (%) | | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | |
| $CS_0$ | 0 | 227 | 0,0 | 0.00 | 0.0 | 4.30 | 0.0 | 0.00 | 0.0 | 3.495 | 0.0 | 3.65 | 0.0 | 2.39 | 0.0 | 1.53 |
| $CS_1$ | 48 | 167 | -26.4 | 3.52 | 26.9 | 4.86 | 13.0 | 0.33 | 44.0 | 3.428 | -1.9 | 2.67 | -26.8 | 2.32 | -2.9 | 1.15 |
| $CS_2$ | 96 | 129 | -43.2 | 5.76 | 44.0 | 5.45 | 26.7 | 0.41 | 54.7 | 3.487 | -0.2 | 2.25 | -38.4 | 2.24 | -6.3 | 1.00 |
| $CS_3$ | 144 | 97 | -57.3 | 7.65 | 58.4 | 6.68 | 55.3 | 0.57 | 76.0 | 3.524 | 0.8 | 2.05 | -43.8 | 1.78 | -25.5 | 1.15 |
| $CS_4$ | 192 | 61 | -73.1 | 9.78 | 74.7 | 6.64 | 54.4 | 0.63 | 84.0 | 3.602 | 3.1 | 1.90 | -47.9 | 1.56 | -34.7 | 1.22 |
| $CS_5$ | 240 | 28 | -87.7 | 11.72 | 89.5 | 6.51 | 51.4 | 0.68 | 90.7 | 3.668 | 4.9 | 1.75 | -52.1 | 1.45 | -39.3 | 1.21 |
| $CS_6$ | 600 | 4 | -98.2 | 13.10 | 100.0 | 6.35 | 47.7 | 0.75 | 100.0 | 3.725 | 6.6 | 1.37 | -62.5 | 1.34 | -43.9 | 1.02 |

*Table 3 (continued)*

| Sample | Time (hours) | Succinic acid | | Lactic acid | | Citric acid | | Other acids | | Potassium ($K^+$) | | Calcium ($Ca^{2+}$) | | Sodium ($Na^+$) | IPT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | g/L | $\delta_r$ (%) | mg/L | $\delta_r$ (%) | mg/L | $\delta_r$ (%) | mg/L | $D_{280}$ |
| $CS_0$ | 0 | 0.00 | 0.0 | 0.00 | 0,0 | 0.00 | 0.0 | 0.00 | 0.0 | 1340 | 0.0 | 114 | 0.0 | 23 | 14.6 |
| $CS_1$ | 48 | 0.24 | 33.3 | 0.29 | 26,9 | 0.13 | 44.8 | 0.16 | 31.4 | 1090 | -18.7 | 112 | -1.8 | 23 | 23.4 |
| $CS_2$ | 96 | 0.48 | 66.7 | 0.45 | 41,7 | 0.16 | 55.2 | 0.20 | 39.2 | 990 | -26.1 | 107 | -6.1 | 23 | 297 |
| $CS_3$ | 144 | 0.65 | 90.3 | 0.83 | 76,9 | 0.21 | 72.4 | 0.33 | 64.7 | 960 | -28.4 | 98 | -14.0 | 23 | 36.6 |
| $CS_4$ | 192 | 0.67 | 93.1 | 0.94 | 87,0 | 0.23 | 79,3 | 0.45 | 88.2 | 930 | -30.6 | 90 | -21.1 | 23 | 45.2 |
| $CS_5$ | 240 | 0.68 | 94.4 | 1.02 | 94,4 | 0.24 | 82.8 | 0.48 | 94.1 | 890 | -33.6 | 89 | -21.9 | 23 | 45.0 |
| $CS_6$ | 600 | 0.72 | 100.0 | 1.08 | 100,0 | 0.29 | 100.0 | 0.51 | 100.0 | 800 | -40.3 | 88 | -22.8 | 23 | 44.6 |

Real acidity or pH had an evolution as follows: at the Fetească albă and Muscat Ottonel samples decreases with 9.6 respectively 4.8% in the first 72 hours, followed by a slightly increasing by the end of fermentation; at the Cabernet Sauvignon samples, decreases with 1.9 % in the first 24 hours (samples $CS_0$ and $CS_1$) after which it raises with 6.6 % at the end (sample $CS_6$).

Data about the evolution of the acids originating from the initial grape musts and those formed during the alcoholic fermentation at the studied samples are presented in table 4.

*Table 4*

**Acids content variation (% meq/L) originating in must ant of those formed during alcoholic fermentation (A.F.) for studied varieties**

| No. | Sample | Time (hours) | Must acids | Acids from (A.F.) |
|---|---|---|---|---|
| **Fetească albă** | | | | |
| **1.** | **$FA_0$** | 0 | 100.00 | 0.00 |
| **2.** | **$FA_1$** | 24 | 93.53 | 6.47 |
| **3.** | **$FA_2$** | 48 | 85.90 | 14.10 |
| **4.** | **$FA_3$** | 72 | 80.75 | 19.25 |
| **5.** | **$FA_4$** | 120 | 74.70 | 25.30 |
| **6.** | **$FA_5$** | 168 | 70.97 | 29.03 |
| **7.** | **$FA_6$** | 216 | 66.12 | 33.88 |
| **Muscat Ottonel** | | | | |
| **1.** | **$MO_0$** | 0 | 100.00 | 0.00 |
| **2.** | **$MO_1$** | 24 | 90.03 | 9.97 |
| **3.** | **$MO_2$** | 48 | 77.61 | 22.39 |
| **4.** | **$MO_3$** | 72 | 71.73 | 28.27 |
| **5.** | **$MO_4$** | 120 | 67.38 | 32.62 |
| **6.** | **$MO_5$** | 168 | 62.89 | 37.11 |
| **7.** | **$MO_6$** | 216 | 57.35 | 42.65 |
| **Cabernet Sauvignon** | | | | |
| **1.** | **$CS_0$** | 0 | 100.00 | 0.00 |
| **2.** | **$CS_1$** | 48 | 80.02 | 19.98 |
| **3.** | **$CS_2$** | 96 | 71.04 | 28.96 |
| **4.** | **$CS_3$** | 144 | 58.26 | 41.74 |
| **5.** | **$CS_4$** | 192 | 52.77 | 47.23 |
| **6.** | **$CS_5$** | 240 | 49.40 | 50.60 |
| **7.** | **$CS_6$** | 600 | 43.41 | 56.59 |

As it can be seen in table 4, for the studied varieties (Fetească albă, Muscat Ottonel, Cabernet Sauvignon) the percentage value of the acids that originate from must (tartaric, malic) expressed as meq/L, decreases at 66.12, 57.35 and 43.41% and those formed during the alcoholic fermentation (succinic, lactic, citric, volatile, other acids) increases to 33.88, 42.65 respectively 56.59%. it can be seen that from one grape variety to another, directly proportional with the increase of sugar content registered at harvest, increases the content of the acids formed during the alcoholic fermentation.

The variation of the acids at the studied varieties, presented in table 4, suggests the identification of technological methods to preserve the acids at the grape level and especially during the alcoholic fermentation when biochemical imbalances occur.

## CONCLUSIONS

1. During the sugar metabolism in must, generally, it can be observed that to around 7-8% vol. alcohol there is an increase of total acidity, dependent of the evolution of the acids (tartaric, malic, succinic, citric, lactic and the volatile ones) and the cations (potassium, calcium), after which it slowly decreases until the end of alcoholic fermentation.

2. In direct correlation with the acids and cations above mentioned, the real acidity (pH), at all the fermented must, decreases in the first part of the fermentation, until the alcohol reaches the value of 5-7 % vol., after which it rises to the end of alcoholic fermentation.

3. Knowledge of mutual dependence between acids and cations of must and wine allows assessing the position of the equilibrium of many ionization reactions of acids present that facilitate theoretical understanding of phenomena, occurring during fermentation of musts, relating in particular to the tartaric compounds insolubilization.

4. Data regarding the variation of acids derived from both initial musts and those formed during alcoholic fermentation can be used to optimize the technologies for winemaking.

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# INFLUENCE OF GRAPE SEEDS ON THE CHEMICAL COMPOSITION OF RED WINES

## INFLUENȚA SEMINȚELOR DIN STRUGURI ASUPRA COMPOZIȚIEI CHIMICE A VINURILOR ROŞII

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***Abstract.*** *The fact that, to enriche whith phenolic compounds the red wine three main uvologic elements are participating, namely: grapes skins, grapes and seeds, it is known. At the moment, the enrichment process of wines with tannins extracted was studied, more detalied, from the peel and bunch and less from seeds. In this context samples of wines Merlot obtained from maceration-fermentation of must whith seeds extracted in an amount of 25, 75 and 100% and from must whith seeds added to the existing ones in an amount of 30, 60 and 100% were researched. The wine sample produced from the usual must served as a martor. The result of research showed that variants in which the seeds were found in small amounts or were absent are distinguished by their high content in phenolic compounds. Adding wet seed in different proportions reduces the content of phenolic substances and anthocyans.*
***Key words:*** *maceration–fermentation, seeds, phenolic substances, anthocyans.*

***Rezumat.*** *Este cunoscut faptul că la îmbogățirea vinurilor roșii cu compuși fenolici participă trei elemente uvologice principale și anume: pielița boabelor, ciorchinii și semințele. În lucrarea de față a fost studiat procesul de îmbogățire a vinurilor cu taninuri extrase din pieliță și ciorchini și mai puțin din semințe. În acest context au fost supuse cercetărilor mostrele de vin din soiul Merlot obținute prin macerarea–fermentarea mustuielii din care s-au extras semințele în proporții de 25, 75 și 100% și în care s-au adăugat semințe la cele existente în proporții de 30, 60 și 100%. În calitate de martor a servit mostra produsă din mustuiala obișnuită. În urma cercetărilor s-a constatat, că variantele în care semințele s-au aflat în cantități mici, sau au lipsit, se disting printr-un conținut mai mare în compuși fenolici. Adăugarea semințelor umede în diferite proporții diminuează conținutul în compuși fenolici și în substanțe colorate.*
***Cuvinte cheie:*** *macerare–fermentare, semințe, substanțe fenolice, substanțe colorante.*

## INTRODUCTION

Red wine, unlike the white ones are much richer in chemical composition due to the longer contact of the must with the solid parts of grapes.

Besides the characteristics of both types of wine compounds (alcohols, organic acids, nitrogenous substances, minerals, glycerol etc.) they have in large

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amounts phenolic and coloring substances.

It is known that red wine enrichment with coloring and phenolic substances (tannin) participates three elements of grapes constituent namely the skin, clusters of grapes and seeds (Rusu, 2006; Valuico, 1973). Coloring substances are located in the skin, while some varieties, very few in number, in pulp too. The tannins are concentrated in large quantities in the skin and clusters of grape. According Valuico G. (Valuico, 1976) - fermentation maceration in the presence of clusters of grape allows the extraction of phenolic substances in 84% of its potential in grape. In this case, red wines contain more phenolic substances with P-vitamin properties (tannins, leucoanthocyans, flavanols, catechins, phenolic acids, etc.) and vitamins of group B. Although fermentation with clusters of grape has a beneficial effect on the chemical composition of wine this process now does not have a wide application in the production of red wines. Note that until now has been studied in more detail the enrichment process of red wines with tannin extracted from skin and clusters of grape and less from seed. As mentioned Cotea, 1985, seed chemical composition differs from the other elements of grape. The water content is 28-40% by weight, cellulose - up to 28% nitrogenous substances varies between 0.8 and 1.2%, the tanning substances - 4-6%, oils - 10-25% and minerals - 2-4%. From the chemical composition of seed main technological importance for red wines have tanning substances.

However, many authors consider that seed from must influence bad the quality of red wine and recommend that they to be removed. Thus Prida I. et al. 2012 proposes that the first 3-5 days after the formation of the hat, the sedimented seeds to be eliminated. On the other hand, in the modern oenology it practice use of enotannin obtained from grape seeds in the production of various types of wines (Cozub and Rusu, 1996; Țârdea *et al.,* 2010).

From the above it can be concluded that, on the role of seed in the production of red wines, among specialists oenologists there is no single opinion. The purpose of the investigations is to determine the influence of the seed from must on enrich with phenolic compounds red wine in fermentation maceration process

## MATERIAL AND METHOD

The research was conducted in the laboratory of oenology and wines with denomination of origin of Practical Scientific Institute of Horticulture and Food Technology.

As experimental samples were served wines produced in the wine region of Centre Merlot grape variety, vintage year 2014.

Table 1 presents the basic indices of Merlot grape. Sugars content is 242 g / L, and titratable acids 7.4 g / L. These data show that the grapes used for the experiment were harvested at full maturity, in which the seeds are ripe. The potential total phenolic substances is 2448 mg / L and 872 mg coloring / L, higher values for Merlot.

*Table 1*

**Basic indices of grape used to obtain experimental samples**

| Variety name | Harvest Year | Physico-chemical | | | | |
|---|---|---|---|---|---|---|
| | | Sugars, g/L | Titratable acidity, g/L | pH | Phenolic substances, mg/L | Coloring matters, mg/L |
| Merlot | 2014 | 242 | 7,4 | 3,36 | 2448 | 872 |

To determine the effect of seeds on the extraction process during maceration-fermentation were fitted experiments with different amounts of seed.

The experiments scheme is the following variants: Variant 1 - adding wet seeds in the must in a proportion of 30% from the the quantity of seeds in must; Variant 2 - adding to the must 60% wet seeds; Variant 3 - adding to the must wet seeds at a rate of 100%; Variant 4 - the separation of seeds from the must 30%; variant 5 - separation from the must of seeds 60%; variant 6 - separation of the seeds from must at a rate of 100%. As a witness sample served wine made from the usual must.

Experimental samples were obtained under microvinification. For each sample were taken 3 kg of grapes that were manual crushed with removal of clusters of grape and the must obtained was sulfided with 75 mg / kg total $SO_2$. The removed seeds from must were released from pulp, which is then added to the respective variant must. In maceration-fermentation variants that provide seeds surplus were added seeds results from previous vinification. Must maceration-fermentation was carried for 8 days at 25-28oC, after the wine has been separated from solids in the laboratory press. The wine obtained was subjected post fermentation, clarification, and then peeled from the yeast sediment, and poured into bottles.

In the obtained wine samples were determined major physico-chemical indices being used standard methods; total phenolic substances with the Folin-Ciocalteu method reagent and coloring substances with OIV method. After 3 months of pulling off sediment samples were subjected tasting, using scores of appreciation from 7.80 till 8.00 points. Besides the physical and chemical clues wine samples were investigated in spectrophotometer in the range of 400-600 nm wavelength.

## RESULTS AND DISCUSSIONS

The results of physicochemical analysis of samples of wine obtained by maceration-fermentation of must with differing seeds content are shown in Table2.

Wine samples investigated are distinguished by a high alcohol content, ranging from 13.75 till 14.71% vol., A fact that confirms their use in the production grapes with a high degree of maturity. Residual sugar not exceed 1.55 g / L, indicating that falls within the limits set for dry red wines.

Wine samples investigated are distinguished by a high alcohol content, ranging from 13.75 till 14.71% vol.

*Table 2*

**Physicochemical indices of samples of wine obtained by maceration-fermentation of must with differing seeds content**

| Experimental variant | Alcohol, %vol. | Sugars, g/L | Titratable acidity, g/L | Volatile Acidity, g/L | pH | Phenolic substances, mg/L | Coloring substances, mg/L | Total soluble salts, mg/L | Conductivity, µS/cm | organoleptic Note,points |
|---|---|---|---|---|---|---|---|---|---|---|
| V-1 | 13,88 | 1,23 | 7,10 | 0,26 | 3,60 | 1663 | 256 | 757 | 1520 | 7,80 |
| V-2 | 13,97 | 1,11 | 7,15 | 0,26 | 3,63 | 1419 | 233 | 758 | 1515 | 7,85 |
| V-3 | 14,40 | 1,43 | 7,42 | 0,33 | 3,66 | 1822 | 271 | 801 | 1602 | 7,80 |
| V-4 | 14,16 | 1,28 | 7,35 | 0,26 | 3,75 | 1914 | 353 | 819 | 1637 | 7,90 |
| V-5 | 14,24 | 1,17 | 7,27 | 0,26 | 3,53 | 1888 | 325 | 761 | 1525 | 7,90 |
| V-6 | 14,71 | 1,07 | 7,2 | 0,33 | 3,73 | 2052 | 374 | 796 | 1594 | 7,85 |
| Witness | 13,75 | 1,55 | 7,05 | 0,26 | 3,32 | 1762 | 290 | 784 | 1552 | 7,85 |

A fact that confirms their use in the production grapes with a high degree of maturity. Residual sugar not exceed 1.55 g / L, indicating that falls within the limits set for dry red wines. Titratable acidity in wine samples is 7.05 to 7.42 g / L - large enough values in red wines that did not happen malo-lactic fermentation. Volatile acidity does not exceed 0.33 g / L, which confirms that the must maceration-fermentation was done in good condition. Total soluble salt content of experimental samples have values that do not deviate much from the witness. In experimental samples this index ranging from 757 up to 819 mg / L, while in the witness he is 784 mg / L. This is due to the fact that in the experimental samples and the witness content of the acid in and acid salts the wine do not vary widely. The conductivity of the wine is to the totally soluble salts content. For the experimental samples, variants 3 and 4, which is characterized by a higher content of total soluble salts have a high electrical conductivity - 1602 and 1637 respectively mS / cm.

Note that in both cases, when must maceration- fermentation proceeds in the presence of an excess of seed or when in the must is a smaller amount of seed or missing them entirely, basic indices of experimental samples did not differ essentially from those of control sample.

As regards the content of phenolic compounds obtained results indicate a more pronounced change between the experimental samples and compared with the control (see fig. 1).

Research has shown that in variants 1 and 2, the production of which have been added seeds in a proportion of 30 to 60%, the content of phenolic compounds is lower than in witness- 1663, 1419 mg / l and 1762 mg / L. A higher content of phenolic substances (1822 mg / L) compared with the witness was documented in experimental sample obtained by adding seed at 100% (variant 3). Regarding the content of the coloring substances results are similar to literature wich confirm on the adsorption of anthocyanins by seeds during maceration -fermentation process.

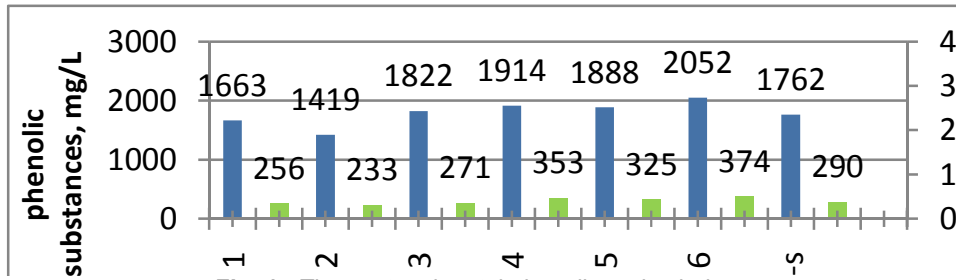


**Fig. 1 -** The content in total phenolic and coloring substances in experimental and witness wines

In variants 1, 2 and 3 obtained with added seeds coloring matter content is lower than in witness.

In the variants obtained by separating the seeds from the must (4, 5 and 6) as can be seen, the content of the coloring is higher compared to the witness and reduction is due to the quantity of seeds which, as already mentioned, serve as adsorbents them. I variant 4 - the content of coloring is 353 mg / L, variant 5 - 324 mg / L and variant 6 (seedless) - 374 mg / L, while in witness this index is only 289 mg / L. Note that in these samples was found and a higher content in phenolic substances. For example, the sample obtained seedless value of this index is 2052 to 1762 mg / L in witness. And the other two experimental samples this index is higher compared to the control.

Note that currently authors do not have reasoned explanations regarding the increase of the amount of phenolic substances in samples of wine produced seedless or with small quantities of seed. In this chapter we assume that seeds have an unfavorable role on the process of extraction of phenolic compounds from the skin. But to confirm this hypothesis is necessary to conduct further research. Another explanation for this phenomenon can be decreased volume as a result of the abolition must seeds, lead to the concentration of phenolic compounds in it and the wine respectively.

Figure 2 presents spectrograms of absorption experimental wine samples and witness. In the visible spectrum at a wavelength of 520 nm, anthocyanins give maximum absorption. Spectrograms obtained show that the experimental samples obtained by reducing the amount of seeds is characterized by a high content of anthocyanins. Spectrograms character samples of wine produced surplus seeds they demonstrate lower content of anthocyanins, especially in variant 3 (adding seed at 100%).

The most interesting gustatory qualities distinguish variants 4, 5 and 6, partial or total removal products seeds. These samples have a round taste, soft, moderate astringency and a dark ruby beautiful color, very intense, and their flavor is predominant shades of red fruit. Sensory appreciation of these wines was at the level of 7.85 (variant 6) and 7.90 points (variants 4 and 5).

The research results can be concluded that the excess seed is not beneficial to the quality of red wines.

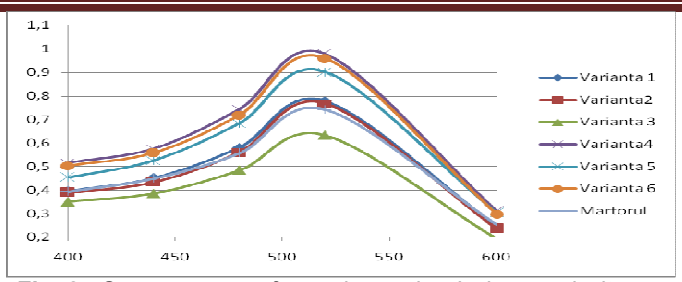


**Fig. 2 -** Spectrograms of experimental red wines and witness

Making must fermentation maceration from which it was removed some of the seeds positively influence the quality of wine. In this chapter the research will continue.

## CONCLUSIONS

1. Both the surplus, as well as the small amount of seeds from the must not influence the basic indices of red wines.

2. When fermentation maceration surplus seeds constituting 30-100% lead to the minimization of coloring matter and not contributing to the increase in phenolic substances.

3. Elimination of the seeds from must in the ratio of 30-60% allows retention of coloring substances because removing a portion of the absorption substrate.

4. The red wines produced by removing a quantity of seeds are distinguished by high olfacto-gustatory quality. They are round, well-structured taste and moderate astringency.

5. As a practical guideline proposes in process of maceration-fermentation of the must to eliminate the amounts of seeds as much as possible bigger, which will increase the quality of red wines.

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# RESEARCHES ON THE INFLUENCE OF THE DIFFERENT TECHNIQUES OF MACERATION ON THE COMPOSITIONAL CHARACTERISTICS OF THE BUSUIOACĂ DE BOHOTIN WINE

## CERCETĂRI PRIVIND INFLUENȚA DIFERITELOR TEHNICI DE MACERARE ASUPRA CARACTERISTICILOR COMPOZIȚIONALE ALE VINULUI DE BUSUIOACĂ DE BOHOTIN

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***Abstract.*** *This study pursues the influence that have various techiques of maceration (maceration fermentation, thermomaceration, cryomaceration - microwave maceration,carbonic maceration) on the compositional characteristics of the Busuioacă de Bohotin wine. This four techniques were applied to the two biotypes of Busuioacă de Bohotin variey, respectively dark violet biotype androse biotype. The main parameters that will be followed to determine the physico-chemical composition of the wine studied are : sulfur dioxide , volatile acidity, total acidity, density, concentration of alcohol , reducing sugars , total dry extract , non-reductive extract , phenolic compounds and chromatic characteristics.*
***Key words:*** *physico-chemical analysis, maceration, thermomaceration, cryomaceration, microwave maceration, carbonic maceration.*

***Rezumat.*** *In acest studiu se urmărește influența pe care o au diferitele tehnici de macerare (macerare-fermentare, termomacerare, criomacerare - macerare cu microunde, macerație carbonică) asupra caracteristicilor compoziționale ale vinului de Busuioacă de Bohotin. Cele patru tehnici de macerare s-au aplicat pe cele două biotipuri ale soiului Busuioacă de Bohotin, respectiv biotipul roz și biotipul vânăt.Principalii parametrii care se vor urmări pentru determinarea compoziției fizico-chimice ale vinului studiat sunt: dioxidul de sulf, aciditatea volatilă, aciditatea totală, densitatea, concentrația alcoolică, zaharurile reducătoare, extractul sec total, extractul nereducător, compușii fenolici și caracteristicile cromatice.*
***Cuvinte cheie:*** *analiză fizico-chimică, macerație, termomacerație, criomacerație, macerație cu microunde, macerație carbonică.*

## INTRODUCTION

The Busuioacă de Bohotin variety is one of the most valuable local varieties, but with limited ecological plasticity that is designed for obtaining

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aromatic wines, characterized by specific organoleptic qualities that gives a special note of originality (Rotaru *et al.,* 2011).

Busuioacă de Bohotin being an aromatic variety it is mandatory the maceration operation.

Maceration is a technological operation by which the marc is maintained for a time in contact with the juice for extraction of certain components from the solid parts of the grapes (Cotea *et al.,* 2012).A number of methods are available to the winemakerto adjust extraction levels during maceration. The various results obtained by applying different methods of maceration demonstrates the utility of a better chemical and gustatory understanding of the molecules involved in maceration phenomenon (Ribereau Gayon *et al*., 2006).

The main objective of this study is to evaluate the influence of different techniques of maceration on the physico-chemical composition of the Busuioacă de Bohotin wine.

## MATERIAL AND METHOD

For this experiment there were used two biotypes of the Busuioacă de Bohotin variety, namely: dark violet biotype and rose biotype. The grapes were harvested at technological maturity in the year 2014, both biotypes being from Huși vineyard.

To obtain the wine samples taken in this study, there were applied four types of maceration processes, specifically:

**V0-Control sample**- it was applied a classical maceration.

**V1-Maceration fermentation**- this type of maceration it is similar with the control sample, with the difference that was added yeast and enzymes.

**V2-Thermomaceration-** for this variant of maceration, after the crushing and destemming operations, the juice was pumped over from the plastic can and heated to a temperature of 70ºC. After reaching this temperature, the juice was brought back into the plastic can and homogenized with the remained pomace.

**V3- Cryomaceration - Microwave maceration-**this technique of maceration is based on two types of methods. In a first stage it was applied cryomaceration method, where the whole grapes were slowly freezed at a temperature of -20ºC in 24 hours. The frozen grapes were manually destemmed and crushed. In the second stage the obtained pomace was iraddiated with microwaves at a power of 750 W for 15 minutes. Meanwhile, thepomace was homogenized from 3 to 3 minutes to equally heat the pomace.

**V4-Carbonic maceration-**it was conducted in a plastic can, where the whole grapes were introduced on a grill, situated at a height of about 10 cm from the bottom of the container.The carbonic maceration was done with the carbon dioxide eliminated in the process of alcoholic fermentation of the must, situated at the bottom of the can.

The maceration time for all the techniques used was seven days and it was added yeast- Fermactive Rouge Primeur and pectolitic enzyme-Zymovarietal Aroma G, both from Sodinal company.

## RESULTS AND DISCUSSIONS

Analyzing the physico-chemical parameters from the table 1, it can be observed that there aren't significant differences between the values obtained for

the variants of maceration applied.Thus, in what concerns alcoholic concentration, the dark violet biotype of the Busuioacă de Bohotin variety registred an average value of 14.67 %. The maximum was reached by the V1-Maceration fermentation variant with 14.85 % in contrast with control sample V0 with 14.75 %, while the minimum was registred by the V4 variant-Carbonic maceration with a value of 14.5 %. In therms of reducing sugars content, all the wine samples prezented values situated below 4 g/l, with an average value of 2 g/l, therefore they can be included in the category of dry wines.

Observing table 2, it can be stated that for the rose biotype of the Busuioacă de Bohotin varietyit exists wide variations in what concerns total acidity parameter, the maximum value being registred by the control sample with 6.14 g/l and the minimum was reached by the V7-Carbonic maceration variant with 5.15.

In what concerns total dry extract (T.D.E) and non-reductive extract (N.E) parameters, it can be observed that almost all variants of maceration prezented higher values. The only exception was found at the V4 Carbonic maceration, which showed lower values with 19.3 T.D.E and 17.58 N.E. for the rose biotype and 19 T.D.E and 16.83 N.E for the dark violet biotype.

pH and conductivity parameters had similar values for all the variants of maceration applied to the both biotypes, with an average value of 3.82 for pH and 2.33 for conductivity.

Analyzing the parameters that describes the content in phenolic compounds, namely total polyphenol index IPT (D280) and Folin Ciocâlteu Index (IFC) there were observed wide variations on the values obtained for the techniques of maceration applied for both biotypes.

Thus, for the IPT parameter (Fig.1) the minimum was reached by V1 sample with 8.44 for the dark violet biotype and 6.16 (V4 variant) for the rose biotype, while the maximum was expresed by the control sample V0 of the dark violet biotype with 15.93 and V1 sample of the rose biotype with a value of 12.25.

In what concerns I.F.C parameter (Fig.2), the minimun was reached by V4 sample with 6.79 for the dark violet biotype and 8.79 (V3 variant) for the rose biotype, while the maximum was expresed by V2 variant of the dark violet biotype with 10.38 and V1 sample of the rose biotype with a value of 15.79.

By comparing these two biotypes regarding phenolic compounds content, it can be observed some differences. Thus, in therms of IPT parameter it can be showen that the highest values wereachieved by the dark violet biotype with an average value of 11.03, while the rose biotype had an average value of 9.52.

The IFC parameterhas higher values for the rose biotype with an average value of 11.49 in comparison with the dark violet biotype with an average value of 8.87.

*Table 1*

Physico-chemical analysis of the Dark violet biotype

| Analyzed wines | $SO_2$ mg/L | | Vol. Acidity (g/L $C_2H_4O_2$) | Total acidity (g/L $C_4H_6O_6$) | Rel. density | Conc Alc. (% vol.) | Red. subst. (g/L) | TDE (g/L) | NE (g/L) | pH | Conductivity mS/cm² |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | free | total | | | | | | | | | |
| BBV - V0 | 50.78 | 147.09 | 0.35 | 5.23 | 0.990 | 14.75 | 2.14 | 24.5 | 22.36 | 3.86 | 2.29 |
| BBV - V1 | 43.66 | 134.70 | 0.36 | 5.30 | 0.990 | 14.85 | 2.14 | 25 | 22.86 | 3.88 | 2.37 |
| BBV - V2 | 33.75 | 130.68 | 0.32 | 5.36 | 0.991 | 14.51 | 1.94 | 25.5 | 23.56 | 3.93 | 2.49 |
| BBV - V3 | 41.18 | 131.92 | 0.37 | 5.84 | 0.989 | 14.75 | 1.96 | 22.9 | 20.94 | 3.89 | 2.22 |
| BBV - V4 | 40.56 | 123.87 | 0.62 | 5.55 | 0.988 | 14.50 | 2.17 | 19 | 16.83 | 3.77 | 1813 µS/cm |

*Table 2*

Physico-chemical analysis of the Rose biotype

| Analyzed wines | $SO_2$ mg/L | | Vol. acidity (g/L $C_2H_4O_2$) | Total acidity (g/L $C_4H_6O_6$) | Rel. density | Conc Alc. (% vol.) | Red. subst. (g/L) | T.D.E (g/L) | N.E (g/L) | pH | Conductivity mS/cm² |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Free | Total | | | | | | | | | |
| BBR - V0 | 40.25 | 112.10 | 0.41 | 6.14 | 0.991 | 14.15 | 2.04 | 24 | 21.96 | 3.83 | 2.48 |
| BBR - V1 | 43.04 | 133.47 | 0.35 | 5.84 | 0.990 | 14.31 | 2.18 | 22.9 | 20.72 | 3.80 | 2.37 |
| BBR - V2 | 34.99 | 114.27 | 0.33 | 5.94 | 0.990 | 14.01 | 2.10 | 23.5 | 21.4 | 3.78 | 2.45 |
| BBR - V3 | 27.39 | 102.72 | 0.33 | 5.45 | 0.990 | 13.88 | 1.69 | 21.1 | 19.41 | 3.82 | 2.35 |
| BBR - V4 | 23.51 | 104.09 | 0.41 | 5.15 | 0.989 | 13.88 | 1.72 | 19.3 | 17.58 | 3.72 | 2.02 |

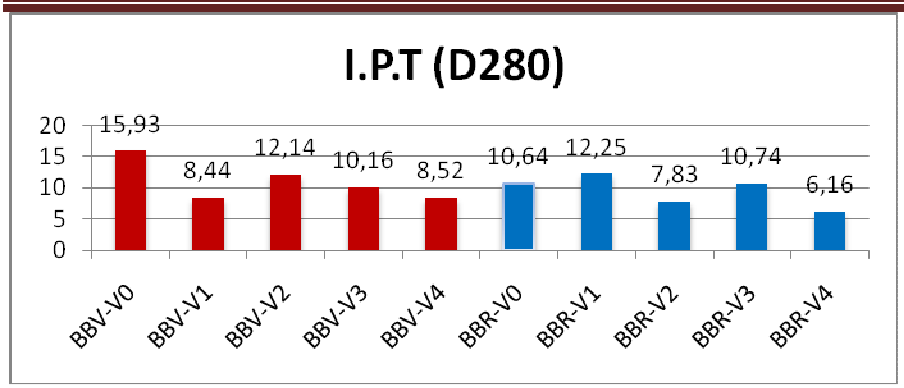


**Fig. 1-** Total poliphenolic index (I.P.T) for the dark violet and rose biotype of Busuioacă de Bohotin variety.

Where:
BBV- is the dark violet biotype
BBR-is the rose biotype

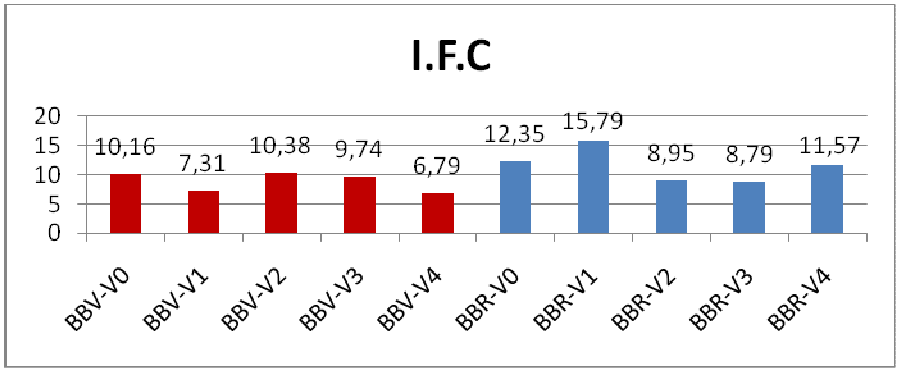


**Fig. 2** - Folin Ciocâlteu Index for the dark violet and rose biotype of Busuioacă de Bohotin variety.

Analyzing the wine samples obtained, from a chromatic point a view it can be observed that the best values for the both biotypes were obtained for the V2 variant where was applied thermomaceration technique, these samples having the most intense color (Table 3 and 4).

Thus, for these samples, the value of L parameter (clarity) was the lowest, predominating shades of red (+a) and yellow (+b), the level of saturation (C) being higher for these samples.

At the opposite pole lies the wine samples obtained from carbonic maceration (V4), where the shades of red and yellow were fade, a-parameter having even negative values for the rose biotype, prevailing green shades.

*Table 3*

**Chromatic parameters of the dark violet biotype**

| Analyzed wines | CieLab-76 | | | | | | | Digital Colour Atlas 3.0 |
|---|---|---|---|---|---|---|---|---|
| | Tristimulus | | | Crome (C) | Tonality (H) | Lighteness | Hue | |
| | L (clarity) | a | b | | | | | Colour |
| **BBV -V0** | 89.06 | 10.05 | 12.67 | 16.17 | 51.58 | 0.50 | 1.41 | |
| **BBV -V1** | 93.81 | 5.03 | 9.01 | 10.32 | 60.81 | 0.29 | 1.65 | |
| **BBV -V2** | 87.77 | 10.81 | 15.07 | 18.54 | 54.33 | 0.57 | 1.47 | |
| **BBV -V3** | 90.93 | 7.42 | 11.10 | 13.35 | 56.21 | 0.41 | 1.55 | |
| **BBV -V4** | 97.42 | 1.02 | 5.05 | 5.16 | 78.50 | 0.14 | 2.25 | |

*Table 4*

**Chromatic parameters of the rose biotype**

| Analyzed wines | CieLab-76 | | | | | | | Digital Colour Atlas 3.0 |
|---|---|---|---|---|---|---|---|---|
| | Tristimulus | | | Crome (C) | Tonality (H) | Lighteness | Hue | |
| | L (clarity) | a | b | | | | | Colour |
| **BBR - V0** | 98.29 | 0.76 | 6.66 | 6.71 | 83.42 | 0.12 | 3.10 | |
| **BBR - V1** | 96.21 | 2.69 | 11.21 | 11.53 | 76.48 | 0.23 | 2.39 | |
| **BBR - V2** | 95.63 | 2.47 | 11.85 | 12.11 | 78.20 | 0.26 | 2.34 | |
| **BBR - V3** | 97.91 | 0.25 | 6.34 | 6.35 | 87.73 | 0.13 | 3.02 | |
| **BBR - V4** | 98.65 | - 0.56 | 5.06 | 5.09 | -83.65 | 0.10 | 4.10 | |

## CONCLUSIONS

1. Analyzing the main physico-chemical parameters of the wine samples obtained there weren't found significant differences between the techniques of maceration applied on compositional characteristics. However, it was observed that the carbonic maceration technique presented the lowest values in what concerns alcoholic concentration, total dry extract and non-reductive extract for both biotypes used.

2. By comparing the two biotypes used regarding phenolic compounds content, it was observed that in what concerns total phenolic compound index, the highest values were achieved by the dark violet biotype and in terms of phenolic compounds with reducing proprieties, the rose biotype obtained the highest values.

3. From a chromatic point of view, it was pointed out that the wine samples obtained by thermomaceration technique had the most intense color and those obtained by carbonic maceration were the most fade in color.

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# RESEARCH ON THE INFLUENCE OF CARBON DIOXIDE-RICH ATMOSPHERE OF SHORT TERM STORAGE OF GRAPES

## CERCETĂRI PRIVIND INFLUENȚA ATMOSFEREI BOGATE ÎN DIOXID DE CARBON ASUPRA PĂSTRĂRII DE SCURTĂ DURATĂ A STRUGURILOR

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***Abstract.*** *The research aimed at determining the optimal concentration of carbon dioxide in the short-term storage in correlation with decrease temperature to approx. 20 C. At the same time we intended to see the effect of carbon dioxide on the metabolic activity and on pathogens. After conducting experiments we established that all modified atmospheres with carbon dioxide have reduced losses. The high content in carbon dioxide of the storage environment exerts a direct influence of inhibition on developing pathogens and carbon dioxide concentrations do not produce in fruits the essential chemical transformation that can influence their quality in a negative way. The total losses were minimal for the variant when it has been used $CO_2$ concentration of 20%, both at hot and at cold.*
***Key words:*** *carbon dioxide, grapes, storage*

***Rezumat.*** *Cercetările au urmărit determinarea concentrației optime de dioxid de carbon necesară păstrării de scurtă durată în corelare cu scăderea temperaturii la cca. $20^0$ C. Totodată s-a urmărit efectul dioxidului de carbon asupra activitătii metabolice şi asupra agenților patogeni. In urma efectuării experimentărilor s-a stabilit că în variantele cu atmosferă modificată cu dioxid de carbon se reduc pierderile. Conținutul ridicat în dioxiod de carbon al mediului de păstrare exercită o influență directă de inhibare a dezvoltării agenților patogeni, iar concentrațiile de dioxid de carbon nu produc în fructe transformări chimice esențiale care să le influențeze negativ calitatea. Pierderile totale au fost minime pentru varianta în care s-a utilizat concentrația de 20% $CO_2$, atât la cald cât şi la frig.*
***Cuvinte cheie:*** *dioxid de cabon, struguri, păstrare*

## INTRODUCTION

Using the gas with inhibitory effect on the metabolic activity of the product and pathogens during transportation has been studied for many researchers. Both, the low $O_2$ level and high $CO_2$ level of concentrations reduce the breathing rate.

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Ever since 1963, based on laboratory tests, are recommended the transportation og strawberries, raspberries and cherries in modified atmosphere with carbon dioxide in concentration of 20-25% (Smith and Skog,1992).

There have been numerous efforts to create the necessary gas mixture around the fruits and to establish the mathematical equations for calculating the concentration of $O_2$ and $CO_2$ from the microatmosphere in a polymeric film for packaging (Jurin and Karel, 1963). It were used computerized methods to solve the mathematical equations for calculating the gas concentration in the packaging system for transportation (Henig and Gilbert, 1975; Rohrbach and all, 1984).

All the abovementioned models require the knowledge of the $O_2$ consumption rate and $CO_2$ evolution concerning the respiration rate, which are based on $O_2$ and $CO_2$ composition from medium.

In 1984, several researchers tested pre-cooling with liquid carbon dioxide for blackberries and grapes packed in conventional and unconventional packaging materials. They found that dehydration of fruits was not significant during pre-cooling and was reduced to half in conventional packaging material's using and for the unconventional material's the reduction was 5-25% more.

## MATERIAL AND METHOD

Through the developed experiments it was mainly aimed to establish the optimal concentration of carbon dioxide which contributes to maintain the quality of grapes during transportation, by organizing experiments both "at warm" (ambiental temperature in working space - approx. $25^0$ C) and" at cold - approx. $20^0$ C).

There was used grapes from Afuz-Ali, Muscat d'Adda şi Alphonse Lavalée varieties.

Apparature, devices and installations:

- hermetically sealed containers for keeping the concentration of experimental carbon dioxide
- TBV – 2000 climate chamber
- INFRALIT gas analyzer equipped with (measuring range 0-50% $CO_2$ )
- CARLO ERBA gas chromatograph for measuring the concentration in ethylene
- laboratory equipment

The research method consisted in an analytical and comparative analysis regarding the grapes's behavior in normal and modified atmosphere with carbon dioxide, to "cold " (in climate chamber) and "warm" (in laboratory).

Were developed the following variants:

$V_{01}$ – Witness- environment in laboratory atmosphere (approx. $25^0$ C)
$V_{02}$ – Witness- environment in climate chamber atmosphere (approx. $2^0$ C)
$M_1$ – Normal atmosphere in laboratory container
$M_2$ – Normal atmosphere in container from climate chamber
$V_1$ – Modified atmosphere with 10% concentration of $CO_2$ in laboratory
$V_2$ – Modified atmosphere with 20% concentration of $CO_2$ in laboratory
$V_3$ – Modified atmosphere with 30% concentration of $CO_2$ in laboratory
$V_4$ – Modified atmosphere with 10% concentration of $CO_2$ in climate chamber
$V_5$ – Modified atmosphere with 20% concentration of $CO_2$ in climate chamber

$V_6$ – Modified atmosphere with 30% concentration of $CO_2$ in climate chamber

Were made observations and determinations in dynamics regarding:

- fruit aspect and organoleptic analysis
- firmness
- weight loss
- evolution of $CO_2$ concentration related with temperature and time
- evolution of ethylene concentration
- chemical composition
- fruit's health

For each variant were done at least 3 measurements. Samples ,,at warm" were removed after 3 days and those ,,at cold" after 6 days. Measuring the concentration was made after 3 days in both situation and after 6 days just for ,,at cold ".

## RESULTS AND DISCUSSIONS

The evolution of total losses in the case of grapes, as is shown in Table 1, it is different from the other studied fruits. The total losses are much smaller and present the minimum value in the case of the variants with 20% $CO_2$, both ,,at warm" and ,,at cold". Unlike the other fruits, losses (depreciation) by softening are non-existent. Samples from "at warm" had presented mold attack but at low intensity, especially on the Afuz-Ali variety's cob/axis. In case ,,at cold "was not found mold attack in any variant.

*Table 1*

**Weight losses achieved in experiments regarding the behavior of grapes from Afuz-Ali variety in modified atmospheres with carbon dioxide ,, at warm " (after 3 days and a temperature of approx. $16^0$ C) and ,,at cold" (after 6 days and a temperature of approx. $2^0$ C)**

| Variant | Symbol | Total losses | | of which: | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Weight losses | | Softening losses | | Mold attack losses | |
| | | warm | cold | warm | cold | warm | cold | warm | cold |
| Witness-environment | $V_{01}$ | 7,09 | - | 6,66 | - | - | - | 0,43 | - |
| | $V_{02}$ | - | 3,8 | - | 3,8 | - | - | - | - |
| Normal atmosphere in container | $M_1$ | 3,44 | - | 0,6 | - | | - | 2,84 | - |
| | $M_2$ | - | 0,71 | - | 0,71 | - | - | - | - |
| Modified atmosphere with 10% $CO_2$ | $V_1$ | 3,42 | - | 0,67 | - | - | - | 2,75 | - |
| | $V_4$ | - | 0,48 | - | 0,48 | - | - | - | - |
| Modified atmosphere with 20% $CO_2$ | $V_2$ | 1,74 | - | 0,66 | - | - | - | 1,08 | - |
| | $V_5$ | - | 0,13 | - | 0,13 | - | - | - | - |
| Modified atmosphere with 30% $CO_2$ | $V_3$ | 3,86 | - | 0,43 | - | - | - | 3,33 | - |
| | $V_6$ | | 0,73 | - | 0,73 | - | - | - | - |

The carbon dioxide has reduced the losses in situation with 20% $CO_2$ by 4 times ,,at warm" comparing with witness case and 29 times ,,at cold".

The evolution of $CO_2$'s concentration at grapes shows a difference comparing with the other studied fruits. „At warm" the concentration in carbon dioxide increases more slowly as in the other fruits, while "at cold„ it is produced a decrease of concentration (Table 2).

*Table 2*

**Evolution of $CO_2$'s concentration in grapes**

| Symbol | Initial $CO_2$'s concentration | Warm (approx. $16^0$C): | | | Cold ($2^0$ C) : | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 days | 6 days | 9 days | 3 days | 6 days | 9 days | 12 days | 15 days |
| $M_1$ | - | 6,5 | 12,5 | 16,7 | - | - | - | - | - |
| $M_2$ | | - | - | | 3,1 | 2,5 | 3,1 | 3,8 | |
| $V_1$ | 10 | 17 | 28,5 | 38 | - | - | - | - | - |
| $V_2$ | 20 | 31 | 39,5 | 47,5 | - | - | - | - | - |
| $V_3$ | 30 | 34 | 40,5 | 50 | - | - | - | - | - |
| $V_4$ | 10 | - | - | - | 8,2 | 7,3 | 8,1 | 9,3 | - |
| $V_5$ | 20 | - | - | - | 11,1 | 10,0 | 10,7 | 11,7 | - |
| $V_6$ | 30 | - | - | - | 14,3 | 13,0 | 13,8 | 14,0 | - |

After a longer storage of grapes was repeated the experience (Table 3 and 4), the conclusion being that the concentration decreases to a minimum value (in the $6^{th}$ day) and after that increases slightly. The grapes have not released ethylene.

*Table 3*

**Analytical changes produced in grapes during the experiences regarding their behavior in modified atmospheres with carbon dioxide after 9 days at approx. $16^0$ C temperature**

| Symbol | Dry substance % | | | Total sugars % | | | Acidity % | | |
|---|---|---|---|---|---|---|---|---|---|
| | prim. | end | dif. | prim. | end | dif. | prim. | end | dif. |
| $V_{01}$-white | 19,9 | 18,1 | -1,8 | 186,3 | 167,2 | -19,1 | 0,61 | 0,58 | -0,03 |
| $V_{01}$ -red | 20,9 | 11,9 | -9 | 196,9 | 101,4 | -95,5 | 0,61 | 0,63 | 0,02 |
| $M_1$- white | 19,9 | 17,4 | -2,5 | 186,3 | 159,7 | -26,6 | 0,61 | 0,59 | -0,02 |
| $M_1$ - red | 20,9 | 19,1 | -1,8 | 196,9 | 177,8 | -19,1 | 0,61 | 0,62 | 0,01 |
| $V_1$ - white | 19,9 | 16,6 | -3,3 | 186,3 | 151,2 | -35,1 | 0,61 | 0,54 | -0,07 |
| $V_1$ - red | 20,9 | 13,1 | -7,8 | 196,9 | 114,1 | -82,8 | 0,61 | 0,58 | -0,03 |
| $V_2$ - white | 19,9 | 17,6 | -2,3 | 186,3 | 161,9 | -24,4 | 0,61 | 0,49 | -0,12 |
| $V_2$ - red | 20,9 | 13,3 | -7,6 | 196,9 | 116,3 | -80,6 | 0,61 | 0,62 | 0,01 |
| $V_3$ - white | 19,9 | 16,1 | -3,8 | 186,3 | 147,0 | -39,3 | 0,61 | 0,65 | 0,04 |
| $V_3$ - red | 20,9 | 15,3 | -5,6 | 196,9 | 137,4 | -59,5 | 0,61 | 0,53 | -0,08 |

From Tables 3 and 4 result that in grapes stored for 9 days „at warm" and 12 days „at cold" had produced no essential chemical transformation able to improve its quality. The significant changes were noticed to sugars, between 62.6% for witness variant and 1.1 for the variant with 20% $CO_2$ „at cold" and

between 95.5% for witness variant and 19.1 for the witness variant in container „at warm".

It doesn't change the taste unlike the other fruits where occurred flavor anomalies due to carbon dioxide impregnation.

Phytosanitary condition of grapes stored in modified atmosphere is reflected in Table 5.

*Table 4*

**Analytical changes produced in grapes during the experiences regarding their behavior in modified atmospheres with carbon dioxide after 12 days ($2^0$ C)**

| Symbol | Dry substance % | | | Total sugars % | | | Acidity % | | |
|---|---|---|---|---|---|---|---|---|---|
| | prim. | end | dif. | prim. | end | dif. | prim. | end | dif. |
| $V_{02}$-white | 19,9 | 14,0 | -5,9 | 186,3 | 123,7 | -62,6 | 0,61 | 0,59 | -0,02 |
| $V_{02}$ - red | 20,9 | 19,5 | -1,4 | 196,9 | 182,0 | -14,9 | 0,61 | 0,62 | 0,01 |
| $M_2$- white | 19,9 | 20 | 0,1 | 186,3 | 187,4 | 1,1 | 0,61 | 0,53 | -0,08 |
| $M_2$ - red | 20,9 | 20,5 | -0,4 | 196,9 | 192,7 | -4,2 | 0,61 | 0,54 | -0,07 |
| $V_4$ - white | 19,9 | 15,7 | -4,2 | 186,3 | 141,7 | -44,6 | 0,61 | 0,48 | -0,13 |
| $V_4$ - red | 20,9 | 20,4 | -0,5 | 196,9 | 191,6 | -5,3 | 0,61 | 0,57 | -0,04 |
| $V_5$ - white | 19,9 | 18,6 | -1,3 | 186,3 | 172,5 | -13,8 | 0,61 | 0,40 | -0,21 |
| $V_5$ - red | 20,9 | 21 | 0,1 | 196,9 | 195,8 | -1,1 | 0,61 | 0,56 | -0,05 |
| $V_6$ - white | 19,9 | 21 | 1,1 | 186,3 | 174,6 | -11,7 | 0,61 | 0,43 | -0,18 |
| $V_6$ - red | 20,9 | 22 | 1,1 | 196,9 | 185,2 | -11,7 | 0,61 | 0,53 | -0,08 |

*Table 5*

**Phytosanitary condition of grapes in mofified atmosphere with carbon dioxide**

| Grapes affected by pathogens after 9 days of storage "at warm" | | | | | | |
|---|---|---|---|---|---|---|
| **Variety** | **Initial** | **Normal atmosphere** | **Normal atmosphere in container** | **10% $CO_2$** | **20% $CO_2$** | **30% $CO_2$** |
| Muscat d'Adda | 2,12 *Botrytis* | | 21,5 *Botrytis* | 0 | | |
| Afuz-Ali | 4,16 *Botrytis* | | 11,7 *Botrytis* | | | |
| **after 12 days „at cold"** | | | | | | |
| Muscat d'Adda | 2,12 *Botrytis* | | 14,28 *Botrytis Penicillium* | 18,18 *Botrytis* | 17,5 *Botrytis* | 30,7 *Botrytis* |
| Afuz-Ali | 4,16 *Botrytis* | 14,2 *Botrytis Penicillium* | 11,4 *Botrytis* | 10,0 *Botrytis* | | 13,5 *Botrytis* |

It was noticed a *Botrytis* attack on all witness samples from container, after 5 days of exicator keeping in the relative humidity conditions (approx. 90%) and laboratory temperature (approx. 160° C). The Muscat d 'Adda variety was attaked by *Botrytis* in all samples with modified atmosphere „at cold". *Botrytis* appeared „at warm" on the cob/ axis of bunches in atmospheres with 20% and 30% $CO_2$. In 30% $CO_2$ atmosphere „at cold" it was noticed the occurrence of *Rhizopus mycelium* on Afuz-Ali variety.

## CONCLUSIONS

Laboratory tests on grapes were made to create the possibility of substantiating their behavior in different atmospheres with carbon dioxide (10% $CO_2$, 20% $CO_2$and 30% $CO_2$).

The purpose of these measurements was to establish the usefulness and optimal percentage of carbon dioxide in modified atmospheres for transportation of fresh fruits and storage on short term of the fruits.

Conclusions resulting from experience:

- all modified atmospheres with carbon dioxide reduce losses;
- $CO_2$ concentrations ,,at cold" decrease to a minimum in the $6^{th}$ day and then increase very slightly, but without reaching the initial value in the studied period;
- $CO_2$ concentration from fruit's respiration decreases with increasing the $CO_2$ percent initial introduced in modified atmosphere;
- grapes have not released ethylene;
- high content of $CO_2$ atmospheres exerts a direct influence in inhibiting the evolution of pathogenic fungi existing on the fruit's surface;
- $CO_2$ concentrations do not produce essential chemical transformation in fruits which can negatively influence their quality;
- with increasing of $CO_2$ concentration do not appear flavor anomalys at grapes .

Considering the obtained results in the laboratory determinations, the optimal conditions for the transportation of grapes are considered to be: low temperature of approx. $20^0$ C associated with a controlled atmosphere between 10% and 20% of $CO_2$ (for 2 or 4 days).

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# THE DYNAMIC OF SUBMISSION AND HATCHING EGGS TO THE COLORADO BEETLE AFTER TREATMENS WITH BIOPESTICIDE

## DINAMICA DEPUNERILOR DE OUĂ ŞI A PROCESULUI DE ECLOZARE LA GÂNDACUL DIN COLORADO ÎN URMA TRATAMENTELOR CU BIOINSECTICIDE

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***Abstract.*** *The Dynamic of eggs submission treated with Neem Azal T/S variants,at the three doses used (0,33%, 0,41% şi 0,50%) gradually decreases, goes that after about a week the number of egg / potato bush to be practically zero. The other biological product, Laser 240 SC, or efficacy was very good on the clutch meaning that eggs come in direct contact with dried immediately, and while there was a radical reduction of eggs deposits treated variants.*
***Key words:*** *eggs, consumption, repellent, mortality*

***Rezumat.*** *Dinamica depunerilor de ouă la variantele tratate cu NeemAzal T/S la cele trei doze folosite (0,33%, 0,41% şi 0,50%) scade treptat, ajungându-se ca după circa o săptămână numărul de ouă/tufa de cartof sa fie practic zero. Celălalt produs biologic, respectiv Laser 240 SC a avut eficacitate foarte bună asupra pontelor în sensul că ouăle care au venit în contact direct cu produsul s-au uscat imediat, iar în timp s-a observat o diminuare radicală a depunerilor de ponte la variantele tratate.*
***Cuvinte cheie:*** *pontă, eclozare, consum, repelenţă, mortalitate*

## INTRODUCTION

Colorado potato beetle (*Leptinotarsa decemlineata* Say) is one of the most dangerous pests in potato. Control of this pest population is developed with chemical insecticides, which diminishes the economic damage (Dubis *et al.,* 1995).

A big drawback of long-standing use of insecticides to the emergence of pest resistance to these substances. It was noted that this resistance to insects occurs after continuous treatment with the same compound for 15-20 generations, against the same pest species.

Organic food market is booming especially in industrialized countries. Requests increasing quantities of potatoes produced with low levels pesticide, causes many farmers who produced the conventional system to turn to organic potato culture (Bruda *et al.,* 2008).

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## MATERIAL AND METHOD

This paper aims to determine the influence of bioinsecticids products Laser 240 SC and NeemAzal SC 240 T / S on Colorado potato beetle eggs. The experiments were carried out both in the laboratory and in the field, using conventional technology for culture of potatoes.

Leaves potato with eggs were collected from experimental field whitout pesticides and were analyzed in a laboratory to determine the number of eggs.

Subsequently, the leaves were immersed for three seconds in the solution prepared bioinsecticids , then placed in Petri dishes at a temperature close to the field. It followed the dynamics of hatching process by ovicidal action . .

At variants of the experimental field, the treatment was done bioinsecticide same as in laboratory, after previously determined number of eggs on potato bushes.

## RESULTS AND DISCUSSION

*a) The dynamics of deposits eggs in genotype cultivated and product*

To Santé variety, the variants treated with NeemAzal T / S regardless of the dosage used is a decrease of egg laying, reaching that after about a week the number of eggs / bush potato is practically zero. This was observed during the entire period of 17 days was monitored as experience.

Similarly, the product Laser 240 SC had strong efficacy on clutch that came in contact with bioinsecticide, meaning that they practically dried, and while there was a decrease radically laying eggs (tab. 1).

In comparison to the untreated solution and subsequently the number of eggs hatching percentage remained within the normal range.

As Santé the other two varieties, Claudiu and Magic were shown the same tendencies to halt the laying eggs on treated bioinsecticide versions, no significant differences between them.

*Table 1*

**Dinamycs of the average number of eggs available on a bush**

| Var. | Product | Conc. (%) | Number of days after treatment | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 | 4 | 7 | 14 | 17 |
| Santé | | | | | | | |
| 1. | NeemAzal T/S | 0,33 | 29 | 4 | 0 | 0 | 0 |
| 2. | NeemAzal T/S | 0,41 | 21 | 9 | 0 | 0 | 0 |
| 3. | NeemAzal T/S | 0,5 | 16 | 0 | 6 | 0 | 0 |
| 4. | Laser 240 SC | 0,033 | 33 | 0 | 5 | 0 | 0 |
| 5. | Untreated | - | 43 | 40 | 27 | 25 | 12 |
| 6. | Calypso 480 SC | 0,4 | 7 | 0 | 17 | 0 | 0 |
| Claudiu | | | | | | | |
| 1. | NeemAzal T/S | 0,33 | 10 | 0 | 0 | 0 | 0 |
| 2. | NeemAzal T/S | 0,41 | 13 | 0 | 1 | 0 | 0 |
| 3. | NeemAzal T/S 0, | 0,5 | 8 | 0 | 0 | 0 | 0 |
| 4. | Laser 240 SC | 0,033 | 16 | 0 | 0 | 0 | 0 |
| 5. | Untreated | - | 21 | 0 | 20 | 15 | 0 |
| 6. | Calypso 480 SC | 0,4 | 14 | 0 | 27 | 0 | 0 |
| Magic | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1. | NeemAzal T/S | 0,33 | 0 | 0 | 0 | 0 | 0 |
| 2. | NeemAzal T/S | 0,41 | 0 | 0 | 0 | 0 | 0 |
| 3. | NeemAzal T/S 0,5% | 0,5 | 0 | 0 | 2 | 0 | 0 |
| 4. | Laser 240 SC | 0,033 | 0 | 0 | 0 | 0 | 0 |
| 5. | Untreated | - | 0 | 0 | 53 | 0 | 0 |
| 6. | Calypso 480 SC | 0,4 | 0 | 0 | 7 | 0 | 0 |

The variant treated with Calypso 480 SC is a decrease of laying eggs after 14 days in all varieties. Even if the product does not act ovicidal direct compensated by the number of larvae eradicated so that after 17 days of treatment, the average percentage of larvae from bush potato Santé and Claudiu was zero, except the variety Magic with an average load of a larva / bush.

The highest density of larvae cargo / plant (tab. 2), is in Santé, which is preferable to the other two, this observing and higher value of the attack: 17.0% (NeemAzal T / S 0.33%) and 11.0% of what was the second concentration used (fig. 1). At a concentration of 0.5%, the degree of attack in all three varieties was low, ranging between 6 and 7%. The lowest rate was recorded in Laser 240 SC and Calypso 480 SC (5%). NeemAzal T / S at 0.5% showed the lowest number of larvae and is recommended to be applied under production conditions.

*Table 2*

**Dynamics of the average number of larvae existing on a bush**

| Var. | Product | Conc. (%) | Number of days after treatment | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 | 4 | 7 | 14 | 17 |
| Santé | | | | | | | |
| 1. | NeemAzal T/S | 0,33 | 25 | 16 | 42 | 9 | 1 |
| 2. | NeemAzal T/S | 0,41 | 27 | 18 | 27 | 6 | 3 |
| 3. | NeemAzal T/S | 0,5 | 24 | 17 | 22 | 3 | 1 |
| 4. | Laser 240 SC | 0,033 | 43 | 3 | 5 | 4 | 1 |
| 5. | Untreated | - | 32 | 21 | 51 | 29 | 13 |
| 6. | Calypso 480 SC | 0,4 | 13 | 5 | 25 | 5 | 0 |
| Claudiu | | | | | | | |
| 1. | NeemAzal T/S | 0,33 | 18 | 9 | 18 | 5 | 2 |
| 2. | NeemAzal T/S | 0,41 | 15 | 3 | 9 | 3 | 2 |
| 3. | NeemAzal T/S | 0,5 | 21 | 3 | 8 | 3 | 1 |
| 4. | Laser 240 SC | 0,033 | 18 | 1 | 1 | 1 | 0 |
| 5. | Untreated | - | 21 | 29 | 38 | 26 | 10 |
| 6. | Calypso 480 SC | 0,4 | 2 | 1 | 22 | 2 | 0 |
| Magic | | | | | | | |
| 1. | NeemAzal T/S | 0,33 | 28 | 14 | 12 | 10 | 3 |
| 2. | NeemAzal T/S | 0,41 | 33 | 12 | 7 | 9 | 1 |
| 3. | NeemAzal T/S | 0,5 | 21 | 7 | 7 | 5 | 0 |
| 4. | Laser 240 SC | 0,033 | 4 | 0 | 3 | 1 | 1 |
| 5. | Untreated | - | 22 | 27 | 60 | 38 | 8 |
| 6. | Calypso 480 SC | 0,4 | 3 | 1 | 8 | 2 | 1 |

*b) The dynamic process of the Colorado beetle hatch after treatment with bioinsecticide*

In laboratory conditions, the first larvae hatched (20%) the day of the execution of the treatment ,were those treated with Laser 240 SC (13.06). These, after hatching the mobility is reduced and leaf consumption virtually nonexistent.

To NeemAzal T / S variants treated hatching process lasted two days.

After 2 days (16.09) mortality NeemAzal T / S ranged from 5.8 to 10.9 after a very low, surviving larvae were removed from potato leaves to the edge of the Petri dish, indicating this effect us repellent product.

The product Laser 240 SC percentage of mortality after two days after hatching was 100% (Fig. 2-b). The untreated variant, the percentage was 91.5% hatch, the larvae exhibiting at the outset a very intense feeding activity.

At variants of field treatment was executed on 06.09.2013 after previously determined number of eggs.

Compared to the untreated control larvae that hatch rate of 80% took place within two days, the plots treated with the products NeemAzal T / S, 60-70% hatching has been apparent for several days.

Of the two types of bioinsecticide product Lasser 240 SC 0.033% has a remarkable capacity to reduce adult population that is in the period of copulation, with an estimated 80-85% of their mortality.

## CONCLUSIONS

1. Regard to the dynamics of the deposition of eggs from all the three varieties (Santé, Claudius and Magic) in the variants treated with NeemAzal T / S at all the doses used ,there is a reduction in the deposition of eggs, reaching that after about a week ,number of egg by potato bush to be practically zero.

2. Laser 240 SC product was very good efficacy on clutch that came in contact with bioinsecticide, meaning that they were dry and there was a decrease in time deposits eggs radical variant treated.

3. In laboratory conditions the larvae hatch and mortality is extended to NeemAzal variants treated with T / S, compared to those treated with Laser 240 SC. This is verified in field conditions, where treatment with NeemAzal T / S, neeclozate clutch frequency was between 8 and 32% even after 7-8 days of the completion of hatching in the control plots.

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# RESULTS CONCERNING THE EFFECT OF SOME PLANT PROTECTION PRODUCTS IN THE CONTROL OF *ERIOSOMA LANIGERUM* SPECIES

## REZULTATE PRIVIND EFECTUL UNOR PRODUSE DE PROTECȚIA PLANTELOR ÎN COMBATEREA SPECIEI *ERIOSOMA LANIGERUM*

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***Abstract:*** *In 2014, at SCDP Iași we carried out researches regarding the chemical control of Eriosoma lanigerum species (wooly apple aphid). The experiment was performed on Generos breed and the tested products were MCW 2222, Mospilan, Movento and Actara .*
***Key words****: insecticides, phytoprotection, control, apple, wooly apple aphid*

***Rezumat****: În anul 2014, la SCDP Iași s-au efectuat cercetări cu privire la combaterea chimică a speciei Eriosoma lanigerum(păduchele lânos). Experiența s-a efectuat la soiul Generos iar produsele testate au fost: MCW 2222, Mospilan, Movento și Actara.*
***Cuvinte cheie****: insecticide,fitoprotecție, combatere,măr, păduchele lânos*

## INTRODUCTION

Wooly apple aphid is one of the most dangerous pests of apple tree, its attack resulting in the debilitation of trees and even their death. (Babuc *et al.,* 2013). Woolly aphid colonizes stems, branches, shoots and roots by stinging and sucking the tissue sap (Cârdei, 1992). The parts attacked hypertrophy and there appear swellings just like nodosities or cancer tumors. The attack may be seen by the presence of some whitish-waxy filamentous secretions at the level of wounds (Beșleagă and Cârdei, 2009; Paşol, 2007)

In recent years, the presence of this pest has been more significant. Thus, in 2014 we carried out researches for the chemical control of this pest.

## MATERIAL AND METHOD

The researches regarding the chemical control of wooly apple aphid were conducted by testing the efficiency of some plant protection products in the experimental polygon, on an apple tree plantation for Generos breed, trees that were planted at 4x3 m distance and guided on fan-shaped espalier.

We tested four products which also represented the experiment variants:

V1 – MCW 2222 – 0.250 l/ha

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V2 – Movento – 1.875 l/ha
V3 – Actara – 0.15 kg/ha
V4 – Mospilan – 0.3 kg/ha
V4 – Untreated control sample

Each variant contained 5 apple trees, each tree being considered as a repetition. Observations and determinations were carried out through sample drawing and analysis under the stereomicroscope of the samples harvested from each variant. 2. Observations were made 3 days, 7 days and 10 days, respectively after each treatment. The two treatments were made during the vegetation period, depending on insect biology. The efficiency of treatments was also influenced by the climatic conditions (table 1).

*Table 1*

**Climatic characterization of the yeaers 2014 – SCDP Iasi**

| Specification | Temperatura °C | | | Precipitations l/mp | No. raing days | U.R. % |
|---|---|---|---|---|---|---|
| | average | Absolute minimum | Absolute maximum | | | |
| january | -1,9 | -19,9 | 10,6 | 12,8 | 5 | 86 |
| february | -1,0 | -18,5 | 10,7 | 26,8 | 15 | 88 |
| march | 7,7 | -1,4 | 22,5 | 23,8 | 11 | 67 |
| april | 10,9 | -0,6 | 24,0 | 73,0 | 10 | 70 |
| may | 15,6 | 0,1 | 30,5 | 113,0 | 16 | 80 |
| june | 17,9 | 8,9 | 29,3 | 35,2 | 8 | 76 |
| july | 20,7 | 10,3 | 31,2 | 61,6 | 13 | 75 |
| august | 21,0 | 8,2 | 34,5 | 19,6 | 5 | 68 |
| september | 16,5 | 1,5 | 30,4 | 9,4 | 3 | 61 |
| octomber | 9,1 | -6,9 | 24,0 | 59,4 | 7 | 77 |
| november | 3,8 | -5,3 | 18,4 | 64,2 | 8 | 85 |
| december | -0,1 | -16,0 | 14,2 | 13,8 | 8 | 84 |
| Total | 10,0 | -19,9 | 34,5 | 512 | 109 | 76 |

The climatic conditions represent the factor having the highest influence on the evolution and attack of pests and pathogens of fruit trees. Thus, in 2014 these conditions were very favorable for the evolution of pests and pathogens.

We mention that high temperatures were recorded ever since the first months of the year which favored the outbreak of the first aphid colonies (21.03.2014). For example, in January-March interval, average temperatures ranged between -1.9$^{o}$C and 17.7$^{o}$C, and the maximum temperatures were between 10.9$^{o}$C (January) and 22.5$^{o}$C (March). During the vegetation period extremely high temperatures were also recorded favoring the evolution and development of the pest. Thus, in June-August, maximum temperatures were 29.3$^{o}$C in June and 34.5$^{o}$C in August. As it is generally known, Eriosoma lanigerum species may have up to 8-10 generations/year during the vegetation period.

## RESULTS AND DISCUSSIONS

*Eriosoma lanigerum* species hibernates in the larva state on trunks, frameworks and branches (Herțug, 1992). The biological reserve from the previous year was quite high and colonies located mostly on collar and roots. The active presence of the pest in the orchards was noticed as early as March (fig. 1).

Tests regarding the efficiency of plant protection products such as MCW 2222, Movento, Actara and Mospilan were conducted in August 2014. Treatments specific to the control of the wooly apple aphid were carried out until the application of treatments with the mentioned products.

**Fig. 1** - *Eriosoma lanigerum* (original)

The results regarding the efficacy of products tested to control the wooly apple aphid are given in table 2.

*Table 2*

**The results regarding the efficacy of products tested to control the wooly apple aphid (*Eriosoma lanigerum*)**

| Variety | Dose/ha | Dose 10l/aqua (1500 l solution) | Nr living aphides before tratament | Efficacy of treatments | | |
|---|---|---|---|---|---|---|
| | | | | 3 days | 7 days | 10 days |
| V1- MCW2222 | 0,250l/ha | 2,5 ml | 352 | 61,3 | 90,4 | 98,7 |
| V2 - Movento | 1,875l/ha | 18,75 ml | 310 | 50,1 | 85,2 | 96,4 |
| V3 - Actara | 0,15kg/ha | 1,5 g | 294 | 75,4 | 84,6 | 95,2 |
| V4 - Mospilan | 0,3kg/ha | 2,5 g | 323 | 57,2 | 83,0 | 91,4 |
| V5 – Control untrated | | | | | | 9,5 |

Two phytosanitary treatments were applied during the vegetation period, namely the first treatment was applied on August 4$^{th}$ and the second on August 15$^{th}$.

The observations regarding the efficacy of treatments were made 3, 7 and 10 days respectively after the treatment by registering the number of living and dead aphids (fig. 2 and 3).

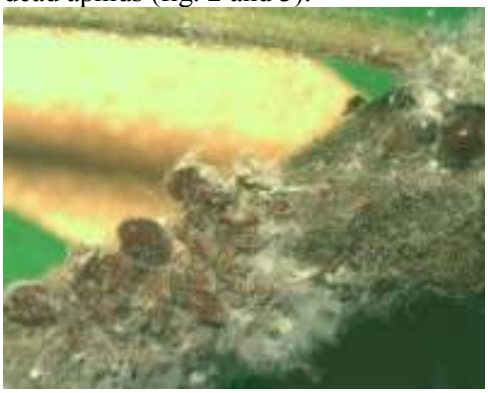

**Fig. 2** - *Eriosoma lanigerum* – living aphids (original)

**Fig. 3** - *Eriosoma lanigerum* colonies of dead aphides(original)

The data in the table attest the value of products used to control the wooly apple aphid. From the determinations made, the best results were registered by Actara insecticide 3 days after application which was applied in a concentration of 0.15 kg/ha, and having a 75.4% efficacy as compared to Movento product whose efficacy was 50.1%, since it has a slower action.

7 days after the treatment, the highest efficacy of 90.4% was registered by MCW 2222 product applied in concentration of 0.250 l/ha, as compared to Mospilan that had an efficacy of 84.6%.

10 days after the treatment, the best results were registered by MCW 2222 insecticide having an efficacy of 98.7%, followed by Movento with an efficacy of 96.4%, an insecticide characterized by double system and long-term efficiency. Comparable results were obtained by Actara product that also had an efficacy of 95.2% and Mospilan insecticide of 91.4%.

The results obtained attest the value of the products tested to control the wooly apple aphid, as compared to the untreated control sample, where the efficiency percentage was 9.5% and whose mortality was caused by the parasitic wasp *Aphelinus mali*.

## CONCLUSIONS

The climatic conditions registered in 2014 influenced the evolution and attack of *Eriosoma lanigerum* species.

To control the wooly apple aphid we tested the newest plant protection products such as MCW 2222, Movento, Actara and Mospilan. Following the determinations carried out, they had a high efficiency with a percentage ranging between 91.4% and 98.7%. 3 days after the treatment the best results were obtained by Actara insecticide whose efficiency was 75.4%, and 10 days after the treatment MCW 2222 had a high efficiency of 98.7%, followed by Movento with 96.4%.

All products tested to control the wooly apple aphid had a very good efficiency, a thing that recommends them to be used on a large scale in the apple tree phytoprotection.

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# RESEARCH ON THE ENTOMOFAUNA STRUCTURE OF INVERTEBRATES BELONGING TO A PLUM ORCHARDS OF FRUIT TREES STATIONARY FROM IAŞI

## CERCETĂRI CU PRIVIRE LA STRUCTURA ENTOMOFAUNEI DE NEVERTEBRATE APARȚINÂND UNEI PLANTAȚII POMICOLE DE PRUN DIN BAZINUL POMICOL IAŞI

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***Abstract**: In this paper, we bring new contributions to the knowledge of invertebrate species in a plum plantation fruit growing in the Bârnova county from Iaşi.Harvesting the material was made between May and September throughout the research period, use to traps type Barber. The biological material collected were identified and determined all species collected.Throughout the period of observations were collected a total of 1397 of pests and useful samples, so 2013 were collected 317 belonging to a number of 33 species in 2014 were collected 338 belonging to a number of 33 species and 2015 were collected 742 specimens belonging to 37 species numbers.*

*Analyzing the collected material it was found that the most commonly collected species were Carabus coriaceus L., Pseudophonus rufipes Mull., Opatrum sabulosum L., Phyllotreta vittula F., Phyllotreta nemorum L., Formicomus pedestri, Harpalus distinguendus Duft., Tomoxia bigutatta, Hymenoptera, Gastropoda and Orthoptera.*
***Key words:** invertebrate species, traps type Barber, plum.*

***Rezumat:** În lucrarea de față se aduc noi contribuții la cunoaşterea speciilor de nevertebrate dintr-o plantație pomicolă de prun din comuna Bârnova din jud. Iaşi.Colectarea materialului s-a făcut în perioada mai- septembrie, pe tot parcursul perioadei de cercetare, cu ajutorul capcanelor de tip Barber. Din materialul biologic colectat, au fost inventariate şi determinate toate speciile colectate.Pe întreaga perioadă de observații, au fost colectate un număr de 503 exemplare de insecte dăunătoare şi utile,astfel în anul 2013 au fost colectate 317 aparținând la un număr de 33 de specii, în anul 2014 au fost colectate 338 aparținând la un număr de 33 de specii, iar în anul 2015 au fost colectate 742 de exemplare aparținând la un număr de 37 de specii. Analizând materialul colectat s-a constatat că, speciile cel mai frecvent colectate au fost: Carabus coriaceus L., Pseudophonus rufipes Mull., Opatrum sabulosum L., Phyllotreta vittula F., Phyllotreta nemorum L., Formicomus pedestri Rossi, Harpalus distinguendus Duft., Tomoxia bigutatta, Himenoptere (viespi), Gastropode (melci), Orthoptere (lăcuste), Orthoptere (Gryllus).*
***Cuvinte cheie**: speciil de nevertebrate, capcane de tip Barber, prun.*

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## INTRODUCTION

The plum culture intensification in Europe took place in the eighteenth century that it is assumed that the same conditions it has developed more in the Romanian countries.

In the intensive orchards was observed that it may produce abundant and constant in the first years, with pronounced adaptability to various climatic zones, is rustic and sturdy enough, without the need of pollinators in particular.

The pest and disease control is a vital segment in plum culture technology. Failure to follow this technological links may compromise the total production in that year, and in coming years.

## MATERIAL AND METHOD

The collection was done using soil traps type Barber in each of the 3 years of observations from May to September in a plum plantation at Stanley variety, Pietrarie stationary.

We can use it as a trap pot of soil to 400 or 800 ml pot which are buried in the ground up to the top so that it is level with the superficial layer of the soil. Above pot setting a cap designed to protect trap rainfall penetration inside.

Location traps may be in line at equal distances between them, diagonally, or evenly over the entire research.

They have used plastic boxes with a volume of 500 ml, having a diameter of 10 cm and a height of 8 cm, and the fixing liquid was used as a solution of formalin in a concentration of 4% (Fig. 1) (Talmaciu, 2004, Talmaciu, 2001).

**Fig.1. Soil trap type Barber**

In studied stationary, they were placed by 6 traps on two rows, installed from from the margin to the inside in a straight line at a distance of 10 m between rows and 6-8 m between traps to row. At harvest contents of the box was placed on a sieve and collected material was collected in bags of mull and then was brought into the lab, separate traps, whichever is all collected species. The research are made to continue in 2012 and 2013 to establish a structure of parasitic and predatory insect species, and useful depending on the parking surveys were conducted and by default depending on the degree of pollution. They were determined and inventoried.

## RESULTS AND DISCUSSIONS

The summary of results obtained in Pietrarie stationary in 2013**.**

In 2013, in Pietrarie stationary (Table 1) were collected a total of 49 species with a total of 733 samples. Of the 50 species collected, a total of 14 species were common in the two years. They were: *Dermestes laniarius*, *Heteroptera* (bedbugs), *Polydrosus sericeus* Schall, *Homoptera* (cycads), *Harpalus distinguendus*, *Hymenoptera* (wasps), *Harpalus calceatus* Duft., *Anisodactylus binotatus* F., *Ontophagus ovatus*, *Homoptera* (aphids), *Calathus fuscipes* and *Carabus coriaceus*. The specie with the largest number of samples collected were: *Araneida* 103 samples, *Hymenoptera* (wasps) with 100 samples, *Ontophagus ovatus* and *Hymenoptera* (bees) with 88 samples *Orthoptera* and *Gastropoda* each with 78 samples, *Homoptera* (cycads) 62 samples, A*nisodactylus binotatus* F 57 samples *Opatrum sabulosum* and *Hymenoptera* (ants) with the 39 copies *Pseudophonus rufipes*, *Galeruca pomonae* and *Orthoptera* (*Gryllus*) with every 37 samples, *Heteroptera* (bedbugs) 35 samples, *Carabus scabriusculus* with 33 samples each, *Harpalus calceatus* Duft 31 samples, *Diptera* (adults) with 29 samples, *Polydrosus sericeus* Schall. 26 samples, *Homoptera* (aphids) and *Lepidoptera* (larvae) with 24 samples, *Tomoxia biguttata* 23 samples, *Dermestes laniarius* with 20 samples, (Chatened du Gaetan, 1990, Panin I., 1951). The other species had between 1 and 19 samples.

The structure, dynamics and abundance of species collected of research period 2013-2015 in Pietrarie stationary

*Table1*

**The structure research species collected inresearch period from Petrarie stationary**

| No. | Name of species | Pietrarie stationary/ Stanley variety | | | Total number |
|---|---|---|---|---|---|
| | | 2013 | 2014 | 2015 | |
| 1. | *Dermestes laniarius* | 18 | - | 2 | 20 |
| 2. | *Heteroptera(bedbugs)* | 30 | 2 | 3 | 35 |
| 3. | *Polydrosus sericeus Schall* | 13 | 4 | 9 | 26 |
| 4. | *Coccinella septempunctata* | | - | 5 | 5 |
| 5. | *Homoptera(cycads)* | 12 | 6 | 44 | 62 |
| 6. | *Polydrosus amoenus Schall* | 12 | - | - | 12 |
| 7. | *Tomoxia biguttata* | 8 | - | 15 | 23 |
| 8. | *Amara aenea* | | 12 | 6 | 18 |
| 9. | *Harpalus distinguendus* | 14 | 15 | 28 | 57 |
| 10. | *Hymenoptera (wasps)* | 1 | 31 | 68 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| 11. | *Necrophorus vespillo L.* | 2 | - | - | 2 |
| 12. | *Harpalus calceatus* | 9 | 5 | 17 | 31 |
| 13. | *Armadillidium vulgare* | 9 | - | - | 9 |
| 14. | *Anisodactylus binotatus F.* | 27 | 14 | 16 | 57 |
| 15. | *Galeruca pomonae* | | 5 | 32 | 37 |
| 16. | *Stomodes gyrisicollis* | 3 | - | - | 3 |
| 17. | *Anisodactylus signatus* | | 6 | - | 6 |
| 18. | *Ontophagus ovatus* | 55 | 6 | 27 | 88 |
| 19. | *Carabus scabriusculus* | 6 | - | 27 | 33 |
| 20. | *Ontophagus taurus* | 15 | - | - | 15 |
| 21. | *Silpha obscura* | 3 | - | - | 3 |
| 22. | *Hister purpurascens* | 9 | - | - | 9 |
| 23. | *Homoptera(aphids)* | 2 | 2 | 20 | 24 |
| 24. | *Coccinella 14 punctata* | 6 | - | 3 | 9 |
| 25. | *Ceuthorrhynchus crucifer* | 5 | - | - | 5 |
| 26. | *Calathus fuscipes* | 6 | 3 | 3 | 12 |
| 27. | *Pseudophonus rufipes Mull.* | 8 | 29 | - | 37 |
| 28. | *Blaps lutifera* | 2 | - | - | 2 |
| 29. | *Opatrum sabulosum* | 3 | - | 36 | 39 |
| 30. | *Calopus serraticornis* | 4 | - | - | 4 |
| 31. | *Carabus coriaceus L* | 4 | 3 | 3 | 10 |
| 32. | *Pseudophonus griseus* | 15 | 3 | - | 18 |
| 33. | *Notiophilus palustris* | 2 | - | - | 2 |
| 34. | *Obera oculata* | 1 | - | - | 1 |
| 35. | *Ptosima 11 maculata* | 3 | - | - | 3 |
| 36. | *Ontophagus semicornis* | 8 | - | - | 8 |
| 37. | *Dorcadion fulvum* | 8 | - | - | 8 |
| 38. | *Himenoptere albine* | | 17 | 71 | 88 |
| 39. | *Ortoptere (lacusta)* | | 24 | 54 | 78 |
| 40. | *Araneida* | | 28 | 75 | 103 |
| 41. | *Galeruca tanaceti* | | 6 | - | 6 |
| 42. | *Lepidoptere larve* | | 10 | 14 | 24 |
| 43. | *Diptere (adults)* | | 12 | 17 | 29 |
| 44. | *Ortoptera* | | 14 | 23 | 37 |
| 45. | *Himenoptere (ants)* | | 23 | 16 | 39 |
| 46. | *Lepidoptere (adults)* | | 2 | 8 | 10 |
| 47. | *Silpha obscura* | | 12 | - | 12 |
| 48. | *Gastropoda* | | 15 | 63 | 78 |
| 49. | *Phyllotreta vittula* | | 5 | - | 5 |
| 50. | *Podagrica fuscicornis* | | 9 | 3 | 12 |
| 51. | *Dibolia nitida* | | 3 | - | 3 |
| 52. | *Mordella aculeata* | | 4 | - | 4 |
| 53. | *Colembola* | | 3 | | 3 |
| 54. | *Coleoptere (larva)* | | 6 | 3 | 9 |
| 55. | *Agabus affinis* | | | 3 | 3 |
| 56. | *Anatis ocellata* | | | 9 | 9 |
| 57. | *Diptere larve* | | | 16 | 16 |
| 58. | *Dermestes lardarius* | | | 10 | 10 |
| 59. | *Copis lunaris* | | | 5 | 5 |
| 60. | *Cantharis fusca* | | | 5 | 5 |
| **Total 60 species** | | **323** | **339** | **759** | **1421** |

In the research period (2013-2015) were collected 1421 samples belonging to 60 species. The highest number of collected samples was recorded in 2015 with the 795 samples and copies lowest number was recorded in 2013 (323).

In 2014, were collected 339 samples.

A total of 12 species were collected during the three years of research, they were *Dermestes laniarius*, *Heteroptera* (bugs), *Polydrosus sericeus*, *Homoptera* (cycads), Harpalus distinguendus, Hymenoptera (wasps), Harpalus calceatus, *Anisodactylus binotatus*, Ontophagus ovatus, Homoptera (aphids), Carabus coriaceus and *Calathus fuscipes*.

A total number of 27 species (Rogojanu V., Perju T., 1979) were collected in a single year as: *Coccinella 7punctata, Polydrosus amoenus, Necrophorus vespilo, Armadillidium vulgare, Stomodes gyrosicollis, Anisodactylus signatus, Ontophagus taurus, Silpha obscura, Hister purpurascens, Blaps lutifera, Obera oculata , Phyllotreta vittula, Mordella aculeata, Agabus affinis,.Dermestes lardarius, Copis lunaris* and *Cantharis fusca*.

The number of species and collected samples in research period of Stanley variety (Table 2)

In 2013 was collected from Pietrarie Stanley variety stationary 323 samples belonging to a number of 33 species;

In 2014 the number of ssamples collected from the Stanley variety from Pietrarie stationary was 339 samples belonging to 33 species numbers;

In 2015 the total samples collected was the759 samples at Stanley variety from the Pietrarie stationary and belong to 36 species.

*Table 2*

**The number of species and collected samples during the period of observations**

| Harvested year | Pietrărie Stanley | |
|---|---|---|
| | No. of samples | No. of species |
| 2013 | 323 | 33 |
| 2014 | 339 | 33 |
| 2015 | 759 | 36 |
| Total | 1421 | |

In total in the Pietrarie stationary at Stanley variety in research during were collected 1421 insect samples belonging to useful and harmful entomofauna of plum plantations.

## CONCLUSIONS

1. In the Pietrarie stationary was studied using soil traps type Barber invertebrate fauna belonging to different classes and phyla, most collected species belonging to the class Hexapoda(insects);

2. The collection of biological material has been made in each of the three years 2013, 2014, 2015 throughout the growing season (May to September), sampling being made at intervals contained in most cases between 10 and 20 days

3. The method of biological material collecting was the method of the soil traps type Barber to identify to determine the structure and dynamics of epigenous entomofauna plum orchards.

4. The investigations was conducted in the PIETRARIE stationary at Stanley variety were collected in total 1421 samples. In 2013 was collected 323 samples belonging to 33 species. In 2014 the total number of 339 samples have belonged to a number of 33 species. In 2015 we recorded a total of 759 samples belonging of 36 species.

***Acknowledgements**: This paper was published under the frame of European Social Found, Human Resources Devlopment Operational Programe 2007-2013, project no. POSDRU/159/1.5/S/132765.*

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# THE IMPORTANCE OF RESEARCH ON THE MOLECULAR LEVEL APPROACH ON PLANT DEFENSE AGAINST PHYTOPHAGUS

## IMPORTANȚA ABORDĂRII CERCETĂRILOR LA NIVEL MOLECULAR PRIVIND APĂRAREA PLANTELOR ÎMPOTRIVA FITOFAGILOR

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***Abstract:*** *To deal with at the biotic stress produced by the attack of phytophagous insects and pathogens have developed mechanisms (signaling molecular, such as phytohormones - jasmonate) to alert all of the plant via a ion transmission, fast, like the nervous system of animals organisms to trigger the some defensive defense. These triggered as a result of complex interactions, many plant hormones, transcription of many genes and sensitive to these signals. The following presents the research carried out in recent decades that have revolutionized knowledge of plant defense mechanisms against fotofage species through the discovery of the role of phytophagous jasmonate. Jasmonates (JAS) are the main signals for adjusting resistance to phytophagous insects, representing new weapons and quick responses against insect attack produced. The relationship trophic plant-fitofagus (insect in this case) -entomophagus, attacked plants increasingly volatile launches more, as the plant is attacked and attract the entomophagous parasitoids. Although ethylene, salicylic acid and other plant hormones play an important role in plant-insect interaction and contribution to the emergence of resistance to these signals what appears to be minor in comparison with jasmonic acid . Using the sequenced genomes plant, as Arabidopsis thaliana, tomatoes, tobacco etc, the mutant gene silencing may clarifies the of chain signals and biotic stresses preparedness measures and inducing defense.*
***Key words****: the entomophagous, parasitoids, jasmonates*

***Rezumat:*** *Pentru a face față la stresul biotic produs de atacul unor insecte fitofage şi patogeni, s-au dezvoltat mecanisme (semnalizare moleculară, cum ar fi fitohormonii – jasmonați) care alertează toată planta, printr-o transmisie ionică, rapidă, asemănătoare sistemului nervos din organismele animale, în vederea declanşării unor apărări defensive. Acestea declanşate în urma interacțiuni complexe, a multor fitohormoni, şi a transcripției a numeroase gene sensibile la aceste semnale.În cele ce urmează prezentăm cercetări realizate în ultimele decenii, care au revoluționat cunoaşterea mecanismelor de apărare a plantelor împotriva speciilor fitofage, prin*

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*descoperirea rolului fitohormonilor jasmonați. Jasmonații (JAs) sunt principalele semnale pentru reglarea rezistenței la insectele fitofage, constituind arme noi şi răspunsuri rapide împotriva atacului produs de insecte. Prin relația trofică plantă-fitofag (insectă în cazul de față) –entomofagi, plantele atacate lansează volatile din ce în ce mai multe, cu cât planta este atacată şi atrag speciile de entomofagi parazitoizi. Deşi etilena, acidul salicilic şi alți fitohormoni au un rol important în interacțiunea plantă-insectă, contribuția acestor semnale în apariția rezistenței apare să fie minoră în comparație cu a acidului jasmonic. Utilizarea plantelor cu genomurile secvenționate, ca Arabidopsis thaliana, tomate, tutun etc, prin mutante cu gene silențiate sau în superexpresie pot aduce clarificări în lanțul semnalizărilor stresurilor biotice şi măsurile de pregătire şi de inducere a apărărilor.*
***Cuvinte cheie**: entomofagi, parazitoizi, jasmonati.*

## INTRODUCTION

Particularly prolific through a stage in the development of basic and applied research at the molecular level. Technology developed in recent decades gives us another dimension on the phenomenology that takes place at the microcosmic level of ultrastructural cellular chromatography, HPLC site and PCR - site, highlighting the nano-molecules, enzymes, metabolites, and transcription of the genes by microarray technology.

These modern means of investigation have created a tumult in the scholarly world and move on from experimentation aimed at biological activities at macro level intrusion in the privacy of phenomena vital, so that the plant be seen as a body extremely complex, with activities very coordinated by various plant hormones. There are new branches of science dealing Molecular, ending with the "omic" such as genomics, proteonomica, metabolomics, transcriptomics, lipidonomica etc preoccupied research all phenomena that occur at the molecular level, factors coordinators outbreak these biological processes. For these achievements in the development of various branches of science and research in the area have benefited entomological, fundamental research and management of insects in different ecosystems, especially agriculture and forestry.

To deal with the stress biotic produced by the attack of phytophagous insects and pathogens have developed mechanisms (signaling molecular, such as phytohormones - jasmonate) to alert all of the plant via a transmission ion, fast, like the nervous system of organisms animals to trigger some defensive defense.

The use of modern equipment would not have the desired success if it would not use plants with genomes sequenced as Arabidopsis thaliana, tomato, tobacco, mutant gene silencing or overexpressing, who clarified the chain of signs stress biotic and measures training and induction of defense.

The following presents the research carried out in recent decades that have revolutionized knowledge of plant defense mechanisms against fotofage species through the discovery role of jasmonate plant hormones.

## MATERIAL AND METHOD

*The evolution of research on jasmonate (JA).*

Not long ago, in 1962, it was isolated Jasmonic acid methyl ester (Meja) Jasminum grandiflorum volatile oils L. (Demo *et al.,* 1962), *Rosmarinus officinalis* L. He then there was a long period of time up to jasmonatul determined that acid and methyl Jasmonic are omnipresent, they are superior plant growth regulators, responding to external stimuli by the expression of genes.

A beneficial step in characterizing the role jasmonaților mutants of *Arabidopsis* was used thaliana, then tomato and tobacco, acid biosynthesis study Jasmonic phases, the transduction of injury, signaling pathways, etc. using mutants in overexpressing or silencing of genes dependent JA sets.

It was established that the enzymes that converge 12$^{th}$ OPDA acid (12-oxo Phytodienoic) and dnOPDA (dinor-OPDA), also called octadecanoizi are acid precursors acid Jasmonic and present in many plants, indicating that it may be a metabolic pathway general plants (Vick and Zimmerman, 1984). Jasmonații are oxilipine, signaling molecules derived from oxygen and polyunsaturated fatty acids are widespread in living organisms. In 1990, Farmer and Ryan, that as a result of injuries caused by a fitofagus by methyl-jasmonatului activity (Meja) in tomato plants occurs accumulation of protease inhibitors (PIN2) that disrupts digestion in the stomach of insects. The same phenomenon is happening and applying air treatment with Jasmonic acid methyl ester (Meja). Subsecvențional, we studied gene expression and induction changing pattern JA metabolites (fig. 1), the study of protein deposits accumulate in vegetative (Staswick *et al.,* 1992) and alkaloids (Gundlach *et al.,* 1992).

Another study phase is cloned enzyme biosynthesis acid Jasmonic, alenoxid synthase (AOS), laboratory Brash (Song *et al.,* 1993) and the isolation of the first mutant insensitive to JA (from the plant Arabidopsis thaliana) in the laboratory of Turner (Feys *et al.,* 1994). Further investigations were performed, using the mutants for determining the factors receiving signals injury and transcription of the genes for the synthesis of jasmonaților and other plant hormones that act ethylene and salicylic acid and, finally, transcription factors, gene-dependent jasmonate, role in inducing defense and "preparation" (priming) plants.

In the last 20 years the Jasmonic acid (JA) and its metabolites, such as its methyl ester (Meja) and acids conjugated acid Jasmonic were included under the term jasmonate, recognized as the first signs of an injury and the defense against biotic (Wasternack, 2006).

Following the attacks occur defensive answers - through induction of resistance: the synthesis of proteins, such as PIN, which are harmful to insect feeding; fitoalexine synthesis, such as nicotine or other alkaloids; synthesis and release of volatile compounds (VOCs) that attract parazitoizii and predators (tritrofice relations plant-plant feeder-parasite); the formation and release of green leaf volatiles (GLVs); formation of honeydew nectar (EFN); mechanical strategies to increase the cell walls (Wasternack, 2006). Also launching volatile place and preparation (priming) or being prepared (primed state) organs are not challenged or neighboring plants.

Unlike defenses that have a genetic component, ie the production of toxic alkaloids, which is produced by continuously energy consumption of the plant, preparation is the most inexpensive, metabolic energy and thus allocated for growth and multiplication and maintaining populations as numerous .


**Fig. 1 -** Relationship-parasite trophic plant-plant feeder. The attack of thentredinid of the *Pteronidea melanopsis* who attract ichneumonids entomophagus *Bassus* sp. (original).

Unit living organisms, plant and animal, is highlighted by the presence oxilipinelor, signaling molecules derived polyunsaturated fatty acids and oxygen. In the animal world, eicosanoids are similar jasmonaților and function as signaling molecules in vertebrates, invertebrates and eukaryotic microbes.

Answers defensive ways Jasmonic acid constituent is a conserved mechanism for promoting defense responses in many phytophagous arthropods (Howe and Jander, 2008). Although ethylene, salicylic acid and other plant hormones play an important role in plant-insect interaction, the contribution of these signals the emergence of resistance appears to be minor in comparison with acid Jasmonic (Koornneef and Pieterse, 2008; Zheng and Dicke, 2008). Many experiments reveal that jasmonații (JAS) are the main signals for adjusting resistance to phytophagous insects. After Browse and Howe (2008), jasmonații (JAS) are new weapons, with quick responses against insect attack produced.

## RESULTS AND DISCUSSIONS

*As primary metabolites and defensive signals.*

Protection of plants against phytophagous triggers changes both in the primary and secondary metabolism. Basically there is a channeling of resources and energy in reducing attack produced, and the possibilities that have resources and to ensure multiplication of the population. The responses of plant resistance to insect feeding is coordinated by various signaling pathways that depend on primary metabolism. In addition, the integration of different signals induced by insect injury and provocateurs specific results from a complex of primary and secondary metabolism rearranged (Schwachtje and Baldwin, 2008). JA has a key role in signaling kinases defensive and

calls WIPK and WRKYs Sipka and transcription factors.

Recently, the role of sugar signaling is assessed, as they have found many resistance genes induced by sucrose. For example, sucrose (Suc), glucose (Glc) and fructose (Fru) plays role in inducing the expression of specific regulatory signals of injury Glycine max; storing a probable role of defensive proteins sucrose it is also induced by Jasmonic acid. Moreover, transcripționarea of hexokinases that can function as a sensor or repressor photosynthetic is induced injury, and is responsive to threalozе-6-P, which itself is involved in the regulation feedback photosynthesis transitions development (Ramon and Roland, 2007). Trehalozele proteins SnRK kinases have been shown to interact, as they have sugar and lectin-induced JA, suggesting that lectins play a role in signal transduction.

Many metabolites that play a role in primary metabolism has thoroughly studied defensive positions. Dual functions of them have been discovered due to the accumulation in plants at high levels or patterns that their induction by herbivores attacks, are similar defensive secondary metabolites. For example threonine deaminase, an enzyme that works to degrade threonine, leading to the hypothesis that operates in essential amino acids degrade insect stomach, causing hunger amino acids. Two isoforms are known threonine, one stable insect stomach and the S. lycopersicum N. attenuator with double functions in primary and secondary metabolism.

High levels of oxalate, calcium (Ca), a primary metabolite, is accumulated in the plant (more than 80% of the dry matter), some synthesis is induced by herbivores. Adjusts the size of the calcium oxalate Ca levels, which are involved in cell signaling and in various biochemical processes. Crystals can be placed around the tissues, eg vascular bundles, producing a physical barrier against insects sucking the abrasive effect of blunting the mandibles. TAC defensive role play and anti-nutritive intake by decreasing feed conversion efficiency. Also, proteins and lectins stored vegetative has a dual role in primary metabolism and resistance, some acid-induced Jasmonic (Zhu *et al.,* 2008).

The knowledge of the molecular phenomena can not be achieved without the use of proper laboratory techniques, improved in recent decades, supported by bioinformatics. Observations at the macro level, biometric evaluations, size, weight etc can not satisfy us. Using the genomes sequenced plant, *Arabidopsis thaliana* as tomatoes, tobacco etc, the mutant gene silencing or overexpressing may clarify the signaling chain and biotic stresses preparedness measures and inducing defense. Research present and future to establish signaling pathways of the biotic environment of receptor proteins stimulated the factors connecting the nodes of interference of various plant hormones, to decision makers in triggering transcripts (activation sets genes dependent on plant hormones, plant defense default) in the production of secondary metabolites involved in defense.

In preparing students during the license, but especially by masters must increase the proportion of subjects with levels of molecular genetic approaches, biochemical, environmental, etc., for a real knowledge of the phenomena of life.

## CONCLUSIONS

1. Entry into the molecular size is the only way to develop fundamental research and practical interest insect management.

2. Jasmonații (JAS) are the main signals for adjusting resistance to phytophagous insects, representing new weapons and rapid responses against insect attack produced.

3. Biotic receptors signals by connecting factors mediating or regulating plant hormones biosynthesis genes and their expansion biosynthesis of secondary metabolites mediate transcription. These events include a reconfiguration transcriptomic with changes in gene transcription levels of defensive and growth; release of volatile organic compounds (VOCs) that functions as an indirect defense and disturbing accumulation of secondary metabolites herbivores or pests nicotine, trypsin and protease inhibitors (TPIs).

4. Jasmonates plays an important role in the production of protease inhibitors (PIN2), resulting in overproduction of digestive proteases in the insect stomach, and reducing the growth of essential amino acids exhausted. Reducing combinations increase may be due to toxic effects of anti-nutritive or antifeedant.

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# THE TREE. SIMBOL AND REPRESENTATION IN THE ARCHITECTURE OF THE SACRED SPACE

## ARBORELE. SIMBOL ȘI REPREZENTARE ÎN ARHITECTURA SPAȚIULUI SACRU

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***Abstract.*** *The tree is an element present from the beginning of the world, a symbol of verticality, the world's axis linking in the same substance heaven, earth and hell. Fountain of thought, the tree summarizes its entire importance as the main symbol of the Mother Goddess, of natural regeneration and of time's renewal. Viewed from the perspective of the transcendence of temporary cyclical death, the tree can be regarded as the «Tree of Life». This study aims at identifying and decoding the representations of the «tree» in the Christian architecture of the sacred space: the Forest, the Cross-Tree, the Tree with the roots in Heaven, etc.*
***Key words:*** *symbol, tree, forest, cross-tree, sacred-space, sacred architecture*

***Rezumat.*** *Copacul sau arborele este un element prezent încă de la începuturile lumii, simbol al verticalității, axă a lumii care leagă în aceeași substanță cerul, pământul și infernul. Izvor al gândirii, arborele rezumă întreaga importanță a lui, ca principal simbol al zeiței Mame, al regenerării naturii și al reînnoirii timpului. Arborele privit din perspectiva transcenderii morții ciclice temporare, poate fi considerat ca «Arbore al vieții». Acest studiu urmărește identificarea și decodificarea reprezentărilor «arborelui» în arhitectura spațiului sacru creștin: pădurea, arborele-cruce, arborele cu rădăcinile în cer, etc.*
***Cuvinte cheie: simbol,*** *arbore, copac, pădure, copacul-cruce, spațiu sacru, arhitectură sacră*

## INTRODUCTION

The tree is an element present from the beginning of the world, a symbol of "*verticality*", *the world's axis* linking in the same substance heaven, earth and hell. Placing it in the position of "*Fountain of thought"* (Behaeghel, 2010), the tree summarizes its entire importance as the main symbol of the Mother Goddess, of natural regeneration and of time's renewal. By excellence the tree is a vertical existence, *"the man thinks with it or through it"* (Behaeghel, 2010) and viewed from the perspective of the transcendence of cyclical and temporary death, it can be considered being the *"Tree of Life"*.

Christian sacred space has an architectural component at the same time a symbolic one. The symbol implies representation, being most often a mark or an image associated with a few words or abbreviations of the item that it

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represents. Symbols have always helped in finding or identifying certain values, beliefs, ideas or creed. (Cehan and Gheorghiță, 2014)

## MATERIAL AND METHOD

"The tree" or "the forest" appear frequently represented in the architecture of Christian sacred space, not only as decorative motifs, but as a defining element of the space. This paper identifies and interprets these representations of the tree and the forest in the architecture of sacred space. As research methods were used: theoretical and photographic documentation, observation and case study.

## RESULTS AND DISCUSSIONS

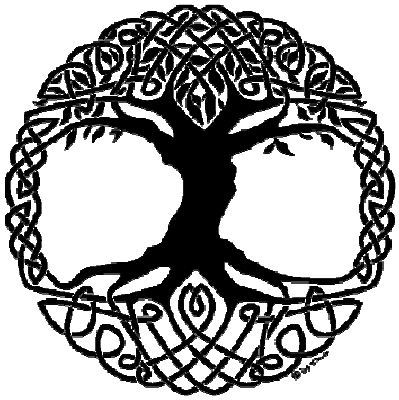
Because there is a universal link expressed through analogic solidarity of the symbolic chain represented by *Axis Mundi, the Cosmic Tree – the Cross - Universal Man,*the model represented by the symbolic geometry *the Tree with the arms into the sky* (Fig.1) describe best the relationship between macrocosm and microcosm.

Both in the East and the West, the universe is sometimes represented by an inverted tree rooted in the sky and with branches spread all over Earth (Chevalier and Gheerbrant, 1994).This reversal might have its origin in a certain conception of the role of the sun and the light in the development of beings: they draw their life from above, from the sky, and try to get it down on Earth.Hence, the overturning of images: the branches act as roots and the roots act as branches. Life comes from the sky and enters the Earth. According to René Guénon, this tree grows beyond the cosmic world, reversed, as a reflection in the mirror.What is at the top or above the surface of the water is reflected down and backwards. In Hindu symbolism, the roots of the inversed tree appear as a principle of manifestation, and the branches, as development of these. René Guénon discerns here a different meaning: the tree stands above the reflection plane, that limits the reversed cosmic domain from below; it goes beyond the limits of manifestation entering the sphere of reflection, in which it insert the inspiration (Guénon, 2008). The Hebrew esoterism resumes this idea: *the Tree of Life extends from top to down and the sun illuminates it entirely*. In Islam, the Tree of Happiness plunges its roots into the last sky and spreads its branches over and under the Earth (Guénon., 2008).In Chinese symbolism, this tree has its branches anastomosed so that their ends get together in pairs to reveal the synthesis of opposites, or the resorption of duality in unity. Thus we find either a unique tree whose branches are splitting and joining, either two trees having the same root, but united through their branches (Guénon, 2006).

Fulcanelli emphasizes - before moving to decrypt the proper alchemical symbolism, that in Gothic cathedrals (Fig. 2), there were many elements that transcended the Christian religion, some being downright pagan: *"The bold vaults, the nobility of the vessels, the magnitude of the proportions and the beauty of construction make the cathedral an original work, a unequaled harmony, that*

*the sequencing of liturgies fails to fill it entirely. If the silence and the introspection under the spectral and colorful light and of the tall stained glass urge to prayer and drive to meditation, the inner curdling, the structure, the ornaments scatter and reflect as a unprecedented force, some feelings not just pious, a more laic spirit and, to call it by name, almost pagan."* (Fulcanelli, 2005)

**a)**



**b)**

**Fig. 1** - a) – The Walnut Tree reversed, Poitier, sec. XVI, France (*https://blogostelle.files.wordpress.com/2015/02/daprc3a8s-le-thc3a8me-de-larbre-renversc3a9-enseigne-du-noyer-inversc3a9-poitier-vers-xvie-sic3a8cle-illustration-marsailly-blogostelle.jpg?w=600&h=450*)
b) – The Tree in the mirror *(http://38.media.tumblr.com/4c24e17575eb1bd358427af56386d220/tumblr_mf1ewefGOJ1rlphuqo1_500.gif)*

**a)**

**b)**

**Fig. 2 -** a) - Santa Maria dos Jeronimos Church, Lisbon (*http://www.madeleine-et-pascal.fr/IMG/jpg/DSC_0462-3.jpg*)
**b) –** The Tree Roots *(https://writervsworld.files.wordpress.com/2015/01/tree-root-photo-1.jpg)*

The forest is an archetypal symbol, associated to the natural temple as exuberance of life, but also to the dangers of fallacies or of death. In the model of specific archaic mentalities, the forest or the wooded mountain occupy an

intermediary role in the triad of *water, forest/mountain* and *sky* or between *the here* and *the beyond*. The forest represents the pre-human nature, symbolizing Chaos, being populated by all kinds of monsters or it can be assimilated to the house or to the protective maternal womb (Evseev I., 1994).

The forest can also be interpreted as a sacred space, this valence of sacredness being part of the composition and of the diversity of trees, as symbolic representatives of the vital substance of the universe, endowed with a regenerative capacity of nature. The custom of consecrating forests and groves was quite widespread in antiquity, being attested by archaeological or historical sources in many nations. The Greeks and Romans considered the forests and the meadows from within, paternilized and protected by various deities, as places for holding some sacred rituals of thank-offering. The heart or the center of the forest, represented by the image of openings, indicates the sacred space where the encounter with the divine is manifested.

*The Dark Forest*symbolizes the disorientation, as an area of the unconscious, in which the man enters only with fear. The wandering in the *labyrinthforest,* as an ambivalent symbol of death and regeneration, it is for the initiated one, only a symbolic stage of his becoming. Thereby*going through the maze* becomes a journey of initiation that becomes harder as the obstacles are more numerous. The transformation of the self in the center of the labyrinth attests at the end of the return journey, at the end of the transition from darkness to light, the victory of the spiritual over the material, of the eternal over the ephemeral, of the intelligence over the instinct.

*The light* that comes on the path of the legendary characters and that glitters though the tree trunks symbolizes the hope in the existence of a shelter. The forest itself, as wildlife nature, it is seen as being threatening, the imagination inhabitating it with wild beings and spirits, but also with fairies that may be benevolent. *"The metaphor of the forest is significant. A forest is not ordered by some binary separation of paths, but is rather a maze"* (Eco, 2009), whose *center* should be sought. So, without changing the original meaning and function of the maze, it's undoubtedly that it includes the idea of defending this *center*. Not *everyone* could get through the maze and get out of there; entering into the labyrinth can have the value of an initiation. The *center* assumes very diverse figurations; thus the way through the center of the maze can be compared with a ritual of defensing a city, a tomb or a shrine, in any case, it defends a magical-religious space that should have been made impenetrable and inviolable to the uninitiated. The military function of the labyrinth was, therefore, just a variant of its essential functions of defense against *"evil"*, hostile spirits and death. In other words, *"the role of the labyrinth was to defend a "center" in the prime and rigorous acceptation of the word, thus representing the initiatory access to the sacredness, to the immortality, to the absolute reality."* (Eliade, 1995)

The sacred architecture synthesized in its forms the characteristics of the forest. While the Romanesque church was considered a refuge for the environment, the Gothic cathedral was gaining subtle signification, its verticality of columns looking like tree trunks, the nervures of the vaults resembling dense branches, the subtle relationship with the filtered light, the sinuous course through the portals to the place of transcendence turn it into a real initiating path of finding the truth search (Fig. 2). The cathedral is a synthesis of opposing ideals. The light filtered through the stained glass windows was the final ingredient in this process. All plastic mediums that could produce these amazing effects, served the symbolic function of the cathedrals that explained visually to the mortals, that the meaning of life on Earth is to prepare you through a pure and full of humility life, for the glowing afterlife. Space, light, sculpture and painting, all worked together to create a mystical atmosphere in this large and complex work of art that that is the Gothic cathedral.

**a)** **b)**

**c)**

**Fig. 3 -** The interior of the cathedral Sagrada Familia, Arch. Antonio Gaudi
**a**) - (*http://www.sagradafamilia.org/wp-content/uploads/2014/12/Arquitectura_8.jpg*);
**b) -** *(http://www.sagradafamilia.org/wp-content/uploads/2014/12/Arquitectura_2.jpg);*
**c)** – *(http://www.sagradafamilia.org/wp-content/uploads/2014/12/Arquitectura_10.jpg)*

Starting from the principle of Gothic and Byzantine architecture, Antonio created *Gaudi's Sagrada Familia* Cathedral (Fig. 3), using a personal architectural style, generated by forms and geometries inspired by nature, light and color. His intention was to express the Christian faith through an architecture that involves the symbiosis between natural form and Christian iconography. The multitude of pillars in the central nave recalls the image of a forest, their verticality leading our sight toward heights, where, the pyramidal growth of the structure reminds of foliage that seems to merge with the sky. These structural columns, decorated with grooves, which appear to be made of wood, reflect Gaudi's idea that you are inside a palm forest that leads you to prayer. To reduce the load of the roof and to bring light into the building dormers or skylights between columns, of hyperbolic shape, made of green and gold glass pieces, which reflect daylight inside, were designed. All the stained glass windows of the apse follow a plan of gradual tones in order to create a suitable atmosphere for introspection.

Tucked away in the forests of the Ozark Mountains, *Thorncrown Capel* (Fig. 4), Eureka Springs, is one of the greatest architectural accomplishments of the twentieth century. Built on a wooden structure that mimics a forest canopy, the church fits perfectly in the American specific landscape due to the perimeter enclosing made solely of glass.

**a)** **b)** **c)**

**Fig. 4 -** Thorncrown Chapel, Eureka Springs, Arkansas, SUA, arh. E. Fay Jones
a) Thorncrown Chapel, exterinal view (https://en.wikipedia.org/wiki/Thorncrown_Chapel);
b) Thorncrown Chapel, interior view (http://www.go-arkansas.com/AR-Thorncrown-Chapel);
c) Alley in Bruges, Belgia (http://31.media.tumblr.com/tumblr_lwnrbylucW1r8fegbo1_500.jpg)

The italian artist Giovanni Mauri demonstrated by its *Vegetal Cathedral* (Fig. 5) that a space of worship, of contemplation and (re)connection with the divine should not be in competition with nature, it may be nature itself. Therefore the artist builds an entirely cathedral just by using trees. The entire structure is made up of 42 columns with a cage type structure built of vegetal and woody elements (pine, chestnut, hornbeam and hazel), that are expected to degrade in time, while the cathedral trees are developing. These live columns are delimiting the space of a basilica with five naves. In total, 80 hornbeam seedlings are planted

in between these columns-structures, and in time they will exceed the existing temporary structure, forming a veritable living cathedral.

In the United States, New Hampshire, *Cathedral of the Pines,* founded in 1945 by Sibyl and Douglas Sloane, represents a place of interfaith worship. *Cathedral of Pines* was intended to be a memorial to those who lost their lives in the Second World War. The church is open every year, from May to October, and hosts various cultural and religious events, as well as lectures on sustainable development and environmental protection. (Fig. 6)

**a)** **b)**

**Fig. 5** - Vegetal Cathedral by Italian artist Giovanni Mauri, Vasugana, Italy
a) *http*://virtualsacredspace.blogspot.com/2012/03/giuliano-mauri-cathedral-bergamo-italy.html
b) *http://www.giulianomauri.com/test/cattedrale-vegetale-orobie/*

**a)** **b)**

**Fig. 6** - Cathedral of the Pine, New Hampshire, SUA
a) *http://discovermonadnock.com/wp-content/uploads/2015/01/Cathedral_of_the_Pines_-_DSC014861.jpg*
b) *http://images.fineartamerica.com/images-medium-large-5/cathedral-of-the-pines-john-poltrack.jpg*

## CONCLUSIONS

The sacred spaces represented by churches, places of prayer or commemoration and introversion, etc., though the program that they serve are worship spaces that exists as a ways of mediation between the human dimension

and the high spiritual one, their architecture being primarily designed to have a psycho-emotional influence over those that use them. In addition to the physical ordering, which dependent on rites, liturgy or dogma, such spaces are emphasizing themselves high symbolicand aesthetic values which, through specific plastic encodings and artistic means - shapes, light, materials, iconography, narrate and communicate ideas, stimulate the spiritual experience and the sense of sacredness.

Over time, the universe was enveloped in a sacred perspective, beginning with the rocks and flowers up to wildlife and humans. Desacralizing himself, the man desacralized the cosmos too.

This world, which belongs to man, represents a perpetual motion. The four elements that form the universe, but are also found in humans, are aware of a continuous movement and suffer various transformations. Earth, water, fire and air are mixed and converted mutually. The movement that animates the man is similar to the universe, being assimilated as an echo of it. In this ever-changing state, the mater is not separated from the spirit, it is, as Schelling said, *"a sleep of the mind"* (Clement *et al.,* 2000),that the man wants to awaken.

*The four elements,* water, fire, air and earth, as essential elements in the creation of the universe, are found in the cosmological myths of the world, setting in motion specific groups of images that help the intimate assimilation of the real dispersed in its forms. Over the time, through different ways of thinking, the humanity has shown its sensitivity, the becoming or existence, aided by sufficient images, drawings, paintings or adjacent poses of those, from the almost mystical desire to bring close in any way the nature, the transcendent, or to explain his being. The Man, being a universe in miniature, he has in himself the four elements: he draws his body from *earth; water* is represented by his blood; *air* is his breath; and the *fire* is the heat which animates his existence.

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# OUTDOOR ENVIRONMENTS: LANDSCAPING (HIGH)SCHOOLYARDS – ANALYSIS, QUALITY FACTORS AND HIGHLIGHTS FOR IMPROVING THE QUALITY

## AMENAJĂRI PEISAGISTICE ÎN SPAȚII DE ÎNVĂȚĂMÂNT IEȘENE - ANALIZĂ, FACTORI DE CALITATE ȘI EVIDENȚIEREA DIRECȚIILOR DE INTERVENȚIE PENTRU CREȘTEREA CALITĂȚII ACESTOR AMENAJĂRI

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***Abstract.****The landscape, an urban design and development tool, is being defined by the form and the historical evolution, the individual perception and the social groups that inhabit it. All these elements are overlapping on society's cultural pattern, thus being born the human-landscape interaction, within the meaning of subjective perception of the existing target. This study aims landscaping the precincts of some schools and high-schools from Iasi. The chosen sites were selected through considering the great importance they play in educating children and adolescents and because they have a coherent structure established in connection with the architectural object(s), being at the same time subject to various degradation processes.*
***Key words:*** *urban landscape, educational space, landscaping, spaces for pupils, landscape design*

***Rezumat.*** *Peisajul, instrument al proiectării și dezvoltării urbane, are ca elemente definitorii forma ș ievoluția istorică, percepția individuală și a socio-grupurilor care-l locuiescși care-l parcurg. Toat eaceste elemente se suprapun peste modelul cultural al societății, născându-se astfel o interacțiune om-peisaj, în sensul percepției subiective a obiectivului existent. Acest studiu vizează amenajările peisagistice din incintele instituțiilor ieșene de învățământ tpreuniversitar. Amplasamentele alese au fos talese prin prisma următoarelor considerente: prezintă o mare importanta în formarea copiilor și adolescenților, au o structură închegată constituită în corelare cu obiectul (obiectele) de arhitectură, fiind supuse diverselo rprocese de degradare.*
***Cuvintecheie:*** *peisaj urban, spații de învățământ, spații pentru elevi, amenajări peisagere*

## INTRODUCTION

The landscape is a tool for urban and regional planning and development having as key elements shape and historical evolution, but also the perception of the individual and of the social groups that inhabit and go through it, overlaid on

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the cultural model of society. The landscape is constituted as landscaping units, as areas perceived with a significantly different character from the next picture. Thus there is a human-landscape interaction in the meaning of subjective perception of the existing objective, but also it reifies through intervention and modeling of landscape performed subjectively by human or social groups. From this perspective, for architects the landscape becomes important also as a process in a logic of connecting that allows communication, interaction and individual adaptation of the subject to the landscape, but also of the landscape to the identity of the subject(Țurlea, 2008).

## MATERIAL AND METHOD

Landscaping generates a communication processes through a varied repertoire of signs (indices, icons, symbols), a complex ensemble of signs and meanings which are communicated through an appropriate and specific language and assembled by specific codes determined by the culture that produced them. This study aims landscaping the precincts of some schools and high-schools from Iasi. The chosen sites were selected through considering the great importance they play in educating children and adolescents and because they have a coherent structure established in connection with the architectural object(s), being at the same time subject to various degradation processes. For this study the following sites had been chosen: Grup Școlar Tehnic de Transporturi Căi Ferate, Colegiul Tehnic „Dimitrie Leonida", Școala Gimnazială nr.11 „Otilia Cazimir" and Liceul teoretic „Dimitrie Cantemir".

The above-mentioned study was under review between October 2014 and May 2015. The study was completed by using analytical and visualization methods and has been divided into 2 phases. The 1st phase of the study involved designing an evaluation grid for evaluating the studied sites and also the proper analysis of the pilot area mentioned above by applying the evaluation grid developed. Thus, we determined the impact of urban, structuring, morphology, identity, vegetable, mineral, water criteria, etc. on public space and urban image. The analysis of landscape designs and urban areas according to established quality criteria was done by allocating each criterion one of the three impact values: low, medium, high. Photomontages and three-dimensional computer simulations completed the analysis report. Thus, at the end of the 1$^{st}$ phase of the study, students managed to establish new directions for revitalizing and redevelopment of the studied area.

The 2$^{nd}$ phase of this study aimed to propose new landscape designs for the studied sites in order to eliminate as much as possible of the specific malfunctions of the site, and increase the quality of this space. In other words, the goal was to revitalize and redevelop the studied schoolyards.

## RESULTS AND DISCUSSIONS

Generally, the proposals for improving the area's urban image and visual aesthetics aimed creating new compositions of the space allowing the materialization of more attractive pathways, with one or more of composition axes sustained by centers of interest grouped by their importance. It was also noticed that introducing temporary or permanent structures, structures that can accommodate different cultural and social activities needed in the learning

process (designated areas for outdoor classes, painting workshops and workshops for technological education classes, sports, jogging track and other facilities required for P.E. classes, etc.), and designing special lightning systems considerably increase the quality of landscaping, and therefore public space and urban image.

**a)** **b)** **c)**

**Fig. 1** - Proposal for Grupului Școlar Tehnic de Transporturi Căi Ferate
a) – concept ; b) – masterplan; c) – urban furniture

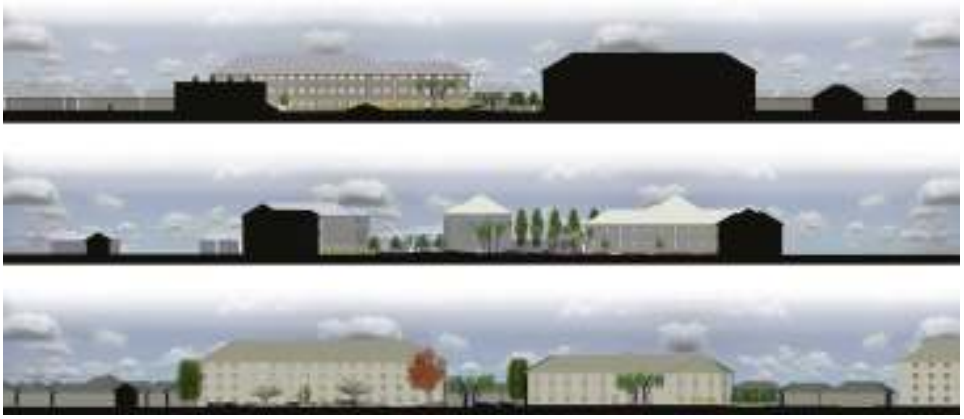


**Fig. 2 -** Proposal for Grupului Școlar Tehnic de Transporturi Căi Ferate – cross-sections

The proposed landscapedesign for the high schoolyard of Grupului Școlar Tehnic de Transporturi Căi Ferate uses the concept of *flowability* (Fig. 1). Within the project, the interstitial space of the buildings which have a rigid geometry, becomes organic. The element that unifies the entire composition, the deck that by its texture invites to human interaction and relaxationis developed only on horizontal plane. The resulted areas, although of different type, are clotted around a central space in which the mineral blend itself with the vegetal, it vertical development underlining the main pathways of the landscape design (Fig. 2).

Due to the immediate vicinity with the project presented before, the landscape designed proposed for the yard of Colegiul Tehnic „Dimitrie Leonida" (Fig. 3) follows the same concept, *flowability,* but materializing it under a different expression. In this case, the element that unifies the entire composition is

a structure which has the form of a linear surface made of four strips, very dynamic as form but also as function. This structure starts containing the sport's field footings, becoming then a generous pergola, floors and urban furniture.

**a)** **b)**

**c)**

**Fig. 3 -** Proposal for Colegiului Tehnic „Dimitrie Leonida"
a) – masterplan; b), c) – perspectives

For Școala Gimnazială nr. 11 „Otilia Cazimir", the new landscape design suggests a diversifications of the spaces, offering to pupils more possibilities for spending their break time, but also more opportunities to diversify the teaching-learning process (Fig. 4). Thus, the entire project identifies the needs of a pupil: relaxation, creativity, playing by sport and movement. Therefore some special areas were designed. These areas bare simple names: sport area, play area, creative area, quiet area.

Therefore, the central location was chosen for the playground, a multipurpose area that can be used for organizing various school events. Around it are the sport area, the creativity area, which includes an area for asphalt drawing and the relaxation area. The relaxation area is placed near the second entrance and by its furniture layout encloses a picnic-area.

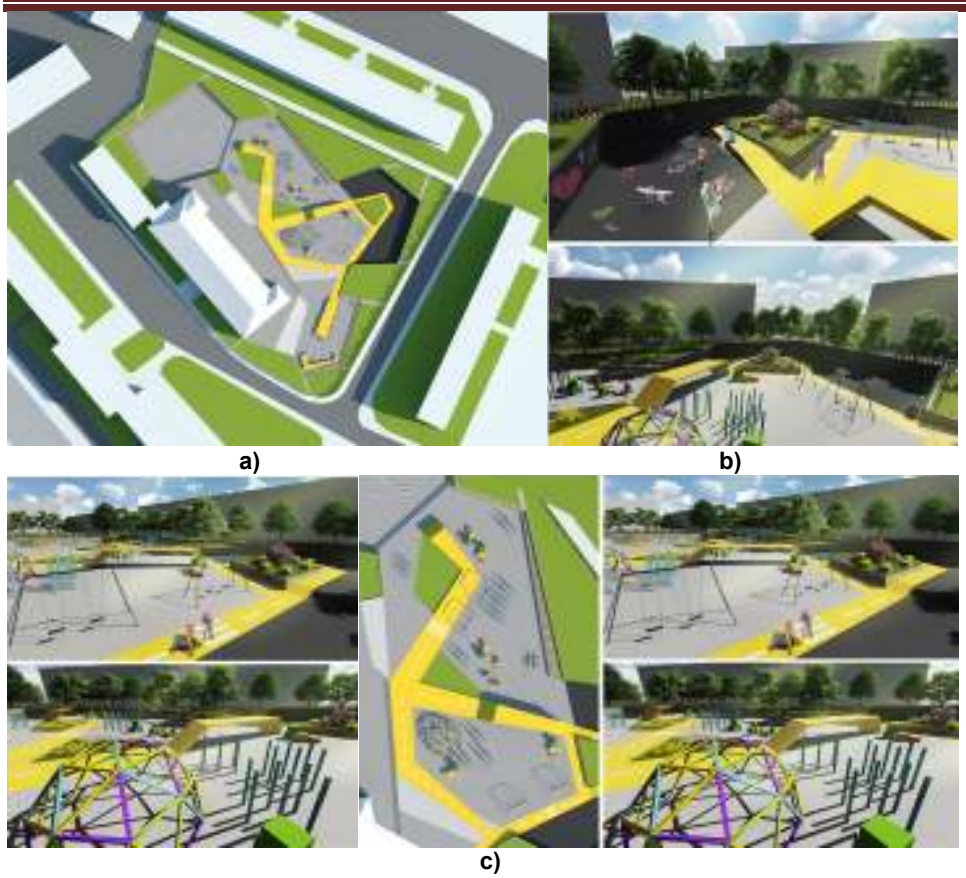
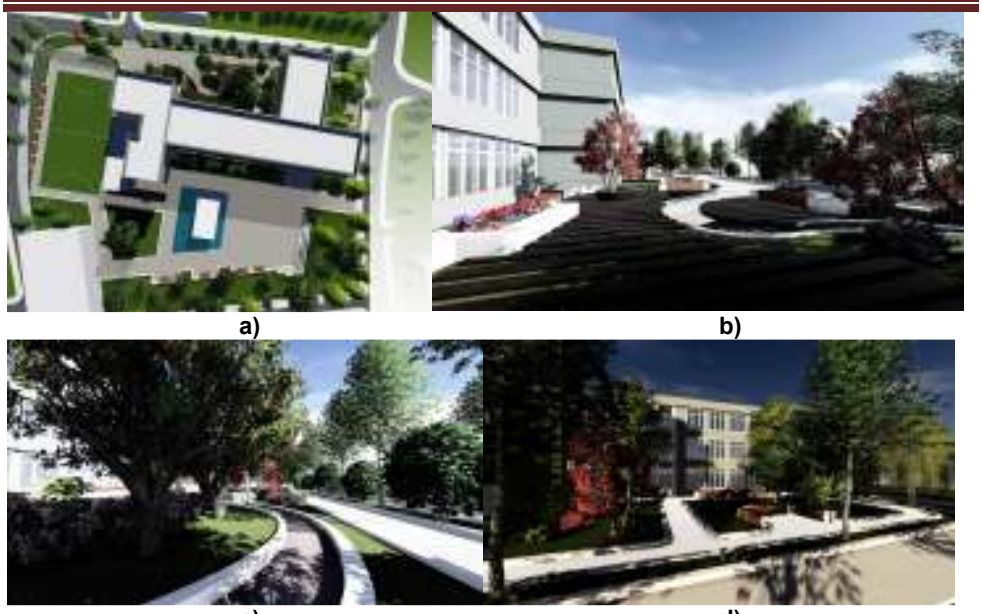


**Fig. 4 -** Proposal for Școala Gimnazială nr. 11 „Otilia Cazimir"
a) – masterplan; b) – creativity area; c) – play area.

The proposed landscape design concept for the Dimitrie Cantemir's High school courtyard (Fig. 5) aims to create a continuous and fluid space, which enfolds many areas, each one having a specific purpose. Hereby, the existing courtyard related to the main access was kept, representing a reception and a cluster area with multiple roles: rest and relaxation, study, meeting and outdoor celebrations for school events. Regarding this, three small footings recalling a small amphitheater and a pavilion with multiple destinations (stage, shelter, sitting and study place) were provided to the area. The yard is surrounded with green spaces and planted areas with trees and shrubs, the connection with the other areas being realized through a small number of circulations. The sport area was narrowed; only one wide sport field was kept.



a) b)

c) d)

**Fig. 5 -** Proposal for Liceul Teoretic „Dimitrie Cantemir"
a) – masterplan; b), c), d) – perspectives

## CONCLUSIONS

By reference to an image, the analysis and evolution of landscape are by nature imbued with subjectivity. Evaluation of landscaping involves aesthetic judgments that may vary depending on social groups, ages, etc. Consequently, there is no fixed standard or absolute measurement of aesthetics of a landscaped area. The existing methods for assessing the landscape, and so by default evaluation of landscaping, have raised and will raise critics for a long time, occasionally powerful, going up to denying the possibility of landscape quality assessment.

***Acknowledgments***. Special thanks to the students of the 5th year of the "G. M. Cantacuzino" Faculty of Architecture of Iași without which we couldn't elaborate this study: Bălinișteanu Ciprian, Ioniță Mihaela, Hodea Magda (Grup Școlar Tehnic de Transporturi Căi Ferate), Cadiș Marius Cosmin, Iftime Adnana, Plăiașu Smaranda (Colegiul Tehnic „Dimitrie Leonida"), Betiuc Sonia, Miluț Ruxandra, Mocanu Iulia (Școala Gimnazială nr. 11 „Otilia Cazimir"), Bofu Adriana, Galearschi Sorina, Răileanu Bianca (Liceul Teoretic „Dimitrie Cantemir").

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# DESIGNS OF CHILDREN PLAYGROUNDS

## AMENAJĂRI ALE JOCURILOR EXTERIOARE PENTRU COPII

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***Abstract***. *Specialized designing playgrounds for children is based on a series of rules on materials, structure and configuration of outdoor spaces for children's play. Materials used in the construction of appropriate furniture abide the ergonomic character game, nontoxic, harmless forms, but also ecological. This paper brings an overview of the methods of planning playgrounds for children in our country compared to those built in other countries.*
***Key words*****:** *outdoor playgrounds, landscape design, children play equipment*

***Rezumat.*** *În proiectarea de specialitate a spațiilor de joacă pentru copii se au în vedere o serie de reguli privind materialele, structura și configurarea spațiilor exterioare destinate jocului copiilor. Materialele utilizate în construcția unui mobilier adecvat jocului se supun rigorilor ergonomicității, nontoxicității, formelor necontondente, dar și ecologicului. Lucrarea de față aduce o privire de ansamblu asupra metodelor de amenajare a spațiilor de joacă pentru copii de la noi din țară, comparativ cu cele construite în alte țări.*
***Cuvinte cheie*****:** *spații de joacă exterioare, amenajări peisagere, echipamente de joacă pentru copii*

## INTRODUCTION

In their games, the children face the reality, check their physical and mental capabilities. As Albert Einstein used to say "the game is the lofty type of research", so the child must be given the freedom to play as you want them, so it can test its limts and develop self-knowledge.

Since before apparition of the concept of "playground" there were various structures that were used by children in their games, but emergence of this concept has made children's games to be concentrated to one particular area in that their supervision is much easier .

## MATERIAL AND METHOD

With the development of prehistoric civilizations have developed also people's perception of public spaces and education, ever since the ancient Greeks and Romans may notice changes in urban living concepts, developing parks, gardens, outdoor theaters. For example Plato (427-347 BC) claimed that children's games are methods of developing skills for adult life and maintain physical health and psychological development.

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Playgrounds have a long history, but the first full illustration of this concept appeared in 1848 (Henry Bernard). This site was designed as a pedagogical space focused on games.(fig.1)

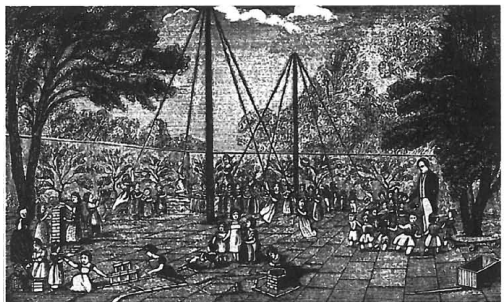

**Fig.1 -** The image of a playground for children ninth century (after H. Berard, 1848) (1)

In the United States, in Boston, in 1885, at the German doctor Marie Zarkrzewska urge, have start to development parks for children as we know them today in the initial formation of "sand gardens" where children could play; mean while in Berlin the same concept has been updated by adding boards to maintain sand in inițial space. Soon, swings and other playground equipment was added for this space to be used by older children. Until 1912 were added also adult recreational places, thus stimulating desire for free movement.

In 1923, to encouraje games as an integrant part of family life, Charles Wicksteed designed a set of equipment that could be used successfully in playgrounds. They were located in Kettering Wicksteed Park in UK, which is considered the first park of its kind in whole the country.(fig.2)

**Fig.2 -** Image of playground equipment in Kettering Wicksteed Park, UK, 1923 (2)

During the Second World War, a new concept of playgrounds developed n Denmark and then was spread in most of the Europe. The architect Carl Theodor Marius Sørensen developed the concept of playgrounds made from waste, also designed by children themselves while they were enjoing the space.(fig.3)

**Fig.3 -** Images of playgrounds for children: in Denmark (during the Second World War) (left)(3), Quarry Hills Estate Playground, Leeds 1937 (right)(4)

Since the beginning of the XX$^{th}$ century, the playgrounds were becoming increasingly industrialized so that the exploatation costs be minimized. As equipment playgrounds have evolved and have been observed involving child safety issues, changes were introduced n their design.

Between 1900 - 2000, no major changes have occurred in the development of playground equipment, but instead, there was an explosion in the number of traders and the variety of playing styles and activities. There was an innovation in constructing elements, creative shapes and colors of playing equipment. Wooden equipment were popular from 1970-1980 and also have written rules for safety. Since 2000, there have appeared equipment for parks that focus on fitness. Handrails were taken off and physical activity encouraged.

Manufacturers have predicted that the metal and equipment-oriented action will remain a priority, plastic that will replace the metal on some plans, sculptures shaped animals will become popular, equipments will be tailored to age groups, will be less high and installed on pebbled or mulch surfaces for added security.

Since 2010, we experience a new trend - the return to nature.

The trend was divided in two directions:

- The first is that which emphasis the constructing playground equipment whith natural elements - trees tower with slides, prefabricated rock for climbing, diferent tipes of structures hanged on the trunk of the trees, standardized form of mushrooms, flowers, insects etc. (fig.4)

**Fig.4 -** Images of playgrounds in classic style (5).

- A second direction, led by landscape architects, creates play areas without equipment, but with natural elements like wood beams for balance over ponds, small hills to climb and slide down, a warren of tall vegetation, houses in trees areas with sandy, natural paths, free materials from wich the childrens can build forts.(fig.5)

**Fig.5 -** Images of playgrounds in naturalist styles.(6),(7)

Today, children's play parks is a good business in most civilized countries, and the design, construction and their elements are too diverse for a general description.

For manufacturing playground equipment should be used durable materials that are resistant and easy to maintain and must comply with applicable European standards and rules of classification, labeling and packaging of substances and mixtures.

Minimum distances to be observed will be measured from the outer perimeter of the playground area to the road and parking area boundaries:

- 10 m for local roads without urban continuity or parking area. A minimum distance of 5 m maximum is permitted only when vehicles are physically limited to run at very low speeds and are provided effective technical solutions to ensure protection against trafficking;
- 20 m for local distribution roads with urban continuity and parking area.
- 50 m for other more intensive traffic roads where playgrounds will be physically separate from these roads.

## RESULTS AND DISCUSSIONS

In Romania after 1990, the design playground has evolved slowly from communist society specific equipment to the modern facilities equipped to meet the latest safety and comfort standards, but also to stimulate children's creativity.

Other areas of the world, depending on the degree of economic and cultural development have known various concerns of local communities, architects and designers in recreational spaces dedicated to children and adolescents, at different levels of ingenuity or complexity.

Researchers have long known that playing with blocks helps children develop cognitive and social skills. Children need free play at home and at school. During free play, children increase their imagination and creativity, organize their own games, develop their own rules, learn problem solving skills, and practice leadership (fig.6,7,8).

Playgrounds today are ultra safe. Stringent codes and a fixation on injury prevention are reducing the fun in public playspaces. The safety preoccupation is shortchanging kids diminishing their ability to assess risk and exercise their own judgment (11).

**Fig.6 -** Images of playgrounds from Dulwich College Singapore, 2014 (8)

**Fig.7 -** Images of playground from condominium
D'leedon,151A KING'S RD, Singapore, 2014 (9)

Imagination Playground blocks are made of a lightweight foam that is non-toxic and microbe and mold resistant. They're soft and friendly to the touch yet dense and firm enough to build structures and shapes. Imagination Playground blocks meet American Society for Testing Materials (ASTM) and U.S. Consumer Products Safety Commission (CFSC) standards.

**Fig.8 -** Equipment of childres playground(10)

## CONCLUSIONS

Integration of our country into the European Union made possible the emergence of internal markets for the sale of various types of modern and advanced products, including those used in the construction playgrounds for children. What there is not in our country is european mentality, and financial capability to purchase such equipment. Major investments in this area there is only in big cities or with the contribution of European funds raised by local authorities.

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# GEOMETRIC DESIGN FOR BUILDINGS WITH INNER COURTYARDS

## PROIECTAREA GEOMETRICĂ A CLĂDIRILOR CARE AU CURȚI INTERIOARE

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***Abstract.*** *In the case of large public buildings, inner courtyards provide optimal solutions for space lighting and ventilation. In relation to height and surface area parameters, space geometry has to take into account that vegetation must develop under good conditions. Shaded and sunlit areas, determined by courtyard's geometry, have direct implications on the spectrum of plant species that can develop. In turn, grown tree canopies cast shadows that hinder the ground vegetation development. Landscaping design of inner courtyards should take into account the force fields specific to the basic shapes and their impact on human behavioral patterns. The article presents general considerations on the topic of inner courtyard's geometry, in relation to landscaping possibilities, as well as a case-study.*
***Key words:*** *geometric design, inner courtyards, landscaping solutions.*

***Rezumat.*** *Curțile interioare reprezintă soluții optime pentru asigurarea iluminării şi ventilării naturale pentru clădirile publice cu o amprentă la sol mare. Geometria spațiului trebuie proiectată în relație cu înălțimea pereților şi aria desfăşurată la sol, astfel încât vegetația să aibă condiții bune de creştere. Zonele umbrite și însorite, determinate de geometria curții, are implicații directe asupra spectrului de specii de plante care se pot dezvolta. La rândul lor, coroana copacilor care au crescut, proiectează umbre care limitează evoluția vegetației de pe sol. Amenajarea curților interioare trebuie să țină cont de câmpurile de forță specifice fiecărei forme geometrice și impactul acestora asupra tiparelor comportamentale umane. Articolul prezintă considerații generale privind tema geometriei curților interioare, în ceea ce privește posibilitățile de amenajare peisagistică, precum și un studiu de caz.*
***Cuvinte cheie:*** *proiectare sustenabilă, clădiri sociale, curți interioare, geometria spațiului, soluții peisagistice.*

## INTRODUCTION

Geometric patterns fulfill an important role in the composition of architectural space, structuring and representing information. Even from antiquity, the basic models have marked the limits of spaces which were a symbolic landmark to a certain culture or civilization. Starting from the irregular shapes of

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the first spontaneous settlements until the abstract lined spaces of the modern times, the historical analysis individualizes certain basic geometric patterns which are repeated throughout time: the circle, the square and the triangle. These suffer materializations and geometric transformations which differ according to various social, spiritual and aesthetic premises of the era that generated them.

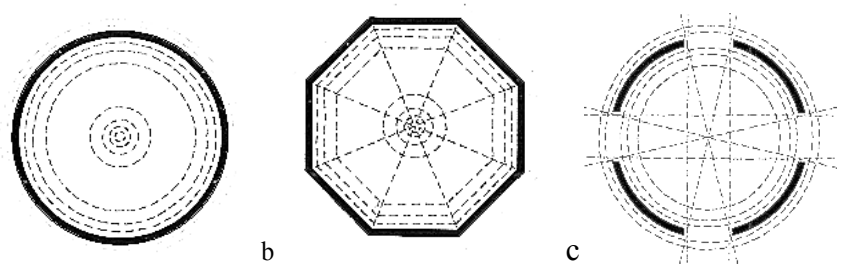
## MATERIAL AND METHOD

Each basic geometric shape generates specific force fields and spatial patterns. Thus, according to each inner courtyard form, the design will propose optimal visual perspectives, areas of interests and circulation directions. The landscaping design is determined by the overall proportions of the space and consequently proposes possible activities within an courtyard and the ratio of plated and paved areas.

Starting from the principle according to which the image of architecture can be reduced to its essential elements which, in turn, can be represented through basic geometric models, an argument can be made for the existence of repetitive patterns, found in various historical stages. The phenomenon is referred to as *"shape persistency"* in the field of visual arts and architecture (Conversano *et al.,* 2011).

## RESULTS AND DISCUSSIONS

Apart from their purely scientific characteristics, the primary models of the circle, square and triangle have the capacity to relay a state or idea, having significations associated to them which denote the profound side of the human being and which have, in time, concentrated ideals, superstitions and various forms of abstraction. After a formal synthetic evaluation of the progress of geometric patterns and transformations in the architecture of inner courtyards, various manifestations of the primary geometries of the circle, square and triangle are particularized.



a b c

**Fig. 1** - Centralization degree for the circle and octagon: a. Closed circle model; b. Closed octagon model; c. Symmetrical opened circle model (Von Meiss, 1986)

Considering these three basic patterns, Pierre von Meiss analyzes separately the radiance (force) fields inherent to each geometric shape.

In the case of octagon and circle patterns, the force fields act in the centre and at the periphery. So if we intervene on the basic model by creating symmetrical openings, more subspaces will be defined as disk sectors (Fig 1).

Three-dimensional extensions of the circle may be represented by models such as cylinder, sphere or dome, all of these maintaining the concept of centrality for the interior space (Fig. 2) .


**Fig. 2** - Traditional houses of Hakka, China, Seventeenth century - earth architecture

The square holds different intensity radiance fields, which are acting in the corners, on the outline, diagonally, and in the centre, Fig. 3a. If the square corners are explicit we are witnessing a perfect closed space definition and its default subspaces are also squares that, through similarity, help focusing and emphasizing the basic model, Fig. 3b. When the square corners are missing, the resulting shape suggests space openness and the basic model is no longer as conclusive, Fig. 3c. The vertical spatial extensions of the square are the cube (Fig.4), tetrahedron and prism.

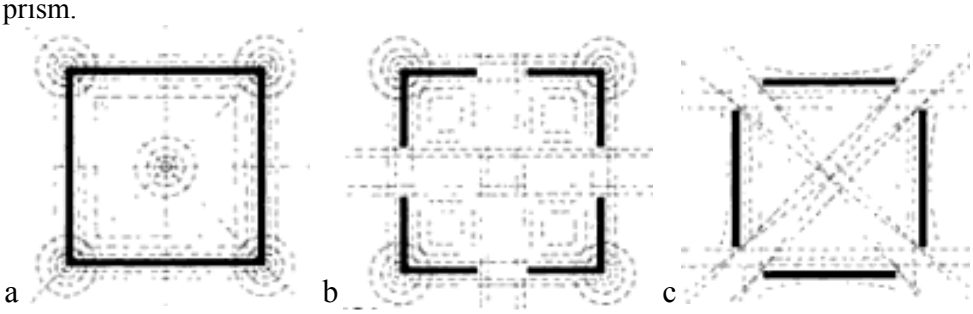



**Fig. 3** - Centralization degree for the square: a. Closed model; b. Model emphasizing the centrality of the basic pattern due to elimination of median part sides; c. Decentralised model (Von Meiss, 1986)

**Fig. 4** - Traditional roman villa

The equilateral triangle features a hidden centre because it lacks diagonals and the bisectors have no precise reference to the midpoint of the opposite sides. Therefore, the triangle is less centralized than the octagon or square, but at the same time, defines an enclosed space, almost claustrophobic due to its sharp angles (Von Meiss, 1986), Fig. 5a. Pierre von Meiss also mentions the difficulty of designing spaces with explicit sharp angles. When the angles are kept and the mid sections of sides are eliminated, the centre of the triangular pattern is emphasized, Fig. 5b. If the angles disappear and mid sections of sides are maintained, the concept of the triangle becomes elusive, referring rather to the geometric model of hexagon, Fig. 5c. Depending on the edges inclination degree, the final image can either remind of the triangle pattern, or the hexagon. (Fig. 6)

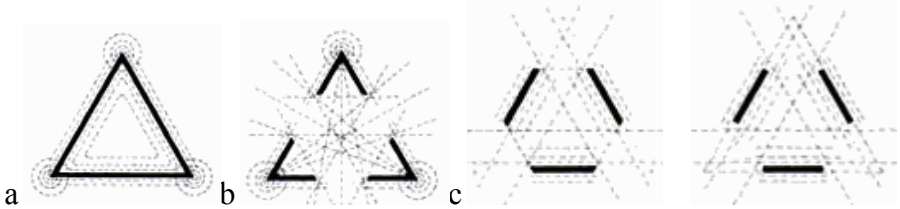



**Fig. 5** - Centralization degree for the triangle: a. Closed model; b. Emphasizing the triangle centre by abolishing the mid section sides; c. Elimination of angles – definition of hexagon and triangle models (Von Meiss, 1986)

**Fig. 6 -** Kyoko Ikuta house, Nagano, Japan (2010) - Ozeki Architects & Associates

In the modern and contemporary period, the evolution of the circle, square and triangle models oscillates between primary, abstract (legible) and composite, complex (difficult to decipher), these geometric materialization states being found at a plan, facade and volumetric level. Considering these aspects, we can state that the circle, square and triangle patterns cross an initiation path, passing through different stages of geometric experimentation, from the purity imposed by machine standardization, to complex methods of mapping the virtual reality.

The geometric pattern of the circle is the most frequent one in the context of digital design, constituting a departure point for spatial materializations based on curved surfaces.

The results of our research on geometry and space proportions can contribute to the sustainable design of an landscaping project. A case study on the interior courtyard of the Faculty of Architecture of Iași is designed to illustrate the role of geometry in landscaping projects. The preliminary phase of the project consists of a sun study meant to determine the possible location of planted areas and the suitable plant species.

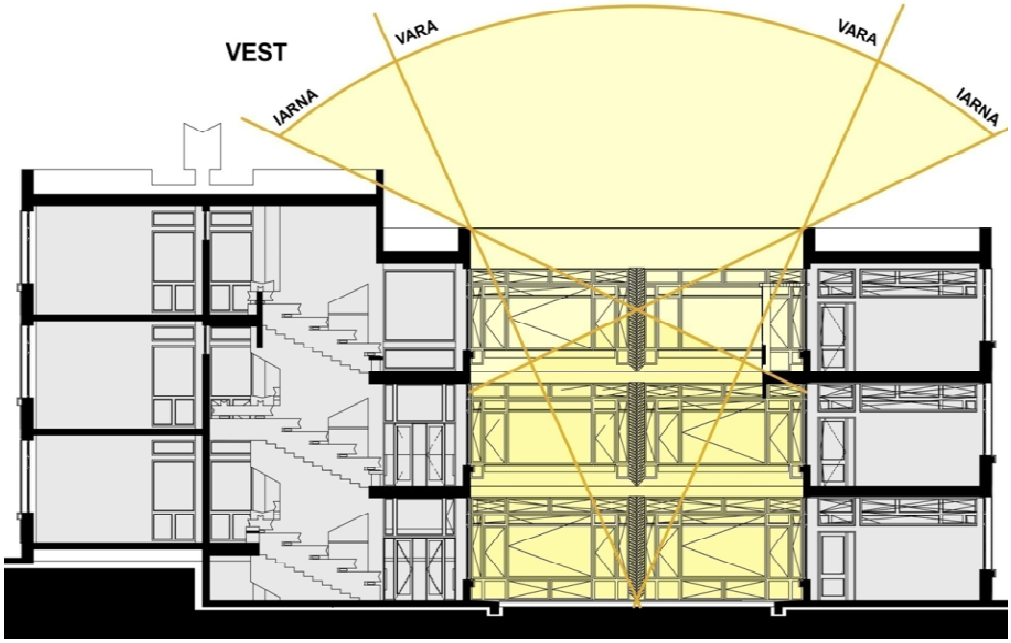



**Fig. 7 -** Sun study of the interior courtyard of the Faculty of Architecture from Iași

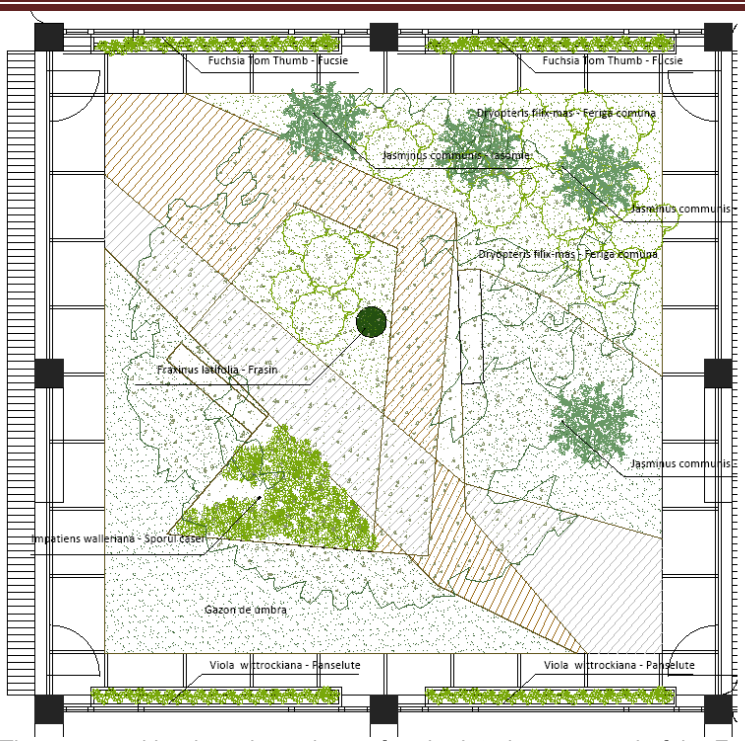



**Fig. 8 -** The proposed landscaping scheme for the interior courtyard of the Faculty of Architecture - Iași

## CONCLUSIONS

The perception of a model depends on the visual contrast that exists along the separation line between figure and background, or between form and its field. Taking into account theories enunciated by Gestalt theory, which state that the human mind simplifies visual environment for a better understanding, the conclusion is that a model is easier to be perceived, the more simpler and regular (Ching, 2007). This category includes the primary models of the circle, square and triangle, each with its own derivatives, whose presence has been continuous in architecture (Thiersch, 1883).

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# ASPECTS REGARDING THE ORNAMENTAL VALUE OF SOME ROSE VARIETIES IN BOTANICAL GARDEN NURSERY CONDITIONS - IASI COUNTY

## ASPECTE PRIVIND VALOAREA ORNAMENTALĂ A UNOR SOIURI DE TRANDAFIRI ÎN CONDIȚIILE GRĂDINII BOTANICE IASI

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***Abstract.*** *The paper present aspects regarding the ornamental value of some rose varieties, in the botanical garden nursery conditions. The observations were made at the next rose varieties: Foc de Tabără, Luchian, Betty Prior, Crimson Glory.There were studied six features defining the decorative value of these varieties, respectively: the foliage, the diseases resistance, the flowering intensity, the form of the flower, the color of the petals and the odor.*
***Key words****: rosa, estimation, decorative value.*

***Rezumat.*** *În lucrare sunt prezentate aspecte privind valoarea ornamentală a unor soiuri de trandafiri în condițiile grădinii botanice din Iaşi. Observațiile au fost făcute la următoarele soiuri de trandafir: Foc de Tabără, Luchian, Betty Prior, Crimson Glory. Au fost luate în studiul sase însuşiri ce definesc valoarea decorativă a acestor soiuri şi anume: frunzişul, rezistența la boli, intensitatea înfloritului, forma florii, culoarea petalelor, parfumul.*
***Cuvinte cheie****: trandafir, bonitare, valoare decorativă.*

## INTRODUCTION

One of the basic components of the green spaces ensuring the aesthetic aspect of the localities and which contributes to the health and good state of mind of people and to insuring a favourable work climate, is constituted by the floral and rose arrangements (Haenchen, 2003; Wagner 2001, 2002).

The rose has been considered ever since the past "The Queen of Flowers", because of its multiple qualities and especially the richness and beauty, perfume and the different colours and shapes (Eckart, 2003; Popescu, 1986).

They are characterized through a series of characteristics, among which we mention:

1. - the bush shape;
2. - the vigour;

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3. - the leafage;
4. - the resistance to diseases;
5. - the shank and the floral peduncle;
6. - the blooming intensity;
7 -the rose bud shape ;
8. - the flower shape;
9. -the durability of the flower in the field;
10. - the colour at opening;
11. - the colour at blossoming ;
12. - the manner of petals fall;
13. - the perfume.

## MATERIAL AND METHOD

The observations regarding the roses were made within the nursery botanical garden Iaşi.

The nursery also has a rich variety of roses, among which we mention: *Foc de Tabără, Luchian, Betty Prior, Crimson Glory, Acapulco, Super Star, Karen Blixen* etc.

The researches were made within the nursery, on 6 varieties of roses and namely: *„Foc de Tabără", „Luchian", „Betty Prior", „Crimson Glory".* They consisted in studying 6 more important characteristics that define to the greatest extent the decorative value of these varieties and namely: 1: the leafage, 2. the resistance to diseases; 3. the blooming intensity; 4. the flower shape; 5. the colour of petals; 6. the perfume.

The observations were made in 6 period, starting with the date of June 5$^{th}$ approximately every 2 weeks. In the end, we calculated the arithmetical mean for each characteristic in part and the total of points for the characteristics analyzed in each variety.

## RESULTS AND DISCUSSION

For the 4 varieties of roses studies we drew up quality evaluation sheets that comprise the reliability characteristics and the data regarding which we made the observations (tables no. 1, 2, 3, 4).

The results regarding the studied characteristics are the following:

1. The leafage: This characteristic was appreciated according to the density of leaves on the sprouts, the colour, the shininess, the manner it maintains on the bush during the vegetation period, the maximum grade being 10. Among the varieties taken in the study the highest score was registered by the varieties *„Foc de Tabără", „Luchian", „Betty Prior"* with 54 points ,and the lowest score was registered by the variety*„Crimson Glory"* with 48 points.

*Table 1*

**The quality evaluation sheet for the *„Foc de tabără”* variety**

| Crit. No. | Evaluated characteristic | Max. no. of points | Date of observations | | | | | | Total points | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5 VI | 26 VI | 12 VII | 30 VII | 13 VIII | 1 IX | | |
| 1. | Leafage | 10 | 9 | 9 | 9 | 9 | 9 | 9 | 54 | 9.0 |
| 2. | Resistance to diseases | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 48 | 8.0 |
| 3. | The blooming intensity | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 60 | 10.0 |
| 4. | The bud shape | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 54 | 9.0 |
| 5. | The colour at blossoming | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 36 | 6.0 |
| 6. | The perfume | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 6 | 1.0 |
| TOTAL POINTS | | | | | | | | | | **43.0** |

*Table 2*

**The quality evaluation sheet for the *„Luchian”* variety**

| Crit. No. | Evaluated characteristic | Max. no. of points | Date of observations | | | | | | Total points | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5 VI | 26 VI | 12 VII | 30 VII | 13 VIII | 1 IX | | |
| 1. | Leafage | 10 | 9 | 9 | 9 | 9 | 9 | 9 | 54 | 9.0 |
| 2. | Resistance to diseases | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 48 | 8.0 |
| 3. | The blooming intensity | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 60 | 10.0 |
| 4. | The bud shape | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 54 | 9.0 |
| 5. | The colour at blossoming | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 36 | 6.0 |
| 6. | The perfume | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 6 | 1.0 |
| TOTAL POINTS | | | | | | | | | | **43.0** |

*Table 3*

**The quality evaluation sheet for the *„Betty Prior"* variety**

| Crit. No. | Evaluated characteristic | Max. no. of points | Date of observations | | | | | | Total points | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5 VI | 26 VI | 12 VII | 30 VII | 13 VIII | 1 IX | | |
| 1. | Leafage | 10 | 9 | 9 | 9 | 9 | 9 | 9 | 54 | 9.0 |
| 2. | Resistance to diseases | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 48 | 8.0 |
| 3. | The blooming intensity | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 60 | 10.0 |
| 4. | The bud shape | 9 | 8 | 8 | 8 | 8 | 7 | 7 | 46 | 7.6 |
| 5. | The colour at blossoming | 6 | 5 | 5 | 5 | 5 | 5 | 5 | 30 | 5.0 |
| 6. | The perfume | 7 | 2 | 2 | 2 | 2 | 2 | 2 | 12 | 2.0 |
| TOTAL POINTS | | | | | | | | | | **41.6** |

*Table nr. 4*

**The quality evaluation sheet for the *„Crimson Glory"* variety**

| Crit. No. | Evaluated characteristic | Max. no. of points | Date of observations | | | | | | Total points | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5 VI | 26 VI | 12 VII | 30 VII | 13 VIII | 1 IX | | |
| 1. | Leafage | 10 | 8 | 8 | 8 | 8 | 8 | 8 | 48 | 8.0 |
| 2. | Resistance to diseases | 8 | 8 | 8 | 7 | 7 | 6 | 6 | 42 | 7.0 |
| 3. | The blooming intensity | 10 | 9 | 9 | 9 | 9 | 9 | 9 | 54 | 9.0 |
| 4. | The bud shape | 9 | 8 | 8 | 8 | 7 | 7 | 7 | 45 | 7.5 |
| 5. | The colour at blossoming | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 36 | 6.0 |
| 6. | The perfume | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 42 | 7.0 |
| TOTAL POINTS | | | | | | | | | | **44,5** |

2. Resistance to diseases: This represents one of the most important aspects in the culture of roses. The most frequent diseases are : the black spotting , (*Diplocarpon rosae*), the scab (*Phragmidium disciflorum*) and the mildew (*Sphaerotheca pannosa, var. rosae*), the maximum grade being 8. The tolerance to these diseases is determined by dense, puckered, dark green foliage and with a thick cuticle.

The very good results were registered by three of the analyzed varieties, having the maximum score of 48 points, with the exception of the variety *„Crimson Glory”* which has proven sensitive to diseases, registering 42 points.

3. The intensity of blooming: This characteristic represents one of the most important qualities of the varieties of roses. In the case of this character, the maximum grade is 10.

The varieties *„Foc de Tabără, „Luchian”, „Betty Prior”* distinguished themselves with a maximum score of 60 points. The minimum score was registered by the variety *„Crimson Glory”* with 54 points.

4. The bud shape: It is a trait characterising all varieties of climbing roses; taking into account both the shape and the dimensions of the flowers.

The varieties *„Foc de Tabără” and „Luchian”,* distinguished themselves as having very beautiful flowers: registering a score of 54 points.

5. The colour at blossoming: It can be assessed rather subjectively according to the person executing the pre-operation. The maximum grade for this characteristic is considered 6, and the maximum grade is given to the varieties whose petals have an intense colour, well emphasized and that remains for a longer period of time.

As regards the colour at flowering, the varieties *„Foc de Tabără”, „Luchian” and „Crimson Glory”* distinguished themselves.

6. Perfume: It is also a much appreciated characteristic, the maximum grade being 7.

Of the 4 varieties analyzed, as regards the most perfumed one, we noticed the flowers of the *„Crimson Glory”* variety with a maximum score of 42 points. This variety represents the velvet red claret flower and with a strong perfume of damask roses.

The quality evaluation operation of the rose characteristics offers us a general, subjective orientation, from some points of view regarding the decorative value of roses. Through this we can thus emphasize the very decorative varieties of roses.

## CONCLUSIONS

The analysis of the behaviour of the 4 varieties, regarding the most important decorative characteristics emphasized the following aspects:

1. The most decorative leafage was ascertained in the *„Foc de Tabără”, „Luchian” and „Betty Prior” varieties*.

2. All the varieties analyzed , with the exception of *„Crimson Glory”* variety that has proven to be sensitive to diseases, have proven most resistant to the attack of diseases,.

3. The varieties *„Foc de Tabără”, „Luchian” and „Betty Prior”* distinguished themselves through a great intensity of blooming.

4. The most beautiful flowers have proven to be those from the varieties *„Foc de Tabără”* and *„Luchian”*.

5. As regards the colour of flowers the most valuable varieties distinguished were *„Foc de Tabără”* and *„Luchian”*

6. The most perfumed flowers, of the four varieties analyzed have proven to be the flowers of the variety *„Crimson Glory”*.

7. From the point of view of the total score, the most valuable variety is *„Crimson Glory”,* registering a score of 44.5 points), distinguishing itself through a medium vigour, semi-erected branches, medium-size, dense, normal mat-green leaves. The flowers are of a red claret velvet colour with shades in blue and with a strong perfume of damask roses. The disadvantage of this variety is represented by the slight sensitivity to mildew and frost.

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# STUDY ON THE VULNERABILITY / SENSITIVITY OF THE TREE VEGETATION IN THE IASI URBAN AREA

## STUDIU PRIVIND VULNERABILITATEA / SENSIBILITATEA VEGETAȚIEI ARBORICOLE DIN ZONA URBANĂ A MUNICIPIULUI IAŞI

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***Abstract:*** *The paper's object is to identify tree species with reduced sensitivity to urban environmental factors. The study was performed between 2012 and 2014 and it used as a material totally or partially dried tree species permanently situated in all kinds of green spaces from Iasi and that were cut during the said period. The studied indicators refer to the trees taxa, trees size, the categories of green spaces where the trees are situated and the initial condition of the trees. Our results show that the green spaces from Iasi are mainly populated with about 18 types of deciduous trees and conifers. The high sensitivity to urban environmental factors between 2012 and 2014 showed tree species ranging from softwood, mainly the t and Populus genera, while from the trees belonging to the hardwood categories, species belonging to the Acer genus.*
***Key words: sensitivity, trees, urban, Iaşi***

***Rezumat:*** *Lucrarea are ca scop identificarea speciilor de arbori cu sensibilitate redusă la factorii de mediu urban. Studiul a fost efectuat în perioada 2012-2014 şi a avut ca material toate speciile de arbori uscați, în totalitate sau parțial, care au fost situați pe toate tipurile de spații verzi ale municipiului Iaşi şi au fost doborâți în această perioadă. Indicatorii luați în studiu se referă la taxonii de arbori, dimensiunile arborilor, categoriile de spații verzi unde se situează aceştia şi starea inițială a arborilor. Rezultatele reieşite arată că în cadrul spațiilor verzi din municipiul Iaşi predomină aproximativ 18 genuri de arbori foioşi şi răşinoşi. Sensibilitate ridicată la factorii de mediu urban, în decursul perioadei 2012-2014 au prezentat speciile de arbori din categoria esențelor moi, cu preponderență cele din genurile Tilia şi Populus, iar dintre arbori aparținând categoriei esențelor tari, speciile aparținând genului Acer.*
***Cuvinte cheie:*** *sensibilitate, arbori, mediu urban, Iaşi*

## INTRODUCTION

Once with the cities development, the urban environment worldwide deals with issues like air pollution, industrialization enhancement and growth of the

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road traffic, waste accumulation, agglomerations of built surfaces (Iliescu, 2006). An extra issue regarding the decrease of the quality and quantity of the green space can be added to this, taking into account the great importance it has on the quality of life (Law 24/2007). A very important role as regards the composition of green spaces is played by the wooden vegetation, especially the tree vegetation (Bernardis, 2012, Iliescu, 1998). The climacteric disturbances during the last period together with the urban factors contributed to the decrease of the wooden vegetation resistance of the green spaces from Iasi and to important loses of trees and scrubs. In order to prevent these trees loses of high social, economic etc. impact and a durable development of the landscaping architecture from Iasi, we performed a study meant to identify the tree species of low sensitivity or vulnerability to the urban environmental factors. In order to create green spaces, it is necessary to know about the strong interdependence between wooden vegetation and its development environmental factors, thus conditioning their durability in time *(*Palade, 1994; Sandu, 2008; Sonea *et al*, 1997).

## MATERIAL AND METHOD

The study was performed between 2012 and 2014 and the study material was represented by species of totally or partially dried trees situated in the green spaces of Iasi Municipality and that were cut during this period. The studied indicators refer to the trees taxa, trees size, the categories of green spaces where the trees are situated and the initial condition of the trees belonging to both softwood and hardwood. In order to perform the said research, we made personal assessments directly on site, based on measurements, observations, images, as well as in the production department of Servicii Publice Iaşi Joint Stock Company.

## RESULTS AND DISCUSSIONS

During the study period between 2012 and 2014, 3041 totally or partially dried trees situated in the green spaces of Iasi Municipality were cut. Among these, 1557 trees had a body diameter between 10 and 30 cm, 932 trees had a body diameter between 31 and 50 cm and 552 trees had a body diameter of over 50 cm (*Table 1*).

*Table 1*

**The number and dimensions of the trees in the green space of Iaşi Municipality cut between 2012 and 2014**

| No. | Trees diameter (cm) | Year 2012 (pc.) | Year 2013 (pc.) | Year 2014 (pc.) | Total no. of cut trees 2012-2014 (pc.) |
|---|---|---|---|---|---|
| 1 | 10-30 | 930 | 491 | 136 | 1557 |
| 2 | 31-50 | 487 | 232 | 213 | 932 |
| 3 | >50 | 375 | 127 | 50 | 552 |
| 4 | Total | 1792 | 850 | 399 | 3041 |

By analyzing table 1 we chat in 2012 and 2013 the percentage of partially and totally dried trees that were cut with a body between 10 and 30 cm, meaning young and very young trees, is of 51.89% and 57.76%. In 2014, the percentage of

the cut dried trees with the body between 31 and 50 cm, meaning young trees with physiological functions performed at maximum capacity, was of 53.38%. during the entire studied period the lowest percentage of cut dried trees, meaning under 20%, was registered for the trees whose bodies were over 50 cm, meaning mature trees. The partially or totally dried trees that were cut were mainly situated on the green spaces like parks, gardens, squares, straightway, pertaining to the residential complexes, and green spaces within the institutions, enterprises, social and cultural units, cemeteries and protection curtains. Most of the dried trees were cut in the period between 2012 and 2013 on the green spaces pertaining to the condominium residential complexes, followed by straightway, parks and squares. In the period between 2013 and 2014, most of the dried trees were cut from the straightway areas, followed by the green spaces pertaining to the condominium residential complexes and then from parks, squares, green spaces within the institutions area (Fig. 1).


**Fig. 1 -** Dried trees in the straightway areas of Iaşi

Lacking a Green Space Register of Iaşi, the data could not have been percentually reported for each category of green space and for each trees taxon, in order to observe the loss of tress for each category.

As regards the dried trees taxa, thanks to the anthropic factors of the urban environment, between 2012 and 2014 about 18 types of ornamental dried trees were identified on the green spaces of Iaşi**.** From a percentage point of view, about 58.82%, of it belong to the hardwood category and 41.18% to the softwood category. The ornamental trees species of high sensitivity or vulnerability to the urban environmental factors on the green spaces of Iaşi were those belonging to the *Tilia*, *Populus* and *Acer* genera. In the period between 2012 and 2013, most of the dried trees that needed to be cut were those belonging to the *Tilia* genus – 43.69%, then to the *Populus* genus –27.90% and the *Acer* genus – 14.84%. The other species registered lower percentages, between 5.63% and 1% for the

*Robinia*, *Aesculus* and *Gleditsia* genera, while others even percentages under 1% *(Table 2*).

*Table 2*

**Types of trees cut in the period between 2012 and 2014 on the green space of Iaşi Municipality**

| No. | Tree type | Year 2012 | | Year 2013 | | Year 2014 | | Total no. of cut trees 2012-2014 | |
|---|---|---|---|---|---|---|---|---|---|
| | | (pc.) | (%) | (pc.) | (%) | (pc.) | (%) | (pc.) | (%) |
| 1 | *Acer* | 266 | 14.84 | 122 | 14.35 | 169 | 42.35 | 557 | 18.32 |
| 2 | *Aesculus* | 55 | 3.07 | 128 | 15.06 | 4 | 1.00 | 187 | 6.15 |
| 3 | *Betula* | 1 | 0.05 | 1 | 0.12 | 4 | 1.00 | 6 | 0.20 |
| 4 | *Catalpa* | 1 | 0.05 | 1 | 0.12 | 14 | 3.50 | 16 | 0.52 |
| 5 | *Fraxinus* | - | - | - | - | 7 | 1.75 | 7 | 0.23 |
| 6 | *Gleditsia* | 27 | 1.50 | 40 | 4.70 | - | - | 67 | 2.20 |
| 7 | *Juglans* | - | - | - | - | 1 | 0.25 | 1 | 0.03 |
| 8 | *Quercus* | 15 | 0.83 | 22 | 2.59 | 1 | 0.25 | 38 | 1.25 |
| 9 | *Malus* | - | | 1 | 0.12 | 2 | 0.50 | 3 | 0.09 |
| 10 | *Morus* | 4 | 0.22 | 1 | 0.12 | - | - | 5 | 0.16 |
| 11 | *Picea* | 8 | 0.44 | 1 | 0.12 | 5 | 1.25 | 14 | 0.46 |
| 12 | *Pinus* | 6 | 0.33 | - | - | - | - | 6 | 0.20 |
| 13 | *Populus* | 500 | 27.90 | 219 | 25.76 | 111 | 27.82 | 830 | 27.29 |
| 14 | *Prunus* | 3 | 0.17 | 2 | 0.23 | 1 | 0.25 | 6 | 0.19 |
| 15 | *Robinia* | 101 | 5.63 | 33 | 3.88 | 14 | 3.51 | 148 | 4,86 |
| 16 | *Salix* | 12 | 0.67 | 32 | 3.76 | 4 | 1.00 | 48 | 1.57 |
| 17 | *Tilia* | 783 | 43.69 | 242 | 28.47 | 62 | 15.54 | 1087 | 35.74 |
| 18 | *Ulmus* | 10 | 0.56 | 11 | 1.29 | - | - | 21 | 0.69 |
| **19** | **Total** | **1792** | **100** | **850** | **100** | **399** | **100** | **3041** | **100** |

In 2012, the lowest percentages of dried trees that were cut belonged to the following genera: *Catalpa* by 0.05%, *Betula* cu 0.05%, *Ulmus*, *Quercus* etc. in the period between 2013 and 2014, most of the dried trees that were cut belonged to the *Tilia* genus – 28.47%, followed by the *Populus* genus – 25.76%, *Aesculus* 15.06% and *Acer* 14.35%. Other dried ornamental trees species that registered percentages between 5% and 1% were those belonging to the *Gleditsia* genus by 4.70%, *Robinia* 3.88%, *Salix* 3.76 %, *Quercus* 2.59% and *Ulmus* 1.29%. The other species registered percentages under 1%, and the least dried trees belonged to the following genera: *Morus, Catalpa* and *Betula*. In the period between 2014 and 2015, due to some legal mismatches that led to operational procedure difficulties; very few dried ornamental trees were cut (less than 50% in comparison with the previous years), even if their effective number on the green spaces of Iaşi Municipality was higher. Most of the dried trees cut in 2014 belonged to the *Acer* genus (42.35%), followed by species belonging to the *Populus* genus (27.82%), while on the third place *Tilia* genus by 15.54%. The lowest percentages of dried trees cut in 2014 were registered for the species

belonging to the *Quercus*, *Juglans*, *Fraxinus* and *Robinia* genera. The results of the study performed in the period between 2012 and 2014 show that the green spaces of Iaşi Municipality of high sensitivity or vulnerability to the urban environmental factors are those belonging to the *Tilia* genus, followed by the *Populus* and *Acer* genera (Fig. 2).

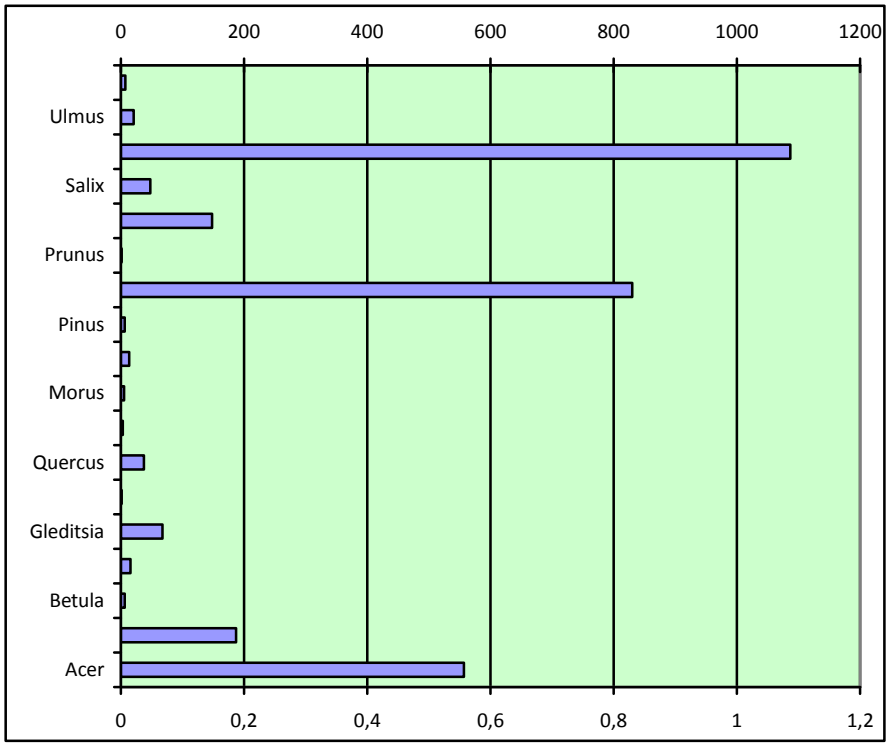


**Fig. 2.** Trees taxon vulnerability in Iaşi Municipality in the period between 2012 and 2014

Figure 2 also shows us that in the period between 2012 and 2014 trees of low sensitivity or vulnerability belonged to the following genera: *Juglans, Betula, Fraxinus, Pinus, Prunus, Ulmus, Robinia, Quercus*. Among these, most of the low vulnerability species belong to the hardwood category, while most of the high vulnerability or sensitivity ornamental trees belong to the softwood category (*Tilia*, *Populus*), indifferently from the green space category they were situated on.

## CONCLUSIONS

1. The green spaces of Iaşi Municipality are mostly populated by about 18 types of deciduous trees and conifers. Among these, 41.18% belong to the softwood category and 58,2% belong to the hardwood category.

2. The high sensitivity to the urban environmental factors of Iaşi Municipality for the period between 2012 and 2014 presented the trees species of the softwood category, mainly those of the *Tilia* and *Populus* genera, which were

situated on the 1st and 2nd positions during the entire period of the study. Among the high sensitivity trees species belonging to hardwood, the *Acer* genus trees species were on the 1st position.

3. During the entire study period, among the low sensitivity/vulnerability trees on all the green spaces categories of Iaşi Municipality the following were signaled: *Ulmus*, *Robinia*, *Quercus* and so on.

4. Most of the dried trees were situated on straightway, followed by the green spaces pertaining to the condominium residence complexes and to the other types of green spaces.

5. Young and very young trees (diameter at the stem between 10-30 cm) have vulnerability/sensitivity increased in urban conditions, compared with mature trees and very mature (diameter at the base of the stems between 31-50 cm and 50 cm).

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# IMPACTS ON CLIMATE CHANGE GENERATED BY POLYVINYL CHLORIDE (PVC) AND POLYHYDROXYALKANOATES (PHAs) PRODUCTION: A COMPARATIVE APPROACH

## IMPACTUL PRIVIND SCHIMBĂRILE CLIMATICE GENERATE DE PRODUCȚIA POLICLORURII DE VINIL (PVC) ȘI A POLIHIDROXIALCANOAȚILOR (PHAs): O ABORDARE COMPARATIVĂ

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***Abstract.*** *The plastic industry has always been considered a major consumer of energy, natural resources, especially non-renewable fossil fuels, causing significant threatens for the environment. This situation is amplified by the impacts generated during the production and utilization of plastics. The processes applied for plastic manufacturing using petroleum-based raw materials have significant implications for $CO_2$, $CH_4$, Volatile Organic Compounds (VOC) emissions occurrence. For this reason, in this paper we have analyzed two plastic production processes: of polyvinyl chloride (PVC) based on non-renewable resources, and polyhydroxyalkanoates (PHAs) biopolymers, respectively using recycled organic waste as raw material transformed by aerobic and/or anaerobic bioprocesses. Environmental impacts of these two processes were evaluated by applying Life Cycle Assessment methodology. The results have shown that PVC production has a considerable contribution to climate change compared with PHAs. Recycling of organic waste for biopolymers manufacturing proved to be a sustainable alternative, since the emissions are significantly lower compared with those from PVC production.*
***Key words:*** *biopolymers, Life Cycle Assessment, polymers, plastic, process*

***Rezumat.*** *Industria de mase plastice a fost întotdeauna considerată un mare consumator de energie, resurse naturale, în special combustibili fosili neregenerabili, având consecințe semnificative pentru calitatea mediului înconjurător. Această situație este amplificată de impacturile generate în timpul*

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*producției şi utilizării materialelor plastice. Procesele aplicate pentru fabricarea plasticelor folosind materii prime pe bază de petrol generează emisii de $CO_2$, $CH_4$, compuşi organici volatili (COV). Din acest motiv, în această lucrare am analizat procesele de producție a două tipuri de materiale plastice: policlorura de vinil (PVC), din materii prime pe bază de resurse neregenerabile şi polihidroxialcanoații (PHAs), din deşeuri organice reciclate, transformate prin bioprocese aerobe şi/sau anaerobe. Impacturile de mediu celor două procese au fost evaluate prin Analiza Ciclului de Viață. Rezultatele analizei au arătat că procesul de producție al PVC contribuie într-o măsură mai mare la generarea de impact în mediul înconjurător asociat schimbărilor climatice decât producția se dovedeşte a fi o alternativă durabilă, deoarece emisiile sunt semnificativ mai mici comparativ cu cele rezultate din producția de polimeri convenționali.*

***Cuvinte cheie:*** *biopolimeri, Analiza Ciclului de Viață, polimeri, plastic, proces*

## INTRODUCTION

Global warming is a phenomenon that occurred as a direct consequence of increased greenhouse gas emissions (GHG) - especially carbon dioxide ($CO_2$) into the atmosphere (Hekkert *et al.,* 2000).

This phenomenon is the result of human industrial activities including extraction and burning of fossil fuels (oil, coal, natural gas) from which significant amounts of $CO_2$ can result. Plastics industry is among the industries using raw materials based on fossil fuels..

The plastics production has become an inseparable part of daily life despite the fact that the plastics manufacturing and their utilization can generate significant impacts in the environment due to necessary raw materials derived from petroleum and also because the finished products are not biodegradable (Gao *et. al.,* 2011). One of the most widely used polymers in the world polyvinyl chloride (PVC) has applications in manufacturing of profiles, pipes and fittings, bottles, cables, flooring, flexible tubes and profiles, film and sheets and others (Shojai and Bakhshandeh, 2011).

Recent studies have highlighted that the plastic materials can affect human health because they contain fairly large proportions of chemical additives that can be endocrine disruptors or carcinogens, or generate toxic reactions in the human body or marine animals (Andrady, 2011; Gavrilescu *et al.,* 2015; Rochman *et. al.,* 2013).

The high consumption of synthetic polymeric materials has brought increasingly into attention the impact that they generate in the environment as a consequence of emissions from the process, leading to increased global warming phenomenon, as well as due to the huge quantities of non-biodegradable polymeric waste (Rochman *et. al.,* 2013).

On the other hand, the interest for alternative solutions that could ensure the perspective for GHG emissions reduction has increased, in parallel with a decline in the volume of wastes derived from non-biodegradable synthetic polymers as well as of biodegradable organic waste (Gao *et. al.,* 2011).

In this context intense research are developed for the synthesis of organic polymeric biomolecules from organic raw materials, in particular biodegradable waste, which can subsequently be used in bioplastics manufacturing process.

In this way it is intended to diminish the environmental impacts that the production processes of traditional plastics can generate in the environment as well as those induced by the presence of non-biodegradable plastic waste.

In this paper, a sustainability analysis of two plastics production processes: a classical one, which uses raw materials from fossil fuels (polyvinyl chloride, PVC), and an unconventional one, which produces biopolymers from bio-organic waste (polyhydroxyalkanoates, PHAs) is performed.

The analysis is based on Life Cycle Assessment methodologies (LCA) and considers a number of impact categories which both production processes can generate into environment.

## MATERIAL AND METHOD

### 1. Description of the studied processes

The chemical industry produces, among others, various types of plastics such as polyethylene terephthalate (PET), high density polyethylene (HDPE), low density polyethylene (LDPE), polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), acrylonitrile butadiene styrene copolymer (ABS). PVC it is considered to be the polymer with the lowest environmental impact (Ognedal *et al.,* 2014).

Vinyl chloride is the monomer used in large quantities in the plastics industry, especially for manufacturing of vinyl polymers and copolymers with great importance for the industry and consumption.

Polyhydroxyalkanoates (PHAs) are biodegradable polymers obtained from biomolecules produced by microbial biosynthesis using available and renewable carbon sources. PHAs are biodegradable and can be used in the production plastic biomaterials (Cyras *et al.,* 2007).

Assimilation at industrial level of bioplastics production could generate a sustainable solution for reducing the consumption non-renewable resources and the impacts caused by their exploitation during plastics production processes. Moreover, this could lead to the reduction of waste plastic quantities bearing in mind that currently about 1.3 billion tons of plastic waste are recorded every year at a global level, according to the United Nations Environment Programme (UNEP) (Melikoglu *et al.,* 2013).

Table 1 presents the characteristics of PVC and PHAs production processes.

### 2. Life Cycle Assessment (LCA)

Life cycle assessment is an objective method used to evaluate the environmental impacts associated with a product, process or system, on the whole life cycle, including the extraction and purchase of raw materials, production, distribution, use, reuse/recycling, final disposal (Ghinea *et al.,* 2014). For correct application of this impact assessment methodology four stages are necessary to be performed (Ghinea *et al.,* 2014; ISO 14040, 2006).

LCA methodology was applied to evaluate different systems: municipal solid waste management (Ghinea and Gavrilescu, 2010; Ghinea and Gavrilescu, 2011; Ghinea *et al.*, 2012; Ghinea *et al.,* 2014), paper making industry (Petraru *et al.,* 2011; Ghinea *et al.,* 2014), biogas (Cozma *et al.,* 2013), construction and demolition waste management (Simon *et al.,*

2013). By applying LCA methodology, some environmental impacts of the two industrial processes for obtaining plastic materials will be assesses and ranked.

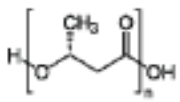
The functional units considered in this paper are represented by 1 kg of PVC and 1 kg of PHAs, respectively. In the inventory stage the necessary data were collected to establish environmental impacts of both processes studied in this context. The inputs and outputs data were determined for each production stage included in the respective processes.

The data were collected from literature, the databases of GaBi software etc. Some data were calculated and estimated based on the existing technologies or Best Available Techniques. In the assessment impacts stage, the environmental impacts for both processes (PVC and PHAs) were obtained. LCA analysis was assisted by GaBi, which is a modular software system that includes plans, flows, processes and their functions. Gabi is a commercial software tool in order to achieve the balance sheets life-cycle products by means of the data for life-cycle modeling.

In addition to the assessment a life cycle products, GaBi software can be used to: eco-design, material flow analysis, environmental balance sheets, environmental and development sustainable reports etc.

*Table 1*

**The characteristics of production processes for PHAs and PVC (Comăniță *et al.,* 2014)**

| Caracterization | Processes studied | |
|---|---|---|
| | **Conventional polymer production process** | **Biopolymer production process** |
| Classification *(depending on production way and utilization)* | Rigid polyvinyl chloride (PVC-D); plasticized polyvinyl chloride PVC-M); chlorinated polyvinyl chloride (PVC-C); copolymer vinyl chloride - vinyl acetate (VC / VAC) etc. | polylactic acid (PLA); polyhydroxyalkanoates (PHAs); starch (BMS); cellulose and its derivatives; polyvinyl alcohol (PVOH); biodegradable aliphatic and aromatic copolymers etc. |
| Chemical formula | PVC:<br>$[-CH_2-CHCl-]_n$ | PAH:<br>  |
| Production process | The polymerization (very exothermic reaction) is the most common process for the manufacture of polyvinyl chloride and may be performed by using three methods: bulk; emulsion; suspension. | Polyhydroxyalkanoates are bioppolymers which can be obtained by using a wide range of substrates such as renewable resources (sucrose, starch, cellulose, and triglycerides), by products (syrup, glycerine). These biopolymers can be synthesized by aerobic and/or anaerobic biological processes. |
| Emissions resulted from the process | sulfur dioxide ($SO_2$), nitrogen dioxide ($NO_2$), volatile organic compounds (VOC), methanol ($CH_3OH$), methane ($CH_4$), hydrogen sulfide ($H_2S$) | carbon dioxide ($CO_2$), methane ($CH_4$), nitrogen ($N_2$), ammonia ($NH_3$), carbon monoxide (CO) |

## RESULTS AND DISCUSSION

The evaluation of the environmental impacts for the PVC and PHAs production processes was performed by applying two methods based on LCA methodology included in GaBi software: CML 2001 and ReCiPe. Figure 1 illustrates the environmental impacts of PVC production calculated based on CML 2001 methodology.

Besides the global warming potential (GWP), the following impact categories were also considered: acidification potential (AP), eutrophication potential (EP), human toxicity potential (HTP), photochemical ozone generation potential (POCP).

The results are presented in the normalized values (EU 25+3).

The results obtained for PVC production show that the environmental impacts are negative and can be ranked in the following order: PH> POCP> AP> GWP> EP. Regarding the PHAs production, it can be observed from Fig. 2 that the values of the impact categories are lower than those illustrated in Fig. 1 for the PVC production, but they are also associated with negative environmental impacts. In the case of PHAs production process, the impact categories decrease in the following order: AP> GWP> PH> POCP> EP. The data obtained based on the ReCiPe method show that some of the impacts such as: climate change associated with ecosystems (Cce) and climate change associated with damage human health (CCHh) have positive values, for both PVC and PHAs production process, which means that the evaluated processes generates negative impacts on environment. Analyzing the data presented in Fig. 3 and Fig. 4 it can be concluded that global warming potential values are lower for PHAs production compared with those obtained for PVC production.

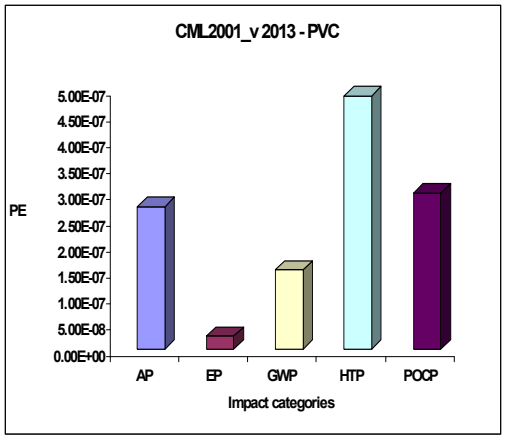



**Fig - 1.** Environmental impacts generated in the PVC production process (CML 2001 methodology)

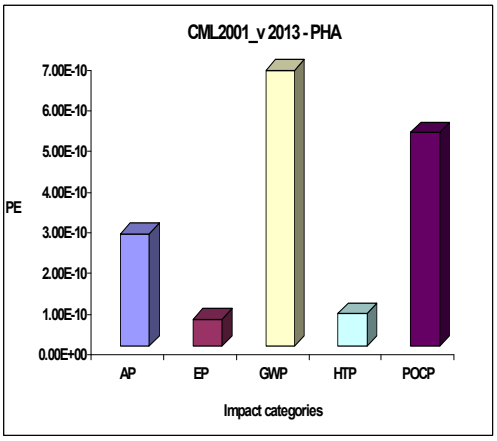



**Fig. 2 -** Environmental impacts generated in the PHAs production process (CML 2001 methodology)

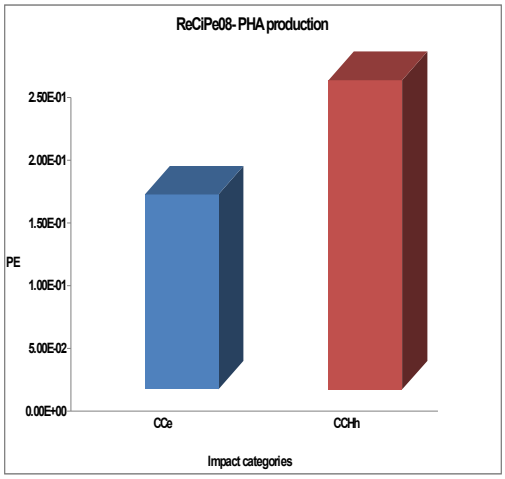



**Fig. 3 -** Global warming potential associated with PVC production - ReCiPe method

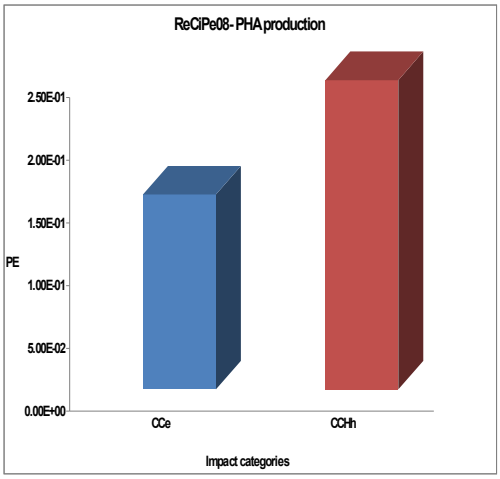



**Fig. 4 -** Global warming potential associated with PHAs production - ReCiPe method

Also CCHh impact category has higher values than Cce. In the case of the production of PVC, the contribution to global warming is the consequence of $NO_2$, VOC, $CH_4$, $SO_2$ emissions from the polymerization step.

The emissions resulted from PHAs production that contribute to global warming are $CO_2$, $CH_4$, CO, which are generated from the anaerobic digestion phase.

## CONCLUSIONS

1. In this paper life cycle assessment methodology was applied for the determination of environmental impacts caused by two industrial processes were evaluated from environmental point of view.

These processes address the manufacturing of obtaining plastics using different raw materials (based on fossil fuels and bio-organic waste, respectively) and having as final product a classic polymer (polyvinyl chloride) and a biopolymer (polyhydroxyalkanoate).

2. The evaluation was performed using CML2001 and ReCiPe LCA methods included in GaBi software.

3. After the evaluation of the impacts generated by the two production processes in the environment based on life cycle analysis methodology (LCA), it has been found that both processes induce negative impacts on environment, with the mention that the impacts generated by PVC production is higher compared to those induced by PHAs production.

4. The higher impact is associated with climate change due to the emissions of greenhouse gases.

***Acknowledgments**: This work was supported by a strategic grant POSDRU/159/1.5/S/133652, co-financed by the European Social Fund within the Sectorial Operational Program Human Resources Development 2007 – 2013 and by a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-IDPCE-2011-3-0559, Contract 265/2011. Also, GaBi Education: Software and database for Life Cycle Engineering.*

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# THE INFLUENCE OF FERTILISATION ON THE ORNAMENTAL CHARACTERS AND ON THE ALANTOLACTONE AND TRITERPENIC ACIDS PROFILES IN *ECHINACEA PURPUREA*

## INFLUENȚA FERTILIZĂRII ASUPRA CARACTERELOR ORNAMENTALE ŞI PROFILULUI ALANTOLACTONEI ŞI ACIZILOR TRITERPENICI LA *ECHINACEA PURPUREA*

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***Abstract.*** *This paper presents the results regarding the ornamental characters and the contents in alantolactone and triterpenic acids of Echinacea purpurea plants fertilized with Osmocote. The fertilisation is positively correlated with the stem ramification, the growth of vegetative mass and the number of inflorescences. Qualitative and semiquantitative analyses for the identification and quantification of alantolactone and for qualitative estimates for triterpenic acids were performed. The general aspect of TLC chromatograms indicated the presence of alantolactone. To confirm the TLC results and to perform a semiquantitative estimate, the samples were analysed through HPLC. The analysis confirmed alantolactone presence. Qualitative analysis also aimed to highlight the profile of certain triterpenic acids (ursolic acid, oleanolic acid), important through the potential farmacodynamic action. Oleanolic acid is present in open inflorescences of Echinacea.*
***Key words****: alantolactone, control released fertiliser, HPLC, morphology, TLC, triterpenic acids.*

***Rezumat.*** *Lucrarea prezintă rezultate privind caracterele ornamentale şi conținutul în alantolactonă şi acizi triterpenici la plantele de Echinacea purpurea fertilizate cu Osmocote. Fertilizarea a fost corelată pozitiv cu gradul de ramificare a tulpinilor, creşterea masei vegetative şi cu numărul de inflorescențe pe plantă. Pentru identificarea şi cuantificarea alantolactonei şi pentru estimarea calitativă a unor acizi triterpenici s-au efectuat analize calitative şi semicantitative. Aspectul general al cromatogramelor CSS a indicat prezența alantolactonei. Pentru confirmarea rezultatelor cromatografiei în strat subțire şi efectuarea unei estimări semicantitative, probele au fost analizate prin metoda HPLC. Analiza a confirmat prezența alantolactonei la nivelul unor valori mici, ceea ce avertizează asupra potențialului alergenic al plantelor. Prin analiză calitativă s-a urmărit şi evidențierea profilului unor acizi triterpenici (acid ursolic, acid oleanolic), importanți prin acțiunea farmacodinamică potențială. Acidul oleanolic este prezent în inflorescențele deschise de Echinacea.*

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***Cuvinte cheie**: alantolactonă, acizi triterpenici, CSS, HPLC, îngrăşământ cu eliberare controlată, morfologie.*

## INTRODUCTION

*Echinacea purpurea* (L.) Moench is a hardy perennial plant which belongs to the *Asteraceae* family and is one of the most common species of the genus, used as ornamentals and commercially treated as medicinal plants due to their antiviral, antibacterial and immunostimulatory activities to humans (Chen *et al*., 2008; Draghia, 2004; Hăncianu, 2014; Stanciu, 2008). The aerial parts of the plant contain flavonoids, polyphenolic acids, essential oils, alcohols, pseudoguaianolide, sesquiterpenes, monoterpenes, sesquiterpene oxides, polyacetylenes, alkylamides and other chemical constituents which have been analysed by applying various chromatographic techniques (Diraz *et al*., 2012; Hăncianu, 2014).

Information regarding the effects of genetic diversity, growing climates and cultivation practices on active constituents (secondary metabolites) and biomass production of *Echinacea* is still very limited (Chen *et al*., 2008). It is known that biosynthesis and concentration of secondary metabolites of plants depend on growing sites, climate conditions, cultural practices, vegetation phases and on cultivar and specific organs of the plant (Mircea (Arsene) *et al*., 2015; Chen *et al*., 2008; Druțu *et al*., 2010).

The objectives of the present study were to simultaneously investigate the alantolactone, ursolic and oleanolic acids profiles in the aerial parts of *Echinacea* and to evaluate macromorphology of the plants in relation to local conditions and nutrients availability. A controlled released fertilizer (CRF) was selected in the study due to its long term soil availability, low cost in terms of labour and good environment protection.

## MATERIAL AND METHOD

Local conditions

The research was conducted at the Floricultural Department of the University of Agricultural Sciences and Veterinary Medicine of Iasi, in the 2013-2015 cultivation years. Soil parameters and climatic conditions were evaluated.

Cultivation trial

The experiment was performed as plot design based on randomised complete blocks with three replications. *Echinacea purpurea* plants were studied related to their macromorphology and terpenoid profile (alantolactone, ursolic acid and oleanolic acids), in two nutritional statuses: local (V1) and enriched soil conditions (V2).

Seedlings of echinacea were planted in the field at the beginning of May, at a density rate of 6 plants $m^{-2}$. The chosen fertilizer was osmocote-type, ecofriendly and 5-6 months availability; NPK (Mg): 15-10-12(2), microelements B, Cu, Fe, Mn, Zn, supporting ornamental growth and development with easy maintenance. The product was administered in 75g $m^{-2}$ just before planting in the first year and at the start of vegetative growth in the next years. Biometric parameters measured were: height and width of plant, number of principal ramifications per plant, number and diameter of inflorescences per plant. Ten plants were randomly selected from each plot for all

parameters evaluated and the average was calculated in both nutritional statuses. Statistical significance between mean values was assessed through classic statistical calculations: significance of differences between the variants using LSD test. The variants were compared with their average. Stems and leaves (together) and inflorescences were studied for aforementioned chemical compounds content in two phenophases (budding stage and full flowering stage), in both nutritional statuses.

Chemicals and phytochemical analysis

*Chemicals* - All chemicals used in the present research were purchased from Sigma Aldrich (Germany). Stock solutions were prepared in HPLC grade methanol and stored at 4ºC until the finalization of the tests. All solvents were of analytical grade.

*Extraction methods*- For TLC evaluation dried plant material was extracted twice with ethanol 70% for 30 minutes under reflux (DER=1:10 g/mL) on a thermostatic water bath. An aliquot of 50 mL from each extract was concentrated using a rotary evaporator (Buchi R 210, Switzerland) to remove the solvent. The residues were dried and stored at 4°C for analysis. For HPLC alantolactone evaluation, the test solutions were obtained in acetonitrile R (1,0 g powdered drug in 3mL solvent, three times, sonicated for 15 minutes). The filtrates were reunited and brought to 10mL in a volumetric flask.

Vegetal samples were noted as follow:

**V1f1** – buds from unfertilized plants (budding stage);
**V2f1** – buds from fertilized plants (budding stage);
**V1f2** – inflorescences from unfertilized plants (full flowering stage);
**V2f2** – inflorescences from fertilized plants (full flowering stage);
**V1-1** – leaves and stems from unfertilized plants (budding stage);
**V2-1** – leaves and stems from fertilized plants (budding stage);
**V1-2** – leaves and stems from unfertilized plants (full flowering stage stage);
**V2-2** – leaves and stems from fertilized plants (full flowering stage stage).

Standards used are noted in TLC chromatograms with: **Ala** – alantolactone; **O** – oleanolic acid; **U** – ursolic acid.

*Terpenoids pattern evaluation by TLC*

In the present study were used:

**a**) for sesquiterpene lactones - Standard compound: alantolactone, 12 µL; Solvents: hexane - ether (25:75, v/v); Detection: Zimmermann reagent, VIS evaluation;

**b**) for triterpenes - Standard compounds: ursolic acid, oleanolic acid, 12 µL; Solvents: chlorophorm - glacial acetic acid - methanol - water (60:32:12:8, v/v); Detection: anisaldehyde - sulphuric acid reagent, VIS evaluation;

In both tests, the TLC plates were sprayed with 10mL of the reagent, heated at at 100°C for 5 min, and then evaluated in VIS.

*RP-LC-DAD analysis of sesquiterpene lactones (alantolactone)*

A Thermo-Fischer UltiMate 3000 system coupled with DAD detector was used to assess the profile sesquiterpene lactones. The working conditions were: Accucore XL-C18 column (4.6 x 150 mm, 4 µm); column temperature: 34°C; detection wavelength was set at 225 nm and the flow rate was 1,2 mL/min. The mobile phase consisting of two eluents as A (water) and B (methanol) used the following linear gradient elution: 0-3 min 38%B; 3-20 min 45%B; 20-30 min 45% B; 30-55 min 55%B; 55-57 min 100%B; 70 min 100%B; 90 min 38%B. As standard, alantolactone was used in amount of 20 µL of a 5mg/mL solution. Samples UV spectra registered at 225nm were automatically compared by Chromeleon 7.2 software and the concentration was expressed as % of the standard's aria/concentration.

## RESULTS AND DISCUSSION

The fertilization increases the plant height, number of principle ramifications and number of inflorescences per plant, and diameter of inflorescences but distinctly positive differences were recorded only in three parameters (tab. 1).

*Table 1*

***Echinacea purpurea:*** **the influence of fertilisation on morphological parameters**

| Treatment | Plant height (cm) | Plant width (cm) | Number of principle ramifications/ plant | Number of inflorescences/ plant | Diameter of inflorescences (cm) |
|---|---|---|---|---|---|
| **$V_1$** | 75,00$^{00}$ | 50,00$^{ns}$ | 6,00$^{00}$ | 45,00$^{00}$ | 7,10$^{ns}$ |
| **$V_2$** | 80,00$^{xx}$ | 48,00$^{ns}$ | 10,00$^{xx}$ | 59,00$^{xx}$ | 8,00$^{ns}$ |
| **Average** | **77,50** | **49,00** | **8,00** | **52,00** | **7,55** |
| LSD 5% | 0,43 | 7,17 | 0,66 | 4,97 | 0,66 |
| LSD 1% | 0,99 | 16,55 | 1,52 | 11,47 | 1,52 |
| LSD 0,1% | 3,16 | 52,67 | 4,83 | 36,49 | 4,83 |

*Note*: **00/xx** = distinctly negative/positive significance; **ns** = not significant

The TLC general overview indicated that alantolactone (violet grey zones, Rf 0,74) is present in closed inflorescences of young plants (budding stage) and in stems and leaves of mature plants (especially fertilized ones).

The HPLC analysis confirmed the presence of alantolactone (Fig. 1). Comparative results obtained (TLC/HPLC) are tabulated in table 2.

Semiquantitative quantification showed that alantolactone is present at low levels (some micrograms at 100 gr dried plant). Moreover, the unfertilised plants contain only traces of alantolactone compared to the fertilised samples.

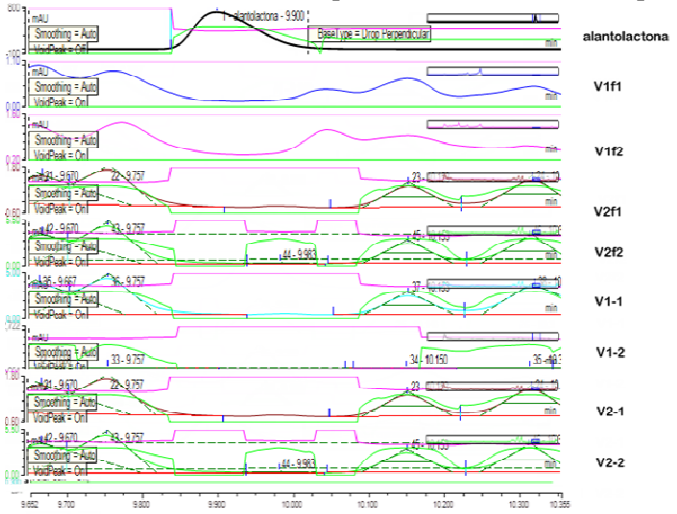


**Fig. 1 -** HPLC alantolactone identification in *Echinacea purpurea* samples

*Table 2*

**Qualitative and semiquantitative identification of alantolactone in *Echinacea purpurea***

| Method | Buds / Open inflorescences | | | | Stems and leaves | | | |
|---|---|---|---|---|---|---|---|---|
| | V1f1 | V1f2 | V2f1 | V2f2 | V1-1 | V1-2 | V2-1 | V2-2 |
| TLC | u | – | u | – | – | u | – | + |
| HPLC | 0,0763 | 0,1010 | 0,3649 | 0,0898 | 0,0135 | 0,1639 | 0,0213 | 0,3065 |

Note: **u** = traces; **+** = present; **–** = not identifiable.

Alantolactone represents a proven allergen and its presence in plants demonstrate that echinacea is potentially allergenic, especially through leaves and stems of fertilised mature plants.

Alantolactone is positive correlated with biomass, especially with ramification and number of inflorescences. These findings are in accordance with Lerdau *et al*. (1997) and Ormeño *et al*. (2008) which have found a positive correlation between the soil and leaves nutrient concentration (especially N) and the concentration of terpenoides from leaves. Gershenzon (1994) stated that nutrient-terpenoid relation has species specificity.

Triterpenic profile confirmed the presence of traces of ursolic acid (Rf 0,40) in open inflorescences while oleanolic acid (Rf 0,58) was confirmed in whole plant. TLC chromatograms are illustrated in figure 2 and the interpretation of results is presented in table 3.

*Table 3*

**Triterpenoides in *Echinacea purpurea* samples**

| Triterpenic acid | Organ | | | | | |
|---|---|---|---|---|---|---|
| | Buds / Open inflorescences | | | | Stems and leaves | |
| | *V1f1* | *V2f1* | *V1f2* | *V2f2* | *V1-2* | *V2-2* |
| ***Ursolic acid*** | - | - | u | u | - | - |
| ***Oleanolic acid*** | u | + | u | u | u | + |

Note: **u** = traces; **+** = present; **–** = not identifiable.

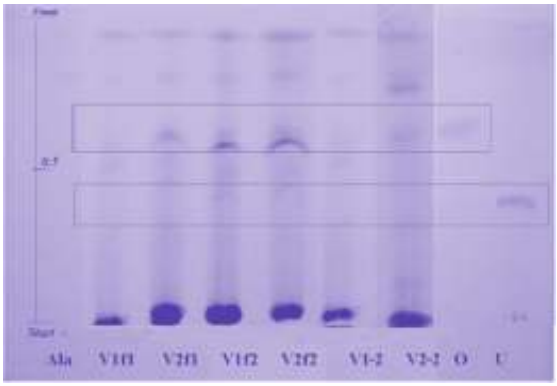


**Fig. 2 -** Triterpenoids in *Echinacea purpurea* samples (TLC)

## CONCLUSIONS

Fertilisation with controlled release fertilizer intensifies plant growth and ramification, also increases the number of inflorescences per plant.

The alantolactone content is positive correlate with biomass of the plants and the concentration of mentioned sesquiterpenlactone is greater in leaves and stems of fertilised mature plants comparative with unfertilized ones.

The TLC triterpenic acid profile in *Echinacea purpurea* confirms the presence of oleanolic acid in all organs of plant while ursolic acid is present only in traces, in open inflorescences.

***Acknowledgments:*** *This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

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# ECOLOGICAL REHABILITATION WORKS RIVER MOLDOVA IN THE "NATURAL SITE ONICEŞTI - MITEŞTI"

## LUCRĂRI DE REABILITARE ECOLOGICĂ A RÂULUI MOLDOVA ÎN „SITUL NATURAL ONICEŞTI – MITEŞTI”

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***Abstract****: Middle and lower course of the river Moldova is integrated into the "Site of Community Importance ROSC10363 River between Oniceni and Miteşti Moldova". It is an integral part of the European ecological network "Natura 2000" in Romania. Part of inactive arms of the river (meander cut) is in a state of ecological degradation. In the area around Sochi are to be accomplished on the river Moldova hydraulic works to rehabilitate pipes under-crossing the water adduction Timişeşti - Iaşi. Positioning the regularization of the river Moldova in the natural site "Oniceni - Miteşti" imposed special conditions on the design of water management. Case study analysis is placed in the area of Moldova under-crossing the river by water adduction Timişeşti - Iaşi. Regularization and defense works on the section of river bank have been designed to provide optimal habitat in the major riverbed and minor riverbed Moldova.*
***Key words****: river, natural site, regularization, protection works, fluvial habitat*

***Rezumat:*** *Cursul mijlociu şi inferior a râului Moldova este integrat în „Situl de importanţă comunitară ROSC10363 Râul Moldova între Oniceni şi Miteşti”. Acesta este parte integrată a reţelei ecologice europene Natura 2000 în România. În zona localităţii Soci sunt prevăzute realizarea de lucrări hidrotehnice pe râul Moldova pentru reabilitarea conductelor de subtraversare a aducţiunii de apă Timişeşti - Iaşi. Lucrările de regularizare a râului sunt poziţionate în sitului natural „Oniceni - Miteşti”. Acest aspect a impus condiţii speciale la proiectarea construcţiilor hidrotehnice. Studiul de caz este amplasat în zona de subtraversare a râului Moldova şi a fost analizat pe o perioadă de 12 ani (2004-2015). Rezultatele cercetării s-au aplicat la proiectarea lucrările de regularizare şi de apărare de mal pe tronsonul de râu considerat. Lucrările au fost proiectate în scopul asigurării condiţiilor optime de habitat în albia minoră şi cea majoră a râului Moldova.*
***Cuvinte cheie****: râu, sit natural, regularizare, apărare de mal, habitat fluvial*

## INTRODUCTION

Hydraulic works regularization changes hydrodynamic balance of the river and surrounding area. Change can be positive or negative. The effect of the regularization may occur immediately or after a longer period of time. The regularization affects existing habitat major and minor river bed (Manoliu, 1973).

The last time there was a change of design concepts to the regularization of the river. New concept aims at collaboration of nature with human activity in modifying

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the hydrodynamic balance of the river.

Follow-up of cooperation with the regularization of the river allowed for useful results for design and execution. The research yielded new theories regularization of rivers. These theories are based on work with construction parameters and habitat requirements of existing river bed. The new concepts aimed at ensuring favorable conditions of life for existing flora and fauna in the river bed and its shores.

Makers involved in the environmental field in the design and implementation of the regularization. Intervention is done by imposing conditions and operating parameters to determine the influence of work as reduced environmental impact. A particular problem requires completion of the regularization of the "natural sites" of rivers. These sites require special environmental conditions in the river bed and the surrounding area.

Protection of flora and fauna zones imposed creating "natural sites" protected by law. In Europe was created the ecological network "Natura 2000". This ecological network was implemented in Romania by a number of community sites protected by law. Community sites include protected areas where human activity is limited. Part of the Moldova River fall into the "site of Community importance ROSC10363 River between Oniceni and Miteşti Moldova" (Fig. 1a).

## MATERIAL AND METHOD

The research was conducted in the Natura "Oniceni - Miteşti". It is located in the basin of river. In the natural site was chosen research sector Moldova River in the town of Soci, Iasi County. The research was conducted in several directions: theoretical studies, field studies and numerical simulations. The theoretical studies were analyzed: a - geo-physical characteristics of research sector; b - site features natural habitat.

The field trials were analyzed: a - Moldova riverbed morphology and stability of shore protection works; b - the influence of hydraulic structures on the natural habitat of the site. The numerical simulations were analyzed behavior shore protection works in various situations of exploitation.

## RESULTS AND DISCUSSIONS

Natural site "Oniceni - Miteşti" is located in the river Moldova and Suceava, Iasi and Neamt County. Site area is 3215 ha. Site coordinates are latitude N 470 17 '22 "; E longitude 260 29 '3 ". Site elevation is 235 mLBS minimum, maximum 339 mLBS and middle 271 mLBS. Biogeographical region is of "Alpine Continental Pannonian Pontic". Natural site „Oniceni-Miteşti" contains habitat type classes rivers, lakes, arable land, grassland and deciduous forests. Natural site contains mammal species listed in Annex II of Council Directive 94/43 / EEC (*Lutra Lutra and Spermophilius citellus*). The site area are present protected species of amphibians and reptiles (*Triturus cristalus, Bombina bombina, Bombina Variegata*). In Moldova and tributary river is protected fish species (*Barbus meridionalis, Rhodeus sericeus amarus, Gobio uranoscopus, Sabanejewia aurata and Cobitis taenia*).

Site location area natural "Oniceni - Miteşti" is characterized by various human activities. The main activities are of agricultural, grazing, water catchments, ballast operations etc. In Moldova River Municipal and industrial waste are discharged. They continuously degrade habitat natural site "Oniceni - Miteşti". Presence of hydraulic structures in the riverbed alters living conditions of flora and fauna (Fig. 1b and 1c).

Moldova River is used as an emissary for the waste generated by human activities and industrial. The Moldova River has the ability to dilute and disperse contaminants reached in the water. But self-recovery possibilities are endless natural quality. Overcoming of some limitations of pollution can cause major and irreversible changes in aquatic ecosystems (Bica, 2000; Tockner, 1999).

The construction of regularization in whites and shore protection affects habitat of species of conservation interest. This results in the need for correlation of design parameters with aquatic environmental conditions. New theories regularization of rivers hydraulic structures based on collaboration with major and minor riverbed habitat.


**Fig. 1 -** Framing research area within the "Natural site Oniceni - Miteşti";
a - natural site map; b, c - the research work analyzed shore.

The work carried out in the riverbed causes morphological and ecological disturbances. Disorders occur in the minor riverbed and floodplain especially. Some

hydraulic works produce an interruption of connectivity between major and minor bed of the river. Interrupting lateral connectivity (floodplain - minor beds) in a watercourse is manifested in various situations: - modification of natural habitats and ecological communities in floodplains land; b - reducing the number of aquatic species; c - achieving a cycle of habitat related to the development of connectivity side (eg reproduction for certain fish).

The research sector is three adduction pipes of the water supply system in Iaşi County. Timişeşti water sources are brought to Iasi through two pipelines adduction: adduction I Timişeşti, diameter 800 mm (put into operation 1911) and adduction II Timişeşti (two pipes with a diameter 1000 mm, in service in 1973). The riverbed Moldova in the Sochi construction is carried out under-crossing of the three adduction pipes.

The section of river regulation work has a bed and shore protection. Coastal defense works were carried out in 1970...1973. Defending the shore is made of massive stone that was laid over concrete slabs. River Moldova in the study presents the current state of a linear channel. On the section of riverbed forming calibrated water flow speeds of the flood. Hydraulic and hydrological regime of the river habitat affects the riverbed (Luca, 2008). Flows and levels of computing probabilities are given in Table 1.

*Table 1*

**Calculus discharges and levels**

| **Calculus probability, *p* %** | **1%** | **2%** | **5%** | **10%** | **50%** |
|---|---|---|---|---|---|
| $Q_{p\%}$ ($m^3/s$) | 1810 | 1555 | 1200 | 940 | 28,8 |
| *H* (m LBS) | 257,15 | 256,95 | 256,50 | 255,90 | 253,10 |

The riverbed Moldova transited in 2005 a flood flow of 1168 $m^3/s$. In 2010 there were two floods: the first summer flow of 660 $m^3/s$; the second in autumn flow 965 $m^3/s$. The effects of floods have resulted in partial degradation of shore defense (Luca, 2012).

**Fig. 2 -** Left bank degradation and flood defense works in 2008

**Fig. 3** - State degradation protection of the bank works (year 2014)

Flooding on the river Moldova for 2010-2015 riverbed morphology changed in the Soci area, Iasi County. In 1970 there was minor riverbed into two branches

branching technological reasons. The flow is divided in two whites and produces increased water velocity. Morphological changes occurred during 2000...2015 partially or completely destroyed buildings shore defenses (Fig. 3 and 4).

On the river Moldova, in the Soci area, have conducted research in the period 2002 ... 2015 (Luca, 2008; Luca, 2012). The research results revealed the following:

- lowering product hydrodynamic erosion riverbed with about 2.0 ... 4.0 m;
- protection of the bank of the left and right have been degraded at a rate of 60 ... 95 % ;
- the left bank of the river is affected by a strong current erosion;
- habitat in the river channel is disrupted and degraded.

**a** **b**

**Fig. 4 -** Comparative analysis of state left bank in 2005 (a) and 2012 (b)

The riverbed in the construction of undercrossing not presented favorable characteristics for the natural habitat of the species in the site. Site negative influences occur through the formation of riverbed under-crossing zone. Influences are produced by high water speed and the presence of concrete buildings. The riverbed calibrated not allows resting areas, breeding and feeding of aquatic fauna. Flora is influenced by the presence of mobile ballast layer of foundation bed. Floodplains provide good habitat conditions on the research.

Research findings require implementation of rehabilitation works. Significant degradation of hydraulic structures affecting the stability of the riverbed in the adduction pipes (Fig. 6). Rehabilitation works sector consistent under-crossing has been designed with the requirements of "natural site". They consist of the following (Luca, 2012):

- protection of the bank replacing rigid concrete slabs made of elastic works (elastic gabion of the plastic material filled with ballast, Fig. 6);
- achieving a groin on the left for directing water flow and limit bank erosion;
- achieving a bottom sill of the last pipe downstream of the supply; threshold will stabilize the longitudinal profile of the river section;
- achievement of a fish ladder bottom threshold for passage by fish species in the river Moldova.

The works regularization construction was designed and undertaken in 2015. Protection of the bank was designed and undertaken in spring 2015 with elastic gabions. Through its vertical and horizontal mounting of these are done to restore

favorable habitat conditions in the minor riverbed Moldova.

**Fig. 5** - Downstream view of the two arms of the river Moldova in the study area

**Fig. 6 -** Protection of the bank with elastic gabions filled with ballast

By the election of the rehabilitation of hydraulic structures in the riverbed Moldova have created favorable conditions for the existence of natural site. In april 2015 began the rehabilitation of pipelines under-crossing Soci area and the regularization works on the river Moldova.

## CONCLUSIONS

1. Design the rehabilitation of hydraulic structures within the natural site "Oniceni - Miteşti" on the section of the river Moldova were considered habitat protection requirements .

2. Hydrotechnics constructions regularization performed on the natural site should provide living conditions and the conservation of species of mammals , which is among the few sites designated for *Lutra Lutra Spermophilius and citellus*.

3. The works included in the rehabilitation project of construction in the riverbed Moldova meet the insurance requirements of the habitat of species listed in Annex II of Council Directive 94/43 / EEC

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# CHEMICAL STATEMENT OF WATER FROM DEEP BOREHOLES FROM THE HYDROGRAPHIC BASIN BÂRLAD

## SITUAȚIA CHIMICĂ A APEI DIN FORAJELE DE ADÂNCIME AFLATE ÎN BAZINUL HIDROGRAFIC BÂRLAD

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***Abstract.*** *The paper presents a chemical characterization of the deep aquifer from hydrographic basin Barlad. It took into account a total of 25 wells with depth between 79 and 289 m from the ground surface. From these boreholes water samples were taken and it was determined a range of chemical indicators. There have been exceeded over the permissible concentration required by Law 458/2002 on Drinking water quality of indicator $Na^+$ for 6 wells (1.05 respectively 2.55 times) and of indicator $SO_4^{2-}$ for 4 wells (1.04 respectively 2.30 times). For these boreholes it is necessary water treatment plants such that the water to be supplied to the population. Thus, from the total investigated depth boreholes, 16 wells have drinking water quality conditions imposed by legislation.*
***Key words:*** *boreholes, quality, hydrology, indicator, chemical characterization.*

***Rezumat.*** *Lucrarea prezintă o caracterizare din punct de vedere chimic a acviferului de adâncime din bazinul hidrografic Bârlad. S-au avut în vedere un număr de 25 foraje cu adâncimea cuprinsă între 79 și 289 m față de suprafața terenului. Din aceste foraje au fost prelevate probe de apă și determinați o serie de indicatori chimici. S-au înregistrat depășiri, peste concentrația admisibilă impusă prin Legea 458/2002 privind Calitatea apei potabile, ale indicatorului $Na^+$ la 6 foraje (de 1.05 respectiv, 2.55 ori) și ale indicatorului $SO_4^{2-}$ la 4 foraje, (de 1.04 respectiv, 2.30 ori). Se impune pentru aceste foraje prevederea de stații de tratare a apei pentru ca apa să poată fi furnizată către populație. Astfel, din totalul forajelor de adâncime investigate, 16 foraje îndeplinesc condițiile de calitate a apei potabile impuse de legislația în vigoare.*
***Cuvinte cheie:*** *foraje, calitate, hidrologie, indicator, caracterizare chimică.*

## INTRODUCTION

Water is one of the most important resources available on planet especially because is the reason for nascent, development, continuity and quality of life.

The main advantages of using groundwater for drinking are: presence near or in the complex areas of consumption, a superior quality instead of the other sources, the need for physical, chemical and biological simple treatment and inexpensive, continuous regeneration (Cojocaru *et al.,* 2011).

On the other hand, underground water sources play an essential role in the hydrological cycle and they are vital for maintaining wetlands and rivers flow, acting

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as a buffer during dry periods.

Groundwater can be polluted by: landfill leachate percolation (Han D. et al., 2014), irrigation or wastewater discharges (Morrissey *et al.,* 2015), water leaching of agricultural land salty and of agricultural land that applies in unreasonable quantities, fertilizers and pesticides (Junior Valle *et al.,* 2014) coastal aquifers intrusion (Tomaszkiewicz *et al.,* 2014) etc.

In order to use groundwater for drinking it must be determined a number of chemical indicators to assess its pollution level. Gautam *et al.,* 2015 concluded that 74%, 95% and 21% of the analyzed water samples are seriously polluted and groundwater is suitable for use in irrigation purpose. Also, Devis et. al., in 2013 has determined 17 groundwater quality parameters taken from 66 different sites. Water quality was classified as class III and IV respectively are not suitable for human consumption. The toxicity of chloride in groundwater was determined by Roy *et al.,* 2015, using juvenile freshwater mussels. The survival rate of the mussels was 80% but, generally, there was poor correlation between survival and individual contaminants.

Groundwater quality can also be evaluated using a number of models. So, a feasible evaluation model and also easy to use is the one based on the radial basis function neural network (Yu *et al.,* 2015). An innovative procedure which allows the rapid processing of large data basis to asses multi-scale temporal trends towards the underground water quality was used by Srinivas *et al.,* 2015. 11 chemical parameters of the ground water taken from 15 wells located in the region of Rajasthan, India.

The paper aims to make a chemical characterization of the deep aquifer from Barlad hydrographic basin.

## MATERIAL AND METHOD

In order to achieve a chemical characterization of the deep aquifer from Barlad hydrographic basin it was identified a number of drilling conducted during:

- Hydrogeological network of investigation and prosecution belonging to the National Hydrogeological System (Water basin Administration Prut - Barlad) that comes under the groundwater body ROGWPR05 - 22 pcs.
- Hydrogeological network of investigation and prosecution belonging to the National Hydrogeological System (Water basin Administration Buzau - Ialomita) that comes under the groundwater body ROGWBI12 - 3 pcs.

In Fig. 1 it can be observed the site of the boreholes national network from Barlad hydrographic basin.

These boreholes have depths between 79 and 289 m from ground surface and the total optimum flow that can be pumped from these wells belonging to national hydrogeological network is 28979.423 $m^3$ / day.

From each borehole water samples were taken and it was determined the following chemical indicators: H, $NH_4^+$, $Na^+$, $Mg^{2+}$, $Ca^{2+}$, $K^+$, $CBO_5$, $Cl^-$, $SO_4^{2-}$, $NO_2^-$, $NO_3^-$, $HCO_3^{2-}$, total hardness and fixed residue. The test results were taken from definitive hydrogeological studies found in the archive of the Water Basin Administration Prut - Barlad. These were compared with the permissible concentration required for each indicator by Law 458/2002 on drinking water quality.

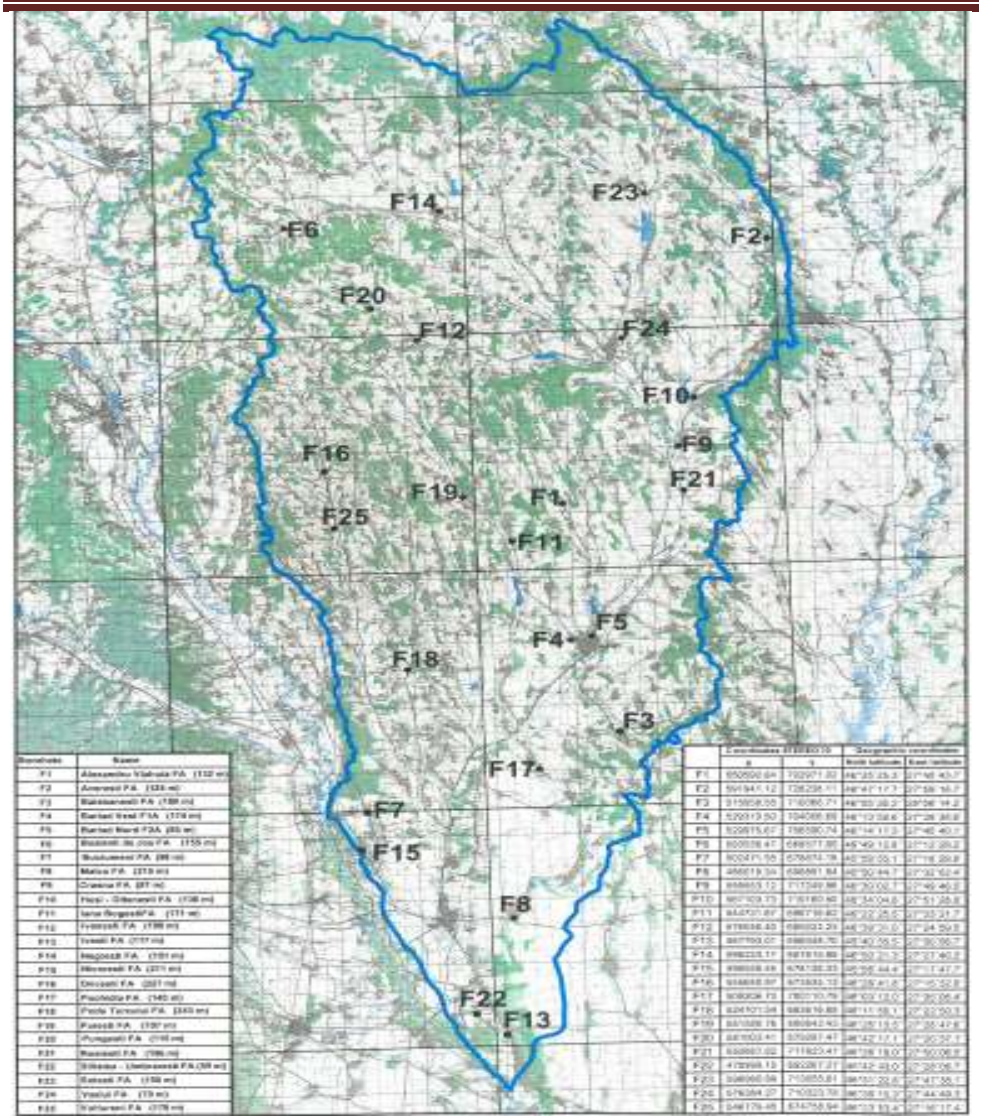


**Fig.1 -** Site of the analyzed 25 boreholes

## RESULTS AND DISCUSSIONS

In EU there is a continuing concern to ensure a unified policy towards the issues related to water ("Framework Directive 60/2000 / EC"), whose purpose is to put into practice integrated management plans in water basins with measures allowing, especially in the case of groundwater bodies, identification, delimitation and characterization of the water bodies based on geological criteria (age of

*Table 1*

**The results of the chemical analyzes of water samples taken from deep wells**

| Bore hole | Measured parameter | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH | $NH_4^+$ (mg/l) | $Na^+$ (mg/l) | $Mg^{2+}$ (mg/l) | $Ca^{2+}$ (mg/l) | $K^+$ (mg/l) | Total hardness | Fixed residue (mg/l) | $CBO_5$ (mg/l) | $Cl^-$ (mg/l) | $SO_4^{2-}$ (mg/l) | $NO_2^-$ (mg/l) | $NO_3^-$ (mg/l) | $HCO_3^{2-}$ (mg/l) |
| F1 | 7 | 0.50 | 170.00 | 5.00 | 28.00 | * | 2.80 | 590.00 | 2.50 | 26.00 | 250.00 | 0.10 | 4.00 | 195.00 |
| F2 | 7.50 | 0.13 | * | * | * | * | 45.00 | * | 14.07 | - | * | 0.11 | 38.00 | * |
| F3 | 8.40 | 0.00 | 117.53 | 0.00 | 5.60 | * | 0.80 | 440.00 | * | 8.00 | 69.93 | 0.00 | 0.00 | 213.50 |
| F4 | 7.00 | 0.71 | 82.00 | 19.00 | 124.00 | 9.00 | 21.80 | 684.00 | * | 48.00 | 226.00 | 0.48 | 16.00 | * |
| F5 | 7.00 | 0.25 | 158.00 | 12.00 | 16.00 | 2.00 | 5.10 | 548.00 | * | 36.00 | 201.00 | 0.025 | 3.00 | 213.00 |
| F6 | 7.50 | 0.13 | * | * | * | * | 4.50 | * | * | 210.00 | * | 0.37 | 0.00 | 957.70 |
| F7 | 7.00 | 2.00 | 112.00 | 31.20 | 60.00 | * | 15.60 | 645.00 | * | 62.00 | 230.00 | 0.80 | 8.00 | 256.00 |
| F8 | 7.50 | * | 80.00 | 101.00 | 160.00 | 6.00 | 45.90 | 1178.00 | 3.60 | 52.00 | 576.00 | 0.00 | 7.50 | 403.00 |
| F9 | 7.50 | 0.25 | 210.00 | 5.00 | 8.00 | 2.00 | 2.24 | * | * | 46.00 | 32.00 | 0.10 | 2.00 | 488.00 |
| F10 | 7.00 | 4.50 | 230.00 | 26.00 | 56.00 | 4.00 | 14.00 | 860.00 | 5.30 | 30.00 | 200.00 | 0.20 | 6.00 | 610.00 |
| F11 | 7.50 | 0.08 | 196.00 | 7.20 | 8.00 | 5.00 | 2.80 | 585.00 | * | 16.00 | 183.00 | 0.62 | 0.00 | 329.00 |
| F12 | 7.00 | 6.50 | 198.00 | 7.00 | 68.00 | 10.00 | 11.20 | 775.00 | 12.00 | 28.00 | 240.00 | 0.00 | 1.00 | 439.00 |
| F13 | * | * | 395.00 | 5.60 | 26.00 | * | * | * | * | 187.00 | 300.00 | * | 0.20 | 810.00 |
| F14 | 7.50 | * | 80.00 | 101.00 | 160.00 | 6.00 | 45.90 | 1178.00 | 3.60 | 52.00 | 576.00 | 0.00 | 7.50 | 403.00 |
| F15 | 7.50 | 0.00 | 45.54 | 27.16 | 80.08 | 45.54 | 45.90 | 1178.00 | * | 20.00 | 41.14 | 0.0004 | 0.00 | 475.80 |
| F16 | 7.50 | 0.50 | 100.00 | 16.00 | 24.00 | 2.00 | 7.28 | 370.00 | * | 30.00 | 5.00 | 0.10 | 4.00 | 366.00 |
| F17 | 7.50 | 0.00 | 69.00 | 50.40 | 60.00 | 12.00 | 20.10 | 527.00 | 1.80 | 36.00 | 207.10 | 0.00 | 0.30 | 390.40 |
| F18 | 7.00 | * | 125.00 | 2.00 | 36.00 | 12.00 | 5.60 | 430.00 | * | 26.00 | 63.00 | * | 4.00 | 329.00 |
| F19 | 7.50 | 0.00 | 210.00 | 7.00 | 12.00 | 0.00 | 3.36 | 640.00 | * | 16.00 | 145.00 | 0.20 | 4.00 | 415.00 |
| F20 | 7.50 | 2.00 | 300.00 | 2.00 | 16.00 | 4.00 | 2.80 | 860.00 | * | 20.00 | 246.00 | 0.00 | 4.00 | 512.00 |
| F21 | 7.50 | * | 180.00 | 17.00 | 36.00 | * | * | * | * | 16.00 | 78.00 | * | 8.00 | * |
| F22 | 7.00 | 1.45 | 237.00 | 22.00 | 52.00 | 19.00 | 12.32 | 913.00 | 6.20 | 25.60 | 211.00 | 0.00 | 0.00 | 220.00 |
| F23 | 7.00 | 4.00 | 510.00 | 2.40 | 16.00 | 5.00 | 2.80 | 1400.00 | * | 230.00 | 260.00 | 0.20 | 2.00 | 707.00 |
| F24 | 7.00 | 0.35 | 68.00 | 41.00 | 92.00 | 14.00 | 22.40 | 667.00 | 3.20 | 56.00 | 182.00 | 0.022 | 12.50 | 329.00 |
| F25 | 7.00 | 0.48 | 159.00 | 38.40 | 8.00 | 18.00 | 10.08 | 640.00 | 1.90 | 16.00 | 192.00 | 0.04 | 1.00 | 402.00 |

Note: The values of permitted concentrations according to Law 458/2002 on drinking water quality, are: $Na^+$ = 200 mg/l; $Cr^-$ = 250 mg/l; $SO_4^{2-}$ = 250 mg/l; $NO_3^{2-}$ = 50 mg/l; $NO_2^-$ = 0.50 mg/l; pH = 6.5-9.5.

*Analysis were not made

water-bearing deposits, petrographic features, structural characteristics, capacity of water storage, etc.) hydrodynamic characteristics (expansion of water bodies), the quantitative status (H-level piezometric Q-flow) and quality (G-generals: conductivity, TDS, pH, alkalinity, SO - organic substances: oxygen dissolved CCO-Mn, N-nutrient nitrogen, nitrates, ammonium SP-MG - heavy metals: the list of priority hazardous substances; SP-O- organic micropollutants; CT- total coliforms, faecal coliforms Type CF, SF, etc faecal streptococci, etc.).

For these reasons now all water basin administrations in our country are concerned with the practical implementation of the framework directive and of Directive 2006/118 / EC on the protection of groundwater against pollution and deterioration. In the coming years it is expected coming over some key stages, the most important being the development of the first management plans for the river basin (surface and underground water body) and also laying down the main practical measures still required to achieve the objective "chemical status good groundwater "(within 2015).

Within Barlad basin there are 44 sources of pollution, of which 39 are wastewater treatment plants. From these the most pollutants are Negreşti waste water treatment plant, Vaslui waste water treatment plant, SC Bearings S.A. Barlad; R.A.G.C.L. Barlad; S.C. Alcohol S.A. Ghidigeni; Tecumseh City; S.C. Sugar S.A. Lieşti etc. (Panaitescu, 2008).

In Tab.1 it is presented the results of chemical analyzes on water samples collected from 25 analyzed deep boreholes. It is noted that in general, the analyzed chemical indicators are within the limits imposed by legislation. Breaches of these limits were recorded for the indicator $Na^+$ at 6 wells: F9, F10, F13, F19, F20 and F23, of 1.05 respectively 2.55 times and for the indicator $SO_4^{2-}$ at 4 wells: F8, F13, F14 and F23, of 1.04 respectively 2.30 times. It is necessary for these boreholes providing water treatment plants for water to be used for drinking and supplied to the population.

The groundwater stock can be an important source of drinking water because there is a shortage of phreatic and surface water in Barlad basin and also the chemical water indicators in most of the boreholes the conditions required by Law 458/2002 on drinking water quality.

## CONCLUSIONS

Following chemical analysis of the deep groundwater situation in Barlad basin, we have reached the following conclusions:

1. Deep groundwater can be an important source of water used for drinking.

2. From the 25 boreholes, 17 have permitted chemical indicators by the limits set out in Law 458/2002 on drinking water quality.

3. Of the nine inappropriate wells 2 wells (F13 and F23) do not achieve the chemical quality requirements on indicators of $Na^+$ and $SO_4^{2-}$ 4 wells (F9, F10, F19 and F20) do not achieve the chemical quality requirements on indicator $Na^+$

and 2 drilling (F8 and F14) do not achieve the chemical quality requirements on indicator $SO_4^{2}$.

4. The boreholes that don't achieve the chemical quality requirements on indicator Na + (F9, F10, F13, F19, F20 and F23) requires a more advanced treatment plant and so that the cost of making the water potable are high.

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# ASSESSMENT OF HEAVY METALS CONTENT ON TOMATOES UNDER ORGANIC SYSTEM

## EVALUAREA CONȚINUTULUI DE METALE GRELE LA O CULTURĂ DE TOMATE ÎN SISTEM ECOLOGIC

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***Abstract:*** *The paper presents a study on heavy metal content on a tomato crop fertilized according to Regulation 834/2007, compared to conventional crop, in a poly-tunnel, in Iasi area. The defined purpose of this study was to evaluate the extent that fertilization regime influence the content of heavy metals in two cultivars of tomatoes. The method for determining the content of heavy metals is based on measurement by atomic absorption spectrometry (AAS).Following the disclosure of this data, we conclude that the bioaccumulation content of heavy metals depends to a great extent and fertilization that are within crop system in which they are grown, and a number of factors such as climate and soil temperature.The Orgevit fertilizer applied on Ismini F1 crop, achieved the highest yield (148.8 t/ha) compared with Brillante F1, where the high production was registered in chemical version (139.7 t/ha). Copper, lead and nickel in larger quantities in tomato fruits were found in chemically fertilized versions, regardless of cultivar.*
***Key words****: organic crop, tomatoes, heavy metals*

***Rezumat:*** *In lucrarea de fata se prezintă un studiu cu privire la continutul de metale grele la o cultură de tomate fertilizată ecologic comparativ cu o variantă fertilizată chimnic (convențional), în solar, în condițiile zonei legumicole Iași. Scopul definit al acestui studiu este de a evalua masura in care regimul de fertilizare influențează conținutul de metale grele din fructe, la două cultivare de tomate. Metoda de determinare a conținutului de metale grele se bazează pe măsurarea prin spectrometrie de absorbție atomică (AAS). În urma prezentării acestor date, putem concluziona că conținutul de metale grele din sol dar și bioacumularea acestora, depinde într-o mare măsură și de fertilizările care se fac în cadrul unei culturi, sistemul în care acestea sunt crescute, dar și de o serie de factori precum, clima temperatura și solul. Ismini fertilizat cu Orgevit a obtinut cea mai ridicata productie (148.8 t/ha), comparativ cu Brillante unde cea mai mare productie a fost inregistrata in varianta fertilizata chimic (139.7 t/ha). Cupru, plumbul si nichelul in cantitati mai mari in fructele de tomate au fost gasite in variantele fertilizate chimic, indiferent de cultivar.*
***Cuvinte cheie:*** *cultura ecologică, tomate, metale grele*

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## INTRODUCTION

Tomatoes belongs the most popular vegetables from consumers, because they are very balanced in terms of the composition in nutrients (Munteanu, 2003).

Thus, it came as tomatoes with Solanaceae vegetables species in the group, have a large surface area for the production of vegetables in our country. However, organic tomato crop is poorly represented among organic vegetable crops because they are not yet established specific technologies to ensure and maintain soil fertility, weed, disease and pest control (Munteanu, 2003; Stefan *et al.,* 2013; Stoleru *et al*., 2014).

The need to protect and respect than to environment and consumers, proved to be necessary emergence of a specific legal framework in the field of organic vegetable, simply to prove that the products obtained are in accordance with the regulations and their quality is one appropriate .

Thus, the term that defines organic farming within the European Community countries use different synonyms, but which all come together and are in compliance with EC Regulations 834/2007 and 889/2008 (Stoleru *et al.,* 2014).

For the determination of heavy metals such as lead (Pb), manganese (Mn), copper (Cu), nickel (Ni) and zinc (Zn), from tomato fruits, it is imperative to make in determining the quality of production obtained in determining the market value of crop technologies (Al-Lahham et al., 2007; Amin et al., 2013; Rodriguez-Iruretagoiena et al., 2015).

In general, the price of vegetable products that are made available to the consumers should be reflected in their quality.

Heavy metals such as Pb, Cu, Mn, Ni and Zn, can have both beneficial effects on plants, but also some negative effects. As is well known, heavy metals are among the elements that exhibit bioaccumulative this encouraging them to increase the amount of plant / fruit (Bulgariu *et al.,* 2006; Amin *et al.,* 2013).

The elements they reach the ground after chemical fertilization, which are then taken up by plants, in turn consumed by humans or animals, thus affecting biological a chain.

Although well-known effects of heavy metals, they are still used by man in order to increase production to meet consumer needs. Crop exposure to these elements excessively, leading to their toxicological and developments in products of plant origin, and their influence on humans and animals (Gu *et al.,* 2015).

The study of heavy metals accumulation in tomato fruit and thus a very important element of study, both the crops in the field, but especially those grown under covered areas.

## MATERIAL AND METHOD

The experiments were organized in Adamachi V. farm that belong UASVM Iasi. To obtain seedlings, the sowing was carried out in 24. 02. 2014. Planting was done around 17/04/2014, using seedlings with 50 days old. The crop was established in 5

beds with 2 rows per each bed. Plants were individual stringhing being run on a single stalk, providing a density of 27770 plants/ha. After planting, each plant was irrigated with 1 l water/pl., having a temperature of 18- 22 °C.

On the tomato crop, were applied specific works care to ensure good plant vegetation, and to obtain early production and an appropriate quality to cover production costs (Munteanu, 2003; Stoleru *et al.*, 2014). The biological material was represented by two hybrid cultivars such, Brillante and Ismini.

The organic crops, exclude the use of chemically synthesized substances, and therefore have been used in organic and biological fertilizers. For this purpose were used substances such as Orgevit and Micoseed that can be used in the organic crop, compared with a chemically version, fertilized with Nutrifine. Samples were collected and prepared for laboratory analysis, according regulations laid down in the legislation, Ministry Order 147/23- 2005.

Soil and vegetable sampling was carried out under good conditions to perform analyzes so that they are representative for the product tested, the identification and assessment of heavy metals.

**The principle of the method used to determine heavy metals.** The method is based on the measurement by atomic absorption spectrometry (AAS) the concentration of an element in a regal aqua sample extract prepared according to ISO 11466. The principle of the method is charring and incineration organic matter in the sample in a furnace at 450 - 500°C, the resulting ashes went into solution by dissolving HCl, diluted (Butnariu, 2014).

The dates collected have been statistical analyzed by one-way analysis of variance (ANOVA) and least significant differences (LSD) at 5%, 1% and 0.1% confidence levels for production. For the contaminants, the statistical significance were carried, compared with maximum accepted limit (MAL).

## RESULT AND DISCUSSIONS

The productivity of a tomato crop can be assessed by: number of fruit/plant, average weight of fruit, fruit numbers from a flowering, fruit weight/plant or early and total production (Tabel 1).

Concerning to the number of fruits per plant, the variations are not very high, having values ranging between 20 to 26 fruits per plant. Average fruit weight varied between 195- 239 g/fruit at the Ismini cultivar and 167 to 205 g of Brillante F1 cultivar.

Determining the average weight of fruit per plant, it has highlighted out that under Ismini F1 cultivar, the highest average weight of a fruit obtained from plants treated with Orgevit and the lowest on control version, compared to Brillante where the high average weight/fruit was obtained under chemical fertilization.

After processing the dates, it was found that the Ismini F1 has achieved the highest production were those fertilized with Orgevit, and the lowest on the unfertilized plants. At the Brillante F1 cultivar, the situation is different in plants fertilized with Nutrifine obtained the highest production and those treated with Orgevit, yielding the lower production.

Following the analysis performed, tomato fruit content of heavy metals varied on the cultivar used and practiced fertilization system (Tabel 2).

*Table 1*

**Tomato productivity indices**

| Experimental versions | Average fruits/plant | Fruit average wight (g) | Weight fruit/plant (kg) | Total yield (kg/ha) |
|---|---|---|---|---|
| Ismini F1+ Orgevit | 26 | 206, 114 | 5, 358 | 148818*** |
| Ismini F1+ Micoseed | 25 | 198, 604 | 4, 965 | 137880** |
| Ismini F1+ Nutrifine | 21 | 239, 714 | 5, 033 | 139794** |
| Ismini F1/ Martor | 20 | 195,702 | 3, 914 | 108692ns |
| Brillante F1+ Orgevit | 22 | 167, 696 | 3, 689 | 102452ns |
| Brillante F1+ Micoseed | 24 | 205, 554 | 4, 933 | 136997** |
| Brillante F1+ Nutrifine | 25 | 201, 246 | 5, 031 | 139715** |
| Brillante F1/ Martor | 21 | 186, 1 | 3, 908 | 108527ns |

LSD 5%=13127 t/ha; LSD 1%=20596 t/ha; LSD 0.1%=33208 t/ha
ns-non significantly; **-positive distinct significantly; ***-positive very significantly

*Table 2*

**Heavy metal contents in tomato fruits**

| Experimental versions | Heavy metal contents | | | | |
|---|---|---|---|---|---|
| | Cu (ppm) | Mn % | Ni (ppm) | Zn (ppm) | Pb (ppm) |
| Brillante+ Orgevit | 36 | 0,29 | 38 | 133 | 22 |
| Brillante+ Micoseed | 25 | **0,56** | 52 | **186** | 5 |
| Birillante+ Nutrifine | 47 | 0,24 | 69 | 147 | **34** |
| Brillante/ Martor | **19** | **0,19** | 37 | **127** | **0** |
| Ismini+ Orgevit | 38 | 0,31 | 40 | 152 | **34** |
| Ismini+ Micoseed | 39 | 0,39 | 34 | 158 | 11 |
| Ismini+ Nutrifine | **52** | 0,42 | **76** | 178 | 28 |
| Ismini/ martor | 27 | 0,24 | **31** | 140 | **0** |

In order to determine the content of heavy metals, the samples have been collected from all experimental variants were determined following elements: Cu, Mn, Pb, Ni and Zn. The content of heavy metals was determined and evaluated in accordance with the maximum admitted limits (MAL) of legislation.

The copper was quite large variations in the two cultivars, but its values were within the rules prescribed by the law organic farming. The copper content varied from 19 ppm in Brillante-Control version to 52 ppm in Ismini fruits chemical fertilized, this version is alone version where the MAL (50 ppm) was exceeded. The content of manganese in the majority of samples was within allowed limits, only in one version the content was higher (Brillante treated with Micoseed), the percent was 0.56 %. The content overcome the MAL is not harm the quality of tomato fruits, because as you well know, manganese plays a role in

intensification of photosynthesis, transpiration and synthesis reactions, thus contributing to increase production and improve quality.

The Ni contents varied from 31 ppm in the Ismini Control to 76 ppm in the same cultivar fertilized with synthetic fertilizers. This fact is confirmed, same in the Brillante cultivar, where the Ni content was over MAL (69 ppm).

In the Brillant version fertilized with Micoseed, the Ni content was over MAL, which shows that favors the passage Ni fertilization of soil microorganisms, in the form of poorly soluble forms accessible for the plant, probably in the form of $Ni^{2+}$.

The Zn content in the experimental study was overcome in more samples. The zinc content varies quite large but is not a real danger, since it is an element that occurs naturally in soil and plants, fulfilling a vital role in the process of flowering, plant growth and fruit.

Regarding the Pb content in tomato fruit, it was found a wide variation in some variants studied, ranging from undetectable in both control versions to 34 ppm in chemical and organic fertilizing variants.

In the experiment carried out on the tomato crop has shown that heavy metal values are greatly influenced by the type of fertilizer and less than cultivar used in crop establishment.

## CONCLUSIONS

1. The results, both on the production and the content of heavy metals of tomatoes, highlight the key role that it has on their fertilization. Ismini and Brillante cultivars react differently to organic fertilization compared to the other two fertilization types.
2. The Orgevit fertilizer applied on Ismini crop, achieved the highest yield (148.8 t/ha) compared with Brillante, where the high production was registered in chemical version (139.7 t/ha).
3. Copper, lead and nickel in larger quantities in tomato fruits were found in chemically fertilized versions, regardless of cultivar.
4. The amount of Zn has been overcome in most experimental versions, except the control variants, where the 150 ppm limit is not exceeded.

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# GIS IN USE OF AGRICULTURE LAND DYNAMICS

## SIG ÎN DINAMICA UTILIZĂRII TERENURILOR AGRICOLE

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***Abstract.*** *The purpose of this article is to present the fact that today, Geografphical Information Systems offers a tool avaible to address various problems to the wind erosion risk areas. To creat the maps presented has been used topografhic map and Corine Land Coverdata. The analysis of the maps can draw conclusions about the land use area in the future. Dabuleni area was chosen because is the only desert of Europe.*
***Key words:*** *GIS, hidrologie, risc, zonă*

***Rezumat.*** *Scopul acestui articol este de a pune în evidență faptul că azi, Sistemele Informatice Geografice oferă un instrument accesibil pentru a aborda diferitele probleme legate de zonele de risc la eroziune eoliană. Pentru a crea hărțile prezentate s-au utilizat hărți topografice și date Corine Land Cover. Din analiza hărților se pot trage concluzii cu privire la modul de utilizare al terenurilor zonei în viitor. Zona Dăbuleni a fost aleasă deoarece se prezintă ca fiind sigurul deșert al Europei.*
***Cuvinte cheie:*** *GIS, hidrologie, risc, zonă*

## INTRODUCTION

In the twentieth century the world there was an increase in global temperature unprecedented for the past 1,000 years. Increasing of this trend is seen by experts as a critical moment for the future evolution of the Earth.

The problem of global warming is considered as a challenge to contemporary society. Problems caused by global climate change are: the accumulation of greenhouse gases, pollutants accumulating and irrational human activity (deforestation, land-use change).

The most important greenhouse emitted by the human activities is carbon dioxide (CO2). Carbon dioxide is responsible for 60-70% of global warming effect. After burning fossil resulting combustion carbon dioxide (43% coal, 38% oil and 19% of natural gas combustion).

Agriculture is responsible for 6% of total carbon dioxide emissions.

The Intergovernmental Panel on Climate Change in the his third report highlights that in the last century there was a temperature rise of 0.6 + -0.2 $^{0}$C. This planetary temperature rise occurred in two phases: between 1910 - 1945 and 1976-2000.

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Highest temperature increases have been registered in Central Europe, the average annual temperature has increased by 0.8 C0, the most important increases were registered in the first and last decades of the XX th century (Busuioc, 2003). Along with global warming manifests an increasing trend of extreme temperatures lowered.

Global temperatures in 2014 have reached a high record and Japan's Meteorological Agency said that this year is the warmest in the history of weather records from 1880 to the present.

Drought is a dangerous phenomenon which leads to aridization and desertification. Installation of permanent phenomenon initially produce dry climate, desertification that occurs after the drastically reduce water availability, lower productivity of crops, decreased areas under forests and the last stage, expansion of sandy soils areas, one quarter of the Earth's surface is affected by this process. (Bălteanu and Șerban, 2005)

In 1931 Acad. Gheorghe Ionescu - Siseşti realized the first study on the drought in Romania - timings with at least 10 days when no rainfall in summer and at least 14 days when not in rainfall in winter.

In Romania, most 220,000 hectares of sand dunes covered with 80000 hectares are located in the area, Dăbuleni form a land covered by sand. The Dăbuleni is the driest area of the country, is considered a desert, Europe's single desert.

United Nations Conference on Environment and Development in Rio de Janeiro in 1992 addressed the problem of desertification. Following this conference, was elaborated Convention to Combat Desertification adopted in Paris on 17 June 1994 and took effect on 26 December 1994. Romania signed the Convention in 1997 by Law 629/1997.

The day of June 17 is dedicated to desertification control, drought and land degradation.

## MATERIAL AND METHOD

To studying effects of the drought on agricultural areas it was chosen Dăbuleni - Potelu Corabia. In these areas it began a process of desertification which is a consequence of the droughts recorded, being also the warmest area of Romania. Dăbuleni - Potelu precinct is characterized by the presence of several lowland areas in the terrace area and the landscape of dunes, in its western part.

This system has a surface of 14.450 ha, which are equipped with embankment, construction drainage works.

The territory is located in the Danube Valley, between primary and lower terrace bed that rises above the meadow with 5-20m. Part of the area is the bottom Potelu Swamp and depressions Potelu Lişteava and Valcov.

Dăbuleni – Potelu- Corabia is part of Olt District. In this district is manifested a temperate continental climate with a wetland area in the north and a arid area in the south. Rainfall amounts for a year are 300 mm in the north and 350 mm in the south. The highest average annual air temperature was recorded at Corabia Station (13.3 $^0$C), and the highest annual amplitude value Caracal Station is recorded.

This can be explained by the geographical location of the meteorologicaly station interior and marginal by the Caracal Station (Olt and Danube valleys) as the river Danube main influence especially in the Corabia Station. The highest temperature was recorded in August 1976 and 1980, 24.6 $^0$C and 20.2 $^0$C in 1985 This .absolute values show the influence of tropical air masses from the South and the influence of arctic air masses from the North. (Achim *et al.,* 2012)

Winds reveal the presence of zones of interference between the eastern part of the Romanian Plain, with dominant winds from the eastern and western part of the same region with dominants winds from the eastern, northern wind in the first case the wind is _Crivațul- and the other is - Austrul. Annual average rainfall is 540 mm, ranging between 300 and 730 mm rainfall in 1-2-3-5 days reached values between 100-140 mm.

The soil has a sandy texture that does not allow water retention and is also subject to wind erosion.

The unfavorable situation which amplifies each year desertification phenomena is the result of massive deforestation and intensive agriculture.

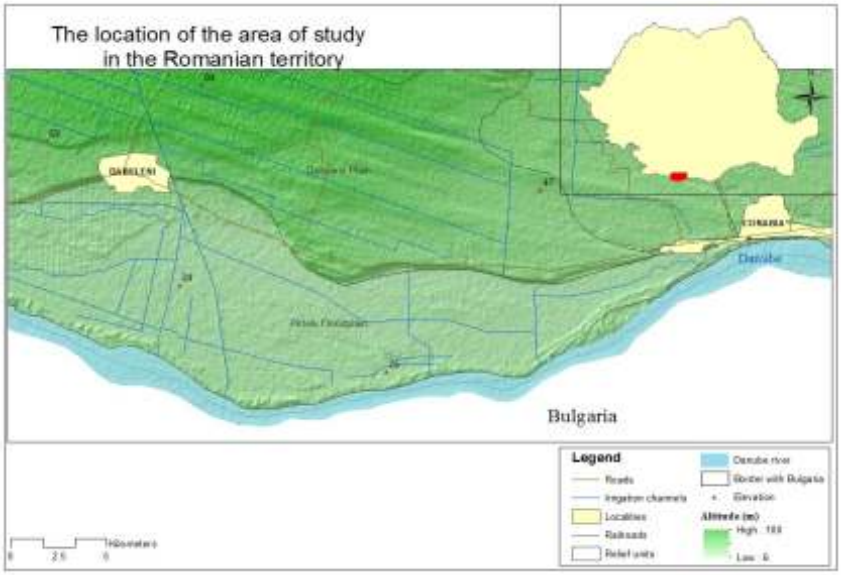


**Fig. 1-** The location of the area of study

## RESULTS AND DISCUSSIONS

To achieve the study goal was used by digital model elevation data wich was downloaded free from the geoportal CGIAR-CSI with spatial resolution of 90 m, these data were interpolated to obtain a 30 m resolution. The software ArcGIS 10.1 was used to vectorized map terrain on scale 1: 25000. And also the Romanian soil map on scale 1: 200,000. Corine Land Cover data were downloaded free on the site of the European Environment Agency, CLC has 44 different classes.

At a global level has been made researches for land and land use blanket, this has been achieved within the project Land Use and Land Cover Changes. The project desired to produce a better understanding of the processes of degradation, desertification and the biodiversity reduction.

In the project we have investigated the relationship between changes in quality and land use, and processes relevant to global environmental changes, urbanization, coastal zone management, water resources and quality. In Figure 2 is the Corine Land Cover map for the Dăbuleni

The map was made with ArcGIS 10.1 program in June of 2105 using data from 2012. By analyzing the map has resulted occupied surface percentages of different uses, as follows: 25% waterways, forests bushes 0.45% berry plantations 0.3%, 0.2% deciduous forests, remaining below 0.1% is occupied by pastures cultivated land occupied by agriculture, natural vegetation areas, and industrial or commercial dune sands.

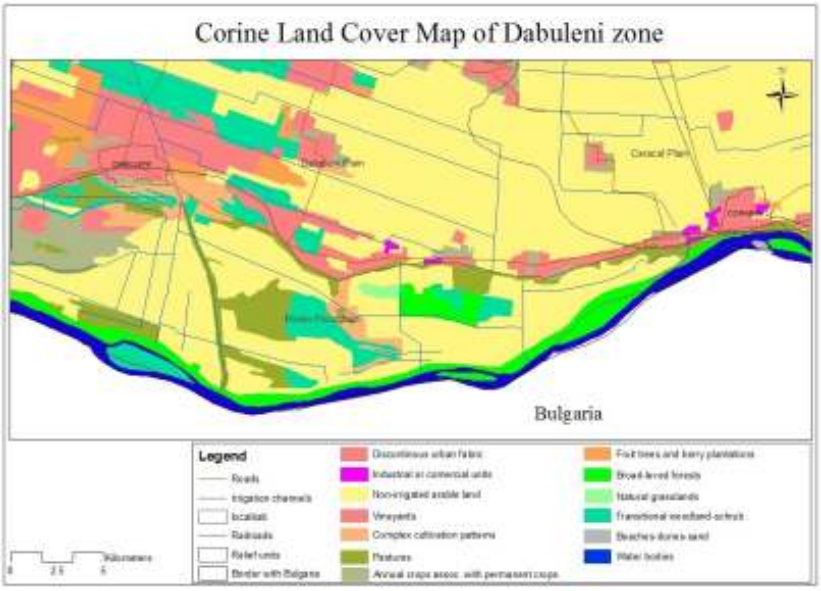


**Fig. 2 -** Corine Land Cover Map of Dabuleni zone

Soil map of the area is presented in Figure 3 and was obtained from Romanian soil map scale of 1: 200,000 over the overlapped Corine Land Cover data. Analysis following map shows the soils areas: soils are poor young formations evolved - protosols and alluvial soils. In the sandbank fluviatil have evolved poor soils and sandy texture textured sandy-loam with a low humus content, or slightly saline gleyzation in the soil profile. In the central area are alluvial soils with medium texture and slightly saline non gleyzation the soil profile. Former swamp areas show alluvial hydromorphic soils with high fertility.

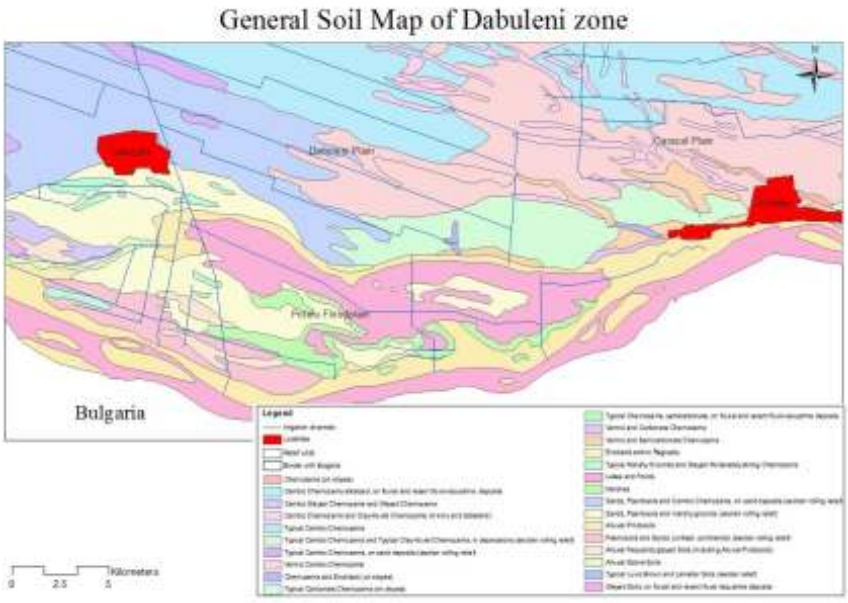


**Fig. 3 -** General Soil Map of Dabuleni

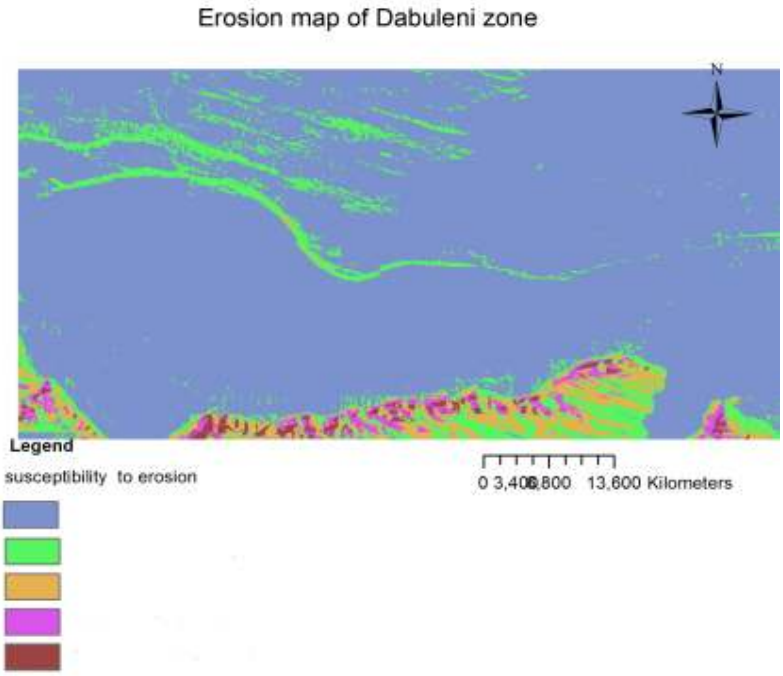


**Fig. 4 -** Erosion Map of Dabuleni

In the figure no 4 is present the map susceptibility to erosion. The map was made using slopes map, slope orientation, relief energy and soils map. Analysis of this map reveals that in the area are five classes of wind erosion risk. The blue colour is high risk, medium risk following the small, insignificant and zero risk.

## CONCLUSIONS

1. Studied area is necessary to reduce the intensity of tillage for soil water retention and to decrease water loss from the soil.

2. Covered areas with vegetal residues on the soil surface, this leads to reduced water loss by evaporation, water is retained in the soil for use by plants.

3. Reduce ground work associated with water conservation in soil mulching ensure losses were reduced and reduce weeds.

4. Curtain forest protection to protect agricultural areas, contribute to reducing dominant wind speed, relative humidity increases, creates a favorable environment for crops, retains moisture in the soil and increase the water supply.

5. The mapping of susceptibility to erosion leads to knowing the accuracy of the areas worst.

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# GROWTH-PROMOTION EFFECT OF *TRICHODERMA* SP. ON HORTICULTURAL SEEDLINGS DEPENDS ON INOCULUM AND SUBSTRATE TYPE

## EFECTUL DE STIMULARE A CREŞTERII AL *TRICHODERMA SP.* ASUPRA RĂSADURILOR HORTICOLE ÎN FUNCTIE DE INOCUL ŞI TIPUL DE SUBSTRAT

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***Abstract.*** *Fungi in the genus Trichoderma have been reported to have biostimulant and biofertilizer qualities in many types of horticultural crops, but the effects are highly variable. Thus, practical use of Trichoderma sp. requires feasible formulated products and suitable substrates. The aim of this study was to evaluate the survival and the growth-promotion effect of a Trichoderma sp. rice formulation compared with the incorporation of this fungi as a nonformulated conidia suspension, both at three different dosis (7 treatments in total), on tomato, pepper and cucumber seedlings grown in two substrates: 1) rich in organic matter (OM) and 2) mineral substrate without OM. Three replicates of each treatment were performed, and the trials were carried out twice. The results showed beneficial effects on seedling growth in the OM-rich substrate when Trichoderma sp. was applied by the formulated form (mainly at maximum concentration), but the effects were opposite when the mineral substrate without OM was used. The effects were closely linked to the survival and growth of the fungus, and therefore to the level of inoculum in the substrate, which was greater on application of the formulated one as opposed to the nonformulated one. These results provide evidence of the complexity inherent in the use of microorganisms in agriculture, while also confirming that the activity of the biofertilizers based on Trichoderma sp. depends on the type of inoculum and its concentration, as well as the properties of the medium in which the fungi develop.*
***Keywords:*** *biostimulant, biofertilizer, in vitro, cucumber, pepper, tomato*

***Rezumat****. Ciupercile genului Trichoderma sunt considerate ca având calități de biostimulator şi biofertilizant, însă cu efecte variabile. Astfel folosirea Trichoderma sp pentru a fi fezabilă necesită utilizarea diverselor substraturi sustenabile. Scopul acestui studiu a fost acela de a stabili influența diverselor substraturi şi tipuri de inocul de Trichoderma sp. A fost studiată dezvoltarea pe inocul de orez în comparație cu o soluție de conidii în suspensie, ambele în diferite doze (7 tratamente în total) administrate pe culturi de răsaduri de tomate,*

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*ardei şi castraveți. Substratul pe care au crescut răsadurile a fost de două tipuri 1) bogat în materie organică (OM) şi 2) substrat mineral fără materie organică (OM). Au fost stabilite trei variate cu două repetiții fiecare. Rezultatele au arătat efectele benefice asupra creşterii răsadurilor în cazul folosirii variantei Trichoderma sp. pe substrat îmbogățit cu materie organică cu inocul pe orez (mai ales la folosirea concentrației maxime), însă efectul a fost opus când a fost utilizat substratul fără materie organică. Rezultatele au fost strâns legate de de supraviețuirea şi creşterea ciupercii, de concentrația ciupercii din substrat care a fost mai ridicată la variata cu orez față de cea cu soluții de conidii. Aceste rezultate oferă dovada complexității inerente a utilizării microorganismelor în agricultură, în timp ce, confirmând că activitatea ca biofertilizator bazat pe Trichoderma sp. depinde de tipul de inocul şi concentrația sa, precum şi proprietățile mediului în care ciupercile se dezvoltă.*

***Cuvinte cheie:*** *biostimulator, biofertilizator, in vitro, castraveți, ardei, tomate*

## INTRODUCERE

Fungi in the genus *Trichoderma* show qualities that allow them their natural presence in all kind of soils (Papavizas, 1985; Widden and Scattolin, 1988; Jackson *et al*., 1991). Likewise, their development is favoured by the presence of roots, to which they colonise quickly, and establish chemical stimulants that modify how the different genes behave in the plants (Harman *et al*., 2012). In this way, species belonging to *Trichoderma* genus are considered as endophytic simbionts with diversity of properties beneficial for plants (Benítez *et al*., 2004; Harman *et al*., 2012). *Trichoderma* spp. has been studied during decades as a biological control agent (BCA) (Weindling, 1934; Mukherjee *et al*., 2013), and specialised literature mainly highlights the existence of several species used for biocontrol of soil phytopathogen organisms, the aerial and post-haverst parts through different mechanisms, to which biostimulant and biofertilizer qualities are shown to generate a growth promoting effect, on seedlings as well as adult plants, and even a production increase (Chang *et al*., 1986; Windham *et al*., 1986; Harman *et al*., 1989, 2004, 2012; Lo *et al*., 1996; Björkman *et al*., 1998; Altomare *et al*., 1999; Harman, 2000, 2011; Yedidia *et al*., 2001; Benítez *et al*., 2004; Savazzini *et al*., 2009; Mastouri *et al*., 2010; Shoresh *et al*., 2010). In this respect, the effects are very variable, and depend mainly on the strain used, the considered crop and its growth conditions, as well as on the rate and type of inoculum, and the substrate used (Lindsey y Baker, 1967; Ahmad y Baker, 1987; Kleifeld y Chet, 1992; Ousley *et al*., 1994; Martínez-Medina *et al*., 2009; Bashan *et al*., 2014; Rajput *et al*., 2014 a, b). In this way, one of the main limitations in the application of BCA such as *Trichoderma* spp., is the development of commercial preparations formulated properly (Fravel, 2005; Bashan *et al*., 2014; Rajput *et al*., 2014a). The development of new effective formulations for the inoculation of microorganisms is a very slow process that has been widely ignored, only another interest shown has been in the evaluation of patent formulations, and such formulations are useless to a large extent, apart from the manufactures' exhibition to the public (Tello *et al*., 2011). While many studies describe plant inoculation with microorganisms, only a few of them study in depth the formulations and the application methods that are hidden between materials and methods. Therefore, the

advantages and/or disadvantages of each formulation are seldom investigated in depth (that is, amounts, conditions, relation of mixtures), nor the improvements in the production process (Bashan *et al*., 2014).

Usually, trials with *Trichoderma* spp. under controlled conditions use solid as well as liquid inocula with concentrations included in the order of $10^4$ and $10^9$, and referred to propagules (mainly conidia) and also to Colony-Forming Units per g or mL respectively (Sivan *et al*., 1984; Ousley *et al*., 1994; Bernal-Vicente *et al*., 2009; Martínez-Medina *et al*., 2009), while in greenhouse and land the recommended concentration must not be lower than $10^8$ (Cooney *et al*., 1997). With reference to the substrate used, one of the main obstacles to use *Trichoderma* efficiently is the maintenance of a high inoculum density after its application (Kleifeld and Chet, 1992; Martínez-Medina *et al*., 2008). For this reason, considering the saprophytic nature of fungi belonging to *Trichoderma* genus, a substrate with high content of organic matter could favour the growth, reproduction and maintenance of the fungus (Rajput *et al*., 2014b). Consequently, the application of the fungus through solid inoculum obtained from its reproduction on organic materials such as cereals (that is, rice, barley, wheat, oats, sorghum), could be favourable because these materials provide food for fungi, which allow them to stay in the source substrate keeping a higher level of inoculum, even under unfavourable conditions for their establishment (Martínez-Medina *et al*., 2009). However, when using liquid inoculum is more important to have favourable conditions and a substrate rich in organic matter (OM) to avoid possible losses (Bernal-Vicente *et al*., 2009; Franco *et al*., 2014).

Therefore, as agriculture cannot support inoculants with high production costs or expensive support materials, it is important to make effective formulations that are also economically profitable. In this way, given that *Trichoderma* spp. has the capacity of being cultivated on different substrates, and rice grain is an economic substrate and one of the most used in mass production under In Vitro conditions (Hjeljord and Tronsmo, 1998), it is interesting to evaluate the potentiality of adding *Trichoderma* sp. through solid rice formulation. As far as we know, there is not any specialised literature that considers this type of inoculum for the evaluation of possible beneficial effects on plants and/or seedlings. Its use would help, among other aspects, to reduce preparation labours of commercial formulations. For this reason, the purpose of this work was to evaluate the effects on tomato, pepper and cucumber seedlings growth after the addition of *Trichoderma* sp. through different inocula (liquid and solid) in two different substrates, one mineral without OM and the other rich in OM and commonly used in commercial nurseries.

## MATERIAL AND METHOD

### Vegetable Material

Assessments were carried out for the three most significant horticultural species in the study area crops: tomato (*Solanum lycopersicum* L. cv. Río Grande; Ramiro Arnedo S.A.), pepper (*Capsicum annuum* cv. Sonar; Clause Spain S.A.) and cucumber (*Cucumis sativus* cv. Marketmore 76; Ramiro Arnedo S.A.).

***Trichoderma* sp. Origin and Inocula preparation**

*Trichoderma* sp. was isolated from the agricultural soils of Almería and sown in Petri dishes which contained PDA medium (Potato-Dextrose-Agar) and incubated during 7 days at 28ºC. After the incubation period, fifteen mL of a preservative solution (KCl at 10%) were added to each Petri dish and was scraped with a lancet on the colony to detach the spores. The resulting solution was deposited in 250 mL flasks to which 3 drops of Tween® 80 (biological detergent) were added to decrease the surface tension and make easier the release of spores or conidia. In this way, a fungus suspension which contained $10^8$ conidia·mL$^{-1}$ was obtained and used for the preparation of the inocula used in the trials. Then, the fungus was multiplied in commercial round grain rice, given that in previous evaluations, differences were not shown in the production of spores when using other types of rice (Table 1). To that end, aluminium trays (146x121x40 mm) which contained 50 g. of rice and 25 mL of distilled water were autoclaved (120ºC/21 minutes) and then inoculated with 10 mL of fungus suspension. After a 21 day period of incubation in darkness at 23-25 ºC until arriving at the stationary phase, a concentration of the order $10^9$ conidia·g$^{-1}$ was reached.

*Table 1*

**Production of *Trichoderma* sp. conidia according to the different types of rice used as solid substrate for the fungus growth and multiplication. The results show the average ± typical deviation. Values obtained from the three counting carried out with Neubauer chamber of the conidia present in each of the three repetitions of each type of rice.**

| Type of rice | Production of conidia (x$10^9$ conidia·g$^{-1}$) |
|---|---|
| Long | 4,7±0,8a[x] |
| Round | 4,1±0,8ab |
| Brown | 3,6±0,6b |
| *p*-value | * |

[x] *Values in the same column with different letters shows significant differences through Tukey's test (HSD) honestly significant difference test. * significance at p≤0,05.*

**Trials description**

Each horticultural species was sown separately at a rate of three tomato seeds, one of pepper, and one of cucumber, in 175 mL volume pots (experimental unit). Seeds were previously disinfected through immersion in a commercial dissolution of sodium hypochlorite at 20% during 15 minutes, and then they were rinsed and soaked during 48 hours in a wet chamber before sowing. Trials were carried out during 30 days in a controlled environmental chamber at 12.000 lux, photoperiod of 14 h light/day and maximum and minimum temperatures of 24,9±0,6 and 21,2±0,8 ºC respectively. Plants were fertilised on demand with a common fertilising solution (NPK 19-19-19) at a rate of 1g·L$^{-1}$.

Sowing was made in two different substrates: 1) vermiculite, mineral substrate without organic matter (OM) and 2) substrate rich in OM (60% blonde peat, 15% black peat, 15% coconut fiber, 10% Perlite) which is commonly used in commercial nurseries for seedling growth. To each substrate *Trichoderma* sp. was added at different concentration using 2 types of inoculum: 1) solid: *Trichoderma* sp. rice formulation and 2) liquid: nonformulated conidia suspension. The treatments with solid inoculum consisted of adding rice colonised by fungus at a rate by weight of 1%, 5% and 10% to the substrates. Treatments with liquid inoculum were prepared from a conidia suspension obtained from the same rice colonised by fungus and adjusted from successive dilutions at $10^8$, $10^7$ and $10^6$ conidia·mL$^{-1}$. For each type of substrate, the control treatment consisted of sowing the horticultural species without adding

*Trichoderma* sp. In this way, seven treatments were considered in the trials (Table 2). Three repetitions of each treatment were carried out for each horticultural species and substrate. Trials with each substrate were repeated twice over time.

*Table 2*

**Treatments with *Trichoderma* sp. carried out in the trials for the evaluation of the growth-promoting effect on tomato, pepper and cucumber seedlings. Treatments consist of adding *Trichoderma* sp. to the substrate (vermiculite and commercial nursery substrate) through solid and liquid inoculum at different concentrations.**

| Treatment | Type of inoculum | Concentration | Dosis[z] |
|---|---|---|---|
| T1 | Control | -- | -- |
| T2 | Solid | 1% p/p[x] | 0,8 g |
| T3 | Solid | 5% p/p | 4,0 g |
| T4 | Solid | 10% p/p | 8,0 g |
| T5 | Liquid | $10^8$ conidia·mL$^{-1}$ [y] | 1,75 mL |
| T6 | Liquid | $10^7$ conidia·mL$^{-1}$ | 1,75 mL |
| T7 | Liquid | $10^6$ conidia·mL$^{-1}$ | 1,75 mL |

[x] *% by weight of solid inoculum ($10^9$ conidia·g$^{-1}$) in the substrate*
[y] *through counting in Neubauer chamber*
[z] *corresponds with the amount of inoculum added, according treatment, in each experimental unit constituted by 175 mL container with 80 g of substrate.*

**Microbiological Analysis of Substrates**

The successive-dilutions method was used to know the microbiological composition of substrates when trials ended (Tello *et al*., 1991). The culture medium used was acidified malt extract agar. Ten replications of each sample for each of the treatments carried out and dilution were made. Incubation was carried out in the laboratory at room temperature during 2-5 days. After the indicated time, the counting of total Colony-Forming Units (CFU) present in each repetition was carried out.

**Evaluated parameters in seedlings**

Several variables (plant height, fresh and dry weight, leaf length and width, among others) were measured in previous trials (Marin-Guirao *et al.,* unpublished data). Finally, plant height and dry weight were taken as being the most representative and constant variable (Guerrero *et al*., 2014)

To obtain the dry weight, the washed plants were dried in a muffle oven at 72°C for 48 h until constant weight as measured in a Mettler Toledo PB 303-S analytical scale with a precision of 0.001 g.

**Statistical Analysis**

The analysis carried out for the comparisons between treatments consisted of simple analysis of variance ANOVA. The method used for the comparison of the average was the procedure of Tukey's test (HSD) honestly significant difference test at 95% of confidence. The results are shown for each horticultural species as the average of the two trials carried out over time and depending on the substrate used. The statistical package used was Statgraphic Plus 5.1 (Manugistic Incorporate, Rockville, MD, USA) for Windows.

## RESULTS AND DISCUSSIONS

The results are classified by sections according to the aspects and parameters considered in the materials and methods.

**Level of inoculum of *Trichoderma* sp. in the substrates**

When trials ended with the mineral substrate made of vermiculite and the substrate rich in OM of the nursery, and in the case of all the horticultural species evaluated, the treatments through solid inoculum showed a inoculum level higher than the treatments with liquid inoculum (Table 3).

*Table 3*

**Level of inoculum of *Trichoderma* sp. present in the nursery and vermiculite substrate 30 days after inoculations. Results according to the treatments carried out with solid and liquid inocula at different concentrations, as well as, of the horticultural species grown during the trial**

| | CFU *Trichoderma* sp.·g$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| | Tomato | | Pepper | | Cucumber | |
| Treatment | Substrate | Vermiculite | Substrate | Vermiculite | Substrate | Vermiculite |
| T1:Control | 7,4·10$^4$ c | -- | 3,0·10$^4$ d | -- | 2,1·10$^4$ d$^y$ | -- |
| T2:Solid 1% p/p$^x$ | 4,5·10$^6$ a | 2,2·10$^6$ c | 5,6·10$^6$ a | 5,2·10$^6$ c | 4,4·10$^6$ a | 2,2·10$^6$ c |
| T3:Solid 5% p/p | 6,0·10$^6$ a | 4,7·10$^7$ b | 8,3·10$^6$ a | 2,8·10$^7$ b | 6,0·10$^6$ a | 8,9·10$^7$ b |
| T4:Solid 10% p/p | 9,0·10$^6$ a | 1,8·10$^8$ a | 9,6·10$^6$ a | 1,7·10$^8$ a | 7,3·10$^6$ a | 3,0 10$^8$ a |
| T5:Liquid 10$^8$ conidia/ml | 4,8·10$^5$ b | 3,1 10$^4$ d | 5,4·10$^5$ b | 1,0 10$^5$ d | 2,4·10$^5$ b | 1,2 10$^5$ d |
| T6:Liquid 10$^7$ conidia/ml | 4,8·10$^4$ c | 2,3 10$^4$ ed | 6,6·10$^4$ c | 3,6 10$^4$ e | 5,4·10$^4$ c | 2,7 10$^4$ e |
| T7:Liquid 10$^6$ conidia/ml | 4,7·10$^4$ c | 1,2 10$^4$ e | 4,7·10$^4$ c | 1,3 10$^4$ f | 3,7·10$^4$ c | 1,6 10$^4$ f |
| *p*-value | *** | *** | *** | *** | *** | *** |

*$^x$ % by weight of Solid inoculum (10$^9$ conidia/g) in the substrate*

*$^y$ Values in the same column with different letters shows significant differences through Tukey's test (HSD) honestly significant difference test. ). *. **. *** significance at p≤0,05. 0,01. o 0,001 respectively. Statistical analysis carried out with the transformation log CFU·g$^{-1}$.*

So that, in the vermiculite, the difference in the solid treatments at 1, 5 and 10% was one, two and three orders of magnitude respectively, compared with the liquid treatment at the maximum concentration (10$^8$ conidia·mL$^{-1}$), while when treatments were carried out in the substrate rich in OM of the nursery, the difference was a magnitude order in the three cases. In both substrates, these differences are increased by one order of magnitude compared with the other treatments through liquid inoculum (10$^7$ and 10$^6$ conidia·mL$^{-1}$). These last ones showed in both substrates a level of inoculum of the order 10$^4$, level similar to that obtained in the control treatment when considering the substrate rich in OM. It is important to highlight that this substrate contained *Trichoderma* and inoculations permitted increasing inoculum density 2 points of power. It would seem that this level would be the maximum acceptable level for OM used in the substrate. Likewise, it is noteworthy that the maximum level of inoculum in the mineral substrate (10$^8$ CFU·g$^{-1}$), which was obtained in the solid treatment by 10% weight, exceeded two orders of magnitude the maximum inoculum density obtained in the substrate rich in OM when applying the same treatment.

*Table 4*

**Height and dry weight of tomato, pepper and cucumber seedlings according to the treatments with *Trichoderma* sp. applied to 2 different substrates (Verm: Vermiculite and Subs: nursery substrate) through solid and liquid inoculum at different concentrations. The results correspond with the average of the two trials carried out in each substrate**

| | Height (cm) | | | | | | Dry weight (g) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tomato | | Pepper | | Cucumber | | Tomato | | Pepper | | Cucumber | |
| **Treatment** | **Subs.** | **Verm.** | **Subs.** | **Verm.** | **Subs.** | **Verm.** | **Subs.** | **Verm.** | **Subs.** | **Verm.** | **Subs.** | **Verm.** |
| T1:Control | 7,32 b | 4,83 a | 6,22 b | 3,30 a | 7,25 ba | 5,40 a | 0,257 b | 0,055 a | 0,089 b | 0,017 a | 0,508 b | 0,113 a |
| T2:Solid 1% p/p[x] | 8,02 ba | 3,05 b | 6,50 ba | 2,10 b | 6,78 ba | 4,13 ba | 0,444 ba | 0,028 b | 0,100 b | 0,012 a | 0,562 ba | 0,073 b |
| T3:Solid 5% p/p | 8,81 a | 2,43 b | 7,07 ba | 2,17 ba | 7,43 ba | 4,22 ba | 0,596 ba | 0,023 cb | 0,196 ba | 0,008 ba | 0,701 ba | 0,069 cb |
| T4:Solid 10% p/p | 8,80 a | 2,28 b | 7,82 a | 1,50 b | 7,88 a | 3,25 b | 0,762 a | 0,018 c | 0,260 a | 0,003 b | 0,849 a | 0,036 c |
| T5:Liquid $10^8$ conidia/ml | 6,83 b | 4,31 a | 6,15 b | 2,87 ba | 6,32 ba | 4,93 a | 0,245 b | 0,052 a | 0,116 b | 0,018 a | 0,462 b | 0,112 a |
| T6:Liquid $10^7$ conidia/ml | 7,12 b | 4,25 a | 6,13 b | 3,18 a | 6,27 ba | 4,80 a | 0,364 ba | 0,054 a | 0,145 ba | 0,016 a | 0,559 ba | 0,108 a |
| T7:Liquid $10^6$ conidia/ml | 7,06 b | 4,33 a | 5,90 b | 2,67 ba | 5,43 b | 5,12 a | 0,323 b | 0,055 a | 0,106 b | 0,014 a | 0,438 b | 0,102 ba |
| *p*-value | *** | *** | ** | *** | ** | *** | *** | *** | ** | *** | ** | *** |

*Values in the same column with different letters shows significant differences through Tukey's test (HSD) honestly significant difference test. *. **. *** significance at p≤0,05. 0,01. o 0,001 respectively.).*

**Plant height**

In general, the length of the aerial part of the seedlings of the three crops studied was higher when using nursery substrate compared with the substrate made of vermiculite (Table 4). The use of solid inoculum on the mineral substrate made of vermiculite had a negative effect on the seedlings of the three horticultural species, obtaining lower height plants at the end of the trials, compared with those grown when the inoculum used was liquid and those grown with the controlled treatment, there being no difference between them. However, the same treatments with solid inoculum applied in the nursery substrate which is rich in organic matter caused an increase of seedling height compared with those obtained from the treatments with liquid inoculum and control treatment. These differences are shown mainly for solid treatment at 10% by weight.

**Dry weight**

In general, the dry weight of seedlings of the three crops studied was higher when using nursery substrate compared with the substrate made of vermiculite (Table 4).

The use of solid inoculum on mineral substrate made of vermiculite did not have a beneficial effect on the seedlings of the three horticultural species. In this way, the aerial dry weight obtained from the treatments with solid inoculum was always inferior, although in some cases was similar to those obtained in the treatments with liquid inoculum and with the control treatment, which in few cases differed between them. However, the seedlings grown from the treatments with solid inoculum at the highest concentration and applied in the nursery substrate rich in organic matter, in general, showed higher dry weight of the aerial part compared with those obtained from the treatments with liquid inoculum and the control treatment.

With the addition of solid formulations, although a decrease approximately of one order of magnitude was registered in the case of commercial substrate of the nursery rich in OM, this decrease was not detected in the mineral substrate, the levels of inoculum of *Trichoderma* sp. were kept close to the initial levels after 30 days. However, in both substrates, in the treatments with liquid formulations, the initial level of inoculum was reduced two or three orders of magnitude, compared with the treatments with the solid formulations and the values of the initial liquid inocula, respectively. Considering the liquid formulations added to the commercial substrate of the nursery, only the addition at the maximum concentration ($10^8$ conidia·mL$^{-1}$) increased one order of magnitude (up to $10^5$ CFU·g$^{-1}$) the initial values of the substrate used, which as they came from a specific nursery of the province of Almería, showed initial values of $10^4$ CFU·g$^{-1}$, a sample from a voluntary inoculation by the same nursery and not from a contaminated one. This low survival rate suggested the *Trichoderma* sp. sensitivity to environmental conditions, although an optimum environment was provided when it was applied. The decrease of *Trichoderma* sp. population which occurred mainly in the treatments with liquid inocula, could be

attributed reasonably, and, in general, to a lack of protection against different factors, including, temperature, humidity and competitors, among others (Jones and Burges, 1998). This last aspect should be considered with interest, because the microbiological analysis carried out, showed the presence of other fungi (*Absidia* sp. and *Penicillium* spp.) and bacteria, and a great amount of actinomycetes that showed a strong antagonistic power compared with the fungus object of study (data not shown). Furthermore, although the nutritive capacity of substrates together with root exudates did not allow the establishment of fungus as it was introduced, the host capacity of substrate increased with the application of solid inoculum made of rice, this could be due the fact that rice supplies food to fungus, which permits it remaining as the source substrate keeping a higher level of inoculum, even under unfavourable conditions for its establishment (Martínez-Medina *et al*.,2009).

On the other hand, and related with what has been previously stated, the results obtained in this work showed that the effectiveness of the application of *Trichoderma* sp. on seedlings of the three species evaluated is directly related with the type of inoculum chosen and its concentration, as well as with the substrate used for the establishment and development of fungus and of the considered host, and therefore, with the survival and establishment of the fungus (Ousley, 1994; Martínez-Medina *et al*., 2009; Bashan *et al*., 2014). The results showed beneficial effects on seedling growth when *Trichoderma* sp. was applied through solid inoculated rice (mainly mixed at a rate of 10% by weight with the substrate) on the commercial nursery substrate rich in OM. These results are consistent with those obtained in other studies, in which the addition of *Trichoderma* sp. using other solid formulations, such as mushroom compost or the combination of oats with bentonite and vermiculite, showed better results in the parameters evaluated in the plants, as well as higher inoculum level in the substrate, compared with those obtained when fungus was applied from liquid formulations (Kleifeld and Chet, 1992; Lo and Lin, 2002; Martínez-Medina *et al*., 2008, 2009; Bernal-Vicente *et al*., 2009; Akoijam *et al*., 2014). The substrate used seems decisive because the development of the seedlings evaluated was always higher when they grew in the nursery substrate rich in OM. According to Rajput *et al*. (2014b) this could be due to nutritionally rich substrates have a longer useful life of microorganism compared with nutritionally poor substrates, however, in this study, a higher inoculum density is shown in the mineral substrate when treatments with solid inoculum are applied on it. On the other hand, it is probably that root exudates vary depending on the substrate where plants grow and that variation has an effect on the physiology of microorganisms that promotes growth in plants, and as a consequence, a possible production of molecules with function of phytohormones, or even capacity of dissolving some beneficial minerals for the plant (Ezziyyani *et al*., 2004). Furthermore, it must be highlighted that the same treatments with solid inoculum that showed beneficial effects on seedlings when using commercial nursery substrate rich in OM, appeared totally contrary, and even in some cases

they caused their death when were applied in the mineral substrate made of vermiculite. In this sense, there are studies in which after inoculating *T. viride* and *T. harzianum* a suppression of seed germination and of growth of wheat, cucumber, tomato and pepper seedlings has been observed (Menzies, 1993; Vujanovic *et al*., 2000), and even the death of forest seedlings caused by *T. viride* (Li Destri Nicosia *et al*., 2015) has been recently registered. However, as far as we know, no references were made to what happened in our trials with vermiculite. In this respect, there is literature that considers vermiculite as a substrate unsuitable for the evaluation of the growth-promoting effect that *Trichoderma* genus has on plants (Kleifeld and Chet, 1992). A possible reason could be the existence of a great competition between fungus and plant, due to the shortage of nutrients in the mineral substrates. In this sense, Martínez-Medina *et al*., (2009) attributed the decrease of nitrogen content in seedlings obtained from treatments with *Trichoderma harzianum*, to the possible competition between the plant and the fungus for the element. This suggests the possibility that under certain conditions of food shortage, *Trichoderma* sp. could show pathogenicity compared with seedlings under the studied conditions.

## CONCLUSIONS

In conclusion, effectiveness of the application of *Trichoderma* sp. is directly related with its formulation, which had a clear influence on fungus survival, as well as on the substrate used for the development of fungus and seedlings. In this way, the use of rice to prepare the inoculum of *Trichoderma* sp. seems to be promising, as well as its application in a substrate rich in OM for seedlings' growth in nursery. The use of this type of inoculum could have great repercussion in different scopes: 1) at industry level it would help to reduce the labour and preparation costs of commercial formulations and 2) at nursery level it would reduce the growth period of seedlings. Future research must be focused on the improvement of formulation and its preservation in time, as on the possible effectiveness under greenhouse conditions.

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**Bun de tipar:** **15.12.2015**
**Apărut:** **2015**
**Format:** **61x86/16**
**Editura:** **„Ion Ionescu de la Brad" Iaşi**
**Aleea M. Sadoveanu, 3**
**Tel.: 0232-407471**
**e-mail: editura@uaiasi.ro**

ISSN–L=1454-7376
(Print)-ISSN 1454-7376
(Online)=ISSN 2069-8275
(CD-ROM) = ISSN 2069 – 847X

**PRINTED IN ROMANIA**

**Editorial Consultant:** **Vasile VÎNTU**

**Technical Editors:** **Liliana ROTARU**
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**Imprimatur:** **15.12.2015**
**Published:** **2015**
**Format:** **61x86/16**
**Publishing House:** **„Ion Ionescu de la Brad" Iaşi**
**Aleea M. Sadoveanu, 3**
**Tel.: 0232-407471**
**e-mail: editura@uaiasi.ro**

ISSN–L=1454-7376
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(CD-ROM) = ISSN 2069 – 847X

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