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2010

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Marina I. Putnik-Delić[1], *Ivana V. Maksimović*[1], *Emilija B. Nikolić-Đorić*[1], *Nevena M. Nagl*[2]

[1] Faculty of Agriculture
Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[2] Institute of Field and Vegetable Crops
Maksima Gorkog 30, 21000 Novi Sad, Serbia

ANALYSES OF STATISTICAL TRANSFORMATIONS OF ROW DATA DESCRIBING FREE PROLINE CONCENTRATION IN SUGAR BEET EXPOSED TO DROUGHT

ABSTRACT: Eleven sugar beet genotypes were tested for their capacity to tolerate drought. Plants were grown in semi-controlled conditions, in the greenhouse, and watered daily. After 90 days, water deficit was imposed by the cessation of watering, while the control plants continued to be watered up to 80% of FWC. Five days later concentration of free proline in leaves was determined. Analysis was done in three replications. Statistical analysis was performed using STATISTICA 9.0, Minitab 15, and R2.11.1. Differences between genotypes were statistically processed by Duncan test. Because of nonormality of the data distribution and heterogeneity of variances in different groups, two types of transformations of row data were applied. For this type of data more appropriate in eliminating nonormality was Johnson transformation, as opposed to Box-Cox. Based on the both transformations it may be concluded that in all genotypes except for 10, concentration of free proline differs significantly between treatment (drought) and the control.

KEY WORDS: tolerance, water deficiency, sugar beet, proline, statistical transformation, Box-Cox transformation, Johnson transformation

INTRODUCTION

According to most authors, for the successful production of sugar beet total annual amount of precipitation should be around 600 mm (Spasić, 1989). Approximately 10—20% of the total water requirements of sugar beet are coming from reserves of water in the soil and the rest is provided by rainfall and irrigation. The amount of evaporated water is 392 mm on average and ranges from 198 mm in dry years to 542 mm in rainy years (Maksimović and Dragović, 2001).

Lack of water has a very complex effect on physiological processes in plants. Research on the physiological basis of drought tolerance in many cases was the first step in the selection for tolerance to drought. It was found that there is variability in responses to water stress in sugar beet (Sadeghian et al., 2000, Ober and Luterbacher, 2002, Maksimović et al., 2004 and 2006, Perčić et al., 2005, 2006, 2007) and in some putative structural and morphological adaptive features of sugar beet to the lack of water (Luković et al., 2009). If stress occurs during the early stages of growth and development, it slows down the growth of roots, which can reduce yield by 46% (Noghabi and Williams, 2000).

The first effects of water stress are expressed in leaves, one of which is accumulation of osmoregulation substances in sugar beet such as glycine betaine, proline, and fructans. This phenomenon is correlated with decreasing of water potential of cells. It is not clear if plants, which in terms of lack of water build up these substances, better tolerate the lack of water or not (Ghoulam et al., 2002; Maksimović et al., 2004 and 2006; Perčić et al., 2005).

When inhomogeneous results are obtained during the measurement of certain parameters, it is difficult to draw reliable conclusions about the effect of applied treatments without additional statistical analyses of row data. Such example of statistical transformation of core values using Box-Cox and Johnson transformations will be shown in this paper. The effect of those transformations on the final conclusion on the effect of water deficiency on free proline concentration in sugar beet leaves is discussed.

MATERIAL AND METHODS

Eleven sugar beet genotypes (1—11), pre-selected for their different ability to maintain turgor in the field, were grown in semi-controlled conditions in the greenhouse. Substrate was a mixture of soil and sand, and plants were watered daily. After 90 days, water deficit was imposed by the cessation of watering, while the control plants continued to be watered up to 80% of FWC. Five days later the concentration of free proline in leaves was determined following the procedure of Bates (1973). Analyses were carried out in three replications.

In order to examine distribution of experimental results basic statistical exploratory methods box-whisker diagram and plot (Q-Q plot) were applied. Box-whisker diagram was obtained on the base of median (M_e) upper (Q_3) and lower quartiles (Q_1) and interquartile range that is a measure of dispersion of the central portion of a distribution ($I_Q = Q_3 - Q_1$). This diagram is very useful in establishing skewness of the distribution and presence of outlier and extreme values. The outlier is defined as the value outside the range of ($Q_1 - 1.5 \cdot I_Q$, $Q_3 + 1.5 \cdot I_Q$), and the extreme value is the value outside the range of ($Q_1 - 3 \cdot I_Q$, $Q_3 + 3 \cdot I_Q$).

In the Q-Q plot, the observed values of the variable are ordered ($x_1 < \ldots < x_n$), and then these values (x_i) are plotted against the inverse probability distribution function (theoretical quantiles). If the observed values fall on the re-

gression line, then it can be concluded that the observed values follow the specified distribution. In our case, specified distribution is normal. The lack of normality was established by means of exploratory analysis and several normality tests based on different approaches. Anderson-Darling and Lilliefors tests are empirical density function (EDF) omnibus tests. Shapiro-Wilk test is based on the squared correlation between the ordered sample values and the (approximated) expected ordered quantiles from the standard normal distribution. Jarque-Bera and D'Agostino omnibus tests measure deviation of empirical skewness and kurtosis from zero.

Applied transformations are preprocessing techniques used to stabilize variance and make the data more normal distribution like. Parametric analysis of transformed data is considered a better strategy than non-parametric analysis because the former appears to be more powerful than the latter (Rasmussen & Dunlap, 1991).

The Box-Cox transformation, introduced by statisticians George E. P. Box and David Cox in 1964, is a family of a power transformations that might be used to convert a general set of *n* observation observations into a set of *n* independent observations from a normal distribution with constant variance. The transformation involves a parameter that can be estimated from the data using the method of maximum likelihood:

$$Y \quad \frac{X \quad 1}{}, \quad 0$$
$$\ln X, \quad 0.$$

Special cases of Box-Cox transformation are log, square root and inverse transformation. The Johnson system (Johnson, 1949) is a very flexible system for describing statistical distributions as it includes four parameters. It is defined by,

$$z = \gamma + \delta \log[f(u)], \text{ with } u \quad \frac{x \quad xi}{}$$

and where function f( ) has four possible forms depending on original data distribution:

SL: f(u) = u for the log normal distribution,
SU: f(u) = u + $\sqrt{u^2 \quad 1}$ for an unbounded distribution,
SB: f(u) = u/(1-u) for a bounded distribution,
SN: f(u) = for e^u the normal distribution.

Three programs Statistica 9, Minitab 15 and R2.11.1 were used to perform statistical analysis.

Exploratory data analysis and normality tests were done by R2.11.1. Johnson transformation was done by Minitab 15 program, and Box-Cox trans-

formation and ANOVA and Duncan test using Statistica 9. Duncan test was applied on original data, and on the both sets of transformed results.

RESULTS AND DISCUSSION

The concentration of free proline showed heterogeneity of variances in genotype and treatment groups according to the tests of homogeneity of variances (Hartley, Cochran, Bartlett, Levene).

On the base of the histogram of row data (mg proline/g DW), it can be concluded that distribution is highly skewed. Normal Q-Q plot and box-plot show deviation from normality, as the right tail of the empirical distribution is heavier than the right tail of normal distribution (first line of Fig. 1). Highly significant deviation from normality was also confirmed by statistical tests (Table 1).

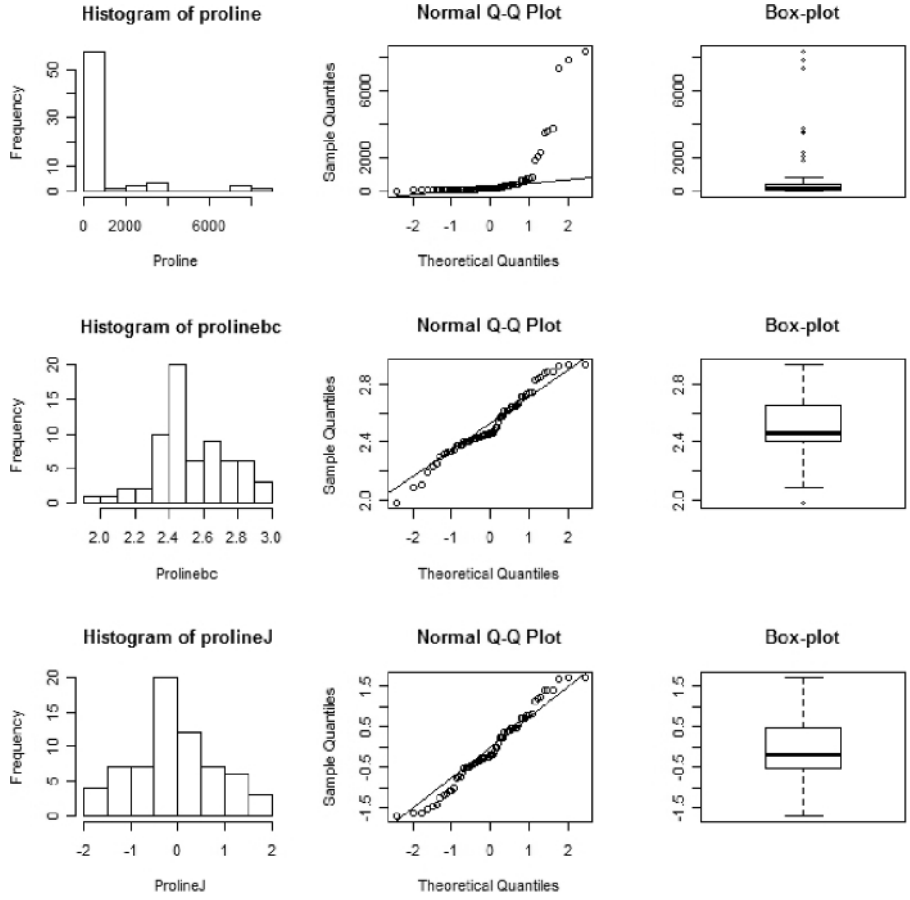

Fig. 1. — Histogram, normal Q-Q plot and box-whisker diagram for the original and transformed data

Tab. 1. — Results of normality tests applied on the raw and transformed data

Normality test	Original data	Box-Cox transformation	Johnson transformation
Anderson-Darling	14.2153	0.8372	0.3697
p-value	< 2.2e-16	0.02932	0.4164
Lilliefors (Kolmogorov-Smirnov)	0.3634	0.1349	0.0882
p-value	< 2.2e-16	0.004512	0.2292
Shapiro-Wilk	0.4555	0.9689	0.976
p-value	3.444e-12	0.09585	0.2290
Jarque-Bera	374.3926	0.2723	1.2122
p-value	< 2.2e-16	0.8727	0.5455
D'Agostino	68.0175	0.1243	1.5037
p-value	1.665e-15	0.9397	0.4715

In order to apply parametric analysis of variance and treatment comparison, transformation of data was necessary.

The distribution of Box-Cox transformed data with maximum likelihood estimate $\lambda = -0.321864$, is much closer to normal distribution. It may be noticed that its left tail is heavier comparing with normal distribution because of presence of several outliers (second line of Fig. 2). The deviation from normality was confirmed by results of Anderson-Darling and Lilliefors test (Table 1). The transformation eliminated heterogeneity of variances in treatment groups and decreased it in genotype groups.

The best Johnson transformation was SU type and transformation function equals

$$f(u) \quad 0.911 \quad 0.3754 \sinh^{1}\left(\frac{x \quad 82.2085}{15.1082}\right), \text{ where } \sinh^{1}(u) \quad \log\left(u \quad \sqrt{1 \quad u^{2}}\right).$$

The Johnson transformation was effective in eliminating nonormality and variance stabilization (third line of Fig. 3, Tab. 1).

Data transformation did not affect the overall F test and F test for interaction genotype x treatment (imposed drought) but it affected pairwise comparisons (Fig. 2, 3, 4).

Figures were all made for $\alpha = 0.05$, because the result was exactly the same at $\alpha = 0.01$. Graphical presentation of the sample mean values of free proline in sugar beet leaves and corresponding 95% intervals suggest that in the case of transformed data, there is greater number of significant comparisons.

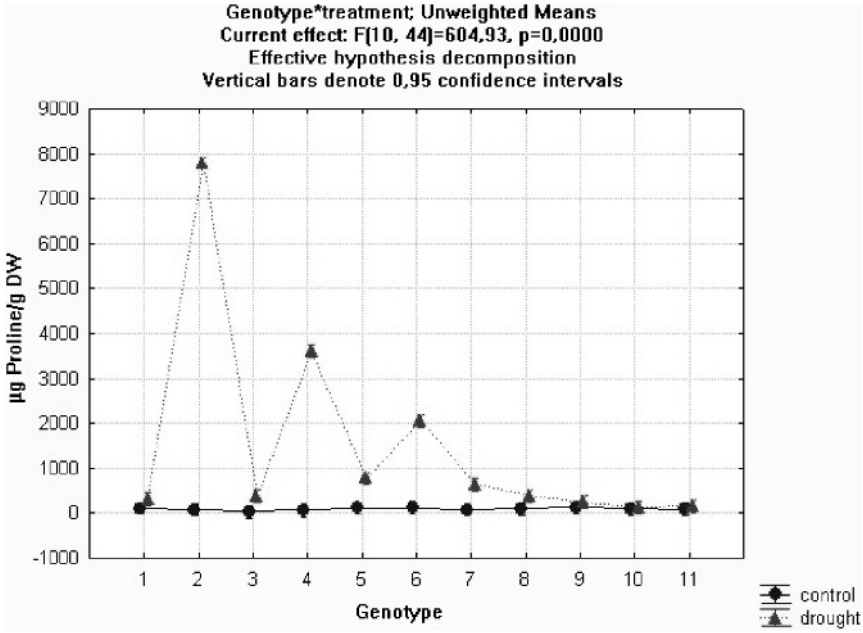

Fig. 2. — The result of analysis of variance for the concentration of free proline in eleven genotypes of sugar beet — the row data

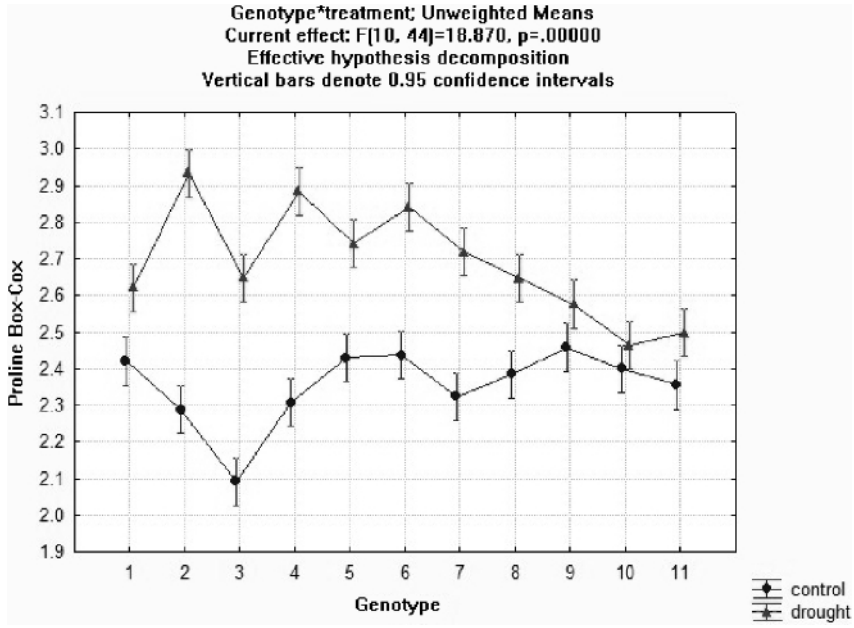

Fig. 3. — The result of analysis of variance for the concentration of free proline in eleven genotypes of sugar beet after Box-Cox transformation of row data

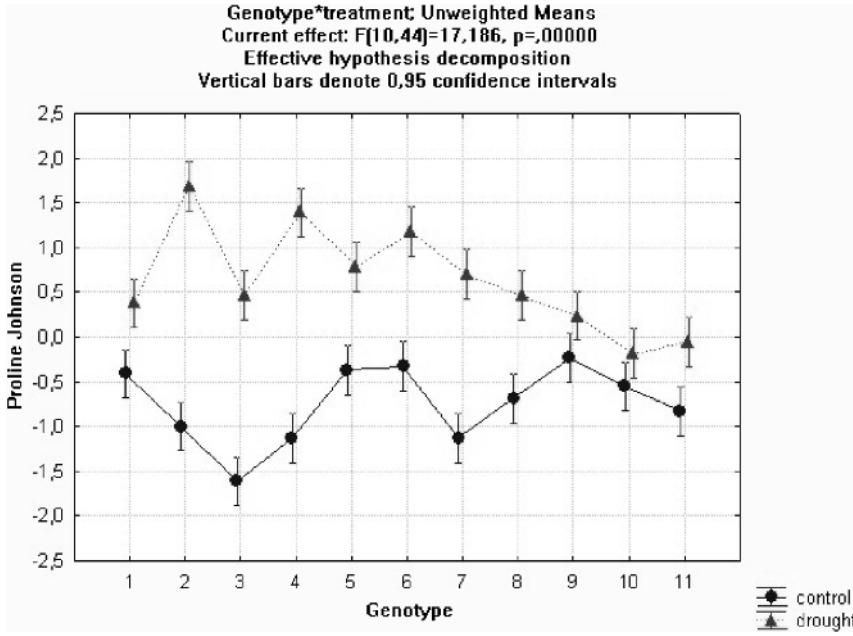

Fig. 4. — The result of analysis of variance for the concentration of free proline in eleven genotypes of sugar beet after Johnson transformation of row data

Tab. 2. — Results of Duncan's test for free proline concentration in sugar beet leaves, row and transformed data. "**c**" stands for control, "**d**" for drought (water deficit in semi control conditions)

Duncan test																					
	1c	1d	2c	2d	3c	3d	4c	4d	5c	5d	6c	6d	7c	7d	8c	8d	9c	9d	10c	10d	11c
1c																					
1d	●Δ																				
2c	●Λ	□●Λ																			
2d	□●Δ	□●Δ	□●Δ																		
3c	●Δ	□●Δ	●Δ	□●Δ																	
3d	□●Δ		□●Λ	□●Δ	□●Δ																
4c	●Δ	□●Δ		□●Δ	●Δ	□●Δ															
4d	□●Δ	□●Δ	□●Δ	□●	□●Δ	□●Δ	□●Δ														
5c		●Λ	●Λ	□●Λ	●Λ	□●Λ	●Λ	□●Λ													
5d	□●Δ	●□	□●Δ	□●Δ	□●Δ	□	□●Δ	□●Δ	□●Δ												
6c		●Λ	●Λ	□●Λ	●Δ	□●Δ	●Λ	□●Δ		□●Δ											
6d	□●Δ	□●Δ	□●Δ	□●Δ	□●Δ	□●Δ	□●Δ	□	□●Δ	□●Δ	□●Δ										
7c	Δ	□●Δ		□●Δ	●Δ	□●Δ		□●Δ	●Δ	□●Δ	●Δ	□●Δ									
7d	□●Δ	□●	□●Δ	□●Δ	□●Δ	□	□●Δ	□●Δ	□●Δ		□●Δ	□●Δ	□●Δ								
8c		●Δ		□●Δ	●Δ	□●Δ	Δ	□●Δ		□●Δ		□●Δ	Δ	□●Δ							
8d	□●Δ		□●Δ	□●Δ	□●Δ		□●Δ	□●Δ	□●Δ	□	●Δ	□●Δ	□●Δ	□	□●Δ						
9c		●Λ	●Λ	□●Λ	●Λ	□●Λ	●Λ	□●Λ		□●Λ		□●Λ	●Λ	□●Λ	Λ	□●Λ					
9d	●Δ		●Δ	□●Δ	●Δ		●Δ	□●Δ	●Δ	□●Δ	●Δ	□●Δ	●Δ	□●Δ	●Δ		●Δ				
10c		●Δ	●Δ	□●Δ	●Δ	□●Δ	Δ	□●Δ		□●Δ		□●Δ	Δ	□●Δ		□●Δ		●Δ			
10d		●Λ	●Λ	□●Λ	●Λ	□●Λ	●Λ	□●Λ		□●Λ		□●Λ	●Λ	□●Λ	Λ	□●Λ		●Λ			
11c		□●Δ		□●Δ	●Δ	□●Δ		□●Δ	Δ	□●Δ	Δ	□●Δ		□●Δ		□●Δ	Δ	●Δ		●Δ	
11d		●Δ	●Δ	□●Δ	●Δ	□●Δ	●Δ	□●Δ		□●Δ		□●Δ	●Δ	□●Δ	●Δ	□●Δ			Δ		●Δ

□ — row data
— Box-Cox transformation
— Johnson transformation

The statistically significant pairwise comparisons based on post-hoc Duncan's test, done on row and on transformed data, are presented in Table 2. The results indicate that in all genotypes except 1, 9, 10 and 11 there was statistically significant differences in free proline concentration between treatment (drought) and control if the analysis is done on the original data. In the case of transformed data, the difference is significant in all genotypes except for 10. If we consider all 231 comparisons presented in Table 2, the 123 comparisons based on original data are significant. For transformed data, there is a greater number of significant comparisons: 173 in the case of Box-Cox and 182 for Johnson transformation.

CONCLUSIONS

The Box-Cox transformation is usually applied in biometrics to eliminate deviation from normality and to stabilize variance. Although this transformation is easy to understand and apply, it often does not find a suitable solution. The Johnson transformation, implemented in statistical software (Minitab, R), may be a good alternative. In this research, results obtained with both, transformations concerning comparison of free proline concentration between treatment (drought) and control are consistent.

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ПРИМЕНА СТАТИСТИЧКИХ ТРАНСФОРМАЦИЈА НА ИЗМЕРЕНЕ КОНЦЕНТРАЦИЈЕ ПРОЛИНА У ЛИСТУ ШЕЋЕРНЕ РЕПЕ У УСЛОВИМА СУШЕ

Марина И. Путник-Делић[1], Ивана В. Максимовић[1],
Емилија Б. Николић-Ђорић[1], Невена М. Нагл[2]

[1] Пољопривредни факултет, Департман за ратарство и повртарство,
Трг Доситеја Обрадовића 8, 21000 Нови Сад, Србија
[2] Институт за ратарство и повртарство Нови Сад, Максима Горког 30,
21000 Нови Сад, Србија

Резиме

Анализирана је толерантност 11 генотипова шећерне репе према недостатку воде. Биљке су гајене у полуконтролисаним условима, у стаклeнику, на супстрату који је био мешавина земље и песка, при чему су биљке свакодневно заливане. После 90 дана, водни дефицит је изазван престанком заливања, док су биљке контролне групе и даље заливане, до 80% ПВК. Пет дана касније утврђена је, у три понављања, концентрација слободног пролина у листовима. Статистичка анализа је извршена коришћењем програма Statistica 9.0, Minitab 15 и R2.11.1. Због велике варијабилности података и одступања од нормалне расподеле анализиран је утицај различитих трансформација експерименталних података на коначан закључак. Разлике између аритметичких средина поређене су применом Данкановог теста. На основу обе примењене трансформације може се закључити да се код свих генотипова, изузев генотипа 10, концентрација слободног пролина у листовима значајно разликује између биљака изложених суши и контроле.

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UDC

Ottó T. Dorogházi
Rudolf R. Kastori
Ivana V. Maksimović

Faculty of Agriculture, Novi Sad, Trg D. Obradovića 8
21000 Novi Sad, Serbia

NICKEL TRANSLOCATION FROM SEED DURING GERMINATION AND GROWTH OF YOUNG MAIZE PLANTS

ABSTRACT: Effect of different concentrations of nickel (0, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} mol Ni/dm^3) present at the time of maize seed imbibition, on concentration, distribution and nickel accumulation coefficient in the root and the shoot, biological value of the seed and growth of young plants was investigated. It was found that during germination the nickel from the seed is intensively translocated to the root and shoot of young plants. With increase of applied concentrations of nickel, its concentration in the root and shoot increased as well. Nickel concentration and accumulation coefficient were higher in the root than in the shoot except at the highest applied concentration when the result was opposite. The highest applied concentration of nickel increased percentage of atypical seedlings and non-germinated seeds and decreased percentage of typical seedlings, germination energy and seed germination ability. Nickel implementation did not affect the growth and mass of the shoot. Root mass and length of the primary root decreased at the highest concentration of nickel, which led to change in shoot and root mass ratio.

Based on the obtained results it can be concluded that only the highest applied nickel concentration affected the biological value of the seed and the growth of young maize plants, regardless of its intensive accumulation in the root and the shoot, which indicates a significant tolerance of maize in initial phases of growth to presence of high nickel concentration. Intensive translocation of nickel during germination into newly formed organs points to its good mobility and potential possibility to enter the food chain from a contaminated seed.

KEY WORDS: maize, nickel imbibition of grain, germination, growth, translocation, distribution

INTRODUCTION

Nickel (Ni) is widespread in the biosphere (Kabata-Pendias and Pendias, 2000). Discovery of nickel being the metal component of the enzyme urease (Dixon et al., 1975) and widespread in the plant world (Welch, 1981, Polacco, 1997), that bacteria require Ni for the synthesis of nickel hydrogenase(s), of nickel-containing carbon monoxide dehygrogena-

se(s), of methyl-CoM reductase and urease (Ankel-Fuchs and Thauer, 1988, Maier et al., 1990), intensified research related to the role of Ni in life processes of higher plants. In the 1970's but also earlier, numerous papers were published that prove favorable effect of Ni on plant growth and metabolism (Kastori and Petrović, 1976). However, it was only recently that its necessity for numerous organisms was confirmed, which led to including Ni in essential, biogene microelements (Eskew et al., 1983, Checkai et al., 1986, Brown et al., 1987, Ankel-Fuchs and Thaure, 1988, Marschner, 1995).

Higher concentrations of Ni, like other heavy metals, have toxic effect on plants (Asher 1991, Seregin and Kozhevnikova, 2006). Nickel became a significant pollutant. In crop plants there is much more concern about Ni contamination and toxicity. The application of sewage sludge which is often high in Ni (Brown et al., 1989) and certain phosphate fertilizers also may by important sources of Ni. Anthropogenic sources of Ni, industrial activity (metal processing operations, combustion of coal and oil) in particular, have resulted in significant increase of Ni content in soils (Kabata-Pendias and Pendias, 2000). In natural conditions, Ni toxicity appears in soils rich in Ni that originated on serpentine as parent rock. Nickel is readily mobile in the xylem and phloem (Kochian, 1991) and some plant species significantly translocate Ni into the seed (Petrović and Kastori, 1979, Petrović and Kastori, 1994). Many environmental stresses (e.g. mineral toxicities, soil acidity, nutrient deficiencies etc.) can directly or indirectly influence seed development including seed vigor and viability (Welch, 1999). The aim of this research was to investigate the influence of Ni contamination of the seed on biological properties of the seed, as well as translocation of Ni from the seed during germination into the root and the shoot of young maize plants.

MATERIALS AND METHODS

The experiment was carried out on a maize hybrid NS 7016 seed. Nickel treatment was performed by soaking the maize seed in $NiSO_4$ solution. It was previously determined that at the temperature of 22°C a seed soaked during 24 h contains 37% of water and that after that period there is no statistically significant increase of water content in the seed. In order to germinate, the seed needs about 30% of water. In the course of the experiment, effect of five treatments was investigated, the seed was soaked in: 0 (control, deionized water), 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} mol Ni/dm^3. The experiment was set up in five replications. After the treatment, the seed was rinsed with deionized water. Than it was determined the biological value of the seed according to standard procedure published in Službeni list SFRJ 47/87 (Official Gazette of SFRY). It was investigated: germination energy, seed germination ability, representation of typical and atypical seedlings and the percentage of non-germinated seeds. After seven days it was measured the length of the shoot, of the primary root and the mesocotile root at 20 plants. It was determined the mass of the dry matter of the root and the shoot after drying the plant material to constant mass.

In the homogenized dry plant material of the shoot and the root it was determined the concentration of Ni and other mineral matter after digestion in cc HNO_3 + cc H_2O_2. Element concentration was determined using ICP. Based on results of chemical analysis there were determined the concentration, distribution and accumulation coefficient of Ni, as well as concentration of some macro- and microelements in the shoot and the root. Accumulation coefficient of Ni was calculated from the difference in Ni concentration between the treatment and the control.

The results were statistically processed by calculating the smallest significant difference between arithmetic means.

RESULTS AND DISCUSSION

Results on Ni concentration in the seed indicate that Ni ions pass with, more or less, no obstruction through the testa and the semipermeable cell membrane of the seed and that in relatively short time they are in all parts of the seed, to which points their intensive accumulation in young plants (Tab. 1). Certain parts of the seed do not absorb water, which is probably why they do not absorb Ni at the same intensity. The germ absorbs water 2—3 times more intensively than the endosperm (Kastori, 1984), which probably contributed to significant Ni accumulation in the root and the shoot of young plants. At lower applied concentrations, the Ni concentration was higher in the root than in the shoot, while at the highest applied concentration it was vice versa. During germination, products of organic matter decomposition of the seed, as well as mineral matter, partly defund into the environment. Probably a part of Ni taken up by the seed got into the environment which enabled the root to uptake it, which reflected on its higher concentration at lower applied concentrations of Ni. With appearance of shoot and transpiration with it, the ascending transport of Ni was probably induced, especially at the highest applied concentration of Ni, which partially explains its higher concentration in the shoot than in the root. Accumulation coefficient which indicates the intensity of increase of Ni concentration with the increase of applied Ni concentration was higher in the shoot than in the root only when applying the highest concentration of Ni. According to Cieslinski et al. (1996) at a higher concentration of cadmium in the soil, out of the total uptaken quantity of cadmium a relatively smaller quantity is being translocated into above-ground organs than at lower concentrations, which they explain as a certain form of protection mechanism. On the contrary, our research results point to a relatively more intensive translocation of Ni to the shoot at a higher applied concentration. Intensive mobilization of Ni from the seed during germination and development of a young plant into newly-formed organs confirms earlier findings about good mobility of Ni in the plant phloem and xylem (Petrović and Kastori, 1979, Neumann and Chamel, 1986, Yang et al., 1996, Page and Feller, 2005).

Tab. 1 — Concentration, distribution and accumulation coefficient of Ni in young maize plants treated with Ni

Organ	Treatment (mol Ni/dm^3)				
	0	10^{-5}	10^{-4}	10^{-3}	10^{-2}
	Concentration (μg/g DM)				
Shoot	4.89	6.90**	10.09**	41.36**	148.85**
Root	4.17	46.35**	52.77**	70.74**	110.29**
	Distribution (%)				
Shoot	46.88	10.43	12.71	32.04	54.83
Root	53.12	89.57	87.29	67.96	46.17
	Accumulation coefficient				
Shoot	0.00	1.41	2.06	8.45**	30.43**
Root	0.00	11.11**	12.65**	16.69**	26.45**

Seed quality, vigor and viability are important characteristics influencing seedling establishment, crop growth, and productivity (TeKrony and Egli, 1991). Only the highest applied concentration of Ni affected biological value of the seed (Tab. 2), which confirms findings of Seregin and Kozhevnikova (2005) of great plant tolerance to high Ni concentration in the germination phase. Appliance of the highest Ni concentration has significantly decreased germination ability, germination energy and percentage of typical seedlings and increased percentage of atypical seedlings and non-germinated seed. Niethammer (1930) was one of the first to observe that Ni affects the seed germination. He established that lower concentrations (0.1% solutions) had stimulant effect, and higher (0.5 to 1.0%) had inhibitory effect. Among the investigated salts, the lowest toxicity was shown by $NiSO_4$ and the highest by Ni $(NO_3)_2$. Effect of Ni on germination thus depended on concentration and type of the salt. It was also observed that a young seed, with well preserved viability, reacts differently to Ni than an old seed having partially damaged germination ability. Namely, Ni concentrations that induced inhibition at a young, vital seed had no effect on an old seed or they even increased germination ability. Also later, a large number of authors investigated the influence of Ni on germination and seed germination ability, and the obtained results were often contradictory (Kastori and Petrović, 1976). Brown et al. (1987) state that imbibition with 1 μM $NiSO_4$ did not improve the percent germination of barley grain, suggesting that the availability of Ni for essential processes in the grain is not limiting germination. However, there is the possibility that Ni in the imbibition treatment was not available for uptake by the embryo. The mentioned authors have concluded, based on results of their own research which showed that plants grown with l mM Ni and nutrient solution produced grain with better germination, vigor and viability, and other authors' results, concluded that Ni is required for normal grain development, maturation and plant senescence. Brown et al. (1987) state that without adequate Ni supply, maternal barley plants developed nonviable grain that did not germinate upon imbibition. The exact metabolic role of Ni in seed germination is not known. It can be assumed that Ni affects the utilization of nitro-

gen stored in compounds in the seed during their catabolism upon germination because Ni is an essential component of the enzyme urease (Welch, 1999). Consequently a question arises at which extent is the nitrogen in the seed of small grains present in the form of ureides. It is obvious that the effect of Ni on seed germination depends on a larger number of factors: concentration, method of use and Ni enrichment of the seed, plant species, seed maturity etc. which could explain often contrary results about this element's effect on biological properties of the seed.

Tab. 2 — Effect of different Ni treatments on biological properties of maize seeds and seedlings

Parameters	Treatment (mol Ni/dm^3)				
	0	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Typical seedlings (%)	83.50	83.75	84.00	83.25	27.50**
Atypical seedlings (%)	10.75	9.50	10.75	13.00	25.25**
Non-germinated seed (%)	5.75	6.75	5.25	3.75	47.25**
Germination energy (%)	81.75	82.25	82.50	80.75	20.00**
Germination ability (%)	83.50	83.75	84.00	83.25	27.50**

Usage of different Ni concentrations did not significantly affect the growth and mass of the shoot (Tab. 3). Higher Ni concentrations decreased growth of the primary root, root mass and increased shoot/root dry mass ratio, which suggests that the unfavorable effect of higher Ni concentrations affected more growth and development of the root system than of the shoot, even the Ni concentration being higher in the shoot than in the root at the highest applied Ni concentration. This contradicts the statement of Seregin et al. (2003) who point out that the growth of organs where Ni is more accumulated is being more intensively inhibited than the growth of organs where the accumulation of Ni is lower. It is reasonable to assume that the effect of toxic concentrations of an element on growth depends also on the length of the treatment. In our case it was short, which can probably partially explain no inhibition of the shoot growth. Significant decrease in the root growth at young maize plants in the presence of Ni was also established by Maksimović et al. (2007).

The critical toxic level of Ni in sensitive cultivated species is 10 μg/g of dry matter and 50 μg/g of dry matter in moderately sensitive species (Asher, 1991). Ni concentration in the shoot and the root when applying the highest investigated concentration of Ni was significantly above mentioned values, which confirm the findings of Seregin (2005) about increased tolerance of plants to surplus of Ni during germination. Toxic concentrations of Ni affect photosynthesis (Singh et al., 1989), as well as the function of cell membranes and the water regime (Llamas et al., 2008), they decrease the chlorophyll content, water potential and transpiration (Pandey and Sharma, 2002) and they affect the concentration and distribution of elements (Petrović et al., 1998, Ilin and Kastori, 1999). Kao and Lin (2005) state that the toxic concentration of Ni increases peroxidase activity, which causes inhibition of synthesis of cellulose and lignin, components of the cell wall, and thus decreases its thickness or even disables normal forming of the cell wall

which prevents cell elongation. L'Huiller et al. (1996) state that unfavorable effect of higher Ni concentrations on the plant growth can, among other, be attributed to its influence on cell division. Findings of these authors can explain inhibition of the root growth and, partially, of the shoot at the highest concentration Ni treatment.

Tab. 3 — Effect of different Ni treatments on growth and dry mass of young maize plants

Parameters	Treatment Ni (mol/dm^3)				
	0	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Shoot length (cm)	6.89	6.42	6.75	7.08	6.19
Primary root length (cm)	11.84	10.88	11.63	10.29*	10.42*
Mesocotile root length (cm)	8.97	8.86	9.08	9.13	8.46
Shoot dry mass (mg/plant)	24.00	22.70	22.90	25.80	23.96
Root dry mass (mg/plant)	31.70	30.10	29.70	32.10	26.63
Shoot dry mass / root dry mass	0.75	0.75	0.77	0.80	0.90

Translocation of some macro- and micro elements was investigated, from the seed to the shoot and the root, during the growth of young plants at a non-treated seed (Tab. 4). Translocation of the investigated elements from the seed during germination into the shoot and the root varied. In the shoots, P, K and B accumulated more intensively, S, Mg, Ca, Fe, Mn, Mo and Al in the root, while accumulation of Zn and Cu was approximately even. Generally, it can be said that translocation of mineral matter during maize seed germination is more intense into the root than into the shoot.

Tab. 4 — Translocation of macro- and microelements during maize seed germination

Organ	Macronutrients (mg/100 g DM)						
	P	**K**	**S**	**Mg**	**Ca**		
Shoot	672.25	1062.50	47.22	159.10	176.50		
Root	451.31	885.28	112.83	245.23	987.95		
	Micronutrients (μg/g DM)						
	Fe	**Zn**	**Cu**	**Mn**	**Mo**	**Al**	**B**
Shoot	29.33	7.27	0.50	2.54	0.05	9.19	1.05
Root	247.30	7.24	0.46	6.84	0.10	99.49	0.64

Obtained results show that due to intensive mobilization of Ni in the seed during germination and its translocation into the root and the shoot, there is a great possibility of Ni entering the food chain from the contaminated seed, which especially has to be taken into account when producing organic food.

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ТРАНСЛОКАЦИЈА НИКЛА ИЗ СЕМЕНА У ТОКУ КЛИЈАЊА И РАСТ МЛАДИХ БИЉАКА КУКУРУЗА

Ото Т. Дорогхази, Рудолф Р. Кастори, Ивана В. Максимовић

Пољопривредни факултет, Трг Д. Обрадовића 8, 21000 Нови Сад, Србија

Резиме

Испитивано је дејство бубрења семена кукуруза у растворима различитих концентрација никла (0, 10^{-5}, 10^{-4}, 10^{-3} и 10^{-2} mol Ni/dm^3) на садржај, дистрибуцију и коефицијент акумулације никла у корену и изданку, биолошку вредност семена и раст младих биљака. Утврђено је да се никал из семена у току клијања интензивно транслоцира у корен и изданак младих биљака. Са повећањем примењене концентрације никла значајно се повећао његов садржај у изданку и корену. Садржај никла и коефицијент акумулације били су већи у корену него у изданку, изузев код највеће примењене концентрације, где је било обрнуто. Највећа примењена концентрација никла повећала је удео атипичних поника и непроклијалог семена и смањила удео типичних поника, енергију клијања и клијавост семена. На раст и масу изданка примена никла није утицала. Маса корена и дужина примарног корена се смањила при употреби највеће концентрације никла, што је довело до промене односа масе изданка и корена.

На основу добијених резултата може се закључити да је само највећа примењена концентрација никла утицала на билошку вредност семена и раст младих биљака кукуруза и поред његовог интензивног накупљања у корену и изданку, што указује на значајну толерантност кукуруза у почетним фазама раста на присуство високе концентрације никла. Интензивна транслокација никла у току клијања у новоформиране органе указује на његову добру покретљивост и потенцијалну могућност да из контаминираног семена улази у ланац исхране.

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UDC

Biljana M. Gorjanović
Marija M. Kraljević-Balalić

Faculty of Agriculture, Trg D. Obradovića 8, 21000 Novi Sad, Serbia

AMMI ANALYSIS OF NITROGEN HARVEST INDEX IN BREAD WHEAT

ABSTRACT: Nitrogen harvest index — NHI is a measure of efficiency of nitrogen translocation from vegetative organs to grain. The goal of this paper is to investigate variability and stability of nitrogen harvest index of twelve bread wheat genotypes, on three nitrogen levels. ANOVA showed that nitrogen harvest index was mostly under influence of the year x genotype interaction, year of investigation and genotype, and in the smallest amount of the nitrogen rate. Increasing doses of nitrogen did not lead to increased nitrogen harvest index. AMMI analysis showed that most genotypes differed in both the main effect and in GxE interaction. The highest stability i.e. the smallest interaction effect, was found in varieties Axis, Ilona, Sonata and Renan on N_0 rate, and in varieties Malyska, Petrana, Axis and Evropa 90 on N_{100} rate. Cultivar Pobeda with the high average values for nitrogen harvest index, also had small interaction effect, i.e. it proved to be a stable variety.

KEY WORDS: AMMI analysis, nitrogen harvest index, wheat

INTRODUCTION

Numerous studies indicate that nitrogen is the key factor of yield and quality in the wheat. Nitrogen harvest index (the ratio of nitrogen content in grain and whole plant) is a measure of efficiency of nitrogen translocation from vegetative organs to grain (Austin et al., 1977, McMullan et al., 1988; Sinclair, 1998). Nitrogen harvest index for wheat usually ranges from 0.70 to 0.80 (Calderini et al., 1995; Brancourt-Hummel, 2003, Andersson, 2005). Van Sanford and MacKown (1987) noted the extreme values of 0.51 to 0.91. Ortiz-Monasterio et al. (1997) have studied the nitrogen harvest index of genotypes recognized between 1950 and 1985 and recorded a significant increase in the newer varieties. Slafer et al. (1990) recorded similar results.

Löffler et al. (1985), Ortiz-Monasterio et al. (1997), and Anderson (2005) noted positive correlations between NHI and HI, as well as between NHI and grain yield (Löffler et al., 1985). McKendry et

al. (1995) and McMullan et al. (1988) did not find significant correlations between NHI and grain yield.

The goal of this paper is to investigate variability and stability of nitrogen harvest index of twelve bread wheat genotypes, on three nitrogen levels.

MATERIAL AND METHODS

The 12 bread wheat cultivars were studied in the three-year (2004—05, 2005—06 and 2006—07 growing seasons) field trial with three nitrogen rates (0, 75, 100 kg/ha N). Five cultivars originated from Serbia (Evropa 90, Nevesinjka, Pobeda, Zlatka, and Sonata), five from Slovakia (Ilona, Malyska, Vanda, Petrana, and Axis), one from France (Renan), and one from Switzerland (Tamaro). The experiment was conducted at the experimental field of the Institute of Field and Vegetable Crops, Novi Sad. The sowing rate was 600 grains/m^2. Plot size was 5 m^2. In all three years 45 kg/ha of each N, P and K before plowing were applied. In spring three N levels were applied (0, 75, 100 kg/ha N). Standard agronomic practices were used to keep the plots free of diseases.

At maturity, ten plants were cut at ground level and they represented one sample/replication in the analysis. At maturity, samples were separated into vegetative (leaf + culm + chaff) and reproductive parts (grains). After drying, all samples were ground in a mill to generate 1-mm particles. The nitrogen concentration was determined by the standard Kjeldahl procedure. Nitrogen harvest index was calculated as the ratio of grain nitrogen content over total nitrogen content. AMMI (Additive main effects and multiplicative interaction) model was used to analyze the genotype x environment interaction (Zobel et al., 1988). The analysis was performed in GenStat 9 program (trial version).

RESULTS AND DISCUSSION

ANOVA showed that nitrogen harvest index was mostly under influence of the year x genotype interaction (52.70%), year of investigation (33.58%) and genotype (10.55%), and in the smallest amount of the nitrogen rate (1.56%) (Table 1) Baldelli et al. (1990) state that they have not recorded a significant difference between genotypes, but the difference between years was significant.

Significant differences were found between N_0 and N_{75} rate, N_0 and N_{100} rate, while there were no significant differences between N_{75} and N_{100} rates (table 1). Increasing doses of nitrogen did not lead to increased nitrogen harvest index; moreover, the majority of genotypes had the highest value in the control, which is in agreement with the results of Le Gouis et al. (2000). Đokić and Lomović (1990) states that the nitrogen harvest index was not significantly changed with increasing doses of nitrogen up to 120 kg N/ha, after which it began to decline.

Tab. 1. — ANOVA for nitrogen harvest index

Source of variation	DF	MS	F	%
Year	2	0.28	219.63**	33.58
Genotype	11	0.016	12.18**	10.55
Nitrogen rate	2	0.013	10.14**	1.56
Replication	1	0.0004	0.28	0.02
Year Genotype	22	0.04	2.84**	52.70
Year N rate	4	0.0008	0.66	0.19
Genotype N rate	22	0.001	1.08	1.32
Error	151	0.001		
Total	216			

Significance of differences between N rates		
N rate		**Differences**
N_0	**N_{75}**	0.019**
	N_{100}	0.026**
N_{75}	**N_0**	–0.019**
	N_{100}	0.006

* $p < 0.05$; ** $p < 0.01$

AMMI analysis was performed for all three nitrogen levels, but the principal component analysis for the N_{75} rate did not show any significant principal component, so in this paper it will be presented only AMMI analysis for N_0 and N_{100} levels of nutrition.

AMMI analysis of variance for N_0 rate showed that both additive sources of variation were highly significant, as well as the year x genotype interaction. Detailed separation of GE interaction variation revealed that explainable agronomic variation had been carried out by the first PC axis in the proportion of 74% of total GE interaction variance (table 2).

Tab. 2. — AMMI analysis of variance for nitrogen harvest index in wheat — N_0

Source of variation	SS	%	DF	MS	F
Treatments	0.319		36	0.0089	11.626**
Replications	0.0008		1	0.0008	1.049
Years	0.221		2	0.111	144.92**
Genotypes	0.063		11	0.0057	7.477**
GxE	0.035	100	22	0.0016	2.058*
PCA 1	0.026	73.96	12	0.0021	2.791**
Residual	0.009	26.04	10	0.0009	1.179
Error	0.027		35	0.0008	
Total	0.346		71		

* $p < 0.05$; ** $p < 0.01$

By analyzing the AMMI1 biplot, it was concluded that most genotypes differed in both the main effect and in interaction. The smallest GxE interaction effect was manifested in the variety Axis, which had a low average value for nitrogen harvest index, as well as in varieties Ilona, Sonata, and Re-

nan with values for the main effect higher than average. Cultivars Zlatko and Pobeda with the highest average values for nitrogen harvest index, also had small interaction effects, i.e. they proved to be stable varieties. The largest interaction effect was recorded in cultivars Nevesinjka and Vanda (Figure 1).

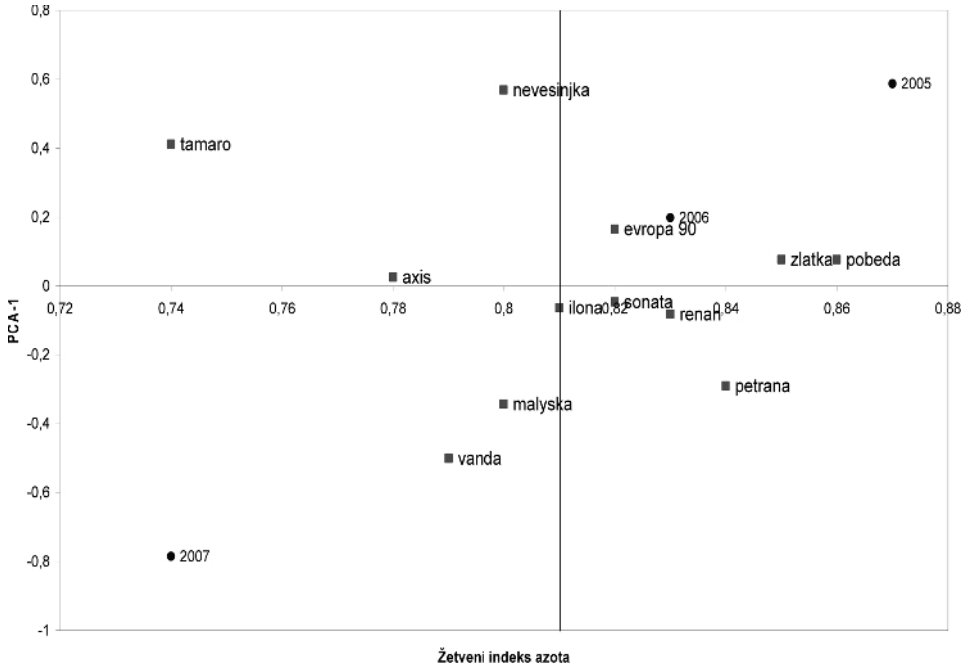

Fig. 1. — AMMI1 biplot for nitrogen harvest index in wheat — N_0

Smallest interaction effect was recorded in year 2006, which is characterized by average values for nitrogen harvest index. A large interaction effect was recorded in year 2005, in which the varieties had the highest mean values for nitrogen harvest index. The largest interaction effect was achieved in year 2007, which is characterized by the lowest values for the main effect, i.e. for nitrogen harvest index (Figure 1).

Cultivars Tamaro, Axis, Nevesinjka, Evropa 90, Zlatko, and Pobeda were in positive interaction with the years 2006 and 2005, while the cultivars Vanda, Malyska, Ilona, Sonata, Renan and Petrana were in positive interaction with the year 2007 (Figure 1).

AMMI analysis of variance for N_{100} rate showed that both additive sources of variation were highly significant, as well as the year x genotype interaction. Detailed separation of GE interaction variation revealed that explainable agronomic variation had been carried out by the first PC axis in the proportion of 80% of total GE interaction variance (Table 3).

Tab. 3. — AMMI analysis of variance for nitrogen harvest index in wheat — N_{100}

Source of variation	SS	%	DF	MS	F
Treatments	0.341		36	0.009	6.840**
Replications	0.0019		1	0.0019	1.374
Years	0.179		2	0.089	64.64**
Genotypes	0.085		11	0.0077	5.552**
GxE	0.075	100	22	0.0034	2.477**
PCA 1	0.060	79.66	12	0.0051	3.618**
Residual	0.015	20.34	10	0.0016	1.109
Error	0.048		35	0.0014	
Total	0.389		71		

* $p < 0.05$; ** $p < 0.01$

By analyzing the biplot, it was concluded that most genotypes differed in both the main effect and in interaction. The highest stability i.e. the smallest effect of the interaction, was found in varieties Malyska, Petrana, Axis and Evropa 90, whereby the first three varieties had a low average nitrogen harvest index, while Europe 90 had a nitrogen harvest index greater than the average. Varieties Renan, Pobeda and Vanda also showed the low interaction effect, with Pobeda and Renan had the highest average values for nitrogen harvest index. The largest interaction effect was achieved by cultivar Zlatko, which had a high average nitrogen harvest index, and cultivar Tamaro, which is the variety with the lowest average nitrogen harvest index (Figure 2).

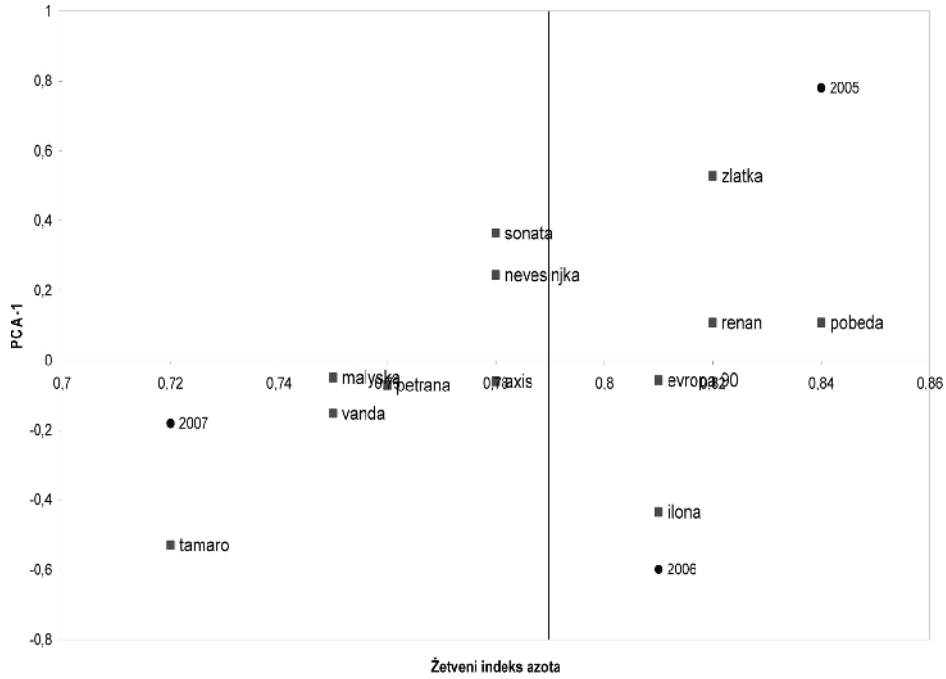

Fig. 2. — AMMI1 biplot for nitrogen harvest index in wheat — N_{100}

The year 2007, in which genotypes achieved the lowest nitrogen harvest index, is characterized by the lowest interaction effect. Year 2006 had high interaction effects, with average values for the main effect, while the year 2005 was characterized by the largest interaction effect and the highest values for nitrogen harvest index. Cultivars Sonata, Nevesinjka, Zlatko, Renan, and Pobeda were in positive interaction with the year 2005, while the varieties Tamaro, Vanda, Malyska, Petrana, Axis, Evropa 90 and Ilona were in positive interaction with the years 2006 and 2007 (Figure 2).

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AMMI АНАЛИЗА ЖЕТВЕНОГ ИНДЕКСА АЗОТА КОД ХЛЕБНЕ ПШЕНИЦЕ

Биљана М. Горјановић, Марија М. Краљевић-Балалић

Пољопривредни факултет, Трг Д. Обрадовића 8, 21000 Нови Сад, Србија

Резиме

Жетвени индекс азота је мера ефикасности транслокације азота из вегетативних органа у зрно. Циљ овог рада је да се испитају варијабилност и стабилност жетвеног индекса азота дванаест генотипова хлебне пшенице, на три нивоа исхране азотом. Анализа варијансе је показала да је жетвени индекс азота у највећој мери био под утицајем интеракције године и генотипа, године испитивања и генотипа, а у најмањој мери примењене дозе азота. Повећање дозе азота није довело до повећања жетвеног индекса азота. AMMI анализа је показала да су се генотипови разликовали како у главном ефекту тако и у GxE интеракцији. Највећа стабилност је забележена код сорти Axis, Ilona, Sonata и Renan на N_0 дози, и код сорти Malyska, Petrana, Axis и Evropa 90 на N_{100} дози. Сорта Pobeda, са високим просечним вредностима за жетвени индекс азота, такође је имала мали ефекат интеракције, тј. показала се као стабилна сорта.

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UDC

Čedomir N. Radenović[1,2], *Milomir R. Filipović*[1]
Mile D. Sečanski[1], *Milica M. Radosavljević*[1]
Zoran F. Čamdžija[1], *Jovan M. Pavlov*[1]
Miloš S. Crevar[1]

[1] Maize Research Institute, Zemun Polje, Slobodana Bajića, 11185 Belgrade—Zemun, Serbia
[2] Faculty of Physical Chemistry, University of Belgrade, Studentski trg 12, 11000 Belgrade, Serbia

SIGNIFICANT BIOPHYSICAL AND GENETIC PROPERTIES OF MAIZE INBRED LINES AND HYBRIDS WITH ERECT TOP LEAVES

ABSTRACT: This study confirms the hypothesis that there are elite maize inbred lines and hybrids with erect top leaves, which have a dominant property of an efficient photosynthetic and fluorescent model that is successfully used in modern breeding programmes and the production hybrid seed and commercial maize. This statement is supported by the displayed results on the erect top leaves, the dynamics of grain dry-down during the maturation period and photosynthetic and florescence parameters: the temperature dependence of the delayed chlorophyll fluorescence intensity, the Arrhenius criterion for the determination of critical temperatures (phase transition temperatures) and the activation energies. The presented results show that properties of observed maize inbreds and their hybrids are based on the nature of conformational and functional changes that occur in their thylakoid membranes and other chemical tissues structures of grain and intact leaves, as well as, on positive effects in maize breeding. Moreover, other relevant significant breeding and seed production properties (commercial maize quality over grain structure, physical and chemical parameters) of maize inbred lines and their hybrids were analysed in the present study.

KEY WORDS: *Zea mays* L., inbred, hybrid, erect top leaf, thylakoid membrane, photosynthetic and fluorescent model, delayed chlorophyll fluorescence

INTRODUCTION

The performance of numerous studies requires a connection of complex and interrelated processes in fundamental, multidisciplinary and applied sciences. The present manuscript presents the results of interrelated studies carried out within breeding, photosynthesis, fluorescence, biophysical chemistry and seed production in maize inbred lines with erect leaves and significant breeding properties.

Maize breeding and seed production have been intensively developed during the last 60 years and because of such activity more than 1300 grain and silage hybrids were derived. Modern equipment and technical and technological prerequisites were provided for carrying out the process of breeding and hybrid maize seed production (Duvick, 1984, Sprague, 1984, Trifunović, 1986, Dumanović, 1986, Hallauer, 1988, Ivanović et al., 1995, Radenović et al., 2000).

Since 1978, the number of plants per area unit (plant density) has been significantly increasing, which mostly affected the increase in grain yields of both, maize hybrids and commercial maize (Radenović et al., 1978, Kojić and Ivanović, 1986, Kojić, 1993). At the same time, a programme on breeding and the seed production of maize hybrids with erect leaves has been performed (Radenović et al., 1978, 2003a, 2003b, 2004a, 2004b, 2007, 2008, Felner et al., 2006). According to our hypothesis, it seems that these observed maize inbred lines with erect leaves are the closest to the assumptive maize photosynthetic and fluorescent model (Radenović and Grodzinskij, 1998).

The studies on maize photosynthesis carried out in the previous period did not have a more important application in breeding and the production of maize hybrid seed. It was almost impossible to present a clear and direct interrelationship among photosynthesis, breeding and the production of maize hybrid seed. On the one hand, such a state is probably a result of the existence of several functional interrelations that unite conformational and dynamic changes within chloroplasts and their thylakoid membranes, but on the other hand, it is a result of effects of numerous environmental factors (Radenović et al., 2000, 2004a, 2004b, 2007).

During the last 35 years, new and important studies within the field of bioluminescence and fluorescence phenomena and processes within the plant systems, including maize, have been carried out. (Govindjee and Papageorgiou, 1971, Barber and Neumann, 1974, Holzapfel and Haug, 1974, Hipkins and Barber 1974, Papageorgiou, 1975, Haveman and Lavorel, 1975, Bukhov et al., 1979, Mccauley and Rubby, 1981, Jurisnić and Govindjee, 1982, Jurisnić, 1986, Marković et al., 1987, 1993, 1996, 1999, Dzhibladze, 1988, Lichtenthaler and Rinderle, 1988, Govindjee et al., 1990, Veselovski and Veselova, 1990, Krause and Weis, 1991, Radenović, 1992, 1994, 1997, 1998, Radenović et al., 1994a, 1994b, Radenović and Jeremić, 1996). The direct dependence of the delayed chlorophyll fluorescence (DF) intensity on changes of photosynthetic processes in thylakoid membranes of maize intact leaves was determined (Radenović, 1994, 1997, Radenović and Jeremić, 1996). Conditions that provided monitoring of complex photosynthetic processes in the maize intact leaf by a photosynthetic and fluorescence model in the form of DF were developed (Radenović et al., 2000, Radenović et al., 2001a, 2001b). During the last 20 years, a group of researchers from the Maize Research Institute, Zemun Polje, have been developing a novel semi-non-invasive photosynthetic-fluore-

scence method that functionally binds processes of photosynthesis, fluorescence and maize breeding (Radenović et al., 2002, Radenović et al., 2003a, Radenović et al., 2004a, 2004b, Marković et al., 1996).

Research methods within the field of biophysical chemistry contributed to diversified connections of studies on photosynthetic and transport processes in the thylakoid membrane and different chemical structures of grain with processes of fluorescence spectroscopy, chemical kinetics and dynamics of grain dry-down in the period of grain maturation (Radenović, 1994, 1998, Radenović et al., 2007, 2008, Rubin et al., 1988).

The objective of the present study was to show that inbred lines with erect top leaves, significant breeding properties and high yielding maize hybrids derived from them, can be an efficient photosynthetic model, meaning that they can contribute to the functional connection of breeding, photosynthesis and florescence, and thereby to the total progress of breeding and the production of hybrid seed and commercial maize of high quality.

MATERIAL AND METHODS

Plant material

The studies were performed with four elite maize inbred lines with erect top leaves, ZPPL 16, ZPPL 218, ZPPL 62 and ZPPL 233, belonging to the collection of the Maize Research Institute, Zemun Polje. Only basic properties of these inbreds are presented.

The inbred line ZPPL 16 was derived from the BSSS population and it belongs to the FAO 700 maturity group. The kernel is classified as a dent type, while the cob is pink. This inbred has been included into the development of more than 20 maize hybrids. Hybrids ZP 578 and ZP 684 have been the most widely grown hybrids in this medium-term period.

The inbred line ZPPL 218 belongs to the Lancaster heterotic group and the FAO 650 maturity group. The kernel is classified as a dent type, while the cob is red. This inbred has been included into the development of over 10 maize hybrids including the hybrid ZP 684.

The inbred line ZPPL 62 was derived from the BSSS population and it belongs to the FAO 350 maturity group. The kernel of this inbred belongs to the dent type and the cob is red. This inbred has been included into over 20 hybrids, including, ZP 341and ZP 434.

The inbred ZPPL 233 belongs to the Lancaster heterotic group and to the FAO 500 maturity group. The kernel of this inbred belongs to the semi-dent/ semi-flint type and the cob is red. This inbred has been included into over 10 hybrids out which the hybrid ZP 578 has been the most widely grown.

Observed maize inbred line with top erect leaves and significant breeding properties are characterised as an efficient photosynthetic model. In addition, these maize inbred lines are characterised by greater grain dry down rates in the maturation period, as well as, by a satisfactory tolerance, resistance, flexi-

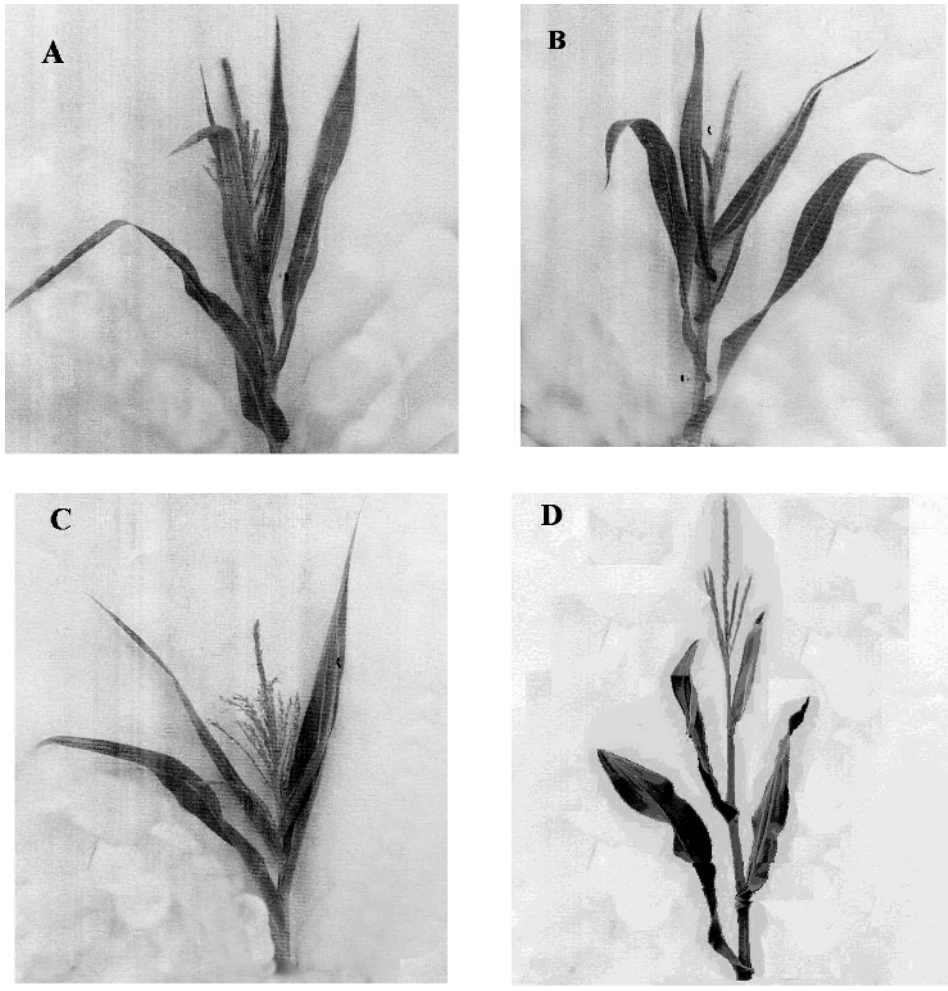

Fig. 1. — Actual appearance of maize inbred lines with top erect leaves and significant breeding properties: ZPPL 16 (A), ZPPL 218 (B), ZPPL 62 (C) and ZPPL 233 (D)

bility and adaptability to high and very high temperatures and drought. The following hybrids were taken into consideration: ZP 341, ZP 434, ZP 578, and ZP 684.

Methods

Overall studies of the stated maize inbred lines with erect top leaves encompassed several series of experiments in which standard and other appropriate methodological procedures were applied.

1. *The measure of an angle and leaf area*

The first series of experiments was related to studying the erect position of top leaves. A specially designed protractor was used to measure the angle between lines of the position of the above-ear leaf and the position of the plant stalk of maize inbred lines. The leaf area was measured using the portable area meter (model LI-3000). Measures of the angle between the above-ear leaf and the stalk and the leaf areas were carried out on 218 plants for each inbred line during the three-year period. These methodical procedures were described in previously published papers (R a d e n o v i ć et al., 2003a, 2004a, 2004b, 2007).

2. *Photosynthetic fluorescence measurements*

The second series of the experiments was related to photosynthetic-fluorescence measurements, including thermal processes of DF, critical phase transition temperatures and activation energies. The test maize inbreds grown in the experimental field of the Maize Research Institute, Zemun Polje, were brought to the laboratory during morning hours (between 7 a.m. and 8 a.m.). Plants sampled in the field were transversally cut in the ground internode. In the laboratory, plants were internode lengthwise placed in water. Prior to the fluorescence experiment, the plants were kept under the black ball glass for two hours. A segment of intact above ear leaves was taken from such plants

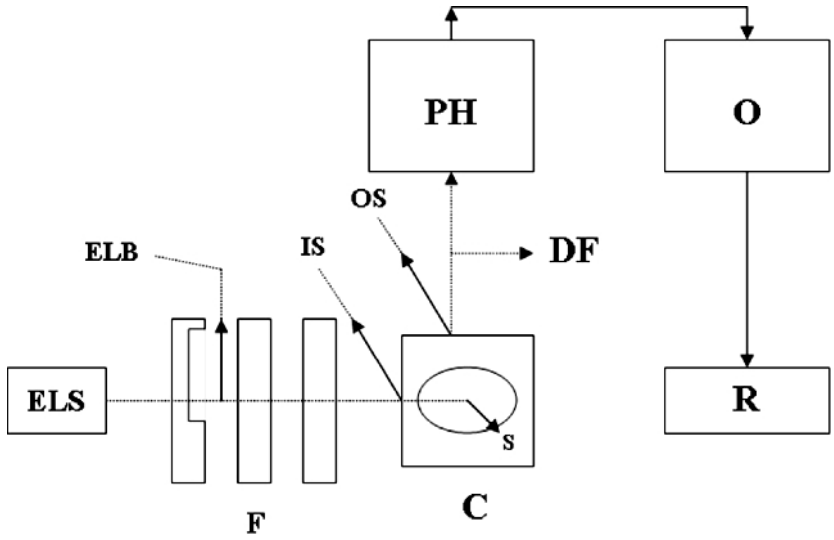

Fig. 2. — Experimental setup of the photosynthetic fluorescence method and the measuring equipment for delayed chlorophyll fluorescence: **ELS** — excitation light source; **F** — filters; **ELB** — excitation light beam, **IS** — input dark chamber slot, **C** — dark chamber with a sample stand; **s** — sample (intact leaf segment), **OS** — output dark chamber slot, **DF** — luminescent light (delayed fluorescence), **PH** — photo-multiplier; **O** — oscilloscope, **R** — printer

and placed into a chamber of the phosphoroscope. The intact leaf segments were kept in the chamber (in the dark) for at least 15 minutes, and then thermal processes of DF were measured. These tests were performed on 268 plants of each inbred line.

The improved non-invasive photosynthetic fluorescence method used to measure DF is schematically presented in Figure 2. This method, developed at the Maize Research Institute, Zemun Polje, has been improved several times. Photosynthetic fluorescence measurements were performed after a method that had been described in the monograph (Marković et al 1996) and our previous papers (Radenović, 1994, 1997, Radenović et al., 2001a, 2001b, 2002, 2004a, 2004b, 2007, 2008).

3. *Dynamics of water status changes in grain*

The third series of the experiments was related to the water status and the dynamics of its grain dry-down rate in the maturation period, which was observed by the application of the thermal method of oven drying at 105°C to the constant weight.

An average kernel sample drawn from five ears was used to perform these measurements. In order to observe the water status, the plants were picked up at the black layer maturity, i.e. at the physiological maturity. Measurements of the grain water status changes were done seven days later and lasted for 35 days. The dynamics of transport processes (grain dry down) during the grain maturation was observed in the course of five years. The long-term measurements were done because of a great instability of this trait in the majority of maize inbred lines (Radenović et al., 2008, 2009).

4. *Grain chemical composition, physical properties and structure of maize hybrids with erect top leaves*

Applied standard methods for the determination of the chemical composition, physical properties and grain structure of maize hybrids with erect top leaves were fully described in previous papers (Bekrić, 1997, Radosavljević et al., 2000, 2002).

RESULTS

1. *The measure of the angle and the area of the above-ear leaf*

Results on the measures of angles between the above-ear leaf and the stalk, as well as, the average leaf areas are presented in Table 1. Based on obtained results on the measures of angles it can be stated that the observed maize inbred lines with significant breeding properties belong to the group of inbred lines with erect top leaves.

Tab. 1. — The angle of the above-ear leaf and the leaf area of maize elite inbred lines with erect top leaves

Inbred line*	FAO maturity group	Heterotic origin of the inbred*	Angle of the above-ear leaf in degrees		Area of the above-ear leaf (x 10^3 cm^2)	
			$\bar{x}$	σ	$\bar{x}$	Σ
ZPPL 16	700	Zemun Polje — BSSS	18.3	1.12	3.63	328
ZPPL 218	650	Zemun Polje — Lancaster	22.1	1.36	3.91	412
ZPPL 62	350	Zemun Polje — BSSS	20.3	1.21	3.33	318
ZPPL 233	500	Zemun Polje — Lancaster	24.5	1.34	5.66	613

* Studied maize inbred lines represent good heterotic pairs, they are characterised as good general combiners for grain yield, they increase well and they are high yielding.

2. *Empirical procedure for photosynthetic fluorescence studies on the above-ear leaf*

The detailed studies on thermal processes of DF of observed maize inbred lines with erect top leaves were performed. The thermal curve is a curve that shows the dynamics of changes in the stationary DF level intensity in depen-

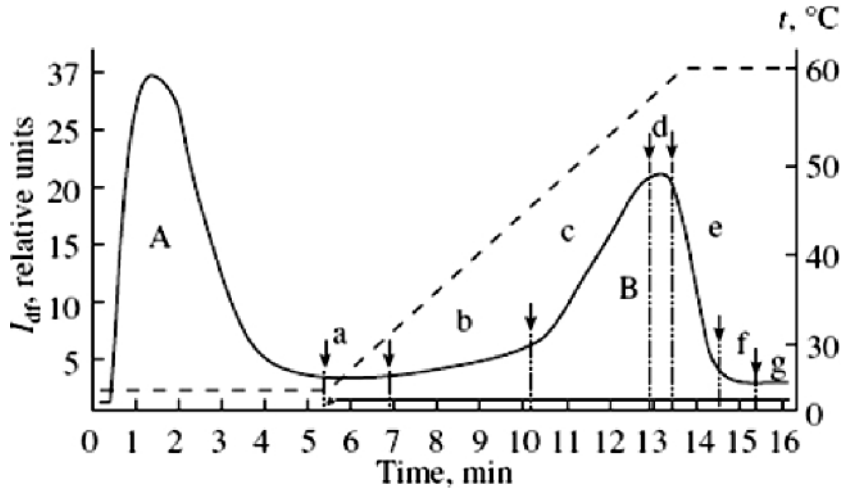

Fig. 3. — Schematic presentation of the empirical procedure for typical changes in DF intensities ($\mathbf{I_{df}}$) on the intact above-ear leaf of the observed maize inbred lines with significant breeding properties (solid line) and changes in temperatures (dashed line): curve **A** indicates induction processes of DF, while curve **B** encompasses photosynthetic fluorescence thermal processes of DF. Typical temporal segments (**a**, **b**, **c**, **d**, **e**, **f** and **g**) on the thermal curve **B** correspond to dynamics of $\mathbf{I_{df}}$ changes at the time of a DF formation. Conformational and functional changes in the thylakoid membrane of observed maize inbred lines with erect top leaves occur at interception points of typical temporal segments

dence on a temperature. The trend of its establishment is usually analogous to changes in the duration in seconds of segments marked with **a**, **b**, **c**, **d**, **e**, **f** and **g** (Figure 3), which was determined by the empirical procedure (Radenović et al., 2008, 2009)

Monitoring the course of the thermal curve and the analysis of the duration of certain segments provided data on the existence of a greater number of critical temperatures (phase transition temperatures) at which greater or smaller structural and functional changes occurred in the thylakoid membrane of observed maize inbred lines with erect top leaves.

3. *The temperature dependence of the delayed chlorophyll fluorescence intensity for the thylakoid membrane of the maize inbred lines with erect top leaves*

Figure 4 A, B, C and D, presents changes in the stationary DF level in a function of the temperature, ranging from 25 to 60°C, in the thylakoid membrane of the maize inbred lines with erect top leaves: ZPPL 16, ZPPL 218, ZPPL 62 and ZPPL 233. The temperature dependence of observed inbreds varies over dynamics of increasing and decreasing occurrence, as well as, over the temperature peaks.

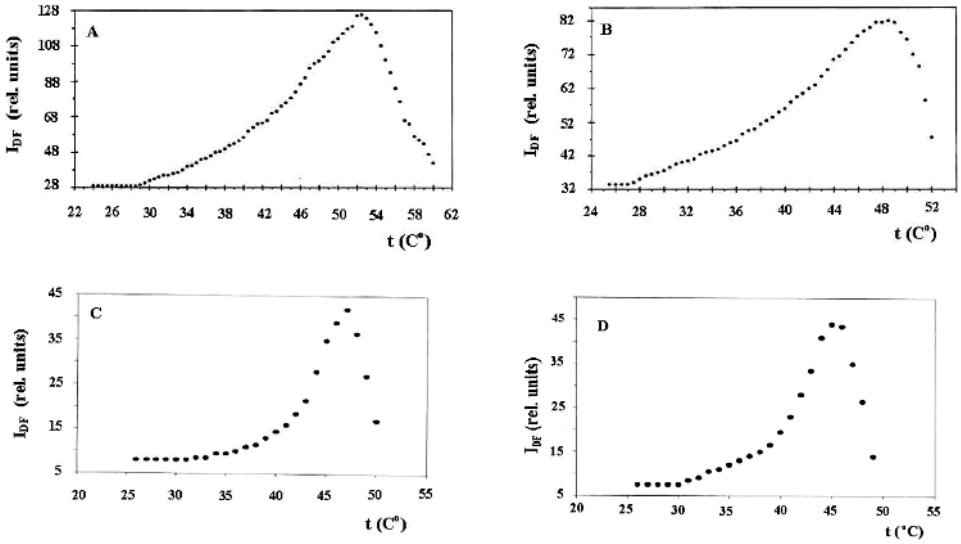

Fig. 4. — A, B, C and D Changes in the intensity of the delayed chlorophyll fluorescence (I_{DF}) of thermal processes in dependence on the effects of temperatures in the thylakoid membrane of the intact above-ear leaf of the maize inbred lines with significant breeding properties and erect top leaves: ZPPL 16(A), ZPPL 218(B), ZPPL 62(C) and ZPPL 233(D)

4. *The Arrhenius plot for the determination of critical temperatures and conformational changes in the thylakoid membrane of the maize inbred lines with erect top leaves*

The Arrhenius plot is based on the linearisation of the DF temperature dependence of observed maize inbred lines (Figure 5, A, B, C and D). Critical temperatures (phase transition temperatures) at which conformational changes occur in the thylakoid membrane are determined by the application of the Arrhenius plot. Results of the Arrhenius plot application to maize inbreds with significant breeding properties and erect top leaves are presented in Figure 5 A, B C and D. The Arrhenius plot shows the dynamics and spots of critical temperature occurrences of studied inbreds.

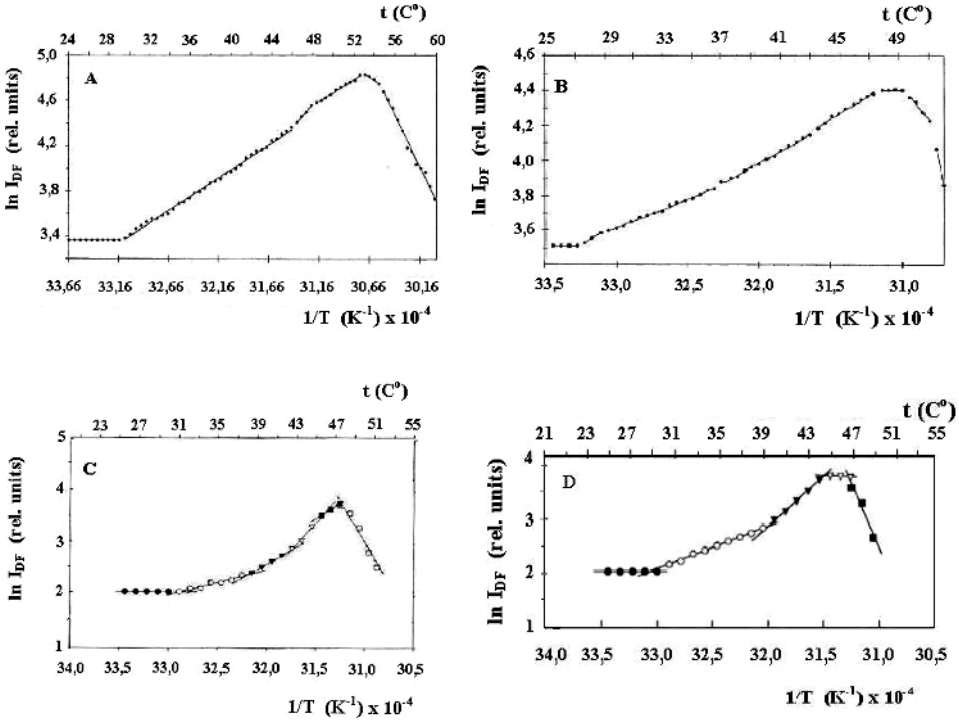

Fig. 5. — A, B, C and D The Arrhenius plot for the determination of critical temperatures and conformational changes in the thylakoid membrane of the above-ear leaf of maize inbred lines with significant breeding properties and erect top leaves: ZPPL 16(A), ZPPL 218(B), ZPPL 62(C) Z and ZPPL 233(D)

5. *Activation energy and critical temperatures in the thylakoid membrane of the observed maize inbred lines with erect top leaves*

Detailed studies on the thermal processes of DF, and especially on the analysis of thermal curve, encompassed not only the temperature dependence and the Arrhenius plot, but also the estimation of values of activation energies for critical temperatures (phase transition temperatures) in the thylakoid membranes of the observed maize inbreds with significant breeding properties and erect top leaves: ZPPL 16, ZPPL 218, ZPPL 62 and ZPPL 233. Obtained results are shown in Table II.

Tab. 2. — Changes in activation energies (Ea) and critical temperatures (t °C) in the course of thermal processes in the thylakoid membrane of the intact above-ear leaf of studied maize inbred lines with erect top leaves

ZPPL 16		ZPPL 218		ZPPL 62		ZPPL 233	
Ea, kJ/mol	t, °C	Ea, kJ/mol	t, °C	Ea, kJ/mol	t, °C	Ea, kJ/mol	t, °C
—	29.5	—	27.0	—	28.0	—	25
48.4	45.9	43.1	29.0	45.0	36.0	32	30
84.3	48.0	27.3	36.9	91.8	41.0	100.3	38
46.7	53.0	37.0	43.5	119.7	46.9	176.7	42
49.2	54.8	42.5	47.8	132.0	49.0	259.9	47
—	60.0	51.1	49.9	—	—	—	50

6. *Dynamics of changes in the grain water status in the maturation period of the studied maize inbred lines with erect top leaves*

Dynamics of changes in the grain water status and dry down during the maturation period of the studied maize inbred lines with erect top leaves are important properties of these inbreds to which a great attention is paid in the processes of contemporary breeding and the production of high-quality hybrid maize seed. Obtained results are presented in Table III.

Tab. 3. — Water content (%) in initial and subsequent measurements

Inbred lines	I initial measure-ment	II measure-ment after 7 days	III measure-ment after 14 days	IV measure-ment after 21 days	V measure-ment after 28 days	VI measure-ment after 35 days	Daily dry down
ZPPL 16	31.40±3.22	28.11±3.11	24.82±3.05	21.53±2.98	18.24±2.81	14.95±2.41	0.47±0.06
ZPPL 218	29.44±3.06	26.29±2.91	23.14±2.77	19.99±2.51	16.84±2.31	14.20±1.76	0.45±0.08
ZPPL 62	28.09±3.28	25.29±3.09	22.49±2.88	19.69±2.56	16.89±2.04	14.09±1.94	0.40±0.07
ZPPL 233	27.44±3.80	24.36±3.51	21.28±3.33	18.20±3.07	15.12±2.81	12.04±2.24	0.44±0.09

7. *Chemical composition, physical properties and grain structure of maize hybrids with erect top leaves*

Results on studies of grain structure, physical properties and chemical composition of maize hybrids with erect top leaves are presented in Tables IV, V and VI.

Tab. 4. — Grain structure of maize hybrids with erect top leaves

Hybrid	Pericarp (%)	Germ (%)	Endosperm (%)
ZP 341	6.49	12.05	81.46
ZP 434	6.49	11.86	81.65
ZP 578	6.08	11.35	82.56
ZP 684	6.39	11.79	81.82

Tab. 5. — Physical properties of the grain of maize hybrids with erect top leaves*

Hybrid	TKW	TW	D	FI	MR	HEF	SEF	WAI
ZP 341	331.4	784	1.25	33.7	19.9	57.3	42.7	0.231
ZP 434	355.1	780	1.26	22.3	16.6	58.8	41.2	0.225
ZP 578	304.8	751	1.27	30.2	16.9	61.9	38.2	0.242
ZP 684	344.8	772	1.26	18.9	13.7	54.5	45.5	0.246

* TKW = 1000-kernel weight (g), TW = test weight (kg m^{-3}), D — density (g cm^{-3}), FI — floatation index (%), MR — milling response (s), HEF — hard endosperm fraction, (%), SEF — soft endosperm fraction (%), WAI — water absorption index

Tab. 6. — Grain chemical composition of maize hybrids with erect top leaves

Hybrid	Starch (%)	Protein (%)	Oil (%)	Fibre (%)	Ash (%)
ZP 341	69.00	9.33	5.75	1.98	1.33
ZP 434	69.02	9.42	5.87	1.99	1.37
ZP 578	72.99	8.64	5.08	1.82	1.33
ZP 684	70.52	8.84	4.82	2.06	1.37

DISCUSSION

The second half of the 20th and the first decade of the 21st century are characterised by a great success achieved in maize breeding and the production of fundamental and hybrid maize seed. The number of plants per area unit has been growing since 1978. This programme was referred to as a "plant density" programme and it further directly affected the yield increase of high quality fundamental and hybrid maize seed (R a d e n o v i ć et al., 1978). In addition, a programme on the development of maize inbred lines with erect top leaves was established at the same time as the "plant density" programme. It was considered that inbreds with the erect top leaves were the closest to the proposed efficient photosynthetic model (R a d e n o v i ć et al., 1978, 2000, 2001a, 2003a, 2004a, R a d e n o v i ć and G r o d z i n s k i j, 1998). The complementary and mass implementation of these programmes led to very important re-

sults in both, maize breeding and the hybrid seed production (Ivanović et al., 1995, Trifunović, 1986, Trifunović et al., 2000, Dumanović, 1986, Kojič, 1993). New and numerous hybrids with high grain and silage yields were developed and grown on large areas due to their high yielding potential and the appropriate quality of the plant and the grain (Duvick, 1984, Russell, 1986, Dumanović, 1986, Hallauer, 1988, Kojić, 1993, Ivanović et al., 1995).

The special contemporary studies have been performed on top maize leaves. The ear leaves have been particularly observed, but also other top leaves up to the tassel. The most efficient and the longest photosynthetic processes necessary for the maize plant have been achieved by these leaves (Radenović and Grodzinskij, 1998). According to the stated, a new hypothesis that precisely top leaves (above-ear leaves) achieve the efficient photosynthesis has been proposed.

This study was an attempt to answer the following questions by using different tests and analyses: (1) are there reliable and dominant traits of maize inbred lines with erect top leaves by which planned and satisfactory progress in maize breeding and the high-quality hybrid seed maize production can be achieved?, and (2) which traits should maize inbred lines have?

The gained results of experimental studies can offer at least a partial answer to asked questions. The first series of experiments included the measure of the angle and the leaf area of observed maize inbred lines with significant breeding properties and erect top leaves. The results obtained on these traits classify them into important seed breeding and seed production traits (Radenović et al., 2003a, 2004a, 2004b, 2007, 2008). The second series of experiments encompassed photosynthetic fluorescence studies on conformational and functional changes in the thylakoid membrane of the intact above-ear leaf of studied maize inbred lines with significant breeding traits. The temperature dependence of thermal processes of DF for the studied maize inbred lines is presented in a form of the empirical procedure (Figure 3). The presented results show that the temperature dependence of DF in each of the four maize inbred lines with erect top leaves is characterised with typical intersection points of two segments on the thermal curve (Figures 3 and 4). The first typical point occurred on the intersection of the segment **a** and the segment **b**, and it represented the lowest critical temperature at which the initial change in the DF intensity was observed. The second typical point occurred on the intersection of the segment **b** and the segment **c** and it was related to a linear monotony with the angle of the increasing part of the DF intensity curve. Evident changes in the structure of the thylakoid membrane occurred in this region. The third typical point reflected a smaller or a greater rotundity of DF intensity peaks. The "breaking" conformational changes occurred in two intersection points of the segments **c** and **d** and the segments **d** and **e**. The fourth typical point was related to the linear monotony and the inclination angle of the declining part of the DF intensity curve. This segment of the thermal curve bore the last conformational changes that had occurred in the thylakoid membrane. These changes can hardly be described as characters of functioning of a living leaf. The typical intersection points designated as **f** and **g** almost had no

physiological role. The analysed typical intersection points (Figure 3 and Figure 4 A, B, C and D) can be considered the points characterising inbred lines with erect top leaves, as these points are precisely the points of conformational and functional changes in the thylakoid (R a d e n o v i ć et al., 2003a, 2003b, 2004a, 2004b, 2007).

All critical temperatures (phase transition temperatures) at which even the slightest conformational changes had occurred in the thylakoid membranes of studied maize inbred lines with erect top leaves were determined by the Arrhenius criterion and the linearisation of the DF temperature dependence. The values of critical temperatures (°C), their frequency and intermediate distance characterise observed maize inbred lines with erect top leaves in relation to their tolerance, resistance, flexibility and adaptability not only to increased and high temperatures, but also to drought (R a d e n o v i ć et al., 2001a, 2001b, 2002, 2003a). The Arrhenius criterion is based on the existence of straight lines. Each Arrhenius straight line represents its activation energy (Ea). The intersection point of two straight lines is determined by a critical temperature. Results of the Ea values in the inclining and declining part of the thermal curve are explained by lesser or greater conformational changes that occur in the molecules of pigments (chlorophyll) in the thylakoid membrane with the temperature increase. Due to such changes, these molecules become more reactive and thereby gain the additional energy that is used in the recombining process of the DF occurrence (R a d e n o v i ć, 1994, 1997, R a d e n o v i ć et al., 2003a, 2003b, 2004a, 2004b).

Presented photosynthetic fluorescence traits of studied maize inbred lines with erect top leaves can contribute to more exact, rational and expeditious proceedings of breeding processes and the production of high-quality hybrid maize seed, which makes them exceptionally important.

The third series of experiments encompassed the thermal studies of the specific grain water status and grain dry down rates in the maturation period. Transport processes and dry down rates at grain maturation are very important and significant properties to which a great economic and scientific importance is ascribed, not only in the process of studying and the development of maize inbreds and hybrids with erect top leaves, but also in the organisation of the hybrid maize seed production (R a d e n o v i ć, 1998, R a d e n o v i ć et al., 2008, 2009). The grain dry down rate in the maturation period is a very complex process and depends on the following several parameters: a) the osmotic pressure in the grain in the maturation period, which is prone to the external atmospheric pressure, as well as, to frequency and intensity of air currents and significant changes in relative air humidity; furthermore, the osmotic pressure in the grain depends on the structural properties of chemical compounds and the nature of their chemical bonds with water; b) the pericarp structure and thickness and its water permeability, that is water transport capacity through such a structure; c) the content and structure of starch grains and protein bodies, including their affinity to bind water; d) morphological properties of the ear; e) morphological properties of the grain; and f) other physical and chemical parameters of a chemical structure of the grain, which interact with water.

Gained results presented in Tables IV, V and VI, point out to structural grain parameters (pericarp, germ and endosperm), physical traits and chemical composition that indicate exceptional quality of commercial maize of observed hybrids with erect top leaves. Commercial maize is of appropriate quality that provides diversified utilisation (Bekrić, 1997, Radosavljević et al., 2000, 2002).

CONCLUSION

According to the gained results it can be established that semi-non-invasive photosynthetic fluorescence method can be applied in breeding and the maize hybrid seed production and that the estimation of maize inbred lines with significant breeding traits for their tolerance, resistance, flexibility and adaptability to increased and high temperatures, as well as, to drought, can be performed. The application of the stated method provided the determination of many properties and parameters of the photosynthetic apparatus of observed maize inbred lines with significant breeding properties and erect top leaves:

— The temperature dependence was monitored within the range of 24—60°C,

— Different monotonies of the increasing part of the thermal curve, which points out to uneven tolerance, resistance, flexibility and adaptability of observed maize inbred lines to increased and high temperatures, as well as, to drought, were established,

— Values of critical temperatures at which smaller or greater structural and functional changes occurred in the thylakoid membranes of observed maize inbred lines with erect top leaves were determined,

— Values of activation energies (Ea, kJ mol^{-1}) were determined before and after the occurrence of critical temperatures in the thermal process of DF,

— It was shown that observed inbred lines had a trait of the erect position of top leaves and efficient photosynthesis model,

— A greater dry down rate in the grain maturation period was estimated and analysed in maize inbred lines with significant breeding properties and erect top leaves,

— Relevant physical and chemical traits of maize hybrids developed from the stated inbred lines with erect top leaves were analysed; these analyses point out to good quality of commercial maize and its diversified utilisation.

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БИОФИЗИЧКА И ГЕНЕТИЧКА СВОЈСТВА ПРЕСТИЖНИХ ИНБРЕД ЛИНИЈА И ХИБРИДА КУКУРУЗА СА УСПРАВНИМ ПОЛОЖАЈЕМ ВРШНИХ ЛИСТОВА

Чедомир Н. Раденовић[1,2], Миломир Р. Филиповић[1], Миле Д. Сечански[1], Милица М. Радосављевић[1], Зоран Ф. Чамџија[1], Јован М. Павлов[1], Милош С. Цревар[1]

[1] Институт за кукуруз „Земун Поље", Слободана Бајића 1, 11185 Београд—Земун, Србија
[2] Факултет за физичку хемију, Универзитет у Београду, Студентски трг 12, 11000 Београд, Србија

Резиме

Проучаване су четири престижне инбред линије кукуруза са усправним положајем вршних листова: ЗППЛ 16, ЗППЛ 218, ЗППЛ 62 и ЗППЛ 233. Ове линије су, као мајка или отац, укључене у стварање више од 50 хибрида кукуруза. У

текућем средњерочном периоду широку комерцијалну примену нашао је већи број хибрида кукуруза: ЗП 341, ЗП 360, ЗП 434, ЗП 578, ЗП 606, ЗП 677, ЗП 684 и други. У овом раду анализирани су само репрезентативни хибриди кукуруза: ЗП 341 (ФАО 300), ЗП 434 (ФАО 400), ЗП 578 (ФАО 500) и ЗП 684 (ФАО 600).

Овим радом потврђује се наша хипотеза да постоје елитне инбред линије и хибриди кукуруза са усправним положајем вршних листова, који поседују доминантно својство ефикасног фотосинтетично-флуоресцентног модела и успешно се користе у савременим програмима оплемењивања, производњи хибридног семена и меркантилног кукуруза. Овај закључак добијен је уз примену неинвазивног фотосинтетично-флуоресцентног метода погодног за оцену ефикасности фотомодела. Добијене фотосинтетичне карактеристике проучаваних престижних инбред линија кукуруза са усправним положајем вршних листова засноване су на ефектима и природи промена закаснеле флуоресценције хлорофила које се одигравају у њиховим тилакоидним мембранама, чији су показатељи температурна зависност интензитета закаснеле флуоресценције хлорофила, Аренијусов критеријум за утврђивање критичних температура (температуре фазних прелаза) у тилакоидним мембранама и енергије активације. Изложени резултати о величини угла између правца простирања првог листа изнад клипа и правца простирања стабљике, као и резултати о динамици ослобађања воде из зрна у периоду његовог сазревања додатно показују да су својства проучаваних инбред линија кукуруза са усправним положајем вршних листова погодни критеријуми за егзактнији, рационалнији и бржи процес оплемењивања.

Такође су анализирана и друга релевантна селекциона и семенарска својства проучаваних престижних инбред линија и хибрида кукуруза (квалитет меркантилног кукуруза преко структуре зрна, физичких и хемијских параметара).

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UDC

Ana T. Selamovska
Olga V. Najdenovska

The Faculty of Agricultural Sciences and Food
Skopje, Republic of Macedonia

THE INFLUENCE OF THE RUNNER ORDERING OF THE PLANTS ON THEIR QUALITY, ROOTING AND YIELD OF STRAWBERRIES

ABSTRACT: In this work, we presented the influence of the runner ordering of the plants on their quality, percentage of root hair formation, and yield of two varieties of strawberries: Senga Sengana and Pocahontas depending on the time of planting. The runner ordering of plants and time of their planting have influence on the quality of plants, the percentage of root hair formation after the planting, and the yield. There has been established strong correlation among the diameter of the crown and the rooting when planted and the yield. Primary plants have higher quality, with bigger diameter of the crown, they take root better, and they have higher yield than secondary plants. Rosettes planted in August have higher yield than the ones planted later.

KEY WORDS: primary, secondary, plants, quality, root hair formation, yield

INTRODUCTION

Depending on the method of production and the storing of the planting material, and the development of the plants, they can be classified in several categories: green, frigo, waiting bed and container. No matter the way the planting material is obtained, it needs to be of good quality, with developed root, with variety, and healthy. High quality planting material is prerequisite for obtaining high yield and high quality fruits. When evaluating the quality of the plants, besides the development of the root system, the thickness of the crown is taken into account. Plants of first and second class have been used most frequently in the production. The number of obtained high quality plants, by the plant and by the square unit is a characteristic of the cultivar (Bulatović, 1970), depending on the cultivation method (Mićić et. al., 1983, Ristevski et. al. 1986, Selamovska, 2006, Mratinić, 2000), the cultivar and runner ordering of the plants (Lootchoomun, 1999, Selamovska, 2006), the age of the plant (Popov et. al., 1963, Ristevski

et. al., 1986, Mićić et. al., 1983), the time of the planting (Ozdemir et. al., 2002, Ristevski et. al., 1986), the used agrotechniques (Paunović et. al., 1974, Mićić et. al., 2003,), the protection (Ristevski et. al., 1986), etc.

For the production of plants, original plants should be used only. A small number of plants is obtained from the production plant, with poorly developed root system, smaller vegetative potential, and poor health condition. The plants with the highest quality are obtained in the first year after the planting. The first class plants have the highest quality. During ageing of the plant, its vegetative potential reduces, as well as, the quality and quantity of obtained plants.

The yield is influenced by several factors: genotype, time of planting, cultivation method, age of the plant, health condition of the plant, etc. Due to its biological characteristics, strawberry can be planted during whole year, but if speaking of high intensity production, the time of planting, on which depends the rooting and later profitability of growing strawberries, needs to be considered. When growing strawberries on an open field, the most appropriate time for planting should be determined having in mind that the plant needs to be as much developed as possible before the beginning of the winter, in order to obtain higher yield next year. If the strawberries are planted earlier, in July and August they obtain 30% higher yield. In September and October, the yield consequently decreases. The plants planted in July and in the first half of August, in the next year they provide five to ten times higher yield compared to those plants that are planted later, in the autumn or spring. When planted in the spring the yield could be left out (Hristov et al., 1967, Šoškić, 1998, Mratinić, 2000, Tešić, 1970, Stančević et al., 1986, Ozdemir et al., 2002, Pasini et al., 1982).

MATERIAL AND METHODS

The examinations were done on the experimental strawberry plantation, located in Dolno Lisiče village near Skopje, Republic of Macedonia. The planted soil was fluvisol, with maize as a previously planted culture, low carbonate, easy to cultivate, moderately to well supplied with phosphor and potassium, but considering that it contained low content of humus and it was moderately alkaline, it was necessary to do some corrections.

To examine the influence of the runner ordering of the plants on their quality, root formation, and the yield depending on the time of planting, green plants of first and second class of two strawberry cultivars Senga Sengana and Pocahontas were planted in different time periods: every 15 days from August 15 to November 15 and in March next year. Strawberry cultivars were planted in black foil, distanced at 80 x 25 cm.

The diameter of the neck of the plant, the rooting, and the yield, in years of 2001 and 2002, were examined. For every period of plating there has been determined an average value for the required parameters.

Regarding the climate indexes (data obtained from Hydro Meteorological Institute — Skopje, measuring station Petrovec) in region of Skopje, Republic

of Macedonia, the average values of air temperature during the vegetation are favorable for growing strawberries except for the high temperatures during the summer, especially in August, which have negative impact on rooting of the plants. All the values of hydrothermal indexes indicate that the climate is semiarid. The relative air humidity is low and unfavorable for growth of strawberries. The annual and vegetative sum of rainfalls is insufficient for development and fruitfulness of the strawberries in this region and because of that, it is necessary the strawberries to be cultivated in irrigated conditions. The results of the examination have been statistically processed, using correlative analysis, analysis of the variance, and t — test.

RESULTS AND DISCUSSION

Data on influence of the runner ordering of the plants on the diameter of the crown, the rooting, and the yield, in different periods of the planting, are presented in Table 1.

Tab. 1. — The influence of runner ordering of the plants on diameter of crown, rooting percent and yield

Date of planting	Primary rosettes				Secondary rosettes			
	Crown diameter mm	Rooting %	Yield g/plant	Yield kg/ha	Crown diameter mm	Rooting %	Yield g/plant	Yield kg/ha
15.08	9.0	55.6	693.1	19268	7.6	55.9	591.0	16518
30.08	9.7	65.0	575.8	18694	8.1	56.2	503.0	14135
15.09	11.3	56.2	567.2	15939	8.9	41.2	410.5	8456
30.09	12.4	88.7	529.9	23501	10.2	67.2	468.6	15745
15.10	12.7	95.0	425.0	20187	10.3	78.7	486.6	19148
30.10	12.6	83.7	375.9	15731	10.6	60.0	376.0	11280
15.11	12.6	90.0	447.6	20141	11.1	91.2	397.4	18121
15.03	13.1	92.5	381.6	17649	11.0	90.0	364.3	16393
X	11.8	79.0	498.9	19706	9.7	69.6	449.6	15645
r	0.903	0.821	0.903		0.971	0.707	0.712	
LSD 0.05	2.29	24.10			1.89	23.89		
LSD 0.01	3.11	32.67			2.56	32.73		

We made comparison between primary and secondary plants related to these parameters. According to the data, the runner ordering of the plants had impact on their quality, the root formation after the planting, and their yield. Plants of the first class had higher quality, they had 17.8% bigger diameter of the crown, 12% better rooting, and 10% higher yield, more exactly 21% higher yield by square unit compared to the plants of second class, which is supported by *considerable statistical differences* (Table 2).

Tab. 2. — Comparison between primary and secondary rosettes

Parameters	Primary rosettes	Secondary rosettes	Difference between primary and secondary rosettes %	t
Diameter of the crown mm	11.8	9.7	+17.8	1.3 +
Rooting %	79.0	69.6	+12.0	1.2 +
Yield/plant g	498.9	449.6	+10.0	1.03 +
Yield kg/ha	19.706	15.645	+21.0	1.13 +

We determined positive correlation between the diameter of the crown and the root development and the yield (plants of the first class r = 0.872, plants of the second class r = 0.752). Plants with bigger diameter of the crown had better rooting when planted, and they had higher yield. In the same period of planting, primary plants had higher values for the diameter of the crown, the rooting development, and the yield, than secondary plants. Because of the earlier organogenesis primary plants were more developed, they started to form earlier, after the planting they adapted quicker and better, and they had better rooting (S e l a m o v s k a, 2006, 2007). Related to this, when producing the planting material it is important to leave 1 to 3 plants on a plant, and in that way a smaller quantity of plants will be obtained, but with higher quality and with bigger diameter of the crown (M i ć i ć et al., 2000).

Besides the runner ordering of the plants, the diameter of the crown and root development was also influenced by the time of planting, on which there are correlative values. We determined considerable statistical differences in the diameter of the crown, rooting, and yield, between earlier and later planted plants (Table 1). Plants planted in August had smaller diameter of the crown and poorer rooting, but they had higher yield, compared to later planted plants.

Primary and secondary plants, planted on the 15th of August, in average had 17.5% smaller diameter of the crown, than plants planted on the 15th of September, 27.5% than rosettes planted on the 15th of October and 31.0% than plants planted on the 15th of March (Table 3).

Tab. 3. — Comparative values of the parameters in different planting periods

Parameters	Difference %								
	15.08/15.09			15.08/15.10			15.08/15.03		
	I	II	X	I	II	X	I	II	X
Crown diameter	–20.0	–15.0	–17.5	–29.1	–26.0	–27.5	–31.0	–31.0	–31.0
Rooting	–1.0	+26.0	+12.5	–41.0	–29.0	–35.0	–40.0	–38.0	–39.0
Yield/plant	+18.0	+31.0	+24.5	+39.0	+18.0	+28.5	+45.0	+38.0	+41.5
Yield kg/ha	+17.0	+49.0	+33.0	–5.0	–14.0	–9.5	+8.0	+1.0	+4.5

Regarding the rooting, plants planted on the 15th of August had 12.5% better rooting, than plants planted on the 15th of September, but 35% — 39% poorer rooting than plants planted on the 15th of October and 15th of March. Regarding the yield, plants planted on the 15th of August have 24.5%, 28.5% to 41.5% higher yield than the plants planted on the 15th of September, 15th of August, and 15th of March. In every period of planting, separately, primary

plants had higher values of every index compared to those of the secondary plants.

Because of the higher yield of the plants planted in summer months, higher values of the yield by square unit were expected in those months. However, it did not happen. Plants planted on the 15th of August had lower yield by square unit compared to later planted plants, from October to March. The reason for the poor yield was the extremely high air temperature. In August and September 2001, in the time of planting and after planting, there were measured extremely high air temperatures, more exactly absolute maximums of 38.7°C in August, and 32.8°C in September. In the year 2002, high midday air temperatures were measured in September with absolute maximum of 27.9°C. Extremely high air temperatures in the summer months, have caused dehydration of the plants, which had negative effect on the rooting, which have been reduced to 50%. In addition, poor rooting of the plants in August had negative effect on the total yield by square unit.

CONCLUSION

Based on the performed experiments we conclude:

— The runner ordering of the plants has influence on their quality, rooting after plating, and yield.

— Primary plants have higher quality, bigger diameter of the crown, they form better root, and provide higher yield than secondary plants.

— The yield of the strawberries depends on the quality and runner ordering of the plants, the rooting after planting, and the time of planting. To obtain higher yield they should be planted in summer, but irrigation is required.

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УТИЦАЈ РЕДОСЛЕДА РОЗЕТА НА КВАЛИТЕТ, УКОРЕЊАВАЊЕ И ПРИНОС ЈАГОДЕ

Олга В. Најденовска, Ана Т. Селимовска

Факултет за земјоделски науки и храна, Скопје, Р. Македонија

Резиме

У раду је приказан утицај редоследа розета на квалитет, проценат формирања изданака и принос две сорте јагоде: зенга зенгана и покахонтас у зависности од времена садње. Редослед розета и време садње утиче на квалитет розете, проценат укорењавања после садње и на принос. Утврђена јака корелација између пречника кореновог врата, и укорењавања након садње и приноса. Примарне розете имају висок квалитет, са већим пречником кореновог врата, боље се укорењавају и имају већи принос него секундарне розете. Розете засађене у августу имају већи принос у односу на розете које се саде касније.

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Milan N. Mataviulj[1], *Milan L. Martinov*[2]
Maja A. Karaman[1], *Branislav V. Veselinov*[2]
Dušan S. Adamović[3]

[1] Faculty of Natural Sciences, Trg D. Obradovića 2, Novi Sad, Serbia
milan.matavuljŽdbe.uns.ac.rs
[2] Faculty of Technical Sciences, Trg Dositeja Obradovića 6, Novi Sad, Serbia
[3] Institute for Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia

MICROBIOLOGICAL INVESTIGATION OF PEPPERMINT AND PUMPKIN SEED KERNELS DRYING IN BATCH DRYER

ABSTRACT: The goal of this research was to investigate improvement of drying method of peppermint and pumpkin kernels (hull-less pumpkin seed) with the aim to get as best microbiological quality of products as possible. Small and medium scale producers of medicinal plants in most cases perform it in batch dryers, which offer optimal output rate. Disadvantages of this dryer type are slower through-heating of upper layers, with the resulting increase of microbial count. In this paper, results of investigation of drying characteristics in batch dryer expressed through the changes of count of bacteria and microfungi are presented. The drying regime was applied in three different phases, and different material batch heights.

Obtained results show that microbial count has increased in the upper layer during process of peppermint drying, while the temperature was less than 45°C for a long period. However, during the final drying phase in this layer, the temperature was also over 45°C, and the final microbial count was almost the same for upper and lower levels. During the treatment of pumpkin kernels in all of cases number of microorganisms indicated that the treated pumpkin grains belonged to the 3B qualitative group or to the 4B for phase drying respectively, and to the 4A for control group according to the Europaean Pharmacopoeia. The results obtained suggest that the treatment of hull-less pumpkin seed with even higher temperatures of drying agents, for example 70°C or 80°C, in the first phase should be investigated. After that phase, the second phase would follow, using temperature of 60°C, and the method with shifting mode should be applied.

KEY WORDS: Drying, microbiological quality, peppermint, pumpkin seed kernel

INTRODUCTION

Production of medicinal plants and oil pumpkin kernels (hull-less pumpkin seed) is economically profitable and the market is assured. Compliance with the microbial count limits in these products, based on European Pharma-

copoeia (Anonymous, 2002, 2005) is mandatory in many countries. The most often, drying is being performed in batch dryers and in practice various parameters are being applied. In developing countries, the use of batch dryers presents a compromise between investments and effects. The control of drying process is very poor in conventional batch dryers, resulting in lower quality and higher input of energy. New development of control equipment enables control in batch dryers, which is not only satisfactory but is almost comparable to that in big band dryers.

Quick evaporation of physically bond moisture takes place at the beginning of the drying process. For the removal of moisture located inside of material is, beside the energy for evaporation, the energy for moisture transport to the surface also needed (Bruin and Luyben, 1980, Mühlbauer, 1989). This process largely depends on material structure and it differs. Essential oils are active ingredients of many medicinal and aromatic plants, and their thermal sensitivity is a limiting factor for setting up of drying temperature (Mimica-Dukić et al., 2003).

Rise of temperature over 45°C results in considerable reduction of microbial count. Contemporary procedures for reduction of microbial count, treatment with steam or microwaves, are used only in big band dryers (Heindl, 2005). As a conclusion, too high temperatures cause losses of essential oils, while too low increase the microbial count. Due to this contradictory requirement the range of auspicious drying agent temperatures is very narrow, especially for final drying phase, e.g. for moisture content range from 18—20% to the final ones, e.g. 11%. The upper limit, due to essential oil losses is about 50°C, while the lower limit, due to desired reduction of microbial count, is 45°C. Temperature control can be performed relatively easy in contemporary band dryers (Müller, 2004, Martinov et al., 2007b, Martinov et al., 2008), as opposed to batch dryers. The through-heating of whole material layer, upper levels, is very slow, causing increase of microbial count (Graf et al., 2002, Martinov et al., 2006a, b).

The goal of this research was to investigate the possibilities of improvement of drying method with the aim to get as best microbiological quality of products as possible.

MATERIALS AND METHODS

Experiment of drying of mint and pumpkin grain was performed in the Department of hops sorghum and medicinal plants in Bački Petrovac (of the Institute for Field and Vegetable Crops, Novi Sad), and microbiological analyses in Laboratory of microbiology of the Faculty of Sciences of Novi Sad.

Cultivar of *Mentha x piperita* L. Danica in first year of vegetation, first and second harvest, August and October, was used. Whole plant, i.e. herb, was dried in two heights of fresh material batch: 80, and 100 cm. The tests without turning and with turning of material layer, after finishing of second drying phase, were performed. Material was weighted by integrated balance, accuracy 0.2 kg. Material samples for determination of moisture content and microbial

count were taken before drying. In the course of drying, changes of moisture content in the upper and lower levels, 10 cm above the grate, and 10 cm below surface were taken for analysis of moisture content and microbial count.

Oil pumpkin seed kernel of the sort Olinka was used. After harvesting and washing, samples, were collected from different sampling sites where they were grown, and period of time from washing to the beginning of drying did not exceed three hours. Samples of untreated plant material have been also sampled for determination of microbial contamination of fresh material.

Experimental dryer and drying procedures

The experimental batch dryer, SD-16 MGA, produced by *Termoplin*, Mladenovac was described earlier in Martinov et al. (2006a, 2006b), but for this experiments was placed on the frame carrier-17 and four weighing SG sensors, position-18. This enables continuous measuring of dried material weight with accuracy 2 N. The burner capacity was 15 kW, and ventilator engine 0.78 kW. The drying agent flow through material layer was adjusted to 0.2 m/s, and measuring was performed with anemometer at overpressure vents opening. The surface of drying grate was 1.6 m^2.

During the course of drying, three working regimes, combination of maximum temperature and working mode, open or circulated, were programmed. In comparison with preliminary testing, Martinov et al. (2006a, 2006b, 2008), where the time of changing the phase was predicted, integrated balance enables change of drying regime according to measured material mass and calculated moisture content.

Relative humidity of air after passing through peppermint material layer was calculated based on temperatures measured by "wet" –9 and "dry" –10 bulbs. The change from open to circulating mode was performed, after reaching the programmed upper value of the relative humidity of the agent, by opening side flaps-4 and closing circulation opening flap-8 using a servo-motors. For the drying agent temperature was used thermostat of the burner and automatic control of drying mode, switching from circulating to open mode was used common PLC for tobacco dryers.

Material temperature was measured by Ni-CrNi thermocouples in four points at different levels. For all peppermint layer height was always measured the temperature at 10 cm above the grate and 10 cm below material layer surface in fresh material. The accuracy of temperature measurement was 1 K. For temperature recording, data acquisition device *Acurex Autodata Nine* was used. Specially developed software was used for data processing.

Samples from the upper level, 10 cm below surface, and lower level, 10 cm above the grate, were taken every two hours and used for moisture content determination. Microwave oven was used for quick determination of moisture content. According to the previously provided testing and comparison with the common method, the accuracy was assessed to be 2% (Martinov et al., 2007a).

The research of oil pumpkin kernels has been performed in dryer corresponding to those applied in routine practice. As a control, the samples subjected to the constant 50°C and permanently open mode, when the drying agent passes through the grain layer and leaves the dryer, as the most often method performed in practice, have been used. For comparison, the multi-phase drying method was performed, when the open mode has been used in order to reduce the moisture content to 32%, and further on, for next two phases of drying, lower temperatures were used, as well as another, open — recirculating mode was applied.

Tab. 1. — Applied temperatures for phases of drying of pumpkin kernels

Sample	1. phase		2. phase		3. phase	
	t [°C]	W [%]	t [°C]	W [%]	t [°C]	W [%]
A	65	32	60	18	55	8
B	65	32	55	18	50	8
C	60	32	55	18	45	8

For investigation, two control groups were analyzed too: a) drying in open mode, in one single phase, with agent (air) temperature of 50°C, as a procedure the most often applied in practice, and b) "natural" drying in shadowed drafty place, as the procedure also often used in practice.

Determination of microbial count

Samples for determination of microbial count were taken from the fresh material before drying and after changing of drying phase. The microbial count was determined by standard methods. The number of particles of microfungi (molds and yeasts) on Sabouraud agar was counted. The total number of aerobic mesophyllic bacteria on nutrient agar was counted. Coagulase positive streptococci, sulphite-reducing clostridia, *E. coli*, *Salmonella* and *Proteus* species were determined according to the national regulations (*Pravilnik o mikrobiološkoj ispravnosti namirnica u prometu*, Sl. list SRJ, br. 26/93, 53/95. and 46/2002; *Pravilnik o mikrobiološkoj ispravnosti namirnica u prometu*, Sl. list SRJ br. 26, 1993).

Determination of microbial metabolic activity

Since the level of phosphatase activity of heterotrophic part of microbicenosis present in harvested or collected plant material indicate their biochemical, metabolic activity, the acid, neutral and alkaline phosphomonoester-hydrolase activity of samples were measured simultaneously with the determination of microbial count. Results were shown as phosphatase activity index (PAI), the average value of acid, neutral, and alkaline phosphatase activity (M a t a - v u l j et al, 1990), as a good indicator of the level of microbial contamination of plant material.

RESULTS AND DISCUSSIONS

Temperature course in layers

Examples of measured material temperature courses in three layers are shown in Fig. 1. It is evident that material in the upper layers reach temperature over 45°C after a significant period of drying. Until this time, there exist favorable conditions for increase of microbial count. The higher the material batch, the longer the time for warming up to 45°C of upper layers. Supposed reduction of warming up of upper layers as result of turning of peppermint has not been confirmed. No significant reduction has been recorded.

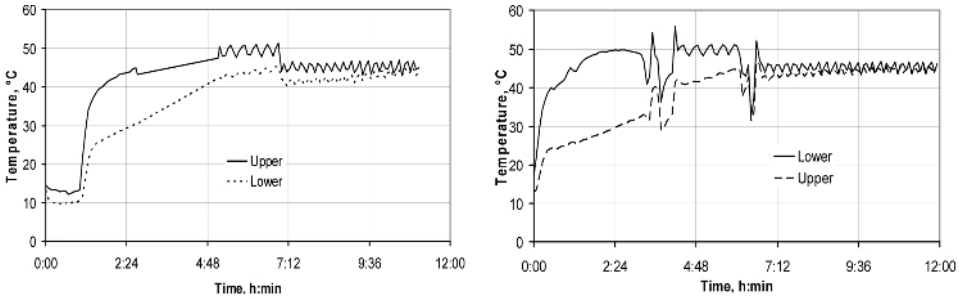

Fig. 1. — Examples of recorded temperature changes in material layers for peppermint a) 80 cm and, b) 100 cm batch height

Moisture content course

Fig. 2 present examples of courses of material moisture content of peppermint. The reduction of drying time is as expected, whereby the relative drying duration is lower for higher batch height.

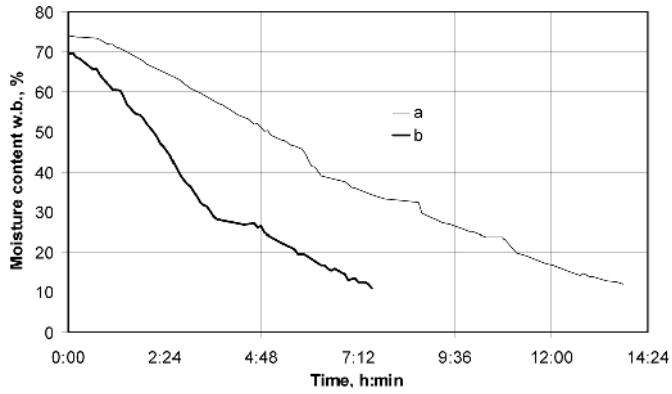

Fig. 2. — Course of material moisture content of peppermint, batch height 1 m: a) without turning b) with turning of batch after first drying phase

The drying time of peppermint without (a) and with (b) turning of batch after first drying phase shows considerable difference, although here are given extreme examples. The start moisture content of turned batch was also about 4% lower. Specific drying rate was for turning of batch about 4.45, and for no turning 2.80 kg/h. Due to relatively small number, only two, one for each batch height, experiments with turning the batch it cannot be concluded that could so huge reduction of drying time can be expected. This effect should be proved in future investigations.

The other positive effects of batch turning are also equalization of moisture content of material layers. Fig. 3 shows measured moisture content in upper and lower layers. It is clear that in the case of material batch turning final moisture content of peppermint is more equalized, Fig. 3b). This effect should be also more approved in future investigations.

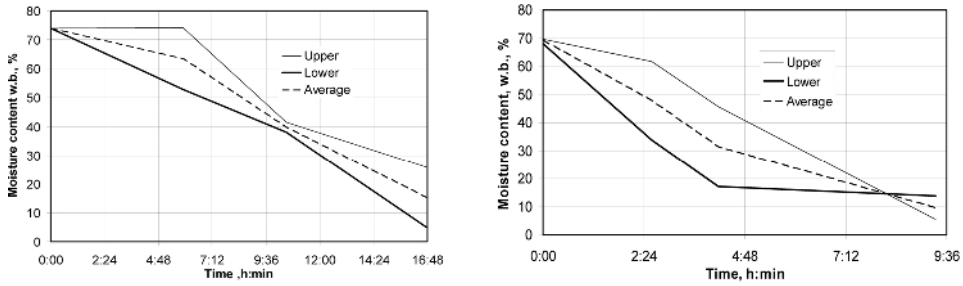

Fig. 3. — Measured (using microwave oven) moisture content of dried peppermint, 1 m batch height, in lower and upper batch layers; a) procedure without turning, b) procedure with turning

Microbial count dynamics

The change of microbial count during drying process, in layers is for selected examples shown in Fig. 4 for peppermint

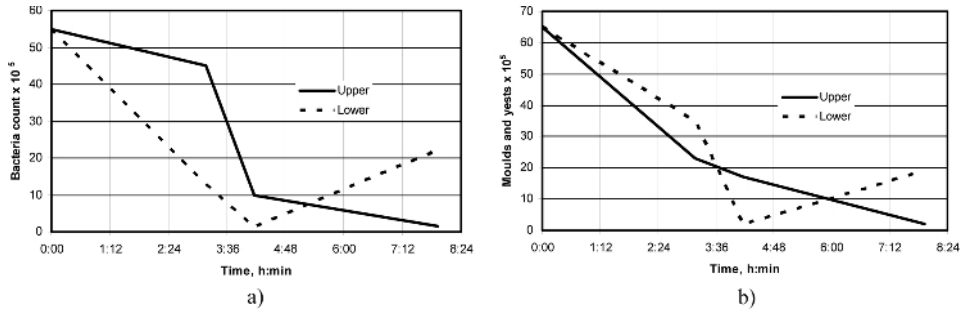

Fig. 4. — Change of microbial count in upper and lower layers, peppermint 0.8 m batch height: a) Bacteria (aerobic mesophylic heterotrophs), b) Microfungi (moulds and yeasts, microfungal particles)

Presented results of drying of batch turning, Fig. 4 show that there is no significant difference of upper and lower layer. This is one more benefit of batch turning. Reached microbial count turned to be lower than that of fresh material, and comparable of those of naturally dried material, and corresponds to the level of 4A category according to European Pharmacopoeia (A n o n y m o u s, 2002).

Tab. 2. — Microbial count and microbial phosphatase activity of pumpkin kernels

Sample	Number of particles of microfungi	Bacterial count	Phosphatase activity index (PAI)
	g^{-1}	g^{-1}	$\mu mol/s^{-1}/g^{-1}$
A	0	33,600	0.76
B	0	40,700	0.88
C	23	69,200	0.93
K	0	142,000	0.94

In accordance with Europaean pharmakopoeia (A n o n y m o u s, 2002) pumpkin kernel A and B samples, dried using method of phase drying, fulfill the criteria for belonging to the 3B quality group. Samples C and K (control group) treated in the same way, fulfilled criteria for 4B quality group.

Particles of microfungi were found only in samples C, when temperature of drying in the third phase was 45°C. Dominated species represented lipophyllic *Mucor* and *Rhizopus* genera. Representatives of xerophyllic *Penicillium, Cladosporium, Aspergillus* and *Fusarium* genera were recorded too.

Number of microorganisms, as well as level of their metabolic activity, testify that bacteria, found as contaminants on oil pumpkin grains cannot be eliminated by applied temperatures higher than 45°C. Further decrease of their number could be achieved only either by using steam treatment or exposing them to microwaves.

In all of cases number of microorganisms indicated that the treated pumpkin grains belonged to the 3B qualitative group or to the 4B for phase drying respectively, and to the 4A for control group according to the Europaean pharmacopoeia.

The results obtained suggest that in future research the treatment with even higher temperatures of drying agents, for example 70°C or 80°C, in the first phase should be investigated. After that phase, the second phase would follow, using temperature of 60°C, and the method with mode shifting would be applied.

CONCLUSIONS

Positive effects of drying in three phases drying have been confirmed. Turning of peppermint batch showed positive effects, and only the needed additional labor could be limitation factor for its application. The more accurate effects of this should be achieved by future investigation.

Three phases drying can be recommended, whereby for phases timing rapid moisture content estimation using microwave oven can be applied. Starting

of drying without heating, use of ambient air, whereby is prolongation of drying time expected.

During the treatment of plant material in all of cases qualitative and quantitative composition of microorganisms indicated that drying process resulted in satisfactory reduction of microbial count according to the Europaean Pharmacopoeia.

ACKNOWLEDGEMENT

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МИКРОБИОЛОШКА ИСТРАЖИВАЊА СУШЕЊА ПИТОМЕ НАНЕ И СЕМЕНКИ УЉНЕ ТИКВЕ У ШАРЖНОЈ СУШАРИ

Милан Н. Матавуљ[1], Милан Л. Мартинов[2], Маја А. Караман[1], Бранислав В. Веселинов[2], Душан С. Адамовић[3]

[1] Природно-математички факултет, Трг Д. Обрадовића 2, Нови Сад
[2] Факултет Техничких наука, Нови Сад, Србија
[3] Институт за ратарство и повртарство, Максима Горког 30, Нови Сад, Србија

Резиме

У раду је постављен циљ да се истражи могућност побољшања поступка сушења питоме нане и ољуштених семенки уљне тикве са циљем да се добије што бољи микробиолошки квалитет. Мали и средњи произвођачи биље најчешће суше у шаржним сушарама које дају најбоље економске ефекте. Лоша страна тих сушара је споро прогрејавање горњих слојева, што ствара услове за почетно повећање броја микроорганизама. У овом раду приказани су резултати испитивања особина сушења у шаржној сушари индикована променама броја бактерија и микрогљива. Режим сушења примењен је у три различите фазе. Добијени резултати сушења питоме нане указују на пораст броја микроорганизама у горњим слојевима биомасе, за релативно дуг период времена када температура није прелазила 45°C. Међутим, за време завршне фазе сушења температура такође није прелазила 45°C и финални број микроорганизама је био скоро изједначен у горњем и доњем слоју.

Истраживање поступка сушења ољуштених семенки уљне тикве је спроведено у условима који одговарају онима које се примењују у пракси. Рад са константном температуром 50°C и стално отвореним модом, при којем агенс једном пролази кроз слој зрна и напушта сушару, што се најчешће користи у пракси, коришћен је као контролна група. За поређење је обављено фазно сушење, при чему је отворени мод коришћен до достизања садржаја влаге око 32%, а надаље су, за две фазе сушења, примењене ниже температуре, и промена мода рада: отворени — рециркулациони. Показано је да је трајање сушења краће и специфична енергија нижа при примени виших температура и промени мода рада у другој и трећој фази. Постигнути квалитет у погледу садржаја броја микроорга-

низама био је такав да се зрно сврстава у квалитетну групу 3Б или 4Б за фазно сушење, а 4А за контролну групу, према Европској фармакопеји.

У будућем раду треба да се испита ефекат још виших температура агенса (ваздуха) за сушење семенки тикве у првој фази (70 и 80°С), после чега би следила друга фаза у којој би температура била до 60°С, а примењивао би се рад са променом мода.

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UDC

Mirjana N. Jarak
Simonida S. Đurić
Biljana D. Đorđević

Faculty of Agriculture, Novi Sad, Trg D. Obradovića 8, Serbia

BENEFITS OF INOCULATION WITH AZOTOBACTER IN THE GROWTH AND PRODUCTION OF TOMATO AND PEPPERS

ABSTRACT: The aim of this research was to investigate the effects of *Azotobacter chroococcum* in tomato and pepper growth and production by using two types of inoculation — seed inoculation and seedling inoculation. The effect of inoculation was observed thirty days after sowing, thirty days after transplanting, and in the phase of technological maturity. The following were measured: height of the plants, dry matter of the plants and number and the weight of the fruits. Inoculation had a positive effect on these in both plants. With tomato, better results were achieved when seedlings were inoculated. With pepper, the length of the plant and the dry matter were greater with seedling inoculation, whereas the number and the weight of the fruits were greater with seed inoculation.

KEY WORDS: inoculation, azotobacter, tomato, pepper

INTRODUCTION

Azotobacter strain is a free-living nitrogen-fixer. It lives freely in soil, growing in abundance in the rhizosphere with a higher concentration of organic matter secreted by plants (Berkum and Bohlool, 1980). Sometimes azotobacter lives on the very surface of the root, making a mucous cover and thus creating a stronger bond with the plant (association). The amount of atmospheric nitrogen fixed by azotobacter ranges from 50 to 80 kg/ha a year, depending on the conditions in soil. Apart from fixing elementary nitrogen, azotobacter produces biologically active substances-auxins, gibberellin, pyridoxine, biotin, and nicotinic acid which can all contribute to plant growth. Therefore, azotobacter can also be used as a microbiological fertilizer in the production of non-legumes (Milić et al., 2004). Several inoculation treatments can be applied: introduction of azotobacter into soil before sowing it, seed inoculation before sowing, seedling inoculation, and introduction of azotobacter into soil by means of irrigation during the vegetation period.

The aim of this research was to observe the possibilities of using *Azotobacter chroococcum* in the growth and production of tomato and pepper by inoculating the seed and the seedling.

MATERIAL AND METHODS

The experiment was conducted in chernozem soil with two kinds of plants: tomato (Novosadski jabucar variety) and pepper (Kalifornijsko čudo variety). In autumn, manure was introduced in the soil. The variants in the experiment were seed inoculation, seedling inoculation, and control (no inoculation).

Seed inoculation consisted of immersing 50 g of tomato and pepper seeds in 15 ml of an inoculum containing 10^8/ml of *Azotobacter chroococcum* cells. The seeds were left in the inoculum for three hours.

Seedling inoculation was performed by immersing the root of seedlings in an inoculum containing 10^8/ml of *Azotobacter chroococcum* cells.

The sowing of inoculated and non-inoculated seed was performed in early April. The distance between seeds was 5 cm.

Thirty days after sowing, the plants were dug out and divided into three groups of thirty plants. The group I consisted of the plants whose seed was inoculated. The group II was the plants whose roots were inoculated, and the group III was the control plants that were not inoculated at all. All the plants were planted into rows. The distance between each plant was 30cm.

The effect of inoculation was observed thirty days after sowing, thirty days after transplanting and in the phase of technological maturity. The length and the dry matter of the plants were measured and the number and weight of fruits of tomato and pepper was determined.

RESULTS AND DISCUSSION

Thirty days after sowing there was no effect of *Azotobacter chroococcum* on the length of the plant above ground, root, or the whole plant of tomato (Table 1).

Tab. 1. — The effect of inoculation on the length of tomato plants

Variants		30 days after seeding		30 days after transplanting	
		Above ground (cm)	Root (cm)	Above ground (cm)	Root (cm)
Control		6.820	1.540	29.300	13.233
Inoculation of seed		6.800	2.060	35.000	20.033
Inoculation of seedlings				39.233	27.267
LSD	1%	2.523	0.937	0.814	0.928
	5%	1.521	0.565	0.491	0.560

Thirty days after transplanting, however, the effect of inoculation was statistically significant. The above ground part of the inoculated plants was lon-

ger by 6—10 cm than in the control plants, and the root of the inoculated plants was longer by 7—14 cm than the root of the control plants. A better effect was achieved when the seedlings were inoculated (table 1).

Thirty days after sowing the pepper, the length of the above ground part of the inoculated plants was smaller than the length of the control plants. The length of the root was the same in both inoculated and non-inoculated plants. However, thirty days after transplanting, the total length of the inoculated plants was greater by about 10—17 cm. As in tomato, the better effect was achieved with seedlings inoculation.

Tab. 2. — The effect of inoculation on the length of peppers plants

Variants		30 days after seeding		30 days after transplanting	
		Above ground (cm)	Root (cm)	Above ground (cm)	Root (cm)
Control		4.360	2.520	19.133	5.553
Inoculation of seed		2.580	2.540	27.200	7.433
Inoculation of seedlings				32.233	9.267
LSD	1%	2.358	2.358	1.243	2.497
	5%	1.422	1.422	0.750	1.505

Thirty days after transplanting, the dry matter mass of the inoculated plants, especially tomato, was greater than the dry matter mass of the non-inoculated plants. Seedlings inoculation had a better effect with both plants (table 3).

Tab. 3. — The effect of inoculation on dry matter mass of tomato and peppers plant

Variants		Dry matter mass of tomato plant (g plant^{-1})		Dry matter mass of peppers plant (g plant^{-1})	
		30 days after seeding	30 days after transplanting	30 days after seeding	30 days after transplanting
Control		0.11	12.25	0.069	7.17
Inoculation of seed		0.11	25.37	0.033	8.24
Inoculation of seedlings			52.43		9.45
LSD	1%	0.001	1.93	0.003	0.112
	5%	0.000	1.16	0.001	0.067

Inoculation with *Azotobacter chroococcum* had a significant effect on the number and weight of pepper and tomato fruits (Table 4). With tomato, the results were better when seedlings were inoculated. With pepper, seed inoculation was more effective.

Tab. 4. — The effect of inoculation on the number and weight of tomato and peppers fruits

Variants		Tomato		Peppers	
		Number of fruits per plant	Average weight of one fruit (g)	Number of fruits per plant	Average weight of one fruit (g)
Control		5.77	59.46	5.33	188.36
Inoculation of seed		7.72	78.50	6.36	231.53
Inoculation of seedlings		10.20	105.56	5.83	211.50
LSD	1%	1.35	0.788	1.42	15.12
	5%	0.81	0.475	0.86	7.43

The use of *Azotobacter chroococcum* in plant production was also justified in many earlier researches. Milošević et al. (1994) concluded that the same strains of *Azotobacter chroococcum* did not produce the same effect in different varieties of pepper. Govedarica et al. (1996) achieved faster germination when cucumber seed was inoculated before sowing. Govedarica et al. (1997) achieved a positive effect of azotobacter on the growth of pepper. The researches of Mrkovački and Milić (2001) showed that this bacterium increases the sugar content of sugar beet. In the researches of maize production by Govedarica et al. (2002) and Hajnal et al. (2004), an even germination, a 3% higher yield and an increased microbiological activity were achieved when *Azotobacter chroococcum* was applied. Jarak et al. (2006) achieved a positive effect of azotobacter on yield of wheat and on microbiological activity in wheat rhizosphere. Azotobacter can be used as an alternative to conventional nitrogen fertilizers. The use of *Azotobacter chroococcum* in tomato and pepper production could reduce the need for nitrogen mineral fertilizers, which is important both economically and ecologically.

CONCLUSION

— Thirty days after sowing there was no effect of *Azotobacter chroococcum* on the length and on the dry matter mass of tomato and pepper plants.

— Inoculation of tomato and pepper with *Azotobacter chroococcum* had a statistically significant positive effect on the length and weight of the plants thirty days after transplanting. A better effect was achieved when seedlings were inoculated.

— The number and weight of pepper and tomato fruits were significantly higher in inoculated variants. Seedling inoculation was more effective with tomato. Seed inoculation was more effective with pepper.

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КОРИСНОСТ ИНОКУЛАЦИЈЕ С АЗОТОБАКТЕРОМ НА РАСТЕЊЕ И НА ПРОДУКТИВНОСТ ПАРАДАЈЗА И ПАПРИКЕ

Мирјана Н. Јарак, Симонида С. Ђурић, Биљана Д. Ђорђевић

Пољопривредни факултет, Нови Сад, Трг Д. Обрадовића 8, Србија

Резиме

Азотобактер спада у слободне азотофиксаторе. У зависности од соја и услова средине, азотобактер може фиксирати 50 до 80 кг азота по хектару годишње. Осим што усваја елементарни азот, он продукује и материје које поспешују раст биљака. Због тих својстава азотобактер се примењује као биофертилизатор. Постоје различити начини примене азотобактера — у земљиште пре сетве, на семе и на расад.

Циљ истраживања је био да се испита ефекат примене *Azotobacter chroococcum* у производњи парадајза и паприке коришћењем два начина инокулације — инокулација семена и инокулација расада. Утицај инокулације испитиван је тридесет дана после сетве, тридесет дана после расађивања и у фази технолошке

зрелости. Испитивани су висина биљака, сува маса биљака, број плодова и маса свежих плодова.

Тридесет дана после сетве није било утицаја *Azotobacter chroococcum* на дужину и суву масу биљака. Инокулација парадајза и паприке имала је значајан утицај тридесет дана након расађивања. Бољи ефекат имала је инокулација расада. Број и тежина плодова код обе биљне врсте били су значајно већи на инокулисаним варијантама. Инокулација расада имала је бољи ефекат код парадајза док је код паприке бољи ефекат имала инокулација семена.

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UDC

Vladimir V. Valchev[1]
Daniela I. Stoeva[2]

[1] Institute of Botany, BAS, str. Akad. G. Bonchev, Bl. 23, 1113 Sofia, Bulgaria
[2] Administration of Vrachansky Balkan Nature Park, str. Ivanka Boteva 1 3000 Vratsa, Bulgaria

STUDY OF AQUATIC MACROPHYTES IN THE WETLANDS ON THE TERRITORY OF VRACHANSKI BALKAN NATURE PARK

ABSTRACT: An overall study of the species composition and abundance of macrophytes in all open water bodies of a large protected territory was made for the first time in Bulgaria. Four high-mountain eutrophic lakes and the upper stretches of three rivers within the boundaries of Vrachanski Balkan Nature Park were investigated. Thirty-nine macrophyte species (higher plants and mosses) were identified scattered around, or forming more or less distinct vegetation groups. Three of the lakes are new for the country locations of the species *Elatine alsinastrum* and *Peplis portula*. The processes of eutrophication are more advanced in the lakes. The habitats formed around the investigated water bodies have been determined. They are three habitats of European Community interest for Bulgaria. The major threats for the investigated wetlands on the territory of the Park are pointed out.

KEY WORDS: Vrachanski Balkan Nature Park, macrophytes, species composition, running waters, lakes

INTRODUCTION

This study of macrophytes has been carried out within the framework of the project *Observations on populations of conservationally important plant and animal species on the territory of Vrachanski Balkan Nature Park and data base completion*.

The main objective of the study was to investigate macrophyte diversity (of species and in quantitative terms) in zones of high conservational value, with priority importance for protection of the herpetofauna. Such zones on the territory of the Park are predominantly the natural open water bodies. The macrophytes have been investigated in these zones as a fundamental group within the food chains and interrelations in their ecosystems.

The objective was implemented thanks to the fulfillment of the following tasks:

— Study of the macrophytes — species composition, plant communities formed by them and the degree of their formation
— Habitats in which these communities participate
— Status of the investigated wetlands
— Threats to the wetlands

MATERIAL AND METHODS

Vrachanski Balkan Nature Park is situated in Northwest Bulgaria and it covers most of the Vratsa Divide and the massive of Lakatnik Rocks. Vratsa Divide rises to the south of the Vratsa Plain and branches off the Balkan Range. The Park covers 28 803.0 ha. Vegetation of the Vrachanski Balkan Nature Park is dominated by two belts: of mixed oak forests (700—1000 m a.s.l.) and of beech forests (700—800 m and up to 1200 m a.s.l.). Above the beech forest belt a small, deforested stretch is inhabited by grassy communities.

Two types of natural open water bodies occurring on the territory of the Park have been object of our study: standing eutrophic lakes and the uppermost stretches of the rivers Leva, Cherna, and Zlatitsa. The river currents have been studied above the Zgorigrad, Lyutadzhik, and Zverino villages, in order

Photo 1. — Panoramic view of the lake region

to avoid the effect of the anthropogenic load caused by them. The lakes lie at altitudes of about 1200 m—1300 m and the investigated river stretches somewhere between 320 m and 600 m a.s.l.

The lakes are situated above the upper timberline, in an area westwards of Parshevitsa chalet (Photo 1). They are sunlit almost all day long. The water mirror area in three of them varies from 0.08 ha to about 0.1 ha, while in the fourth it is about 25—30 sq.m. Their depth is between 25 cm and 150 cm. The lakeshores are bare, covered only by herbal vegetation and occasionally this grassy cover is completely destroyed by the livestock herds reared in this region. The floors of these water bodies are covered with a thick layer of mud and silt. There is mass blooming of green and red algae in two of the lakes, which is a clear sign of a strongly advanced process of eutrophication. The reason for overdevelopment of algae in them is their small depth, rapid warming of water and zoogenic pressure by the livestock.

The investigated rivers have stony beds: about 90% of stones and 10% of coarse-grained sand. They are lit up partially by the sun during different hours, or are completely shaded. The width of water currents varies from 1.5 m to 2.0 m and their depth is 30 cm. Small pools form occasionally in them. The current is fast and the water is transparent.

The transect method of study was applied, with fixed spots, as requested by the "Water quality: Guidance standard for surveying of aquatic macrophytes in running waters" EN 14184 CEN/01. 08. 2003 for running waters and "Water quality: Guidance standard for surveying of macrophytes in lakes — complementary element", CEN TC 230/TG 3/N72 for standing water bodies.

Macrophytes (vascular plants and mosses) were described in each fixed spot. Plant abundance was assessed according to the 5-point Braun-Blanquet scale (G u i n o c h e t, 1973). Mosses in the descriptions were designated by (+), without assessment of abundance. Macrophyte species composition in the four lakes is presented in a table, and there is a general table for each river, with data on the macrophytes from all fixed spots designated on their respective currents.

Latin names of the vascular plants are given according to the *Guide to the Higher Plants in Bulgaria* (1992), and of mosses according to P e t r o v (1975) and S m i t h (1980).

The habitats were defined according to the *Guide for Identification of Habitats of European Community Interest in Bulgaria* (2009).

RESULTS AND DISCUSSION

Macrophyte species of higher plants are referred to 22 species from 22 genera, belonging to 19 families. The group of mosses is represented by 18 species, referred to 17 genera from 12 families. These data testify to a relatively great floristic diversity, considering the modest size of the water bodies.

Of all 22 macrophyte species, 20 are widely spread in Bulgaria. Two species — *Elatine alsinastrum* and *Peplis portula* — occur very seldom and the three lakes in which they were found represent new locations of these plants in

Bulgaria. This fact enhances the botanical value of the water bodies situated on the territory of the Park. Irrespective of the extremely high zoogenic pressure on the water bodies, the populations of both species (*Elatine alsinastrum* and *Peplis portula*) were numerous (in all cases over 100 specimens) and the plants were in a very good condition. At the time of study, both species were flowering (Photos 2 and 3).

Photo 2. — A specimen of *Elatine alsinastrum* L.

Photo 3. — A specimen of *Peplis portula* L.

Tab. 1. — List of the macrophytes

Higher plants	Bryophytes
Alisma plantago-aquatica L.	*Aulacomnium palustre* (Hedw.) Schwägr.
Butomus umbellatus L.	*Brachythecium rutabulum* (Hedw.) Schimp.
Callitriche hamulata Kütz.	*Bryum argenteum* Hedw.
Carex pendula Huds.	*Bryum dichotomum* Hedw.
Elatine alsinastrum L.	*Chilosciphus polyanthos* (L.) Corda
Eleocharis palustris (L.) R. Br.	*Cratoneuron filicinum* (Hedw.) Spruce
Epilobium hirsutum L.	*Ctenidium molluscum* (Hedw.) Mitt.
Equisetum palustre L.	*Didymodon* sp.
Juncus effusus L.	*Fontinalis antipyretica* Hedw.
Lycopus europaeus L.	*Funaria hygrometrica* Hedw.
Lysimachia nummularia L.	*Hygroamblystegium tenax* (Hedw.) Jenn.
Lythrum salicaria L.	*Isothecium alopecuroides* (Lam. ex Dubois) Isov.
Mentha spicata L.	*Marchantia aquatica* (Nees) Burgeff
Myosoton aqaticum (L.) Moench	*Palustriella commutata* (Hedw.) Ochyra
Peplis portula L.	*Pellia* sp.
Persicaria hydropiper (L.) Spach	*Platyhypnidium riparioides* (Hedw.) Dixon
Potamogeton natans L.	*Pohlia nutans* (Hedw.) Lindb.
Ranunculus sardous Crantz	*Schistidium rivulare* (Brid.) Podp.
Rorippa sylvestris (L.) Bess.	
Solanum dulcamara L.	
Sparganium erectum L.	
Veronica beccabunga gr.	

Phytocoenotically, most interesting were the communities of *Potamogeton natans*, formed in the water mirrors of two of the lakes. *Alisma plantago--aquatica* was comparatively abundant in three of the lakes, but most of its specimens were strongly suppressed and did not flower (Table 2, Photo 4).

Tab. 2. — Lakes

Species	Abundance
Potamogeton natans L.	4
Alisma plantago-aquatica L.	1-3
Peplis portula L.	1-2
Juncus effusus L.	1-2
Callitriche hamulata Kütz.	1-2
Butomus umbellatus L.	2
Eleocharis palustris (L.) R. Br.	1
Sparganium erectum L.	1
Elatine alsinastrum L.	1
Persicaria hydropiper (L.) Spach	1
Mentha spicata L.	1

Photo 4. — A community of *Potamogeton natans* L. in lake Krugloto

The species composition of macrophytes has shown rather advanced processes of eutrophication of the four lakes: the indexes of these plants were 5, or above (V a l c h e v, 2009). This was confirmed by the strong presence of

Alisma plantago-aquatica. Emergence of *Sparganium erectum* in one of the lakes (index 5—7) confirmed the lake as highly eutrophic. The processes of accumulation of organic and inorganic substances in the water body of the smallest lake were most advanced and this has led to intensive blooming of the green algae in it. They did not allow other plant species to settle there.

The species composition of macrophytes in the rivers was comparatively poor and the different species had low abundance (Tables 3—5, Photo 5). The low abundance of macrophytes was conditioned by the stony riverbeds and the high speed of the water current. These two factors did not allow more structured macrophyte groups to be formed and that is why the plants were more or less scattered. The above-mentioned factors (riverbeds and the speed of the water flow) were also the reason for macrophytes (higher plants) to be represented only by the group of Helophytes.

Tab. 3. — River Leva, above Zgorigrad village

Species	Abundance
Equisetum palustre L.	1
Persicaria hydropiper (L.) Spach	1
Mentha spicata L.	+
Aulacomnium palustre (Hedw.) Schwägr.	+
Chilosciphus polyanthos (L.) Corda	+
Cratoneuron filicinum (Hedw.) Spruce	+
Ctenidium molluscum (Hedw.) Mitt.	+
Didymodon sp.	+
Isothecium alopecuroides (Lam. ex Dubois) Isov.	+
Palustriella commutata (Hedw.) Ochyra	+
Pellia sp.	+
Platyhypnidium riparioides (Hedw.) Dixon	+

Tab. 4. — River Cherna, above Lyutadzhik village

Species	Abundance
Mentha spicata L.	3-1
Persicaria hydropiper (L.) Spach	2-1
Equisetum palustre L.	2-1
Rorippa sylvestris (L.) Bess.	1
Veronica beccabunga gr.	1
Lythrum salicaria L.	1
Juncus effusus L.	1
Ranunculus sardous Crantz	1
Epilobium hirsutum L.	1
Lysimachia nummularia L.	1
Lycopus europaeus L.	1
Carex pendula Huds.	1
Myosoton aqaticum (L.) Moench	1
Brachythecium rutabulum (Hedw.) Schimp.	+
Hygroamblystegium tenax (Hedw.) Jenn.	+
Cratoneuron filicinum (Hedw.) Spruce	+
Platyhypnidium riparioides (Hedw.) Dixon	+
Schistidium rivulare (Brid.) Podp.	+

Tab. 5. — River Zlatitsa, above Zverino village

Species	Abundance
Mentha spicata L.	4-2
Equisetum palustre L.	3-2
Ranunculus sardous Crantz	2-1
Epilobium hirsutum L.	3-1
Juncus effusus L.	2-1
Lythrum salicaria L.	2
Veronica beccabunga gr.	1
Persicaria hydropiper (L.) Spach	1
Solanum dulcamara L.	1
Rorippa sylvestris (L.) Bess.	1
Lycopus europaeus L.	+
Fontinalis antipyretica Hedw.	+
Funaria hygrometrica Hedw.	+
Hygroamblystegium tenax (Hedw.) Jenn.	+
Brachythecium rutabulum (Hedw.) Schimp.	+
Bryum dichotomum Hedw.	+
Marchantia aquatica (Nees) Burgeff	+
Palustriella decipiens (De Not) Ochyra	+
Pohlia nutans (Hedw.) Lindb.	+
Bryum argenteum Hedw.	+
Platyhypnidium riparioides (Hedw.) Dixon	+

Photo 5. — River Zlatitsa above Zverino village

In those parts of the river valleys some species of the mosses group occurred: *Bryophyta* and *Marchantiophyta*. Among these, the most frequent were *Platyhypnidium riparioides* and *Cratoneuron commutatum*. The presence of these mosses and their abundance on the rocks and in the water has shown that the processes of eutrophisation in these water bodies are still in their initial stage. These rivers on the territory of the Park were in a rather good state in terms of the trophic character of their water bodies.

Vegetation in the investigated eutrophic lakes and the communities of *Potamogeton natans* allowed us to refer it to habitat 3150 — Natural eutrophic lakes with ***Magnopotamion*** or ***Hydrocharition*** type vegetation. Species composition of vegetation in that habitat is certainly by far richer, as compared to the concrete case, owing to the fact that the lakes were subjected to a strong zoogenic pressure from the livestock herds grazing there. The lakes themselves are situated in a region with a dominating habitat 6170 — Alpine and subalpine calcareous grasslands.

The species composition of macrophytes in the rivers refers them to habitat 3260 — Water courses of plain to mountain levels with the ***Ranunculion fluitantis*** and ***Callitricho-Batrachion*** vegetation. Here mention deserves the fact that in this case only the uppermost stretches of the rivers on the territory of the Park are discussed. This explains the absence of many aquatic macrophyte species, either floating or submerged (personal observations of Vladimir Valchev in September 2004): in the middle stretches of the same rivers but outside the territory of the Park such species as *Potamogeton nodosus, P. perfoliatus, Myriophyllum spicatum, Ceratophyllum demersum*, etc. have been encountered, which fall among the characteristic plant taxa for habitat 3260).

The origin and formation of the lakes and configuration of the terrain in the region of standing water bodies set a precondition for the advanced processes of eutrophication in them. A combination with the strong zoogenic pressure from the livestock herds in the region explains the degradation of the water bodies: impoverishment of the species composition of macrophytes and presence of poorly structured vegetation groups. The communities of *Potamogeton natans*, which have managed to survive in two of them, are an exception. Greater stretches of the lake shores were completely deprived of any vegetation cover under the impact of the exceptionally strong pasture degradation caused by the watering livestock herds. Macrophyte vegetation in them has survived only in those parts of the water bodies that are inaccessible to the livestock. All this prompts the conclusion that the state of these water bodies is close to critical.

In terms of the trophic character of water, the state of investigated rivers was much better. This was due to the strong water current, very stony riverbeds and very active processes of water aeration.

The greatest threat to the normal existence of the investigated water bodies is the possible drying up of the climate. It could make some of them (the lakes) disappear, or lead to critical depletion of the water flow (the rivers). Great problem are also the sheep herds grazing in the lake region during the warm months of the year.

CONCLUSION

The investigated water bodies are an important element of the natural resources of the Vrachanski Balkan Nature Park and enrich its landscape and habitat diversity. In terms of species diversity and abundance of macrophytes, the investigated eutrophic lakes (standing water bodies) are more interesting. Their botanic value is also enhanced by the fact that they are new locations of two rare for Bulgarian macrophyte species: *Elatine alsinastrum* and *Peplis portula*.

Species diversity of the investigated sites is relatively great, while the habitats to which they are referred by their vegetation are of European Community interest in Bulgaria. Graver is the state of the lakes, because they are located in a region subjected to intensive grazing and zoogenic pressure. The main threat for the lakes is zoogenic pressure (grazing and pasture degradation), while for the rivers is their pollution by tourists or local inhabitants.

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ИСТРАЖИВАЊА АКВАТИЧНИХ МАКРОФИТА У ВЛАЖНИМ ЗОНАМА НА ПОДРУЧЈУ ПАРКА ПРИРОДЕ „ВРАЧАНСКИ БАЛКАН”

Владимир В. Валчев,[1], Данијела И. Стојева[2]

[1] Ботанички институт, БАС, ул. Акад. Г. Бончев, бл. 23,
1113 Софија, Бугарска
[2] Администрација Парка природе „Врачански Балкан”,
ул. Иванка Ботева 1, 3000 Враца, Бугарска

Резиме

По први пут је у Бугарској спроведено целовито истраживање флористичког састава и квантитативне заступљености акватичних макрофита на свим отвореним воденим површинама на великој заштићеној територији. Проучавана су четири високопланинска еутрофна језера и токови трију река у границама Парка природе „Врачански Балкан”. Установљено је присуство 39 врста акватичних макрофита (виших биљака и маховина), које расту местимично или образују веће или нешто слабије, добро формиране биљне заједнице. Три језера представљају нова налазишта на државном нивоу за врсте *Elatine alsinastrum* и *Peplis portula*. Процеси еутрофизације су у језерима веома изражени. Одређени су типови станишта који се формирају у околини истраживаних водених објеката. То су три хабитата европског значаја у Бугарској. Дефинисани су и основни фактори угрожавања за истраживане влажне зоне на подручју парка.

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UDC

Ivan N. Pavlović[1], *Zoran B. Kulišić*[2]
Zoran Ž. Tambur[3], *Nada M. Protić*[4]

[1] Scientific Veterinary Institute of Serbia, Vojvode Toze 14, 11000 Belgrade, Serbia, dr_ivanp@yahoo.com
[2] Department of Parasitology, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, 11000 Belgrade, Serbia
[3] Institute of Hygiene, Military Medical Academy, Crnotravska 17, 11000 Belgrade, Serbia
[4] EKO-LAB ltd. for quality management, 11000 Belgrade, Serbia

SCARABIDAE — INTERMEDIATE HOST FOR *MACRACANTHORHYNCHUS HIRUDINACEUS*

ABSTRACT: *Macracanthorhynchus hirudinaceus* infestation is parasitosis caused by *Macracanthorhynchus hirudinaceus* Adult forms parasite in small intestine of swine. Development of parasite is happening through intermediate hosts — coleopteras from *Scarabaeidae* family (*Melolontha vulgaris*, *Cetonia aurata*, *Polyphilla fullo*, *Anomalia vitis* etc). Infection begins when swines ingest infected coleopteras. *Macracanthorhynchus hirudinaceus* infestation is encountered in swines in extensive breeding, as well as in wild boars.

KEY WORDS: *Macracanthorhynchus hirudinaceus*, coleopteras, swine's, epysootiology

INTRODUCTION

Macracanthorhynchus hirudinaceus infestation is parasitosis induced by acanthocephales belonging to gender *Macracanthorhynchus — Macracanthorhynchus catulinus* (Kostylev, 1927), *M. ingens* (Linstow, 1879) and *Macracanthorhynchus hirudinaceus* (Pallas, 1781) (Crompton and Nickol, 1995, Pavlović et al., 2007b). Disease of swines is most frequently caused by *Macracanthorhynchus hirudinaceus* (Eršov et al., 1963; Vujić, 1976; Lindquist, 1978; Lončarević et al., 1997; Pavlović et al., 1997; Kulišić, 2001). *Macracanthorhynchus hirudinaceus* are cylinder parasites of big growth and explicit expression of sexual dimorphism (Crompton and Nickol, 1995). Male is 5—10 cm long, 3—5 mm wide and usually bended like comma, with bell shaped posterior end. Females are 10—17 cm long, (yet exemplars of 47—53 cm of length are registered, too), and 4—10 mm wide (Eršov et al., 1963; Soulsby, 1977; Crompton and Nickol, 1995). They have dull posterior end and usually are spirally twisted

(Šulc and Grozdov, 1972; Lindquist, 1978). At the anterior end, there is rounded proboscis-feeler 1 mm long, up to 500 microns wide, armed with backwards twisted hooks, placed by 6 in 5—6 transversal rows, or by 3 in 12 longitudinal rows. Body of parasite is painted white with shades of blue, cylindrical with bigger or lesser extensions in different parts, with grooved cuticle (Dunagan and Miller, 1987).

Macracanthorhynchus hirudinaceus parasitizes in domestic and wild swines, and rarely in dogs and monkeys (Corwin and Stewart, 1992; Crompton and Nickol, 1995; Mary Aquin, 2003). Human infections are registered in Asia and Australia (Pradatsundarasar and Pechranond, 1961; Prociv et al., 1990).

INTERMEDIATE HOSTS OF *Macracanthorhynchus hirudinaceus*

Macracanthorhynchus hirudinaceus females lay oval eggs 60—100 micrometers long and 50—56 microns wide, that come to environment excreted with feces. They contain embryo surrounded by 3 membranes, armed with several hooks (hooklets) (Crompton and Nickol, 1995). Their further development takes place in intermediate hosts-coleopteras belonging to Scarabaeidae family (Soulsby, 1977; Lindquist, 1978; Pavlović et al., 2007b). Those are most frequently insects *Melolontha melolontha* and *Melolontha vulgaris*, rose vermin — *Cetonia aurata*, marble insect — *Polyphylla fullo*, May's vermin — *Anomala vitis*, rolling insect — *Scarabeus* (*Ateuchus*) *sacer*, shaggy insect — *Tropinota (Epicometis) hirta Poda*, grain-vermin — *Anisoplia segetum*, *Amphimallon solsititialis*, *Phylophaga vehemens*, etc. (Olsen, 1986; Crompton and Nickol, 1995; Parshad and Crompton, 1997).

MORPHOLOGY OF Scarabidae

Hardalated insects from Scarabidae family belong to class *Insecta*, order Coleoptera and suborder Polyphaga. They are distributed worldwide, ranging in size from 0.4—11 cm. Anterior wings (elitere) are thickened, while posterior wings (alae) are of use for flying. Head and almost entire body has grown strong — is hardened. Facets of daily species are tiny, more flattened, while species active in twilight have convex and massive (Bei-Benko, 1968; Krunić, 1981). Antennas have phalanxes, up to 11 articles, all of different length.

Mouth apparatus of images and larva's is adapted for crunching — mandible and maxilla are well developed and strong (Crompton and Nickol, 1995). It continues into esophagus that is broadening in craw, behind which is muscular stomach with cuticular thickening. Middle intestine is capacious and posterior intestine is twisted, while some species have caecum. Rectum is like broad chamber (Hickman, 1973; Krunić, 1981).

Blood system consists of heart with different number of chambers with ostia on it. Tracheal system is branching, a well developed, and 10 pair of

stigmatic apertures is arranged on thorax and abdomen. Brain, 3 pairs of thoracic, and 6 to 8 pairs of abdominal ganglia constitute nerve system (Hickman, 1973).

Out of three thoracic segments, prothorax is well developed and mobile. Meso- and metathorax are connected forming pterothorax, which is carrying eliteras (almost whole dorsal surface of pterothorax is covered with eliteras) and wings for flying. They fly with aid of posterior wings that are well developed, while eliteras do not have significant role in that. Posterior wings are longer from eliteras, and when insects are resting, they are wrinkled below eliteras (Krunić, 1981).

Extremities of Scarabidae are adapted for walking and running with 35-articles tarsus. They have pygidial glands developed for defense (Krunić, 1981).

DEVELOPMENT OF LARVAE
Macracanthorhynchus hirudinaceus AT Scarabidae

Members of Scarabidae family have complete metamorphosis. They lay eggs in different places, most often on leaves or in plant's tissue. Larva hatches from egg (Bei-Benko, 1968; Šulc and Grozdov, 1972; Krunić, 1981; Nichols et al., 2008). Scarabidae larvae have thoracic extremities without tarsus, but with little claws on them. They do not have abdominal prolongations (Hickman, 1973; Krunić, 1981). Stigmas are placed on thoracic and abdominal segments. They drag out into soil where live for 3—4 years and during that period undergo complete metamorphosis (larva — doll — adult form) (Šulc and Grozdov, 1972; Nichols et al., 2008).

Infection of Scarabaeidae happens in larval stage (Šulc and Grozdov, 1972). When larvae of Scarabidae eat macracanthorhynchus eggs excreted into ground with feces of swine, they release larvae (acantor) that hitch on intestinal wall soon, with their hooklets (Šulc and Grozdov, 1972; Parshad and Crompton, 1997). Acantor is completely developed in 5—20 days, becoming evolutive form well known as acanthela (Crompton and Nickol, 1995). In this shape, it becomes parasite of Scarabaeidae larva, feeding, growing, and developing until stage infectious for original — real hosts (Zhao and Wang, 1992, Parshad and Crompton, 1997; Mary Aquin, 2003). In this stage they stay during whole metamorphosis of Scarabaeidae (Moore, 1984; Crompton and Nickol, 1995; Nichols et al., 2008).

If infection begins before June, acanthelas are created in 3—4 months, whilst with later beginning of infection larvae are created in 12—13 months (Dunagan and Miller, 1987; Mary Aquin, 2003; Nichols et al., 2008). Preparent period of parasite lasts 2—3 months. Larvae of Scarabaeidae live in soil 3—4 years crunching roots of plants (Bei-Benko, 1968; Hickman, 1973; Krunić, 1981). After finishing of development, they are going out from the soil and live on the trees like adult insects, feeding with buds and leaves (Krunić, 1981; Crompton and Nickol, 1995). *Macracanthorhynchus* larvae — acanthelas stay vital during whole life cycle of coleopteras,

so we can find them in larval as well as in doll stage, and in adults of Scarabidae also (Šulc and Grozdov, 1972; Crompton and Nickol, 1995; Pavlović at al. 1997, 2007b).

Considering habitats where we can meet Scarabidae — fields and pasture grounds, it is real to expect that biggest grade of infection is on fields manure with swine feces, or in grassland with swineherd (Vujić, 1976; Lončarević et al., 1997). In some district areas (Posavina, Podrinje, upper parts of Backa to Danube) where swine herding is permanent and number of Scarabidae big, prevalence of infection with acanthelas amounts even 60% (Pavlović et al., 2007b).

In some larval and doll examples, especially May's coleopteras belonging to Melolontha, Cetonia and Polyphylla order, up to 130 acanthelas can be encountered (Olsen, 1986; Parshad and Crompton, 1997). High prevalence of infection of Scarabaeidae certainly correlates with large extension of swine infection, length of parasite life and great resistance of parasite eggs in external environment. Besides, long life of Scarabidae larvae in soil (3—4 years) maintains permanent degree of contamination in above-mentioned region (Nichols et al., 2008).

SWINE INFECTION

Swine infection originates most frequently through larval forms of Scarabidae. Larvae live in soil 12 — 5 cm deep, whilst swines searching for food during digging come to them (Šulc and Grozdov, 1972; Vujić, 1976; Pavlović et al. 1997). Swine could also become infected through adult Scarabaeidae forms that happen during their non-hygienic or pasturage way of feeding (Lindquist, 1978; Olsen, 1986; Nichols et al., 2008).

Adult parasites take rise from acanthelas in digestive system of swine during 2 months. They attach with proboscis to intestinal wall of host. Deep lesions are being formed at those places, sometimes reaching intestinal serosa (serous layer) (Lindquist, 1978; Lončarević et al., 1995; Pavlović et al., 1997; Ivetić et al., 2000). On the exterior side of intestine, convexes little knots at the attachment points could be seen.

Swine with heavy degree of infestation is agitated — (anxious, nervous), thin and skinny, has weaker appetite and sometimes even convulsions may be present (Corwin and Stewart, 1992; Lindquist, 1978). Young pigs may die most often because of peritonitis, caused by perforation of intestinal wall in consequence of parasite activity (Lindquist, 1978; Pavlović et al., 1997, 2007b).

PREVENTION OF INFECTION

In the aim of prevention, it is recommended to avoid contaminated grassland and pasture fields, in consideration of deepness of Scarabidae larva's burying (12—15 cm) and their long life (3—4 years) (Lončarević et al.,

1997). Pre-expulsion method PREGON for leave out (similar way like in sheep) has full justification. Preventive autumnal dehelminthisation is also recommended — being performed 3—4 weeks after dragging, drawing from pasture fields, and spring dehelminthisation before expulsion, exile to pasturage, when all animals are treated (Lončarević et al., 1997; Pavlović et al., 2007b). After the treatment, cleaning and mechanical removal of manure from building is necessary, as well as washing with hot water (above 60°C) and disinfection using 2% NaOH solution as most efficient (Pavlović et al., 2007a).

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SCARABIDAE — ПРЕЛАЗНИ ДОМАЋИНИ ЗА *MACRACANTHORHYNCHUS HIRUDINACEUS*

Иван Н. Павловић[1], Зоран Б. Кулишић[2],
Зоран Ж. Тамбур[3], Нада М. Протић[4]

[1] Научни институт за ветеринарство Србије,
Војводе Тозе 14, Београд, Србија
[2] Факултет ветеринарске медицине, Катедра за паразитологију,
Бул. ослобођења 18, Београд, Србија
[3] Војномедицинска академија, Институт за хигијену,
Црнотравска 17, Београд, Србија
[4] EKO-LAB лтд. За контролу квалитета, Београд, Србија

Резиме

Макраканторинхоза је паразитоза узрокована акантоцефалом *Macracanthorhynchus hirudinaceus*. Одрасли паразити паразитирају у танким цревима свиња. Развој паразита се одвија преко прелазних домаћина — колеоптера из фамилије Scarabidae (*Melolontha vulgaris*, *Cetonia aurata*, *Polyphilla fullo*, *Anomalia vitis* и др). Инфекција настаје када свиње поједу заражене колеоптере. Макраканторинхоза се сређе код свиња у екстензивном држању, као и код дивљих свиња.

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UDC

Prokeš L. Bela

Institute of Occupational Medicine, Futoška 121, Novi Sad, Serbia

SOME LABORATORY BLOOD INDICATORS IN TRACTOR DRIVERS EXPOSED TO PESTICIDES

ABSTRACT: Pesticides represent group of extremely different compounds or mixed compounds. They are produced in the form of powder for direct application, powder for suspension, concentrated suspension, concentration of emulsion, and in other forms as well.

Influence of pesticides to exposed workers mainly depends on technology of pesticide application use.

All poisons, just like pesticides that come in organism in some way and reach the location of their metabolism, accumulation in the body or extraction must get there through blood.

Pesticides influence stem cells in bone marrow, then maturation process of blood elements and can damage mature blood cells in blood circulation.

The sample encompassed 142 tractor drivers employed in state agricultural unions who were exposed to pesticides during their work.

Concerning annual and daily exposure to pesticides in examined persons is determined that daily extent ranged from 3 to 12 hours and annual from 5 to 125 days, 60.93 in average.

In the paper are analyzed following blood count parameters: erythrocytes, hemoglobin, leucocytes and thrombocytes

The aim of the paper is to investigate whether pesticides influence changed blood count of tractor drivers exposed to pesticides during their work.

Analysis of obtained results indicates that exposure to pesticides, like in examined group of workers, has not influenced damage in any of investigated blood count parameter.

KEY WORDS: blood, blood elements, pesticides, tractor drivers

INTRODUCTION

Pesticides represent group of extremely different compounds or mixed compounds. They are produced in the form of powder for direct use, powder for suspension, concentrated suspension and in other forms. They are dissolved in water before use or in some organic solvent. Therefore, during analysis of possible effect of pesticides to health of exposed workers it should be also taken into account analysis of possible effects of compounds used as dissolvents or carriers and dilutors of active substances (benzene, toluene, xylene, al-

cohols, ketones, chlorinated hydrocarbons etc.) (Savić, 1997; Ružić and Poznanović, 2009).

Apart from previously mentioned facts, influence of pesticides to exposed workers extremely depends on technology of pesticide application. It is significant to emphasize that technology of pesticide application in our country develops mush slowly than other agro-technical measures. Technical solutions of equipment for pesticide application very often lag behind the new knowledge in achieving necessary efficiency of newly made compounds/preparations. Each preparation requires certain way of application and in order to increase efficiency control of their application is necessary to be made. Controlled application of pesticides is achieved with modern equipment that is increasingly improved in developed countries day by day. However, in our circumstances this improving of protective equipment is very slow. We are now in the second phase of development while developed countries are in the fifth phase (Đukić et al., 2001).

Pesticides, like all poisons, which reach in human body at the location of their metabolism, extraction or accumulation, have to come there through blood. In addition, circulating cells of peripheral blood and bone marrow except cells of some lymphocyte lines are relatively short-living and quickly renewed, so even slightly changed length of living or rate of their production result in significant disorders in peripheral blood (Jocić and Savić, 2002).

Having in mind these facts, pesticide influence to blood can be manifested as follows:

— In the direction of provoked disorders in reproductive cells that imperil reproduction ability of those cells and results in decreased number of red blood cells, white blood cells and thrombocytes in peripheral blood (carbamates, organo-chlorine compounds, substituted phenols, and rodenticides (Savić, 1997; Fait et al., 1994).

— In the direction of diseases that are characterized by abnormality in maturation and production of certain types of blood elements which consequently result in their increased sensitivity (carbomatic herbicides, dipiridiles (paraquate), copper-sulphate) (Savić, 1997; Fait et al., 1994), and

— In the direction of diseases with main characteristics of exceeded production of cells of all or some of blood lines (Savić 1997; Fait et al., 1994).

In addition, of influence to reproductive cells in bone marrow and maturation process of blood elements, pesticides can also imperil mature blood cells in peripheral blood. Accordingly, copper-sulphate can cause insufficiency of one enzyme (glucose-6-phosphate dehydrogenase) which results in rapid decay and decomposition of red blood cells; due to production of unnatural blood color (methemoglobine) in red blood cells, dinitrophenol and paraquat can cause anemia; organic dissolvent tetrachlorinecarbone and organic nitro and amino compounds cause disease called porphyria etc.

These are reasons why accurate interpretation even of a slight and/or atypical disorder in peripheral blood can indicate to professional poisoning with pesticides.

MATERIAL AND METHODS

The sample included 142 tractor drivers employed in government agricultural organizations who were exposed to pesticides during their agricultural activities.

Blood for analysis of certain parameters of blood count was taken in the period from October 2007 to the end of January 2008. During this period selected tractor drivers had regular periodical check-ups.

Regarding to annual and daily exposure to pesticides of tested persons it was determined that daily length ranged from 3—12 hours and annual from 5—125 days, in average 60.93 days.

During the year was applied great number of preparations with more than 20 various active substances. Mostly were used organo-phosphorous compounds, then triazines and carbamates and fewer phenoacetic acid and urea derivates, piretroides, anilides, imidazolines, phtalimides etc. Organo-chlorine compounds were rarely used.

During work with pesticides each of tested tractor drivers had certain personal protective devices (in accordance to internal regulations of the firm).

The control group encompassed 70 doormen and workers who were exposed neither to pesticides nor to any other substances with poisoning effect to blood and blood elements.

All persons in sample and control group were males.

In this paper were monitored the following parameters of blood count: red blood cells (erythrocytes), blood color (hemoglobin), white blood cells (leucocytes) and blood platelets (thrombocytes).

Counting of observed blood elements, concentration of blood color (hemoglobin) is performed by automatic counter AVL AUTOLYZER AL 818 after usual procedure of preparation of blood samples.

Borderline values of described method are erythrocytes from 3.90—6.50x 10^{12}/L, hemoglobin from 120.00—175.00 g/L, leucocytes from 4.00—11.00x 10^{9}/L and thrombocytes from 15.00—400.00x10^{9}/L.

Blood count and other laboratory analyses are performed 5—6 months after exposure.

In statistical data processing are used the following methods: average value ($\bar{x}$), minimal value (Min) and maximal value (Max), standard deviation (SD), coefficient of variation (CV) and Student's t-test (p).

The aim of the paper was to investigate whether pesticides influence changed blood count of tractor drivers who were exposed to pesticides during their work.

RESULTS

The sample encompassed 142 tractor drivers who were exposed to pesticides during their work.

The average age of tractor drivers was 38.16 and average age in the control group was 39.98 years. Age of living in both groups was homogenous — CV at tractor drivers and control group was identical: 18.14% (Table 1).

Tab. 1. — Age of tractor drivers and the control group

Statistics	Tractor drivers (N = 142)	Control group (N = 70)
Average ($\bar{x}$)	38.16*	39.98*
Minimum	25	22
Maximum	57	55
Standard deviation	6.92	7.25
Coefficient of variation %	18.14	18.14

* There is no statistically significant difference between groups ($p > 0.05$)

Difference of average age of tractor drivers and control group was not statistically significant ($p > 0.05$) (Table 1).

Average length of service spent at the post of tractor driver (exposed length of service — ELS) was 11.18 years, while for control group this data was not statistically significant because they were selected regarding to non--exposure to pesticides.

Tab. 2. — Hematological parameters of the tractor drivers and the control group

Statistics	Red blood cells ($x10^{12}$/L)		Hemoglobin (g/L)		White blood cells ($x10^9$/L)		Platelets ($x10^9$/L)	
	T	C	T	C	T	C	T	C
Average ($\bar{x}$)	4.94	4.99	154.1	154.4	7.12	7.29	246.1*	208.5
Minimum	4.16	4.11	133	112	2.80	4.10	107	116
Maximum	6.94	5.79	182	178	14.50	17.70	440	335
Standard deviation	0.4	0.32	8.56	9.92	1.88	2.01	56.35	41.11
Coefficient of variation %	8.23	6.48	5.55	6.42	26.43	27.65	22.89	19.71

T = tractor drivers C = Control group * $p < 0.05$ (t-test)

Average number of red blood cells registered at tractor drivers was $4.94x10^{12}$/L and at their control group it was $4.99x10^{12}$/L. In both groups was registered very low coefficient of variation (CV) of the number of red blood cells. Difference of the average number of red blood cells between tractor drivers and control group was not statistically significant ($p > 0.05$) (Table 2).

Average concentration of blood color (hemoglobin) at tractor drivers was 154.17 g/L while in their control group it was 154.40 g/L. In both groups was also registered very low coefficient of variation (CV) of blood color (hemoglobin) concentration in blood. Difference of average blood color (hemoglobin) concentration between tractor drivers and control group was not statistically significant ($p > 0.05$) (Table 2).

Average number of white blood cells registered at tractor drivers was $7.12x10^9$/L, while at their control group it was $7.29x10^9$/L. In both groups was registered coefficient of variation (CV) of the number of white blood cells above 20%. Difference of average number of white blood cells between tractor drivers and control group was not statistically significant ($p < 0.05$) (Table 2).

Average number of blood platelets (thrombocytes) at tractor drivers (246.11x 10^9/L) was higher than at their control group (208.55x10^9/L). Coefficient of variation of the number of blood platelets (thrombocytes) in both examined groups also ranged around 20%. Difference of the average number of blood platelets (thrombocytes) between tractor drivers and control group was statistically significant (pœ(Table 2).

Analysis of the range of examined hematological parameters at tractor drivers was carried out within the group, after their classification according to age (6 sub-groups), length of service with exposure to pesticides (4 sub-groups) and according to the number of days of work with pesticides during the year (4 sub-groups).

Obtained statistical analyses indicated that there is not any statistically significant difference of the average values of hematological parameters between groups classified in this way. Therefore mentioned analyses are not presented in this paper.

DISCUSSION

Before analysis of the mentioned blood count parameters it was determined that tractor drivers, although in average younger, were not statistically significantly different from their control group.

Statistical analysis of the length of service of tractor drivers regarding to their control group was not carried out because they were selected regarding to non-exposure to pesticides or some other blood poisons.

Average number of red blood cells registered at tractor drivers and their control group was very similar and within normal limits. In both groups were registered minimal values and they indicated that in the group of tractor drivers was not noticed decreased number of red blood cells under the lowest limit of normal values in any of examinees.

In any of tractor drivers was not registered concentration of blood color (hemoglobin) under the lowest allowed limit, which is opposite to the result of Parron and collaborators (1996) who had confirmed decreased concentration of blood color (hemoglobin) at 38% of exposed tractor drivers.

Statistical analysis of difference of average number of red blood cells and average concentration of blood color (hemoglobin) between tractor drivers and their control group confirmed that, in this case, exposure of tractor drivers to pesticides have not resulted in significant disorder of the number of red blood cells and quantity of blood color (hemoglobin) in peripheral blood of examinees.

Average number of white blood cells is registered in tractor drivers only to some extent lower (p0.05) than average number of white blood cells in their control group. In the group of tractor drivers are registered just two cases of the number of white blood cells under normal limit. In the first case the tractor driver was exposed to pesticides around 100 days per year, and in the second case tractor driver had the exposure of 30 days per year.

Very interesting is data that average number of blood platelets (thrombocytes) at tractor drivers is statistically significant higher (p,ically their control group. However, it is well known that toxic influence of pesticides at blood platelets (thrombocytes) is characterized by their decreased but not increased number. In the group of tractor drivers are registered only four cases (2.82%) with values lower than normal, which would indicate to toxic influence of pesticides to produce blood platelets (thrombocytes). These cases include two examinees exposed to pesticides around 30 days per year, one around 70 days and one around 100 days per year.

These data on the number of blood platelets (thrombocytes) at tractor drivers also indicate that exposure to pesticides haven't resulted in significant disorder in function of this blood line as Savic et al. have determined (1993).

Previous discussion indicates that exposure to pesticides which is registered in analyzed group have not resulted in disorder of any of monitored blood count parameters.

Statistical analyses of average levels of monitored blood count parameters in the analyzed group of tractor drivers are classified according to exposed length of service to four sub-groups and according to the number of days of exposure to pesticides during the year (4 sub-groups). It indicates that there is no significant difference in those levels between mentioned groups established in described way.

Concerning the length of time passed from exposition and performed laboratory analyses, at the very end of discussion should be also mentioned option that immediately after exposition there still happens certain aberrations in some parameters of blood count but also that these damages are already recovered by the moment of taking the blood samples. This hypothesis could not be confirmed by the analysis of monitored parameters of blood count.

In order to exclude similar confusions in further researches, it is necessary to examine parameters of blood count at exposed workers immediately after their exposure to pesticides.

Based on obtained results the following conclusions can be assumed:

— Although statistically insignificant, tractor drivers were younger than their control group ($\bar{x}$ = 38.16 years and $\bar{x}$ = 39.98 respectively).

— Daily length of exposure to pesticides ranged from 3—12 hours and from 5—125 days annually, in average 60.93 days.

— Within work of tractor drivers in the fields during the year great number of preparations with more than 20 various active substances are applied. Mostly are used organophosphorous compounds, then triazines and carbamates, in a smaller amount derivates of phenoxy-acetic acid and urea, pyretroides, anilides, imidazolines, taliloides etc. Organo-chlorine compounds are rarely applied.

Analysis of monitored parameters of blood count of tractor drivers regarding to control group as well as regarding to length of exposed years of service and the number of days of exposition to pesticides per year, indicates that exposition to pesticides, like that which is registered in analyzed group of tractor drivers, have not resulted in significant disorders in their blood count. Laboratory analyses of selected blood count parameters at exposed persons

should be carried out immediately after their exposure to pesticides. Use of all available technical, technological, and organizational protective measures is necessary, as well as necessary use of adequate personal protective measures. Regular follow-up of health condition of exposed tractor drivers is also necessary through periodical/systematic checkups according to the program included in the Regulations on previous and periodical check-ups of employed workers at work places with increased risk (2007).

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Pravilnik o prethodnim i periodičnim lekarskim pregledima zaposlenih na radnim mestima sa povećanim rizikom (Službeni glasnik RS br 120/07).

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НЕКИ ЛАБОРАТОРИЈСКИ ПОКАЗАТЕЉИ КРВНЕ СЛИКЕ КОД ТРАКТОРИСТА ИЗЛОЖЕНИХ ПЕСТИЦИДИМА

Прокеш Л. Бела

Завод за здравствену заштиту радника, Футошка 121, Нови Сад, Србија

Резиме

Пестициди представљају групу веома различитих једињења или смеша једињења. Производе се у виду праха за директну употребу, праха за припремање суспензије, концентроване суспензије, концентрата за емулзију и у другим облицима.

Утицај пестицида на изложене запослене раднике умногоме зависи од технологије примене пестицида.

Сви отрови, па и пестициди, који на било који начин доспеју у организам, до места свог метаболизма, излучивања или складиштења у телу, морају доћи путем крви.

Пестициди делују на матичне ћелије у коштаној сржи, процес сазревања крвних елемената, и могу да оштећују и зреле крвне ћелије у периферној крви.

За узорак је узето 142 возача трактора запослених у државним пољопривредним добрима који су при обављању пољопривредних послова били изложени пестицидима.

У погледу годишње и дневне изложености пестицидима испитиваних особа утврђено је да се дневна дужина кретала од 3 до 12 часова, а годишња од 5 до 125 дана, просечно 60,93 дана.

У раду су праћени следећи параметри крвне слике: црвена крвна зрнца (еритроцити), крвна боја (хемоглобин), бела крвна зрнца (леукоцити) и крвне плочице (тромбоцити).

Циљ рада је био да се испита да ли пестициди доводе до промена у крвној слици тракториста који су приликом обављања свог посла изложени деловању пестицида.

Анализа добијених резултата указује да изложеност пестицидима какву је имала испитивана група није довела до оштећења ни једног од посматраних параметара крвне слике.

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UDC

Aleh I. Rodzkin, Vladimir A. Ivanyukovich*
Svetlana K. Pronko, Elena V. Kresova

International Sakharov Environmental University,
Dolgobrodskaja 23, 220009 Minsk, Belarus
* Author for correspondence. e-mail: aleh.rodzkin@rambler.ru

WILLOW WOOD PRODUCTION ON RADIONUCLIDE POLLUTED AREAS

ABSTRACT: One of the key environmental problems in Belarus is effective use of agricultural lands contaminated by radionuclide due to the Chernobyl disaster. The alternative method to traditional agricultural crops is fast growing willow cultivation. It is possible to use biomass of willow as renewable energy source. The goal of our investigation was the estimation of environmental aspects of willow wood production on polluted areas. The field study experiments (2007—2010) were conducted at Krichev district of Mogilev region in eastern Belarus. This region characterized by high level of Cs-137 contamination as well as high level of heavy metals pollution. In the first stage of experiments, the concentration of cesium-137 in different parts of willow biomass had been measured and transfer factor calculated. The measuring had been done for leaves, roots, and wood. To control cesium-137 accumulation in willow biomass we apply different types (nitrogen N, phosphorus P and potassium K) and dose of fertilizer. The experiments show that potassium mineral fertilizer is the key factor for radionuclide accumulation control. The optimal dose of potassium is 90 kg per hectare. On the base of experimental results the model of cesium-137 accumulation in the wood for a 21 year has been developed. In accordance with calculation to the end of willow cultivation (21 year) concentration of cesium-137 in wood will not be higher than permitted even with the level of cesium-137 contamination in the soil 1480 kBq/m^2 (maximum 140 kqB/m^2 with permitted level for firewood is 740 Bq/kg.). The concentration of cesium-137 in the roots increases gradually and get maximum in 21 year (3000 kqB/m^2).

Our results confirm that in the sum about 0.8 million hectares of radionuclide polluted arable lands partly excluded from agricultural practice in Belarus could be used for willow biomass production.

KEY WORDS: biomass, fertilizer, potassium, radionuclide-contaminated soils, willow

INTRODUCTION

The Republic of Belarus does not have an adequate potential of its own fossil fuels supplying and nowadays we use about 5% of Belarus demand of energy at the cost of local renewable recourses. The National State Program was approved in order to increase this input to 25% until 2012. The most pers-

pective resources of renewable energy in Belarus are bioenergy, wind, and hydroenergy. Belarus has about 9.5 million hectares of forests, 5.7 million hectares of arable lands, and 3 million hectares of pastures. Part of lands may be used for biomass production on the base of cultivation of fast-growing crops like willow. The yield of willow biomass crops may achieve 10—15 tons of dried wood or 5—6 toe per hectare. The potential area for willow biomass production in Belarus is estimated in 0.5 millions hectare. It means the annual energy potential of willow biomass systems in Belarus is 2.5—3 millions toe.

Willow biomass cropping systems simultaneously produce not only power and economic, but also environmental and social benefits. These include reduced SO_2 and NO_x emission, less extraction of additional CO_2 to the atmosphere, reduced soil erosion, and pollution from non-point source of agricultural lands, and enhanced agricultural landscape diversity. Willow plants may be successfully grown on different types of lands and have the potential in reclamation of degraded and polluted soils.

Because of the Chernobyl disaster the area of radionuclide contaminated agricultural soils in Belarus is about 1.3 million ha, including 0.8 million ha of arable lands. The optimal system of cultivation of this type of soils on contaminated area is a serious problem, because traditional crops such as grass and cereals may accumulate extra radionuclide (Abagyan et al., 1996). The willow does not accumulate a lot of radionuclide and it can be used as wood for bioenergy purpose.

There are some publications concerning cultivation of willow on radioactive polluted soils.

The experiments in Palesse district, a typical rural area located close to Chernobyl, were fulfilled for modeling of SRC biomass production and estimations of Cs-137 soil-to-wood transfer. The results shown that from the radio-ecological viewpoint, and according to the local legislation, the SRC biomass produced on loamy sand, sandy loam and loamy soils is suitable for firewood. SRC biomass from the highly productive peaty soils (39.4% of the land area of Palesse) may also be used but only if its conversion into heat or electricity is carefully managed (Goor et al., 2001).

Gommers, A., and others (2000) get the same conclusion in experiments with radiocesium uptake by one-year-old willows planted as short rotation coppice (Gommers et al., 2000). Radiocesium uptake and distribution were measured in a willow (*Salix viminalis* L. var. Orm) short rotation coppice (SRC) stand. This system allows production of energy from the harvested biomass. Even at this high soil contamination level, radiocesium concentrations in wood do not exceed appreciably the naturally occurring K-40 content in the wood (135 Bq/kg).

The Norway scientists compare the accumulation of radionuclide by different types of trees. The results showed that willow accumulate radionuclide not as intensive as birch or alder (Brittain and Bjoernstad, 1996).

These publications confirm that it is possible to get comparatively clean biomass of willow on radionuclide-polluted areas. The problem is the development of adequate technology of willow production for polluted soils and esti-

mation of pollutants accumulation in the wood for future period. The goal of our investigation is the estimation of cesium-137 in willow biomass to model opportunity of wood utilization as a bioenergy.

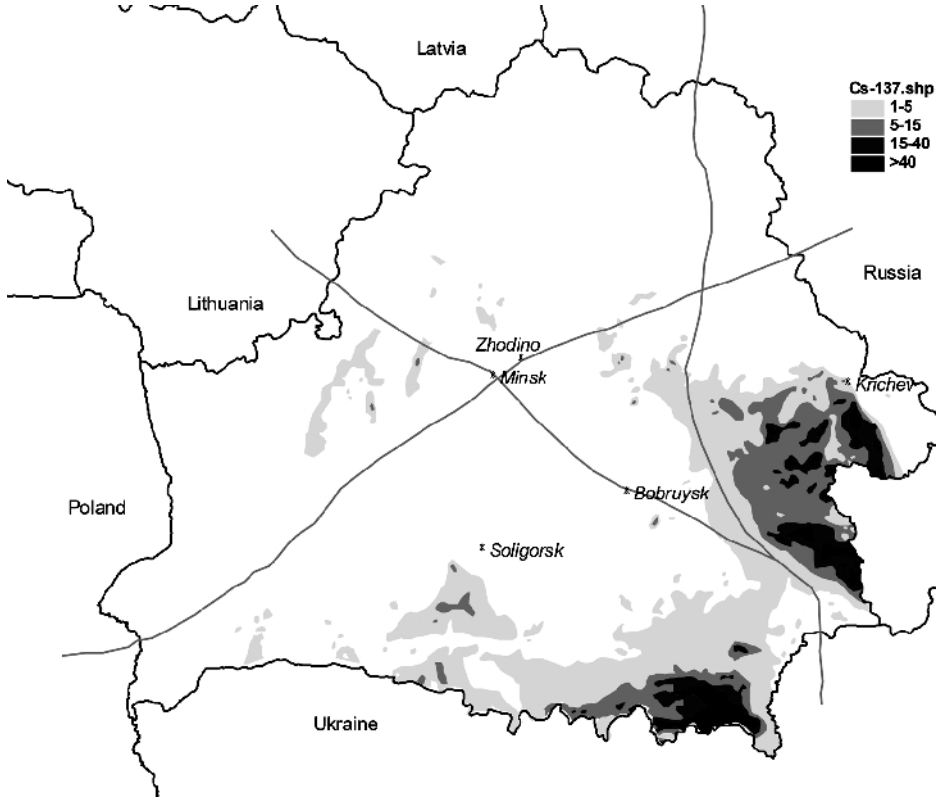

Fig. 1. — The map of Belarus with Cs-137 contaminated territories (2000 year)

MATERIALS AND METHODS

The field study experiments (2007—2010) were conducted at Krichev district of Mogilev region in eastern Belarus, close to the Russian border. This region characterized by high level of Cs-137 contamination as well as high level of heavy metals pollution. The radioactive contamination in the region has been conditioned by precipitating from clouds after the Chernobyl accident. As a result, local cesium "spots" appeared. The level of contamination in the place of our experiment varied from 185 to 370 kBq/m^2 (Figure 1).

The soils of experimental plot were sandy and sandy loams with single grain structure. It was excluded from agricultural practice after Chernobyl disaster. Available water capacity was moderate to high.

The practical experiment included some variants with different dose of fertilizer application:

1. Control (C).
2. Variant $N_{30}P_{60}K_{90}$ (V-2)
3. Variant K_{30} (V-3)
4. Variant K_{60} (V-4)
5. Variant N_{30} (V-5)
6. Variant N_{60} (V-6)
7. Variant K_{90} (V-7)
8. Variant K_{120} (V-8)
9. Variant K_{90} (V-9)
10. Variant K_{150} (V-10)

The different rates of potassium fertilizer were used, as it is chemical analog of cesium. In accordance with our hypothesis, the additional application of potassium enables to decrease the level of accumulation cesium in willow biomass.

The different rates of nitrogen fertilizer in experiment should let us find optimal balance of the element. From one side the rates of nitrogen should be optimal for willow growing but not so high for extra weed development from another side.

The experimental design was randomized by complete blocks of four treatment replicated four or five times. Each elementary plot was 7 m long by 7.2 m wide (50 m^2) and contained 4 double rows of plants.

RESULTS AND DISCUSSION

In the first stage of our experiments, the concentration of cesium-137 in different parts of willow biomass had been measured and transfer factor calculated (Table 1). The measuring had been done for leaves, roots, and wood. The same experiments fulfilled because of different ways of utilizations these components. The leaves go back to the soil every year, wood is using for energy in every 3 year and roots leave in the soils as far as plantation of willow used. We admitted it for 21 year.

To control cesium-137 accumulation in willow biomass we apply different types (nitrogen N, phosphorus P and potassium K) and dose of fertilizer.

Tab. 1. — The transfer factor of cesium-137 from soil to willow biomass

Fertilizer	Transfer factor for roots $10^{-5}m^2/kg$	Transfer factor for wood $10^{-5}m^2/kg$	Transfer factor for leaves $10^{-5}m^2/kg$
Control (C)	93.925	3.175	5.110
$N_{30}P_{60}K_{90}$	33.712	1.315	2.999
K_{30}	56.425	2.875	5.088
K_{60}	39.325	1.5	4.105
K_{90}	20.425	0.9	3.163
N_{60}	54.375	2.6775	4.782
N_{80}	55.911	2.8	5.139

The highest rates of transferring took place in roots. The transfer factor for leave was approximately twice as compared to wood.

Our experiments also shown that accumulation of cesium-137 in willow biomass the mostly depends on potassium application. This dependence has linear character for roots and wood but not for leaves.

It is obviously that potassium mineral fertilizer is the key factor for radionuclide accumulation controlling and for cesium-137 modeling in willow biomass. The application of doze potassium above 90 kg per hectare (120—150 kg/ha) not delayed accumulation of cesium-137.

It was admitted that for modeling of cesium-137 accumulations for long time it is necessary to identify following factors:

— the level of cesium-137 contamination of the soil
— the period of cesium-137 half life
— yield of willow wood
— transfer factor

On the base of these factors the model of cesium-137 accumulation in the wood for a 21 year has been developed. The level of soil contamination by cesium-137 was choosing as for experimental plots (Figure 2). The 3 year is the period between harvesting time for biomass in willow in accordance with technology.

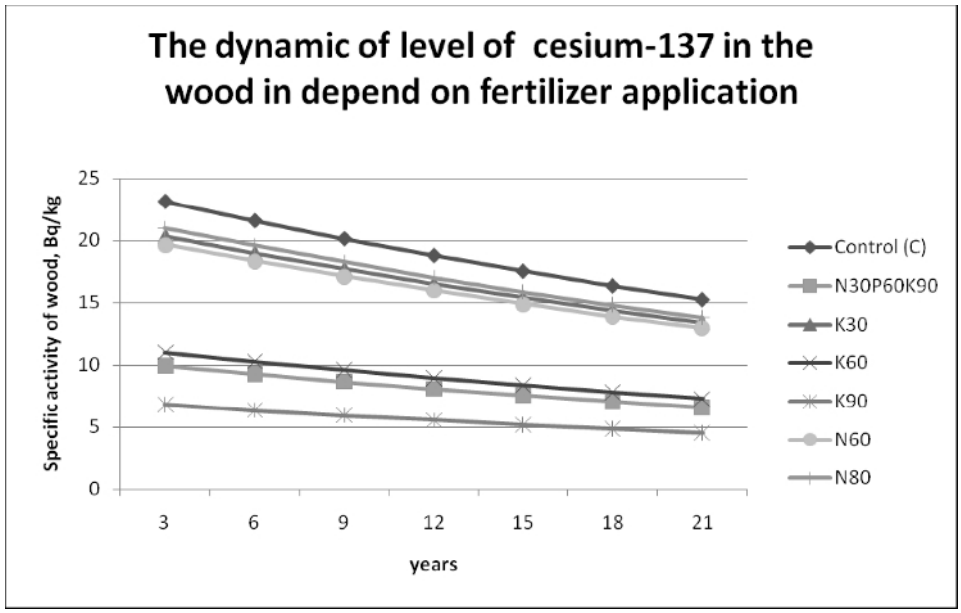

Fig. 2. — The dynamic of cesium-137 level in wood depending on fertilizer application; the level of cesium-137 contamination in the soil is 294 kBq/m^2

The results of modeling identify that for this level of the soil contamination cesium-137 activity in wood was several times lower than Belarus permitted level for firewood (740 Bq/kg).

The following task was confirming by opportunity of willow wood production at the soils with higher level of radionuclide contamination. The results of modeling for soils contaminations from 185 to 1480 kBq/m^2 presented in the Figure 3.

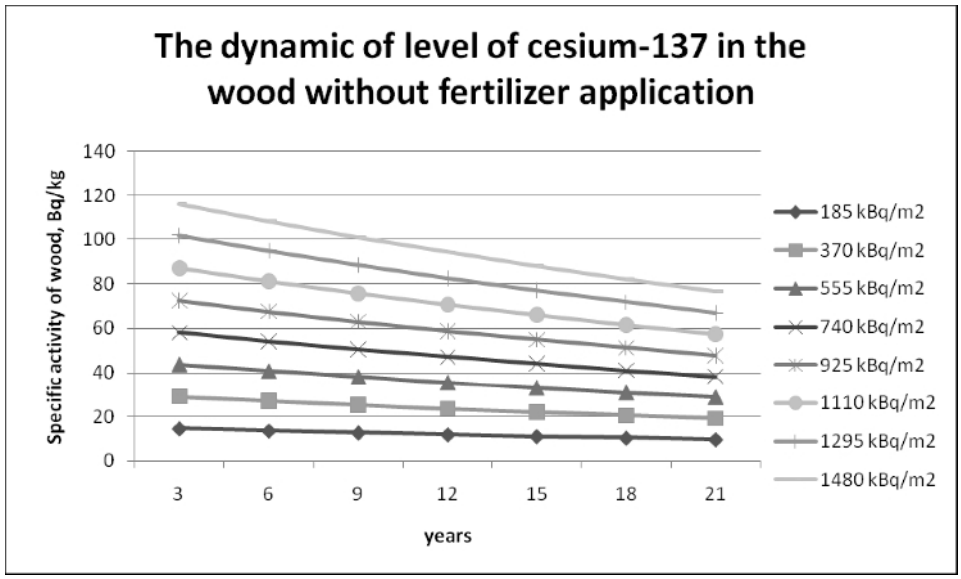

Fig. 3. — The dynamic of cesium-137 level in wood without fertilizer application

It was found that it was possible to get normatively "clean" wood even without additional fertilizer application for the level of cesium-137 contamination of the soil as high as 1480 kBq/m^2. The application of the 90 kg of potassium let us decrease of cesium-137 activity in the wood in 3 times.

As it was shown, earlier the roots accumulate biomass more actively than wood. As a result, to the end of willow plantation existing (21 year) the level of cesium-137 in biomass of root will be higher than permitted level for firewood. The application of 90 kg of potassium decrease of cesium accumulation more than in 4 times. Results of the modeling are shown on the Figure 4.

The future problem will be roots utilization. It is possible to use two directions. The first is to recultivate the plantation in 12—15 year, not after 21. It will be possible to use part of roots as firewood. The alternative way is to leave it in the soil to plough the plot and do new plantation willow or other perennial crop. The optimal decision may be adopted after economy and ecology calculation.

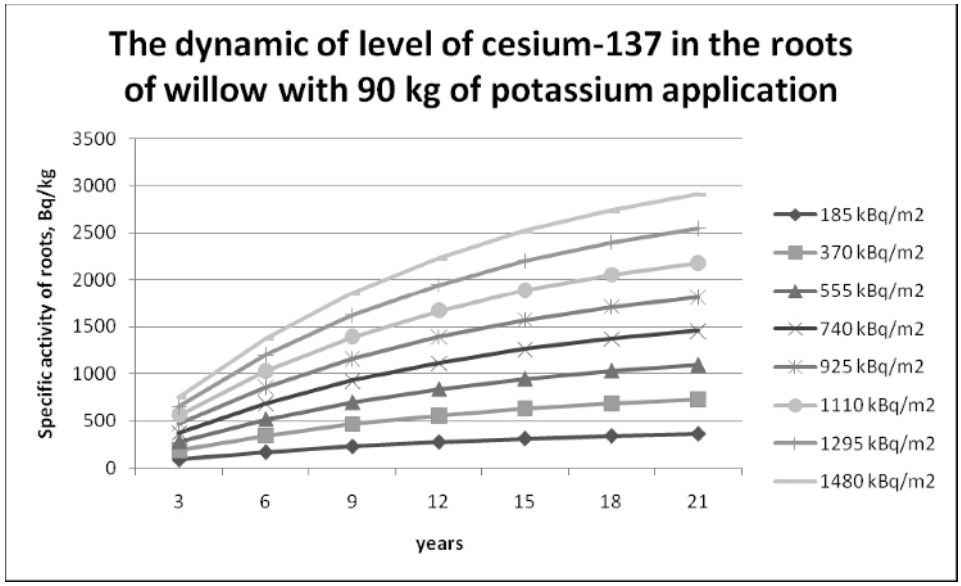

Fig. 4. — The dynamic of level of cesium-137 in the roots of willow with 90 kg of potassium application

CONCLUSION

It was confirmed that potassium application might control accumulation of cesium-137 to biomass. It is possible to get relatively "clean" biomass especially with application additional rates of K fertilizer on the site with the extra level of cesium-137 contamination. The optimal dose of K for delaying of cesium-137 accumulation to the willow biomass is 90 kg/ha. The higher dose of potassium application not stimulate and adequate accumulation of cesium-137.

There are some difference in accumulation of cesium-137 in wood, leaves, and roots. The level of cesium in wood biomass after three years of willow cultivation varied from 5 (with high rates of fertilizer application) to 25 (control plants) Bq/kg with the level of cesium-137 contamination in the soil 294 kBq/m^2. In Republic of Belarus, the permitted level for firewood is 740 Bq/kg. On the base of field experiments the model of cesium-137 accumulation for long time has been developed. In accordance with calculation to the end of willow cultivation for firewood (21 year) concentration of cesium-137 in wood will not be higher than permitted even with the level of cesium-137 contamination in the soil 1480 kBq/m^2 (maximum 140 kBq/m^2).

The concentration of cesium-137 in the roots increases gradually and get maximum in 21 year (3000 kBq/m^2). The future problem will be roots utilization because it is not possible to use root's residues as energy biomass. We believe that two directions may be admitted. The first one is to recultivate the plantation in 12—15 year, not after 21. It will be possible to use part of roots as firewood this way. The alternative decision is to leave it in the soil to

plough the plot and do new plantation willow or other perennial crop. The optimal decision may be adopted after economy and ecology calculation.

The accumulation of cesium-137 in the leaves was not as active as in other parts of biomass.

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ДРВНА ПРОИЗВОДЊА ВРБА НА ЗЕМЉИШТИМА ЗАГАЂЕНИМ РАДИОНУКЛИДИМА

Олег И. Родкин, Владимир А. Иванукович,
Светлана К. Пронко, Елена В. Кресова

Међународни државни еколошки Универзитет „А.Д. Сахарова",
Долгобродскаја 23, 220009 Минск, Република Белорусија

Резиме

Један од кључних еколошких проблема Белорусије је коришћење пољопривредног земљишта контаминираног радионуклидима током катастрофе у Чернобилу. Алтернатива традиционалним пољопривредним културама је садња брзорастућих биљака, као што је врба (*Salix* spp.), чија се биомаса може користити као обновљиви извор енергије. Циљ истраживања био је процена утицаја животне средине на дрвну производњу врба гајених на загађеним земљиштима. Пољски огледи постављени су 2007—2010. године у области Кирцхев, региона Могилев, источна Белорусија. Овај регион карактеришу висок ниво контаминације Цс-137 и висока контаминираност тешким металима. У првој фази експеримента мерена је концентрација цезијума — 137 у листу, стаблу и корену врбе и рачунат је трансфер фактор. Биљкама гајеним на загађеном земљишту додаване су различите врсте (N, P, K) и дозе ђубрива. Резултати су показали да је калијумово минерално ђубриво кључни фактор контроле акумулације радионуклида и да је оптимална доза ђубрења 90 kg/h. На основу добијених резултата направљен је модел акумулација цезијума — 137 током 21 године који показује да би по истеку тог временског рока врбе садржале мање цезијума од дозвољене границе, чак и на зе-

мљиштима са онтаминацином од 1480 kBq/m^2 (највише 140 kBq/m^2, а дозвољени ниво је 740 kBq/m^2). Концентрација Cs-137 у корену расла би и достигла максимум током 21. године (3000 kBq/m^2).

Резултати су потврдили да би се око 0.8 милиона хектара пољопривредног земљишта загађеног радионуклидима, које се не користи за пољопривредну производњу, могло користити за гајење врба, као извора обновљиве енергије.

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UDC

Jovan P. Šetrajčić[1]
Ljubiša D. Džambas[2]
Stevan Armaković[1]

[1] Department of Physics, Faculty of Sciences, University of Novi Sad, Trg D. Obradovića 4, 21000 Novi Sad, Vojvodina — Serbia
[2] Department of Stomatology, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Vojvodina — Serbia

PARTICULARITIES IN PHYSICAL CHARACTERISTICS OF MOLECULAR CRYSTALLINE NANOFILMS

ABSTRACT: In this paper, the alterations and changes in dielectrical properties of different nanofilm molecular crystals, caused by the presence of boundaries were theoretically investigated and analyzed. By combined analytical and numerical calculations, allowed exciton states were found, and their spatial distribution along the axis limit (by layers of film) and the surface localization was examined. The relative permittivity of the observed ultrathin film was determined, and the impact of (five) boundary parameters on resonant absorption phenomenon: discrete (by frequencies) and selective (by layers of film) was examined. The conditions for the emergence of single-resonant absorption lines were found.

KEYWORDS: Excitons, Ultrathin film, Green's function, Permittivity, Resonant absorption

INTRODUCTION

It is very well known that nanostructure: ultrathin films, quantum wires, points, etc. owe their excellent properties to dimensionality of their constituents. In order to gain fundamental information concerning extremely different physical and chemical properties of these low-dimensional crystalline samples it is crucial to intensify their experimental and theoretical research. Such important class of materials is experiencing extensive practical application in nano-, opto- and bio-electronics (Wood, 2008). What is special to these "tiny" structures is that presence of close border surfaces changes generally known properties of these materials and eventually leads to some non-specific phenomena (a consequence of effects of dimension quantizing, Tringides, 2007) comparing to analogues in the large scale samples (Davison, 1996). Interest in studying exciton sub-system was brought about by the fact that the excitons are "responsible" for dielectric, optical (absorption, light dispersion, luminescence), photoelectrical and other properties of crystals (Agra-

n o v i c h, 1979). In this paper, we observed ultrathin dielectric films (the thickness of which does not exceed a dozen atomic planes). Typical representatives of these structures include molecular crystals in which elementary excitations — excitons occur as a result of interaction of external electromagnetic field with electrons of crystal. Using the law of exciton dispersion and their density of states, we can define relative permittivity (by means of theory) and use it to analyze optical properties of the observed system.

MODEL AND METHODS

Effective exciton Hamiltonian in harmonic approximation (A g r a n o v i c h, 1979; M i r j a n i ć, 1982) is given by standard expression in following form:

$$H \quad \vec{n} \quad \vec{n} B_{\vec{n}} B_{\vec{n}} \quad \vec{n},\vec{m} \quad X_{\vec{n}\vec{m}} B_{\vec{n}} B_{\vec{m}}, \tag{1}$$

where $B_{\vec{n}}$ and $B_{\vec{n}}$ represent creation and annihilation exciton operators on the site of crystal lattice, $\vec{n}$ represents energy of isolated exciton on that site, and $X_{\vec{n}\vec{m}}$ are matrix elements of exciton transfer from site $\vec{n}$ to site $\vec{m}$. The model presumes that the energy of excitons on site is 10^2 times higher than the energy of its transfer.

Microtheoretical analysis will be conducted by using the method of two--time temperature Green functions (R i c k a y z e n, 1980; M a h a n, 1990) due to the convenience provided by this method. For that purpose, let us observe Green's function

$$G_{\vec{n}\vec{m}}(t) \quad \left\langle\left\langle B_{\vec{n}}(t) \middle| B_{\vec{m}}(0)\right\rangle\right\rangle,$$

which satisfies the following equation of motion:

$$i\hbar \frac{d}{dt} G_{\vec{n}\vec{m}}(t) \quad i\hbar\delta(t)\delta_{\vec{n}\vec{m}} \quad \vec{n} G_{\vec{n}\vec{m}}(t) \quad \vec{l} \quad X_{\vec{n}\vec{l}} G_{\vec{l}\vec{m}}(t). \tag{2}$$

A form of Green's function in $\vec{k}$ — space can be obtained by full time and space Fourier transform, and later from its pole, wanted exciton dispersion law:

$$\hbar\omega_{\vec{k}} \quad 2(X_x \cos a_x k_x \quad X_y \cos a_y k_y \quad X_z \cos a_z k_z), \tag{3}$$

which, if: $X_x \quad X_y \quad X_z \quad |X|$ and $a_x \quad a_y \quad a_z \quad a$, can be written in non-dimensional form:

$$E_{\vec{k}} \quad \frac{\hbar\omega}{|X|} \quad R_{xy} \quad S_z; \qquad R_{xy} \quad 2(\cos ak_x \quad \cos ak_y); \quad S_z \quad 2\cos ak_z \tag{4}$$

and which can be presented in a graph as in Figure 1.

General expression of relative dielectric permittivity is given by (D z i a - l o s h i n s k i, 1959; P e l e m i š, 2008):

$$\varepsilon^{-1}(\omega) \quad 1 \quad 2\pi i F[G(\omega) \quad G(-\omega)], \tag{5}$$

where F is a an internal structure parameter. Substituting Green's functions in this expression, we obtain an expression for dynamic permittivity of bulk:

$$\varepsilon \quad 1 \quad 2F\frac{\omega_{\vec{k}}}{\omega^2 \quad \omega_{\vec{k}}^2}. \tag{6}$$

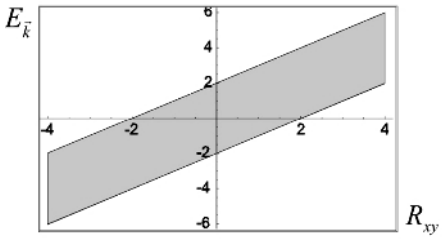

Fig. 1. — Dispersion law of excitons in bulk

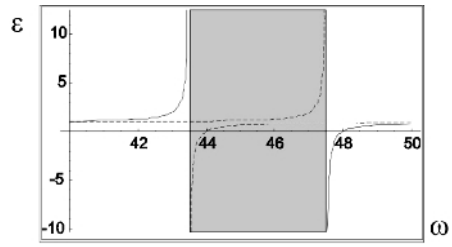

Fig. 2. — Relative permittivity of bulk-crystal

Dependence of that permittivity is shown in Figure 2.

In the case of the bulk, we can see that energies take continuous values, within a certain range of allowed energies, which results in dependence of bulk permittivity, i.e. dielectric response. In other words, absorption zone/interval is formed in which bulk "swallows" all energies (i.e. frequencies of electromagnetic field) of exactly determined ranges.

ULTRATHIN DIELECTRIC FILM

Films present systems, which are bordered/limited by two parallel surfaces (C o t t a m, 1989; Š e t r a j č i ć, 2008). Dimensions of crystal nano-film are such that XY is unlimited, while in z-direction it has final and very small thickness $L = N_a$ ($N < 10$).

We will observe ultrathin (nano) crystal film (Figure 3), which can be practically made by means of controlled reaching of massive dielectric samples (Š e t r a j č i ć, 2008). Due to the existence of border surfaces, the energies of excitons on nodes and transfers of energy within borders ($n_z = 0$; $n_z = N$) and their adjacent planes ($n_z = 1$; $n_z = N - 1$) are perturbed, which can be presented in the following manner (P e l e m i š, 2008; Š e t r a j č i ć, 2008; Š e t r a j - č i ć, J. P., 2008; Š e t r a j č i ć, 2005; M a r k o s k i, 2009).

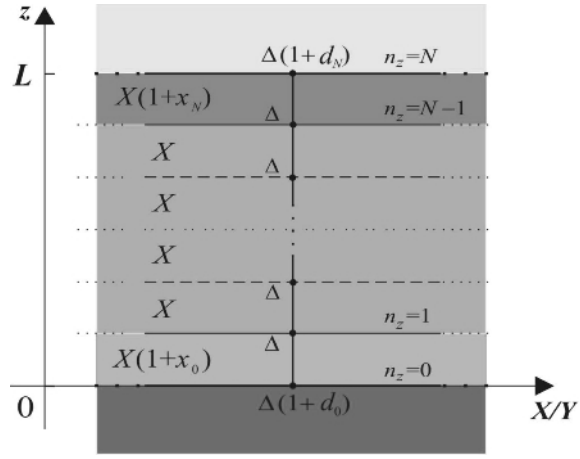

Fig. 3. — Model of dielectric nanofilm

$$\begin{aligned} & \vec{n} \quad 1 \quad d_0\delta_{n_z,0} \quad d_N\delta_{n_z,N})]; \\ X_{\vec{n},\vec{n}\ \vec{\lambda}} & \quad X[1 \quad (x_0\delta_{n_z,0} \quad x_N\delta_{n_z,N\ 1})]; \\ X_{\vec{n},\vec{n}\ \vec{\lambda}} & \quad X[1 \quad (x_0\delta_{n_z,1} \quad x_N\delta_{n_z,N})], \end{aligned} \tag{7}$$

where parameter d defines perturbation on node of border surfaces, and parameter x perturbation of transfer in border layers along z — direction.

Micro theoretical analysis of exciton sub-system in ultrathin (crystal) molecule films can be conducted in the same manner as it is for the bulk — by applying Green's functions. We will observe the same Green's functions and use the same procedure for their determination. Taking into account the border conditions (7) and the expressions for Hamiltonian (1) as well as the equation of motion (2) we derive the equation for requested Green's functions (Šetrajčić, 2008; Šetrajčić, J. P., 2008; Šetrajčić, 2005; Markoski, 2009). After full time, and now only partial spatial Fourier transform of these equations, we obtain the following:

$$\begin{aligned} & G_{n_z,m_z} \quad \rho \quad \frac{}{|X|}(d_0\delta_{n_z,0} \quad d_N\delta_{n_z,N}) \quad G_{n_z\ 1,m_z}[1 \quad (x_0\delta_{n_z,0} \quad x_N\delta_{n_z,N\ 1})] \\ & G_{n_z\ 1,m_z}[1 \quad (x_0\delta_{n_z,1} \quad x_N\delta_{n_z,N})] \quad \frac{i\hbar}{2\ |X|}\delta_{n_z,m_z}, \end{aligned} \tag{8}$$

where the next denotation is introduced:

$$\rho \quad \frac{\hbar\omega}{|X|} \quad 2(\cos ak_x \quad \cos ak_y).$$

An N 1 non-homogenous algebra-differential equations for Green's functions are presented by equation (8). The same as for deriving dispersion laws we need only the poles of these functions, it is sufficient to find the determinant of the system (8) and equalize it with zero (Šetrajčić, 2008; Šetrajčić, J. P., 2008; Šetrajčić, 2005; Markoski, 2009). In this way, we obtain N 1 solutions ρ ρ_v; v 1,2,…,N 1.

Non-dimensional form of exciton dispersion law is chosen again and values of reduced energies are:

$$E_v \quad \frac{\hbar\omega}{|X|} \quad R_{xy} \quad \rho_v ,$$

depending on the function R_{xy} 2 (cosak_x cosak_y).

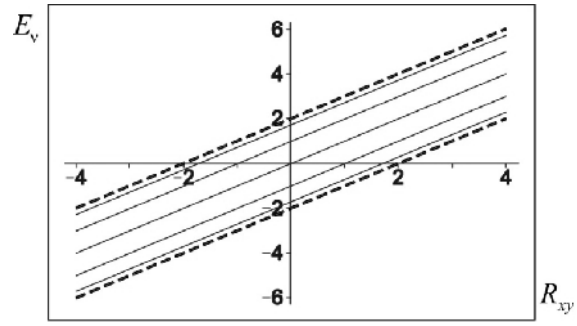

Fig. 4 — The law of dispersion of ideal (d = 0,0; x = 0,0) five-layer film

The dispersion laws are presented in Figures 4 and 5, more precisely: in Figure 4 for non-perturbed (ideal) five-layer film, and in Figures 5 for d- and x- symmetrical perturbed five-layer film, respectively. Full lines present energy levels of excitons in the observed film, while dotted lines determine the borders of continual energy areas of excitons in the bulk. Immediately, one can see the absence of zero energies and discreetness of exciton energies in the film.

The number of possible exciton states corresponds to the number of crystallographic planes of this film along the z-axis. Increased d parameter expands energy area in a way that one or two energy levels move toward higher energies and leave bulk borders, which can be seen in Figure 5. These energy conditions are known as localized or Tamm states (Agranovich, 1979). Based on analysis of Figure 6 it is clear that increased x parameter expands the spectrum and two or four energy levels leave bulk borders, i.e. there is an emergence of localized conditions. Since ideal film structure are analyzed in this work it is clear that applied perturbation is symmetrical and further analysis shows that localized levels overlap. This happens only for d-perturbation, which is decisive/dominant in other cases too. This is illustrated on Figure 5.

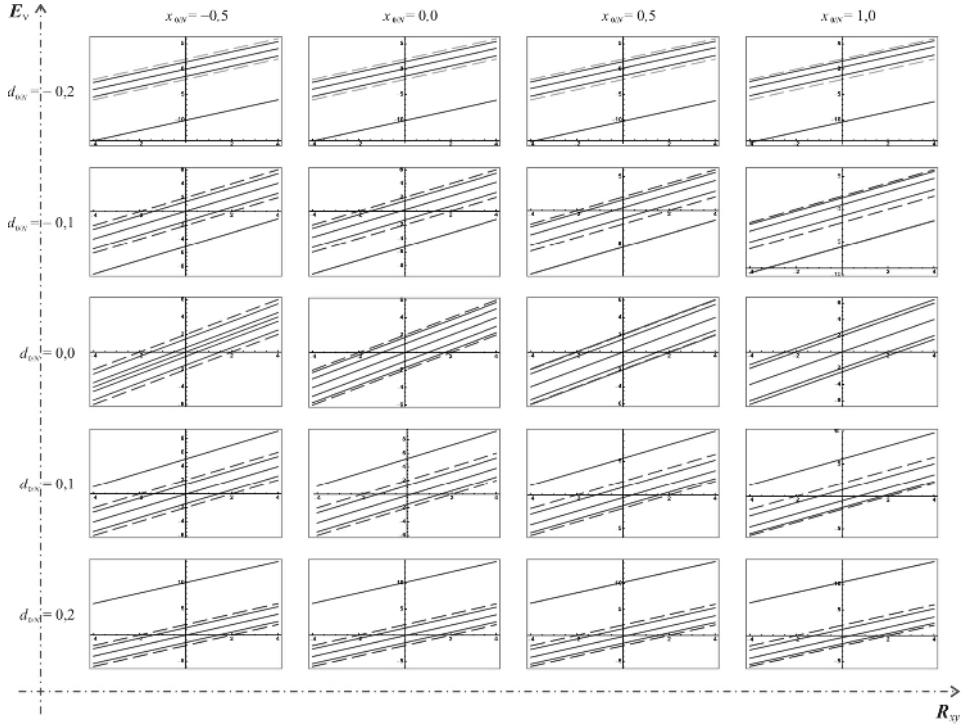

Fig. 5. — Dispersion law of d- and *x*- perturbed five-layer film

Calculating of spectral weights of individual Green's functions is essential for finding the probability of occurrence and spatial distribution of exciton states in the film. The starting point is the system of equations for Green's functions (8), written in matrix form:

$$\hat{D}_{N\ 1}\tilde{G}_{N\ 1} \quad \hat{K}_{N\ 1}, \tag{9}$$

where $\hat{D}_{N\ 1}$ is a matrix which corresponds to the system determinant and $\tilde{G}_{N\ 1}$ and $\hat{K}_{N\ 1}$ are the vectors of Green's function and of Kronecker deltas, respectively. If we act with inverse matrix $\hat{D}^{1}_{N\ 1}$ in (9) from the left side, and knowing that inverse matrix may be expressed in a way of adjunct matrix which members D_{ik} are co-factors of elements d_{ik} of direct matrix, by further calculations we can obtain Green's functions, in which numerators figurate spectral weights $g_{n_z}(\rho_v)$, i.e. probabilities to find exciton states ρ_v (P e l e m i š, 2008; Š e t r a j č i ć, 2008; Š e t r a j č i ć, J. P., 2008; Š e t r a j č i ć, 2005; M a r k o s k i, 2009):

$$G_{n_z} \quad \frac{i\hbar}{2\pi|X|}^{N\ 1}_{v\ 1}\frac{g_{n_z}(\rho_v)}{\rho \quad \rho_v}. \tag{10}$$

For determining dynamic permittivity of film we use general expression (5), but taking strict account that Green's functions, as well as permittivity, depends on (the count, i.e. the position) crystallographic planes of symmetric film n_z:

$$\varepsilon_{n_z}^{1}(\omega) \quad 1 \quad 2\pi i F [G_{n_z}(\omega) \quad G_{n_z}(\ \omega)], \tag{13}$$

where F is the same structural factor as in (5). By incorporating the expression for Green's functions (10), we obtain:

$$\varepsilon_{n_z}^{1}(\omega) \quad 1 \quad \frac{\hbar F}{|X|}^{N\ 1}_{\ v\ 1\ s\ ,} \frac{g_{n_z}^{v}}{\rho_s \quad \rho_v}, \tag{14}$$

where: $\rho \quad \dfrac{\mp \hbar\omega}{|X|} \quad 2(\cos ak_x \quad \cos ak_y)$, and when arranged in analytical way, the expression (14) is transformed to:

$$\varepsilon_{n_z}(\omega) \quad 1 \quad \frac{2\hbar F}{|X|}^{N\ 1}_{\ v\ 1} g_{n_z}^{v} \frac{\rho_v \quad \overline{|X|} \quad 2(\cos ak_x \quad \cos ak_y)}{\dfrac{\hbar\omega}{|X|}^{2} \quad \rho_v \quad \overline{|X|} \quad 2(\cos ak_x \quad \cos ak_y)}^{2}}^{1} \tag{15}$$

This expression represents dependence of relative dynamic permittivity on frequency of initial electromagnetic excitation, i.e. dielectric response of the observed symmetrical molecular film to external electromagnetic field. Dependences of relative dynamic permittivity on reduced energy of external electromagnetic radiation for five-layer film are presented in Figure 6.

All Figures present dependencies of permittivity on reduced frequency of external electromagnetic field for external (border) surfaces, for the first internal and central crystallographic plane. Occurrence of resonant peaks is visible, the count and distribution of which is very much dependent upon border perturbation parameters. Dependences of relative permittivity on parameter D changes by perturbation of d_0 (or d_N) are given in rows of Figure 6. It is clear that the number of resonant peaks (ensuing when $\varepsilon(\omega_r)$) depends on the number, i.e. position, of atomic planes n_z (for which permittivity is calculated), and on the value of parameter $d_{0/N}$. Dominant resonant peak can be seen only at the border surface of the film ($n_z = 0$ or $n_z \quad N = 4$) when this parameter is increased, i.e. when the expanding of the absorption area occurs. This is an expected result, because the analysis of spectral weights of exciton conditions (Š e t r a j č i ć, J. P., 2008) shows that this is exactly the point where probability of finding excitons in localized state is the highest. It is interesting to notice that occurrence of selective resonance peaks is asymmetric in comparing with changes of parameter Δ. That also can be noticed from mirror symmetry of first and last row ($d_{0/N} = -0{,}2$; $d_{0/N} = +0{,}2$), or second and fourth ($d_{0/N} = -0{,}1$; $d_{0/N} = +0{,}1$) in Figure 6.

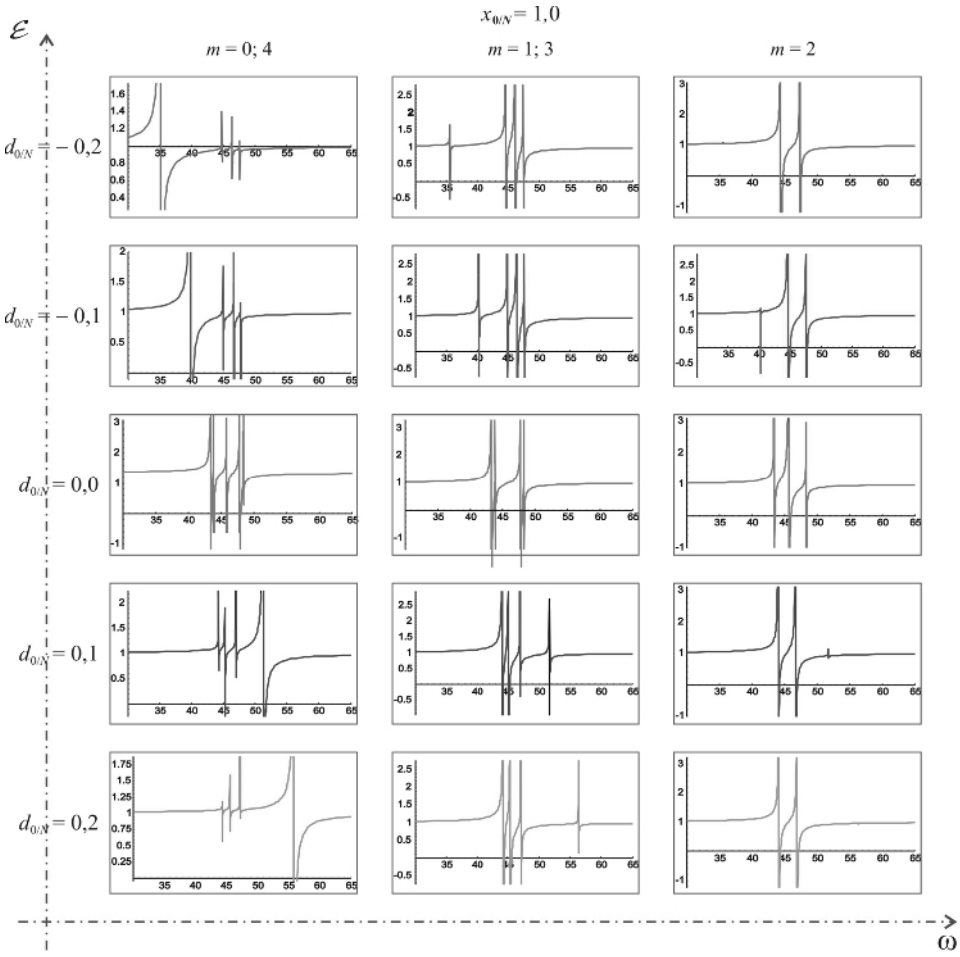

Fig. 6. — Relative permittivity of perturbed five-layer film depending on reduced frequency

Analysis of relative permittivity changes with changes of transfer factor X was conducted in former paper (Š e t r a j č i ć, J. P., 2009), where we showed that influence of parameter d is evidently much "stronger" and that it also "shifts" the whole energy and, selects, optical picture of observed film-structures, while here we focused on extreme case (x = 1,0).

This result may be explained by experimental facts regarding resonating optical peaks in similar molecular layered nanostructures (S c h u s t e r, 2007; C o m b e s c o t, 2008). In papers (F r i e d l e n, 2003; R ö g e r, 2007) this was evident in perylene chemical compounds (PTCDA, PTCS and PTFE) and explained by resonating effects at specific unoccupied levels (D i e n e l, 2008). These effects are manifested by narrow optic absorption in close infrared band. Complete review of optic propertied of PBI in visible band is given in paper (H i p p i u s, 2008).

In comparison with our results, which belong in deeper infrared band in electromagnetic radiation, it may be concluded that these differences are effect of differences in crystalline (chemical and physical) structure of samples investigated. Good agreement in resonating absorption may be attributed and explained by presence of boundary conditions and quantum size effects. Differences in profile of absorption lines appear because in our work we observed only electron line spectra, disregarding oscillatory and rotational contributions. In this regard, here we defined the position of central absorption peak as the resonating line depending on relative permittivity, which depends from external electromagnetic field.

CONCLUSION

Significant differences in dispersion law (microscopic, i.e. quantum properties) and in dielectric response (macroscopic, and also dimension-quantum properties) of excitons between bulk and ultrathin film-structures are presented by this analysis. These differences are consequences of film limitation along the z-direction and existence of perturbation parameters on border surfaces and border layers of the structure. Results of our work can be given in next few terms:

— Discreteness of exciton energy spectra in symmetrical film — number of levels that is equal to the number of film layers.

— Increase in the energy of excitons on border layers, i.e. nodes moves the spectrum toward higher energies, while the increase in energy transfer between border layers and their adjacent internal layers expands the spectrum symmetrically toward higher and lower energies.

— Existence of localized (Tamm's) conditions is very possible, and its probability grows rapidly with the increase of border perturbation parameters.

— Selectivity of dielectric response of the film, i.e. occurrence of discrete resonant absorption peeks at exactly specified energies, the count and distribution of which is directly dependant on the number of the layers in the film and the number of perturbation parameters. These properties give an advantage to the films compared to bulk structures (dielectric response of which is continual in certain energy spans), because in that case the films can be used as certain filters of external radiation.

ACKNOWLEDGMENTS

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СПЕЦИФИЧНОСТИ ФИЗИЧКИХ СВОЈСТАВА МОЛЕКУЛСКИХ КРИСТАЛНИХ НАНОФИЛМОВА

Јован П. Шетрајчић[1], Љубиша Д. Џамбас[2], Стеван Армаковић[1]

[1] Департман за физику, Природно-математички факултет,
Универзитет у Новом Саду, Трг Д. Обрадовића 4,
21000 Нови Сад, Војводина — Србија
[2] Медицински факултет, Департман за стоматологију,
Универзитет у Новом Саду, Хајдук Вељкова 3,
21000 Нови Сад, Војводина — Србија

Резиме

У раду су теоријски истражене и анализиране измене и промене диелектричних својстава, различитих нанофилм молекулских кристала, изазване присуством граница. Дозвољена екситонска стања су нађена аналитичко-нумеричким прорачунима и испитане су њихова просторна расподела дуж осе ограничења (по слојевима) и површинска локализација. Одређена је релативна пермитивност посматраног филма и утицај (пет) граничних параметара на феномен резонантне апсорпције: дискретне (по фреквенцијама) и селективне (по слојевима филма). Пронађени су услови за појављивање једне резонантне апсорпционе линије.

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UDC

*Svetlana Trifunschi**
Dorina Ardelean

Facultatea de Medicină, Farmacie şi Medicină Dentară,
Universitatea de Vest "Vasile Goldiş" din Arad,
Str. Feleacului nr. 1, Arad, Romania
* Author for correspondence: svetlana_cacig@yahoo.com

STUDIES ON THE OPTIMAL EXTRACTION OF FLAVONOIDS FROM THE FRUIT OF *JUNIPERUS VIRGINIANA* L.

ABSTRACT: The isolation and quantitative determination of flavonoid compounds in fruit of *Juniperus virginiana* L. (Cupressaceae) are described. A method for the detection of those flavonoids was high performance liquid chromatography (HPLC). Rutin and kaempferol were determined in accordingly extracts and quercetin only in hydrolysated extracts.

KEY WORDS: flavonoids, extract, Soxhlet extraction, *Juniperus virginiana* L.

INTRODUCTION

Juniperus virginiana L. (red cedar, juniper) which belongs to genus *Juniperus* of family to Cupressaceae, is a species of juniper native to eastern North America, from southeastern Canada to the Gulf of Mexico, east of the Great Plains.

Juniper is used in the treatment of arthritis, asthma, colds, cough, cystitis, diabetes, diarrhea, sore throat, tuberculosis. The berries are being studied for their anticancer and anti-tumor properties. Topical use is applied for acne, burns, dandruff, hemorrhoids, herpes, rheumatism, and warts. In food industry the berries are used in jams, pepper substitutes, beer and gin, and made into a coffee substitute, an also are used in composition of bitter and other energetics.

Juniper fruit contains volatile oil (myrcene, cineole, terpineol, camphen), resin, saccharides, organic acids, ascorbic acid, tannins, vitamins and minerals. Apart from these, juniper fruit contain flavonoids (L e u n g et al., 1996). It was reported that flavonoids could remove $O_2\cdot$ in human bodies, improve blood circulation, and lower blood pressure (F a n g, 1998; L i u et al., 2002; W a n g at al., 1996).

At present, studies on the extraction of flavonoids from juniper fruits have not been reported. In this study, optimum conditions to extract flavonoids from red cedar fruits were studied in order to achieve scientific evidence for the processing and utilizing of juniper fruits.

MATERIAL AND METHODS

Plant Material: The juniper fruits were collected from The Macea Bothanical Garden of West University Vasile Goldiş (Arad, Romania) and were dried at 20°C in dark place.

Solvents and reagents: Quercetin, rutin, and kaempferol were purchased from Sigma-Aldrich and all HPLC-grade solvents were purchased from Merck (Germany). All other chemicals were of analytical grade and were purchased from Chimopar Bucharest.

Extraction and preparation of extracts: The dried and finely ground samples of juniper fruits (10 g each) were extracted with 100 mL solvent for 4 h in a Soxhlet apparatus (C a c i g, 2007). The extracts were concentrated at 15 mL and then stored at 4°C for further analysis. The solvents used were methanol, ethanol, dichlormethane, tetrachlormethane, benzene, and toluene.

HPLC analysis (C a c i g et al., 2006): Flavonoids were measured at 365 nm by a HPLC Agilent 1100. Separation was carried out on a Lichrospher 100-RP-18 column (5 μm, 250 x 4 mm). A gradient elution was performed with eluent acetonitril: water = 1:1. The flow rate was 1 mL/min and the injection volume was 20 mL. Identification of the flavonoids was carried out by comparing their retention times to those of standards.

Hydrolysis conditions: The total amount of each flavonoid in the extracts was determined after hydrolysis of its glycosides by refluxing samples of extract in HCl 25% for 30 min. According procedures (H a s l e r et al., 1990) and its injection into the HPLC. The analytical data of each flavonoid detected were compared with datum of an authentic standard.

RESULTS AND DISCUSSION

Figure 1 shows the structures of the compounds under study.

The content of flavonoid according to HPLC method was calculated as rutin type compound and kaempferol. Results given as rutin varied from 0.2364 mg/mL extract in tetrachlormetane extract to 11.7365 mg/mL extract in methanolic extract of juniper fruit.

Flavonoid content was the higher for methanolic and ethanolic extracts (Table 1).

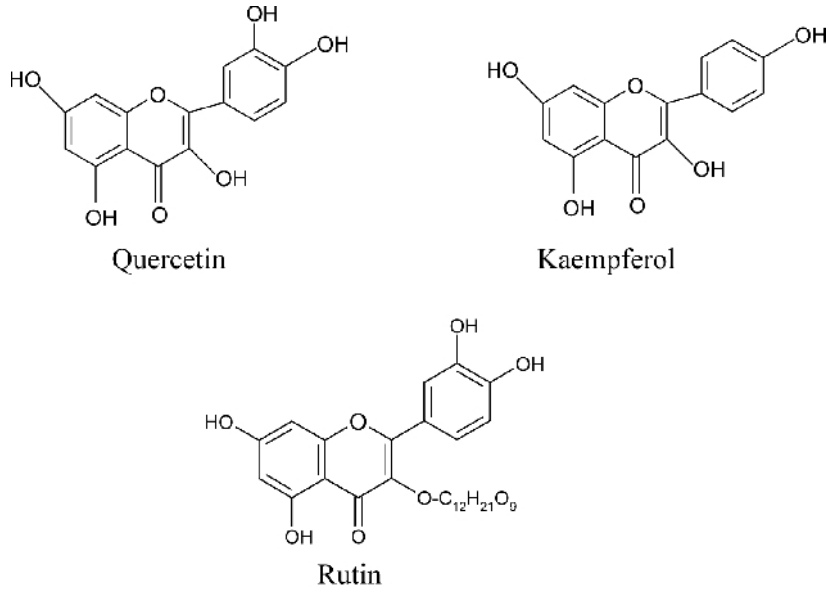


Fig. 1. — The structure of flavonoids analyzed in *Juniperus virginiana* extracts

Tab. 1. — Samples and results of the determination of flavonoids in *Juniperus virginiana* L. by using the HPLC method

Sample	Solvent for extraction	Content mg/mL extract	
		Rutin	Kaempferol
E1	Methanol 94%	11.7365	0.7892
E2	Ethanol 96%	9.0525	trace
E3	Dichlormethane	5.0885	trace
E4	Benzene	4.6880	trace
E5	Toluene	1.8335	trace
E6	Tetrachlormethane	0.2364	trace

At same time flavonoid compound was determined after acid hydrolysis, frequently applied to standardize flavonoid material. Kampferol, rutin and quercetin were used as standards.

Quercetin was found to dominate in alcoholic extracts after acid hydrolysis (Table 2).

Tab. 2. — Results of the quantitative determination of flavonoids in *Juniperus virginiana* L. by using the HPLC method after acid hydrolysis

Sample	Content mg/mL extract	
	Rutin	Quercetin
E7*	0.2460	5.2356
E8**	0.0841	5.6630

* methanolic extract after hydrolisis
** ethanolic extract after hydrolisis

The content of quercetin in other extracts is a much lower quantity than flavonol type compound.

HPLC chromatograms are presented in Figures 2, 3, and 4.

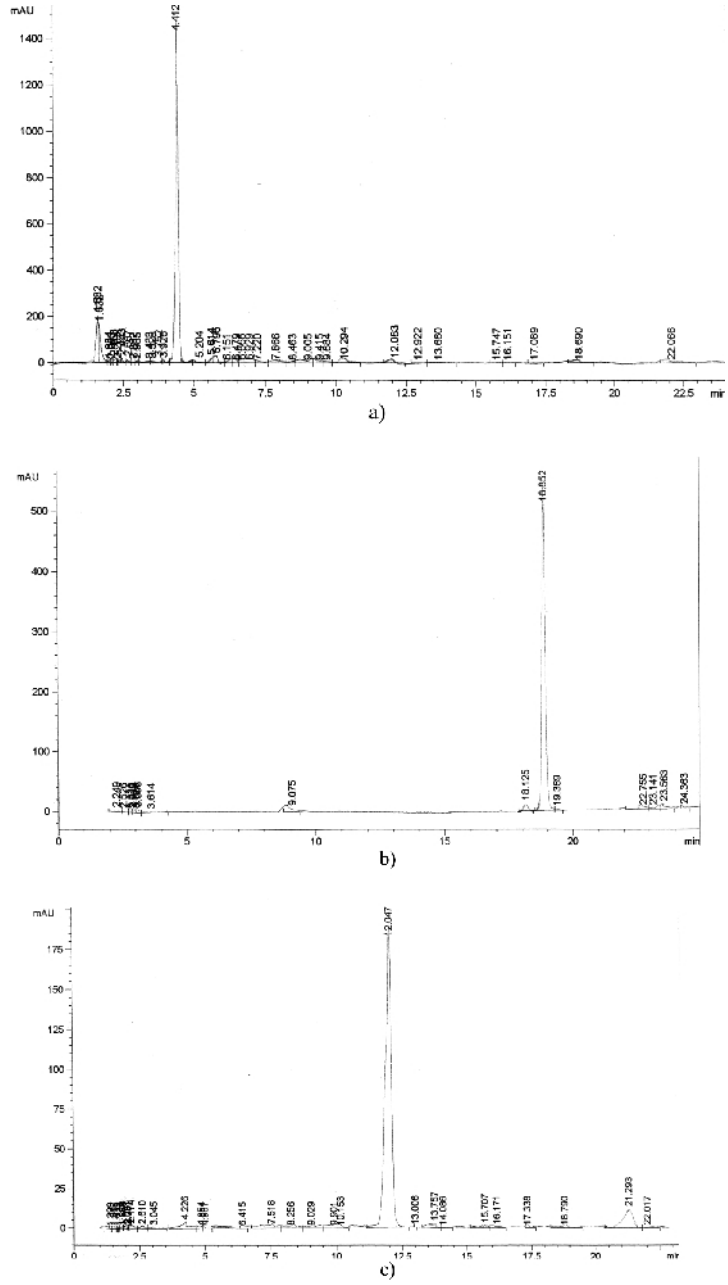
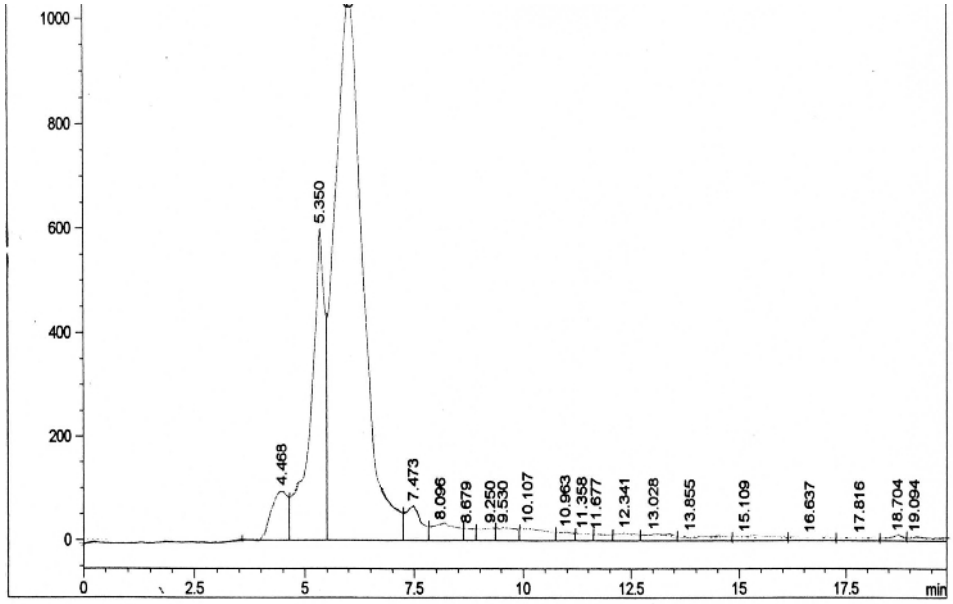
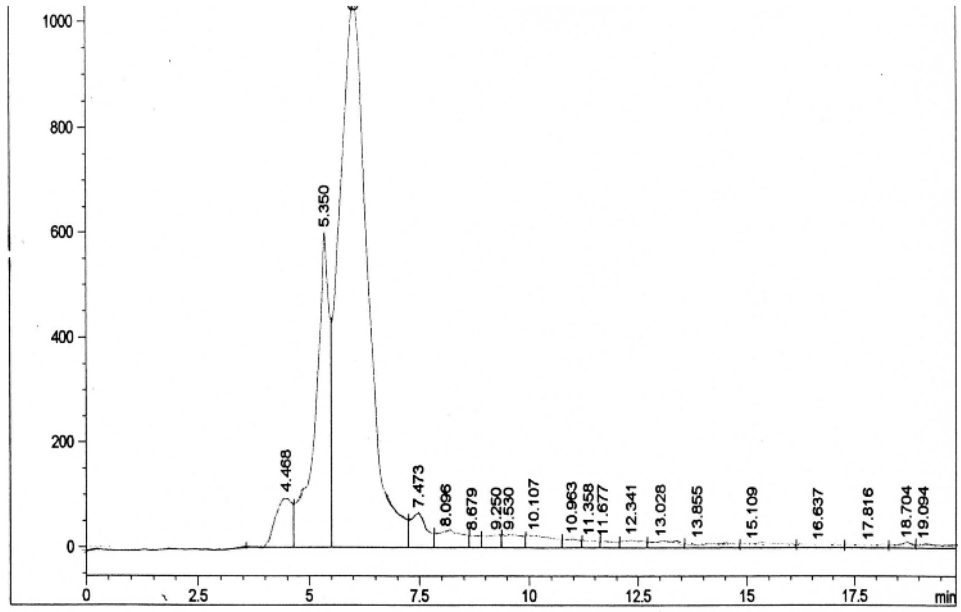


Fig. 2. — Chromatograms of standards: rutin (a), kaemferol (b) and quercetin (c)

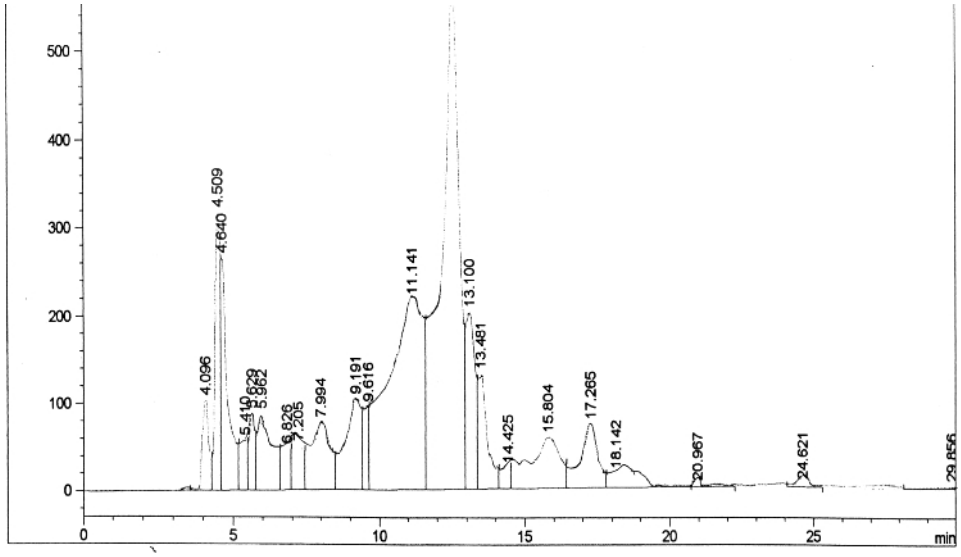


E1

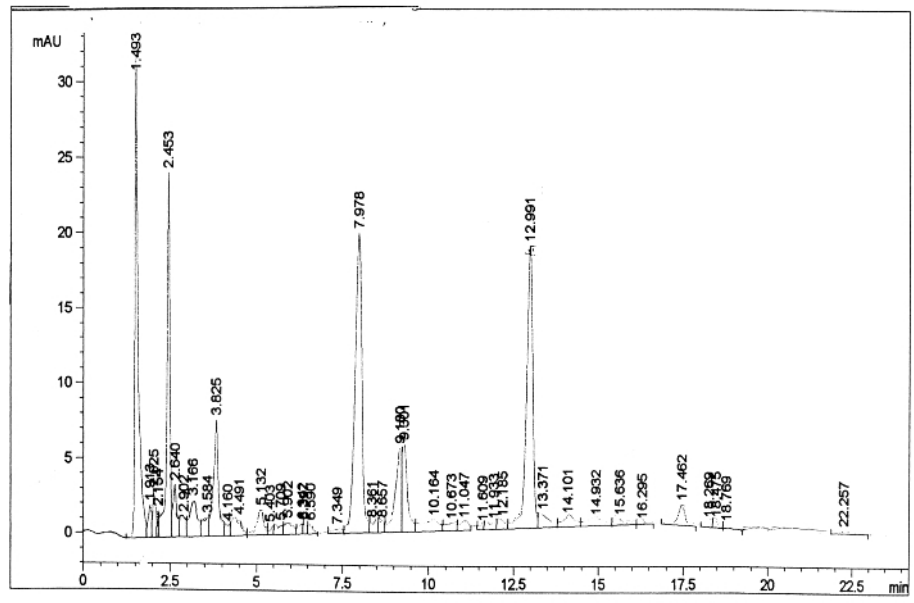


E2

Fig. 3. — Chromatograms of alcoholic extracts (E1, E2)



E7



E8

Fig. 4. — Chromatograms of hydrolisated extracts (E7, E8)

CONCLUSION

The optimum process to extract flavonoids from junipers fruit were obtained, namely extracted for 4 h by using 94% methanol solution in a Soxhlet apparatus with the material ratio of 1:10 (w:v).

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СТУДИЈА ОПТИМАЛНЕ ЕКСТРАКЦИЈЕ ФЛАВОНОИДА ИЗ ПЛОДА *Juniperus virginiana* L.

Светлана Трифунчи*, Дорина Арделеан

Медицински факултет, фармација и стоматологија,
Универзитет „Vasile Goldiș", Арад, ул. Feleacului nr. 1, Арад, Румунија

Резиме

Juniperus virginiana L. која припада генусу *Juniperus* и фамилији Cupressaceae користи се врло често у медицинске сврхе код различитих врста болести, а екстракти из ове биљке имају и антиканцерогено дејство. Активна супстанца у овој биљци су флавоноиди.

У раду су изучавани изолација и квантитативно одређивање флавоноида из плода *Juniperus virginiana* L. (Cupresaceae).

Метода за одређивање фловоноида је хроматографија HPLC.

Кверцентин, који преовладава у алкохолном екстракту, одређен је после хидролизе, а рутин и каемпферол су одређени у различитим екстракционим растварачима (метанол 94%, етанол 96%, дихлорметане, бензен, толуен, тетрахлорметан). Као најбољи растварач показао се метанол 94%.

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