

# **ISIRR 2003**

**VII<sup>th</sup> INTERNATIONAL SYMPOSIUM  
INTERDISCIPLINARY REGIONAL RESEARCH - ISIRR 2003  
HUNGARY – SERBIA & MONTENEGRO – ROMANIA  
Hunedoara, ROMANIA  
25<sup>th</sup> – 26<sup>th</sup>, September, 2003**

**Section IV**

**– USES OF NATURAL RESOURCES IN REGIONS –**

**25<sup>th</sup> September, 2003  
11<sup>00</sup> – 13<sup>00</sup>**

**25<sup>th</sup> September, 2003  
15<sup>00</sup> – 19<sup>00</sup>**

**26<sup>th</sup> September, 2003  
11<sup>00</sup> – 13<sup>00</sup>**

**"SIDERURGISTUL" CLUB - AMPHITHEATER No.1 – HUNEDOARA**

*Ștefan MAKSAY – President & Chairman*

*Gallia BUTNARU – Member*

*Cosmina ANDRĂȘESCU – Member*

**Name of the Authors**

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## SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF COPPER(II), COBALT(II) AND NICKEL(II) COMPLEXES WITH 2-AMINOBENZIMIDAZOLE

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**Abstract:** The preparation and properties of some copper(II), cobalt(II) and nickel(II) complexes with 2-aminobenzimidazole (L) are reported. The synthesized complexes are of the general formula:  $[ML_2Cl_2] \cdot nH_2O$ ;  $M = Cu, Co \text{ or } Ni$ ;  $n = 0 \text{ or } 2$ ). The complexes were characterized by elemental analysis (metal), molar conductivity, magnetic susceptibility measurements and IR spectra. The antimicrobial activity of the benzimidazoles and their complexes were evaluated against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus sp.* and *Saccharomyces cerevisiae*. It is found that some complexes have shown higher antimicrobial activity than ligand.

### **Keywords:**

benzimidazole, complexes, copper(II), cobalt(II), nickel(II), antimicrobial.

## **1. INTRODUCTION**

A wide variety of benzimidazole derivatives have been described for their chemotherapeutic importance. These compounds have shown biological activity against parasites and bacteria. Also, some of them are known to possess antibacterial, antiviral, antiinflammatory, antihypertensive, arteriosclerosis and fungicidal properties. Various benzimidazoles are effective inhibitors of the growth of lactobacilli, vaccinia virus, influenza virus and HIV-virus [2-6,8-12].

In our previous studies [8-11], we also observed that benzimidazole derivatives, as well as their complexes with transition metal ions, have shown considerable biological activity. Following our synthesis and antibacterial works on benzimidazole derivatives and their complexes,

we now report the synthesis, physico-chemical characteristics and antimicrobial activities of copper(II), cobalt(II) and nickel(II) complexes with 2-aminobenzimidazole.

## 2. EXPERIMENTAL

All chemicals used to prepare the complexes were of analytical reagent grade, commercially available from different sources.

### Synthesis of complexes

All the complexes were prepared following the same procedure. A solution of 5mmol  $MCl_2 \cdot 6H_2O$  ( $M = Cu, Co$  or  $Ni$ ) in  $20cm^3$  of EtOH was added into a solution of 10mmol of the 2-aminobenzimidazole (L) in  $20cm^3$  EtOH. The resulting mixture was boiled under reflux on a water bath for about 2h and then cooled. The complexes were separated from the reaction mixture by filtration, washed with EtOH and dried *in vacuo* over  $CaCl_2$ . The yield of the complexes varied in the range 50-55%.

### Measurement methods

Elemental analysis was carried out by standard micromethods. Magnetic susceptibility measurements were made at room temperature using an MSB-MKI magnetic susceptibility balance (Sherwood Scientific Ltd., Cambridge, England). Molar conductivities of freshly prepared  $1 \cdot 10^{-3} mol dm^{-3}$  solutions (DMF) were measured on a Jenway 4010 conductivity meter. Infrared spectra (KBr pellets) were recorded on an Infrared 457 Perkin-Elmer spectrophotometer.

### Antimicrobial investigations

For these investigations the filter paper disc method was applied. Each of the investigated isolates of bacteria were seeded in the tubes with nutrient broth (NB). The seeded NB ( $1cm^3$ ) were homogenized in the tubes with  $9cm^3$  of melted ( $45^\circ C$ ) nutrient agar (NA). The homogenous suspension was poured into Petri dishes.

The discs of filter paper (diameter 5mm) were ranged on cool. After cooling on the formed solid medium,  $2 \cdot 10^{-5} dm^3$  of the investigated compounds were placed with micropipette. After incubation for 24 hours in thermostat at  $25-27^\circ C$ , inhibition (sterile) zone diameters (including disc) were measured and expressed in mm. Inhibition zone diameter over 8mm indicates the tested compound is active against bacteria under investigation. Every test was done in three replications.

The antimicrobial activities of the investigated compounds were tested against four strains of bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus sp.* and *Saccharomyces cerevisiae*). In parallel with antimicrobial investigations of the complexes, ligand was

tested too, as well as the pure solvent. The concentration of each solution was  $5 \cdot 10^{-2} \text{ mol dm}^{-3}$ . Commercial DMF was employed to dissolve the tested samples.

### 3. RESULTS AND DISCUSSION

The elemental analysis of complexes, magnetic moments and molar conductance data are summarized in Table 1.

**Table 1.** Some physical characteristics and analytical data of the complexes

Complex	Mr	Colour	M.p. (°C)	$\mu_{\text{eff}}$ ( $\mu_{\text{B}}$ )	$\lambda_{\text{M}}^*$	Metal Found (Calcd.) %
[CuCl <sub>2</sub> L <sub>2</sub> ].2H <sub>2</sub> O	436.45	green	201	1.83	35.6	14.01 (14.56)
[CoCl <sub>2</sub> L <sub>2</sub> ]	395.83	blue	192	4.57	39.9	14.52 (14.88)
[NiCl <sub>2</sub> L <sub>2</sub> ]	395.60	green	196	3.40	79.1	14.27 (14.84)

\* In DMF,  $1 \text{ mmol dm}^{-3}$  solution at  $25^\circ \text{C}$ ; in  $\text{Scm}^2 \text{mol}^{-1}$

All the complexes are sparingly soluble in common organic solvents such as alcohols or acetone, but highly soluble in dimethylformamide and dimethylsulphoxide. The complexes were synthesized in the reaction of warm ethanolic solution of the  $\text{MCl}_2 \cdot 6\text{H}_2\text{O}$  ( $\text{M} = \text{Cu, Co or Ni}$ ) with L (2-aminobenzimidazole) in a mole ratio 1:2. It should be noticed that the reaction of the all metal ions yielded bis(ligand) complexes.

The molar conductance values of copper(II) and cobalt(II) complexes in DMF solutions, compared with the values of non-electrolytes, are higher, but considerable less than the molar conductances of 1:1 type of electrolytes. It indicates that one coordinated chloride anion has been replaced by DMF molecule. The molar conductances of nickel(II) complexes fall in the range of  $65\text{--}90 \text{ Scm}^2 \text{mol}^{-1}$ . These values correspond to a 1:1 type of electrolytes in DMF, which also indicates a partial substitution of the coordinated chloride with solvent molecules [11].

#### *Magnetic properties*

An indication of the most probable geometric configuration of the synthesized Co(II) complexes may be their colour and magnetic moments (Table 1). Namely, the blue cobalt(II) complexes usually have a tetrahedral configuration. The magnetic moment values (Table 1) of the cobalt(II) complexes are in the expected range ( $4.2\text{--}4.7\mu_{\text{B}}$ ) for tetrahedral stereochemistry [11]. The room temperature effective magnetic moments of the nickel(II) complexes are in the range which also

support their tetrahedral geometry. The room temperature effective magnetic moments of the copper(II) complexes are in the range of  $1.73\text{--}2.20\mu_{\text{B}}$ , which corresponds to one unpaired electron typical for tetrahedral geometry.

#### *Infrared spectra*

The infrared spectra of the ligand exhibit a band at  $3300\text{--}3100\text{cm}^{-1}$ , assigned to  $\nu(\text{NH})$ . The lowering of this band frequency is due to association through the intermolecular hydrogen bonding [7]. The band appearing at about  $1550\text{ cm}^{-1}$  may be assigned to  $\nu(\text{C}=\text{N})$  vibrations [7]. The infrared spectra of the complexes investigated are similar to those of the corresponding ligand. An upward shift ( $10\text{--}15\text{ cm}^{-1}$ ) of  $\nu(\text{C}=\text{N})$  in the IR spectra of the complexes as compared to their values in the free ligand, suggests coordination through pyridine nitrogen of 2-aminobenzimidazole [1]. The other bands in the spectrum of each complex are similar to those in the corresponding ligand spectrum except for slight shifts in their positions and changes in their intensities due to coordination.

The presented results (molar conductivity, magnetic moments and IR spectra) suggest that all Cu(II), Co(II) and Ni(II) complexes are tetrahedral which is realized by participation of the pyridine nitrogen of two organic ligand molecules and two chloride anions, typical for these classes of organic ligands [8-11].

#### *Antimicrobial investigations*

All the complexes were screened for their antimicrobial activities against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus sp.* and *Saccharomyces cerevisiae*. The relevant data are presented in Table 2.

**Table 2.** Antimicrobial activity of the benzimidazole derivatives and their complexes

Compound	<i>Pseudomon. aeruginosa</i>	<i>Staphylococc. aureus</i>	<i>Bacillus sp.</i>	<i>Sacchar. cerevisiae</i>
L	++	Ø	Ø	Ø
$[\text{CuCl}_2\text{L}_2]\cdot 2\text{H}_2\text{O}$	+++	+	+++	Ø
$[\text{CoCl}_2\text{L}_2]$	Ø	+++	+++	+++
$[\text{NiCl}_2\text{L}_2]$	Ø	+	Ø	Ø

- Ø - no activity  
 + - low inhibitory activity  
 ++ - middle inhibitory activity  
 +++ - high inhibitory activity

From the data, it is evident that the most active compounds are copper(II) and cobalt(II) complexes. In the case of the *Sacharomyces*



*cerevisiae* only the cobalt(II) complex is antimicrobial active. On comparing the biological activity of the ligand and its complexes, it was found that some complexes are more effective against the bacteria. From the results, it can be concluded that nickel(II) does not increase the general antibacterial activity of the organic ligand.

The higher activity of the some complexes, as compared to the free ligand, can be understood in terms of the chelation theory. This theory explains that a decrease in the polarizability of the metal could enhance the lipophilicity of the complexes.

#### 4. CONCLUSION

2-aminobenzimidazole with copper(II), cobalt(II) and nickel(II) formed complexes of the general formula  $[ML_2Cl_2] \cdot nH_2O$ ;  $M = Cu, Co \text{ or } Ni$ ;  $L = 2\text{-aminobenzimidazole}$ ;  $n = 0 \text{ or } 2$ ). All the complexes are tetrahedral. The configuration being realized by coordination of the pyridine nitrogen of the two organic ligand molecules and two chloride anions.

The results of antimicrobial investigations indicate that the most active compounds are copper(II) and cobalt(II) complexes. On comparing the biological activity of the ligand and its complexes, it was found that some complexes are more effective against the bacteria. From the results, it can be concluded that nickel(II) does not increase the general antibacterial activity of the organic ligand.

The higher activity of the some complexes, as compared to the free ligands, can be understood in terms of the chelation theory. This theory explains that a decrease in the polarizability of the metal could enhance the lipophilicity of the complexes.

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#### **ACKNOWLEDGMENT**

*These results are the part of project No. 1318 "**Physico-chemical, structural and biological investigations of complex compounds**", which are financial supported by Serbian ministry of science and technologies.*



## **2,2'-DIPHENYL-1-PICRYLHYDRAZYL RADICAL-SCAVENGING ACTIVITY OF DIFFERENT *Teucrium montanum* L. EXTRACTS**

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### **Abstract:**

*The sequential extraction of *Teucrium montanum* L. was realized with five solvents of different polarities (70% methanol, petroleum ether, chloroform, ethyl acetate, n-butanol). The scavenging activity (SA) of obtained extracts was tested by measuring their ability to scavenge stable 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical using electron spin resonance (ESR) spectroscopy. The results demonstrated that the SA depended on the type and concentration of applied extracts. In investigated range of concentrations (0.10-0.15 mg/mL) petroleum ether and chloroform extracts did not show any SA. Other extracts exhibited SA in the following order: n-butanol > methanol > ethyl acetate > water extracts. The antioxidant properties were in correlation with the contents of total phenolic compounds (0-296 mg/g) in investigated extracts.*

*The investigated *Teucrium montanum* L. extracts probably had the SA due to the hydrogen donor ability of the flavonoids (quercetin, rutin) and phenolic acids (chlorogenic, caffeic, gallic, ellagic) which were identified by TLC. Another mechanism is "scavenging" activity (one DPPH<sup>•</sup> molecule forms complex with one aryl radical formed from phenolic compounds).*

### **Keywords:**

*Teucrium montanum* L. extracts; polyphenols; scavenging activity; DPPH; ESR; TLC.

## **1. INTRODUCTION**

Nowadays there is an increasing interest in antioxidant activity of phytochemicals present in the diet. Antioxidants are believed to play a very important role in the body defence system against reactive free radical species, which are harmful by products generated during normal cell aerobic respiration [1].

Among the recognized antioxidants (vitamins C and E, carotenoids, tocopherols, etc.) there is an extensive family of diverse components that are found in all food-stuffs of plant origin, known generally as polyphenolic compounds [2]. Herbals and especially herbal extracts, which contain different classes of polyphenols, such as

flavonoids and phenolic acids, are very attractive not only for the modern phytotherapy but also for food industry.

*Teucrium montanum* L. (mountain germander) is a grass crop that has long been consumed both as an herbal medicine and as a nourishing food. It is widely used as diuretic, stomachic, analgesic and antispasmodic agent and also possesses antibacterial, antifungal, antiinflammatory and antioxidative activity. From the aerial parts of *Teucrium montanum* L. diterpenoids 19-acetylnaphthalin, montanin B, D, E, teubotrin (teulamifin B), flavone cirsiolol, *neo-clerodane* diterpenoid, montanin H (4 $\alpha$ , 18:15, 16-diepoxy-6 $\alpha$ -hydroxy-7-keto-*neo-cleroda*-13(16), 14-diene, -12 $\xi$ -acetoxy, 20R, 19-hemiacetal), have been isolated [3].

The task of this study is to investigate the free-radical scavenging activity of methanol, petroleum ether, chloroform, ethyl acetate, *n*-butanol and water extracts of *Teucrium montanum* L. on stable 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical by ESR spectroscopy.

## 2. EXPERIMENTAL

Methanol, ethyl acetate, petroleum ether, chloroform, *n*-butanol, formic acid and acetic acid were purchased from "Zorka" Šabac. 2,2'-Diphenyl-1-picrylhydrazyl, DPPH, quercetin, rutin, chlorogenic acid, caffeic acid, ellagic acid and gallic acid were from Sigma Chemicals Co., USA. Folin-Ciocalteu reagent was from Fluka, USA.

*Teucrium montanum* L. was collected from the region of Zlatibor.

**Extraction.** Dried plant of *Teucrium montanum* L. (20 g) was extracted with 70 % methanol (2 x 500 mL) at room temperature for 2 x 24 h. 20% v/v of obtained extract was evaporated to dryness under reduced pressure and used further as methanol extract. The rest (80 % v/v) of the extract was concentrated under reduced pressure. After removing methanol, the extract was successively treated with petroleum ether (2 x 20 mL), chloroform (2 x 20 mL), ethyl acetate (2 x 20 mL) and *n*-butanol (2 x 20 mL). The petroleum ether, chloroform, ethyl acetate, *n*-butanol and remained water extract were evaporated to dryness under reduced pressure. The yields of extracts were: methanol, *m* = 0.8488 g; petroleum ether, *m* = 0.1195 g; chloroform, *m* = 0.1554 g; ethyl acetate, *m* = 0.1065 g; *n*-butanol, *m* = 1.1132 g and water, *m* = 2.2759 g.

**Total Phenolic Content (TPh).** Total phenolic compounds in extracts were determined spectrophotometrically using the Folin-Ciocalteu reagent. The results are expressed as chlorogenic acid equivalents per g dry weight [4].

**Thin-layer chromatography (TLC).** Thin-layer chromatography was performed on 20 x 20 cm plates precoated with microcrystalline cellulose (Camag, Muttanez, Switzerland). A volume of 1  $\mu$ L of 1% of methanolic solutions of standards and investigated extracts was spotted on the plates. Analysis was performed with ethyl acetate : formic acid : acetic acid : water in volume ratio 100:11:11:26 as mobile phase. Spots were observed by spraying with 1% FeCl<sub>3</sub>.

**DPPH radical assay.** A volume of *x*  $\mu$ L of 1% of the methanolic solutions of investigated extracts was added to (200-*x*)  $\mu$ L methanol and 600  $\mu$ L 0.4 mM methanolic solution of DPPH. The final concentrations of the investigated extracts were: 0.1, 0.125 and 0.15 mg/mL. After that the mixture was stirred for 2 min., transferred to a quartz flat cell ER-160FT and analysed by ESR spectroscopy. Blank probe was

obtained by mixing the 600  $\mu\text{L}$  0.4 mM methanolic solution of DPPH and 200  $\mu\text{L}$  of methanol.

The scavenging activity (SA) of extracts was defined as:

$$\text{SA} = \frac{100\%(h_0 - h_x)}{h_0} \quad (1)$$

where:

$h_0$  - the height of the second peak in the ESR spectrum of DPPH radical of the blank

$h_x$  - the height of the second peak in the ESR spectrum of DPPH radical in reaction mixture with the addition of the extracts

The ESR spectra were recorded on a Bruker 300E ESR spectrometer (Rheinstetten, Germany) under the following conditions: field modulation 100.00 kHz, modulation amplitude 0.226 G, time constant 40.96 ms, conversion time 671.089 ms, centre field 3440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW, temperature 23°C.

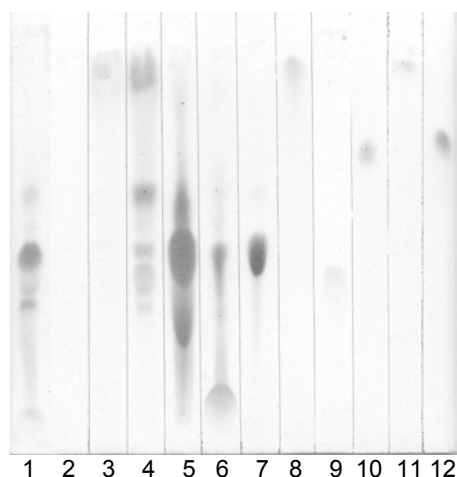
### 3. RESULTS AND DISCUSSION

**Total Phenolic Content.** The TPh values of different *Teucrium montanum* L. extracts are shown in Table 1. n-Butanol extract had the highest phenolic content (296 mg/g). Petroleum ether did not extract any of the phenolic compounds. This fact is in correlation with polarity of the solvents used for extraction and solubility of phenolic compounds in them.

**Table 1.** Total Phenolic Content in *Teucrium montanum* L. extracts

Extract	Total Phenolic Content ( mg/g )
Methanol	154
Petroleum ether	0
Chloroform	0.0956
Ethyl acetate	20.4
n-Butanol	296
Water	59.8

**Thin-layer chromatography (TLC).** Qualitative characterization of TLC chromatogram was performed comparing the  $R_f$  values and colour (green/grey) of separated components of the examined extracts and the standard compounds (Fig. 1.). Methanol, n-butanol and ethyl acetate extract were very rich in phenolic compounds. n-Butanol extract had chlorogenic, gallic acid, rutin and ellagic acid. Ethyl acetate extract contained quercetin, caffeic, chlorogenic, gallic acid, rutin and ellagic acid. In methanol extract the same substances were detected. Water extract contained rutin and ellagic acid. The smallest amount of phenolic compounds was detected in petroleum ether (quercetin) and chloroform (quercetin and caffeic acid) extract. Also, some other unidentified substances were spotted.



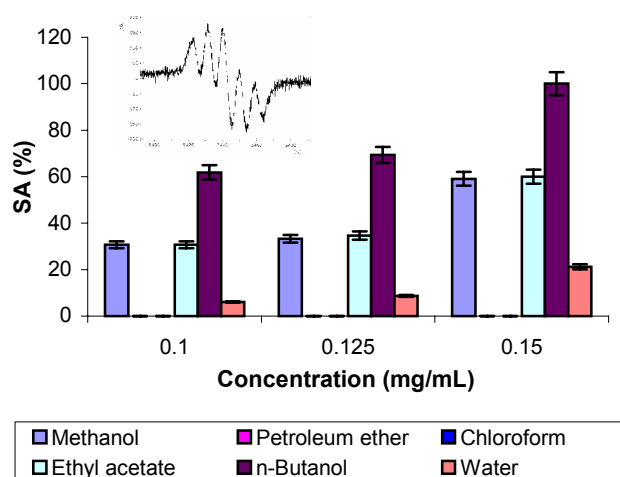
**Fig. 1.** TLC chromatogram of *Teucrium montanum* L. extracts

1 - methanol extract, 2 - petroleum ether extract, 3 - chloroform extract, 4 - ethyl acetate extract, 5 - *n*-butanol extract, 6 - water extract, 7 - rutin, 8 - quercetin, 9 - ellagic acid, 10 - chlorogenic acid, 11 - caffeic acid, 12 - gallic acid

**DPPH radical scavenging activity of extracts.** Most of the methods described in the bibliography for determining antioxidant activity are based on the study of a reaction in which a free radical is generated and how this reaction is inhibited by the addition of the sample that is object of the measurement of antioxidant power. In this paper we used direct, rapid, simple and reliable method - DPPH method in combination with ESR spectroscopy.

The ESR spectra of DPPH radicals in the blank and in probes with investigated extracts were characterized by their five lines of relative intensities 1:2:3:2:1 and hyperfine splitting constant  $a_N=9.03$  G (Fig. 2).

The scavenging activity (SA) of different concentrations of methanol, petroleum ether, chloroform, ethyl acetate, *n*-butanol and water extracts of *Teucrium montanum* L. on DPPH radicals is evident from Fig. 2.



**Fig. 2.** The scavenging activity (SA) of different concentrations of methanol, petroleum ether, chloroform, ethyl acetate, *n*-butanol and water extracts of *Teucrium montanum* L. on DPPH radicals. Values are means  $\pm$ SD of three independent experiments.

On the basis of the obtained results it is evident that the SA on DPPH radicals depended on the type and concentrations of the investigated extracts.

n-Butanol extract had the strongest effect and also exhibit very rapid increase of SA in a narrow range of concentrations. n-Butanol extract scavenged 61.85%, 69.36% and 100% DPPH radicals at concentrations 0.10 mg/mL, 0.125 mg/mL and 0.15 mg/mL, respectively. The same concentrations of ethyl acetate and methanol extract had similar activities, but lower than n-butanol extract, ranging from 30.64% to 60% and 30.64% to 59.09%, respectively. The water extract exhibited much lower SA, from 6.06% to 21.21%. SA of petroleum ether and chloroform extract was not evident at any of tested concentrations..

The antioxidant properties (Fig.2) were in correlation with the contents of total phenolic compounds (Table 1).

Natural phytochemicals such as phenolic compounds found in numerous herbs, commonly involve an aromatic ring as a part of the molecular structure, with one or more hydroxyl groups. They can act as antioxidants as their extensive conjugated  $\pi$ -electron systems allow ready donation of electrons or hydrogen atoms from the hydroxyl moieties to free radicals. The less reactive aroxyl radicals were obtained during this reaction, and stabilized their structure by electron delocalization (forming aryl radicals) [5,6].

The antiradical efficiencies based on this mechanism are typical for different phenolic acids (chlorogenic, syringic, gallic and ellagic acid) and flavonoids (possessing two or three hydroxyl groups on the carbon in the B or C ring of the molecules), whose presence was proved in *Teucrium montanum* L extracts. Another mechanism proposed for SA of extracts on DPPH radicals is "scavenging" activity (one DPPH<sup>•</sup> molecule forms complex with one aryl radical).

#### 4. CONCLUSION

The antioxidant properties of *Teucrium montanum* L. extracts were assessed by electron spin resonance (ESR) spectroscopy.

SA depended on the type and concentration of applied extracts.

In investigated range of concentrations (0.10-0.15 mg/mL) petroleum ether and chloroform extracts did not show any SA.

Other extracts exhibited SA in the following order: n-butanol > methanol > ethyl acetate > water extracts.

Antioxidant properties were in correlation with the contents of total phenolic compounds (0-296 mg/g) in investigated extracts.

The investigated *Teucrium montanum* L. extracts probably had the SA due to the hydrogen donor ability of the flavonoids (quercetin, rutin) and phenolic acids (chlorogenic, caffeic, gallic, ellagic) which were identified by TLC. Another mechanism was "scavenging" activity (one DPPH<sup>•</sup> molecule forms complex with one aryl radical formed from phenolic compounds).

#### Acknowledgement

This research is a part of the project "Biologically Active Constituents of Growing wild Plants as Natural Sources for Pharmacy, Cosmetics and Foodstuff Industry" (Project No.1862), which is financially supported by the Ministry of Science, Technologies and Development of the Republic of Serbia.

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## PHYSICO-CHEMICAL CHARACTERISATION AND BIOLOGICAL ACTIVITY OF ZINC(II) COMPLEXES WITH 2-AMINO AND 2-METHYLBENZIMIDAZOLE DERIVATIVES

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**Abstract:** *The preparation and characteristics of some zinc(II) complexes with 2-amino and 2-methylbenzimidazole derivatives are reported. The synthesized complexes are of the general formula:  $[ZnL_2Cl_2] \cdot nH_2O$ ; (L=2-aminobenzimidazole, 1-benzyl-2-aminobenzimidazole, 1-(4-methylbenzyl)-2-aminobenzimidazole, 2-methylbenzimidazole, 1-benzyl-2-methylbenzimidazole or 1-(4-methylbenzyl)-2-methylbenzimidazole; n=0, 0.5 or 1). The complexes were characterized by elemental analysis, molar conductivity and IR spectra. The antimicrobial activity of the benzimidazoles and their complexes were evaluated against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus* sp., *Saccharomyces cerevisiae* and *Sarcina lutea*. It is found that amino group at position 2 of the benzimidazole ring increases the general antibacterial activity of the relevant benzimidazoles.*

**Keywords:** benzimidazole, complexes, zinc(II), biological activity.

### 1. INTRODUCTION

Benzimidazole and its derivatives have received much attention because of their biological activity and commercial application. They are present in many naturally occurring products and various drugs. Some of these compounds have antibacterial, antifungal, antiviral, antiinflammatory, antihypertensive, arteriosclerosis and anti-HIV activities [3-6,8-11]. Recently, a class of compounds which have a benzimidazole nucleus were reported as a new group of antitumor agents [2].

Considerable attention is being paid in recent years to the study of transition metal complexes of benzimidazoles and related ligands because of their biological significance and interesting spectral, magnetic and structural aspects. We have reported earlier the isolation and

characterisation of different metal ion complexes with 2-substituted benzimidazoles [8-11]. A similar study of physico-chemical characteristics and antimicrobial activities of zinc(II) complexes with 2-amino and 2-methylbenzimidazole derivatives is being report here.

## 2. EXPERIMENTAL

All chemicals used to prepare the complexes were of analytical reagent grade, commercially available from different sources.

### Synthesis of complexes

All the complexes were prepared following the same procedure. A solution of 2.5mmol  $\text{ZnCl}_2$  in  $10\text{cm}^3$  of EtOH was added into a solution of 5mmol of the ligand (L) ( $\text{L}^1$ =2-aminobenzimidazole,  $\text{L}^2$ =1-benzyl-2-aminobenzimidazole,  $\text{L}^3$ =1-(4-methylbenzyl)-2-aminobenzimidazole, 2-methylbenzimidazole,  $\text{L}^4$ =2-methylbenzimidazole,  $\text{L}^5$ =1-benzyl-2-methylbenzimidazole or  $\text{L}^6$ =1-(4-methylbenzyl)-2-methylbenzimidazole) in  $10\text{cm}^3$  EtOH. The resulting mixture was boiled under reflux on a water bath for about 2h and then cooled. The complexes were separated from the reaction mixture by filtration, washed with EtOH and dried *in vacuo* over  $\text{CaCl}_2$ . The yield of the complexes varied in the range 45-50%.

### Measurement methods

Elemental analysis was carried out by standard micromethods. Molar conductivities of freshly prepared  $1 \cdot 10^{-3} \text{mol dm}^{-3}$  solutions (DMF) were measured on a Jenway 4010 conductivity meter. Infrared spectra (KBr pellets) were recorded on an Infrared 457 Perkin-Elmer spectrophotometer.

### Antimicrobial investigations

For these investigations the filter paper disc method was applied. Each of the investigated isolates of bacteria were seeded in the tubes with nutrient broth (NB). The seeded NB ( $1\text{cm}^3$ ) were homogenized in the tubes with  $9\text{cm}^3$  of melted ( $45^\circ\text{C}$ ) nutrient agar (NA). The homogenous suspension was poured into Petri dishes.

The discs of filter paper (diameter 5mm) were ranged on cool. After cooling on the formed solid medium,  $2 \cdot 10^{-5} \text{dm}^3$  of the investigated compounds were placed with micropipette. After incubation for 24 hours in thermostat at  $25-27^\circ\text{C}$ , inhibition (sterile) zone diameters (including disc) were measured and expressed in mm. Inhibition zone diameter over 8mm indicates the tested compound is active against bacteria under investigation. Every test was done in three replications.

The antimicrobial activities of the investigated compounds were tested against four strains of bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus sp.*, *Saccharomyces cerevisiae* and *Sarcina lutea*). In parallel with antimicrobial investigations of Zn(II) complexes, all ligands were tested too, as well as the pure solvent. The concentration of each solution was  $5 \cdot 10^{-2} \text{ mol dm}^{-3}$ . Commercial DMF was employed to dissolve the tested samples.

### 3. RESULTS AND DISCUSSION

The elemental analysis of complexes and molar conductance data are summarized in Table 1.

**Table 1.** Some physical characteristics and analytical data of the complexes

Complex	Mr	Colour	M.p. (°C)	$\lambda_M^*$	Metal Found (Calcd.) %
$[\text{Zn}(\text{L}^1)_2\text{Cl}_2]$	402.28	white	199	5.4	15.98 (16.25)
$[\text{Zn}(\text{L}^2)_2\text{Cl}_2]$	582.28	rose	195	34.2	10.97 (11.23)
$[\text{Zn}(\text{L}^3)_2\text{Cl}_2]$	610.28	white	210	5.6	10.08 (10.71)
$[\text{Zn}(\text{L}^4)_2\text{Cl}_2]$	400.28	white	220	4.4	15.87 (16.33)
$[\text{Zn}(\text{L}^5)_2\text{Cl}_2] \cdot 0.5\text{H}_2\text{O}$	589.28	white	212	37.5	10.57 (11.09)
$[\text{Zn}(\text{L}^6)_2\text{Cl}_2] \cdot \text{H}_2\text{O}$	626.28	white	218	14.4	9.96 (10.44)

\* In DMF,  $1 \text{ mmol dm}^{-3}$  solution at  $25^\circ \text{C}$ ; in  $\text{Scm}^2 \text{mol}^{-1}$

All the complexes are sparingly soluble in common organic solvents such as alcohols or acetone, but highly soluble in dimethylformamide and dimethylsulphoxide. The complexes were synthesized in the reaction of warm ethanolic solution of the  $\text{ZnCl}_2$  with  $\text{L}^1$ ,  $\text{L}^2$ ,  $\text{L}^3$ ,  $\text{L}^4$ ,  $\text{L}^5$  or  $\text{L}^6$  in a mole ratio 1:2. It should be noticed that the reaction of all the ligands yielded bis(ligand) complexes.

The molar conductances values of  $\text{L}^1$ ,  $\text{L}^3$ ,  $\text{L}^4$  and  $\text{L}^6$  complexes in DMF solutions fall in the range  $4.4\text{--}14.4 \text{ Scm}^2 \text{mol}^{-1}$  (Table 1). The values indicate that the complexes behave as non-electrolytes in DMF [11]. The molar conductances of  $[\text{Zn}(\text{L}^2)_2\text{Cl}_2]$  and  $[\text{Zn}(\text{L}^5)_2\text{Cl}_2] \cdot 0.5\text{H}_2\text{O}$  in DMF solutions, compared with the values of non-electrolytes are increased. The same values are considerably less compared with the molar conductances of the 1:1 type electrolytes ( $\lambda_M = 65\text{--}90 \text{ Scm}^2 \text{mol}^{-1}$ ) [11], which indicates the partial substitution of coordinated anions with solvent molecules.

### *Infrared spectra*

The infrared spectra of some ligands ( $L^1$ ,  $L^2$  and  $L^3$ ) exhibit band at  $3450\text{--}3330\text{cm}^{-1}$  and ca.  $1650\text{cm}^{-1}$ , assigned to  $\nu(\text{NH}_2)$  and  $\delta(\text{NH}_2)$  of the benzimidazole ring, respectively [7]. The band appearing at about  $1550\text{cm}^{-1}$ , for all the ligands, may be assigned to  $\nu(\text{C}=\text{N})$  vibrations [7]. Substituted phenyl group shows ring vibrations at  $1485$  and  $740\text{ cm}^{-1}$ . The infrared spectra of the complexes investigated are similar to those of the corresponding ligands.

An upward shift ( $5\text{--}15\text{cm}^{-1}$ ) of  $\nu(\text{C}=\text{N})$  in the IR spectra of the complexes as compared to their values in the free ligands, suggests coordination through pyridine nitrogen of benzimidazoles [1]. The bands due to  $\nu(\text{NH}_2)$  and  $\delta(\text{NH}_2)$  in the complexes are shifted to lower frequency in all the complexes. These shifts may be indicative of present hydrogen bonding [1]. The other bands in the spectrum of each complex are similar to those in the corresponding ligand spectrum except for slight shifts in their positions and changes in their intensities due to coordination.

The presented results (molar conductivity and IR spectra) suggest that all the complexes are tetrahedral which is realized by participation of the pyridine nitrogen of two organic ligand molecules and two chloride anions.

### *Antimicrobial investigations*

All the complexes were screened for their antimicrobial activities against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus sp.*, *Saccharomyces cerevisiae* and *Sarcina lutea*. The relevant data are presented in Table 2.

From the data, it is evident that the most active ligand is 1-(4-methylbenzyl)-2-aminobenzimidazole ( $L^3$ ), as well as its zinc(II) complex ( $[\text{Zn}(\text{L}^3)_2\text{Cl}_2]$ ). In the case of the 2-methylbenzimidazole derivatives all the compounds are not antimicrobial active, except 1-(4-methylbenzyl)-2-methylbenzimidazole ( $L^6$ ) and its zinc(II) complex. On comparing the biological activity of the ligands and their complexes, it was found that some complexes are more effective against the bacteria. The higher activity of the complexes, as compared to the free ligands, can be understood in terms of the chelation theory. This theory explains that a decrease in the polarizability of the metal could enhance the lipophilicity of the complexes.

From the results, it can be concluded that amino group at position 2 of the benzimidazole ring, as well as methyl group at position 4 of the benzyl substituent increase the general antibacterial activity of the relevant benzimidazoles.

**Table 2.** Antimicrobial activity of the benzimidazole derivatives and their complexes

Compound	<i>Pseud. aerugin.</i>	<i>Staph. aureus.</i>	<i>Bacillus sp.</i>	<i>Sacch. cerevis</i>	<i>Sarcina lutea</i>
L <sup>1</sup>	+	Ø	Ø	Ø	Ø
[Zn(L <sup>1</sup> ) <sub>2</sub> Cl <sub>2</sub> ]	+++	+++	+++	+++	++
L <sup>2</sup>	+/-	++	++	+/-	+/-
[Zn(L <sup>2</sup> ) <sub>2</sub> Cl <sub>2</sub> ]	+++	+++	+++	Ø	++
L <sup>3</sup>	+++	+++	+++	+/-	+++
[Zn(L <sup>3</sup> ) <sub>2</sub> Cl <sub>2</sub> ]	+++	+++	++++	+++	++++
L <sup>4</sup>	+/-	Ø	Ø	Ø	Ø
[Zn(L <sup>4</sup> ) <sub>2</sub> Cl <sub>2</sub> ]	+	Ø	Ø	Ø	Ø
L <sup>5</sup>	+/-	Ø	Ø	Ø	Ø
[Zn(L <sup>5</sup> ) <sub>2</sub> Cl <sub>2</sub> ]·0.5H <sub>2</sub> O	+/-	Ø	Ø	Ø	Ø
L <sup>6</sup>	++	++	++	Ø	+++
[Zn(L <sup>6</sup> ) <sub>2</sub> Cl <sub>2</sub> ]·H <sub>2</sub> O	+++	+++	++	Ø	+++

- Ø - no activity  
 + - low inhibitory activity  
 ++ - middle inhibitory activity  
 +++ - high inhibitory activity  
 ++++ - very high inhibitory activity

#### 4. CONCLUSION

Zinc(II) with 2-aminobenzimidazole, 1-benzyl-2-aminobenzimidazole, 1-(4-methylbenzyl)-2-aminobenzimidazole, 2-methylbenzimidazole, 1-benzyl-2-methylbenzimidazole and 1-(4-methylbenzyl)-2-methylbenzimidazole formed complexes of the general formula [ZnL<sub>2</sub>Cl<sub>2</sub>]·nH<sub>2</sub>O; n=0, 0.5 or 1). All the complexes have a tetrahedral configuration, which is realized by coordination through pyridine nitrogen of one or two organic ligands and two chloride anions.

The results of antimicrobial investigations indicate that the most active ligand is 1-(4-methylbenzyl)-2-aminobenzimidazole (L<sup>3</sup>), as well as its zinc(II) complex. On comparing the biological activity of the ligands and their complexes, it was found that some complexes are more effective against the bacteria. From the results, it can be concluded that amino group at position 2 of the benzimidazole ring, as well as methyl group at position 4 of the benzyl substituent increase the general antibacterial activity of the relevant benzimidazoles.

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### ACKNOWLEDGMENT

These results are the part of project No. 1318 "**Physico-chemical, structural and biological investigations of complex compounds**", which are financial supported by Serbian ministry of science and technologies.



## **PRESENT STATE OF FLOOD CONTROL AND WATER STREAMS IMPROVEMENT IN VOIVODINA**

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### **Abstract:**

*Historical development of flood control and regulation of water streams are considered as a set of constructions, activities and measures yielding a decrease of unfavourable effects (damages) caused by floods, as well as a planned water resources management, and environmental protection. To advance further the functioning of the flood control system, some additional measures of the investment and noninvestment character have been proposed on the basis of techno-economical and ecological analyses. At that, some specificities of Voivodina have to be taken into account, and especially the unfinished reconstruction and inadequate maintenance of the existing control systems.*

### **Keywords:**

*Flood, protection, control, mitigation, river restoration.*

## **1. INTRODUCTION**

As is the case with many engineering undertakings, in the domain of water streams regulation, the decisions about acceptance of the majority of projects have been mainly made on the basis of the results of techno-economical analyses in which the parameters of technical reliability, functionality and economy of the proposed solution are of the crucial importance. However, the awareness of the increasing risk to the environment in general suggests that all the engineering projects, including the projects of river improvement for the control of floods, have also to take into account the parameters concerning environmental protection and its advancement [7, 9]. In fact, the word is about bringing into concordance the modern ecological and traditional techno-economical concepts in taking the engineering decisions. In other words, it is a rational compromise between human activities and nature itself, which should ensure a sustainable development of the society with an appropriate protection and advancement of the environment. The problem thus formulated is of general significance, and its solution is sought in terms of the new scientific-technological discipline called "engineering ecology" [2, 6, 8].

Previous activities on flood control and improvement of watercourses in Voivodina have primarily ensured the basic conditions for physical existence of the

major part of population, and then also for general economic and cultural development of the region. In relation to this, the flood protection structures, together with the network improvement of watercourses (natural and artificial), represent for Voivodina the infrastructure systems of crucial importance, whose proper functioning influences almost all economic and other activities. However, the word is not only about the protective function of the system but also of ensuring the conditions for the development of some new activities, as well as of the enhancement of the utilization value of the land, water, and other resources in Voivodina [5]. It should be especially pointed out that, without flood control objects and water resources management in Voivodina, there would be no possibilities for intensive agricultural production on the reclamation systems based on modern methods of irrigation and drainage.

In view of the above it is obvious that for an effective flood protection it is necessary to analyze both the natural and anthropogenic factors which can yield an increased flood risk. This assumes the continuous co-operation with all the neighbouring countries, systematic measurements, monitoring and research of water streams regime on our territory, as well as the joint studies of different undertakings on the catchment areas and along the parts of water streams on our territory and the territories of the neighbouring countries (Hungary, Romania, Bulgaria, Croatia, etc.).

## **2. EVALUATION OF THE PRESENT STATE OF FLOOD CONTROL AND WATER STREAMS IMPROVEMENT IN VOIVODINA**

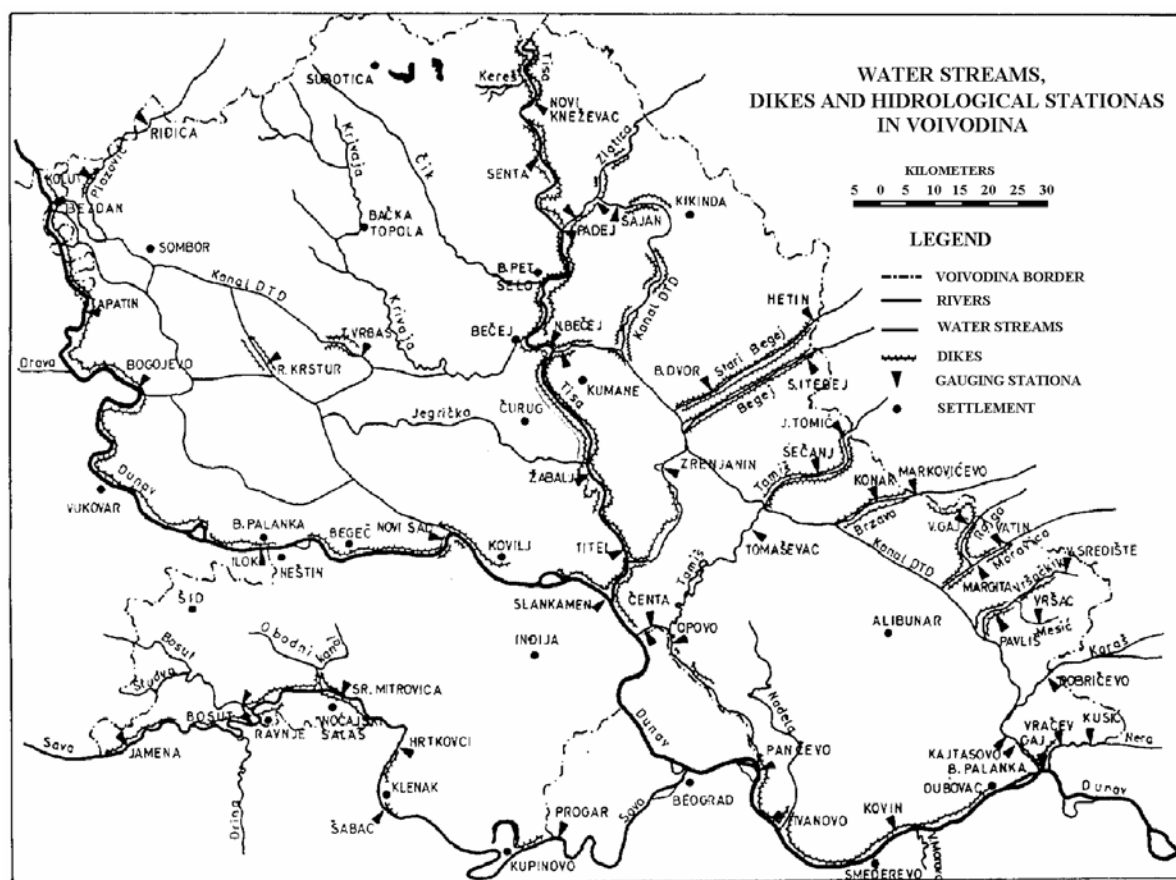
The numerousness of the water streams in Voivodina and their diversity in respect of the hydrological, hydraulic, morphological characteristics, etc., as well as in respect of the purpose and utilization mode, have necessitated construction of the different structures, and undertaking various measures on the existing flood control and regulation systems. However, a crucial role in flood control and water streams improvement have undoubtedly played the so-called passive measures, i.e. protection dikes and classical protection structures in the river bed, whereas the so-called active measures (effects of accumulations and retention) are of the secondary importance. An exception makes the main canal network of the Danube-Tisa-Danube (DTD) Hydrosystem, which in certain circumstances can also play an important active role in flood control.

The Voivodina region has been from ancient times endangered by flood because of the plain character of the terrain, as well as because of a developed network of water streams, with the diversity in their size and hydrological-hydraulic regimes (Fig. 1).

In Voivodina, about 60% of total territory (about 12,900 km<sup>2</sup>) is potentially endangered by flood discharge frequency 1/100 per year. On the endangered surfaces there are about 80% of the most fertile agricultural soil, 260 settlements with 1.2 million of inhabitants, about 4,000 km of traffic routes, and a number of capital objects of national economy. The endangered areas are protected by a system of dikes of a total length of 1,400 km, with the accompanying objects and 14 smaller reservoirs. An active role in flood control has also the network improvement of streams and canals of the DTD Hydrosystem. Out of the numerous flood occurrences which caused great direct and indirect damages in Voivodina, the most harmful were those of the rivers Danube (1965), the Tisa (1970) and the Sava (1974). The greatest damages were caused to agriculture (above 40%), and total damages in some communes exceeded 10% of the gross national product for the previous year.



The dikes along the Danube and Tisa have been reconstructed after the floods of the year 1965 and 1970, respectively, as well as in the frame of coastal belt protection from the backwater of the Hydropower Dam "Djerdap I". Reconstruction of the dikes has been completed on the 90% of their total length for one-hundred year high water and protective height of 1.0-1.7 m. The reconstructed dikes are presently in a satisfactory state. However, it should be noticed that there plans on reconstructing their remaining parts and sanitation of the weak points on about 30 locations in the zones of the objects constructed along the main protection line. In the future period, some new calculations and analyses should be carried out in order to quantify the need for constructing additional dikes along the Djerdap accumulation because of the additional back water due to sediment deposition.



**Fig. 1.** Water streams, dikes and hydrological stations in Voivodina.

As for the smaller plain water streams, with the exception of the Karas river, they have been encompassed by the projects of dike construction, as well as by the projects of partial reconstruction of the dikes along the Stari Begej and Plazovic and some other small water streams. However, the realization of these projects will be probably slowed down because of the shortage of financial resources.

When the Voivodina torrents are concerned, the state of flood control is inappropriate and uneven. Although there are protecting structures on some of such streams, flood damages are evident, especially if the stream is flowing through the settlements [4].

In order to increase the reliability of flood control systems in Voivodina it would be necessary, in addition to other works, to design and construct the border-line dikes, with the aim of preventing the uncontrolled breach of water from the

neighbouring countries to the Voivodina territory during high water occurrence. A considerable attention should also be paid to the protection from ice-caused floods, i.e. to the prevention of formation of ice barriers. However, the ice protection service is not organized and equipped at a satisfactory level. It would be necessary to complete the icebreaker fleet along the Danube, Tisa and Sava rivers, as well as to establish and equip mobile teams to fight ice formation on smaller water streams.

On the whole, the state of flood control system in the region of Voivodina cannot be considered as fully satisfactory, so that it would be necessary to continue the action on realization of the works mentioned above. It would be highly desirable to do prompt sanitation of the weak points along the main protection lines, as well as to pay more attention to the systematic auscultation and maintenance of all the control system objects.

The works on regulation of the water stream beds in Voivodina have been very extensive and diverse in the previous period [4]. The most comprehensive works have been carried on the Danube with the aim of providing waterway traffic and protecting the river banks from deformation. In the period since the end of the 19<sup>th</sup> century to the present, the following works have been carried out on the Danube: 155.9 km of revetments; 27.8 km of groynes; 6.7 km of barriers; 1.1 km of bed fixation by sills ; 21.9 km stone deposition; 15 km cutoff meanders and other structures. The extent of regulation works has been directly influenced by the more stringent requirements for increasing the depth and other characteristics of the waterway. In 1965, in accordance with the recommendations of the Danube Commission, an investment programme was elaborated concerning regulation of the Yugoslav part of the Danube for the river traffic, requiring the minimal waterway depth of 2.5 m (the former minimal depth was 1.8 m and then 2.0 m), minimal width of 180 m, and minimal radius of the waterway bending of 1000 m. Up to now, more than 60% of the works have been carried out in accordance with this programme [5], allowing thus a satisfactory state of the waterway traffic on the section Hungarian-Yugoslav border to Belgrade.

After the construction of the hydroelectric power station "Djerdap I", there has been no need any more for regulation works on the Danube downstream of Belgrade, because of the favourable effect of the back water on the Danube navigation characteristics. However, very extensive works had to be carried out on rising and reconstruction of the embankments and revetments along the accumulation, with the aim of protecting the coastal belt from the increased water level and erosion action of the waves. Also, the works have been carried out on reconstructing of the quays in the settlements, water tapping objects, and on some other coastal structures.

On the river Tisa, significant regulation works were carried out still in the end of the 19<sup>th</sup> century. To the end of 1875, the length of the river bed of Tisa was shortened from 1419 to 962 km, which was due to the realization of 110 cutoffs (13 cutoffs on the Voivodina territory), with simultaneous constructing of a number of regulation objects. In the period of constructing the dam on the Tisa at Novi Becej it was necessary to reconstruct 8.5 km of revetments, to build the quay walls in the urban zones of Novi Becej, Knezevac and Senta (including the bed fixation by sills), and cutoff meander near the village of Sanad.

In comparison with those carried out on the Danube, Tisa, and Sava, the regulation works that have been performed on the smaller water streams have been significantly less extensive in their scope. However, these works have allowed the majority of these water streams be successfully incorporated in the basic canal network of the DTD Hydrosystem, ensuring thus water regime management in the

largest part of Backa and Banat. It should be especially pointed out that very favourable results have been obtained in protection of canal banks of the DTD Hydrosystem from erosion using biological means [1] and new synthetic materials [3]. In the Srem region, the situation in respect of regulation and control of water regime of smaller water streams is more unfavourable, but on the basis of the realization of the water supply project, a significant improvement in this region is to be expected.

Small longitudinal slopes of the majority of the natural water streams and canals in the plain region, as well as the periodic action of back waters, bring about a decrease in the transportation capacity for sediment and formation of deposit layers in the stream bed. As was already mentioned, this problem will be more acute in the near future, especially in the Djerdap accumulation, although some unfavourable consequences have also been evident on a large scale with some other water streams. For example, it was found that, to the year 1987, in the basic canal network of the DTD Hydrosystem there had been about 12 million m<sup>3</sup> of deposit, which had significantly diminished water conducting capacity of the canals, and there had been the instances when the deposit formed had greatly hindered operation of water gates, water locks, and other water management objects. Besides, the unfavourable characteristics of these deposits can cause substantial problems in the functioning of the drainage and irrigation systems [10].

### 3. CONCLUSION

The works and structures involved in flood control and regulation of the natural water streams and canals represent the infrastructure systems of primary importance, crucially influencing the overall economic and social activities in the Voivodina region. Thanks to the structures of flood control and water streams regulation, the whole Voivodina region has become a complex water management and land reclamation system. If it is taken into account that Voivodina represents the most developed region and the granary of Yugoslavia, then it can be concluded that a normal functioning of this system is of vital interest not only to Voivodina but to the country as a whole. Related to this, the auscultation and normal maintenance of the protection objects have to be considered as a permanent obligation of the whole community.

The nature of occurrence of floods, as well as the experience that has been gained in many countries of the world indicate that an absolute flood control cannot be achieved, but the application of a range of mutually compatible measures of the investment and noninvestment character can only yield rational diminishing of flood risks and damages. The largest part of Voivodina will be further protected by dikes (passive measures), with a reasonable utilization of the existing accumulations, retentions, and canal network of the DTD Hydrosystem (active measures). The measures of noninvestment character as a supplement to the investment ones, represent a set of administrative, legislative, and institutional measures aiming at the preventive reduction of flood damages. The key elements of these measures are: specific landscape planning and land reclamation on the flood-endangered territories; regulations related to the mode of utilization of land, objects and other resources on the endangered areas; specific technical regulations in the domain of construction, utilization and maintenance of flood control objects on the flood-endangered areas; specific regulations concerning insurance of the property on the endangered areas. These measures are to be consequently applied in the forthcoming period.

Further advancements in the domain of designing, constructing, and utilizing of the system for flood control and regulation of water streams should be based on modern achievements of science and technology. All this assumes modernization of the equipment for field and laboratory research, application of modern methods for simulation, prediction and control of the processes important for all the activities in the field of flood control and water streams regulation. Besides, it is necessary to strengthen the co-operation with the neighbouring countries and promote joint synchronized actions on flood control and water streams regulation.

**Acknowledgements:** The work was undertaken as a part of the SOIL DETERIORATION, CONSERVATION AND UTILISATION AT LAND RECLAMATION SYSTEMS Research Projects No. 0419, under contract BTR.5.01.0419.B. It has been funded partially by the Ministry of Science, Technology and Development, Government of Serbia and "SRBIJAVODE" WATER AUTHORITY – Beograd (The River Danube Water Resource Center - Novi Sad).

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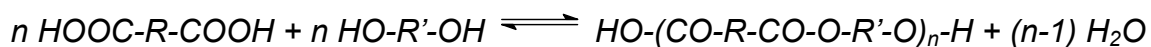
**ESTERIFICATION AND POLYESTERIFICATION REACTIONS  
WITH INDUSTRIAL APPLICATIONS.  
POLYESTERS OBTAINED FROM MIXTURE OF DICARBOXILIC  
ACIDS AND DIOLS**

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**Abstract**

*This paper is continuing the research of authors in domain of polyesterification reactions with industrial applications. It was pursued the obtaining of new polyesteric oils using mixtures of adipic acid and phthalic acid and two different diols.*



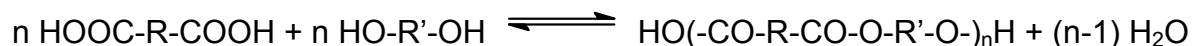
*The polyesterification reaction was realized without catalyst, at 220-225°C temperature, in presence of one chain switch. The obtained oils were physical-chemical characterized. The color of these oils was situated between 4 and 12 mg I<sub>2</sub>/100 ml solution of KI; the densities between 1.080 and 1.160 g/cm<sup>3</sup>, at 20°C and the dynamic viscosities were situated between 2000 and 147400 cP. In this way it's offer the possibility to obtain the esteric oils with a large viscosity domain.*

**Keywords:** polyesterification reaction, dicarboxylic acids, diols

**INTRODUCTION**

Polyesterification reaction is important as scientific as technologic point of view. Flory and co-workers developed the theory of polyesterification reaction first. [1,2]. About this type of reaction was realized a lot of other study for the kinetic and for the mechanism [3-7].

Polyesterification reaction is between a dicarboxylic acid and a diol, with water elimination and formation of polycondensation product:



The polyesterification can take place in the presence of an external catalyst. In the absence of this, the diacid monomer acts as its own catalyst for the reaction.

The polyesterification reaction can take place in the presence or in the absence of switches of chains, too.

Through this paper, it's continuing our research in domain of polyesterification reactions with industrial application [8]. We used as monomers mixtures of adipic (AA) and phtalic (FA) acid, respectively mixtures of two diols: 1,2-propylene glycol (PG), monoethylene-glycol (MEG) or 1,3-butane-diol (1,3 BD) with neopenthy-glycol (NPG). This type of polyesterification proposes to obtain better plasticizers.

## EXPERIMENTAL

The installation used in polyesterification it was a classical installation, described in previos paper [8]. The reactants used in polyesterification reaction are mixture of two dicarboxilic acids (adipic acid and phtalic acid) and two diols (monoethylene-glycol, 1,2-propylene-glycol, 1,3-butane-diol and neopenthy-glycol) in different molar ratio. As switch of chain we used 2-ethyl-hexanol and decanol. The characteristics of these raw materials were discussed in previos paper [8].

In stead of phtalic acid we used phtalic anhydride, an industrial product, purity 98.4%, melting point 131-132°C, density at 20°C 1.530 g/cm<sup>3</sup>.

The polyesterification reaction was realized in melting, at temperature until 220-225°C, in nitrogen atmosphere. At the beginning the reaction was guided at normal pressure and ultimately in vacuum. The water is stripped off continuous, during the process. When the acid number reached the expected value, it was realized the devolatilization of polyesters. By this way it's stripped out the excess of diols, too.

In Table 1 are containing the molar ratios of reactants and the conditions of polyesterification of acids and diols mixtures.

**Tabel 1.** *The molar ratios of the reactants and of switch of chain and the conditions of polyesterification reaction*

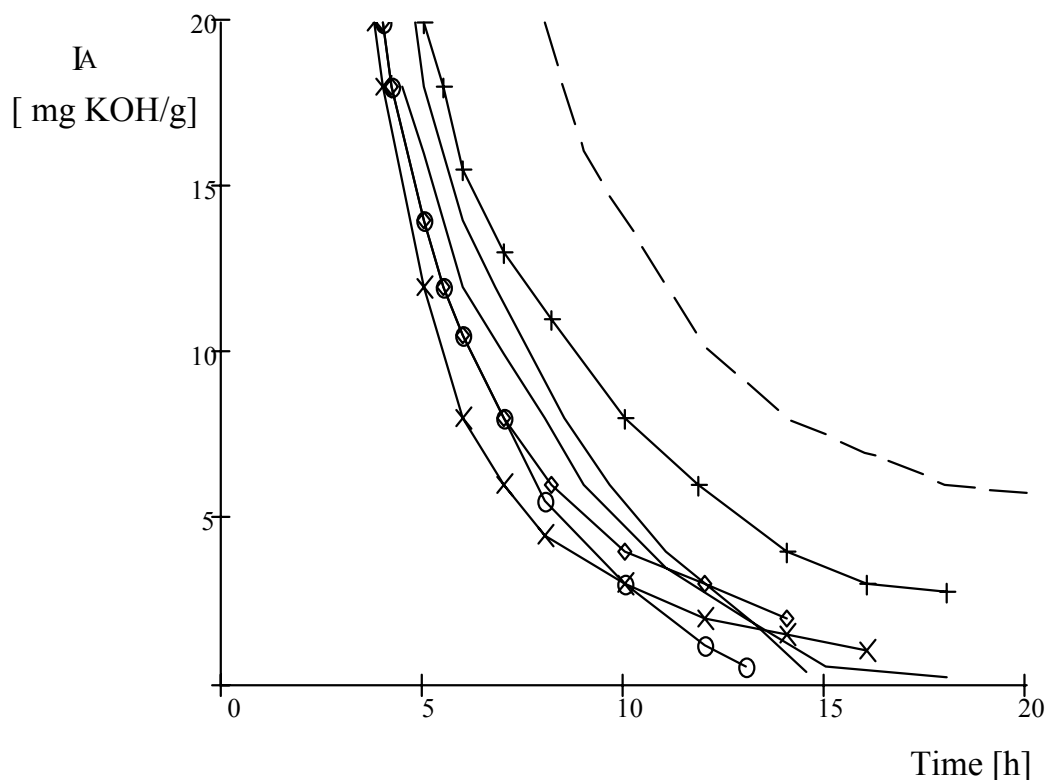
No	Molar ratio		Temp. of work [°C]	Time of reaction [h]
	Raw materials	Switch of chain		
1	AA : AF : PG : MEG 1 : 0.32 : 0.768 : 0.678	2-Ethyl-hexanol 0.1	225	21
2	AA : AF : PG : MEG 1 : 0.33 : 0.611 : 0.611	2-Ethyl-hexanol 1.047	230	22
3	AA : AF : PG : MEG 1 : 0.33 : 0.58 : 0.58	2-Ethyl-hexanol 0.786	220	24
4	AA : AF : NPG : 1,3 BD 1 : 0.71 : 1.23 : 1.33	Decanol 0.08	220	24
5	AA : AF : NPG : 1,3 BD 0.68 : 0.405 : 0.972 : 0.70	Decanol 0.51	225	28
6	AA : AF : NPG : 1,3 BD 1 : 0.71 : 1.63 : 1.77	Decanol 0.01	220	24

The physic-chemical determinations of compounds and the methods used were described in previous paper [8].

## RESULTS AND DISCUSSIONS

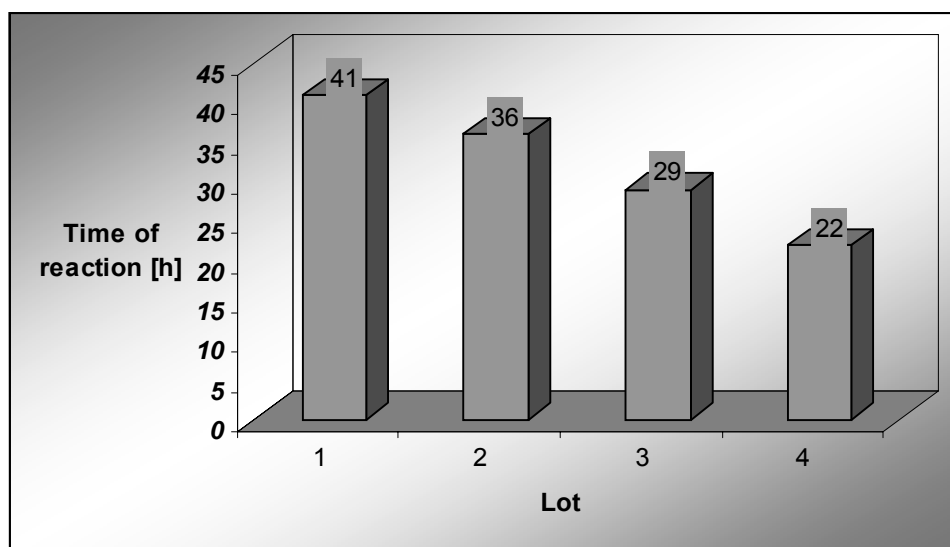
Continuous determining the acid number respectively the dynamic viscosity of reaction mass pursued the polyesterification process.

Figure 1 represents the variation of the acid number versus time. We observe a diminution of acid number more pronounced in the lots where we worked with excess of diol. In this case, after 25 hours the acid number presents a reduced value (less than 3 mg KOH/g). In lots with excess of dicarboxylic acids, the diminution of acid number is slower in time. In final, the value was between 10-23 mg KOH/g.



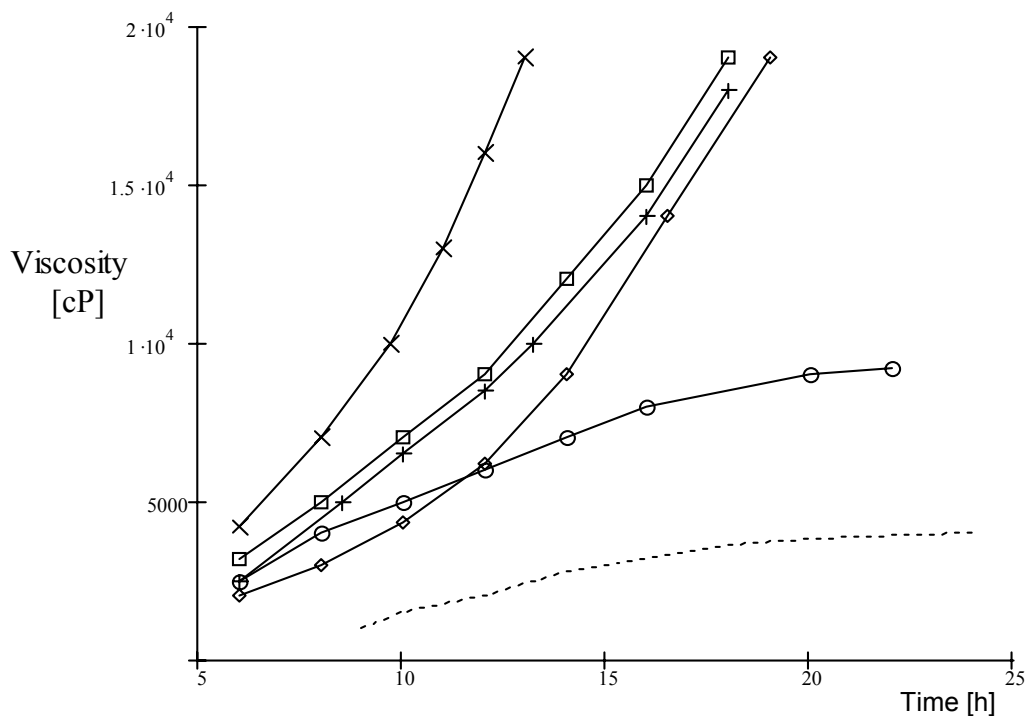
**Fig. 1** Acid number versus time

The diminution of acid number to the small value (2-3 mg KOH/g) is require long times of reaction. We observe this in figure 2 where is considering the lots with excess of diols.



**Fig. 2** Time of reaction in hours necessary for an polyesterification at 225-230°C until the acid number touch the value 2 mg KOH/g

Figure 3 represent the variation of dynamic viscosity (in cP) versus time. The viscosity increases more pronounced in the lots 1 and 2 where the quantities of chain switch are small (less than 0.1 molar). In the lots, which much quantity of chain switch, the increase of viscosity is slower, and the final value of viscosity is less than 2000 cP.



**Fig. 3** The variation of dynamic viscosity (cP) versus time of reaction (h)



In Table 2 are containing the physic-chemical properties of obtaining polyesters. There are viscous oils, with color on iodine scale between 4-12 mg iodine/100 ml solution KI.

**Table 2** *The physic-chemical properties of obtaining polyesters according with table 1*

No	The characteristic	The polyesters charges					
		1	2	3	4	5	6
1	Color, iodine scale [mg I <sub>2</sub> /100 ml sol KI]	12	4	8	8	4	8
2	Acid number [mg KOH/g]	23	0.5	18	10.6	3.8	3.02
3	Hydroxyl number [mg KOH/g]	31	20.95	28	22	6	7
4	Refraction index, at 20°C	1.488	1.472	1.473	1.478	1.487	1.487
5	Viscosity at 20°C [cP]	147422	678	1800	7400	1387	2000
6	Density at 20°C [g/cm <sup>3</sup> ]	1.11	1.10	1.15	1.08	1.16	1.15
7	Flash point [°C]	210	220	230	235	230	230

The color of polyesters is influenced by the quality of raw materials. While the polyesterification process, the air has an oxidative influence to the oils, determining their coloring.

For avoiding this fact, the reaction was realized in presence of nitrogen atmosphere.

The refraction indexes of studied polyesters are between value 1.470 -1.488 at 20°C and the density between 1.080-1.160 g/cm<sup>3</sup>.

The dynamic viscosity, determinate at 20°C, is varying in large limits, between 680-148000 cP (mPa·s).

The flash points of this esteric oils is higher than flash points of esters of dicarboxylic acids used as plasticizers. There are between 210-235°C.

## CONCLUSIONS

The polyesterification reaction of mixture of adipic and phthalic acid and two different diols was realized in melting, at 220-230°C, with chain switch and without catalyst. The obtained polyesters were characterized from physico-chemical point of view.

The dynamic viscosity values of this, in large limits, show the multiples possibility for obtaining and capitalizing this polyesters.

In our next paper we intent to present the evaluation studies of the esteric oils as plasticizers for plastic masses.

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## COLOUR CHANGES OF DRIED ONION TREATED IRRADIATION AND OZONATION

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### **Abstract:**

*Food industry generally uses dehydrated spices and vegetables in manufacture of soups, sauces, packet foods, etc.. Colour and quality changes during antimicrobial treatments or storage may cause serious loss of value. In recent work the effect of gamma irradiation and ozone treatment on colour of dried sliced onion was investigated. It was found that the storage and gamma irradiation caused unbeneficial dark colour changes, while ozone significantly caused favourable lighter colour. The aim of our work was to prove what kind of chemical transformations cause the colour and quality changes of dried onion.*

### **Keywords:**

*Allium cepa*, colour changes, ozonation, irradiation,  
dried onion , reducing sugars

## **1. INTRODUCTION**

There are several methods for microbial decontamination of dried foods, for example fumigation with ethylene oxide, gamma irradiation or ozone treatment [1]. Gamma irradiation in food processing has been extensively studied. It is very effective in reducing postharvest food losses, ensuring hygienic quality, thus it is extensively used method for food decontamination [2,3]. On the other hand numerous costumer particularly in Europe are prejudiced to irradiated foodstuff because of the creation of radiolytic products, including „free radicals” by food irradiation. Ozone is effective against microorganisms, it is used for sterilization and deodorization of several places: water supplies, hospital rooms, and

recently, it is increasingly used in agriculture and food industry [4]. This method seems to be very effective for food decontamination without any health or environmental risks.

Our earlier experiments have showed that both irradiation and ozone treatment are appropriate for reducing microbial count below the permitted limit, but the gamma irradiation was more effective than ozone treatment. Gamma irradiated dried onion showed changes in colour. Ozone treatment have not caused any colour change, the colour and organoleptic properties of ozonated dried onion was the same as the untreated sample [5].

The aim of recent work is to investigate the quality changes in treated matter, thus the presence of free radicals in irradiated and ozone treated dried onion by means of ESR method and the colour changes caused by irradiation.

The colour change of the irradiated dried onion may be caused by the Maillard reaction. It is generally initiated by a condensation between amino acids and reducing sugars. The reaction proceeds to form hundreds of products by a series of consecutive and parallel reactions including oxidations, reductions and aldol condensations, among others. The presence of reducing sugars (i.e. fructose and glucose) in an onion bulb can have major effect on the non-enzymatic browning. In this work the results of preliminary studies are presented which are aimed to prove the presence of Maillard reactions in this system.

## 2. Materials and methods

Common bulb onion (*Allium cepa* L.) were purchased from a local vegetable-drying company (Droginvest Kft, Szeged). The onion was dried after slicing operation by a multi-stage conveyor-type dryer. The moisture content of dried onion slices was about 50 g/kg. For gamma irradiation 0.05 kg of the samples were aerobically packed in PVC containers. Gamma irradiation was carried out in a  $^{60}\text{Co}$  irradiator operated at a dose rate of  $0.3 \text{ Gy s}^{-1}$ . The applied dose levels were 0, 2, 4, 6 and 8 kGy. Absorbed doses were checked by Fricke method [6].

Ozone was generated from oxygen by a flow-type ozone-generator operating silent electric discharge. The ozone containing gas continuously was flowing throughout a reactor containing 0.05 kg of the sample during the treatment. The treating time was 60 min in all cases, the flow rate was  $1 \text{ dm}^3 \text{ min}^{-1}$ . The final ozone concentration was followed by an UV spectrophotometer (WPA Lightwave 2000) at 254 nm and it was  $3,6 \times 10^{-5}$  and  $3,6 \times 10^{-6} \text{ mol} \times \text{dm}^{-3}$ . The treated samples were packed in a polyethylene container.

Microbiological analysis: Total aerobic bacteria were enumerated by the spread plate method using standard plate count agar, and by incubating plates at  $37^\circ\text{C}$  for 48 h.

The ESR measurements were carried out on 0,1 g powder form samples with a BRUKER 106 ESR spectrometer at room temperature. The

parameters used in the ESR measurements were chosen on the basis of the CEN standard (CEN 1787, 1996)[7].

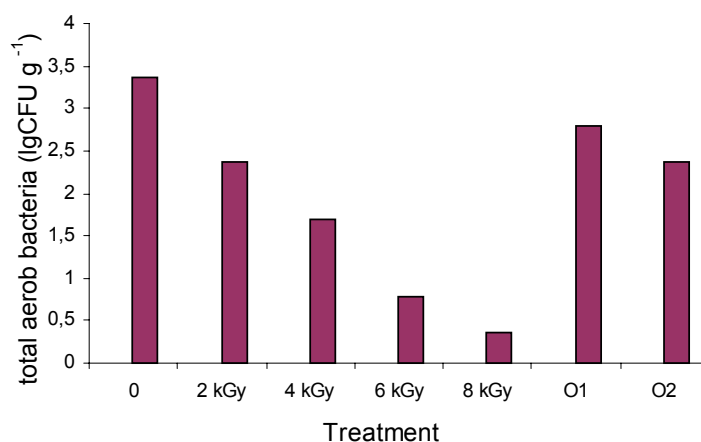
The colour of the untreated and treated samples was determined by a Hunter Labscan type colorimeter. 10 g onion samples were placed into a standard glass container to measure CIE Lab values. CIE  $L^*$ ,  $a^*$  and  $b^*$  values were instrumentally calculated. Lightness,  $L^*$ , is a quantity that measures the percentage of total solar spectral reflectance in relation to a pure white surface;  $a^*$  is a measure of the degree red-green; and  $b^*$  characterise the quantity yellow-blue. Colour difference values of treated samples against untreated samples (CIE  $\Delta E^*$ ) were calculated on the basis of CIE  $L^*$ ,  $a^*$  and  $b^*$  values. Measurements were performed 10 times, than the results were averaged.

For the chromatographic analysis the aliquots of 1 g dry samples were diluted in 50 ml of a 20/80 (v/v) water – ethanol solution and extracted by reflux for 1 h. The extract was filtered through a Whatman paper filter (No.1), adjusted to 50 ml with 70% ethanol and concentrated in a rotary evaporator. Raffinose was added as internal standard. [8]

HPLC analysis was carried out with the Varian LC Star system, incorporating a pump (9012), an autosampler (9100), a diode-array detector (9065) and a PC computer with Varian Star 5.3 software. Separations were made on a BST Rutin 10 APS column, (4.0 × 250 mm, 10  $\mu$ m particle size), with acetonitrile-water (80-20 v/v) as mobile phases. The solvent flow rate was 1.00  $\text{cm}^3 \text{min}^{-1}$ ; the sample volume was 20  $\text{mm}^3$ . The absorbance of the eluate was recorded in the range 190–367 nm.[9]

### 3. Results and discussion

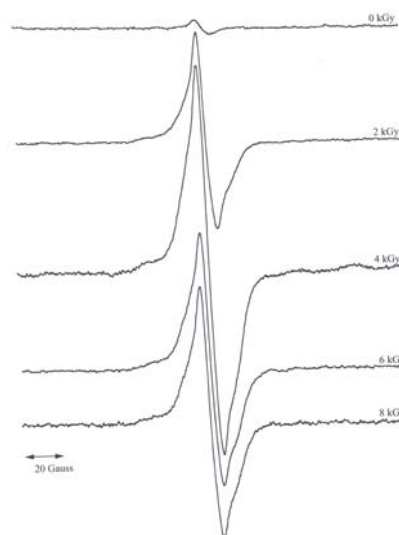
The cell counts of total aerobic bacteria of untreated samples ranged  $2.3 \times 10^3$  colony forming unit (CFU)  $\text{g}^{-1}$  was not higher than the standard allowed maximum values for dried onion. Results of the microbiological analysis of the irradiated samples (Fig. 1.) show that in the function of irradiation dose the total number of colonies were decreased significantly.



**Fig.1.** Mean total aerobic microorganism populations ( $\log_{10}$  CFU/g) in irradiated and ozone treated (O1:  $3,6 \times 10^{-6}$  mol  $\text{dm}^{-3}$ , O2:  $3,6 \times 10^{-5}$  mol  $\text{dm}^{-3}$  ozone concentrations)

The ozone treatment caused only slight reduction of counts – it is corresponding to 2 kGy gamma irradiation.

The ESR measurements were carried out on the untreated, gamma irradiated and ozone treated samples too. Fig. 2. and Table 1 show that irradiation increases the ESR intensity of dried onion, while the ozonation has no significant effect on the number of free radicals. This means, that ozone treatment of foods have less health risk than irradiation.



**Fig. 2.** ESR spectra of untreated and irradiated dried onion.

**Table 1.** Average ESR intensity values of treated and untreated dried onion samples.

	ESR intensity						
	Control	2 KGy	4 KGy	6 KGy	8 KGy	O1	O2
average	2,008	20,898	38,036	32,598	25,013	1,741	1,012
dev.	0,610	3,243	2,972	6,575	9,327	0,753	0,466

Colour changes of irradiated onion were observed. The results show (Fig. 2.) that the lightness ( $L^*$  values) and yellow colour co-ordinates ( $b^*$  values) of onion samples decreased with increasing dose, while green-red colour co-ordinates ( $a^*$  values) increased. This means that the colour of irradiated onion became darker and more brown, causing quality damage of the samples.

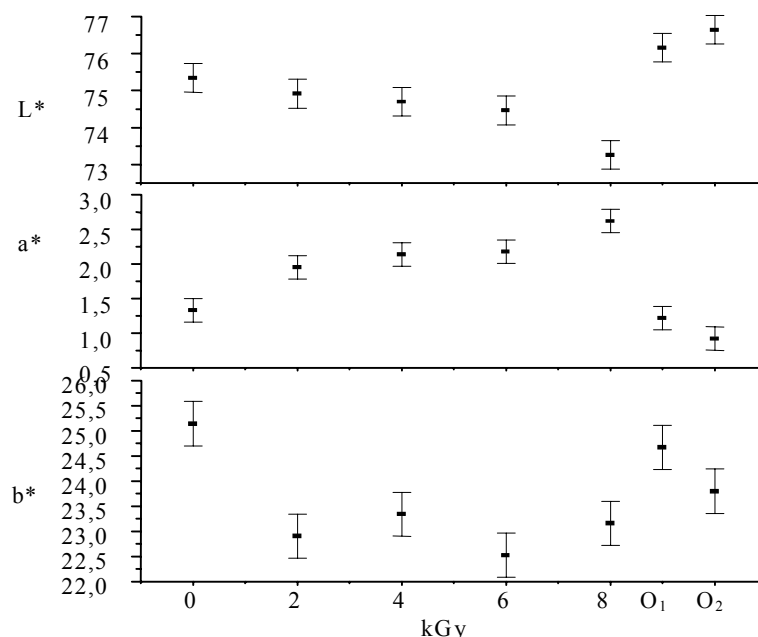
At the same time only slight colour change was observed during ozonation, colour co-ordinates  $L^*$ ,  $a^*$  were changed. The ozone treated onion became lighter and less red, while there were no significant changes in yellowness of the samples.(Fig.3.)

The lighter colour means better quality, thus in an other series of experiments it was examined that the ozone could disappear the negative colour changes caused by irradiation. The ozone treatment caused lighter and less brown colour in the irradiated samples too. The effect of

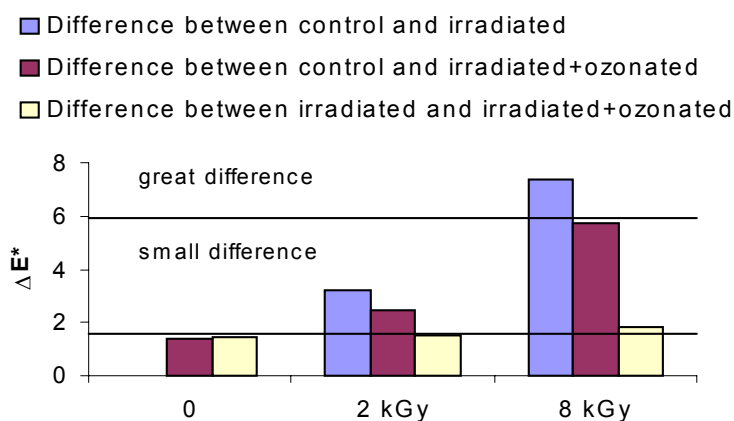
irradiation and ozonation on the colour can be demonstrate by the total colour difference  $\Delta E^*$ , calculated according to Eq. 1.:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where  $\Delta L^* = L^*(t) - L^*(0)$ ;  $\Delta a^* = a^*(t) - a^*(0)$ ;  $\Delta b^* = b^*(t) - b^*(0)$  are the differences calculated for treated dried onion (t) and the original untreated dried onion (0). (Fig. 4.)



**Fig. 3.** CIE Lab values of the untreated and treated dried onion in the function of applied dose.



**Fig. 4.** Total colour difference ( $\Delta E^*$ ) between of the control and irradiated, the control and the irradiated+ ozonated, and the irradiated and irradiated+ozonated samples

These results show, that the ozonation slightly can compensate the colour changes caused by irradiation, but this compensation effect of ozone is smaller than the browning effect of irradiation. Nevertheless the

effect of ozone could be enough to improve the quality of poor dried onion.

The presence of reducing sugars (i.e. fructose and glucose) in an onion bulb can have major effect on the non-enzymatic browning. The effect of the irradiation and the ozonation on amount of reducing sugars was investigated. It was found that the amount of reducing sugars did not changed significantly, the sugar content of the samples practically unchanged. At the same time in the chromatogram of non-structural carbohydrates of treated samples some new peak were observed, and the evaluation of the chromatograms showed that these components are colour (yellow). These components may be originated from the Maillard reaction. Identification of these components is in progress.

#### 4. Conclusions

The effect of gamma irradiation and ozonation on the quality of sliced, dried onion was investigated. Both method decrease the total microbial count, the ozone treatment equivalent to about 2 kGy irradiation dose. However the irradiation is more efficient sterilizer, it has negative effect too: it causes quality damaging colour changes, and increase the of free radicals content. At the same time the ozone has positive effects: it causes lighter colour, and does not forms radicals. Therewith ozone may diminish the colour changes caused by irradiation.

#### 5. Acknowledgements

The authors are grateful to Lajos Kovács (Droginvest Kft., Szeged) for suggesting, and to Foundation for Hungarian Higher Education and Research, for supporting this work by Magyary Zoltán Postdoctoral Grant.

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## ***Glu-1* AND *Glu-3* ALLELIC VARIABILITY OF GENUS *Triticum* – GENETIC RESOURCES IN WHEAT BREEDING**

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### **Abstract:**

*SDS-PAGE was used in characterization of HMW and BLMW GS composition of 40 Triticum genotypes. Six alleles at Glu-A<sup>m</sup>1 expressing four x and three y type subunits were scored. At Glu-A<sup>m</sup>3 seven alleles were found. Tetraploid accessions showed three HMW GS of different electrophoretic mobility as well as a null Glu-A1 allele and ten HMW GS encoded by seven Glu-B1 alleles. Analysis of B LMW GS revealed eight specific electrophoregrams. Three to six LMW GS, encoded by Glu-A3 and Glu-B3 loci, characterized each phenotype. The obtained results represent a rich source of new variation in wheat storage proteins.*

### **Keywords:**

*Wheat, glutenins, allelic variability, electrophoresis*

## **1. INTRODUCTION**

The unique properties of the wheat grain residue primarily in the gluten- forming storage proteins of its endosperm. Gliadins and glutenins are the main groups of storage proteins in wheat. Glutenin group members are classified as high- and low-molecular-weight glutenin subunits (HMW and LMW GS) according to their migration on SDS-PAGE. The synthesis of HMW and LMW GS is controlled by *Glu-1* and *Glu-3* loci, respectively. Both loci have been mapped to the homeologous group 1 chromosomes, together with the *Gli-1* loci, controlling the synthesis of major gliadin fractions. Several studies of storage proteins (5, 8, 14) have provided evidence of significant amount of genetic variation present at these loci in wild and less widely cultivated wheat genotypes.

As with most other crops, intensive breeding has lead to gene pool erosion within modern wheat cultivars. With the current availability of gene introgression methodologies (1,9), insights into the *Glu* and *Gli*

variation in wild relatives of cultivated wheats will enable the rapid transfer of genes encoding amenable glutenin and gliadin polypeptides.

The aim of the present paper was to assess the *Glu-1* and *Glu-3* allelic variability within and between different diploid and tetraploid wheat genotypes.

## 2. MATERIAL AND METHODS

Fifteen *T. monococcum*, five *T. turgidum* var. *polonicum*, seven *T. turgidum* var. *dicoccum* accessions, and thirteen durum wheat cultivars (Tab. 1) originating from the Research Institute of Field and Vegetable Crops, Novi Sad (Serbia and Montenegro) and the Vavilov Institute, Sankt Petersburg, (Russia) were used in this study. Identification of individual alleles was assisted by the use of standard bread wheat cultivars (Chinese Spring, Hope, and Nizija).

Table 1. Analyzed wheat genotypes

Accession	Label	Accession	Label	Cultivar	Label
<i>T. monococcum</i>		<i>T. turgidum</i> var. <i>polonicum</i>		<i>T. turgidum</i> var. <i>durum</i>	
K-20984	M1	S 1302/95	P1	NSD 1	NSD 1
S 1312/95	M2	GK 330/91	P2	NSD 2	NSD 2
S 1313/95	M3	GK 331/91	P3	NSD 3	NSD 3
S 1314/95	M4	GK 332/91	P4	NSD 9	NSD 9
1315/95	M5	GK 333/91	P5	NSD 15	NSD 15
K-8555	M6	<i>T. turgidum</i> var. <i>dicoccum</i>		NSD 18	NSD 18
K-31566	M7	S 1303/95	D1	Waha	W
K-23650	M8	S 1304/95	D2	Herson	H
K-35914	M9	S 1306/95	D3	Omruv	O
K-1729	M10	S 1307/95	D4	Korifla	K
K-29603	M11	S 1308/95	D5	Stojocki	Stoj
K-20491	M12	S 1309/95	D6	Cham	C
K-14237	M13	S 1310/95	D7	Stork	S
K-23032	M14				
K-14379	M15				

Extraction and SDS-PAGE procedure for analysis of HMW GS followed the one by Vapa and Savic (12). Extraction of LMW GS followed the procedure of Gupta and MacRitchie (3). Gels were stained in Commasie Brilliant Blue R-250, or in the case of LMW GS, in silver (14).

## 3. RESULTS AND DISCUSSION

Analysis of *T. monococcum* seed storage proteins revealed six *Glu-A<sup>m</sup>1* alleles, assigned as *Glu-A<sup>m</sup>1a* to *Glu-A<sup>m</sup>1f* (Fig.1). Expression of both x and y type of glutenin subunits was observed in all except accessions

M13 and M14 where only a single x or y subunit was scored, respectively. The mobility of x subunits was within or below the range of subunit 5 encoded by *Glu-D1* locus of the reference cultivar Hope (Fig. 1). Three products of active *Glu-A<sup>m</sup>1-2* genes exhibited minor size differences and were all slightly slower than the subunit 7 of the reference cultivar Nizija. Variability of *Glu-A1* locus in bread wheat is significantly lower when compared to the variation of *Glu-B1* and *Glu-D1* loci. In addition, only x-type *Glu-A1* genes were active (7, 13). The results reported here provide further evidence that *T. monococcum* possesses novel allelic forms at *Glu-A<sup>m</sup>1* locus (Tab. 2) and that the silencing of *Glu-A<sup>m</sup>1* genes encoding y type subunits is not a common feature of *Glu-1* genes in diploid relatives of bread wheat.

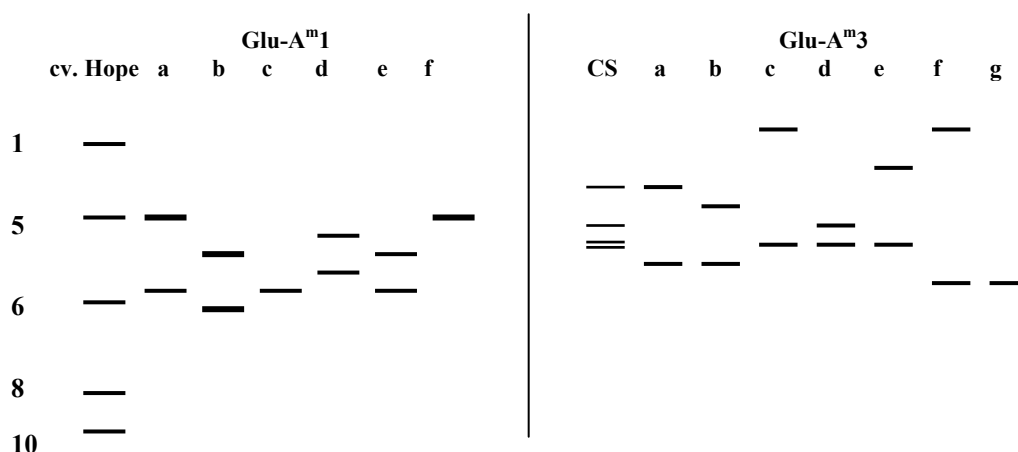


Figure 1. Diagrammatic representation of HMW and B LMW GS from *T. monococcum* accessions separated by SDS-PAGE. Control cultivars were Hope and Chinese Spring

SDS-PAGE analysis revealed seven different electrophoregrams of B LMW GS in *T. monococcum* all presumably coded by *Glu-3* alleles (Fig. 1). Presence of two subunits was observed in all except for M11 accession. Lee et al. (4) reported 60 different B LMW GS patterns among einkorn wheats accessions. In spite of a significantly smaller number of accessions analyzed, our results confirm a large extent of variation present at *Glu-3* locus of *T. monococcum*.

SDS-PAGE analysis of HMW GS in tetraploid wheats revealed three subunits of different electrophoretic mobility encoded by genes at *Glu-A1* as well as a null *Glu-A1* allele and ten HMW GS encoded by seven *Glu-B1* alleles (Fig. 2). The subunit 1 (*Glu-A1a*), has been found in majority of bread and durum wheat cultivars (2). In contrast, subunits 1' and 1'' of slightly higher electrophoretic mobility have been scored only in accession D1 and NSD3, as well as D6 and D7, respectively. These subunits represent novel allelic forms at *Glu-A1* locus.

Out of ten HMW GS encoded by *Glu-B1* alleles, the most frequent was the subunit 20, the product of *Glu-B1e* allele (Tab. 3). Another *Glu-B1* allele, *Glu-B1d*, present in worldwide durum wheat has been found in the analyzed accessions, solely among the cultivated ones. The *Glu-B1*

encoded HMW GS found in wild tetraploid accessions (subunits 8, 7', 14'+15' and 20') represent rare and presumably novel HMW GS (Fig 2).

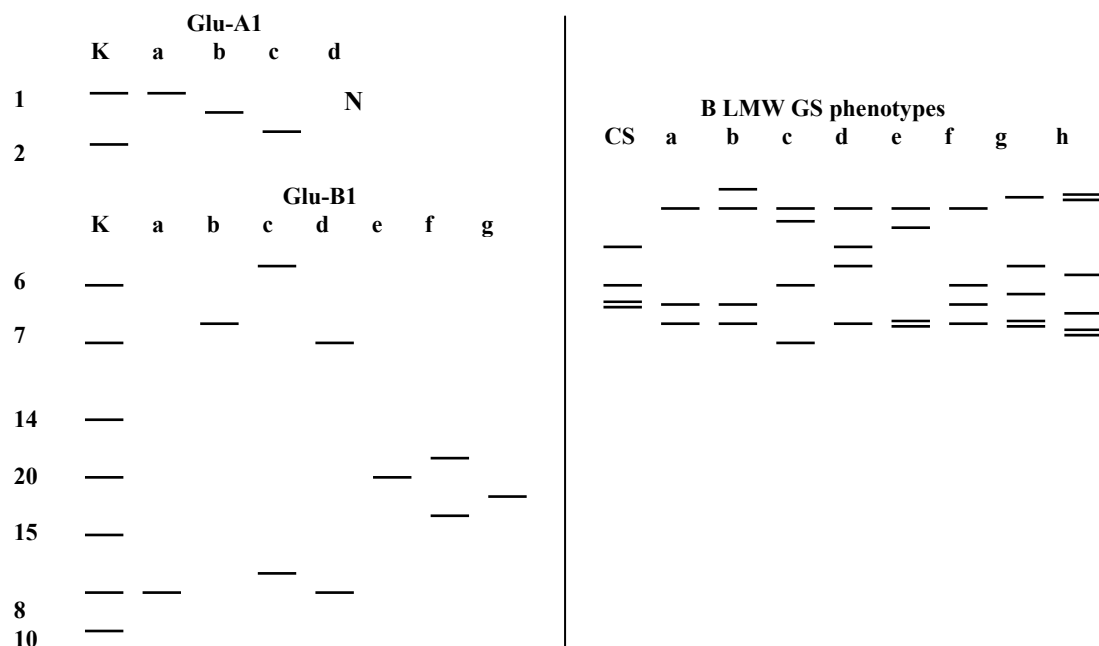


Figure 2. Diagrammatic representation of HMW and B LMW GS from *T. turgidum* accessions and cultivars separated by SDS-PAGE. **K** stands for reference glutenin subunits, and **CS** stands for cv. Chinese Spring

Table 2. HMW and B LMW GS composition of *T. monococcum* accessions

Locus	Allele	Accessions
<i>Glu-A<sup>m</sup>1</i>	a	M2, M3
	b	M4, M5
	c	M14
	d	M11
	e	M1, M6, M7, M8, M9, M10, M12, M15
	f	M13
<i>Glu-A<sup>m</sup>3</i>	a	M1
	b	M2, M3
	c	M4, M6, M7, M 8, M9, M10, M12
	d	M5
	e	M13, M15
	f	M14
	g	M11

Electrophoretic analysis of reduced and alkylated B LMW GS revealed the presence of eight specific B LMW GS electrophoregrams (Fig 2). Each *Glu-3* phenotype was characterized by three to six LMW GS subunits,

encoded by *Glu-A3* and *Glu-B3* loci (Fig 2). Durum cultivars originated from Novi Sad, with the exception of NSD18, all had the same B LMW GS composition.

Table 3. HMW GS composition and B LMW phenotypes of *T. turgidum* genotypes

Locus	HMW GS (allele)	Accession	B LMW phenotype	Accession
<i>Glu-A1</i>	1 (a)	D2, D3, D4, D5, P1, NSD15	a	P2
	1' (b)	D1, NSD3	b	H, D6, D7
	1'' (c)	D6, D7	c	P1, D1, D3, D4
	N (d)	P2-P5, NSD1-NSD3, NSD18, W, H, O, K, C, S, Stoj	d	C, W
<i>Glu-B1</i>	8 (a)	P1, D1, D3	e	NSD1, NSD2, NSD3, NSD9, NSD15
	7' (b)	D4, P3, P4	f	Stoj, S, P5, NSD18, O, K
	6'+8' (c)	D5, D2, NSD15, K	g	D2, D5
	7+8 (d)	NSD1, NSD9, W, H, C, S	h	P3, P4
	20 (e)	P5, NSD2, NSD3, NSD18, O, Stoj		
	14'+15' (f)	D6, D7		
	20' (g)	P2		

The high levels of LMW GS variation in tetraploid wheats among the accession analyzed in this paper are no exception to the findings of other authors (1, 5). Recently, five alleles at *Glu-A3* and 14 alleles at *Glu-B3* have been characterized in durum cultivars (6). Current views into the influence of different classes of seed storage proteins stress the HMW and LMW GS composition as the main factor in bread and pasta making quality (10).

#### 4. ACKNOWLEDGEMENTS

This research was partially funded by Ministry of Science, Technology and Development of republic Serbia, grant N° 1773

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## THE PRESENCE AND THE ORIGIN OF 1BL/1RS TRANSLOCATION IN BREAD WHEAT CULTIVARS BRED IN SERBIA

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### **Abstract:**

*Bread wheat cultivars with a 1BL/1RS translocation are used in many breeding programs throughout the world to improve grain yield and cultivar stability and adaptability. Unfortunately, serious defects in bread-making quality such as poor mixing tolerance, superficial dough stickiness, and low bread volume have been associated with this translocation. The presence of 1BL/1RS translocation in 68 bread wheat cultivars bred in Serbia was detected by SDS-PAG electrophoresis. 1BL/1RS translocation was present in 17 cultivars, although the two of them were heterogenous. Pedigree analysis revealed that the rye genes were introduced in to analysed cultivars from Aurora, Kavkaz and Skorospelka 35.*

### **Keywords:**

*1BL/1RS translocation, bread-making quality, wheat, gel electrophoresis*

## **1. INTRODUCTION**

The most widely spread translocations in wheat are those in which the short arm of rye chromosome 1 replaces the short arm of wheat group-1 chromosome. These whole-arm translocations developed from breakages of wheat and rye univalent chromosomes at the centromeres, followed by exchange and fusion of the respective chromosome arms (8). In European breeding programs, univalent 1R and 1B chromosomes would have coexisted in the initial hybrids between 1R(1B) substitution lines and euploid wheat (14). A long-term goal of breeders and geneticists has been to suppress the undesirable quality effects associated with having 1RS whole arm translocations. 1RS carries resistance genes to rusts (Lr26, Sr31, Yr9), (1, 4) and to powdery mildew (Pm8), (6), and to insects (10). Also, 1RS may directly increase yield (18). Conversely, depending of wheat genotype, 1RS chromatin can negatively impact wheat end-product, meaning reduction of flour yield (11) or production of undesirable 'sticky dough' (3). It was shown that two physically separated loci for rye seed endosperm proteins are present on 1RS,  $\alpha$  and  $\omega$

secalins. Sec-1 locus is rather complex, and it has been estimated that at least 10 to 30 copies of the  $\omega$  secalin structural gene are present, which appear to be associated with negative wheat end-product quality (1).

At least two, and perhaps three independent origins of 1BL/1RS translocations are known. The substitution line 'Zorba' gave rise to a number of 1BL/1RS translocations in former West Germany. The other substitution line 'Salzmünder Bartweizen' gave rise to a number of 1BL/1RS translocations in Eastern European breeding programs. Various breeding programs throughout the world distributed Kavkaz, a 1BL/1RS derivative of 'Salzmünder Bartweizen'. The third possible translocation was produced in Japan in the wheat 'Salmon' (4).

The aim of this study was to identify the presence and trace the origin of 1BL/1RS translocation in bread wheat cultivars bred in Serbia using SDS-PAG electrophoresis.

## 2. MATERIAL AND METHODS

**Material:** Sixty-eight bread wheat cultivars originated from Research Institute of Field and Vegetable Crops, Novi Sad, Serbia and Montenegro were used in the analysis. Cultivars Chinese Spring and Proteinka were used as negative or positive control, respectively.

**Methods:** The presence of 1BL/1RS translocation in different wheat cultivars was detected by sodium dodecyl sulphate polyacrylamide gel (SDS-PAG) electrophoresis of unreduced total seed proteins (15). After protein separation gels were stained using Commasie Brilliant Blue R-250 and electrophoregram was analyzed for presence of secalines, controlled by Sec-1 locus.

## 3. RESULTS

Proteins of single grain of 68 different bread wheat cultivars bred in Serbia were analyzed by method of SDS PAG electrophoresis. If 1BL/1RS translocation is present a secaline protein bands were visible at the upper part of the gel.

Among 68 wheat cultivars, 51 didn't expressed secaline protein subunits, meaning didn't posses 1BL/1RS translocation. Fifteen cultivars possessed 1RS rye chromatine, detected as secaline bands on the electrophoregram. Two cultivars, Anastasija and Selekt, showed intracultivar heterogeneity. Cultivar Anastasija had 1BL/1RS translocation at 36% frequency, while Selekt showed the presence of translocation at 25% frequency (Table 1).

*Table 1. The presence of 1BL/1RS translocation in bread wheat cultivars bred in Serbia*

	Cultivar	Year	1BL/1RS presence
1.	Danica	1990	-
2.	Evropa 90	1990	-
3.	Pobeda	1990	-
4.	Proteinka	1990	+
5.	Kratka	1991	+

6.	Novosadska 330	1991	+
7.	Novosadska rana 5	1991	-
8.	Bojana	1992	-
9.	Desa	1992	+
10.	Dicna	1992	-
11.	Draga	1992	-
12.	Jovana	1992	-
13.	Kosuta	1992	-
14.	Milica	1992	+
15.	Slavija	1992	+
16.	Srna	1992	-
17.	Zlatica	1992	+
18.	Atina	1993	-
19.	Eva	1993	+
20.	Fortuna	1993	+
21.	Jarebica	1993	-
22.	Rusija	1993	-
23.	Slava	1993	+
24.	Sloga	1993	-
25.	Stepa	1993	-
26.	Alfa	1994	-
27.	Dejana	1994	-
28.	Dina	1994	-
29.	Divna	1994	-
30.	Lira	1994	-
31.	Luna	1994	-
32.	Neva	1994	-
33.	Renesansa	1994	-
34.	Sasanka	1994	-
35.	Stotka	1994	-
36.	Stela	1994	+
37.	Struna	1994	-
38.	Suvaca	1994	+
39.	Laguna	1995	-
40.	Kremna	1995	-
41.	Omega	1995	-
42.	Pesma	1995	-
43.	Prima	1995	-
44.	Sila	1995	-
45.	Silna	1995	+
46.	Tera	1995	-
47.	Tiha	1995	-
48.	Bajka	1997	-
49.	Dobra	1997	-
50.	Galija	1997	-
51.	Mina	1997	-
52.	Prva	1997	-
53.	Selekta	1997	75% -/ 25% +

54.	Senica	1997	-
55.	Sreca	1997	-
56.	Super rana	1997	-
57.	Zlatka	1997	-
58.	Delta	1998	-
59.	Milena	1998	-
60.	Sirena	1998	-
61.	Sofija	1998	-
62.	Sonja	1998	-
63.	Anastasija	1999	64% -/ 36% +
64.	Ivanka	1999	-
65.	Sara	1999	+
66.	Stamena	1999	+
67.	Ljiljana	2000	-
68.	Sonata	2000	-

Pedigree analysis for 15 cultivars that possessed 1BL/1RS translocation was performed, in order to trace the origin (Table 2).

*Table 2. The pedigree of cultivars possessing 1BL/1RS translocation*

	Cultivar	Pedigree
1.	Proteinka	NS 2726-2/Macvanka 1
2.	Kratka	Skopljanka/ZG 2463-74
3.	Novosadska 330	Zl. dol./NSR-2//Partizanka
4.	Desa	((L-69-68/NS7000)/Mironov. Jubil 50)/NS7005
5.	Milica	Zelengora/Macvanka2//Partizanka
6.	Slavija	NS 1987/Jugoslavija
7.	Zlatica	NSR-2/Mutant 48//Sutjeska
8.	Eva	Macvanka 2/VM 705-140
9.	Fortuna	Balkan/ZG 2597-76
10.	Slava	NS 27-97/VM 701-41
11.	Stela	NS 51-15/Balkan//Posavka 2
12.	Suvaca	MV 22-72/NS 32
13.	Silna	NS 7000/Zvezda
14.	Sara	Partizanka/Jedina
15.	Stamena	Lasta/Rodna

#### 4. DISCUSSION

The presence of 1BL/1RS translocation in cultivars bred in Serbia was proved in previous research, using different cytogenetical and biochemical methods (9, 12). SDS PAG electrophoresis confirmed the presence of 1BL/1RS translocation in 27% of analyzed cultivars bred in Serbia until 1990 (16). This research revealed the presence in 22% of cultivars bred in Serbia during last decade. Some cultivars possessing 1BL/1RS translocation are also known as cultivars resistant to leaf rust (17).

This research revealed 15 cultivars possessing translocation. Pedigree analysis revealed that at least one of parents of these also possesses 1BL/1RS translocation. Moreover, pedigree analysis revealed that the rye genes were most probably introduced into analyzed cultivars from Aurora, Kavkaz and Skorospelka 35.

Although alien chromatin carrying beneficial genes controlling resistance can be useful in wheat breeding programs (4), it also may have negative impact on wheat bread-making performance, as low specific loaf volume, the production of sticky dough, and lack of tolerance to overmixing (2, 7).

Careful selection of parents for crosses with known 1RS lines, coupled with rigorous selection for end-use quality, can diminish negative impact of 1BL/1RS translocation. In addition, the tools of genetic engineering can now be used to improve the quality of 1RS lines.

## 5. CONCLUSION

The presence of 1BL/1RS translocation in 68 bread wheat cultivars originated from Research Institute of Field and Vegetable Crops, Novi Sad, Serbia and Montenegro, was detected by SDS-PAG electrophoresis. 1BL/1RS translocation was discovered in 17 cultivars, although the two of them were heterogenous.

Pedigree analysis revealed that at least one of parents of cultivars with 1BL/1RS translocation originated from crosses involved Aurora, Kavkaz and Skorospelka 35 cultivars.

## 6. ACKNOWLEDGEMENTS

This research was partially funded by Ministry of Science, Technology and Development of Republic of Serbia, grant No. 1773.

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## SEED OF *AMARANTHUS CRUENTUS* AS BREAKFAST CEREAL AND SNACK PRODUCT

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### **Abstract:**

*This paper presents the possibility of extrusion, expanding and popping of *Amaranthus cruentus* seed. The seed and the snack products were analyzed for chemical composition, energy value and sensory evaluation. Results demonstrated that the Amaranth snack has high nutritive value. The sensory evaluation proved that these products have similar rank in comparison with other commercial snack products.*

**Keywords:** *Amaranthus cruentus*, snacks, chemical composition, energy value

### **INTRODUCTION**

*Amaranthus cruentus* is one of 60 different species of the genus *Amaranthus*. This plant was a staple crop of the Aztecs and other pre-Columbian cultures (8). As it is not a true cereal, Amaranth grain has characteristics and properties similar to those of the cereal grains and is often called a pseudocereal. Investigation with *Amaranthus* are a relatively modern endeavour and were undertaken in the 1970s in the United States of America (10). The importance of growing *Amaranthus* was overemphasized by the fact that it is a C<sub>4</sub> plant undergoing the Hatch-Slack CO<sub>2</sub> reduction (6).

Considering the biological as well as nutritional quality of *Amaranthus* seed it is of key importance to establish a gene bank in Serbia. Our study on the possibility of growing *Amaranthus* sp. in a temperate continental climate under the precipitation regime of the Danube River basin has started in 1994 (3,2). Based on recent survey on the chemical content of the Indian *Amaranthus* in Yugoslavia it has been shown that it was a relatively high protein value in comparison to the average cereal protein values (9,3). Amaranth protein has been shown in numerous studies to be superior in quality to that of the cereals. It is high in the amino acid lysine, which is characteristically low in cereal proteins. Thus it is well suited for blending with cereals (5). High level of dry matter, protein, mineral content (calcium and iron) and fat gave importance to seed of *Amaranthus* sp. (1,3,9)

The *Amaranthus sp.* seed can be expanded and milled similar to corn. Extrudates have a pleasant specific taste and can be used as a separate snack, addition to müsli, cake decoration or as raw material for further processing (4).

It has long been known that Amaranth seeds expand or "pop" when heated. Popped Amaranth is used as an ingredient in snack, sometimes along with popped popcorn and in caramel corn or comparable sweet snacks (1,4,5).

The small size of the seeds and their oil content of approximately 8% allows extrusion without grinding or adding additional water. By selecting the proper screw configuration along with a range of barrel temperatures and screw speeds, extruded products with varying degrees of expansion can be obtained (5).

## MATERIAL AND METHODS

Seed of selected lines of *Amaranthus cruentus* No. A-17 used in investigation, were popped, expanded and extruded.

Popping of *A.cruentus* seed was on aluminium hot plate at 200 °C for 15-20 sec. *A.cruentus* seed was expanded using Expander LGUN with pressure of 11,5 A.

In this paper we also investigated pellets which were produced by extrusion using "Brabender " 20 DN extruder with a range of barrel temperature (100°C) and screw speeds of 150-200 o/min.

Proximate chemical analyses were determined according to the official regulations in force (7). Protein content (N x 5,8) was estimated by the Kjeldahl method. Carbohydrate content was estimated determining the total starch content according to Ewans and the reducing sugar content by Luff-Schoorl. Fat content was determined by Soxhlet extraction. Cellulose content was determined according to Wender. Moisture and ash content were determined by standard methods according to the official regulations.

## RESULTS AND DISCUSSION

Chemical composition of *Amaranthus cruentus* seed and the snack products is presented in Table 1. The average protein content of *Amaranthus cruentus* seed was 17.6% (Table 1). The protein content of the snack products ranged from 12.2% to 16.5%. The protein content of snack products are similar to the protein content of the raw seeds, except of the extruded pellets (Table 1). The protein content fall within ranges obtained by other authors.

The ash, fat and cellulose content of *Amaranthus cruentus* seed and snack products are higher than in cereals (Table 1).



Table 1.- Composition of Amaranth seed and snack products

Amaranth seed, and snack products	Moisture (%)	Protein % d.m.	Ash %d.m.	Fat %d.m.	Cellul. %d.m.	Carbohydrates
seed of <i>A.cruentus</i> No. A.17	11.3	17.6	3.26	7.2	5.5	64.3
Expanded pellets 100% Amaranth seed	7.9	16.5	3.04	7.0	5.2	68.2
Extruded pellets 100% Amaranth seed	6.6	12.2	1.56	4.5	1.7	77.2
Popped <i>A.cruentus</i> seed	1.1	16.1	3.05	7.3	5.0	67.2
Average	6.7	15.6	2.73	6.5	4.4	69.2

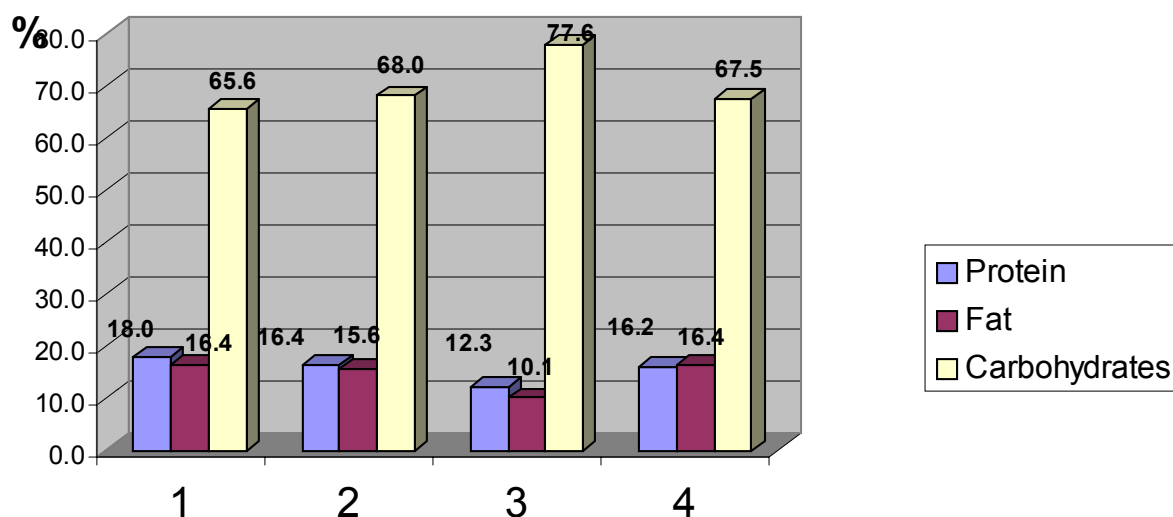
The average ash, fat and cellulose content are 2.73%, 6.5% and 4.4% respectively (Table 1).

Data on energy and calorie distributions are reported in Table 2. Energy values of *Amaranthus cruentus* seed and snack products are similar and range between 1665.9 kJ and 1705.9 kJ/100g dry basis (Table 2).

Table 2.- Energy and caloric value of *Amaranthus cruentus* seed and the snack products

Energy value	seed of <i>A.cruentus</i> No. A.17	Expanded <i>A.cruentus</i> seed,	Extruded <i>A.cruentus</i> seed,	Popped <i>A.cruentus</i> seed,
kJ /100g.d.m.	1665,9	1705.9	1690.8	1693.5
kcal. /100g.d.m.	392.4	401.8	398.1	398.9

Energy distribution has shown that more than 65% energy is obtained from carbohydrates (Fig.1)



1- Seed of *A.cruentus* No. A.17

2- Expanded *A.cruentus* seed

3- Extruded *A.cruentus* seed

4- Popped *A.cruentus* seed

Fig.1.-Energy distribution of *Amaranthus cruentus* seed and the snack products

The sensory evaluation proved that these products have similar rank in comparison with other commercial snack products (Table3).

Table 3.- Sensory evaluation of *Amaranthus cruentus* simple and there snack products

	seed of <i>A.cruentus</i> No. A.17	Expanded <i>A.cruentus</i> seed,	extruded <i>A.cruentus</i> seed,	Popped <i>A.cruentus</i> seed,
Taste	weak on Amaranth	neutral	similar to corn	specific crunchy
Aroma	weak on Amaranth	pleasant	pleasant	very pleasant

## CONCLUSION

The snack products of *Amaranthus cruentus* seed have high nutritive value (high protein, fat, cellulose content). Sensory evaluation of *Amaranthus cruentus* seed and the snack products have neutral, specific taste and a pleasant aroma. *Amaranthus cruentus* seed and snacks blended with other raw materials and cereals are suitable for creating new formulations of snack food.

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## ENERGY PRODUCTION FROM SHORT ROTATION POPLAR PLANTATIONS

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### **Abstract:**

*The objective of this work was to estimate the dry matter production and energy potential of 6 poplar clones (*P.x euramericana* cl. Ostia, *P.nigra* cl.53/86, *P deltoides* cl. PE 19/66, *P.x .euramericana* cl.I-214, *P.x deltoides* cl. S6-7, *P.x euramericana* cv. Robusta), with two different plant densities (38461 plant/ha and 83333 plant/ha) aged one year rooted cuttings. Average dry matter biomass yields reached 21 t ha<sup>-1</sup> year<sup>-1</sup> (38461 plant/ha), and 12 t ha<sup>-1</sup> year<sup>-1</sup> (83333 plant/ha). Based on calorific values of oven dry wood and bark of each clone, average energy potential of researched poplar clones was estimated up to 395 GJ ha<sup>-1</sup> year<sup>-1</sup>, and for denser plantation up to 222 GJ ha<sup>-1</sup> year<sup>-1</sup>.*

### **Key words:**

*Poplar clones, biomass production, biomass characteristics, combustion.*

## **1. INTRODUCTION**

Shortage of wood in general, especially in last decades, has led to excessive exploitation of forest resources. Intensive cultivation of fast growing forest species on short rotations can solve this problem, especially in those regions where there are large areas suitable for poplar and willow growing. Biomass is considered to be one of the key renewable resources of the future at both small and large scale levels. It already supplies 14% of the world's primary energy consumption. On average, biomass produces 38% of the primary energy in developing countries (90% in some countries). Biomass is likely to remain an important global source in developing countries [5]. Even in developed countries, biomass is being increasingly used. A number of developed countries use this source quite substantially. e.g. in Sweden and Austria 15% of their primary energy consumption is covered by biomass. Sweden has plans to increase further use of biomass as it phases down nuclear and fossil fuel plants [17,1].

Plantations help ease shortage of forestry wood. In 1995 the industrial plantation area was estimated to be 103 million ha and the non-industrial plantation

area to be 20 million ha [14]. Over 50% of the plantations are assessed to be less than 15 years and 25% are less than five years [2,18]. The establishment of new plantations is assumed to increase between 160 and 235 million ha in year 2050 [18]. Thus, the above-identified regional and global shortages of wood supply would be much worse without the establishment of plantations.

Short rotation intensive culture or tree biomass cropping refers to woody biomass production in carefully tended plantations using fast growing hardwoods of good coppicing ability, for rotations of less than 15 years [6]. Management objectives centre on maximising annual woody biomass yield per unit area. The success of the biomass production concept depends, in part, on the efficient production systems. Agricultural management practices (plant spacing, high density, use of herbicides, short rotation and regular harvests) are applied to fast growing tree species (such as hybrid poplar, willow or eucalyptus). Poplar appears to be a model species and prototype for such tree biomass plantations. The idea of producing large amounts of wooden biomass by cultivation of fast growing tree species with different rotation periods is a well known approach [12,13,14,15].

The objective of this work was to estimate the dry matter production and energy potential of six poplar clones with two different plant density (38461 and 83333 plant/ha) aged one year rooted cuttings. Interclonal differences of the poplar clone potential for biomass yield were determined based on the study of plantations with a high number of seedlings per unit area.

## 2. MATERIAL AND METHOD

An experimental field plantations were established in experiment estate "Kacka suma". In the field trial 6 poplar clones (*P.x euramericana* cl. Ostia, *P.nigra* cl.53/86, *P deltoides* cl. PE 19/66, *P.x .euramericana* cl.1-214, *P.x euramericana* cl. S6-7, *P.x euramericana* cv. Robusta), with two different plant densities (38461 plant/ha and 83333 plant/ha) are being tested. Trees were planted using 25cm long hardwood cuttings obtained from the Poplar Research Institute Novi Sad. The cuttings were stored at 4<sup>0</sup> and then soaked in water for 24 hours prior to planting. Cuttings were planted to a depth of 22-23cm. Above ground biomass was harvested at the end of the first growing season. Measurement trees were weighted fresh separately for wood and bark in the field and a random sub sample of trees was taken from each plot to estimate moisture content. Samples were dried to a constant weight at 60<sup>0</sup>C and at 105<sup>0</sup>C in a forced air drying oven. Biomass production was calculated on an oven dry weight per ha basis.

After natural seasoning of samples for one month at room temperature, wood was ground into wood flour suitable for pellet pressing. Pellets were made in a special device, and pellets weight ranged between 0.35 and 0.64g. The pellets were combusted in the adiabatic calorimetric bomb (DIN 51 708). The changes in calorimetric bomb were measured in three repetitions for each sample. Correction factors for the formation of acids were not included in the gross heat of combustion (higher heating value) calculations.

## 3. RESULTS AND DISCUSSION

Diameter at breast height and seedling height was measured on the selected samples in field tests. Immediately after felling, the mass of freshly cut trees was

measured and the bark was measured after barking in the green state. The specimens were taken for moisture content measurement. After the biomass drying in the laboratory, it was kiln dried and its oven-dry weight was measured (Table 1)

**Table 1:** Average tree parameters and oven dry weights of stem and bark

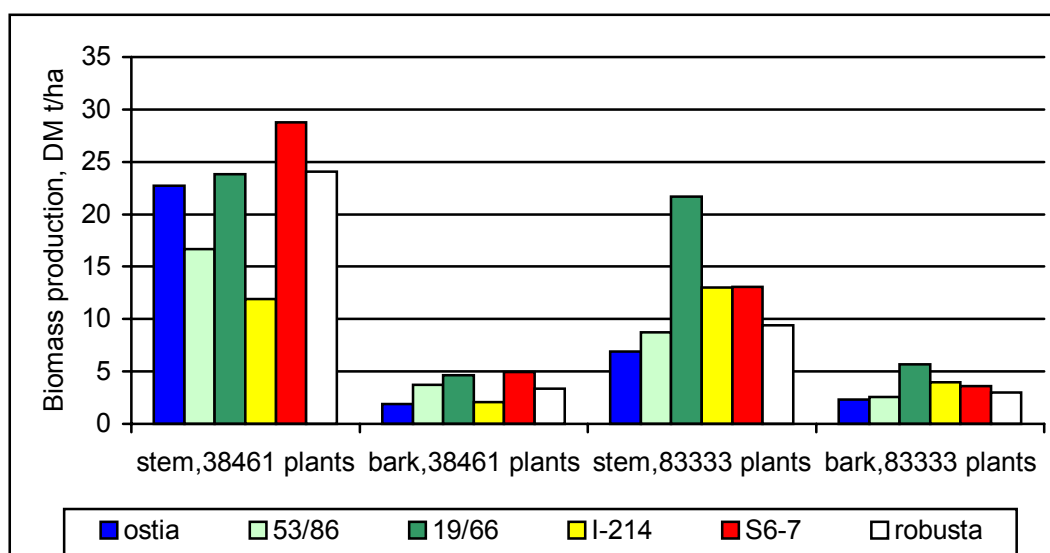
Clone	Stem dimensions		Average weight, DM kg		
	Diameter, cm	Height, m	Stem with bark	Bark	Wood
Plant density 38461 trees/ha					
Ostia	2.8	2.95	0.590	0.049	0.541
53/86	1.9	3.30	0.433	0.097	0.336
19/66	2.5	3.30	0.620	0.120	0.500
I-214	1.9	2.60	0.310	0.054	0.256
S6-7	2.5	3.65	0.748	0.129	0.619
Robusta	2.1	3.50	0.625	0.088	0.537
Plant density 83333 trees/ha					
Ostia	1.0	2.40	0.083	0.028	0.055
53/86	1.2	2.90	0.105	0.031	0.074
19/66	1.8	3.27	0.260	0.068	0.192
I-214	1.6	2.70	0.156	0.048	0.108
S6-7	1.6	2.50	0.157	0.043	0.114
Robusta	1.1	2.30	0.113	0.036	0.077

The results in Table 1 show significant differences in tree diameters and heights, depending on planting density. The consequences of denser plantings are significantly lower diameters, especially cl. Ostia (drop for about 64%) and Robusta (for about 48%). Other clones range in the interval between 16% for the cl. I-214 (min) and 37% (cl. 53/86 and S6-7). The changes of seedling height are not so prominent, and the maximal values are attained by cl. S6-7 and Robusta. The changed tree sizes, which are the consequence of significantly greater planting density, result also in a significantly lower biomass yield. Based on the weights of measured plants, biomass ranges up to 85% (cl. Ostia and Robusta), i.e. more than 70% for cl. 53/86 and S6-7. The minimal value (cl. I-214) amounts to only a half of the biomass weight reached in the lower-density plantation.

Biomass yield per unit area depending on the plantation density was calculated based on the weight of test trees and the number of plants. The quantity of bark per hectare of plantation was calculated based on the bark percentage. Due to the fact that this study deals with the biomass of very young trees, practically one-year-old seedlings in which bark percentage is very high, and because of great differences in diameters of the study mean trees, bark weight per unit area is presented separately, disregarding the fact that the bark is not removed from so young plants, i.e. the trees are not barked before chipping. However, as the bark has a relatively high upper calorific value, it is significant to present the percentage of bark in the total energy released by biomass combustion

The study results of biomass yield after the first year show (Fig.1.) that the increase of planting density has not the same effect on all the study clones. Namely, cl. I-214 shows the rise of biomass yield for about 8%, i.e. if only the bark yield is taken into account, it is the increase of more than 90%. Biomass yield of the clone

PE 19/66 has a downward tendency for about 9% (higher yield of bark for about 23%). The clones Ostia and Robusta are significantly behind, because their yield is lower for 60%. Maximal values of biomass yield in the plantations with 38,461 plants/ha were attained by the clones S6-7 (28,769 t/ha year) and PE 19/66 (23,846 t/ha year). It should be noted that PE 19/66 had the maximal yield also in a denser plantation (21,667 t/ha year). Clone S6-7, with 13,083 t/ha year, is the second by the yield in a denser plantation, although this is only cca 55% of its yield attained in the thinner plantation.



**Fig.1. Biomass production after first growing season**

In spite of the higher plant density the biomass production figure is generally in accordance with other studies, reporting biomass production of 10 to 12t dry wood/ha year [15]; the one-year-old shoots of willow clones (52500 plants/ha) also produced about 12t dry wood/ha [14]. Jiranek and Weger [7] referred that natural clones grow slower than the hybrids, and in good natural conditions annual yield of best poplar clones is expected to be over 15 t/ha of dry biomass. The yield after first year (18000 cutting/ha) ranges from 2.2 to 3.6t DM/ha for poplar clones and 2 to 2.5 t DM for willow clones [8]. After the first of four years rotation cycle in medium density poplar plantations (10000 stems/ha) mean annual increment was 10 to 12 t DM/ha [11]. Riddel-Black et al. [16] reported that yield of six poplar clones (16500 stools/ha) after first growing season was 4.88 to 9.54 t DM/ha. The greatest production of 11.25 t/ha annually can be achieved in experimental plantations with one-year rotations in the production process of 9 years with 40000 trees/ha [9], as well as the fact that plantation establishment and tending, felling, manipulation and preparation for combustion is far simpler and economical than biomass resulting from other forms of production.

To be able to assess the amount of energy obtained from the unit area in two study planting densities, by plantation clear cutting after one-year rotation, the calorific values – higher heating values, were determined for wood and bark specimens of the study clones [9,10].

The analysis of the calorific value of the study poplar wood and bark shows that the calorific value ranges within the interval from 15.68 MJ/kg (min) for the clone I-214, to 21.145 MJ/kg (max) for the clone S6-7. The bark calorific values have a

narrower range, between 15,539 MJ/kg and 19,808 MJ/kg and they also have both positive and negative deviations from the respective wood calorific values. The values calculated for unbarked wood show that cl. I-214 (15,787 MJ/kg) has the min value and that the max value was recorded for clone S6-7 - 20,505 MJ/kg.

The amount of energy that could be produced by the combustion of wood of the study clones was assessed based on the number of trees per unit area and the mass of mean trees of each individual clone, separately for wood and bark, and for the whole tree (based on bark percentage). It is presented in Table 2.

**Table 2:** Calculated amounts of energy per unit area of plantation

Clone	Energy, GJ/ha		
	Wood	Bark	Stem with bark
Plant density 38461 trees/ha			
Ostia	360.169	37.338	398.993
53/86	242.267	62.520	315.144
19/66	335.004	71.712	407.051
I-214	154.385	33.741	188.728
S6-7	503.420	87.735	589.908
Robusta	406.823	64.599	470.159
Plant density 83333 trees/ha			
Ostia	78.511	46.212	121.622
53/86	115.613	43.283	160.064
19/66	278.703	94.962	396.354
I-214	141.104	64.964	205.215
S6-7	199.820	63.365	268.267
Robusta	126.402	57.233	184.187

The calculated amounts of energy show that there is a similar tendency as in the calculation of biomass yield. Max value is recorded for the clone S6-7 (589,908 GJ/ha) in the plantation with 38,641 trees per hectare. The minimal amount of energy is produced by cl. I-214 (188,728 GJ/ha). Robusta and PE19/66 have the advantage over the other study clones in the plantations with a lower number of trees. It is interesting that the heating value liberated by the combustion of PE19/66 trees is similar also in a denser plantation (396,354 GJ/kg) and that the drop is only 3%, which is minor compared to the drop of almost 70% for Ostia or 61% for Robusta. Clone I-214 showed a slight increase (cca 8%) in the denser planting, which is explained by insignificant changes of biomass yield.

#### 4. CONCLUSIONS

The analysis of results obtained by measuring and computing the yield of biomass (and energy) in two field tests with different planting densities, i.e. in the tests with a great number of plants per unit area, shows that in such studies it is necessary to know the characteristics of individual clones. The reaction of the clones to increased planting density is very different. Evidently, the clones I-214 and PE 19/66 are the least susceptible to higher planting density. Of course, due to its significantly higher basic density, clone PE 19/66 is much more interesting, because its biomass yield is in both cases significantly higher.

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## **MORPHO - AGRONOMIC VARIABILITY OF *Triticum monococcum* L. LAND RACES IN THE TIMISOARA AREA**

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### **ABSTRACT:**

*In the Timisoara area 37 accessions of *Triticum monococcum* land races and genotypes collected in Romania (Ro) from 1999 to 2001 and by the Institute for Agrobotany, Taposzele, Hungary (Hu) were evaluated. The genetic variation of this diploid cultivar ( $2n=14$ ) was characterized for 6 morphological characters and for 5 agronomic peculiarities. The statistical data obtained for 2 years pointed out a broad variation for all traits. The grain number varied between  $17.0 \pm 1.81$ – $32.5 \pm 5.59$  in 2002 at the 498 and 689 Romanian land races respectively. In 2003, a very droughty year the grain number varied from  $20.68 \pm 1.29$  to  $36.08 \pm 1.44$  at the 687 and 444 land races respectively. The variation of Hungarian land races was  $6.0 \pm 0.01$ – $55.0 \pm 0.001$  during 2002 at the 1158 and 1153 respectively. In 2003 the amplitude of variation was from  $22.64 \pm 0.88$  to  $33.93 \pm 2.7$ . In 2002 the grain weight varied from  $2.2 \pm 0.2$  to  $7.3 \pm 0.5$  g/10 in Romanian land races and from  $1.5 \pm 0.4$  to  $9.4 \pm 4.7$  g/10 at the Hungarian entries.*

### **Keywords:**

*Triticum monococcum*, Romanian Hungarian land races, evaluation.

## **1. INTRODUCTION**

Cultivated einkorn (*Triticum monococcum* L.) like wild einkorn has a diploid constitution ( $2n=14$ ; AA-genome). Wild einkorn was harvested in the late Paleolithic (16,000 – 15,000 BC; **23**) continued to be a popular cultivated crop during Neolithic and early Bronze Age, being the first cereal cultivated for food (10,000 – 4,000 BC; **10**). Its importance decreased when higher yielding and free-threshing tetraploid and hexaploid wheats replaced it. Today, einkorn production is limited to small isolated regions of the Mediterranean region (**11**) and in the West Romanian Carpathian Mountains (**2**). The wild form was still common in some locations in Turkey during the 1960s (**23**).

The diploid wheat are used in bread making and also in breeding programs for durum wheat ( $2n=28$ ) and Triticale tetraploid forms ( $2n=28$ ), due too its resistance to several biotic and a biotic stresses (**7;12;5**). Therefore, the evaluation, identification and description of the genetic variation of einkorn accessions stored in germplasm collections is of utmost importance for the prevention of genetic erosion and to promote its use in breeding programs (**16**).

Several studies on limited numbers of accessions have revealed high variability for morpho-physiological traits (15;21), yield (9;19;4), pathogen resistance (8), storage proteins (22;14), starch characteristics (17;6), bread-making quality (1) and molecular markers (20;18;3;10).

The Banat University of Agricultural Sciences Timisoara, Romania, in collaboration with the Institute for Agrobotany, Tapioszele, Hungary assembled 37 einkorn accessions from the Tapioszele gene bank and Romanian land races collected in 2001 in the *Metaliphereous Mountains* in 2001.

The objectives were:

- to evaluate the genetic variation among accessions collected in different places and preserved in gene bank conditions in comparison to “native” *in situ* grown land races from Romania;
- to evaluate the agronomical characteristics and the homeostasis in the Timisoara area.

## 2. MATERIALS AND METHODS

37 einkorn accessions studied, divided by provenience classification and origin is presented in **Table 1**.

Table 1: Origin of einkorn land races accessions characterized in Timisoara, Romania

Romanian		Hungarian	
accession no.	Origin	accession no.	Origin
444	Mada	1150	ESP
495	Almas de Mijloc	1155	DDR
498	Almasul Mare	1160	DDR
672	Almasul Mare	1163	DDR
673	Almasul Mare	1164	UNKNOWN
687	Almasul de Mijloc	1165	DEU
688	Almasul de Mijloc	1168	CHE
689	Almasul de Mijloc	1171	HUN
690	Almasul de Mijloc	1175	HUN

One hundred seeds of each accession were hand planted in the field in autumn 2001 on October 17<sup>in</sup> the Romanian and on December 5<sup>th</sup> the Hungarian accessions, in single rows 1.50 m long and 0.25 cm apart.

Plants were harvested by hand between 15 and 30 July 1992. In 2002, 600 seeds were sown in mini plots (5m<sup>2</sup>) in October 10<sup>th</sup> 2002 and harvested with Hege combine at July 15<sup>in</sup> 2003.

Every year mineral fertilizer was applied before planting (45N:45P:45K kg/ha active substance) and in January (on the snow Nitrogen 66 kg/ha active substance was applied).

For analysis land races these showed the best 1000 seed weight was selected.

The einkorn land races were characterized using 21 morpho-agronomic descriptors. In addition, the total protein content and protein fractions were done.

Statistical analysis was carried out using the statistical package VA-S (1999).

### 3. RESULTS AND DISCUSSIONS

Mean, minimum and maximum values and standard deviations of the seven continuously variable descriptors were analyzed. The characters showed a high variability.

The most important characters are pointed out.

An earlier **sprouting** revealed the Romanian land races (78%) in comparison with the Hungarian ones (56%). In the Romanian land races the germination varied from 20.0% (Ro.690) to 93.3% (Ro.498) and in Hungarian from 26.7% (Unknown-1164) to 100% (ESP-1150).

The **frost resistance** differentiated very much the Romanian land races from the Hungarian ones. Two land races Ro.495 and Ro.689 (22.2%) were affected by frost (10% and 12% respectively) and five (56%) were affected too from 9.09% to 75% (DEU-1165 and DDR-1160 respectively).

The physiological traits as well as sprouting, vegetative period (days from sprouting to heading), heading data, and maturity (days from sprouting) pointed out the almost the same values.

The earlier **heading** took place at Ro.495, after 181 days from sprouting. Late heading after 190 days was equal in both Romanian and Hungarian land races (66.7%).

The **maturity time** was divided in 3 periods early, under 220 days, medium between 221- 225 days, and late more than 225 days. 22.2% of the foreign land races were early (Unknown-1165 and DDR-1163) and 55.6 of them were late. The Romanian land races pointed out medium maturity (88.9%). In the Timisoara climate conditions (2003) the einkorn maturity was like in wheat, triticale and oats.

**Plant height** had a high variability in 2002 being from  $70.67 \pm 3.54$  cm BGR-1154 to  $107.88 \pm 3.43$  cm Ro-689, and a narrow variability in 2003 ( $80.44 \pm 0.89$  cm ESP-1150 to  $98.20 \pm 1.01$  cm DDR-1163).

Despite their height, many accessions (16.7%) showed little **lodging**.

The **number of spikelets** per spike was very variable in 2002. The distribution revealed a marked bimodal one 69.2% in small class and 30.3% in medium class (25-33 spikelets). At the same time 50% of Romanian cultivars pointed out 50% high number of spikelets. In 2003, 88.7% and 22.2% Romanian and Hungarian land races respectively belonged to high and medium classes.

In 2003 the variability of **grain weight** was less in Romanian land races ( $5.0 \pm 0.2$ - $8.0 \pm 0.5$  g/10) in comparison to Hungarian ones ( $3.7 \pm 0.3$ - $8.2 \pm 0.8$  g/10).

Average of **1000-seed weight** was 21.23 g and 20.96 g in Hungarian and Romanian land races respectively (2003), much lower than that of bread or durum wheat (commonly 40-45 g/1000 seeds). No accession with a 1000-seed weight above 40 g was founded. The most frequent seed colour was white or yellowish.

All einkorn observed did not show a correlation between morphological and physiological peculiarities and yield. The highest physical yield was 3,400 kg/ha and 2,800 kg/ha in Ro.495 and DEU-1165 respectively. The *theoretical yield potential* (TYP) calculated from main spike grains weight multiplied with the number of viable plants in the spring and no. of tillers was far from the real yield (**Fig.1**). It was 5,498 and 4,390 kg/ha in Ro.495 and DEU-1165 respectively. In other land races the TYP was higher than 7,812kg/ha and 4,829 kg/ha in Ro.673 and DDR-1163 respectively. It is clear that the einkorn has a high *theoretical yield potential*.

#### 4. CONCLUSIONS

Identification and description of the genetic variability available in germplasm collections are the basis of improved plans designed to control genetic erosion; they are also a preliminary requirement for the exploitation of useful traits in plant breeding.

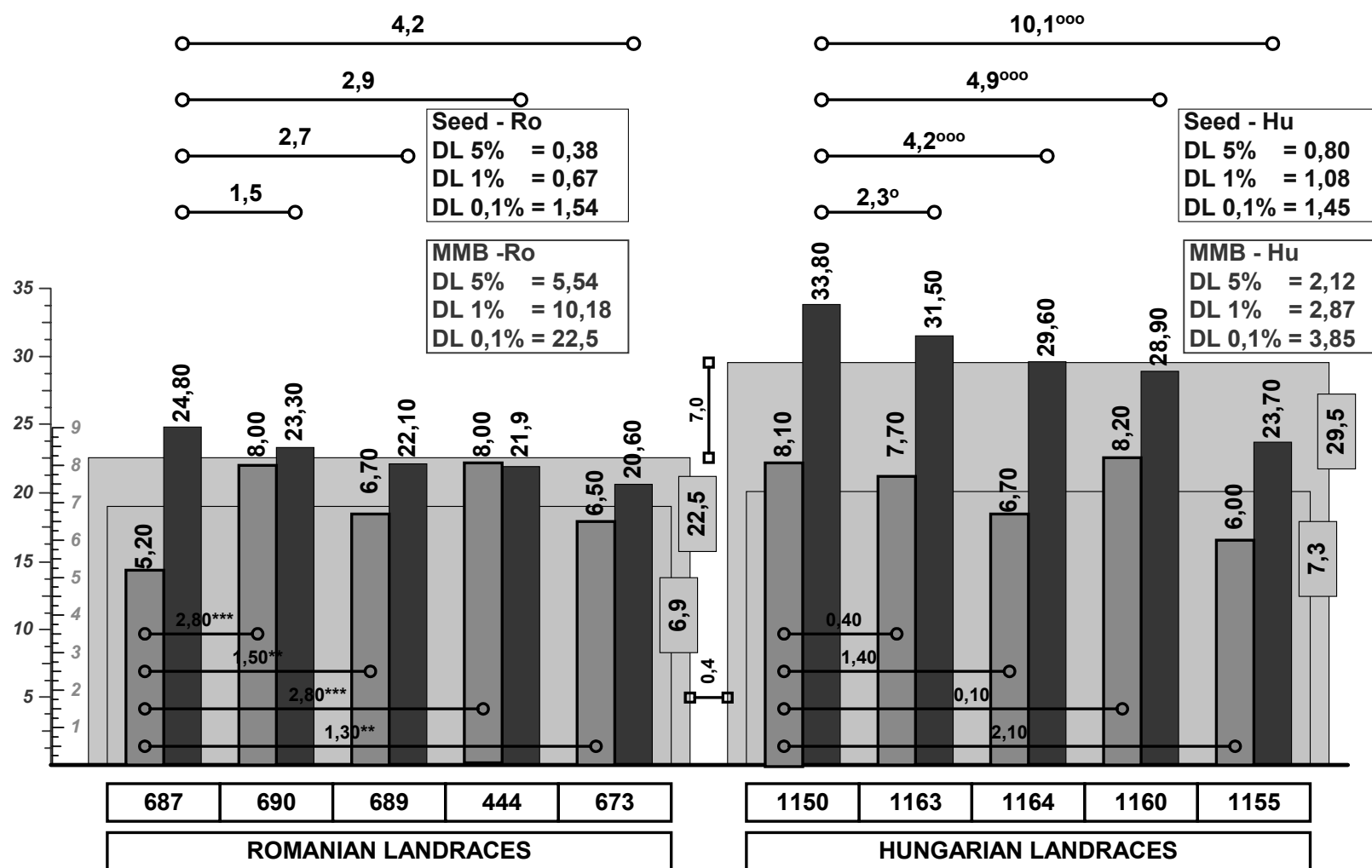
Even if einkorn generally revealed late maturity and small seeds prevalent characters, limiting einkorn cultivation in many places all over the world, in *Metalipherous Mountains* it is a common cultivar. The broad variation for these and other traits, especially the theoretical yield potential allows identification of promising accessions for einkorn breeding.

A few samples (Ro.495, Ro.689 and DEU-1165) showed good physical yield.

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**Fig.1:** The differences in grain weight and 1000 grain mass at Romanian against Hungarian einkorn landraces (2003)

■ 1000 seed weight (g); ■ Seed weight / main spike (gx10); ■ Average of seed weight / main spike (gx10); ■ Average of 1000 seed weight



## THE INFLUENCE OF CYCOCEL IN THE PROCESS OF WHEAT'S IMPROVEMENT AND DEVELOPMENT

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### **Abstract:**

*The experimental research that was done aimed at proving that the cycocel had an action on some morphological, physiological and qualitative characteristics, but also on productive capacities of some wheat varieties, cultivated around Lugoj city (Timiș county). This kind of action depends on: the climatic variables (precipitations, temperature), the soil characteristics (type, pH and contents), the applied dose, the number of treatments, the application stage and the quantity of active substance used.*

### **Keywords:**

*cycocel, growth retarders, quaternary ammonium combinations, wheat*

## **1. INTRODUCTION**

As early as the beginning of the last century, it had been inferred that the development of the plants could be chemically influenced [1]. The discovery of vegetal hormones, seven decades ago, represented a new stage in the knowledge and management of plant's development regarding their improvement.

The identification of vegetal hormones had been followed by research that observed the reaction that the plants had to a series of synthetic compounds having the structure and the physiological characteristics similar to the natural hormones.

Nowadays, a great number of researchers confirmed the multilateral action of the growth retarders.

In a more general way, the retarders belong to the group of morpho-regulative substances. By their action, the retarders differ from the stimuli and inhibitors of growth.

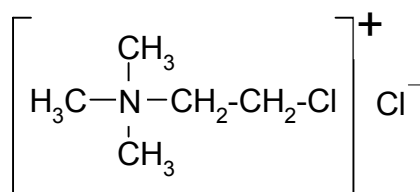
The growth retarders cause some morphological and physiological changes by which influence the rhythm of growth, although, through their

action, they do not irreversibly stop the metabolically processes that are vital for the plant, they do not completely suppress the growth and do not affect the formation and the development of the reproductive organs, so that the plant's growth and their fertility potential are not diminished.

The group of retarders contains [2]:

- nicotine derivates;
- quaternary phosphorus combinations;
- quaternary ammonium combinations [3].

Cycocel is a quaternary ammonium combination that has a similar structure to that of the choline.



**Fig. 1.** The structure of cycocel

The cycocel contains a chloride atom placed to the second carbon of the ethylene group, while the choline contains a hydroxyl group. The substitution of the hydroxyl group of the choline with a chloride atom has a great importance in what regards biological processes, because cycocel can not form esters, because it is short of -OH group.

Cycocel,  $\text{C}_5\text{H}_{13}\text{Cl}_2\text{N}$ , is a yellowish crystalline substance, with density of  $1,15 \text{ g / cm}^3$ , soluble in water in which forms an acid solution ( $\text{pH} = 5,14$ ), soluble in ethanol and insoluble in ether. It is volatile in proportion of 88 %; it has a molecular mass of 158,11 and melts at  $245^\circ\text{C}$  [4, 5].

## 2.METHODS AND MATERIALS

The cycocel's application can be done using one of the following methods:

- its introduction in the soil in pure state or mixed with ammonium fertilizer;
- the treatment of seeds before the sowing time;
- the sowing splash.

The action of cycocel depends on climatic variables and especially on precipitations.

The average annual quantity of precipitations between 2000 – 2002 had been of 761 mm, which compared to the average quantity of 606 mm registered during a decade, is 115 mm less.

The average annual value of temperature between 2000 – 2002 had been of  $10,6^\circ\text{C}$ ,  $0,3^\circ\text{C}$  less than the average value of the temperature registered during ten years ( $10,9^\circ\text{C}$ ), with average annual variations between  $10,2 - 11^\circ\text{C}$ .



The researches that had been done were placed on the soil of the second terrace of the Timiș river. This soil contains a poor acid pH (4,98 – 5,60) and superior layers of 3,6 % clay. The soil contains an average quantity of nitrogen, mobile phosphor and potassium. The water level is 2 – 3 m underground.

The cycocel used contained 40 % active substance and its administration was done on a foliage way, by adding a quantity of 400 liters water per ha.

With a view for establishing the best dose of active substance, the application stage, there had been different treatments. When it comes to the field experiences, the doses of cycocel were of 3, 6 and 9 kg per ha (1,2, 2,4 and 3,6 kg active substance). The treatment was done in three stuffing stages, when the plants had only 4 – 5 leaves and 10 – 40 cm height.

**Table 1.** *The characteristics of the wheat varieties, treated with cycocel*

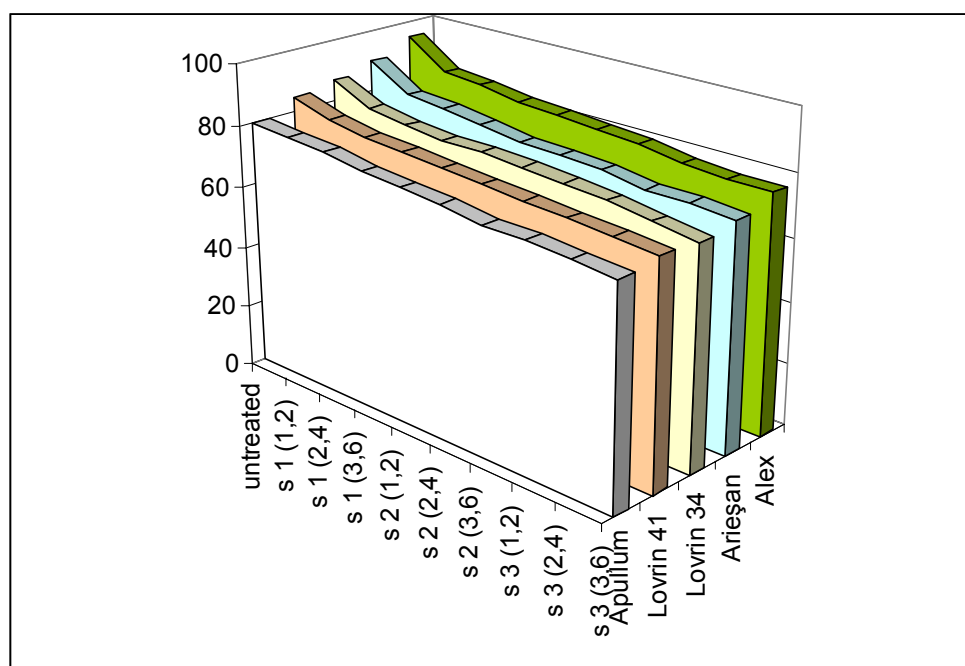
The name of the wheat variety	The keeper of the variety	The year of registration	The length of the straw
Alex	Stațiunea de Cercetări Agricole Lovrin	1994	95 cm
Apullum	Stațiunea de Cercetări Agricole Turda	1992	80 cm
Arieșan	Stațiunea de Cercetări Agricole Turda	1985 (1995)	90 cm
Lovrin 34	Stațiunea de Cercetări Agricole Lovrin	1981 (1999)	87 cm
Lovrin 41	Stațiunea de Cercetări Agricole Lovrin	1987 (1997)	85 cm

### 3. RESULTS AND DISCUSSIONS

Among the actions of retarders, the most specific one is the shortening of the length of the wheat's straw by which the resistance potential is increased and prevents the falling phenomenon.

Out of the results that were obtained to the application of cycocel, it had been noticed that the length of the straw had shortened between 10 cm (Apullum) and 18 cm (Alex). The result was that the straw's length had significantly shortened to all the varieties researched in what regards the doses applied and the application stages of cycocel.

In the case of the wheat varieties that had been studied, it proved that the best dose was of 2,4 kg cycocel (active substance) per ha, applied at the stuffing stage, when the plants had 4 – 5 leaves and 20 – 30 cm height.



**Fig. 2.** The influence of cycocel on the shortening of the straw's length regarding the dose and the application stage (s)

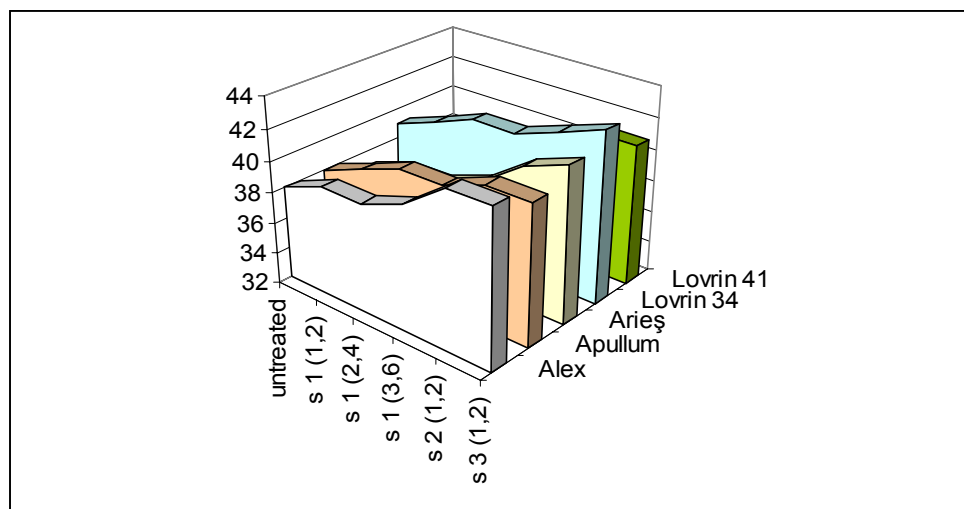
When the treatment had been many times repeated, there had been different results depending on the number of treatments. When there was applied only one treatment, the average shortening of the straw's length

**Table 2.** The wheat production under the influence of cycocel in different doses and application stages

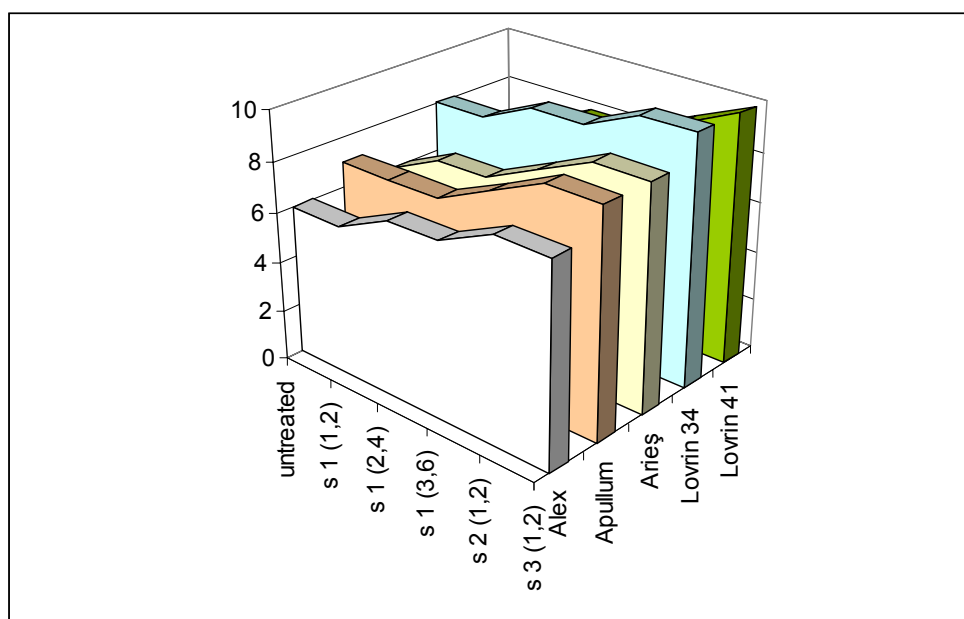
The wheat's variety	The year	The application stage	The dose		
			1,2 kg / ha	2,4 kg / ha	3,6 kg / ha
Lovrin 34	2000 – 2001	1	3,322	4,012	4,087
		2	4,150	3,765	3,970
		3	3,785	3,765	3,790
	2001 – 2002	1	4,367	4,122	4,120
		2	3,762	4,010	4,122
		3	3,847	3,772	3,762
Lovrin 41	2000 – 2001	1	3,500	3,725	3,650
		2	3,725	3,600	3,550
		3	3,650	3,725	3,662
	2001 – 2002	1	3,993	3,845	3,953
		2	3,898	3,881	3,987
		3	3,750	3,857	3,642

was 7,07 cm, when there were applied two treatments was 10,53 cm and to three treatments was 13,8 cm. These results show a high efficiency of the retarders to the shortening of straw's length when the treatment is repeated.

In what regards wheat's production we observed and noticed in table 2 the values for Lovrin 34 and Lovrin 41 varieties. About the qualitative characteristics, we observed the absolute mass of 1000 grains and the gluten content regarding the dose and the application stage and noticed them in figures 3 and 4.



**Fig. 3.** The influence of cycocel on the absolute mass of 1000 grains regarding the dose and the application stage (s)



**Fig. 4.** The influence of cycocel on the gluten content regarding the dose and the application stage (s)

In our researches, the influence of cycocel on the wheat's resistance to hibernation and its tolerance to frost had been observed using doses of 1,2 and 2,4 kg per ha and the number of viable plants had registered an increase of 2 and 2,1% in comparison to the plants that were not treated with cycocel. These indicate a poor action of the retarder on the wheat's resistance to hibernation.

In what regards its resistance to drought, during springtime, when the plants are at the stuffing stage, they were given drought conditions for 45 days. The resistance to drought had been appreciated depending on the number of viable plants compared to those not treated with cycocel.

#### 4. CONCLUSIONS

The results that we obtained after this research, allow us state that:

- The most evident action of the cycocel is that of the effect it has on the shortening of the straw's length by modifications of the anatomical structure.
- The action done by cycocel influences only in a little degree the technological quality of the wheat.
- The shortening of the straw's length influences indirectly the resistance capacity to drought by modifying the transpiration coefficient.

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## THE ROLE OF MYCORRHIZAE IN AFFORESTATION

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### **Abstract**

*Hungary is facing to perform intensive afforestation on 700 thousand – 1 million hectares during the next 40 years. New forests and wood plantations will be planted mostly on dry, poorly fertile soils nonprofitable for agricultural use to be found firstly on the Great Hungarian Plain. Applying artificially mycorrhized seedlings partly may considerably increase the effectivity of afforestation assuring of more intake of nutrients and water for the seedlings, partly we hope that may decrease the total costs of afforestation.*

### **Keywords**

*Mycorrhiza, afforestation*

## **1. INTRODUCTION**

Hungary had always belong to the European forefront of quantitative afforestation. Therefore after the Second World War the forest area could be increased from 11 % by the present to 19 %. The extent of agricultural territories suitable for afforestation in the country is calculated to be between 700 thousand and 1 million hectares [1,2,3].

The agricultural territories possibly involved in afforestation belong partly to the very dry, sandy areas where the drought is often raised by high lime content. The other part of land potentially usable for afforestation is the steep slopes of mountains and hills previously covered by woods. These areas have been cultivated for centuries but exhaustion and erosion degraded the soils, so their agricultural use is nonprofitable. The humus content of these soils is very low, usually below 1 %. In addition agricultural soils miss the normal microbiota of forest soils the trees are adapted to and contain highly different microbe communities disadvantageous for the development of planted seedlings.

It can be stated that the roots of tree seedlings planted into agricultural soils get into a hostile environment which a part of the plantlets cannot cope with. That is one

reason why new plantations must be planted in average 1,6 – 1,7 times or even twice.

According to our results merely in Bács-Kiskun County there are more than 300 thousand hectares of unpritable agricultural land can be proceeded. In such case afforestation seems to be the most reasonable land use [4].

Rapid ecological changes of the last years (e.g. warming up, drying and sink of underground water level) warn us to look for new ways of afforestation succesful also in disadvantageous circumstances. Establishing artificial mycorrhizae on the roots of seedlings is such a new and in addition a natural method.

## 2. THE EFFECT OF MYCORRHIZAE ON THE NUTRITION AND WATER UPTAKE OF FOREST TREES

Mycorrhiza, a symbiotic relationship between roots and fungi, is widespread all over the world. Different types of mycorrhizae, characteristic to plant communities having evolvel in different geographical and climatical zones, exist. In the deciduous and needle woods under temperate climate trees typically form ectomycorrhizal connections mainly with basidiomycetes, less with some ascomycetes. There are of course some broadleaved species having endomycorrhiza connections (*Fraxinus sp.*, *Acer sp.*, *Prunus sp.*, *Sorbus sp.*, etc.)

Diversity of fungal communities in forest ecosystems is determined by the following factors:

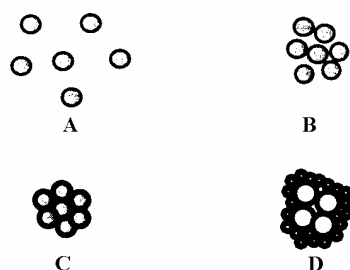
- *age of trees in the stand;*
- *composition of natural plant association, the occurence of host plants, specificity of host-symbiont connection;*
- *soil factors (pH, chemical composition, organic contents, etc.);*
- *climatic and microclimatic factors*

The mycorrhiza fungi have a great influence on the growth, water and mineral uptake of the trees to be as host plants.

According to the findings of several experiments on seedlings, mycorrhizal plants can better take up the water and show more drought-tolerant than non-mycorrhizal trees. Moreover, fungal strains differ widely in this respect. Several mechanisms are involved: a direct effect on water uptake through various strategies of soil exploration by the mycelium, an indirect effect through the modification of water status regulation by the tree and changes in the water-use efficiency of photosynthetic carbon.

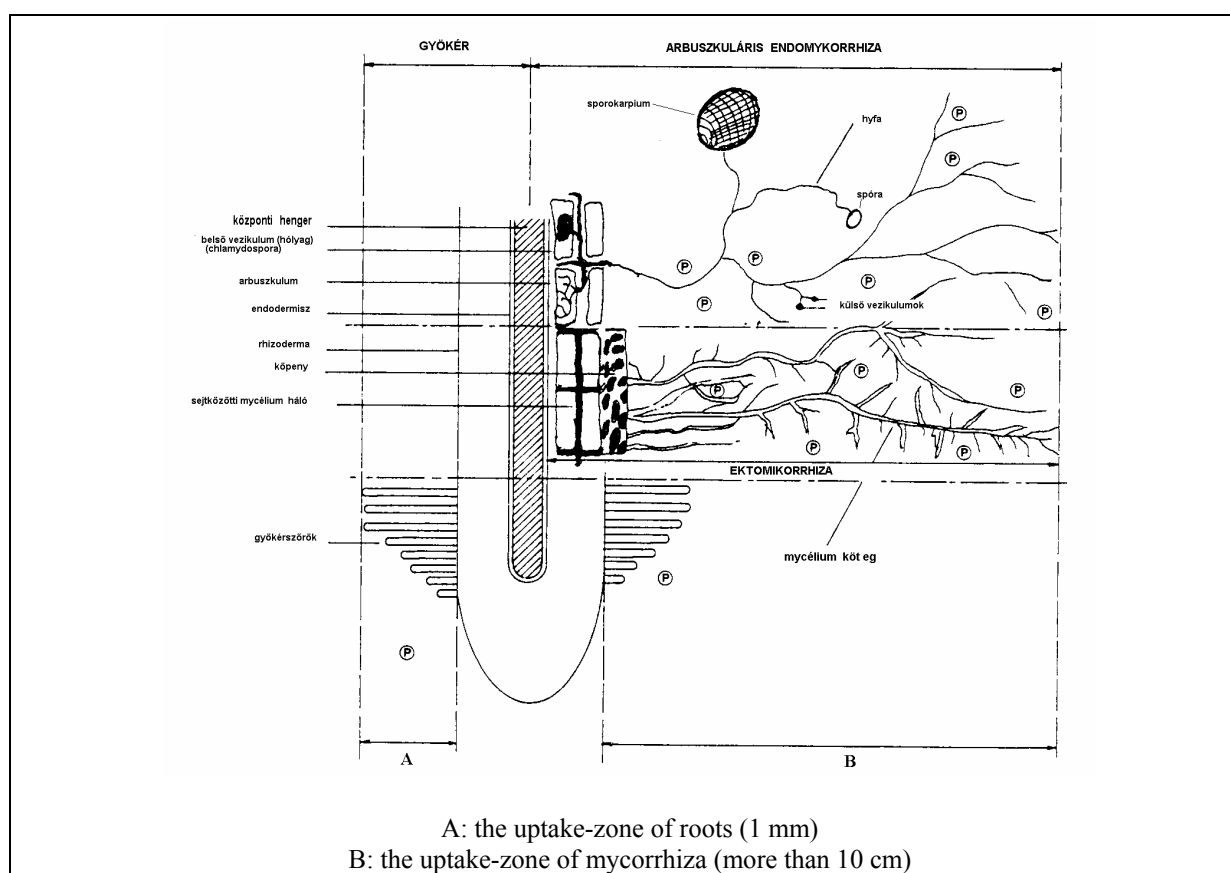
In ectomycorrhizae, a dense sheet of fungal mycelium, the so called *mantle*, is covering the root tips. Emanating hyphae, growing from the mantle into the soil, multiply the covered soil volume, and permit the increased water and mineral uptake. It happens the same way in the case of endomycorrhizae (Fig. 1-2.)

The advantage of mycorrhizae compared to non-mycorrhized plants is more distinctly manifested in dry soils, poorly supplied with phosphorous and nitrogen. Mycorrhization increases growth (Table 1.) as well as P and N content of plants (Table 2.)



- A: myceliums consisting dispersed, individual hyphae  
 B: myceliums organised loose bundle  
 C: mycelium bundle containing closed connected hyphae  
 D: complex, water-conduit rhyzomorpha consisting thick, hollow hyphae inside and thin, cortical hyphae outside

**Fig. 1. Organization of hyphae bundle [7]**



**Fig. 2. The extension of mineral uptake-zone surroundings of mycorrhiza [PLENCHETTE et al. 1982 cited in 10]**

Mycorrhiza help plants to survive dry periods and adapt to limy [8, 9, 13]. Mycorrhizal seedlings can tolerate higher soil temperature and lower pH conditions. Mycorrhizae increase tolerance of plants against inorganic and organic toxic substances, protecting them from heavy metal stress [5, 6, 14, 15]. This is extremely

significant economically in afforestation and reafforestation of dry, poor and polluted areas.

**Table 1. The effect of ectomycorrhiza on overground growth of *Pinus pinaster* seedlings 10 months after planting out (fresh plant mass in g)**  
[MOUSSAIN et al. 1979 cited in 10]

Soil	Non-mycorrhized control	Natural mycorrhiza	<i>Pisolithus tinctorius</i>	<i>Hebeloma cylindrosporum</i>
1.	1,4 ± 0,2	3,5 ± 1,2	10,6 ± 1,6	13,5 ± 2,1
2.	0,6 ± 0,3		7,3 ± 2,6	8,9 ± 1,7
3a.	1,4 ± 0,7	3,5 ± 0,5	7,7 ± 0,7	6,9 ± 1,1
3b.	1,5 ± 0,3		4,6 ± 0,8	3,8 ± 0,4

The test was realised partly in controlled conditons. One part of the 3.5 months age seedlings were inoculated with artificialy produced mycelium, the other part of them were inoculated with naturaly mycorrhized root extract.

The soil samples were taken from the A level.

1. humous podzol; 2. eluviated adobed sand; 3a. slightly humous sand (humous content < 0,55 %); 3b. slightly humous sand (humous content = 0,08 %).

**Table 2. The effect of mycorrhization on the N and P content on overground parts of *Pinus pinaster* seedlings 10 months after planting out (expressed in % of dry mass)**  
[MOUSSAIN et al. 1979 cited in 10]

Content	Non-mycorrhized control	Natural mycorrhiza	<i>Pisolithus tinctorius</i>	<i>Hebeloma cylindrosporum</i>
Total P	0,09	0,17	0,21	0,32
Total N	1,79	2,19	2,10	2,77

### 3. POSSIBILITIES OF APPLICATION OF MYCORRHIZAE

In the nurseries, the underground parts of the plants are often damaged by different root pathogenic fungi belong to the following genera.

- *Phytophthora*
- *Pythium*
- *Fusarium*
- *Rhizoctonia*
- *Cylindrocarpon*

Some of them can cause diseases only when plants are stressed (alcalic pH, bad water draining, too low/high temperature or irradiation). In contrary, *Phytophthora*, *Fusarium* and *Rhizoctonia* are aggressive pathogens damaging also healthy plantlets.

The most simple protection methods are the traditional crop-rotation and the soil disinfection. The sensibility of phytopathogenic fungi against chemicals are highly different. Many pathogenic fungi are resistant to commonly used fungicides. Worldwide tendency of decreasing the use of pesticides from human health and environmental protection reasons helps biological control methods to expand.



The plant protection effect of ectomycorrhizal fungi have been demonstrated [14]. These fungi may play a significant role in the biological control of nurseries. The main advantage is that, in contrast of pesticides which must be applied repeatedly, mycorrhizae have to be applied only once. However, it is important that inocula get into the soil before phytopathogens can spread [12].

The protection mechanism of ectomycorrhizal fungi consists of the following elements:

- *fungus mantle itself serves as a mechanical defense barrier to root;*
- *the mycorrhizal fungus degrades toxins and enzymes of the pathogens and produce acids and antibiotics inhibiting the enemy;*
- *mycorrhizal fungi compete with pathogenic species for the use of root carbohydrates;*
- *the rhizosphere of mycorrhized roots are about ten times richer in other microorganisms than non-mycorrhized ones;*
- *some microbes enhance mycorrhization, they are so called Mycorrhiza Helper Bacteria (MHB) and some show an additional inhibition against pathogens*

According to experiments carried out in Germany the shoot volume of beech seedlings mycorrhized with *Pisolithus tinctorius* was by 72 % higher than that of the non-mycorrhized control. The same value in the case of *Paxillus involutus* was 58 % [11].

### 3. CONCLUSIONS

We hope that using mycorrhized plants for the afforestations, we can partly establish healthier forest ecosystems and more productively wood stands, partly may decrease the total costs of afforestations because it will be no need to repeat the plantations.

After some years of preparation, this year we have begun the field trial to produce more and more mycorrhized forest seedlings for the afforestations.

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## NEW STRATEGY FOR WATER SUPPLY IN VOJVODINA

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### **Abstract:**

*The great intensity of groundwater exploitation for water supply in Vojvodina Province (northern part of Serbia) has been provided from deep aquifers. More than 6,5 m<sup>3</sup>/s comes from those aquifers, which belong to a subartesian and artesian type, and from depths varying from 60 m to more than 250 m. Due to intensive long-term groundwater production, the actual pumping levels at numerous deep wells, used for supplying cities, have declined more than 30m in. The new strategy of water supply will be contributed by radical restriction of groundwater exploitation of deep aquifers and by saving the available reserves for future generations.*

### **Keywords:**

*Water, supply, well, aquifer, alluvium, GIS.*

## 1. EXISTING STATE OF WATER SUPPLYING IN VOJVODINA

Water from basic water-bearing complex, mostly from deep aquifers is, even today, the most significant resource of potable water supply in Vojvodina.

Exploitation of underground water in Backa and Banat intensively started in 1960's. Today, ground water is tapped from the formed sources in water-bearing areas of BWC (basic water-bearing complex), water-bearings from younger Quaternary in coastal belt of the Danube and locally, from deep water-bearings from Pliocene. The depths of underground water tapping are different and depend on position of the water-bearing systems. Generally, from the water-bearings of BWC and younger quarter, water is tapped on the deep of 25 m in coastal belt of the Danube and in South Banat. According to middle and northeast Backa the tapping depths are from 75 to 190 m, but in northeast Banat (Kikinda-Mokrin) water is tapped even from 230 m. Water-bearing of Pliocene is available on 140-230 m, locally and in the deeper areas (from 255-272 in Novi Knezevac, from 306-395 m in Opovo).

Today's average underground water consumption from some sources of city centres in Backa, Banat and Srem is from 10-50 l/s to 0.5 m<sup>3</sup>/s. Most of the sources daily exploit less than 100 l/s (33 springs). There are no precise information for spring exploitation in rural areas. It is believed that there are over 250, from which is tapped 2-10 l/s, mostly less than 5 l/s.

Total amount of collected water for public water supply for inhabitants and industry, or total average daily exploitation of underground water in Vojvodina is: in area of Backa  $Q=3870$  l/s, Banat  $Q=2140$  l/s and Srem  $Q=800$  l/s [2].

According to this, total average daily exploitation of underground water for water supply of inhabitants and industry in Backa, Banat and Srem is almost  $7 \text{ m}^3/\text{s}$ . About 92% of this amount is tapped from Quarter water-bearing complex, and only 8% from Pliocene water bearing.

Over abstraction effects of deeper aquifers, actually the main water-bearings from BWC, is at first manifested as a fall of piezic level in water sources, with tendency of spreading of depression cone. These effects are mostly expressed in basic aquifer, actually in polycyclic sand-gravel packs in layers from Pleistocene, then in formed aquifers in Pliocene sandy water-bearings. Progressive fall of piezic level in the system of deep aquifers is especially expressed in Backa and in North Banat.

According to data of occasional measuring in the time of 1960. -1990. general fall of piezometric levels in wider aquifer zone in North Backa was 14 m, in the water source of Becej, and over 30 m in the spring source of Vrbas and Kula. Some falls of piezic levels were registered even to 1.5 m a year, in source area. Big falls of underground water level are also registered in North Banat, where the general fall in wider water source area is about 17 m. This state of deep aquifers is affected by an exploitation of underground water on the border area of Romania. As a reliable evidence of overexploitation of these aquifers stands the fact that there are no artesian wells in Northern Banat today, but before 1960. every borehole, which collected these water-bearings, had a positive piezic level [3, 4, 7, 8, 9, 10].

## 2. REGIONAL WATER SUPPLY SYSTEM IN VOJVODINA

Condition of the high quality water supply in Vojvodina can be characterized as the least favourable in Serbia. Needed amounts of water today are supplied by exploitation of alluvial sources and basic aquifer, which caused a big fall of underground water level, with all negative consequences. By its quality, potable water in Vojvodina is certainly the worst in Serbia (together with some small communities in Central Serbia and Kosovo). On the other side, the biggest three rivers in our region the Danube, the Tisa and the Sava run through this plain. In alluvium of the Danube, zones marked as suitable for regional sources are between Bezdán and Bogojévo, **between Kovin and Dubovac** and some smaller zones in the middle flow of this European river through our country. Alluvium of Sava gives suitable conditions in some parts from Jamena to Lacarak and from Jarak to Grabovac. Tisa does not have such suitable sectors (in hydrological way) for high quality waters, but the water quality of the Tisa is worse than the water quality of The Danube and Sava. The main advantage of the Tisa is its position near the most endangered drinking water consumers (area of North Banat and Northeast Backa). Its water can be used as potable only with the most contemporary purification procedures. Other waters in this area are not reliable now, and it will probably be the same in future. On the North of Central Serbia in the riparian zone of the Drina and the Velika Morava, in the mouth of the Sava and the Danube, these locations give significant opportunities as sources of high quality water [2].

Water supply problem of Vojvodina, cannot be solved separately. Many alternative solutions have been considered in the case that some of the considered water sources work with a small capacity or that some do not work at all. Proposed

solutions for this whole part of Serbia include many alternative solutions, which strengthen the sureness of water supply for the whole region. Developing of regional systems, which will use its own water supplies and the water from mentioned sources is inevitable for Vojvodinian region. Besides regional systems, some smaller communities should strict bond to the lows on the exploitation of local sources.

### 3. NEW WATER SUPPLY STRATEGY

It is obvious that the problem of water supplying in Vojvodina cannot be solved as an extension to over exploitation of deep aquifers of BWC. This kind of underground water usage causes destroying of this precious natural resource as a consequence. This is why it is needed to start a completely different way for solving the problem of water supply, which would be based on the usage of surface and underground waters in coastal riparian of big Vojvodinian rivers: the Danube, the Sava, the Tisa, the Tamis. This is, actually, all about making restorable water sources in free aquifers of alluvial water-bearings whose feeding is ensured by the interaction of the river and water-bearing system. Coastal sector Kovin-Dubovac gives remarkable opportunities, and its restorable sources of underground water have bigger capacity per a year than any other water supply object in Vojvodina [1, 5, 6].

This approach to the problem represents a new strategy in water supply policy in Vojvodina, which would contribute to the radical limiting of drinking water usage from deep aquifers and also its protection and keeping water for longer terms.

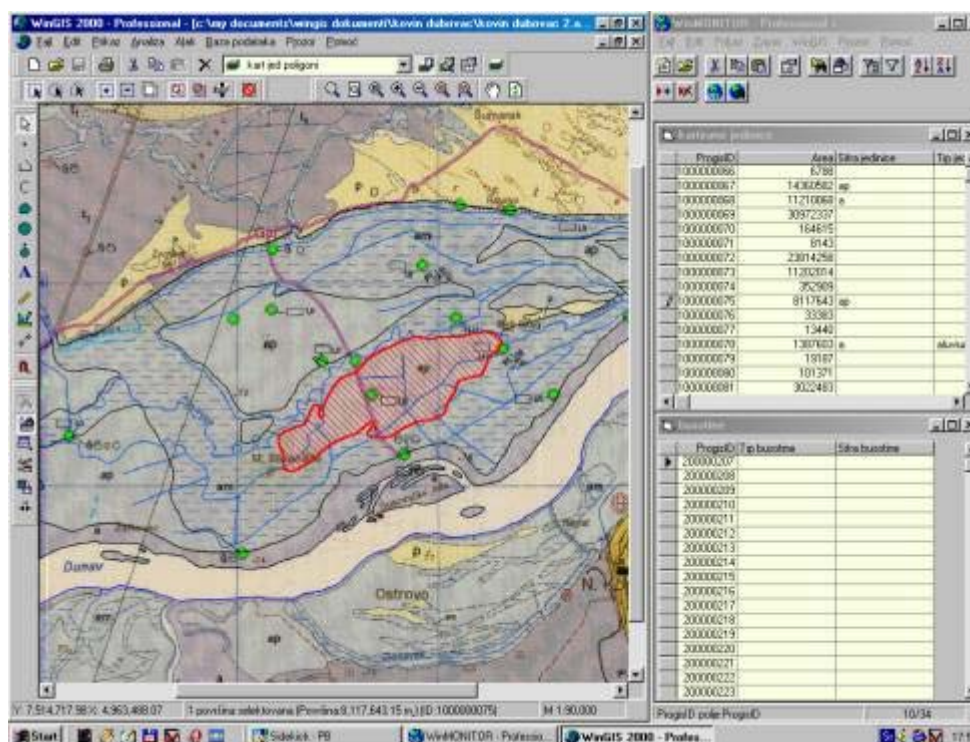
#### DEVELOPMENT OF OPERATIVE-COMMUNAL CENTER

New information technologies, although they ask for investitions, positively affect the integrating processes-connecting big number of smaller waterworks, what enables forming of better material and technical conditions for integral planning, managing and protection of water sources, in other words the total water system. It is usually hard for waterworks to provide needed means and personnel for the researches of water source, but it is a different situation with the whole water resources. At first, it is related to hydrological and hydro geological researches, researches related to water quality measuring etc.

**Operative-communal center**, based on modern technology and current water legislature, must fulfil the aims:

- *Operative-communal center must be a part of communal organization information system, and then part of integral managing of water sources information system, which can enable data, experience and knowledge exchange.*
- *Communal organizations will enable to a Communal Center: expanse data, demographic data, hydro geological data, available sources data and possibilities of its usage, amounts and water quality that can be tapped from available sources and other general data.*
- *Operative-communal center will enable the softwares connected to GIS technology, working with database, information about available water sources and contaminators, then, statistical data works, specialized software for water systems, surface and underground water managing etc.*

In the aim of developing GIS database, within **Balkan University Support Programme, Project 6, GIS Education in Forestry and Agriculture** started to work on a project “**Competence transfer and institutional contact and co-operation between faculties of Agriculture, Forestry and Veterinary Medicine in South Eastern Europe**”. Institution from Norway, responsible for realization is **Agricultural University of Norway, Noragic centre for International Environment and Development studies**; agreement was signed by the Faculty of Agriculture in Novi Sad. The project has started with its realization in 2002. There was a coordination meeting in Sarajevo, in March 2002., where the ways of cooperation, aims and criterions of donation distributions were defined. In September 2002. there was a 7-day course for using GIS. Lecturers were Mr. Knut Bjorkelo, MSc, Norwegian institute of land inventory. P.O. Box 115, N-1430 As, Norway and Mr. Ahmet Lojo, MSc, Faculty of Forestry, Zagrebacka 20, 71000 Sarajevo, Bosnia and Herzegovina. Donation was 150.000 NOK (12.460 EUR), 4 computer configurations, scanner, etc. Project is a result of three-year working, but it is planed to be continued in next 2 years. Fig. 1. shows the work-surrounding of **ProGIS** Programme package, which includes **WinGIS** - graphical part and **WinMonitor** - database.



**Fig. 1.** Work-surrounding of **ProGIS** Programme package (**WinGIS** - graphical part and **WinMonitor** - database).

#### 4. CONCLUSION

Modern water supply strategy implies to rational organizing of perception and measuring different meteorological and hydrologic phenomena, establishing geological and hydrological field characteristics, establishing physical, chemical and biological water characteristics, dynamic and changes of time parameters etc. During the observation, measuring and analysis of natural phenomenon it is necessary to

use modern information systems for integral managing of water sources, which enable quick and efficient data, experience and knowledge exchange.

However, available data about the characteristic of intake areas are generally poor, dissatisfying and extremely unequal. In the work of communal water supply system, main problem is an equipage level. Measuring of exploited water amounts is done by the direct measuring with different kinds of flow measurers or indirect evaluation based on payments. Laboratory equipage level cannot response to all law regulations in the meaning of the analyses that need to be conducted. All this influences the final quality underground water researching results.

**Final selection of the way of using the potential source Kovin-Dubovac will be taken based on detail and all-inclusive technical-economic analysis, which must be included in the Expanse Plans conformably to the interests of the state.**

For the mentioned reasons, there is a proposition in this work, for starting an initiative for the study: **“FIZBILITI STUDY OF THE POTENTIAL UNDERGROUND WATER SOURCE KOVIN-DUBOVAC”**.

Therefore, due to overexploitation of underground waters in some hydro geological areas of Vojvodina or agravation (naturally unsatisfied) of drinking water quality, according to current standards, here is a preposition for undertaking studies and pre investment researches for protection and establishing underground water reserves in the area of potential regional source Kovin-Dubovac. It is known that (in the aim of protection of left Danube coast) in this zone there are constantly working drainage objects (artesian wells) with acceptable quantitative and qualitative parameters of water sources, which could be used in a new conception of regional water supply of Middle and South Banat. Proposed study will verify the opportunity of using this area in the aim of collecting and distribution of existing amounts of underground water from existing drainage line, or in the aim of underground exploitation in the whole area of Kovin-Dubovac.

Based on this study and researches of Kovin community, preparing of the base is enabled:

- To cede the area and mineral resources for usage (clay, sand, gravel, coal and water):
- Charging the taxes for an expanse and resources usage
- Easier selection for interested users or for tenants of expanse for using an alternative pure energy sources in the extent of its activity (wind, sun)
- Selection of different area purpose (ecological food production, chemical industry, food industry, secondary raw materials...)
- Research conditions
- Protecting conditions
- Extra research conditions (assignative) for corresponding expanse usage, which is also an obligation for future owners and tenants

Besides, the development of **OPERATIVE-COMMUNAL CENTRE**, as a long term aim in the realization of result application, will enable transition to new power sources in free aquifer systems of alluvial type, based on the usage of total water resources in coastal areas of big Vojvodinian rivers. Accordingly, considerate example of coastal sector Kovin-Dubovac, on the left Danube coast, has manifold significance and it presents a big contribution to the promotion of **new strategy for water supply in Vojvodina**.

**Acknowledgements:** The work was undertaken as a part of the SOIL DETERIORATION, CONSERVATION AND UTILISATION AT LAND RECLAMATION SYSTEMS Research Projects No. 0419, under contract BTR.5.01.0419.B. It has been funded partially by the Ministry of Science, Technology and Development, Government of Serbia and "SRBIJAVODE" WATER AUTHORITY – Beograd (The River Danube Water Resource Center - Novi Sad).

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## COMPARATIVE STUDY FOR DETERMINATION OF NITRATE IN MEAT PRODUCTS

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### **Abstract:**

*The aim of the present study is to offer alternatives regarding the determinations of nitrate from meat products. In this respect, the potentiometric determination of nitrate from these products was experimented, using a nitrate ion-selective liquid membrane electrode (NO<sub>3</sub>-ISME). This method was compared with classical methods such as spectrophotometry and HPLC as regarding precision and determination speed. Based on the obtained results, we can conclude that for the meat products having a nitrate content situated in the linearity domain, from the point of view of electrode's Nernstian response, the potentiometric method is more accurate, less prodigious and faster than classical methods.*

### **Keywords:**

*nitrate, potentiometry, NO<sub>3</sub>-ISME, meat product*

## **1. INTRODUCTION**

Nitrate represents one of the most poisoning vectors from food, where it can be found because of an indirect way, as a consequence of nitrogen fertilization of plants, or by direct way, as a result of using it as additive in meat food products. As nitrite, nitrate poisoning can cause discoloration of the blood, due to the presence of methemoglobin. Nitrates in blood can also cause blood vessels to dilate and are responsible for peripheral circulatory failure. Other physical signs of nitrate poisoning include difficult and rapid breathing, muscle tremors, low tolerance to exercise, incoordination, diarrhea, frequent urination, which is why its use was severely restricted in many countries.

The numerous analytical applications of ISME, as well as the fact that potentiometric method based on such electrodes is very fast and accurate,

encouraged us to experiment the use of the NO<sub>3</sub>-ISME for the determination of nitrate ion in various food products.

## 2. EXPERIMENTAL

### *Equipment:*

- HITACHI U 1100 spectrophotometer ;
- Merck-Hitachi L 6200A HPLC system ;
- pH/mV digital Hanna Instruments HI 8817;
- NO<sub>3</sub>-ISME provided by Senzorom Cluj;
- Double junction reference electrode RA (0.3 M, Na<sub>2</sub>SO<sub>4</sub> in the 2<sup>nd</sup> salt bridge) provided by Senzorom Cluj.

### *Reagents:*

- 10<sup>-1</sup>M and 5·10<sup>-2</sup> M, NaNO<sub>3</sub> stock solutions (with constant ionic force J = 0.1), prepared from NaNO<sub>3</sub> p.a., dried for 2 hours at 105°C;
- Solution for making dilution: 0.033 M, Na<sub>2</sub>SO<sub>4</sub> solution ;
- Nitrate etalon solutions, prepared from stock solutions by diluting, with concentrations between the range 10<sup>-2</sup> M÷2.5·10<sup>-4</sup> M.

*Extract preparation:* 10 g of homogeneous sample, weighted with 0.001 g precision, is quantitatively passed through a calibrated 200 mL volumetric flask, by adding of 100 mL water. The content of the flask is warmed up to 60-70°C on a water bath, for 30 minutes, under vigorous stirring. The sample is cooled to room temperature, and than 2 mL Carrez I reagent and 2 mL Carrez II reagent are added one by one (for deproteinization), the solution being well stirred after each adding. The sample is leaving to repose for 30 minutes and than is filled with water to mark. The content of the volumetric flask is well homogenized and than is filtered [1].

The extract obtained by this procedure is used for the determination of nitrate from meat products by all three above mentioned methods.

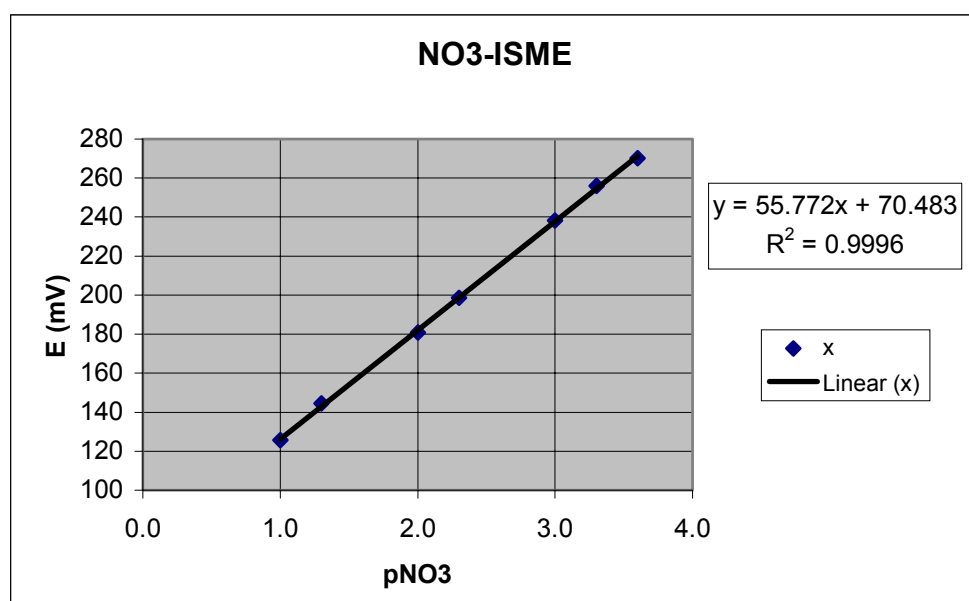
## 3. RESULTS AND DISCUSSIONS

The potentiometric determination of nitrate ion from meat with NO<sub>3</sub>-ISME was done by multiple standard addition, using 25 mL extract.

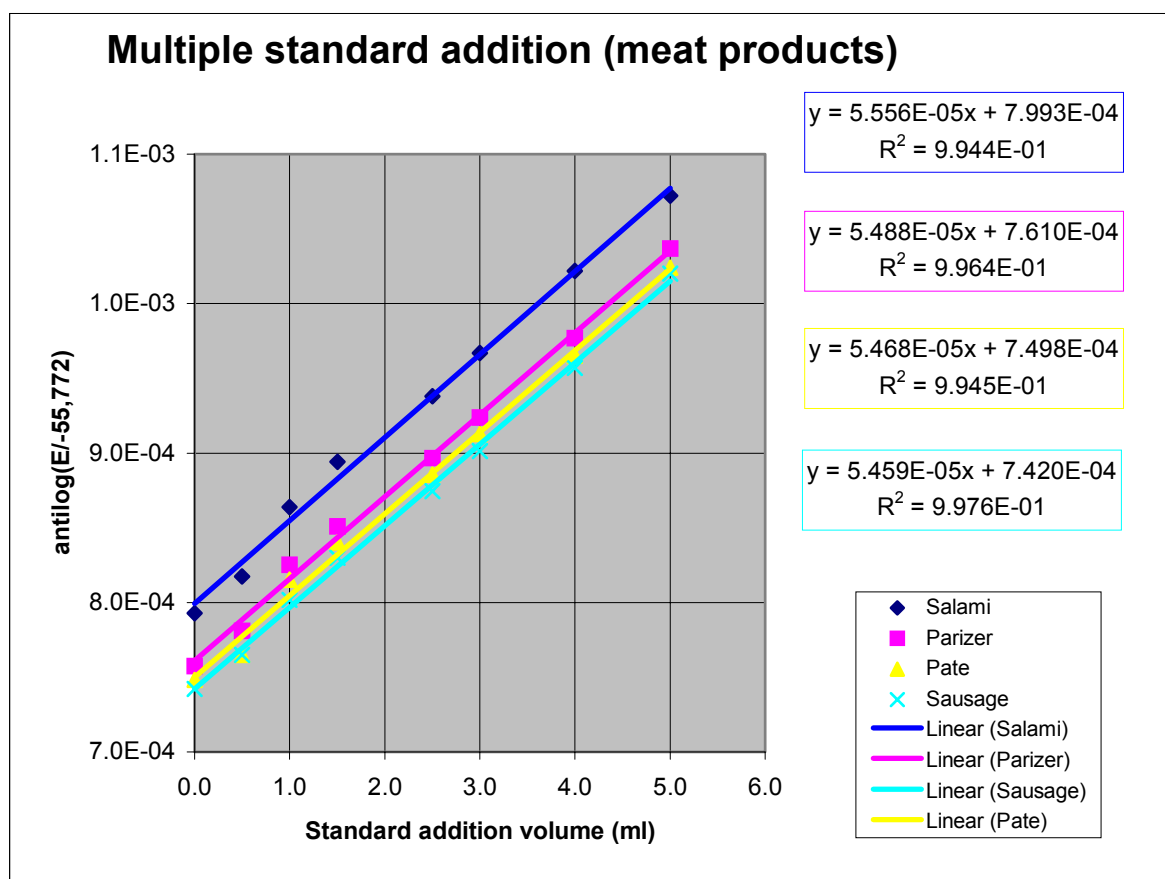
In case of potentiometric determination of nitrate, the main interference is given by chloride anion. In order to eliminate this interference, a 0.16 M, Ag<sub>2</sub>SO<sub>4</sub> solution in ammoniac was used. The interference of hydrogen-carbonate is avoided by changing the pH of the solution to the value of 3.5 (with concentrated sulfuric acid solution). Other disturbing agents are nitrite and carboxyl anions, their action are removed with the help of a solution made up from: aluminum sulfate, sulfanilic acid and boric acid, having the pH=3.5 (fixed with a 0.1 N natrium hydricum solution) [2].

The calibration curve for the electrode and the plots resulted from experimental data by multiple standard addition [3, 4] is presented in figures 1 and 2.

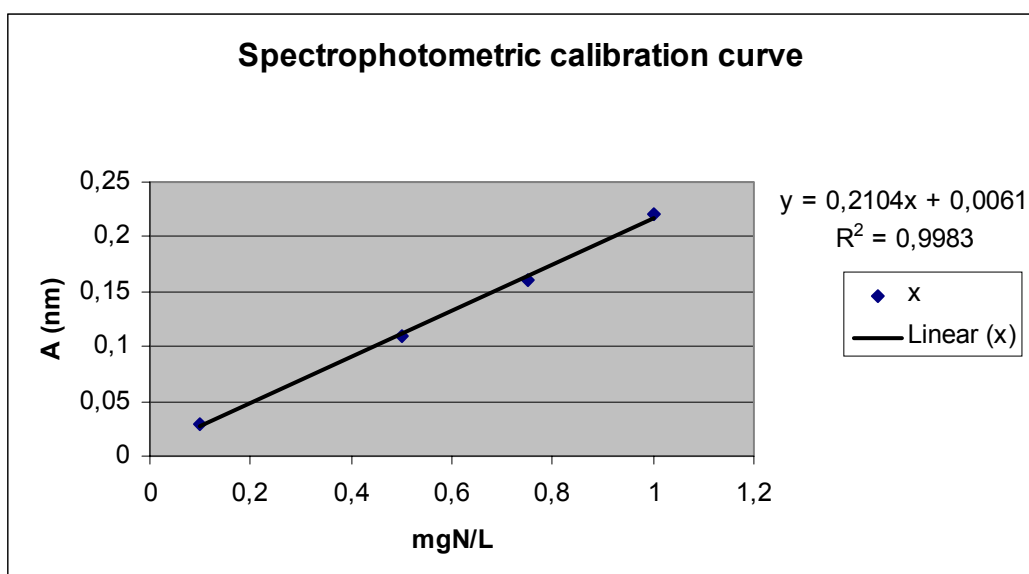
The spectrophotometric determination of nitrate from meat products with brucine was done using 10 mL extract, prepared as mentioned above. The absorbance was measured at 410 nm comparatively with control [5, 6, 7]. The nitrate ion concentration from meat products was established by the help of a calibration curve plotted in the same experimental conditions (figure 3).



**Fig. 1.** The transfer function of NO<sub>3</sub>-ISME

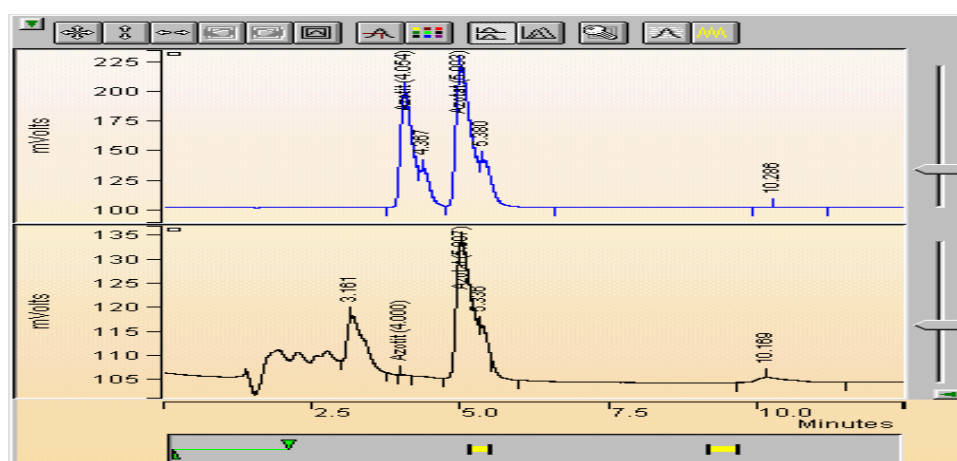


**Fig. 2.** The determination of nitrate ion from meat products by means of multiple standard additions

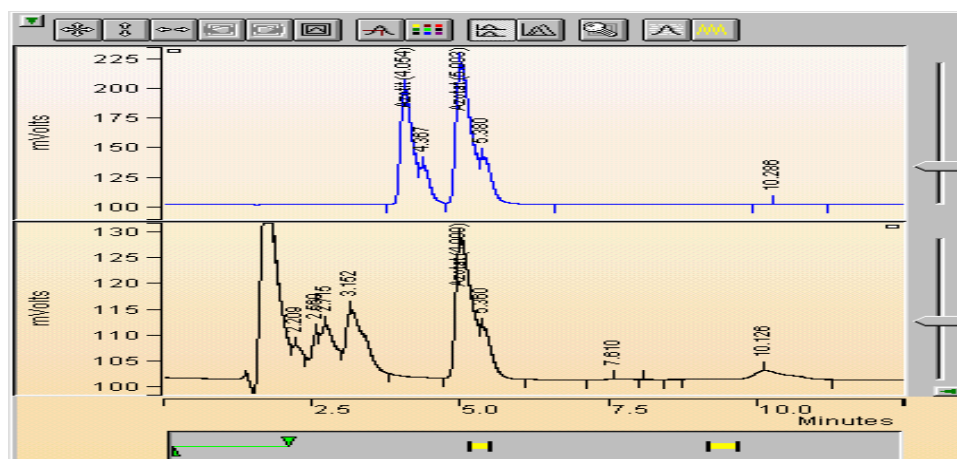


**Fig. 3.** Spectrophotometric curve for the determination of Nitrate ion with brucine

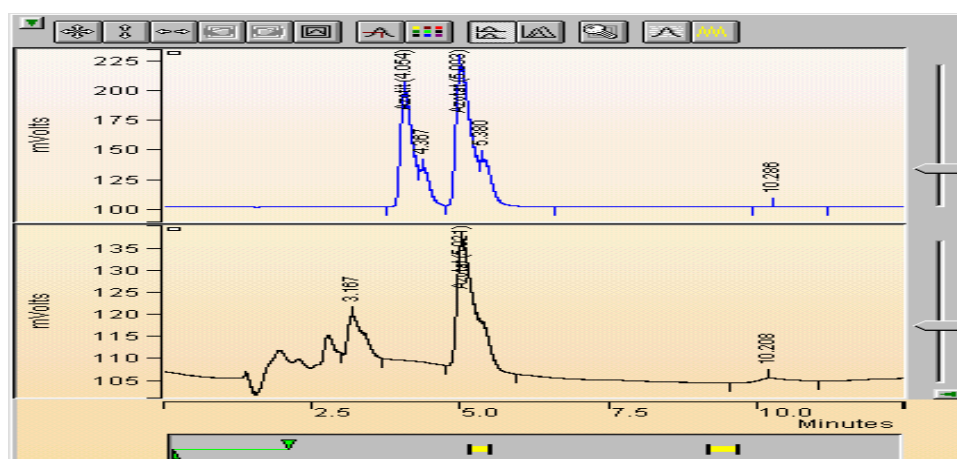
*HPLC method* for chromatographic determination of nitrate from meat, based on 50 $\mu$ L aqueous samples, and the results are illustrated in figure 4:



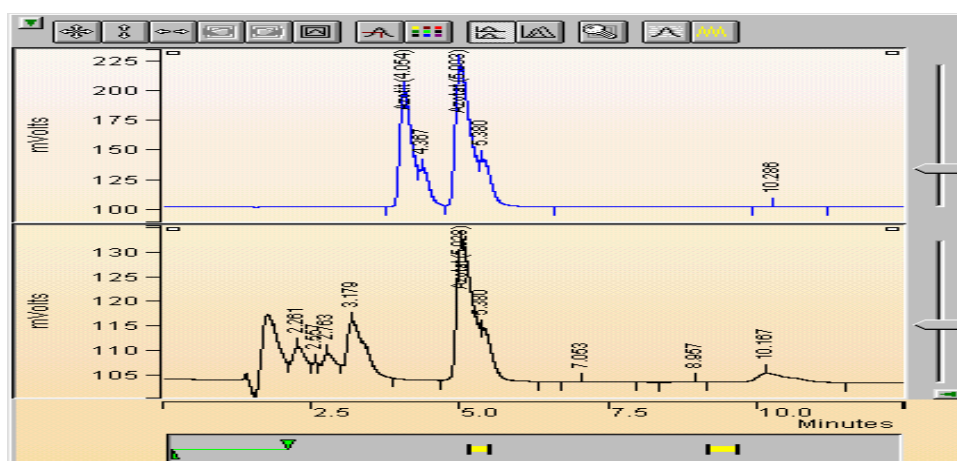
a)



b)



c)



d)

**Fig. 4.** Up panel – mixture  $\text{KNO}_2$  (252mg/L) /  $\text{KNO}_3$  (249.5mg/L);

Down panel – extract sample from:

a) sausage; b) parizer; c) pate; d) salami, 10 times diluted.

Column: SAX Nucleosil 100 SB, 150x4 mm,  $\Phi = 5 \mu\text{m}$ . Mobile phase: 6.25% MeOH, 93.75% Phosphate buffer 45 mM ( $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ ), pH 6. Detection UV 210 nm, flow of mobile phase: 1 ml/min.

Comparative results at the determination of nitrate ion (mg NO /kg meat) by all the three methods: potentiometric, spectrophotometric and chromatographic, are presented in table 1:

**Table 1.** Nitrate content in different meat products

Meat product	Potentiometric	Spectrophotometric	HPLC
Salami	$713.6 \pm 3.1$	$713.5 \pm 9.8$	716.4
Pate	$680.2 \pm 2.3$	$681.3 \pm 9.1$	682.9
Parizer	$687.8 \pm 2.5$	$689.7 \pm 9.4$	686.8
Sausage	$674.2 \pm 2.1$	$668.6 \pm 8.7$	675.6

#### 4. CONCLUSION

From the obtained data we can state that all the three methods lead to similar results.

The proposed method for the determination of nitrate from meat products using an  $\text{NO}_3$ -ISME represents an advantageous method due to its speed, the low cost of required equipment and also to its high precision.

The spectrophotometric determination method presents the drawbacks of a low reproductiveness, the need of higher cost reagents as well as a more laborious work. Besides, the reagent we used, brucine, is highly toxic.

The results obtained by HPLC are similar to those recorded by potentiometric and spectrophotometric methods and we have to mention that this method is easy to do and a small volume of the sample is needed, but the cost of the apparatus is too high.

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## **BETULA POLLEN SEASON IN THE DANUBE-KRIS-MURES-TISA EUROREGION (2000-2002)**

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### **Abstract:**

*Betula* is considered the most important aeroallergen among tree species.

The aim of this study was to compare *Betula* pollen season in three cities situated in the Danube-Kris-Mures-Tisa euro region. Novi Sad (Serbia and Montenegro), Szeged (Hungary) and Timisoara (Romania) routinely monitor aeropollen concentrations using Hirst type pollen and spore trap. Duration of the *Betula* pollen season, seasonal peak concentrations and season start dates were compared for the period 2000-2002.

Three year average pollen season duration were 71 days in Novi Sad and 46 and 47 days in Szeged and Timisoara, respectively. Szeged measured the highest peak concentrations, except in 2002, when peak concentration in Novi Sad reached extremely high level (1034 PG/m<sup>3</sup>). For Szeged was characteristic high number of days with pollen concentrations higher than 30 PG/m<sup>3</sup> (39% of pollen season), while this number was lower for Novi Sad and Timisoara (18% and 13% of pollen season, respectively).

### **Keywords:**

*Aeropollen, Betula, pollen concentration, allergy*

## **1. INTRODUCTION**

The onset of pollen allergy symptoms has been correlated to atmospheric concentrations of allergenic pollen. Knowledge of yearly-fluctuations in pollen count is useful in clinical correlations and development of effective patient treatment regimes [5]

*Betula* pollen is one of the main European aeroallergens triggering symptoms of asthma and seasonal rhinitis [6]. It was shown that major *Betula* pollen allergen (antigen Bet v 1) makes cross reactivity with major apple allergen (antigen Mal d 1), which could cause anaphylactic shock [7]. Further knowledge of *Betula* pollen season in Europe is therefore very important to clinicians and patients.

The aim of this study was to present and compare *Betula* pollen season in three cities situated in the Danube-Kris-Mures-Tisa Euroregion.

## 2. MATERIAL AND METHODS

Pollen samples were collected in 2000, 2001 and 2002 by Hirst volumetric method with a Burkard or Lanzony pollen and spore trap. [The collector pump was aspirating a volume of 10 l/min of air through a 14 x 2 mm slit, which was permanently oriented to windward. The resulting air stream hits onto a tape covered with a thin layer of petroleum jelly [3]. The tape was fixed around the drum, which was rotated by a seven-day clockwork at a speed of two mm/h [4]. At the end of the collecting period, this tape was cut into 48 mm segments, each corresponding to 24 hours. The tape segments were stained with phenylated glycerol-gelatin and fuchsine, and the pollen grains were identified and counted in an optic microscope [3]. Five scans were performed over each segment with the magnification of 400x (in accordance with the recommendations of the International Aerobiology Association)]. The daily pollen grain count per cubic meter of air ( $\text{pg}/\text{m}^3$ ) could be calculated by multiplying the cumulative observed count of the five scans by a correction factor, resulting from the proportion between the scanning area of the optical field of the microscope to the collecting tape area (14mm x 48mm). Pollen identification was based on the comparison with the reference slides and photographs. Start of pollen season was calculated as first day when  $1\text{pg}/\text{m}^3$  occurs in atmosphere.  $30\text{pg}/\text{m}^3$  was chosen as critical concentration of *Corylus* pollen because this concentration is considered as a threshold for the start of allergic symptoms by patients sensitive to *Betula* pollen, which have similar antigens [1])

## 3. RESULTS AND DISCUSSION

*Betula* pollen season in Danube–Kris-Mures-Tisa starts at the end of February and during March. Three year average pollen season duration were 71 days in Novi Sad and 46 and 47 days in Szeged and Timisoara, respectively (Table 1). *Betula* pollen could be considered as important allergy inducer in observed region, because of high number of days when its pollen occurs in atmosphere. Such long pollen season duration enlarges risk of occurrence of cross-reactions between *Betula* pollen and fruit allergens.

Table 1. *Betula* pollen season duration and start date (in parenthesis)

	Novi Sad	Szeged	Timisoara
2000	64 (1. March)	37 (23. March)	20 (27. February)
2001	67 (13. March)	62 (13. March)	55 (13. March)
2002	82 (27. February)	38 (8. March)	66 (5. March)
Average season duration	71 days	46 days	47 days

Szeged measured the highest peak concentrations, except in 2002, when peak concentration in Novi Sad reached extremely high level (Table 2.).



Table 2. Peak concentrations (in  $\text{pg}/\text{m}^3$ )

	Novi Sad	Szeged	Timisoara
2000	97	148	9
2001	95	219	46
2002	1034	185	106

For Szeged was characteristic high average number of days with pollen concentrations higher than  $30 \text{ pg}/\text{m}^3$  (39% of pollen season), while this number was lower for Novi Sad and Timisoara (18% and 13% of pollen season, respectively) (Table 3).

Table 3. Number of days when *Betula* pollen concentration reached critical values

	Novi Sad	Szeged	Timisoara
2000	10	20	0
2001	8	22	8
2002	22	13	10
Average	13 days	18 days	6 days
Part of season when <i>Betula</i> pollen reached critical concentrations	18%	39%	13%

It should have been considered that priming effect of *Corylus* and *Alnus* pollen and increasing air pollution could decrease the reactivation threshold in patients with *Betula* allergy [8].

Further studies should provide knowledge about meteorological factors that cause start and duration of *Betula* pollen season, in order to make accurate forecasts for occurrence of *Betula* pollen in atmosphere. These forecasts could help pollen sensitive patients to organize their life stile, in order to diminish problems caused by pollen allergy.

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## ***Brassica napus* ETHYLIC EXTRACT AS ECOLOGICAL PHYTOREGULATING AGENT**

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### **Abstract**

*It is known that some of the important natural plant growth regulating substances are the Brassinosteroids. We have studied the phyto regulating effect of *Brassica napus* pollen ethylic extract on monocotyledonous (wheat) and dicotyledonous (cucumber) in laboratory conditions. We used different concentrations between 5-1000 ppm. The plant extract presents an important phytostimulating activity.*

### **Keywords:**

brassinolide, phytostimulating effect, monocotyledonous, dicotyledonous.

## **INTRODUCTION**

Brassinosteroids are a group of naturally occurring polyhydroxysteroids [2]. Brassinosteroids was originally isolated from *Brassica napus* pollen [1,4]. In the present, more than 60 brassinosteroids have been identified, 31 of these have been completely characterized.

Brassinosteroids may thus be regarded as a new group of plant hormones with regulatory function in cell elongation and cell division [3].

In the present paper is presented the biostimulating effect of *Brassica napus* pollen extract on monocotyledonous (wheat) and dicotyledonous (cucumber) in laboratory condition.

## **EXPERIMENTAL**

For the obtained *Brassica napus* pollen extract the solvent used was 87% ethylic alcohol in a Soxhlet extractor at constant temperature for 6-7 hours.

For the determination of the biological activity of this extract, the laboratory test applied was the general biotest Tsibulskaya – Vasslev [5,6] on monocotyledonous and dicotyledonous. With this assay we can establish the biological activity as stimulator or inhibitor of the plant growth.

Wheat caryopses from Alex species were used as monocotyledonous seeds and cucumber from Cornichon species as dicotyledonous. The used concentration

were 20, 50, 100, 200, 500, 1000 ppm for monocotyledonous and 5, 10, 20, 50, 100, 200, 500 ppm for dicotyledonous, compared with control.

The average height of the plans, the average length of the main root, the number of the roots, estimated the effect of *Brassica napus* pollen extract on the growth of the plants.

## RESULTS AND DISCUSSIONS

The data obtained from the experiments were calculated in percentage compared with the control. The results are listed in the figures 1,2.

On monocotyledonous the *Brassica napus* pollen extract stimulate the height growth of the plants with 13-19% at 50 – 100 ppm concentration.

The average length increases with 9% at 100 ppm.

The number of the roots increases with 22 – 36% at 20 – 500 ppm.

On dicotyledonous the height growth of the plants increases with 7% at 200 ppm concentration.

The length of main root increases with 11 – 26% at 5 – 200 ppm concentration.

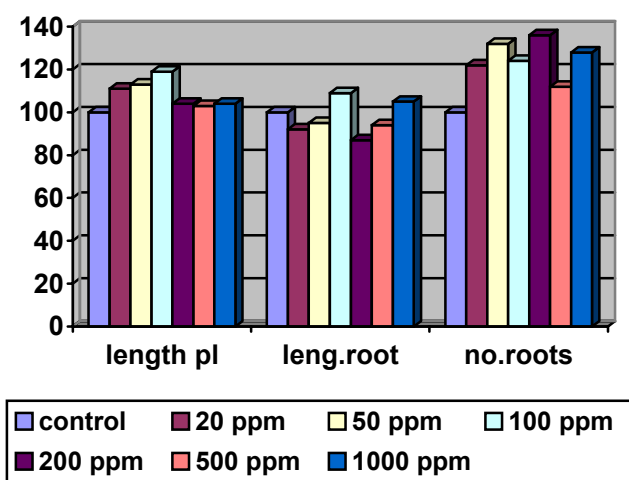


Fig.1. The effect of rape pollen extract on monocotyledonous

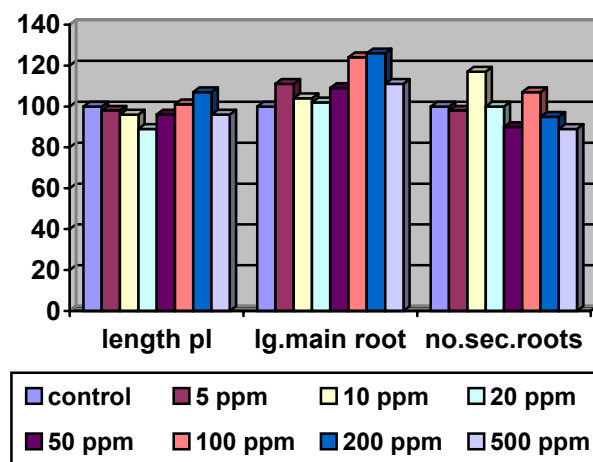


Fig.2. . The effect of rape pollen extract on dicotyledonous

The number of the secondary roots increases with 17% at 10 ppm concentration.

## CONCLUSIONS

Applying the treatment with *Brassica napus* pollen extract on monocotyledonous and dicotyledonous lead to the following effects:

- The extract stimulating the growth of plants with 13 – 19% at monocotyledonous and with 7% at dicotyledonous.
- The extract has a rooting effect, the number of the roots increases with 22 – 36% at monocotyledonous and on the dicotyledonous the secondary roots increases with 17%, and the length of the main root growth with 11 – 26%.

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## THE VALORIZATION OF A RESIDUAL PRODUCT FROM THE SYNTHESIS OF BIS (DIISOPROPYLAMINE) CHLOROPHOSPHINE AS GROWTH REGULATOR

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### Abstract:

*The purpose of the present study is to create a link between the chemical research and the agriculture, taking into account the environmental care. The paper presents the biological tests made upon wheat (Alex variety) as monocotyledonous plant and cucumber (Cornichon cultivar) as dicotyledonous plant, with diisopropylamine hydrochloride, an undesired byproduct in the synthesis of bis(diisopropylamine)chlorophosphine, which finally proved useful as growth regulating agent, functioning similar to auxine. The auxinic effect activity was established with the Tsibulskaya-Vassilev general biotest, by using concentrations between 5-100 ppm and we can conclude that the product is stimulating the risogenesis process, especially on dicotyledonous plants.*

### Keywords:

*bis(diisopropylamine)chlorophosphine, diisopropylamine hydrochloride, Tsibulskaya-Vassilev general biotest, risogenesis process, auxinic effect*

## 1. INTRODUCTION

This work is promoting responsible research and application of chemistry science stepping up the science / agriculture dialogue.

A chemical substance can act as a plant hormone [1,2] when it shows specific biological activity in very low concentration and also must play a fundamental role in regulating physiological phenomena in vivo, such as: cell enlargement, vascular tissue differentiation, root initiation, gravitropic and phototropic responses, and apical dominance.

This paper presents the biological tests made upon wheat (monocotyledonous) and cucumber (dicotyledonous), with a substance named *diisopropylamine hydrochloride* which was an undesired byproduct in the synthesis of

bis(diisopropylamine)chlorophosphine and which finally proved to be useful as a growth regulating agent, functioning similar to auxine. The idea of searching diisopropylamine hydrochloride as a biological active substance, started from the previous literature studies [3] which proved that compounds with secondary amino groups have local anaesthetic activity, are not toxic, and above all, their use in agriculture contribute to the reduction of environmental pollution caused by necessary packaging for removing an unnecessary product.

Auxins are responsible in promoting cell elongation [4], a process that is required before differentiation of a cell. Auxins are able to do this by promoting the intake of water, increasing the elasticity of the cell to cope with the increase of water taken in by the cell.

Some of the other effects [5-9] that auxins are known to cause are: stimulates cell elongation, stimulates differentiation of phloem and xylem, stimulates root initiation on stem cuttings and lateral root development in tissue culture, delays leaf senescence, can inhibit or promote (via ethylene stimulation) leaf and fruit abscission, can induce fruit setting and growth in some plants, involved in assimilate movement toward auxin possibly by an effect on phloem transport, delays fruit ripening, stimulates growth of flower parts. A few of these effects are to be seen as results of our presented study.

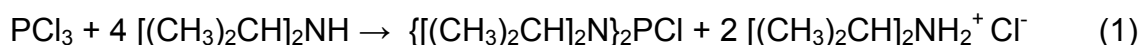
On the other hand, plants require some essential elements to function properly, mainly carbon, oxygen and hydrogen and also small quantities of nitrogen, phosphorous, potassium and magnesium, as additional elements. For example, a lack of nitrogen causes an excessive growth of roots and a red leaf base. The compound we have been tested has benefic effect from this point of view too.

## 2. EXPERIMENTAL

- Reactions, reagents and all operations were carried out with protection from atmospheric moisture, using Schlenk glassware and purging inert gas.
- Reagents: phosphorus trichloride and hexane were Merck reagents for synthesis; diisopropylamine is from Sigma Aldrich Division, Germany. All chemicals used were predried and distilled from appropriate drying agents [10].
- Melting points were determined on a Böttius apparatus.
- $^1\text{H-NMR}$  spectrum was determined in  $\text{DMSO-}d_6 + \text{CDCl}_3$  solutions with a Bruker Avance DRX 400 apparatus Chemical shifts ( $\delta$ ) are given in ppm downfield from internal TMS.
- IR spectra were determined on a SPECORD M80 JENA.
- Elemental analysis was carried out on a CARLO ERBA 1106 analyzer.

### *Procedure for synthesis of diisopropylamine hydrochloride*

We obtain the diisopropylamine hydrochloride as a byproduct in our attempt to synthesize bis(diisopropylamino)chlorophosphine [11], according to equation (1):



*A solution of 10 mL (13.7g, 0.1 mol) of phosphorus trichloride in 20 mL hexane was added dropwise to a solution of 56 mL (40.4g, 0.4 mol) diisopropylamine in 180 mL hexane with continuous stirring and cooling in an ice bath. The reaction mixture*

was allowed to warm to room temperature and then boiled under reflux for 30 hours. After cooling to room temperature, the reaction mixture was filtered and the precipitate, which is diisopropylamine hydrochloride, Mp: 212-214 °C, was washed and recrystallized from hexane. It was obtained 17g diisopropylamine hydrochloride, as byproduct. Concentration of the hexane filtrate gave 13.5 g of  $(i\text{-Pr}_2\text{N})_2\text{PCl}$ , mp: 98-99°C

**Diisopropylamine hydrochloride:** white crystals, m.p. 212-214 °C; IR (KBr,  $\text{cm}^{-1}$ ): 3432( $\nu_{\text{NH}}$ ); 2976 ( $\nu_{\text{CH}_3}^{\text{as}}$ ); 2912 ( $\nu_{\text{CH}}$ ); 2836 ( $\nu_{\text{CH}_3}^{\text{s}}$ ); 1588 ( $\delta_{\text{NH}}$ ); 1462 ( $\delta_{\text{CH}_3}^{\text{as}}$ ); 1376 ( $\delta_{\text{CH}_3}^{\text{s}}$ ); 1168 ( $\nu_{\text{CN}}$ ).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$  +  $\text{CDCl}_3$ ): 3.29(m, 2 H); 1.1(dd,  $J=6.2$  Hz,  $J=3.1$ Hz, 12 H); Elemental analysis for  $\text{C}_6\text{H}_{16}\text{NCl}$  (137.5) (%): calc. C, 52.36; H, 11.63; found C, 52.61; H, 11.79.

### ***The method for testing growth regulator activity***

To establish the auxinic effect activity of diisopropylamine hydrochloride, the Tsibulskaya-Vassilev general biotest [12,13] was used.

For the determination of the biological activity of the diisopropylamine hydrochloride, as stimulator of the plant growth, laboratory tests were carried out on monocotyledonous - wheat caryopses (*Alex variety*) and on dicotyledonous - on cucumber (*Cornichon cultivar*) using the general Tsibulskaya-Vassilev biotest method comparatively with water control.

The concentrations we used were for monocotyledonous: 10 ppm, 50 ppm, and 100 ppm and for dicotyledonous 5 ppm, 10 ppm and 20 ppm. The seeds treated with bioactive compounds were held in Petri dishes on agar medium at 22° C during six days. After that, the biometrics measurements were carried out, watching of: the average height of plants, the average number of the roots for one plant, the average length of the roots and the dry substance on monocotyledonous and the average height of plants, the average length of principal roots, the average number of secondary roots and the dry substance for dicotyledonous.

The obtained data were calculated in percentage and compared to the water control. The results are presented in Tables and Figures 1 and 2. STATGRAPHICS PROGRAM carried out the statistical processing of the data.

## **3. RESULTS AND DISCUSSIONS**

From the data presented it could be observed that the average length of the plants increases with 3% at the concentration of 50 ppm. When the concentrations are equal with 10 and respectively 100 ppm, the registered values are below the values belonging to the water control.

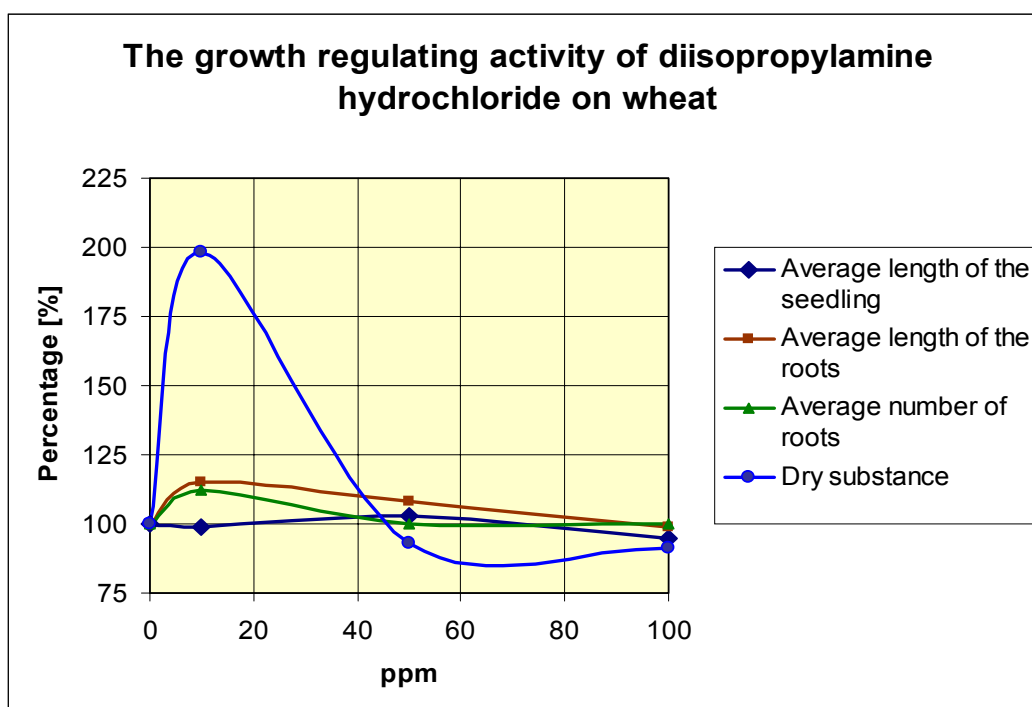
By watching of the average length of the roots, we registered an increase of 15% when the concentration was 10 ppm and an increase of 8% for the concentration of 50 ppm. The concentration of 100 ppm proved to have inhibition effect (99%).

The average number of the roots on one plant increases with 12% at 10 ppm and for concentrations of 50 and 100 ppm is equal with the water control.

The dry substance reaches a significant distinct increase of 98% at the concentration of 10 ppm.

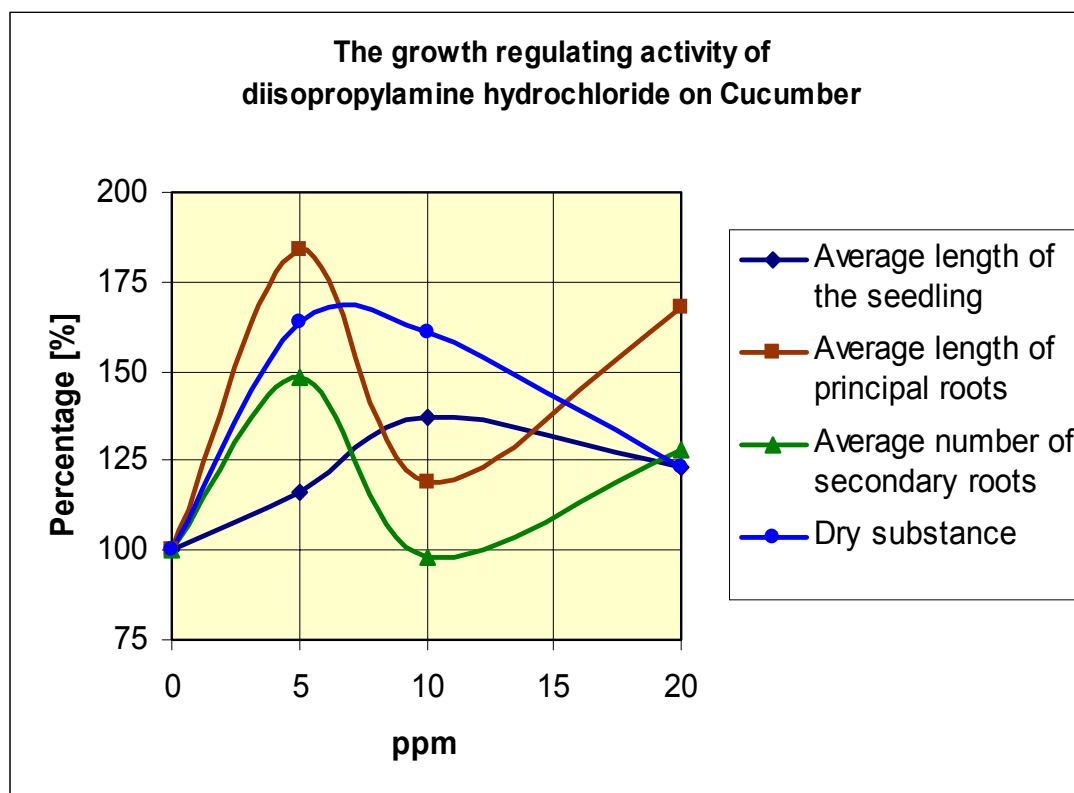
**Table 1.** The growth regulating activity of diisopropylamine hydrochloride on wheat

Variant	Average length of the seedling		Average length of the roots		Average number of roots			Dry substance		
	cm	%	cm	%	No.	Difference	%	g	Difference	%
Water control	8.13	100	7.26	100	3.76	N/A	100	0.0119	N/A	100
<b>Diisopropylamine hydrochloride</b>										
10 ppm	8.02	99	8.37	115	4.21	+0.45	112*	0.0236	+0.0117	198***
50 ppm	8.34	103	7.87	108	3.75	-0.01	100	0.0111	-0.0008	93
100 ppm	7.74	95	7.17	99	3.75	-0.01	100	0.0108	-0.0011	91
DL					5%	= 0.32907		= 0.00046		
					1%	= 0.73113		= 0.00103		
					0.1%	= 1.11062		= 0.00239		

**Fig. 1.** Biological activity of diisopropylamine hydrochloride on wheat**Table 2.** The growth regulating activity of diisopropylamine hydrochloride on Cucumber

Variant	Average length of the seedling			Average length of principal roots			Average number of secondary roots			Dry substance		
	cm	Diff.	%	cm	Diff.	%	No.	Diff.	%	g	Diff.	%
Water control	8.26	N/A	100	4.63	N/A	100	6.75	N/A	100	0.0156	N/A	100
<b>Diisopropylamine hydrochloride</b>												
5 ppm	9.56	+1.3	116*	8.54	+3.91	184***	10.0	+3.25	148***	0.0256	+0.01	164***
10 ppm	11.35	+3.09	137***	5.5	+0.87	119*	6.60	-0.15	98	0.0251	+0.0095	161***
20 ppm	10.13	+1.87	123**	7.77	+3.14	168***	8.63	+1.88	128**	0.0192	+0.0036	123
DL												
5%				= 1.29442			= 0.64130			= 1.28157		
1%				= 1.70145			= 2.03891			= 1.70034		
0.1%				= 2.23141			= 2.61773			= 2.12419		
										= 0.00291		
										= 0.00470		
										= 0.00883		





**Fig. 2.** Biological activity of diisopropylamine hydrochloride on cucumber

With respect to the dicotyledonous plant (cucumber) the average length of the seedling increases with 16% at the concentration of 5 ppm. At concentration of 10 ppm a significant distinct increase of 37% is registered, and when a concentration of 20 ppm was used the significant increase noticed, decreases to 23%.

The average length of the principal roots increase with 84% at 5 ppm, is highly with 19% at 10 ppm, and when we used a concentration of 20 ppm a distinct significant increase of 68% is noticed.

From the point of view of the average number of the secondary roots it is to be reported an increase of 48% at 5 ppm and an increase of 28% at a concentration of 20 ppm.

The statistically assured cumulative effect of protein substances is registered as significant distinct increase of 64% for 5 ppm and with an increase of 61% at 10 ppm.

#### 4. CONCLUSIONS

By comparison of the obtained data we could remark the following:

The product we have tested is stimulating the risogenesis process of monocotyledonous plants with 12% and when referred to dicotyledonous with an increase between 28-48% with respect to the average number of roots, and an increase between 19-84% with respect to the average length of the roots.

The product has a stimulative effect on dicotyledonous seedlings when an increase of the growth of plants between 16-37% can be specified.

So we can say that the product has a significant biological activity, especially when it is applied on dicotyledonous plants.

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## NOTES ON ANTHROPOHILOUS FLORA AND VEGETATION IN THE CITY OF TIMISOARA

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### **Abstract:**

*We present the spontaneous flora and vegetation in the city of Timisoara on the ground of our own researches made by the phytosociological method. Vegetal associations that occupy the largest areas and that are most frequent are Trifolium repenti - Lolium perennis and Lolium - Plantaginietum majoris. We have also detected a concentrical zoning of vegetation from downtown to uptown. We point out vascular plant species whose presence has an impact on urban environment quality, especially allergy-generating plants (e.g. Ambrosia artemisiifolia). Researches have also underlined some new species not mentioned in literature about flora of the Timisoara city.*

### **Keywords:**

flora, vegetation, urban environment quality, *Ambrosia artemisiifolia*, *Panicum dichotomiflorum*.

### **1. Introduction**

Timișoara is the largest cities in southwestern Romania. Situated in the Banat's Plain, at an altitude of 80-90 m, the city has a moderate continental climate with mean yearly temperatures of 10.9<sup>0</sup> C and mean rainfalls of about 630 mm. During the last 10 years, there have been great variations both in temperature and rainfall. The soil is of the leached chernozem type, with great *intra muros* changes due to building and infrastructure maintenance activities.

Spontaneous flora and vegetation of the city have been little studied [3, 4], as they have been maybe considered dull. Nevertheless, anthropophilous vegetation has a great impact on urban ambiance at least from an aesthetic and humans' health points of view [1]. In addition, phytocenoses occupying ruderal habitats (*sensu* Grimme, 1979) represent good study models of the first steps of vegetation dynamics.

The data in the present paper have been collected during the years 1999-2002. They are a picture of synanthropic flora and vegetation

biodiversity and offer good hints for occasional maintenance measures of the urban environment.

## 2. Material and Methods

Field research was done on spontaneous phytocenoses in the following types of stations: lanes, roadsides, ditch sides, spaces between roads and sidewalks, slopes, railway and tramway roads, un-worked lawns. According to urban subsystem classifications [8], the areas we surveyed are mainly within the following subsystems:

- grouped buildings, characteristic of the first half of the 20<sup>th</sup>;
- industrial and warehousing sites, with different buildings and facilities separate by relatively wide spaces;
- residential areas.

The floral compendium was defined by marking species we came across in the different tras-sections but also by reuniting all the species we met in the field.

Vegetation study was done with the method og phytosociological land survey [2]. The main features of the method we appllied are:

- variable size of samples (2-100 m<sup>2</sup>), due to field conditions;
- awarding each species an abundance-dominance grade according to the Braun-Blanquet scale (1964), from „+” to „5” and a local frequency grade on a scale from „1” to „5”;
- average height and general coverage of the vegetal cover (visual assessments in %);
- land survey data were gathered in phytosociological synthetic tables.

Vegetation units were identified according to Sanda *et al.* [7] system on the ground of processing with the help of the 105 relévés.

## 3. Flora compendium

**Urticaceae** - *Urtica dioica* L., *Urtica urens* L.; **Polygonaceae** - *Fallopia convolvulus* (L.) A. S. Löve. *Polygonum aviculare* L., *Polygonum hydropiper* L., *Polygonum lapathifolium* L., *Polygonum persicaria* L., *Reynoutria japonica* Houtt., *Rumex acetosella* L., *Rumex crispus* L., *Rumex obtusifolius* L.; **Cannabaceae** - *Humulus lupulus* L.; **Amaranthaceae** - *Amaranthus retroflexus* L.; **Chenopodiaceae** - *Atriplex patula* L., *Atriplex tatarica* L., *Bassia scoparia* (L.) A. J. Scott, *Chenopodium album* L., *Chenopodium hybridum* L., *Chenopodium murale* L., *Chenopodium polyspermum* L.; **Portulacaceae** - *Portulaca oleracea* L.; **Caryophyllaceae** - *Arenaria serpyllifolia* L., *Cerastium arvense* L., *Cerastium glomeratum* Thuill., *Saponaria officinalis* L., *Silene alba* (Mill.) E. H. L. Krause, *Stellaria media* (L.) Vill.; **Ranunculaceae** - *Ranunculus arvensis* L., *Ranunculus ficaria* L., *Ranunculus repens* L., *Ranunculus sardous* Crantz; **Aristolochiaceae** - *Aristolochia clematitis* L.; **Papaveraceae** - *Chelidonium majus* L., *Papaver rhoeas* L.; **Fumariaceae** - *Fumaria officinalis* L., *Fumaria schleicheri* Soy-Will.;

**Brassicaceae** - *Alliaria petiolata* (M. Bieb.) Cavara & Grande, *Arabidopsis thaliana* (L.) Heynh., *Armoracia rusticana* P. Gaertn., B. Mey & Schreb., *Berteroa incana* (L.) DC., *Capsella bursa-pastoris* (L.) Medik., *Cardaria draba* (L.) Desv., *Descurainia sophia* (L.) Webb. ex Prantl, *Diplotaxis muralis* (L.) DC., *Erophila verna* (L.) Chevall., *Lepidium ruderae* L., *Myagrum perfoliatum* L., *Raphanus raphanistrum* L., *Rorippa austriaca* (Crantz) Besser, *Rorippa sylvestris* (L.) Besser, *Sinapis arvensis* L., *Sisymbrium orientale* L., *Thlaspi arvense* L., *Thlaspi perfoliatum* L.; **Resedaceae** - *Reseda lutea* L.; **Violaceae** - *Viola arvensis* Murray, *Viola elatior* Fr., *Viola odorata* L.; **Rosaceae** - *Geum urbanum* L., *Potentilla anserina* L., *Potentilla argentea* L., *Potentilla reptans* L., *Prunus cerasifera* Ehrh., *Prunus spinosa* L., *Rosa canina* L., *Rubus caesius* L.; **Fabaceae** - *Astragalus glycyphyllos* L., *Coronilla varia* L., *Lathyrus sativus* L., *Lathyrus tuberosus* L., *Lotus corniculatus* L., *Medicago lupulina* L., *Medicago sativa* L., *Medicago sativa* L. subsp. *falcata* (L.) Arcang., *Melilotus alba* Medik., *Melilotus officinalis* (L.) Pall., *Robinia pseudacacia* L., *Trifolium arvense* L., *Trifolium campestre* Schreb., *Trifolium pratense* L., *Trifolium repens* L., *Vicia cassubica* L., *Vicia cracca* L., *Vicia grandiflora* Scop., *Vicia lathyroides* L., *Vicia sativa* L., *Vicia tetrasperma* (L.) Schreb.; **Primulaceae** - *Lysimachia numularia* L.; **Zygophyllaceae** - *Tribulus terrestris* L.; **Malvaceae** - *Abutilon theophrasti* Medik., *Althaea officinalis* L., *Hibiscus trionum* L., *Malva neglecta* Wallr., *Malva pusilla* Sm., *Malva sylvestris* L.; **Oxalidaceae** - *Oxalis acetosella* L., *Oxalis corniculata* L., *Oxalis stricta* L.; **Geraniaceae** - *Erodium cicutarium* (L.) L'Her., *Geranium dissectum* L., *Geranium pratense* L., *Geranium pusillum* L.; **Vitaceae** - *Parthenocissus quinquefolia* (L.) Planch.; **Apiaceae** - *Anthriscus cerefolium* (L.) Hoffm., *Anthriscus sylvestris* (L.) Hoffm., *Conium maculatum* L., *Coriandrum sativum* L., *Daucus carota* L., *Eryngium campestre* L., *Pastinaca sativa* L., *Torilis arvensis* (Huds.) Link.; **Euphorbiaceae** - *Euphorbia cyparissias* L., *Euphorbia maculata* L.; **Caprifoliaceae** - *Sambucus ebulus* L.; **Valerianaceae** - *Valerianella locusta* (L.) Laterr.; **Apocynaceae** - *Vincetoxicum minor* L.; **Convolvulaceae** - *Calystegia sepium* (L.) R. Br., *Convolvulus arvensis* L.; **Cuscutaceae** - *Cuscuta europaea* L.; **Boraginaceae** - *Cynoglossum officinale* L., *Echium vulgare* L., *Myosotis arvensis* (L.) Hill, *Symphytum officinale* L.; **Solanaceae** - *Lycium barbarum* L., *Solanum dulcamara* L., *Solanum nigrum* L.; **Scrophulariaceae** - *Linaria vulgaris* Mill., *Verbascum blattaria* L., *Verbascum phlomoides* L., *Veronica chamaedrys* L., *Veronica hederifolia* L., *Veronica persica* Poir.; **Verbenaceae** - *Verbena officinalis* L.; **Lamiaceae** - *Ajuga genevensis* L., *Ajuga reptans* L., *Ballota nigra* L., *Glechoma hederacea* L., *Lamium amplexicaule* L., *Lamium purpureum* L., *Leonurus cardiaca* L., *Lycopus europaeus* L., *Mentha arvensis* L., *Mentha longifolia* (L.) Huds., *Prunella vulgaris* L., *Salvia nemorosa* L., *Salvia pratensis* L., *Stachys annua* (L.) L.; **Dipsacaceae** - *Knautia arvensis* (L.) Coult., *Scabiosa ochroleuca* L.; **Cucurbitaceae** - *Bryonia cretica* L. subsp. *dioica* (Jacq.) Tutin; **Plantaginaceae** - *Plantago lanceolata* L., *Plantago major* L., *Plantago media* L.; **Rubiaceae** - *Galium album* Mill., *Galium aparine* L., *Galium tricornutum* L., *Galium verum* L.; **Asteraceae** - *Achillea millefolium* L.,

*Achillea setacea* Waldst. & Kit., *Ambrosia artemisiifolia* L., *Anthemis arvensis* L., *Anthemis austriaca* Jacq., *Arctium lappa* L., *Artemisia vulgaris* L., *Bellis perennis* L., *Bidens tripartita* L., *Carduus acantoides* L., *Carduus nutans* L., *Centaurea biebersteinii* DC., *Centaurea scabiosa* L., *Chamomilla recutita* (L.) Rauschert, *Chamomilla suaveolens* (Pursh) Rydb., *Chondrilla juncea* L., *Cichorium intybus* L., *Cirsium arvense* L., *Cirsium vulgare* (Savi) Ten., *Conyza canadensis* (L.) Cronquist, *Crepis foetida* L., *Crepis setosa* Haller f., *Erigeron annuus* (L.) Pers., *Galinsoga parviflora* Cav., *Helianthus tuberosus* L., *Inula hirta* L., *Lactuca serriola* L., *Leontodon hispidus* L., *Matricaria perforata* Merat, *Onopordum acanthium* L., *Picris hieracioides* L., *Senecio vernalis* Waldst. & Kit., *Senecio vulgaris* L., *Solidago canadensis* L., *Sonchus arvensis* L., *Sonchus asper* (L.) Hill, *Tanacetum vulgare* L., *Taraxacum officinale* Webber, *Tragopogon pratensis* L., *Tussilago farfara* L., *Xanthium spinosum* L., *Xanthium strumarium* L., *Xanthium strumarium* L. subsp. *italicum* (Moretti) D. Löve; **Juncaceae** - *Juncus inflexus* L.; **Lemnaceae** - *Lemna minor* L.; **Liliaceae** - *Gagea arvensis* (Pers.) Dumort., *Gagea lutea* (L.) Ker Gawl., *Ornithogalum pyramidale* L.; **Iridaceae** - *Iris graminea* L., *Iris pseudacorus* L.; **Cyperaceae** - *Carex hirta* L., *Carex vulpina* L.; **Poaceae** - *Agrostis stolonifera* L., *Alopecurus pratensis* L., *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl. & C. Presl., *Avena fatua* L., *Bromus arvensis* L., *Bromus commutatus* Schrad., *Bromus hordeaceus* L., *Bromus inermis* Leyss., *Bromus sterilis* L., *Bromus tectorum* L., *Calamagrostis epigejos* (L.) Roth., *Cynodon dactylon* (L.) Pers., *Dactylis glomerata* L., *Digitaria sanguinalis* (L.) Scop., *Echinochloa crus-galli* (L.) P. Beauv., *Elymus repens* (L.) Gould., *Eragrostis cilianensis* (All.) F. T. Hubb., *Eragrostis minor* Host., *Festuca pratensis* Huds., *Festuca pseudovina* Hack. ex Wiesb., *Festuca rupicola* Heuff., *Hordeum murinum* L., *Lolium perenne* L., *Panicum dichotomiflorum* Michx., *Panicum miliaceum* L., *Poa annua* L., *Poa bulbosa* L., *Poa pratensis* L., *Poa trivialis* L., *Sclerochloa dura* (L.) P. Beauv., *Setaria pumila* (Poir.) Schult., *Setaria verticillata* (L.) P. Beauv., *Setaria viridis* (L.) P. Beauv., *Sorghum halepense* (L.) Pers.

#### 4. Main vegetation units compendium

*Stellarietea mediae* R. Tx., Lohm & Prsg. in R. Tx. 1950

*Eragrostetalia* J. Tx. ex Poli 1966

*Amarantho* – *Chenopodion* Morariu 1943

*Amarantho* – *Chenopodietum albi* Morariu 1943

*Portulacetum oleracei* Felföldy 1942

*Tribulo* – *Tragetum* Soo & Timar in Timar 1954

*Sisymbrietalia* J. Tx. in Lohm et al. 1962

*Sisymbrium officinalis* R. Tx., Lohm. & Prsg. in R. Tx. 1950

*Erigeronto* – *Lactucetum serriolae* Lohm. in Oberd. 1957

*Brometum arvensi* (Șerbănescu 1957) Kiss 1964

*Hordeetum murini* Libbert 1932 em. Pass. 1964

*Plantaginetea majoris* Tx. & Prsg. 1950

*Plantaginetalia majoris* Tx. (1947) 1950

- Polygonion avicularis* Br.-Bl. 1931 em. Tx. 1950  
*Lolio – Plantaginetum majoris* (Linkola 1921) Beger 1950  
*Poëtum annuae* Gams 1927  
*Sclerochloo – Polygonetum avicularis* (Gams 1927) Soo 1940  
*Artemisietea vulgaris* Lohm et al. in Tx. 1950  
*Onopordetalia acanthii* Br.-Bl. & R. Tx. ex Klika & Hadac 1944  
*Onopordion acanthii* Br.-Bl. et al. 1936  
*Onopordetum acanthii* Br.-Bl. et al. 1936  
*Ambrosietum artemisiifoliae* Vițălariu 1973  
*Dauco – Melilotion* Görs 1966  
*Conietum maculati* I. Pop 1968  
*Agropyretalia repentis* Oberd. et al. 1967  
*Convolvulo – Agropyron* Görs 1966  
*Convolvulo – Agropyretum repentis* Felföldy 1943  
*Molinio – Arrhenatheretea* Tx. 1937  
*Arrhenatheretalia* Pawl. 1928  
*Cynosurion* R. Tx. 1947  
*Trifolio repenti – Lolietum* Krippelova 1967

## 5. Conclusions

1. Spontaneous flora under survey is made up of over 250 species of cormophytous, most of which are ruderal and segetal weeds, sometimes accompanied by a few sub-spintaneous species.
2. We therefore confirm the presence of the *Panicum dichotomiflorum* Michx species [4] of which a population was identified at the North Station.
3. The spreading of the *Ambrosia artemisiifolia* L. species is of great concern, as its pollen is highly allergenic [5]. As we have seen, this species spreads very rapidly and makes up compact phytocenoses surrounded by a self-sustaining association (*Ambrosietum artemisiifoliae* Vițălariu 1973).
4. The vegetation under survey proves to be a rich one if we take into account the number of identified vegetal associations, but some associations are represented by phytocenoses that occupy small areas. The most widespread associations are on lawns *Trifolio repenti – Lolietum* Krippelova 1967 and *Lolio – Plantaginetum majoris* (Linkola 1921) Beger 1950, and on lanes *Convolvulo – Agropyretum repentis* Felföldy. On dried soils (and enhanced by the droughts in the last few years) we came across cenoses of *Hordeetum murini* Libbert 1932 em. Pass. 1964. on lanes we identified during the first stages of succession associations of *Stellarietea mediae* E. Tx., Lohm & Pesg. in R. Tx. 1950, almost identical to those on abandoned agricultural lands.

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**GENUS *ALLIUM* L. 1753 (AMARAYLLIDALES, ALLIACEAE)  
ON FLOODED HABITATS IN SOUTHERN PART  
OF THE PANNONIAN PLANE (VOJVODINA, SERBIA)**

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Serbia and Montenegro

**Abstract**

*In humid habitats in Vojvodina, only two species of Allium genus has been recorded – A. angulosum and A. vineale. Their distribution is consequence of their ecological adaptability and their origin. A. angulosum belongs to the primitive Rhizirideum section, and by that could be defined as an inhabitant of humid grasslands and ecosystems connected to the dictation of the higric factor. A. vineale is more developed representative of the Allium genus, and it is not limited to the humid habitats, but in Vojvodina it could be found on similar places, because of its wide ecological valence. Both species grow in vegetation of humid grasslands of classis Molinio-Arrhenatheretea Tx. 1937, as well as in classis of weed vegetation Artemisietea vulgaris Lohm., Prsg. et Tx. 1950. A. angulosum is frequent inhabitant of lowland flooded forest vegetation of order Populetales albae Br.-Bl. 1931, classis Querco-Fagetea Br.-Bl. et Vlieg. 1937.*

**Keywords**

*Distribution, floristic diversity, ecological characteristics*

**1. INTRODUCTION**

Vojvodina province is situated on southern brim of Pannonian basin, characterized with fragmented climate-zonal forest and steppe vegetation. Aside of developed steppe plant associations, especially along Danube and flows of other large rivers, vegetation of habitats with variable water regime have been developed. Permanent negative anthropogenic influence is threat to extinction for ecosystems such as marshes, wetlands, flooded meadows in Vojvodina. Ecological adaptations of species in genus *Allium* L. 1753, aimed to modification subterraneous organs, have improved accumulation of organic matter and survival of arid period. However, some of them are successfully adapted and they are taking part in building of plant communities in humid habitats.

## 2. MATERIAL AND METHODS

During the years of field investigations, completed with literature data and information from Institute of Biology and Ecology in Novi Sad Herbarium (BUNS), presence of species of genus *Allium* on analyzed habitats is defined, with accent on flooded areas along Danube River. Sin taxonomic status of plant communities are grouped in alliances and orders according to Parabućski et al. [13], Kojić et al. [12] and to the Vegetation of Serbia [15]. On UTM map (10x10 km) habitats of recorded species are located (UTM yone T34). Basic ecological demands are shown through ecological indices [5]; while life forms of these species define their basic adaptive characteristics [19].

## 3. RESULTS AND DISCUSSION

For now, two species of genus *Allium* that successfully inhabit humid habitats have been recorded in Vojvodina: *A. angulosum* and *A. vineale*.

*Allium angulosum* L.1753

Distribution of this species in Vojvodina is connected with habitats which are permanently flooded. *A. angulosum* mostly inhabit flooded plains and humid meadows, and can be considered as an edificatory of wet meadows inside climate-zonal vegetation of forests and steppe.

This species is mostly distributed in middle part of river Danube flow in Vojvodina (UTM Code: DR/00;10;11;20;30) [23, 14, 16, 17, 18, 2, 3, 7, 9, 6, 1]. Also *A. angulosum* has been found near Danube in western Backa region (UTM Code: CR46) [10]. Considering that eastern part of Vojvodina, Banat, is relatively pore with water flows, this species has been recorded only on few localities near Begej river (UTM Code: DR/51; 52) [16, 1] and vicinity of Banatska Palanka (UTM Code: EQ26) [1]. Near river Sava, in southern Vojvodina (Srem), it is recorded only in flooded forests of southeastern Srem (UTM Code: CQ67) [9], as well as near Obedska Bara, on locality near Kupinovo village (UTM Code: DQ35), however this plant species has not been recorded on that place since 1916 (Fig. 1).

**Ecological indices:** SBT<sub>s</sub> TB<sub>6</sub> WB<sub>8</sub> RB<sub>8</sub> NB<sub>3</sub> LB<sub>8</sub> SB<sub>0</sub> [5]

**Life form:** a Mac-Meg G bulb/rhiz scap

### Cenological distribution on flooded habitats in Vojvodina:

**Classis** *Querco-Fagetea* Br.-Bl. et Vlieg. 1937

**Order:** *Populetalia albae* Br.-Bl. 1931

**Alliance:** *Salicion albae* Soó 1940

**Alliance:** *Populion albae* Br.-Bl. 1931

**Alliance:** *Alno-Quercion roboris* Ht. (1937) 1938

**Classis:** *Molinio-Arrhenatheretea* Tx. 1937

**Order:** *Molinietalia coeruleae* Koch 1926

**Alliance:** *Molinion coeruleae* Horv. 1949

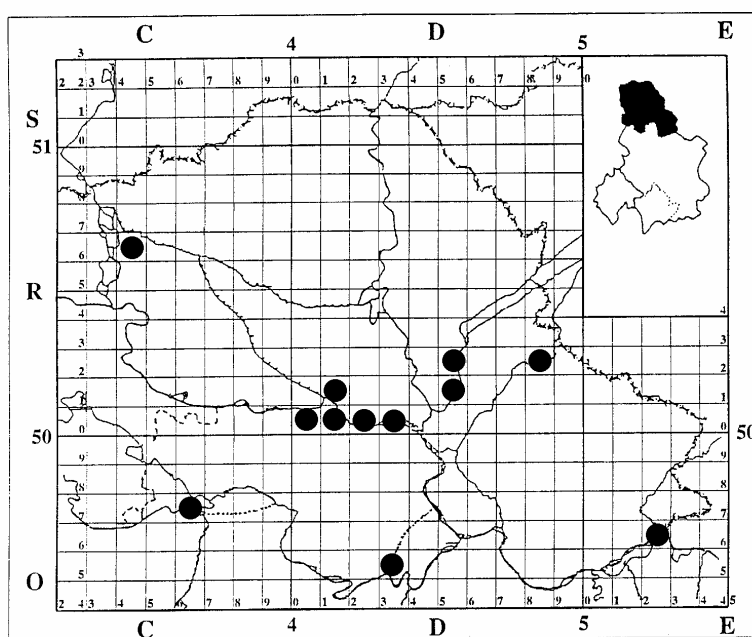
**Order:** *Arrhenatheretalia* Pawl. 1928

**Alliance:** *Agropyro-Rumicion crispae* Nordh. 1940

**Classis:** *Artemisietea vulgaris* Lohm., Prsg. et Tx. 1950

**Order:** *Galio- Alliarietalia* (Txd. 1950) Oberd. et al. 1967

**Alliance:** *Convolvulion sepium* Tx. 1947



**Fig. 1.** Distribution of *Allium angulosum* on humid habitats in Vojvodina

Considering the status of species that inhabit only specific types of habitats, its definition as a Specialists species (SBTs) fulfill general view on a plant that is not tolerant to changes in habitat conditions. Its presence in alliances of classis *Artemisitea vulgaris* is surprising, we have to point out that this data origin from localities near water flows. *A. angulosum* is species of submontane wide leaved forests (TB8), but in Vojvodina it grows in forest-steppe zone in habitats which are occasionally flooded (WB8), so it can be indicator of flooded meadows in steppe area. It demands alkaline (RB8), oligotrophic (NB3) grounds with enough day light (LB8). It is halofobic plant (S0) but it has been recorded on saline soils near Novi Sad [4].

*A. angulosum* belongs to the Rhizirideum section, it also could be seen from the description of its life form: G bulb/rhiz. This quite primitive section of genus *Allium* is characteristic for moderate continental areas, and its rhizome is main accumulation of organic matter. It is flowering during summer (a) and it can be 60 to 100 cm high (Mac-Meg).

In Vojvodina it grows in three vegetation classes. Classis *Querco-Fagetea* is presented with alliances of lowland flooded forests *Salicion albae*, *Populion albae* and *Alno-Quercion roboris*, while in classis of humid meadows *Molinio-Arrhenatheretea* it is present in alliances of hygro-mesophylous meadows *Molinion coeruleae* and mezophylous meadows and pasturages *Agropyro-Rumicion crispi*.

#### *Allium vineale* L. 1753

Species of wide distribution and wide ecological valence according to all factors. Although it is representative of the *Allium* section, which mostly inhabit dry places, *A. vineale* is successfully accommodated to humid areas in Vojvodina, and it could be found along river banks and frequently flooded areas.

In Vojvodina, it has been recorded on few humid localities along Danube river (UTM Code: CR/37; 45; DR/10; 20) [14, 20, ]. It has been also recorded near Plazovic river (UTM Code: CR57) [21], in lower Tisa region, near river (UTM Code: DR32) [8], as well as near Begej (UTM Code: DR51) [11] and in eastern Potamisje (near Tamis river) (UTM Code: DR82) [22] (Fig. 2).

**Ecological indices:** SBT<sub>W</sub> TB<sub>7</sub> WB<sub>4</sub> RB<sub>5</sub> NB<sub>7</sub> LB<sub>3</sub> SB<sub>0</sub> [5]

**Life form:** v-a Mes-Mac G bulb scap

#### Cenological distribution on flooded habitats in Vojvodina:

**Classis:** *Molinio-Arrhenatheretea* Tx. 1937

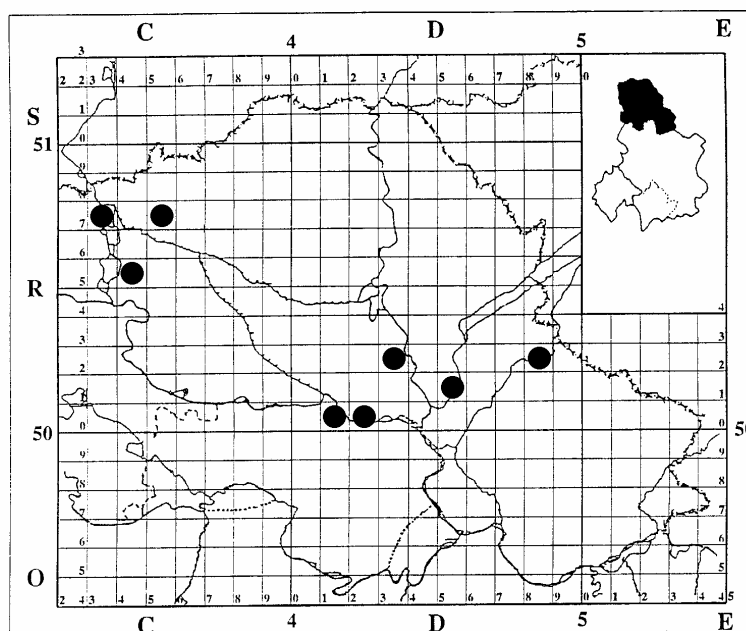
**Order:** *Molinietales* *coeruleae* Koch 1926

**Alliance:** *Molinion coeruleae* Horv. 1949

**Classis:** *Artemisietea vulgaris* Lohm., Prsg. et Tx. 1950

**Order:** *Galio-Alliarietales* (Txd. 1950) Oberd. et al. 1967

**Alliance:** *Convolvulion sepium* Tx. 1947



**Fig. 2.** Distribution of *Allium vineale* on humid habitats in Vojvodina

Species *A. vineale* is wide spreaded, stress tolerant plant. According to its social status, it could be treated as inhabitant of places with intensive anthropogenic influence (SBT<sub>W</sub>). It is characteristic for the thermophilous forest-steppe belt (TB<sub>7</sub>), it grows on moderate humid (WB<sub>4</sub>), weakly acid (RB<sub>5</sub>) soils, rich in nitrogen (NB<sub>7</sub>), with low day light (LB<sub>5</sub>). It is halofobic (S<sub>0</sub>), but it has been found on saline soils in Vojvodina [1].

Belonging to the *Allium* section, it has developed ability for accumulation of organic matter in bulb (G bulb). It is flowering during the spring and summer (v-a), and its high reaches 30 to 60 cm (Mes-Mac).

*A. vineale* is mostly companion species in alliances, with low degree of presence that is consequence of high adaptive plastic and wide ecological valence.

In Vojvodina it has been recorded on humid habitats, only in alliance *Molinion coeruleae*, as well as in few associations of alliance *Convolvulion sepil.*

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## PHYSIOLOGICAL AND GROWTH CHARACTERISTICS OF WHITE WILLOW (*SALIX ALBA* L.) CLONES

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### ABSTRACT:

*The samples from field experiment on five clones of white willow (*Salix alba* L.) in the adult phase, was investigated as follows: net photosynthesis and dark respiration, number and size of stomata and elements of growth (tree diameters and heights). The aim of the research is to determine if there are any relations between these physiological characters and the elements of growth, i.e. if any of them can be utilized in the early selection for growth vigor.*

*The results show that all the characters are characterized by low coefficients of variation, statistically highly significant differences and high coefficients of heritability in a broad sense. As for the net of photosynthesis, the number of stomata on the adaxial and abaxial sides of the leaf is in high correlation with the elements of growth, which indicates that they can be used in the early selection for growth vigor.*

### Key words:

*White willow, physiological characters, growth elements*

### 1. INTRODUCTION

Tree-shaped willows in the conditions of Serbia are very important because they grow on the soils of heavier particle size composition and, in contrast to the other tree species on the alluvium, they can tolerate longer flood periods. In the sites where flood periods are too long for poplars, intensive experimental-production plantations of white willow are established from clonal material obtained by long-term breeding. Because of their high rate of growth, simple methods of regeneration, multiple use primarily in chemical [6] and mechanical processing of wood [7], willows are very suitable for research. Recently, the importance of this species has increased because of the creation of good conditions for the establishment of energy plantations, especially in the Scandinavian countries [15].

Previous work and research of tree-shaped willow breeding [7], was based on the selection for the straightness of the stem and fast growth, utilization of the effects of spontaneous mutations and inter-species hybridization. In addition, much attention was drawn to the research of its adaptive value, i.e. the width of reaction norm [7,

15], requirements of mineral nutrients [5], as well as its ability to form adventitious roots, in the aim of the maximum utilization of this species genetic potential. So far the research mainly included quantitative characters and genetic and phenotypic correlation willows [8], so as to increase the efficiency of selection. According to the available references, insufficient attention was drawn to the possibility of utilization of physiological characters in the early selection for growth vigor of white willow clones. Nowadays, in forest tree breeding, the trend is to make the time needed for the creation of varieties maximally short. In this aim, structurally functional relations are researched (anatomical structure of plant organs and physiological processes) which enables the definition of characters based on which the genotypes with desirable properties can be recognized in the earliest possible ontogenetical phase [13]. The successful resolving of this problem shortens the period necessary for the research and lowers the financials [2]. One of the most desirable properties of willows, along with the low susceptibility to diseases and insects, is growth vigor. It is the result of complex genetic interactions, i.e. interactions of the genotype and the environment [11].

This paper presents the results of the research of variability of several physiological characters of white willow clones, in the aim to research their potential utilization in the selection for growth vigor.

## 2. MATERIAL AND METHOD

The research in field conditions was carried out on the samples from field experiment. The experiment was established from the clones of *Salix alba* in 1983 by deep planting with seedlings 1/0, spacing 4.25 m x 4.25 m on fluvisol at Kac forest (45° 17' N, 19° 53' E, Elevation 76 m). In autumn 1995 the samples of completely formed leaves exposed to light were determined as follows: net of photosynthesis and dark respiration, and the number and size of stomata. Diameters were measured at the height of 1.30 m and tree heights precisely to within 10 cm.

The leaf samples were taken from the top of the crown and all analyses and measurements have been done on the completed formed leaves fully exposed to light.

The net photosynthesis and dark respiration were determined polarographically, by using Clark's electrode. The net of respiration was determined by the quantity of absorbed oxygen (in the dark) and photosynthesis by the quantity of released oxygen in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . In the analysis, very thin leaf slices (up to 0.5 mm). The process of photosynthesis was carried out under complete saturation with white light produced by quartz -iodine lamp. The number and size of stomata was determined by the Wolf [18] method. By this method, microscopic preparations were made and the number of stomata per  $\text{mm}^2$  was determined as well as their length and width in  $\mu\text{m}$ . The data were processed by the standard statistical method, and the table presents the arithmetical means, coefficients of variation, LSD test,

correlations and heritability in the broad sense (by the formula:  $h_{bs}^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_e^2}$  ;  $\sigma_c^2$ -Genotypic variance;  $\sigma_e^2$ =Environmental variance) [19]

## 3. RESULTS

Table 1 presents the results of the research of net photosynthesis and dark respiration intensities. The results show that the highest net (rate) was that of the clone 107/65/6 with  $17.14 \mu\text{mol m}^{-1}\text{s}^{-1}$ , and the lowest was 282 ( $12.52 \mu\text{mol m}^{-1}\text{s}^{-1}$ ). The highest intraclonal variability of this parameter was measured for the clone 377



(11.00%), and the lowest for the clone 107/65/8 (7.77%). LSD test determined that all the clones except the clone 282 occur within one interval of homogeneity, i.e. that between them there are no statistically significant differences.

Respiration net (rate) was the highest for the clone 73/64/8 ( $11.70 \mu\text{mol m}^{-1}\text{s}^{-1}$ ), and the lowest for the clone 107/65/8 ( $7.47 \mu\text{mol m}^{-1}\text{s}^{-1}$ ). The characteristic of this parameter is that three clones (378, 107/65/6, 377) had very low, and the other two clones (73/64/8 and 282) had high coefficients of variation. By the analysis of variance, it was observed that the differences between the clones were statistically highly significant, which is also proved by LSD test, by which the researched clones were grouped into two intervals of homogeneity.

For the net photosynthesis and dark respiration, very high coefficients of heritability in the broad sense were obtained (photosynthesis 0.91 and respiration 0.90).

The number and size of leaf stomata in the researched white willow clones have been shown in Table 1. The results of the research show that all the clones had a higher number of stomata on the abaxial compared to the adaxial surface. The highest number of stomata per  $\text{mm}^2$  of leaf area was in the clone 377 (81 adaxial and 91 abaxial), and the lowest number was in the clone 282 (51 adaxial and 61 abaxial). LSD test produced several intervals of homogeneity, which means that the clones showed the highly significant statistical difference.

The stomatal length on the upper epidermis (adaxial) ranged between  $21.92 \mu\text{m}$  (clone 378) to  $26.60 \mu\text{m}$  (clone 73/64/8). On the lower epidermis (abaxial), stomatal length ranged between  $22.12 \mu\text{m}$  to  $29.76 \mu\text{m}$ . As for this parameter, statistically highly significant differences were observed on both surfaces of the leaf.

The largest width of stomata on both surfaces of the leaf was found for the clone 377 ( $22.60 \mu\text{m}$  adaxial and  $19.76 \mu\text{m}$  abaxial), and the smallest width, for the clone 107/65/6 ( $14.40 \mu\text{m}$ ) on the adaxial and 73/64/8 ( $15.88 \mu\text{m}$ ) on the abaxial epidermis.

All the stomatal characters (number, length and width) are characterized by high coefficients of heritability in the broad sense and low coefficients of variation.

The largest tree diameters (Table 1) were in the clone 377 (15.75 cm), and the smallest - the clone 282 (14.55 cm). As for this parameter, the analysis of variance showed statistically highly significant differences between the clones, which is also proved by the differentiation of the clones in several intervals of homogeneity.

Table 1. Physiological and growth elements of investigated clones

Clone	Net photosynthesis		Respiration		Stoma number per mm <sup>2</sup>				Stoma length (μm)				Stoma width (μm)				Diameter (cm)		Height (m)	
					adaxial		abaxial		adaxial		abaxial		adaxial		abaxial					
	Mean val.	Vk	Mean val.	Vk	Mean val.	Vk	Mean val.	Vk	Mean val.	Vk	Mean val.	Vk	Mean val.	Vk	Mean val.	Vk	Mean val.	Vk	Mean	Vk
378	15.28 a	9.93	8.19 b	2.11	55 c	4.21	76 b	5.18	21.92 c	3.18	22.12 c	4.22	16.44 ab	4.13	19.00 b	5.16	15.60 a	4.93	12.45	5.75
107/65/6	17.14 a	7.77	7.47 b	2.58	71 b	6.16	78 b	6.33	22.90 b	5.11	23.75 b	6.12	14.40 b	5.10	16.48 c	6.16	15.45 ab	10.97	14.05	2.30
377	15.86 a	11.00	9.55 a	2.00	81 a	4.11	91 a	2.82	24.28 b	5.82	24.08 b	5.93	22.60 a	4.18	19.76 a	4.00	15.75 ab	4.95	18.45	7.75
73/64/8	16.24 a	9.55	11.70 a	15.4	57 c	5.17	64 c	5.43	26.60 a	5.66	24.36 b	4.21	18.28 ab	3.82	15.88 c	4.61	14.85 ab	5.55	13.40	7.45
282	12.52 b	11.60	10.12 a	14.4	51 d	4.82	61 d	5.88	24.76 b	4.88	29.76 a	3.16	18.24 ab	3.89	16.40 c	3.33	14.55 b	9.02	13.95	8.24
$h^2_{bs}$	0.91		0.90		0.89		0.90		0.82		0.89		0.73		0.88		0.65		0.54	
Coefficients of correlation between the researched physiological characters and the elements of growth																				
Diameter	0.73		-0.63		0.72		0.94		-0.62		-0.80		0.12		0.79					
Height	0.77		0.06		0.84		0.68		0.22		0.03		0.80		0.53					

Height growth (Table 1) was best for the clone 377 (18.45 m), and the least for the clone 378 (12.45 m). LSD test showed the existence of two intervals of homogeneity, which means that, as regards this parameter, the clones showed statistically highly significant differences.

The correlation analysis was performed in order to research the relationship between the analyzed physiological characters and the elements of growth. Table 1 shows the coefficient of correlation between the researched characters and the elements of growth.

The results show that the following were in high positive correlation with tree diameters: net photosynthesis, stoma number (adaxial and abaxial) and stoma width (adaxial), and with tree height: net photosynthesis, stoma number (adaxial) and stoma width (adaxial).

#### 4. DISCUSSION

The results of the research of several physiological characters and elements of growth indicate that there is a very marked variability within the species *Salix alba*. The greatest number of these characters is characterized by low coefficients of variation and by statistically significant differences between the clones, which leads to a conclusion that these characters are under genetic control to a high extent. The net photosynthesis and respiration were within the limits of previous researches by several authors [14].

The net photosynthesis was in a high positive correlation with the elements of growth, which has so far been observed for several woody species: poplars [4,11,12], larch [9,10]. This means practically that this parameter can be used unfailingly in the selection for growth vigor. The number of stomata on the upper epidermis (adaxial) was also in the positive correlation with the elements of growth, which has already been observed for the clones of black poplar [11,13]. The researched physiological characters are characterized by high coefficients of heritability in the broad sense ( $h^2_{bs}$ ), which has a high significance in the enhancement of selection methods in the aim of maximum utilization of the genetic potential of tree-shaped willows.

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## **GIS IN IRRIGATION SYSTEM MANAGEMENT**

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### **ABSTRACT**

This paper deals with consideration of possible content of Geographical Information System (GIS) for applying in irrigation system management. Spatial data which are content of this GIS can be divided into two groups according to character of their actuality: data which are not changed during longer period of time, like topography, and data which are with short term character, describing quickly changed appearance, like soil moisture.

Possible way for getting spatial data are taken into consideration in order to form GIS. Importance of forming GIS as a step in applying principles of precise agriculture in agricultural production is pointed out.



**ZOOPLANKTON AND MACROZOOBENTHOS COMMUNITIES  
AS WATER QUALITY INDICATORS  
IN DANUBE-TISZA-DANUBE CANAL SYSTEM OF THE  
BACHKA REGION**

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In the frame of the Project No 1945, "Hydrobiological Investigations of the Danube-Tisza-Danube (DTD) Canal Network with the Aim of a Wise Use and the Sustainable Development of the Resources" supported by Ministry of Science and Technologies of the Republic of Serbia, investigations of the, the seasonal surveillance of canal system Danube-Tisza-Danube (Backa region) during 2002 and 2003 included macrozoobenthos and zooplankton analysis. Sampling, sample handling and laboratory analysis were done according to standard hydrobiological methods.

Species richness analysis of benthic fauna revealed the presence of 15 groups. It is worth mentioning the first record ever of the group Polycheta in canal system, represented by one species - *Hyphania invalida*. Besides, the rare groups for this type of aquatic ecosystems were also reported: Trichoptera and Ephemeroptera. The highest diversity was noted at the sampling station downstream of Ruski Krstur and downstream of Kucura fish cage aquaculture - each benthic community consisted of 5 groups. The macrozoobenthos species richness depends mostly on different organic matter load within the canal system. The most significant impairment of the ecosystem integrity was noted at the sampling stations downstream of the Vrbas town and at sampling site on the Mostonga River downstream the Sombor town. The total number of benthic specimens varied between 75 and 15,361 spec./m<sup>2</sup>, while all recorded species indicated, according to their individual indicator value, high organic load of water. The analysis of zooplankton community suggested the uniformity of taxons recorded within the investigated section of canal system.



## WEST ROMANIA SOILS RESEARCHING AND MONITORING AS A PART OF A LASTING DEVELOPMENT

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The paper presents the main aspects regarding the soil resources state, soil cover using, soils quality and the area characteristics of a surface of 3 202 917 ha, from which 1 962 036 ha is presented by the agriculture lands from south-west Romania, Arad, Caras-Severin, Hunedoara and Timis counties.

There are shortly presented the physical-geographical characteristics of the area, and in a more detailed manner the edaphic cover content, some quality restrictive characteristics, soils favorability for some culture species or their capacity for different uses.

For this purpose there were used data from various own themes of scientifically research conducted for many years and from area companies archive data ( Timisoara, Arad, Hunedoara) of more than 50 years of pedological and agrochemical studies and researches and from the monitoring system data organized by ICPA Bucuresti ( M. Dumitru et al.).

The ecopedological conditions characterizing and researching were conducted according to the Pedological Studies Elaboration Methodology elaborated by ICPA Bucuresti in 1987. The analysis were conducted at OSPA (Arad, Deva and Timisoara) laboratories according to the national standards approved by the Romanian Standardization Association

A special attention is given to the evolution of soil degradation under the human activity impact and that of researcher activity, agrochemical survey and soil survey need with the purpose of silviculture and agriculture soils conservation and lasting using.



## **STUDY REGARDING THE GROWTH ACTIVITY OF FLAVONOLS IN ORGANIC AGRICULTURE**

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### **ABSTRACT**

Organic agriculture represents in the last years a research subject with great interest, being imposed by the increased preoccupation in healthy nutrition. There are many research works on growth stimulators from natural sources, which contain natural growth hormones, enzymes, vitamins, amino acids and colloidal minerals.

Flavonoids are natural compounds with phenolic structure (the 3-hydroxy-flavone basic structure with hydroxyl substituents in different positions of aromatic rings), widely spread in vegetal kingdoms. Usually the phenols are known as growth inhibitors, but in this work we intend to study the stimulating or inhibiting activity in the plant growth, depending on application dose,

We have studied the effect of pure quercetin (3,5,7,3', 4'-pentahydroxy-flavone) and morin (3,5,7,2', 4'-pentahydroxy-flavone) over the germination process in biological tests.

The preliminary results show dependence between the administration dose and the stimulating action on the plants, and also the antagonistic action with the growing doses.





## QUANTITATIVE AND QUALITATIVE COMPOSITION OF PHYTO- AND ZOOPLANKTON IN THE DANUBE-TISZA- DANUBE CANAL WATER OF THE BACHKA REGION

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### ABSTRACT

In the frame of the Project No 1945, "Hydrobiological Investigations of the Danube-Tisza-Danube (DTD) Canal Network with the Aim of a Wise use and the Sustainable Development of the Resources", supported by Ministry of Science and Technologies of the Republic of Serbia, investigations of the qualitative and quantitative composition of the phyto- and zooplankton as an indicator of water eutrophication degree have been undertaken.

Twenty sampling sites represented diversity of environmental conditions of Bachka Region Danube-Tisza-Danube canal network: The water eutrophication degree was estimated according to Pantle and Buck (1955) system of saprobity.

In the most of samples, phytoplankton community was dominated by *Cyanobacteria*, *Pyrrophyta*, *Chrysophyta*, *Bacillariophyta*, *Euglenophyta* and *Chlorophyta*. *Bacillariophyta* dominated in the spring period together with algae belonging to the *Pyrrophyta* and *Chrysophyta* groups. *Euglenophyta* were represented by 8 species and *Chlorophyta* with 18 species. Indicative is relative poor qualitative composition of phytoplankton downstream of Vrbas town wastewater discharge.

Zooplankton was represented by species belonging to: *Protozoa*, *Rotatoria*, *Cladocera* and *Copepoda*. *Protozoa* were represented by 12 species, dominating *Rotatoria* by 25. *Cladocera* and *Copepoda* were found to be much less numerous and even totally absent in some sampling sites.

Quantitative composition of both, phyto- and zooplankton varied according to the locality and index of saprobity in the most of water samples was found to be in the frame of  $\beta$ -mezo-saprobity, but shifting towards the  $\alpha$ -mezosaprobity downstream of riparian towns (Vrbas, Bachko Gradishte, Bechey).



**MYXOBACTERIA AS BIOINDICATORS OF WATER  
ORGANIC LOAD OF THE BACHKA REGION DANUBE-  
TISZA-DANUBE CANAL NETWORK**

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**ABSTRACT**

In the frame of the Project No 1945, "Hydrobiological Investigations of the Danube-Tisza-Danube (DTD) Canal Network with the aim of a Wise use and the Sustainable Development of the Resources" supported by Ministry of Science and Technologies of the Republic of Serbia, investigations of myxobacteria as a new microbiological indicator of water organic load have been undertaken.

Due to their multienzymatic nature, myxobacteria are considered as a good bioindicators of organic pollution of water. Results obtained in this investigations show that organic load of canal waters, estimated according to the number of this specific group of bacteria, varied remarkably depending on season and on the sampling site. Using categorization system after Lecianova (1981), quality of water ranged between almost pure water, over slightly and moderately polluted, to the range of extremely polluted water. As a bioindicator parameter, myxobacteria quantitative composition correlated significantly with other classical microbiological indicators of the water organic load.



**CONCERNING ON METABOLIC PROFILE OF PARTRIDGES  
"PERDIX PERDIX" IN CAPTIVITY  
IN WESTERN PART OF ROMANIA**

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Metabolic profile was determined in partridges "Perdix Perdix" with reference to season, age and physiological state, in intensive breeding conditions.

This study was thought to be helpful in distinguishing between intensive and extensive breeding. The distribution of partridges is inequality: 20.78 per cent in intensive breeding and 79.22 per cent in extensive breeding, in western part of Romania, actually.

The possibilities of paraclinical diagnosis of functional disorders of the liver have been verified by effectuated so many tests on some blood biochemical constants.

Early diagnostics of blood serum altered values is extremely important for clinical practice. There are various parameters introduced and used in that connection. The majority of metabolic disorders are related to falling concentrations of serum albumins, of blood sugar, increased concentrations of serum gammaglobulines. In the continuation of the investigations we investigated the changes of calcium (Ca) and phosphorus (P) in the blood serum of partridges, which occur in reproductive period. It is well known that the liver cells, besides the cells of the skeletal muscle and myocardium, contain transaminases and in the cases of disease or cell damage these enzymes come out of tissues, causing the increase of their quantity in the blood.

In all of batches the lowest values were obtain in the winter season and the highest in the autumn.

Although there may be differences in nutrition environment and level of activity between partridges in these conditions, we believe that the results of the present study can be used as a guideline for health evaluation of partridges in intensive breeding in western part of Romania.



## **HUNGARIAN MODEL OF SELF-SUSTAINING RURAL HOMESTEADS**

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### **SUMMARY**

In my work I examine the characteristics and the possibilities of subsistence of the self-sustaining homesteads in the South Plain Region in Hungary. I analyse the problem through the most important plant of the region, the grapevine, using economical examination methods.

Now it can be clearly indicated that the so called 'table wine' types are grown on bigger areas. Therefore the aim of the wine cultivation is the cheap, good quality 'table wine', where while reducing the costs they can produce competitive wine. The domestic consumption is approximately 2,5-3,0 million hl/year, to fulfil this can be the task of the South-Plain of Hungary wine culture. The conditions to produce quality wine can be realized in small areas because of the ecological abilities of the country. This means that in quality wine export the role of the Alföld can be small. Based upon different experiments, even with the traditional species of the Great Hungarian Plain, quality wine can be produced and it can be introduced to the market as Hungaricums. The only national concerned champagne company is in the area, which, with proper support, can undertake the integration of 3000 ha.

For the objective adjudgement of the above mentioned I use the so called SGM calculation. I define the income, the expenses the SGM1 and SGM2 indexes required for the investigation. I examine how much land can a homestead cultivate without alien labour.



## **MARKETING ANALYSIS OF SOME HUNGARIKUM-VEGETABLES IN HUNGARY**

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### **SUMMARY**

Agriculture has been and probably will be in the future as well a significant branch in the south part of the Great Hungarian Plain. Besides the mass products and in many cases instead of them when forming the agricultural structure, this region has to pay more attention to the branches that were important in the past. Hungarian experts who are famous in foreign countries as well deal with these branches and they provide excellent products.

The rules referring to these products are more liberal in the market places of the European Union and their development is not controlled by strict quota systems. In the south part of the Great Hungarian Plain a lot of unique products of excellent quality are produced. Here in this essay we would like to find the answer to the question how the two significant products of the southern part of the Great Hungarian Plain can provide the families with the income that they can live on.

We aim at the economical examination of the asparagus grown in Sand and of the cucumber grown in Méhkerék. To do this we will apply the so called Standard Gross Margin. The agriculture of the states of the European Union is measured with the help of this method. It can also help us in the future to decide whether the different farms belonging to families are economically viable in Hungary.



## **THE SITUATION AND THE RECOMMENDED MARKETING STRATEGY OF THE APRICOT OF KECSKEMÉT IN HUNGARY**

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### **SUMMARY**

Hungary will soon be a member of the EU. Those products that meet the strict requirements of the regulations related to origin protection or the certification system of traditional and unique characteristics can expect a more liberal regulation on the EU markets.

Therefore an effort is made in the South Plain Region of Hungary, instead of the production of mass products, to place a greater emphasis on the industries that have a great history, the Hungarian participants of which possess internationally acknowledged professional skills and that produce excellent quality products.

In our work we searched for an answer about how to increase the popularity and the market volume of the Hungaricums produced in the South Plain Region.



## COMPETITIVENESS OF THE POULTRY BRANCH

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### ABSTRACT

*During my research I revealed the situation of the Hungarian poultry industry with the deep interview. I received replies to the following questions: in what situation the poultry section is in Hungary; how could the position of the companies be improved; how consumer habits have changed in the last decade; what characterises the marketing strategy of the sector*

*I will try to introduce the current situation of the Hungarian poultry industry, its major anxieties, problems and the possible alterations contributing to the improvement with the evaluation of the answers composed by my interviewee.*

### KEYWORDS:

*Poultry, competitiveness, deep interview, marketing strategy*

## 1. INTRODUCTION

The consumption of poultry has gradually increased in recent years - primarily at the expense of pork - which exceeded the quantity of 30 KGs/person last year (2) This tendency can be attributed to two reasons: one is the more health conscious nourishment of the population, the other is the relatively low price of poultry meat. Unfortunately the demand is moving toward the cheap mass products also in case of the further processed products, therefore the price is probably and decisively the most significant factor (4)

I lead my scientific research at the PhD Training of the University of Kaposvár, I examine the marketing-strategy and the consumer attitude on the market of the processed poultry products. The three main areas of my research are:

- Consumer focus group examination,
- Professional deep interviews,
- Representative consumer questionnaire.

I made the deep interviews with the managing director of the first 10 most significant - also producing processed poultry products - Hungarian poultry processing companies.

My interviewees were of the same opinion in most of the issues but in some other themes they disagreed. Therefore I decided to also ask for the opinion of an expert independent of the company.

One of the most important associations of the Hungarian poultry regulation is the Poultry Product Board established in 1991, which assembles those, which produce, process and trade with poultry products. The product council is an interest conciliating organization, which fulfils public duty, and its activity extends all over Hungary.

The objective of the organization is to promote self-regulation and reconciliation of the productive and marketing attitude of all participants of the product range. In order to achieve this, the product council continuously carries out data collection in the field of production and marketing procedures, and after processing these it makes suggestions and decisions (5).

Thus I asked one of the consultants of the product council for the interview who undertook the task with pleasure.

## **2. MEANS AND METHODS OF RESEARCH**

Deep interview belongs to the methods of primary marketing research. The demoscopic research (terrain or primary) directly gathers information about the attitude and opinion of market participants. It can be further divided into two big groups: qualitative and quantitative procedures. The qualitative research - including deep interview - is a discovery one, it primarily wants to know the 'why' and the those reasons, motivations and opinions which determine the attitude and preference of the consumers (1.)

Generally deep interview is applied when the given theme is confidential, embarrassing or sensitive; when it is about questioning professionals or when competitors are interviewed (3).

The following themes were covered during the conversation:

- The current general situation of poultry industry,
- The outstandingly successful and bad periods of the past 10 years,
- The relationship of the processing companies to the trade,
- The changes after joining the European Union,
- The improvement of the position of poultry industry,
- The shaping of the consumer attitude,
- The change of the marketing-strategy of the sector,
- The marketing means and methods applied by the companies,
- Price and price calculation.



I intended to frame the questions in a way that would lead from the general situation to the marketing strategy, or rather, to its four main elements.

### **3. RESULTS AND THEIR EVALUATIONS**

According to the judgement of my interviewee the current situation is not an advantageous one: the efficiency of the factories is lower than 12-14 years ago, though their production structures are more up-to-date. The proprietary structures of the poultry factories have been transformed: bigger co-operating organizations have been established (Hajdú-Bét, Bábolna, Carnex-group), this way these bigger groups can be regarded as competitors. As the consultant argues, the singular smaller companies either come up with a delicate production structure or try to make their livings behind the back of - in the shadow or side-water of - some bigger companies in one way or another. Later on, for them, the only chance will be to produce special: bio, natural or outdoor raised poultry products. According to his report, there is a great dispersion among the factories in terms of technological developments and the same extreme is characteristic of the standard of their food safety activity.

The consultant considers 1988 to be the most significant year, because the biggest production output took place in that year, and that level has not been reached since then yet. The beginning of the 1990s is considered to be a critical period, when the eastern market disappeared. The companies utilizing their capacities moved to the West, where they reduced their prices on the market themselves. As a consequence the companies had to face a series of financial problems. There was a slight mitigation until 1996, the market seemed to become consolidated, some processing factories failed, but all the processing capacity remained untouched. This carries the possibility of a continual war with itself. This is somewhat lessened by the formation of the previously mentioned groups.

In recent years the relationship of the trade and the manufacturers has changed a lot. In Hungary the role, and accordingly, the power of store chains are very significant. They fundamentally determine trade connections. The effective nature of the consumption in great masses forces the companies to link to store chains by all means, and trade chains willingly utilize this possibility, therefore prices are calculated very peculiarly. In the service of the retail shop system consumers make demands for a different production structure, different quality and different food safety.

The consultant also states that we cannot get into an advantageous position with the Hungarian supporting system after joining the European Union. About forty companies or so own union numbers within the sector, but they possess rather extreme capacities, therefore some of them will certainly be unable to operate after the accession. Thus one can imagine that those capacities which could not have been utilized so far, because of the establishment of the quotas will now result in a more effective functioning in the surviving companies.

Hungary has some advantages that are not to be found in every member of the Union, for instance Hungary, besides soya, does not need any imported elementary substance for poultry raising. However, the extent of the integration is not as significant as that of Western countries, and this is an obvious disadvantage.

We could maintain the good position on the Union market with those Hungarian products, such as meat, oaten, fattened goose, with which we have a competitive advantage.

My conversation partner can see several solutions to improve the current not too favourable situation. Firstly, the technique and technology of the fattening and raising farms within the cultivating sectors must be raised to the level that fits into the European standard. Foreign models should be followed in term of trade, where the representatives of huge trade chains are members of the product developing, monetary and economic committees of some processing factories. Thus they have a suitable knowledge of the costs needed for the production of the particular product. They have a great role in deciding the direction of future developments, too. This kind of co-operation is much better than revealing certain facts on the market, for example: the product is unusable, it is not good and it does not comply with the expectations made out by the store chains.

In connection with the attitude changing of the consumer the consultant experienced the following: the consumer demand has cleanly moved toward the fresh poultry products and this seems to reach slowly the Western-European scale. One has to count with the continuous maintenance of the low category product trade, because of the Hungarian income conditions, and with the fact that the most valuable parts of poultry will be exported further on. Of course there has been a growing demand for higher quality products, but this falls behind both in value and quantity compared to lower price category products. Unfortunately there is still only little demand for the so-called natural or bio products in Hungary. This claim is already in existence, but this is not yet observable in relation to the products of poultry and meat industry, but mainly in the baking industry, because of flour-allergy, for example.

The marketing strategy in the industrial sector has gone through significant changes. As my interviewee put it, the marketing strategy of the companies has changed the most which transformed from national companies into share companies. The most important reason for this was that the Eastern markets imported poultry in one hundred thousand tons of magnitude from Hungary, until the end of the 1980s, but at the same time this quantity meant a quality level of mass product requirements. It was easy to select the special quality requirement products from this great quantity. However, this tendency ceased to exist at the beginning of the 1990s and there was a firm launching of the application of food-safety and quality assurance systems first only in the processing factories, later in the producing factories as well. What constitutes the problem is that all this happens to the responsibility of the processing factories: the quality assurance system is supposed to be controlled by the processing factories

and not by the producing ones. The modification of the marketing strategy was supposed to take place with such an uncertain background. This was primarily attempted in case of the processed products, but, as the consultant thinks, a marketing strategy is to comprehend the entire production structure of a company.

He mentioned another very important factor in the change of the marketing strategy: the companies undoubtedly apply the techniques and methods of the Western-European marketing, and they make steps that play a great role in the formation of the strategies toward a direction which fits into the Western-European product structure.

The next question concerned the marketing means and methods applied by the companies. As the consultant sees it, nowadays the companies own some level of a marketing organization, but generally these are of one-person and are only related to the national sales, and if they intend to show some kind of a more serious marketing programme to the consumer, they will have it made by an adequate external organization.

Mainly short-term programmes are typical which are either aimed at one particular group of products. The marketing procedure is dominated by commercials and propaganda. The characteristics of these marketing means are that they are separated, and tied to the companies. The Poultry Product Board has established the trademark "Safe Hungarian Poultry", which was revealed by a former problem at the beginning of last year (Chinese chicken scandal). The expert hopes, that a marketing means like this will promote the national prejudice in the European Union, which will result in consuming our own products with pleasure. If it fulfils this task too, it can put our market in a more profitable position after joining the EU.

In relation to price calculating, my interviewee made the following statements: "Generally manufacturers try to sell "packages" and instead of one lonely product they present themselves with a diverse product range with which they would like to cover their production structure. There is a possibility to vary prices within one package: the manufacturer is obliged to accept lower prices in case of the products that qualified as marketable from the point of view of the stores, but the manufacturer has the opportunity to gain the loss back with higher prices of the rest of the offered package product".

The other main characteristic is that the factories try to use cheaper products to be the base material of their processed products, because the price sensitivity is much lower there. If the consumer is accustomed to, and is the regular buyer of e.g. a breaded, frozen, semi-manufactured product, stuffed with cheese and ham, he will not feel the change of the price as much as in the case of a pre-cooled chicken breast.

#### **4. CONCLUSIONS/SUMMARY**

The field of my research is the examination of marketing-strategy and consumer attitude in relation to the processed poultry products. In my research I made a deep interview with the expert, who is one of the consultants of the Poultry Product Board.

I came to the following conclusions based on his answers:

- Nowadays the poultry section is not in a favourable situation. Firstly, it cannot utilize its capacity, secondly, there is a great difference between the technical and hygienic levels of the factories, and thirdly, the trade chains have a significant superiority.
- The number of the processing companies will probably decrease after joining the EU, which in turn will reduce the competition. However, subsidy is necessary to gain advantageous position on the markets.
- The trademark of the Poultry Product Council serves as a solution for the improvement of the current situation, which urges the consumer to buy the Hungarian poultry. The relationship with trade chains could be settled, if their representatives, following foreign models, would take part in the processing procedure of the factories.
- Consumer attitude has gone through significant changes in the last decade: the demand for fresh, pre-cooled products has increased. Also a health-conscious layer of society is taking shape, preferring bio-products.
- Although the marketing-strategy and the applied marketing means have not reached the Western-European level yet, the companies are on the right track.
- The application of product packages is a good solution in case of price calculation, with the help of which the processing companies also have the opportunity to fix prices.

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