

University of Belgrade  
Faculty of Agriculture

EU Projects Collaborations:

# State-of-the-Art Technologies: Challenge for the Research in Agricultural and Food Sciences

Programme and Abstracts



**AREA**

**"ADVANCING RESEARCH IN AGRICULTURAL AND  
FOOD SCIENCES AT FACULTY OF AGRICULTURE,  
UNIVERSITY OF BELGRADE"**



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Belgrade, 18-20 April, 2016.

**International Conference**  
**State-of-the-Art Technologies: Challenge for the Research**  
**in Agricultural and Food Sciences**  
18-20 April, 2016, Belgrade, Serbia

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# State-of-the-Art Technologies: Challenge for the Research in Agricultural and Food Sciences

18-20 April, 2016  
Hotel Tulip-Inn, Belgrade, Serbia

## PROGRAMME

### Monday, 18<sup>th</sup> April

- 8.30-9.30      The arrival of participants and registration  
9.30-9.45      Conference opening
- Chairmans:      Prof. Radmila Stikić, Prof. Zora Dajić Stevanović, Dr. Tijana Blanuša
- 9.45-10.15      **Advancing research in agricultural and food sciences through the use of DNA based technologies**  
Dimitrije Krstić, *Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*
- 10.20-10.50      **The potential of using advanced approaches for accurate genotyping in grapevines (*Vitis vinifera* L.)**  
Nataša Štajner<sup>1</sup>, Lidija Bitz<sup>2</sup>, Jernej Jakše<sup>1</sup>, Blaž Škrli<sup>1</sup>, Branka Javornik<sup>1</sup>,  
<sup>1</sup>*University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia*; <sup>2</sup>*Natural Resources Institute Finland (Luke), Plant and Forest Genetics and Genetic Resources, Jokioinen, Finland*
- 10.55 -11.05      **Application of next-generation sequencing for selective soybean breeding**  
Marina Tomičić, Vuk Đorđević, Svetlana Balešević-Tubić, Jegor Miladinović, Miloš Vidić, Kristina Petrović, Zlatica Miladionov, *Institute of Field and Vegetable Crops, Novi Sad, Serbia*
- 11.10-11.20      **Metabolic control of tomato fruit growth**  
Milena Marjanović<sup>1</sup>, Radmila Stikić<sup>1</sup>, Zorica Jovanović<sup>1</sup>, Mireille Faurobert<sup>2</sup>, Biljana Vucelić-Radović<sup>1</sup>, Slađana Savić<sup>3</sup>, Ivana Petrović<sup>1</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*; <sup>2</sup>*National Institute for Agricultural Research - INRA, Montfavet Cedex, France*; <sup>3</sup>*Faculty of Biofarming, Megatrend University, Bačka Topola, Serbia*
- 11.20-11.50      Coffee break

Chairmans: Prof. Elena Maestri, Prof. Branka Krstić, Prof. Aleksa Obradović

11.50-12.20 **Grafting: a promising way to manage virus infections in tomato crops**  
Roberta Spanò<sup>1,2</sup>, Tiziana Mascia<sup>1,2</sup>, Donato Gallitelli<sup>1,2</sup>, <sup>1</sup>*Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università degli studi di Bari Aldo Moro, Bari, Italy*; <sup>2</sup>*Istituto del CNR per la Protezione Sostenibile delle Piante, UOS di Bari, Bari, Italy*

12.25-12.55 **Carrot resistance to *Alternaria* leaf blight: from genetics to metabolomic**  
Valérie Le Clerc<sup>1</sup>, Claude Koutouan<sup>2</sup>, Mathilde Piquet<sup>3</sup>, Anita Suel<sup>1</sup>, Sébastien Huet<sup>1</sup>, Latifa Hamama<sup>2</sup>, Linda Voisine<sup>2</sup>, Claire Yovanopoulos<sup>3</sup>, Romain Berruyer<sup>2</sup>, Sabrina Marquès<sup>1</sup>, Pascal Poupard<sup>2</sup>, Mathilde Briard<sup>1</sup>, <sup>1</sup>*IRHS, FR Quasav, Agrocampus Ouest, France*; <sup>2</sup>*IRHS, SFR Quasav, University of Angers, Franc*; <sup>3</sup>*IRHS, SFR Quasav, INRA, France*

13.00-13.10 **Detection limits of different Real-Time PCR protocols for *Clavibacter michiganensis* subsp. *sepedonicus* and *Ralstonia solanacearum***  
Milan Ivanović<sup>1</sup>, Nemanja Kuzmanović<sup>1</sup>, Robert Wreeburg<sup>2</sup>, Robert Bollema<sup>2</sup>, Miriam Kooman-Gersmann<sup>2</sup>, Anđelka Prokić<sup>1</sup>, Nevena Zlatković<sup>1</sup>, Aleksa Obradović<sup>1</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*; <sup>2</sup>*Dutch General Inspection Service, Emmeloord, The Netherlands*

13.15-13.25 **Using qPCR for quantification of *Salmonella* in colonization of wheat seedlings**  
Igor Kljujev<sup>1</sup>, Vera Raičević<sup>1</sup>, Blažo Lalević<sup>1</sup>, Dragan Kiković<sup>2</sup>, Michael Schmid<sup>3</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*; <sup>2</sup>*Faculty of Natural Sciences and Mathematics, Kosovska Mitrovica, Serbia*; <sup>3</sup>*Helmholtz Zentrum, München, Germany*

13.30-14.30 Lunch

Chairmans: Prof. Rafal Baranski, Prof. Valérie Le Clerc, Mr. Dejan Lazić

14.30-15.00 **Raman spectroscopy-based techniques to study secondary metabolites in higher plants, algae and yeast**  
Malgorzata Baranska<sup>1</sup>, Marta Z. Pacia<sup>1</sup>, Grzegorz Zajac<sup>1</sup>, Monika Dudek<sup>1</sup>, Agnieszka Kaczor<sup>1</sup>, Rafal Baranski<sup>2</sup>, <sup>1</sup>*Faculty of Chemistry, Jagiellonian University, Krakow, Poland*; <sup>2</sup>*Institute of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Krakow, Poland*

15.05-15.35 **Raman analysis of foods - emerging sampling approaches and representative analysis**  
Niils Kristian Afseth, *Nofima - Norwegian Institute of Food, Fisheries and Aquaculture research, Ås, Norway*

15.40-15.50 **Fluorescent microscopy in determination of lignified tissues in stems of intercropped white lupin plants**

Lana Zorić<sup>1</sup>, Aleksandar Mikić<sup>2</sup>, Svetlana Vujić<sup>3</sup>, Branko Ćupina<sup>3</sup>, Milica Rat<sup>1</sup>, Dunja Karanović<sup>1</sup>, Jadranka Luković<sup>1</sup>, <sup>1</sup>*Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia*; <sup>2</sup>*Institute of Field and Vegetable Crops, Novi Sad, Serbia*; <sup>3</sup>*Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia*

15.55 -16.25 Coffee break

Chairmans: Dr. Finn Plauborg, Prof. Sofija Pekic Quarrie, Prof. Vera Raičević

16.25-16.35 **Raman spectroscopy of fish fillets - outcome of AREA project**

Božidar Rašković<sup>1</sup>, Aleksandar Nedeljković<sup>1</sup>, Ivana Božičković<sup>1</sup>, Vesna Poleksić<sup>1</sup>, Zoran Marković<sup>1</sup>, Jürgen Popp<sup>2</sup>, Petra Rösch<sup>2</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*; <sup>2</sup>*Institute of Physical Chemistry, Friedrich Schiller University Jena, Jena, Germany*

16.40-16.50 **Raman spectroscopy in determination carotenoids in tomato genotypes during development**

Ilinka Pećinar<sup>1</sup>, Dragana Rančić<sup>1</sup>, Sofija Pekić Quarrie<sup>1</sup>, Nenad Milosavić<sup>2</sup>, Petra Rösch<sup>3</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*; <sup>2</sup>*Faculty of Chemistry, University of Belgrade, Belgrade, Serbia*; <sup>3</sup>*Institute of Physical Chemistry, Friedrich Schiller University Jena, Jena, Germany*

16.55-17.05 **Application of Raman microscopy for control of encapsulation/ immobilization processes**

Steva Lević<sup>1</sup>, Petra Rösch<sup>2</sup>, Jürgen Popp<sup>2</sup>, Vladimir Pavlović<sup>1</sup>, Ana Kalušević<sup>1</sup>, Viktor Nedović<sup>1</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*; <sup>2</sup>*Institute of Physical Chemistry, Friedrich Schiller University Jena, Jena, Germany*

17.10-18.10 Steering Committee Meeting

20.30 Gala Dinner

**Tuesday, 19<sup>th</sup> April 2016**

Chairmans: Prof. Donato Gallitelli, Dr. Panagiotis Gkorezis, Prof. Dragan Nikolić

9.00-9.30 **The role of endophytic bacteria in phytoremediation**

Evdokia Syranidou, Nicolas Kalogerakis, *School of Environmental Engineering, Technical University of Crete, Chania, Greece*

- 9.35 -10.05      **Opportunities for the use of molecular markers in the conservation and use of fruit genetic resources**  
Matthew Ordidge, Edward Venison, Paul Hadley, Richard Ellis, *School of Agriculture, Policy and Development, University of Reading, UK*
- 10.10-10.20      ***Acidovorax citrulli* - casual agent of bacterial fruit blotch on watermelon in Serbia**  
Nevena Zlatković<sup>1</sup>, Nemanja Kuzmanović<sup>1</sup>, Anđelka Prokić<sup>1</sup>, Milan Ivanović<sup>1</sup>, Katarina Gašić<sup>2</sup>, Aleksa Obradović<sup>1</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia;* <sup>2</sup>*Institute for Plant Protection and Environment, Belgrade, Serbia*
- 10.25-10.35      **DNA extraction and applications of SSR markers in identification of grapevine varieties collected from Serbia and UK**  
Zorica Ranković-Vasić<sup>1</sup>, Matthew Ordidge<sup>2</sup>, George Gibbings<sup>2</sup>, Dragan Nikolić<sup>1</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia;* <sup>2</sup>*School of Agriculture, Policy and Development, University of Reading, UK*
- 10.40 -11.10      Coffee break
- Chairmans:      Prof. Matthew Ordidge, Prof. Nicolas Kalogerakis, Prof. Sava Vrbničanin
- 11.10 -11.40      **The concepts of critical period of weed control and biologically effective dose for applied agricultural research**  
Stevan Z. Knežević, *Haskell Agricultural Laboratory, University of Nebraska, Concord, USA*
- 11.45-12.15      **Epigenetic vs. genetic diversity in natural plant populations: a case study of Croatian endemic *Salvia* species**  
Zlatko Šatović<sup>1,2</sup>, Klaudija Carović-Stanko<sup>1,2</sup>, Martina Grdiša<sup>1,2</sup>, Ivan Radosavljević<sup>2,3</sup>, Zlatko Liber<sup>2,3</sup>, <sup>1</sup>*Faculty of Agriculture, University of Zagreb, Zagreb, Croatia;* <sup>2</sup>*Centre of Excellence for Biodiversity and Molecular Plant Breeding, Zagreb, Croatia;* <sup>3</sup>*Faculty of Science, University of Zagreb, Zagreb, Croatia*
- 12.20-12.30      **Root architectural traits under abiotic stress conditions**  
Ivica Đalović<sup>1</sup>, Yinglong Chen<sup>2</sup>, Željana Prijjić<sup>3</sup>, Goran Bekavac<sup>1</sup>, <sup>1</sup>*Institute of Field and Vegetable Crops, Novi Sad, Serbia;* <sup>2</sup>*The UWA Institute of Agriculture, and School of Earth and Environment, The University of Western Australia, Australia;* <sup>3</sup>*Ministry of Agriculture and Environmental Protection, Belgrade, Serbia*

- 12.35-12.45      **Potential pollen-mediated gene flow between herbicide tolerant and weedy sunflower**  
 Dragana Božić<sup>1</sup>, George Gibbins<sup>2</sup>, Markola Saulić<sup>1</sup>, Tijana Blanuša<sup>2</sup>, Sava Vrbničanić<sup>1</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia;* <sup>2</sup>*School of Agriculture, Policy and Development, University of Reading, Reading, UK*
- 12.50-13.00      **DNA extraction and use of PCR based methods in *Calamintha* sp. research**  
 Ivan Šoštarić<sup>1</sup>, Danilo Stojanović<sup>2</sup>, Svetlana Ačić<sup>1</sup>, Marina Mačukanović Jocić<sup>1</sup>, Zora Dajić Stevanović<sup>1</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia;* <sup>2</sup>*Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia*
- 13.05-14.05      Lunch
- Chairmans:      Dr. Laura Scarabel, Prof. Biljana Vucelić Radović, Prof. Predrag Puđa
- 14.05 -14.35      **Advances in polymerase chain reaction technologies for food authenticity testing**  
 Elena Maestri, Davide Imperiale, Nelson Marmioli, *Department of Life Sciences and SITEIA.PARMA, University of Parma, Parma, Italy*
- 14.40-14.50      **Angiotensin-converting enzyme inhibitory activity of selected edible and medicinal mushrooms extracts**  
 Jovana Vunduk<sup>1</sup>, Anita Klaus<sup>1</sup>, Maja Kozarski<sup>1</sup>, Predrag Petrović<sup>2</sup>, Milica Zdravković<sup>1</sup>, Anita Adamović<sup>1</sup>, Miomir Nikšić<sup>1</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia;* <sup>2</sup>*Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia*
- 14.55-15.05      ***Laactococcus lactis* SSP. *lactis* BGBU1-4: Inhibition of *Listeria monocytogenes* ATCC19111 in cheese model system**  
 Nemanja Mirković<sup>1</sup>, Zorica Radulović<sup>1</sup>, Milica Mirković<sup>1</sup>, Dušanka Paunović<sup>1</sup>, Elena Maestri<sup>4</sup>, Milan Kojić<sup>2</sup>, Jelena Lozo<sup>2,3</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia;* <sup>2</sup>*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia;* <sup>3</sup>*Faculty of Biology, University of Belgrade, Belgrade, Serbia;* <sup>4</sup>*Department of Life Sciences and SITEIA.PARMA, University of Parma, Parma, Italy*
- 15.10-15.20      **Bioactive peptides in food of animal origin**  
 Milica Pavličević<sup>1</sup>, Elena Maestri<sup>2</sup>, Nemanja Mirković<sup>1</sup>, Nelson Marmioli<sup>2</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia;* <sup>2</sup>*Department of Life Sciences and SITEIA.PARMA, University of Parma, Parma, Italy*

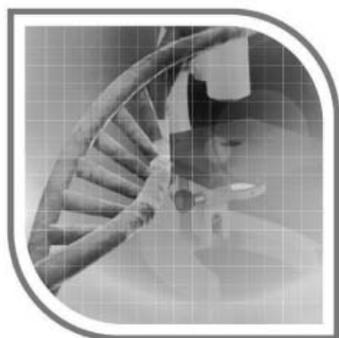
- 15.25-15.35      **The influence of goat milk heat treatment and calcium chloride addition on the rheology of rennet coagulation**  
Milan Stojković, Zorana Miloradović, Predrag Puđa, Jelena Miočinović, *Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*
- 15.40 -16.05      Coffee break
- Chairmans:      Dr. Nils Kristian Afseth, Prof. Malgorzata Baranska, Prof. Zoran Marković
- 16.05-16.35      **Carotenoids in carrot: Raman spectroscopic analysis at organ, tissue and cell levels**  
Rafal Baranski<sup>1</sup>, Malgorzata Baranska<sup>2</sup>, <sup>1</sup>*Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Krakow, Poland;* <sup>2</sup>*Faculty of Chemistry, Jagiellonian University, Krakow, Poland*
- 16.40-16.50      **Characterization of yeasts using Raman spectroscopy**  
Danka Radić<sup>1</sup>, Vera Karličić<sup>1</sup>, Jelena Jovičić Petrović<sup>1</sup>, Dragana Kusić<sup>2</sup>, Blažo Lalević<sup>1</sup>, Vera Raičević<sup>1</sup>, <sup>1</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia;* *Institute of Physical Chemistry, Friedrich Schiller University Jena, Jena, Germany, Germany*
- 16.55- 17.05      **Raman spectroscopy as innovative technique for discrimination of milk from different species**  
Aleksandar Nedeljković, Igor Tomašević, Jelena Miočinović, Predrag Puđa, *Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*

**Wednesday, 20<sup>th</sup> April 2016**

- Chairmans:      Prof. Zlatko Šatović, Prof. Miomir Nikšić, Prof. Vesna Rapić Otrin
- 9.00-9.30      **Evolution and diversity of mechanisms conferring resistance to ALS inhibiting herbicides in *Papaver rhoeas***  
Laura Scarabel, *CNR-Institute of Agro-environmental and Forest Biology, Legnaro (PD) Italy*
- 9.35 -10.05      **Plant growth promoting bacteria: An environmental perspective**  
Panagiotis Gkorezis, *Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium*
- 10.10-10.20      **Molecular characterization of soil *Pseudomonas aeruginosa* populations from the Balkans and their putative role as biocontrol agents in agriculture**  
Bojana Vujović<sup>1</sup>, Vera Raičević<sup>2</sup>, Smilja Teodorović<sup>3</sup>, <sup>1</sup>*Jaroslav Černi Institute for the Development of Water Resources, Belgrade, Serbia;* <sup>2</sup>*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia;* <sup>3</sup>*Forensics Department, Academy of Criminalistic and Police Studies, Belgrade, Serbia*

- 10.25-10.55 Coffee break
- Chairmans: Prof. Radmila Stikić, Bogdan Mladenović, Dr. Marijana Todorčević
- 11.00-11.30 **Knowledge transfer between academy and industry**  
Finn Plauborg, *Aarhus University, Foulum, Denmark*
- 11.35-11.45 **Use of collaborative research between SME and University-research institutes to build new infant formula products**  
Ana Radonjić<sup>1</sup>, Jelena Milić<sup>2</sup>, Nikoleta Lugonja<sup>2</sup>, Snežana Spasić<sup>2</sup>, Milica Ranković-Janevski<sup>3</sup>, Radmila Bućan-Petronijević<sup>4</sup>, Miroslav Vrvčić<sup>1,2</sup>, *<sup>1</sup>Faculty of Chemistry, University of Belgrade, Serbia; <sup>2</sup>Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Belgrade, Serbia; <sup>3</sup>Institute of Neonatology, Belgrade, Serbia; <sup>4</sup>Impamil Ltd, Belgrade, Serbia*
- 11.50-12.00 **Support of traditional food sector via TRAF00N project**  
Tanja Petrović<sup>1</sup>, Mirjana Pešić<sup>1</sup>, Đuro Kutlača, Dijana Štrbac<sup>2</sup>, Radosav Cerović<sup>3</sup>, Biljana Rabrenović<sup>2</sup>, Viktor Nedović<sup>1</sup>, *<sup>1</sup>Faculty of Agriculture, University of Belgrade, Belgrade, Serbia; <sup>2</sup>Institute Mihajlo Pupin, University of Belgrade, Belgrade, Serbia; <sup>3</sup>Innovation Center of Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia*
- 12.15-13.30 Poster session
- 13.30-14.00 General discussion and closing statement





## AREA

“ADVANCING RESEARCH IN AGRICULTURAL AND  
FOOD SCIENCES AT FACULTY OF AGRICULTURE,  
UNIVERSITY OF BELGRADE”

**Plenary lectures**



**ADVANCING RESEARCH IN AGRICULTURAL AND FOOD SCIENCES  
THROUGH THE USE OF DNA BASED TECHNOLOGIES**

Krstić Dimitrije

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With the development of molecular biology, some DNA-based technologies have proved to be of a great potential for advancing research in agricultural and food sciences, e.g. in promoting the efficiency of crop breeding programs, protecting germplasm resources, protecting biodiversity, increasing food safety, improving the quality and outputs of agricultural products, etc., making their roles in modern agriculture increasingly important. Here, it will be briefly reflected on the history of molecular biology and its development with the reference to DNA technologies being applied in agricultural and food sciences. Furthermore, to promote utilization of DNA methods in modern agriculture it will be addressed future challenges and applications of molecular markers, transgenic engineering and gene's information in agriculture. Given today's molecular biology endeavors, knowledge and application of different DNA-based technologies is vital for establishing high quality, up-to-date research. Thus, the need for the exchange of know-how and experience in this field has been recognized within the AREA project. This overview will be complemented by the reference to the three-year DNA-related training activities of AREA research groups with very broad thematic scientific areas including crop physiology and anatomy, biodiversity, weed science, horticulture, plant pathology, aquaculture, food biochemistry and biotechnology. Also, it will signify the importance of the AREA FP7 REGPOT grant for strengthening research capacity and advancement of agricultural and food sciences in Serbia and its surroundings.

*Keywords: molecular biology, DNA-based technologies, agriculture and food sciences, research challenges*

## THE POTENTIAL OF ADVANCED APPROACHES FOR ACCURATE GENOTYPING IN GRAPEVINES (*Vitis vinifera* L.)

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Progress in genome sequencing has resulted in hundreds of polymorphic neutral markers, such as microsatellites, which have proven to be powerful tools for identity, parentage and kinship analysis in a variety of species, including grapevines. Microsatellites are one of the most exploited molecular markers in various research areas, including plant genotyping. The scope of our past analysis was determination of the identity and variability of autochthonous grapevines from the Western Balkan region. In addition, in order to complete this task, we analysed all presumed old and indigenous grapevine material collected in local vineyards, grapevine collections or individual farms from Serbia, Bosnia and Herzegovina, Macedonia, Montenegro and Slovenia. Microsatellite analysis resulted in a set of unique genotypes, which were further compared to the genotypes of Western and Eastern European countries with the aim of linking the data and resolving some additional synonyms/homonyms and identifying true-to-type autochthonous Balkan germplasm. The comparison showed that about 85% of identified unique accessions (40 accessions) that were structured within a group of Balkan and Eastern European genotypes are presumably old traditional grapevines, with a putative Balkan origin, and are therefore worth exploring for their origin and identity. Only a small proportion of analysed genotypes resulted in admixed population assignment.

These tasks were accomplished using efficient and rapid multiplex PCR amplification and a capillary electrophoresis approach. The main challenge when using this methodology is that data from two sub-sets obtained in different laboratories using different techniques need to be compared and their relative values to be standardized against each other. At this step, manual sizing and editing are required, mainly due to the rounding of allele sizes, which must be very precise to avoid false differences among samples from two data sets. Microsatellite databases, including those for grapevines, have been mostly developed based on fast and inexpensive semi-quantitative techniques, such as PCR and capillary electrophoresis, which do not allow the determination of a full sequence of microsatellite loci but limit the information to length polymorphism, which hinders straightforward comparison of two or more data sets. As an alternative, New Generation Sequencing platforms (NGS) offer information about DNA sequences including identification of sequence variants of microsatellite loci and of their flanking regions. This advanced approach might give a deeper insight and more precise evaluation of allele variants applicable for sample identification and parentage analysis. We examined here the suitability of an Ion torrent fragment sequencing approach for microsatellite genotyping, by comparing sequencing data generated on the Ion torrent PGM platform to CE-based methods. The sequences of microsatellites were evaluated by the CLC Genomics Workbench suite using various approaches but the study still needs to address issues regarding stutters and artefacts, which are a particular problem in determination of 'true' alleles and require the development of a bioinformatics tool for unambiguous distinction of these sequences. However, the NGS sequencing approach facilitates high multiplexing of SSR loci and also enables identification of variations, which is only possible to a limited extent when using conventional SSR genotyping based on length polymorphism.

*Keywords: microsatellites, next generation sequencing, NGS, Balkan grapevines*

## GRAFTING: A PROMISING WAY TO MANAGE VIRUS INFECTIONS IN TOMATO CROPS

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Epidemics of Sw5-breaking strains of *Tomato spotted wilt virus* (RB-TSWV) recurrently strike tomato crops grown in Apulia (southern Italy). The exploitation of tomato varieties resistant to the common strains of TSWV was attempted, but it was moderately successful as resistance-breaking strains (RB) quickly emerged replacing extant viral population. Grafting of commercial tomato varieties and hybrids onto an old Apulian tomato variety (*Solanum lycopersicum* 'Manduria', SI-Ma) resulted in a high level of resistance to an RB-TSWV via RNA silencing (RNAi). On the basis of previous experience, the evaluation of SI-Ma RNAi-based response was also analyzed in grafted plants challenged with a recombinant strain of *Potato virus Y* (PVY<sup>C-to</sup>) necrogenic to tomato, and with a strain of *Cucumber mosaic virus* (CMV-77) carrying a necrogenic satRNA. Accumulation of PVY<sup>C-to</sup> RNA was commensurate to the severity of symptoms observed, with a clear decrease in plants grafted on SI-Ma. Transient attenuation of symptoms severity comparable to that of the transgenic tomato line UCTC5.9.2 expressing a benign variant of the satRNA was observed also in grafted plants challenged with CMV-77. However, unlike in UCTC5.9.2 symptom attenuation was not accompanied by a reduction in virus titre and plants died by 30 days post inoculation because of stem and leaf necrosis. Transcriptome analysis of key enzymes of RNAi pathway showed that the graft itself is involved in the response of grafted plants to viral infection. Due to the natural source-to-sink virus movement it accumulates at higher levels in the rootstock than in the scion probably because of a reduced transporting capacity of the vascular tissue at the graft interface. In turn, the high virus titre triggers the activation of a strong RNA silencing signal, which reduces virus accumulation in the rootstock and spreads through the graft junction, limiting virus accumulation and consequent symptom expression also in the scion. Studies in progress in our lab suggest that grafting could be a new and environmentally friendly approach to manage viral diseases in tomato.

**Keywords:** *Tomato grafting, TSWV, PVY, CMV, RNAi*

## CARROT RESISTANCE TO ALTERNARIA LEAF BLIGHT: FROM GENETICS TO METABOLOMICS

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It is well admitted that to answer environmental and economic challenges of pesticide reduction in agriculture, the use of durable resistant varieties is one of the most efficient disease control practices. To improve plant resistance durability, knowledge on the genetic control and resistance mechanisms is of importance. *Alternaria* leaf blight (ALB) is a major foliar disease in carrot production and one of the goals of the breeders is to develop highly resistant varieties. For this purpose, QuaRVeg and FungiSem, two IRHS research teams, worked with private companies to better understand this plant pathogen interaction. Despite the high genetic diversity of this fungus and a large host range, no variety by isolate interaction was identified. This result suggests that the partial resistance of carrot to *A. dauci* is determined by major QTLs that confer resistance to a large number of isolates. Indeed, the study of the genetic determinism of carrot resistance to *A. dauci* allowed the identification of several important QTLs and highlighted the opportunity to combine favorable alleles coming from different genetic backgrounds in one genotype. Some of the genes underlying Quantitative Resistance Loci may be involved in the production of defense compounds. In a previous study, we identified higher contents of faltarindiol in a resistant genotype suggesting that this secondary metabolite may be part of the molecular mechanism involved in resistance against ALB. To go further on this question and to identify other secondary metabolites involved in this resistance, a metabolomic approach was developed. Based on a metabolite QTL strategy, we should highlight some regions in the carrot genome that will colocalize with the QTLs of resistance already identified and we should be able to provide some candidate genes for future functional validation. The impact of breeding for resistance on carrot quality is also under investigation. Combining genetic, phenotypic and metabolic approaches to better understand this plant-pathogen interaction will be underlined.

*Keywords: partial resistance, secondary metabolites, metabolite QTL, quality, Daucus carota*

## RAMAN SPECTROSCOPY-BASED TECHNIQUES TO STUDY SECONDARY METABOLITES IN HIGHER PLANTS, ALGAE AND YEAST

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Spectrophotometric and chromatographic methods have been extensively applied for plant chemical analysis. Today, high-performance liquid chromatography (HPLC) is still mostly used in this context due to its high reproducibility and low detection limit. However, application of HPLC requires a long process of sample preparation including solvent extraction of the analytes, which are usually strongly bonded to other plant constituents. In light of the foregoing consideration, Raman spectroscopy, which is considered as a fast and non-destructive method, can be a useful and powerful tool for plant secondary metabolite analysis.

Information on chemical composition of plant tissue can be obtained using various Raman spectroscopy techniques. The spectrum of Raman measurement consists of several bands, which code for information on chemical composition of the sample. Although biological samples contain hundreds of various molecules, several of these components, like e.g. polysaccharides, fatty acids, carotenoids, polyphenols, alkaloids or polyacetylenes, can be identified due to their predominant key bands, or, when occur in minor concentrations, with the support of chemometrics e.g., Hierarchical Cluster Analysis. Further technical improvements enabled Raman spectroscopy to be combined with microscopy called mapping or imaging. This advanced research tool is suitable for gathering molecular information with a high spatial resolution, even at a cellular level. Moreover, it is possible to collect Raman spectra by measuring either at a single point or by mapping in a specified area of a sample. The results can be presented as a two- or three-dimensional spectroscopic map and directly compared to the corresponding visual image of the investigated tissue providing detailed information on the distribution of the analyzed molecules occurring in a surface layer of the plant sample. Raman spectroscopy appears to be a fast, safe and non-destructive technique. These unique features allow both dried and living tissues to be analyzed without any need for pre-processing.

The paper shows a potential of Raman spectroscopy techniques for analysis of biological systems. First, an application of confocal Raman imaging to monitor various secondary plant metabolites (polysaccharides, fatty acids, carotenoids, polyphenols, alkaloids or polyacetylenes) directly in biological samples is discussed. Additionally, studies on carotenoids produced by algae or many yeast species, responsible for defence against free radicals are presented. Moreover, "Raman signature of life", a band at 1602 cm<sup>-1</sup>, was observed as a unique spectroscopic feature directly related to mitochondria activity.

In the second part, a stereochemistry of natural pigments and their aggregates is reported. Carotenoids dissolved in organic-water media can form two types of aggregates: H (card-packed) and J (head-to-tail) that exhibit hypsochromic and bathochromic shift of chromophore absorption, respectively. With the help of (resonance) Raman Optical Activity spectroscopy detail information about the structure and configuration of chiral, supramolecular carotenoid assemblies are studied here.

*Keywords: vibrational spectroscopy, Raman imaging, Raman Optical Activity, in situ analysis, biologically active compounds*

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## **RAMAN ANALYSIS OF FOODS – EMERGING SAMPLING APPROACHES AND REPRESENTATIVE ANALYSIS**

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For several decades, Raman spectroscopy has been considered a potential and promising tool within the area of food analysis. Raman spectroscopy offers a combination of sampling opportunities and chemical specificity that allows for a variety of applications. The spectral information that could be obtained is close and complementary to the information obtainable from mid-infrared spectra. The sampling opportunities, on the other hand, are close to the standard non-invasive approaches used in near-infrared spectroscopy. However, the widespread use of Raman spectroscopy in food analysis has so far been hampered by several inherent obstacles, including dominating background fluorescence and limited means for representative sampling. In the last ten years, developments like spatially offset Raman spectroscopy, transmission Raman spectroscopy and wide-area illumination schemes have been introduced, providing potential solutions for these challenges by enabling better means for representative analyte sampling, for probing deeper layers of opaque biological materials, and even, to some extent, for the rejection of background fluorescence when originating from near surface layers. In the presentation, the emergence of new sampling approaches and their potential for true representative analyte sampling will be considered. It is convenient to look at the potential of Raman spectroscopy for food analysis and emerging sampling approaches by considering one important class of compounds found in a vast variety of foods, namely the lipids. Thus, in the presentation, the potential of using Raman spectroscopy for qualitative and quantitative probing of fatty acid features and single fatty acids in simplified and complex food matrices, from pure lipids via cell samples to muscle systems and intact salmon, will be discussed.

*Keywords: Raman spectroscopy, lipids, representative sampling*

## THE ROLE OF ENDOPHYTIC BACTERIA IN PHYTOREMEDIATION

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Phytoremediation technologies use plants and their associated microorganisms for the degradation of toxic organic contaminants in soil, air or water. The idea of exploiting endophytes in phytoremediation strategies is becoming increasingly popular in the last few years. The term “endophytes” refers to bacteria or fungi that grow in the internal parts of the plants without causing any negative effects on their host. Endophytic bacteria have been found in numerous plant species and many studies have shown that they can effect beneficially the plant growth by direct and indirect mechanisms. Moreover, they help the plants to tolerate increased biotic and abiotic stress. Metagenome analysis has revealed the diverse endosphere communities that reside in numerous plant species and the impact of various types of contaminants to them. One of the advantages of using endophytic bacteria is their efficiency to colonize both rhizosphere and endosphere despite plant’s immune system. Inside the plant, they can develop a mutualistic relationship with the host, aiding the xenobiotics degradation and amelioration of induced stress. The gene expression of endophytic bacteria catabolic genes can be monitored and the distribution of plasmids carrying specific degradation/resistant genes can be estimated though molecular techniques. As a result, the potential *in planta* degradation of organic toxic compounds could be revealed as well as the enhancement of tolerance to metal stress. Inoculation with specific metal resistant bacterial strains or consortia has accelerated plant’s capacity to accumulate metals from soil or water. In this context, endophytic bacteria that are able to mineralize organic xenobiotics have been exploited in remediation practices and a decrease in evapotranspiration in volatile organic contaminants or an increase in degradation of organic compounds has been demonstrated. However, many aspects of plant-microbiome interactions are yet to be elucidated and high-throughput molecular approaches will aid the investigation towards the characterization of plant-microbe metaorganism. This will allow the description of interactions of the integrated biological systems, which in turn will enhance our ability to interfere and manipulate these systems for enhancing the efficiency and reliability of phytoremediation.

*Keywords: endophytic bacteria, community structure, stress enzymes, xenobiotics, pharmaceuticals, heavy metals*

## **OPPORTUNITIES FOR THE USE OF MOLECULAR MARKERS IN THE CONSERVATION AND USE OF FRUIT GENETIC RESOURCES**

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Plant genetic resources for food and agriculture are widely recognised as offering a range of diversity that will help develop crops to meet the challenges of future food production. A number of international agreements support the conservation and utilisation of these resources and numerous complementary collections are maintained at national levels around the world. The utilisation of these resources is key to their value, and the availability of information on these resources is key to their utilisation. In line with this, many collections have been characterised for a range of phenotypic characteristics of potential use to researchers and breeders.

Increasingly, this essential phenotypic assessment is additionally supported by molecular genetic analysis. Molecular markers are available that can be used in the curation of collections, to both identify duplication and potential synonymy of old varieties and, increasingly to aid in the co-ordination of collections across multiple countries. In the major fruit crops, markers that can be used to reveal important genetic traits (for example relating to ethylene production, and consequent storage potential in apples) are also increasingly becoming available. Modern fruit breeding is beginning to make use of these marker assisted strategies and it is important for the collections to maintain a linkage with current methods if they are to maintain their utility for research and breeding.

A number of recent European initiatives (through the European Co-operative Program on Plant Genetic Resources and the EU FP7 FruitBreedomics project) have built further on the opportunity to utilise genetic marker technologies to support an increase in the co-ordination of national resource collections, and to investigate their potential use for further research through genome wide association techniques.

*Keywords: plant genetic resources, fruit, breeding, conservation*

## THE CONCEPTS OF CRITICAL PERIOD OF WEED CONTROL AND BIOLOGICALLY EFFECTIVE DOSE FOR APPLIED AGRICULTURAL RESEARCH

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The principles of integrated weed management should provide a foundation for developing optimum systems for efficient use of chemical and non-chemical weed control tools. The two key components of such system are: (1) the critical period for weed control (CPWC) for all major crops and (2) the biologically effective dose for potential weed control tools (e.g. chemical and non-chemical).

The CPWC is a period in the crop growth cycle during which weeds must be controlled to prevent yield losses. The CPWC is useful for making decisions on the need for and timing of weed control. The CPWC consists of two components. The first is the critical time for weed removal (CTWR), which determines the point in the growing season when weeds must be removed to prevent unacceptable yield losses, and the second is the end of the critical period, which determines the time in crop growth when late-emerging weeds will no longer cause yield loss. Many studies have been conducted to determine the CPWC and CTWR in various crops under a range of environmental conditions.

Another critical topic is related to the selection of the appropriate dose of chemical or non-chemical material (eg. propane, steam, foam, etc.) for weed control. The importance of dose was recognized more than 500 years ago. In the fifteenth century, Paracelsus (1494–1541), who practiced alchemy, suggested that the poison is in the dose (“All things are poison and are not poison; only the dose makes a thing not a poison”). In recent history, many researchers have examined various aspects of the dose-response in herbicide bioassays, and toxicological and eco-toxicological studies. In weed science research, the most common goal of a biological assay is to measure and compare the response of weeds and crops to physical, chemical, biological, or temporal stimuli. Often, summaries of biological assays require the use of nonlinear regression models with upper and lower limits, which provide information on the dose required to control the plant species of interest. With advances in computer technology, a variety of statistical programs have become available, and many have been utilized to fit nonlinear regressions and estimate their parameter values. Advances in statistical software allow both standard and more complex statistical methods for nonlinear regression analysis of the critical period of weed control and dose-response curves to be carried out by non-statisticians. One such package, drc (dose-response curves), has been developed for the open-source language R and is available at no cost from the Internet.

*Keywords: weed, control, dose-response, herbicide bioassays*

**EPIGENETIC VS. GENETIC DIVERSITY IN NATURAL PLANT POPULATIONS:  
A CASE STUDY OF CROATIAN ENDEMIC *Salvia* SPECIES**

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In plants, epigenetic variations based on DNA methylation are often transmitted across generations. Epigenetic processes may cause significant heritable variation in phenotypic traits without changing the sequence and thus increase considerably the evolutionary potential of plants in response to abiotic and biotic stress. Thus, heritable epigenetic variation could influence the course of evolution in plants, as it can affect the processes of adaptation and divergence through selection of stable epigenetic variants without involvement of genetic variation.

In order to understand the true importance of epigenetic processes, existing epigenetic variation of natural plant populations has been assessed and compared to genetic variation. Three Croatian endemic *Salvia* species including (1) *Salvia officinalis* L., (2) *Salvia brachyodon* Vandas, and (3) *Salvia x auriculata* Mill. have been used as a study system. The species were chosen for having contrasting range sizes and genetic diversity heavily influenced by different phenomena: clonality (*S. brachyodon*) and interspecies hybridization (*S. x auriculata*). *S. officinalis* is widely distributed along the eastern Adriatic coast while *S. brachyodon* is a stenoendemic plant of a very narrow range size. *S. x auriculata* is a natural hybrid between *S. officinalis* and *S. fruticosa*. Preliminary results based on the analysis of 25 *S. officinalis* populations showed that the proportion of epigenetic variance attributable to differences among populations was similar to the proportion of the among-population component of genetic variance. Systematic patterns of epigenetic variation were observed in relation to a number of bioclimatic variables related to amount of precipitation at sampling sites. The investigation of clonal *S. brachyodon* revealed the existence of substantial epigenetic variation among genetically identical plants (clones). Finally, hybrid *S. x auriculata* exhibited higher epigenetic diversity in comparison to parental species.

*Keywords: epigenetic diversity, genetic diversity, population structure, clonality, interspecies hybridization*

## ADVANCES IN POLYMERASE CHAIN REACTION TECHNOLOGIES FOR FOOD AUTHENTICITY TESTING

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The occurrence of food frauds and crimes has necessitated testing of food authenticity, including the determination of ingredients and verification of compliance with the label. The horse-meat scandal of 2013 has to be considered a turning point for DNA analysis in food authenticity. Possible types of fraud have been categorised into three groups: (a) the replacement of an ingredient with a different substance or ingredient that is less expensive, (b) the addition of non authorised, non-authentic substances and (c) the removal of a component which is authentic. Among the many different and relevant analytical techniques, amplification of DNA through the Polymerase Chain Reaction has a central role as a tool for analysis in food authenticity issues. The applications are classified into two groups according to the type of analytical approach which is required: (1) verification of the presence/absence of specific ingredients; (2) identification of the ingredients (species determination). Both types of analyses are needed to detect instances of replacement, addition or substitution. The information available and the recent examples highlight how strong the need is for harmonisation and integration. Even if each food matrix can be seen as a specific new challenge, requiring ad hoc approaches and development or optimisation of techniques for DNA analysis, harmonised protocols are essential.

The European Union has invested millions of EUR for research on food safety, quality, traceability and, recently, integrity of the food supply chains: the “What’s for Lunch?” conference in 2011 summarised the main results and the research project FoodIntegrity ([www.foodintegrity.eu](http://www.foodintegrity.eu)) is currently coordinating efforts for organising and harmonising the knowledge gathered in the past years, building knowledge bases and guidelines for analysts, researchers, and stakeholders. The relevance of research efforts funded by governmental institutions, joining together scientists and operators from different countries is also essential, because food frauds and crimes are trans-national in nature.

*Keywords: DNA barcoding, food authenticity, food fraud, food genomics, food integrity*

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## **CAROTENOIDS IN CARROT: RAMAN SPECTROSCOPIC ANALYSIS AT ORGAN, TISSUE AND CELL LEVELS**

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Carrot is a root vegetable grown in regions of temperate climate worldwide. Roots can be processed, consumed fresh or after a few month storage that makes this vegetable a valuable source of nutrients and vitamins in human diet. In particular, carrot storage root is one of the richest sources of various carotenoids like beta- and alpha-carotenes having pro-vitamin A activity, but can also contain lutein and lycopene. The content and composition of carrot carotenoids vary considerably in carrot roots depending on variety and can be altered by agrotechnical and climatic factors. Carotenoid content also affects carrot commercial value and is an essential character being improved by breeders. As the analytical determination of carotenoids is destructive to plant material and can additionally affect complexes and structure of the analyte, we have applied modern Raman spectroscopy to get complex information on carrot carotenoid composition and their distribution in storage root. Recent advances in Raman spectroscopy have delivered also tools for gathering information at tissue, cell and subcellular levels. We thus demonstrated tissue specific carotenoid distribution and different activity of the carotenoid biosynthesis depending on the cell age. Moreover, the detailed composition of single carotenoid crystals was assessed by direct measurements of chromoplasts without cell disruption. This approach is being extended to measurements of callus lines differing in the carotenoid composition and serving as a model system to study carrot carotenoid biosynthesis.

*Keywords: carotenoids, carrot, mapping, Raman spectroscopy*

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## EVOLUTION AND DIVERSITY OF RESISTANCE MECHANISMS TO ALS INHIBITING HERBICIDES IN *Papaver rhoeas*

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Herbicides have become a major threat to global agriculture. Due to the intensive reliance on chemical control, especially on those herbicides with a specific site of action, resistance in weeds has evolved, jeopardizing crop sustainability. In the vast majority of reported cases, resistance to ALS inhibitors in broadleaves is due to the selection of mutant, herbicide-resistant ALS alleles carrying a mutation at one of a few ALS codons. Instead, herbicide resistance not due to mutations at the herbicide target site (non-target site resistance or NTSR) is the most widespread and major cause of resistance in grass weed species. It includes all mechanisms that cause a compensation for herbicide action and/or a reduction in the amount of herbicide molecules reaching their target protein. Up to now, few data concerning the occurrence of NTSR or the factors influencing its evolution are available for dicotyledonous weed species.

This study investigates the presence and inheritance of NTSR to ALS inhibitors in the broadleaved weed *Papaver rhoeas* (corn poppy) through genetic and molecular approaches. *P. rhoeas* is a troublesome weed infesting winter cereals in Mediterranean Europe that has evolved resistance to ALS, mainly graminicide sulfonylureas used over years. The study will aid in understanding and predicting the selection and spread of *P. rhoeas* plants carrying NTSR in agricultural ecosystems. Results demonstrate that resistance to ALS inhibitors in the broadleaf *P. rhoeas* can be due not only to ALS-based resistance, but also to NTSR mechanisms. NTSR is genetically inherited and the segregation patterns are consistent with an overall polygenic control. Furthermore, mutant ALS alleles can also be present in plants containing NTSR loci, thereby "masking" the presence of NTSR and likely causing underestimation of its significance in *P. rhoeas*.

It thus emerges that NTSR could have a more important role in broadleaved species, as in grass weeds, and all data indicate that the evolution of resistance to ALS inhibitors in *P. rhoeas* is rather complex. The implementation of other genomic approaches such as next-generation sequencing (genomics, transcriptomics) could be a way forward to identify and characterize the genes involved in NTSR.

*Keywords: weed, herbicide, evolution, resistance mechanisms, polygenic control*

## PLANT GROWTH PROMOTING BACTERIA: AN ENVIRONMENTAL PERSPECTIVE

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Microorganisms comprise the biggest part of biomass and biodiversity on our planet. Indeed, according to estimations their number rises up to  $10^{30}$  and this huge number represents a capital for researchers globally that can be further exploited in order to understand the mechanisms and solutions being invented by nature as an auto regulation machinery of its functions. The contribution of bacteria to plant growth promotion relies on their ability to alleviate, through various complex mechanisms, unfavorable abiotic and biotic conditions that plants confront during their life cycles. Although, it has been known for over a century that bacteria can stimulate plant growth and increase productivity, the establishment of the term Plant Growth-Promoting Rhizobacteria (PGPR) did not occur until the mid 1970s when Kloepper and Schroth introduced the term to describe the ability of some bacteria, mainly pseudomonads, to control soil-borne pathogens resulting in the indirect enhancement of plant growth. Thus, PGPR was originally used to describe only bacteria with biocontrol properties. Later, scientists introduced more specific terms to reflect the physical sites on a plant where PGPR reside: Intracellular PGPR reside inside plant cells, produce nodules on the surface of a root hair during infection and being localized inside those specialized structures; and extracellular PGPR live outside plant cells and do not produce nodules, but enhance plant growth through production of signal compounds that directly stimulate plant growth, improve plant disease resistance, or improve mobilization of soil nutrients. There is evidence to suggest that Plant Growth Promoting Bacteria elicit plant growth promotion through indirect and direct action. Both direct and indirect activities resulting in Plant Growth Promotion have been described for a diverse array of bacteria including, most commonly, *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Burkholderia*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Ochrobactrum*, *Pantoea*, *Rhodococcus*, *Rhizobium*, *Serratia*, *Stenotrophomonas* and *Streptomyces*. Unraveling the mechanistic details underlying the myriad of plant-bacterial interactions will have a substantial effect on our abilities to contribute to plant welfare and could lead to the development of useful tools for sustainable approaches to agriculture and bioremediation. In particular for bacteria, information gathered over the last decades from various scientific disciplines has been proven extremely helpful and valuable in order to comprehend in depth their function, as well as the interaction between them and their habitats. At present, a molecular description based on the so-called “omics” technologies has a pivotal role in all areas of microbial ecology. Yet, care must be taken that, while these tools are able to generate a huge amount of data, the genetic composition of a microbial community does not necessarily corresponds to ecosystem function. Conclusively, a sound understanding of the evolved plant-bacteria synergies along with extensive field experiments will fill the gap in knowledge that is still needed in order to design effective schemes with emphasis on increased agricultural yields as well as environmental restoration based on the ingenuity of bacteria and their host plants.

*Keywords: plant growth bacteria, agriculture, bioremediation*

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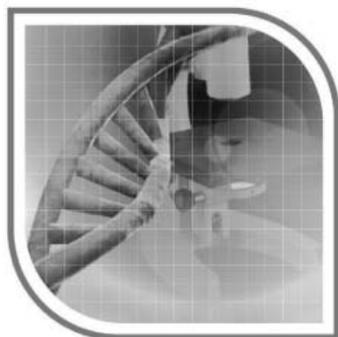
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In all societies food and food production are important for social development and human well-being. In our globalized world food is imported and exported across continents in immense amounts, and it poses a big pressures on both public and private actors to gain knowledge on how to produce and transport healthy products, not only fresh but also processed both animal and plant food. To be successful it is deemed necessary with a strong private and public collaboration among players of the modern society. At the university level a strong collaboration within programmes of education, innovation, and basic and applied research have been developed over the last decades. In Europe various universities offers 32 and 28 Master Degree programmes in Agricultural Science and Food Science, respectively and by this the foundation to achieve the capacity to understand the complex factors that shape agricultural systems and obtain a sound foundation in the scientific principles and analytical skills vital for global plant and animal-derived food and fibre production. Further improving of the quality of the food supply and technologies for food processing and quality management, food analysis, food packaging, food product development is needed. But most importantly, research collaboration shows high activity levels. The scientific performance and collaboration profile of European universities showed from 2007 to 2011, that 303 selected universities within 35 countries of the ERA comprising members of the EU-28, candidate EU countries, members of the EFTA, and Israel a production close to 90,500 papers in Food, Agriculture and Fisheries representing one-third of the world publications in this area in that period. Another important achievement is the effect of the EU Marie Skłodowska Curie Actions (MSCA), which in Horizon 2020 has a budget of 6.2 billion EUR. In the MSCA the private and public research collaboration is facilitated by Innovative Training Networks, where the goals are to: i) Strengthen innovation capacity of universities/research institutes with commercial exploitation of their research, ii) Enhance the research potential and competitiveness of European companies and SMEs, iii) Develop research careers combining scientific excellence with business innovation, iv) Equip PhD holders with skills matching public and private sector needs, v) Create a bridge and build long-term partnerships between universities and businesses.

*Keywords: Research collaboration, Innovative Training Networks, Horizon 2020*





## AREA

“ADVANCING RESEARCH IN AGRICULTURAL AND  
FOOD SCIENCES AT FACULTY OF AGRICULTURE,  
UNIVERSITY OF BELGRADE”

**Oral presentations**



## APPLICATION OF NEXT-GENERATION SEQUENCING FOR SELECTIVE SOYBEAN BREEDING

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In the light of global climate change, the modification of soybean breeding programs and goals is necessary not only to increase the yield, but also to maintain its previous level. Yield and most of the agronomically important traits are quantitatively inherited and controlled by many loci, contributing a small effect to the final expression of the character with a pronounced environmental influence. Genomics approaches are particularly useful when dealing with multi-genic traits, as it is possible to simultaneously estimate the effects of loci or markers across the entire genome, on phenotypic reaction of genotypes in the variable environmental conditions. Genomic profiles and phenotypic data should be combined to build a statistical prediction model. In order to maximize accuracy of the prediction model, the population used for its development must be representative of selection candidates in the breeding program to which genomic selection will be applied. Based on estimated parameters of the prediction model and genotypic data only, the expected phenotype of breeding lines can be predicted by calculating genomic estimated breeding value. Thanks to the rapid progress of next-generation DNA sequencing (NGS) technologies, it is now feasible to provide good genome coverage with a sufficient number of molecular markers, making genomic prediction more affordable which will further lead to the reduction of costs associated with phenotyping. Genotyping-by-sequencing (GBS) is an approach based on reduction of the complexity of a genome by using restriction enzymes, which enables target-enriched genome sequencing on NGS platforms. This is an efficient method for simultaneous discovery and genotyping of thousands of single nucleotide polymorphism (SNPs) in a large number of samples.

A set of 285 soybean genotypes sampled from the IFVCNS existing breeding programs were genotyped using GBS and evaluated for yield and other agronomic traits at multiple locations in Serbia during the previous growing season. Obtained data will be combined to develop statistical models of genomic selection. Validation of model efficiency and model upgrade will be carried out through intensive testing on multiple field trials in the coming years, as well as through the addition of new genotypes. It is expected to obtain the precise tool to increase the efficiency of soybean breeding and development of varieties aiming to overcome the negative effects of climate change and to improve soybean breeding programs using genomic selection.

*Keywords: genomic selection, genotyping-by-sequencing, next generation sequencing, soybean*

*Acknowledgement:* The project “Regional cooperation in overcoming negative effects of global climate changes on soybean production through selective soybean breeding” of Deutsche Gesellschaft für Internationale Zusammenarbeit GmbH (GIZ), Project “GMO-free Quality soya of the Danube region”.

## METABOLIC CONTROL OF TOMATO FRUIT GROWTH

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The analyses of metabolic control of tomato fruit growth were done on the basis of proteomic analyses, concentration of plant hormone abscisic acid (ABA) and activity of cell wall peroxidase as one of the key enzyme for cell growth. To better understand the role of stress hormone abscisic acid (ABA) in these processes, comparative analyses with tomato variety Ailsa Craig (wild type) and *flacca* mutant (ABA-deficient) were done. Experiments were conducted in controlled conditions and traits were measured in the phase of maximal fruit growth rate. For metabolic analyses, pericarps of investigating fruits were collected in the phase of intensive cell expansion (30 days post-anthesis). Proteomic analyses were done by LC-MS/MS mass spectrometry and identification of proteins by using the SGN tomato unigene database. ABA concentration was measured by the Elisa method, while the activity of cell wall-associated peroxidase was determined spectrophotometrically by the guaiacol method.

Obtained results showed that the lower fruit growth rate in *flacca* fruits compared to the fruits of wild type plants resulted in significantly smaller final fruit size. In both genotypes, concentrations of ABA during the development of fruits changed in such a way that there has been a decrease in the phase of intensive growth, while the beginning of the ABA increase coincided with the beginning of the ripening of the fruit. It is also shown that a significant increase in the activity of enzyme cell wall-associated peroxidase in tomato fruit pericarp coincided with the end of cell growth and the beginning of the ripening process. A comparative analysis of the dynamics of changes in peroxidase activity and concentration of ABA in the fruits indicates their antagonistic relationship in all treatments and in both genotypes. Proteomic results showed increased expression for the most proteins (related to carbon, amino acid metabolism and protein translation, processing of the proteins and degradation) in the wild type compared to *flacca*. These results indicate that faster metabolic flux in wild type compared to *flacca* might be responsible for a higher fruit growth rate and final bigger fruit size in wild type than in *flacca*.

*Keywords: tomato, ABA, peroxidase, proteomic, fruit growth*

*Acknowledgement:* This study was supported by the EU Commission (FP7 project AREA) and the Serbian Ministry of Education, Science and Technological Development (project TR 31005).

**DETECTION LIMITS OF DIFFERENT REAL-TIME PCR PROTOCOLS FOR  
*Clavibacter michiganensis* subsp. *sepedonicus* AND  
*Ralstonia solanacearum***

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*Clavibacter michiganensis* subsp. *sepedonicus* (Cms) and *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* race 3 (Rsol) are the causal agents of ring rot and brown rot of potato, respectively. These two diseases represent a serious threat to potato production in temperate climates. Both bacteria can remain latent for a long time in asymptomatic potato tubers. Latent infections in seed potato tubers have led to the spread of these bacteria, both locally and worldwide. Therefore, effective control of brown rot and ring rot of potato is dependent on reliable detection of these two pathogens at the latent stage.

In this study, we tested the sensitivity of two real-time PCR (rt-PCR) protocols for detection of Cms, one rt-PCR protocol for detection of Rsol, and multiplex rt-PCR protocol for simultaneous detection of Cms and Rsol. Bacterial suspensions of representative strains of Cms (PD 406) and Rsol (PD 2762) were serially diluted by 10-fold dilutions from  $2 \times 10^8$  to  $2 \times 10^0$  CFU/ml. The colony forming unit (CFU) number of each suspension was estimated by plating on a corresponding medium. The suspensions were further subjected to DNA extraction, followed by real time PCR reaction according to the author's guidelines.

In this study, rt-PCR protocols of Weller *et al.* (2000) and Massart *et al.* (2014) showed high sensitivity in detecting Rsol. Both methods were able to detect bacteria at concentrations of  $10^4$  to  $10^5$  CFU/ml. However, being multiplex rt-PCR, the protocol described by Massart *et al.* (2014) enables simultaneous detection of Cms in the same reaction, saving both time and cost per reaction. The lowest detected concentration for Cms according to this protocol was  $2 \times 10^2$  CFU/ml. Detection limits for Cms in rt-PCR protocols according to Bach *et al.* (2003) and Mills *et al.* (1997) were lower comparing to detection limits for Rsol. The lowest detected concentration of bacterial cells for both of these protocols was  $2 \times 10^4$  CFU/ml.

The obtained sensitivity of all tested rt-PCRs in our study was close to the requested level of sensitivity for an official detection method described by EU directives. After optimization and inter-laboratory evaluation of the robustness and performances, some of these rt-PCR protocols could be adopted and used in the future in large scale screening of seed potato tubers.

**Keywords:** *real time PCR, sensitivity, detection limit, Ralstonia solanacearum, Clavibacter michiganensis* subsp. *sepedonicus*

**Acknowledgement:** This research was supported by the project III46008 financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, and EU Commission project AREA, No. 316004.

## USING qPCR FOR QUANTIFICATION OF *Salmonella* IN COLONIZATION OF WHEAT SEEDLINGS

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The consumption of vegetables is very important for prevention of cardiovascular diseases and it is recommended by WHO. The fresh vegetables are essential for healthy nutrition and provide minerals and vitamins. The vegetables are mostly consumed raw and it is very important to avoid its microbiological contamination during the production chain. The disease which is caused by human pathogen bacteria is a very big problem for public health. These bacteria are able to contaminate fresh vegetables in any part of the food production chain.

In recent years, there has been an increasing number of outbreaks that are caused by consumption of fresh vegetables which are contaminated with human pathogen bacteria. The application of organic fertilizers, contaminated irrigation water as well as polluted environment, pests, pets, food handler, contaminated equipment are the main reasons of contamination by pathogen bacteria during the food production. According to CDC (Center of Disease Control and Prevention, 2010), the lettuce was the one of the most frequent source of foodborne outbreaks in the USA during 2007. Salmonellosis is a usual foodborne infection which is caused by bacteria *Salmonella* spp. According to the U.S. Public Health Service (2009), *Salmonella* is in the second place as ranks second as a cause of foodborne disease in the USA. Approximately, there are 40,000 cases of Salmonellosis in the USA per year (DFBMD, 2009).

The most common serovars which are found worldwide are *Salmonella enteritidis* and *Salmonella typhimurium* but other serovars are limited to specific regions in the world (OIE, 2005).

Today, there are many methods for detection of human pathogen bacteria in food. The conventional methods are generally time-consuming. The PCR is much faster and with qPCR we can get results in only a few hours. The PCR allows increasing speed, sensitivity, specificity of detection of human pathogen bacteria in fresh vegetables.

The aim of this study is application of the qPCR method for detection of *Salmonella enterica* subsp. *Welteweden* and *Salmonella typhimurium* LT2 in wheat seedlings.

Bacterial strains used in this experiment were *Salmonella enterica* subsp. *Welteweden* and *Salmonella typhimurium* LT2. The model plant was wheat. The bacterial suspension applied for inoculation of seeds was  $\approx 10^8$  CFU. The inoculated plants were left to grow in a phytochamber for 3 weeks. The standard PCR was done for *Salmonella* strains. The primers for *Salmonella* were: rfbJ; fliC; fliB; invA, hilA. Cloning was done for getting the plasmid with the invA gene which was used for preparing standards for qPCR. Isolation of *Salmonella* DNA from plants was done using a kit. The sequencing of invA isolated from plant samples was also done.

The qPCR was done for DNA samples isolated from wheat root, shoot and substrate liquid inoculated with *Salmonella* strains. The Fluorescence *In Situ* Hybridization was done for inoculated plant samples. Specific probes were used for detection of *Salmonella* by CLSM.

The results show that investigated *Salmonella* strains were able to colonize wheat plants. The number of *Salmonella* DNA copies was  $4.01 \times 10^6$  per 1 g root (*S. enterica*) and  $3.32 \times 10^7$  per 1 g root (*S. typhimurium*).

**Keywords:** real-time PCR, *Salmonella*, colonization plants, FISH, CLSM

**Acknowledgement:** This study was supported by the EU Commission (FP7 project AREA) and Serbian Ministry of Education, Science and Technological Development (project TR 31080).

## FLUORESCENT MICROSCOPY IN DETERMINATION OF LIGNIFIED TISSUES IN STEMS OF INTERCROPPED WHITE LUPIN PLANTS

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White lupin (*Lupinus albus* L.) is annual legume crop, widely cultivated and selected as green forage. In companion growing with other legume species it usually plays the role of supporting crop, because of its good standing ability. Intercropping affects the morphology and structure of vegetative organs of both components, due to the competition between them. Proportion of stem lignified tissues of forage plants proved to be especially important for their quality determination. Autofluorescence of lignin enables its precise determination and localization, without staining. In this paper, fluorescent microscopy was applied for determination of stem lignified tissues of intercropped white lupin plants. Following the level of lignification along the stem maturity gradient, the effect of intercropping of white lupin with other annual legumes was tested on anatomical level.

The plants were grown as sole crops and intercropped with *Vicia sativa* L., *V. narbonensis* L., *Pisum sativum* L. and *Lathyrus sativus* L. The stereological method was used, and the point-counting method applied for determination of proportions of stem lignified tissues, and calculation of their volume densities.

Parameters that contributed most to the total variability, and made differences between younger and older stem segments, were volume densities of cortex parenchyma and xylem. The treatments differed significantly only in the proportions of tissues in the youngest stem segments. General variability in tissue volume densities was also higher in younger segments. The strongest lignification was observed in xylem, followed by sclerenchyma tissue. Proportion of lignified tissues was similar in all treatments, except in plants grown with *V. sativa*, where it was the lowest (28.2%). Non-lignified, thick-walled tissues were significantly best developed in plants grown as pure crops (9.5%), whilst non-lignified, thin-walled tissues in plants grown with *V. sativa* (61.3%). The obtained results showed that companion growing of *L. albus* did not significantly affect its stem anatomical parameters which determine forage quality.

**Keywords:** *Lupinus*, anatomy, stereology, intercropping, lignin

## RAMAN SPECTROSCOPY OF FISH FILLETS – OUTCOME OF AREA PROJECT

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Raman spectroscopy has a very broad spectrum of applications in life sciences. Among others, Raman spectroscopy is used for quality assessment of meat, not only fish meat, but from other animals as well. Through the FP7 project AREA, a Raman system was purchased and researchers of the Faculty of Agriculture, University of Belgrade (FA) were trained in Raman spectroscopic methods. One of the groups involved in the project is the AQUACARP group, having a primary interest in introducing Raman spectroscopy to already established expertise in aquaculture. Fish fillets were the main point of interest, as we wanted to exploit the possibility to collect Raman spectra directly from the fish muscle tissue. During training at the Friedrich Schiller University Jena, Raman spectra from intact fish fillets of 12 different species were collected. All fish were raised in aquaculture, and purchased in the supermarket in Jena. Raman scattering was excited by a frequency-doubled Nd/YAG laser at a wavelength of 532 nm using a HR LabRam inverse system (Horiba Jobin Yvon). Samples were not pre-processed in any way, and spectra were collected by directly focusing the laser beam on the muscle tissue, immediately after thawing. Two types of Raman spectra were obtained: lipid spectra and protein spectra. Protein spectra were the most frequently obtained, while the percentage of lipid spectra varied according to the fish species. After a standard pre-processing of the protein spectra which included: removing spikes and fluorescent background, normalisation and wavenumber calibration, a dendrogram from the hierarchical cluster analysis in the spectral region from 500 to 1730  $\text{cm}^{-1}$  was calculated, as well as a PCA analysis of the data. Two out of three groups distinct in the dendrogram undoubtedly reflected the environments in which the fish were reared. The main characteristic of the third group was that it comprised fishes fed with a higher amount of carotenoids in feed. After such a distinct classification, the same methodology was applied with the idea to classify fillets of the common carp reared in pond aquaculture, fed with four different diets. A field study lasted for three months, while diets consisted of altered percentage of fish meal (24%, 16%, 8% and 0%) and vegetable-based ingredients. In this assay a different Raman system was used (Xplora Raman, Horiba JobinYvon), based on FA purchase, equipped with the equivalent Nd/YAG laser at a wavelength of 532 nm. No apparent differences between the groups were established using a PCA analysis. In conclusion, Raman spectroscopy is successfully introduced at the FA. Fillet analysis could be used for classification of fish living in different environments. However, further research is needed to be able to evaluate differences within the same fish species fed with similar diets.

*Keywords: fish meat, Raman spectroscopy, aquaculture, environment*

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## RAMAN MICROSCOPY IN DETERMINATION OF CAROTENOIDS IN TOMATO GENOTYPES DURING DEVELOPMENT

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The main characteristics of tomato fruit ripening process is a significant accumulation of carotenoids, especially lycopene and  $\beta$ -carotene. Lycopene, constituting 80-90% of total carotenoid content present in tomato, has been shown to act as a powerful antioxidant. The total antioxidant activity of tomato fruits varies significantly, depending on tomato genotypes, stages of maturation, and particularly on lycopene content.

The aim of the paper was to investigate the relative and absolute lycopene content in fruits of the wild type 'Ailsa Craig' and its ABA deficient mutant *flacca*, grown under optimal irrigation treatments. The lycopene content in final size fruits was measured as well as fruit antioxidative activity. Standard methods for evaluating lycopene and other carotenoids in tomatoes require complicated procedures for sample preparation, processing, and analysis. The absorbance was measured at 734 nm (SPECTRO UV-VIS RS, 1166, Lambomed, Inc. USA) for two minutes after the initial mixing - in the vortex, with PBS as a blank test. In addition, in this paper, we also applied Raman spectroscopy in order to establish fast and nondestructive methods for detection and observation of luteine, lycopene and  $\beta$ -carotene contents in two tomato genotypes. The Raman spectra were recorded in the range 900-2000  $\text{cm}^{-1}$  with a micro-Raman setup (HR LabRam inverse system, Jobin Yvon Horiba). Raman scattering was excited by a frequency-doubled Nd/YAG laser at a wavelength of 532 nm with a laser power incident on the sample of about 2 mW. For the analysis of tomato carotenoids, the following fruit stages were used: immature green stage (20 DPA, days post anthesis), mature green stage (39 DPA) and ripe fruit stage (52 DPA). The fruit cross sections on three spots (pericarp, gel tissue and collumela) were measured, and in total, a minimum of 30 spectra were made from each fruit developmental stage. Whole spectra were analyzed by Origin Pro 8.6 and peak analyzer tool for peak deconvolution using the Voigh function. For each Raman peak, we observed and compared: area and peak intensity, position and ratio between two main peaks in two tomato genotypes.

Carotenoids in tomato fruit exhibit two strong Raman peaks in two separate spectral regions, 1100-1200 and 1400-1600  $\text{cm}^{-1}$ , due to the stretching vibrations of the C-C and C=C bonds in the polyene chain, which compose the structure of carotenoids lycopene,  $\beta$ -carotene and  $\alpha$ -carotene. Based on the intensity of the spectra at the tomato immature green stage, we can detect lutein as well as at the mature green fruit stage on 1520 and 1523  $\text{cm}^{-1}$ . It was also noted that carotenoids were not detected in the outer pericarp, exocarp, before the first phase of ripening, while in at the mature green fruit stage peaks that indicate the presence of  $\beta$ -carotene and lycopene were observed. Our results showed that absolute concentration of lycopene was similar in *flacca* (125 mg/kg) and wild type, 75 mg/kg, but the antioxidative activity was 2.5 times lower in *flacca* (38.6  $\mu\text{mol TU/g}$ ) than in wild type, indicating a positive effect of ABA on the antioxidant potential of tomato plants.

**Keywords:** *Lycopersicon esculentum*, wild type, *flacca*, Raman spectroscopy

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## APPLICATION OF RAMAN MICROSCOPY FOR CONTROL OF ENCAPSULATION/IMMOBILIZATION PROCESSES

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Protection of valuable food compounds is considered to be an important aspect of modern food processes. During food production, ingredients are exposed to heat, moisture, oxygen or other conditions that may cause an irreversible chemical degradation of food constituents. Also, the post-production conditions such as inappropriate packaging, storage, manipulation and preparation can lead to further damage. Protection of active food compounds by encapsulation is one of the most promising solutions for prevention against negative influences of process conditions on food ingredients. Encapsulation can be defined as a formation of protective layer(s) around active compounds using adequate techniques and protective (carrier) material(s). Also, targeted compound(s) can be retained on the surface of carrier materials in the immobilized form in order to prevent an uncontrollable release and to ensure easier manipulation.

Encapsulates can be produced in different shapes and dimension, depending on application and process requirements. The majority of encapsulates are produced in the form of spherical or irregular shaped particles, but also fibers or agglomerated forms can be formed. The dimensions of encapsulates are in the range of several nanometers (nanoparticles) to over 1000 µm. During encapsulation and post-encapsulation processes such as drying, granulation or storage, an encapsulated compound can undergo damage or loss. Tracking of encapsulated compound stability and optimization of encapsulation processes is critical for the successful application of encapsulates.

We considered the application of Raman microscopy for the evaluation and control of various encapsulation/immobilization processes. Final products (i.e. encapsulates) in the form of beads, microparticles and films as well as carrier materials were tested in order to establish optimal process conditions for efficient encapsulation/immobilization. As active ingredients, we used flavour compounds, edible oils and polysaccharides encapsulated by several different processes. The most important advantage of Raman microscopy compared to other techniques such as scanning electron microscopy (SEM) or conventional light microscopy is the possibility to identify chemical compounds without any sample destruction, simultaneously with a morphological study. Even more, our results showed that the efficiency of encapsulation processes could be evaluated by a surface observation of encapsulates where the local unencapsulated (i.e. free) material is located. Both crystals as well as liquids were identified as free fractions on the surface of encapsulate which indicates poor encapsulation efficiency or carrier saturation with an active ingredient. Based on these findings, we were in the position to change process conditions in order to avoid low encapsulation efficiency and to preserve properties of active compounds.

*Keywords: Raman microscopy, encapsulation, food ingredients*

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***Acidovorax citrulli* - CAUSAL AGENT OF BACTERIAL FRUIT  
BLOTCH ON WATERMELON IN SERBIA**

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*Cucurbitaceae* spp. production contributes significantly to the Serbian agriculture. The data from 2012 shows that Serbia belongs to a group of mid-range producer countries in Europe (FAO statistics). In 2011, watermelon yield reached approximately 190,000 t. However, profitability of the production is often compromised by disease outbreaks, particularly once that they have not been recorded before. *Acidovorax citrulli*, the causal agent of bacterial fruit blotch, is known as an economically important pathogen of watermelon and related plant species worldwide. Under favorable environmental conditions for disease development, yield losses can reach 90% in commercial fields. Watermelon and melon are the most susceptible hosts, but symptoms can be produced in all cucurbits tested, especially at the seedling stage.

In Serbia, *A. citrulli* is a quarantine pathogen and its presence was not detected until summer of 2014. At that time, watermelon growers noticed symptoms on mature fruits, resembling bacterial fruit blotch (BFB), in the district of Srem. The first symptoms were water-soaked, irregularly shaped lesions with slightly raised margins. This was quickly followed by development of the fruit rind cracks and inner tissue softening. Watermelon samples were collected and subjected to the laboratory analysis. Predominantly isolated colonies were white, glistening, convex and circular with regular edges. Representative isolates were purified and ten strains were selected for identification. According to physiological, biochemical tests, PCR assay and 16s rRNA gene sequencing analysis, the causal organism was identified as *A. citrulli*. The bacterium is seed-born and can be easily introduced in countries where its presence was not determined. Trade of infected seed lots and seedlings is the most important pathway for spreading the disease all over the world. In recent years, it has caused considerable damage to the watermelon and melon industry in China and the United States. The following control measures are necessary to apply: using certified seed, inspection of transplants, destruction of all symptomatic plants, sanitation procedures, elimination of plant debris and crop rotation. Growing of resistant cultivars is important step in BFB management, but it is difficult to execute because of a high level of genetic diversity of *A. citrulli*. In addition, a timely and reliable diagnosis has a decisive role in crop protection.

**Keywords:** *Acidovorax citrulli*, bacterial fruit blotch, watermelon, identification

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## DNA EXTRACTION AND APPLICATION OF SSR MARKERS IN IDENTIFICATION OF GRAPEVINE VARIETIES COLLECTED FROM SERBIA AND UK

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Grapevine (*Vitis vinifera* L.) is one of the most valuable horticultural species. Currently, there are a large but imprecise number of grapevine varieties in the world. In many regions varieties have the synonyms (different names for the same variety) as well as the homonyms (different varieties identified under the same name). This number could likely be reduced once all varieties are properly genotyped and compared. In the past decade, molecular characterization has been used more and more, namely DNA technology in ampelography, helping to identify varieties and their origin. Microsatellite markers (SSR markers) are widely used in grapevine genetic research for identification of cultivars, parentage analysis, and genetic characterization of germplasm. The aim of this paper was the extraction of total DNA, primer selection and design, PCR protocols and analysis of DNA sequences with special attention to variability between collected samples of different grapevine cultivars. The material which was used in this study consisted of 34 samples of grapevine leaves of different autochthonous and introduced cultivars from grapevine collection on the Experimental field "Radmilovac" at the Faculty of Agriculture, University in Belgrade from Serbia, and 42 from the National fruit collection "Brogdale" from the United Kingdom (UK). Nine primers for grapevine were used: VrZAG79, VrZAG62, VVMD32, VVMD28, VVMD27, VVMD25, VVMD7, VVMD5 and VVS2. Analyses are performed in molecular-genetics Laboratory in School of Agriculture, Policy and Development, University of Reading, Reading, UK. Extraction and purification of total DNA from fresh and frozen plant material (grapevine leaves) was performed using a DNeasy® Plant Mini Kit following the standard protocol for isolation of DNA from plant leaf tissue outlined in the DNeasy Plant protocol handbook (Qiagen Inc.). The concentration of extracted DNA was measured by NanoDrop and in storage at -20°C until use. Following protocol Type-it Microsatellite PCR Kit which is optimized for use with fluorescent primers and subsequent high-resolution fragment analysis using capillary sequencing instruments also outlined in the Type-it Microsatellite PCR Handbook (Qiagen Inc.), a reaction mix was prepared according to reaction setup for the Type-it Microsatellite PCR Kit and cycling conditions. After the Polymerase Chain Reaction (PCR) which was performed using the standard sets of primers for grapevine, all of genotyping plates were analyzed in Nottingham, UK. Both collections are a source of genetic variability of grapevine. The DNA analyses should be combined with ampelographic descriptions in planning the selection of grapevine varieties with desirable viticultural and enological values.

*Keywords:* grapevine, variety, DNA extraction, SSR markers, genetic diversity

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## ROOT ARCHITECTURAL TRAITS UNDER ABIOTIC STRESS CONDITIONS

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Major problems affecting crop plants are abiotic stresses as: drought, salinity, soil acidity, floods or excess water, extreme temperatures, mineral toxicities and deficiencies, low or high pH, poor edaphic conditions. These adverse environmental factors prevent plants from realizing their full genetic potential. Plant breeding was mainly aimed at the shoot system, and the most important parameters in selection were yield and disease resistance. Although a root is an essential organ to acquire nutrients and water in variable soil environments, due to the inability of the root system, characterization with earlier methods was not in the focus of selection. Root system architecture (RSA) is a fundamental component of plant productivity. The studies of the root system architecture (RSA) and its modelling are in progress, so different approaches and methods have been developed as the result of the analysis of morphological and physiological root characteristics in different stressful conditions. Acquiring knowledge of root phenotyping systems and shoot: root ratio is important for obtaining high and stable yields. Environmental factors significantly affect the shoot: root ratio. Studies have shown a positive correlation with the concentration of nitrogen, as well as the impact of day length and light spectrum on the aforementioned relationship. Drought has an important role in functional balance between the mentioned ratio as well. An understanding of genotypic variation in the responses of root system architecture to the heterogeneous distribution of nutrients in the field will determine the potential for the exploitation of such traits to increase crop yields and maximize nutrient-use efficiency. The importance and strategies of developing plant phenotyping, their advantages and limitations as well as possibilities of practical application to plant breeding and production technology will be discussed.

*Keywords: root system architecture (RSA), plant phenotyping, abiotic stresses*

## POTENTIAL POLLEN-MEDIATED GENE FLOW BETWEEN HERBICIDE TOLERANT AND WEEDY SUNFLOWER

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Weedy sunflower herbicide susceptible population and two sunflower hybrids (RSU- tolerant to tribenuron-methyl and RIMI- tolerant to imazamox) were included in the investigation. Two experiments (E1 – gene flow from RSU to weedy sunflower; E2–gene flow from RIMI to weedy sunflower) were set at two (4km distant) locations (the central part of Serbia where there is no sunflower production) in 2011. In both experiments, sunflower hybrids (E1-RSU and E2-RIMI) were sown in a 5 m diameter circle. Surrounding tolerant hybrids of weedy sunflower were sown in the ring width of 5 m. After the maturity, achenes of weedy sunflower from 40 randomly selected plants per experiment were collected and were sown in the field near Belgrade at The Faculty of Agriculture Experimental Station “Radmilovac” in 2012. Two hundred plants from each experiment were sown into separate two blocks, one for plants from the experiment E1 and the other for plants from the experiment E2. Plants were treated with herbicides at the four-true-leaf growth stage using a knapsack sprayer and TeeJet 1004 flat-fan nozzles to deliver a spray volume of 300 L water per hectare. Plants from E1 were treated with the recommended rate (22.50 g a.i. ha<sup>-1</sup>) of tribenuron-methyl (Express 50-SX, 500 g a.i. kg<sup>-1</sup>, WG, Du Pont, Switzerland) and plants from E2 were treated with the recommended rate (48 g a.i. ha<sup>-1</sup>) of imazamox (Pulsar-40, 40 g a.i. L<sup>-1</sup>, SL, BASF, Germany). After maturity, seeds from the plants that survived were collected and stored at room temperature until used for molecular analysis. Seeds collected from the 2012 field experiment were sown in pots and tissues sampled from 12 randomly selected plants for molecular analyses. The extraction and purification of the total DNA were performed using a DNeasy ® Plant Mini Kit following the standard protocol for the isolation of DNA from plant leaf tissue outlined in the DNeasy Plant protocol handbook (Qiagen Inc.). Two primer pairs were designed using the software Primer 3. The primers Hel ForA (CAATGGAGATCCACCAAGCT) and Hel RevA (AACGCAAGCAACAAATCACT) were used for the amplification of approximately 700bp fragments. Purified products were sent together with the corresponding primer (Hel ForA) to Sorce Bioscience (Osford, UK) for sequencing. Analyses of obtained sequences were done based on comparison with sequences of the amplified region of ALS gene located in GenBank using a multiple sequence alignment program Clustal Omega. Expected mutations responsible for sunflower tolerance to tribenuron-methyl and imazamox were not confirmed.

*Keywords: gene flow, molecular analysis, tolerant sunflower hybrid, weedy sunflower*

*Acknowledgment:* This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project III 46008) and EU FP7 REGPOT-AREA Project No. 316004.

**DNA EXTRACTION AND USE OF PCR BASED METHODS IN  
*Calamintha* sp. RESEARCH**

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In the past 25 years, PCR based techniques became a tool of choice for researchers in systematics. Although numerous commercial kits for DNA extraction exist, crude extraction procedures can still be adopted to prepare a sufficient amount of DNA to allow for multiple end uses. One of the most popular protocols is CTAB DNA extraction protocol. In order to obtain high yield and purity of DNA from *Calamintha nepeta* and *Calamintha sylvatica*, the CTAB protocol was modified.  $\beta$ -mercaptoethanol and PVP were added to CTAB buffer. In species with a high concentration of polyphenols contamination of DNA isolates by polyphenols is expected, and the polyphenol adsorbent PVP has been proven to have a beneficial effect. To remove as many proteins and other carbohydrate contaminants as possible, Sevag treatment step was repeated. The mean DNA yield obtained with our Protocol was 316.74  $\mu\text{g}/\mu\text{L}$  tissue, and the absorbance ratio 260/280 averaged 1.815 revealing lack of contamination. PCR amplifications of two chloroplast loci (*rpl32-trnL* and *ndhF-rpl32*) indicated that DNA isolated using this protocol can be used in *Calamintha* sp. and other aromatic and medicinal plants containing essential oil for PCR based methods used in biodiversity research and conservation surveys in which nuclear or chloroplast genomes would be studied in large numbers of individuals.

*Keywords: CTAB, DNA yield, DNA contamination, MAP*

## ANGIOTENSIN-CONVERTING ENZYME INHIBITORY ACTIVITY OF SELECTED EDIBLE AND MEDICINAL MUSHROOMS EXTRACTS

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As the World Hypertension League reported, just in 2010, an estimated 162 million of life lost were connected to hypertension, thus making it the leading cause of deaths and disability. Moreover, reducing the increased blood pressure became an important target to achieve by the United Nations. Although medications for hypertension are developed and are commercially available, constant search for new natural sources is present due to many side effects expressed by contemporary drug therapy. As angiotensin I converting enzyme (ACE) has a crucial role in hypertension development, it is an important target for new potential active agents.

Mushrooms are already known as biologically active with numerous medicinal effects but hardly known as ACE inhibitors. Several mushroom species (*Polyporus squamosus*, *Ganoderma lucidum*, *Fomes fomentarius*, *Ganoderma applanatum* and *Fomitopsis pinicola*), in form of methanolic extracts (0.5-4 mg/ml), were tested in order to evaluate their ability to inhibit ACE activity.

All tested samples exhibited moderate inhibitory activity in the ACE assay, although not comparable with that of the standard used (captopril) that expressed almost complete inhibition even at the concentration of 10 µg/ml (99.4%). From all of the tested samples *Fomitopsis pinicola* expressed the strongest activity at a concentration of 4 mg/ml (100%). In case of other samples the same concentration effect was never less than 60%. *Ganoderma applanatum* appeared at the weakest ACE inhibitor (61.44%) when applied in concentration of 4 mg/ml.

The observed effect is to be expected due to the high polyphenolic compounds rates that are usually present in polypore mushrooms, especially in their methanol extracts, since methanol extraction favours polyphenolic compounds which are considered as ACE inhibitors.

The obtained results demonstrate that mushrooms could serve as promising sources of compounds that inhibit ACE, thus regulating blood pressure and health conditions connected with it. Moreover, side effects that are connected to the use of existing hypertension drugs are not present if the blood pressure is regulated through naturally derived compounds or food.

*Keywords: Angiotensin I converting enzyme, hypertension, mushrooms*

## **Lactococcus lactis** SSP. *lactis* BGBU1-4: INHIBITION OF *Listeria monocytogenes* ATCC19111 IN CHEESE MODEL SYSTEM

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Bacteriocins are ribosomally synthesized antimicrobial peptides that generally inhibit the growth of closely related microbes, with a narrow spectrum of activity. Some bacteriocins show an inhibitory effect against species or genera different from the bacteriocin producer, including food pathogens. Bacteriocins produced by lactic acid bacteria (LAB) are of special interest since they are found or used in fermented food and feed products. In the last two decades, the interest in the application of bacteriocinogenic LAB and their bacteriocins to food preservation has increased.

The goal of this study was to isolate and identify bacteriocin-producer isolate(s) of lactococci and to investigate the efficacy of bacteriocins against the foodborne pathogen *Listeria monocytogenes* in a cheese model system.

Fifteen randomly selected colonies, isolated using standard procedures for the isolation of lactic acid bacteria, were collected from semi-hard homemade cheese and subjected to bacteriocin testing. For detection of bacteriocin activity, an agar well diffusion assay was used. *Lactococcus lactis* ssp. *lactis* BGBMN1-596 and *Listeria monocytogenes* ATCC19111 were used as indicator strains. Based on tests of bacteriocin activity, for further work, one strain, designed BGBU1-4, was chosen. Identification of a bacteriocin-producer isolate was made by nucleotide sequencing using primers complementary to 16S rDNA. The PCR products were purified and sequenced. The BLAST algorithm was used to determine the most related DNA sequence relatives in the NCBI nucleotide sequence database.

The bacteriocin crude extract was purified from supernatant of the 16h old overnight culture of BGBU1-4, which was submitted to precipitation with ammonium sulfate to obtain 40% of saturation. *L. monocytogenes* ATCC19111 ( $10^5$  cfu/g) was inoculated in the cheese model system, in the presence of bacteriocin (10% v/v) and incubated at the following temperatures: at 16–18°C for 24h, then at 12°C for 7 days, and finally at 4°C for 28 days. Enumeration of *L. monocytogenes* was assessed by serial dilution and plating on Oxford agar after 0, 1, 7, 14, 21, 28 and 35 days. The negative control was *L. monocytogenes* inoculated without bacteriocin.

According to the results of bacteriocin test, isolate BGBU1-4 showed the highest zone of inhibition on indicator strains BGBMN1-596 and *L. monocytogenes* ATCC19111. It was taxonomically classified as *Lactococcus lactis* subsp. *lactis* by sequencing of the gene for 16S rRNA and chosen for further study.

The initial number of *L. monocytogenes* of  $10^5$  cfu/g remained unchanged up to 35 days of incubation of the cheese model system with addition of the bacteriocin from BGBU1-4, while in the negative control, the counts of *L. monocytogenes* were as high as  $10^8$  cfu/g.

The bacteriocin crude extract from strain BGBU1-4 has bacteriostatic activity against *L. monocytogenes* ATCC19111 in the cheese model system that makes it a good candidate for practical application in the food industry to control listeria contamination.

**Keywords:** bacteriocin, *Listeria monocytogenes* ATCC19111, PCR, cheese model system

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## BIOACTIVE PEPTIDES IN FOOD OF ANIMAL ORIGIN

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Bioactive peptides represent short amino acid sequences (usually 2-20 amino acids in length) that exhibit medicinal effect. The type of effect that will be exhibited greatly depends on the type and position of amino acid present in peptide. The first amino acid and amino acid next to opposite terminus will have the biggest effect on activity of peptide. Stability of bioactive peptide depends on numbers of factors among which are the most prominent: number and type of amino acid residues, size and overall charge of peptide, concentration of bioactive peptides, pH, metabolic state and age of studied organism and nutrition. However, it should be pointed out that higher stability of bioactive peptides can lead to toxicity, due to their slower degradation. Although it has been shown that hydrolysates exhibit higher activity when compared to the whole protein, activity of bioactive peptides during processing can be affected by temperature, pressure, changes in pH, extent of hydrolysis and/or duration and type of microorganism used for fermentation. Heat treatment results in denaturation of proteins and subsequent aggregation of peptides due to formation of covalent and/or non-covalent bonds between proteins. This intra- and intermolecular bonding can lead to lower digestibility and absorption of produced bioactive peptides. Since application of high pressure in most cases increases digestibility of peptides, with allergenic proteins (such as casein from cow's milk), this type of treatment might be desired. Although bioactive peptides are often classified on the basis of medicinal effect as antioxidative, immunomodulatory, antihypertensive, antimicrobial, hypolipidemic, opioid, osteoprotective and antithrombotic, it is rare that the bioactive peptide exhibits just one of these activities. This fact might be explained by intersecting metabolic pathways and findings that bioactive peptides might use several mechanisms to achieve their effect. The structure-function relationship in bioactive peptides is especially evident in case of antihypertensive peptides where a high percentage of aromatic amino acids are necessary for formation of complex with the angiotensin-converting enzyme.

*Keywords: bioactive peptides, food processing, medicinal effect, peptide stability*

*Acknowledgment: This study was supported by the EU Commission (FP7 project AREA).*

## THE INFLUENCE OF GOAT MILK HEAT TREATMENT AND CALCIUM CHLORIDE ADDITION ON THE RHEOLOGY OF RENNET COAGULATION

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Due to its valuable and unique composition and properties, goat milk popularity is on the rise for the last decade. In Serbia, goat milk production is increasing and many small dairy plants were established in the last few years. However, knowledge about goat milk is often limited due to its minor quantity compared to cow milk. Production of goat dairy products is usually conducted on the knowledge obtained from cow milk research.

The aim of this study was to investigate the influence of different goat milk heat treatments (65°C/ 30 min; 80°C/ 5 min and 90°C/ 5 min), with or without the addition of calcium chloride on the rheology of rennet coagulation.

Coagulation properties were evaluated using rheometer with vane geometry tool. Coagulation time (CT) was estimated during gel formation for 60 min at 1 Hz and 1% strain. Aggregation rate (AR) was characterized by the slope of the tangent at max increase of storage modulus  $G'$ . Evaluation of curd firmness (CF) was done by subsequent frequency spectra of the resulting gels.

Heat treatment showed a significant effect on all three coagulation parameters. A significant difference was observed between CT of milk samples treated at 80°C/ 5 min and 90°C/ 5 min, without calcium chloride. There was no significant difference in CF and AR observed between samples treated at 80°C/ 5 min and 90°C/ 5 min regardless of calcium chloride addition. Addition of calcium chloride had the influence only on coagulation time (CT) with a significant difference observed between samples treated at 80°C/ 5 min.

This study showed that goat milk behavior was very different compared to cow's milk, especially after higher heat treatments. Obviously, goat cheese production requires some modifications with regard to cow cheese production. According to the results of this study, the addition of calcium chloride after goat milk heat treatment might be unnecessary.

*Keywords: goat milk, heat treatment, calcium chloride, rheology, coagulation properties*

## CHARACTERIZATION OF YEASTS USING RAMAN SPECTROSCOPY

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Raman spectroscopy (RS), vibrational spectroscopic technique, has been used extensively to identify samples of different microorganisms by a careful research of the vibrating modes of the molecules in the microorganisms. Raman spectroscopy has recently gained popularity as an attractive approach for the biochemical characterization, rapid identification, and an accurate classification of a wide range of prokaryote and eukaryote organisms.

The Raman spectra of the microorganisms are the superposition of spectra of the biochemical components inside the cells like e.g. protein, DNA, RNA, lipids, carbohydrates, water, as well as a few components with minor concentrations. Whole cells represent a complex mixture of compounds, and the fingerprints (FPs) of all the molecules are superimposed in a Raman spectrum. For the Raman spectroscopic characterization of eukaryotes like yeasts or fungi, different approaches are required. The main advantage of the use of Raman spectroscopy for yeast identification is the low cost.

Soil was used for the isolation of the representative yeasts. Pure cultures were identified and characterized by the API AUX and API ZYM gallery (bioMérieux, France). Isolates were members of *Candida* sp., *Kloeckera* sp., *Rhodotorula* sp.

Also, for the classification and characterization of yeasts, the Bioparticle explorer, at a wavelength of 532 nm, has been used. The yeast cells were incubated at 28°C in a nutrient-rich YPD medium. Aliquots of the cell suspension were centrifuged (3000 rpm, 2min), washed three times with sterile water, and finally suspended in the new aliquot of water. The obtained spectra were analyzed using the R program.

Different chemical components can be identified in the obtained Raman spectra: the signal at 1746 cm<sup>-1</sup> can be assigned to the C=O double bond of esters, e.g., fatty acid esters. The amide I vibration exhibits a band at 1665 cm<sup>-1</sup>, which can be seen as a shoulder of the 1655 cm<sup>-1</sup> band from a C=C stretching vibration of a *cis*-isomer. Aromatic amino acids and nucleic acid bases (G, A) give rise to Raman signals at 1599 and 1582 cm<sup>-1</sup>, respectively. The broad band at 1440 cm<sup>-1</sup> is probably due to CH<sub>2</sub>, CH<sub>3</sub> deformation vibrations.

Raman spectroscopy can be used in the future experiments as a tool to analyze the response of yeasts when they are cultivated under the different nutrition conditions, or when being exposed to various stress factors (a high concentration of heavy metals, sodium chloride, etc.).

**Keywords:** yeasts, Raman spectroscopy, Raman spectra

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## **RAMAN SPECTROSCOPY AS INNOVATIVE TECHNIQUE FOR DISCRIMINATION OF MILK FROM DIFFERENT SPECIES**

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In this study, we evaluated a rapid Raman spectroscopic method for discrimination of milk from different species. Cow, ewe, goat and jenny milks were examined. Besides these samples, we investigated 25%, 50% and 75% mixtures of cow milk and milk of the other three species. The overall objective was to calibrate a successful multivariate model which could be employed on Raman spectra in order to detect potential fraudulent activities. Principal component analysis (PCA) allowed us to gain insight into highly complex data sets. Afterwards, support vector machines (SVM) classification algorithm was used in order to discriminate different milk samples. High model performance (overall accuracy = 97.5 %) was achieved when milk samples from different species were compared. In like manner, SVM models for cow-ewe, and cow-goat milk mixtures performed very well (90% overall accuracy in both cases). Only for the mixtures of cow and jenny was milk performance of the model somewhat lower (68%).

In conclusion, the results of our work point out that Raman spectroscopy coupled with chemometrics analysis could be potentially used for discrimination of milk samples coming from different species. In addition, effective classification of mixtures of cow milk with milk from other species indicates that this technique may be a possible solution for a rapid, cost-effective analysis of adulterated milk samples.

*Keywords: Raman spectroscopy; cow, ewe, goat, jenny*

## MOLECULAR CHARACTERIZATION OF SOIL *Pseudomonas aeruginosa* POPULATIONS FROM THE BALKANS AND THEIR PUTATIVE ROLE AS BIOCONTROL AGENTS IN AGRICULTURE

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*Pseudomonas aeruginosa* is an aerobic, rod-shaped, Gram-negative bacterium, with ubiquitous environmental distribution worldwide. It is characterized by an outstanding ability to adapt to a wide variety of niches, including soil, aquatic environments, sewage, human tissues, etc. The bacterium has been isolated both from uncultivated and agricultural soils, as well as plants, where its ability to colonize plant tissue is dependent on factors such as temperature and moisture. *P. aeruginosa*'s association with plant hosts has been shown to result in plant growth promotion (PGP), as well as suppression of disease in plants caused by fungi and nematodes (Anjaiah et al., 2003). It has been proposed that this is achieved via successful colonization of plant tissue, induction of plant resistance and the production of antimicrobial (antifungal) compounds (for instance, phenazines) (Haas and Keel, 2003). Given the above, it is not surprising that *P. aeruginosa* is an attractive candidate for biocontrol of soilborne plant pathogens, which represent a notable concern in agriculture. However, *P. aeruginosa* is a well-known opportunistic pathogen, primarily infecting individuals with weakened immune systems, which can occasionally even result in fatal outcomes. This clearly raises concerns regarding its safety as a potential biocontrol agent and the consequent impact it might have on human health. Therefore, pathogenic potential of individual *P. aeruginosa* isolates needs to be closely examined prior to considering their use as plant pathogen suppression agents.

At the very core of addressing this issue lies the molecular characterization of *P. aeruginosa* populations of interest. We have isolated *P. aeruginosa* from soil from the Balkans region using culture-dependent techniques and have employed traditional microbiological and biochemical approaches to species identification. We have, then, performed molecular identification, using internal fragments of five *P. aeruginosa* housekeeping genes (*acsA*, *guaA*, *mutL*, *ppsA* and *trpE*). These loci were selected as part of the modified Multilocus Sequence Typing (MLST) scheme and were further utilized for phylogenetic analyses. In addition to *P. aeruginosa* population distribution, structure and diversity, MLST and phylogenetic data provide indirect insight into isolate's pathogenicity, although future experiments will need to directly address this issue. Further strategies to examine biocontrol potential will also need to be evaluated.

**Keywords:** *Pseudomonas aeruginosa*, MLST, phylogenetics

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## USE OF COLLABORATIVE RESEARCH BETWEEN SME AND UNIVERSITY-RESEARCH INSTITUTES TO BUILD NEW INFANT FORMULA PRODUCTS

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Breast milk is the best food for infants during the first 6 months of life, because of the valuable nutrition composition and presence of biologically active ingredients and therefore is considered the gold standard in feeding a baby. Understanding the role of human milk on infant health is fundamental in realising opportunities in the design and production of next generation infant formula. Scientific institutions have significant research expertise in examination of nutrition effect of food, designing formulas and technologies which can be accessed by food industry including production of infant formulas.

Institute of Chemistry, Technology and Metallurgy (IChTM) has a long history of cooperation with Impamil Ltd, only producer of milk formula and milk baby food, not only in Serbia, but throughout the Balkans. During this cooperation a lot of products have been developed for feeding infants, which are already available on the market, as well as several products still under development. Joint cooperation of IChTM and Impamil is devoted to development of new products in the diet of infants and small children, and has been guided by the principles of physiological and biochemical specificities of infant metabolism. In order to provide the necessary macronutrients and micronutrients, we are researching a new approaches for formulas for healthy infants from the birth to 12 months of age, as well as for young children aged up to 3 years ("toddler formulas"). As well, formulas for specific purposes (such as prematurely born children or children born with low birth weight, children with lactose intolerance or feeding children with acute diarrhea caused by viruses, anti regurgitation formula and fortifier for mother milk) are designed in respect to the principles of healthy children and the characteristics of the target group.

IChTM principles for designing food formulations for infants and young children are deeply based on the latest scientific knowledge, decades of experience in the application of research results in this field, and following of international standards. In cooperation with Impamil Ltd, the company which is strongly devoted to production of high quality products, we are developing the products with undeniable nutrition advantages which make them dietetic products of top quality. Research, development and implementation of new technologies in production of new formulations lead to successful placing of new dietetic products -from baby food category on market. Moreover, the special formulations for specific (medical) purposes (i.e. preterm infants) are developed in cooperation with the Institute of Neonatology in Belgrade, an institution dedicated to health care of newborns and infants, most of them born prematurely. This trilateral cooperation is merging scientific and medical knowledge for continuous improvement of existing products according to the results of modern science of biochemistry, food and nutrition and pediatrics and the demands of the international advanced regulations and legislation. The results of our joint research and development are product line 'MIL', brand of 'Impamil®' company which has success in the domestic market, but first and above all, these products are successful in healthy growth and development of infants and young children!

*Keywords: infant formula, trilateral cooperation, special formulations for preterm infants*

## SUPPORT OF TRADITIONAL FOOD SECTOR VIA TRAF00N PROJECT

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The main objective of TRAF00N Project is to connect European Research Sector and producers of traditional food based on grain, fish, vegetables, mushrooms, sweet fruits and olives by improving transfer of knowledge for innovation. The target groups of this project are the major stakeholders of the traditional food sector, associations of SMEs at the national and EU levels, researchers, knowledge transfer organisations etc.

During the project different training and stakeholder and entrepreneurial workshops have been organised throughout Europe. In addition, web-based information shop has been created in order to provide all the necessary information related to innovations in the sector of traditional food. The key point of this project is the creation of Strategic Research and Innovation Agenda (SRIA) for Traditional Food Sector according to the outputs obtained from all stakeholders.

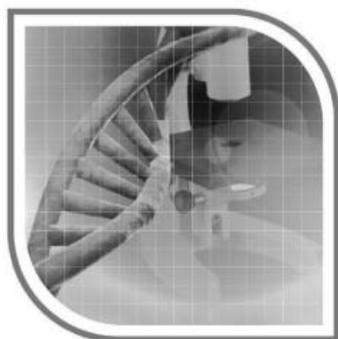
Serbia has been involved in the project in the creation of a sub-network for the "Traditional Products of Fruits" with raspberry and plum recognized as the most important traditional fruits. Throughout the second and third year of the Project, the University of Belgrade, Faculty of Agriculture organized four training workshops devoted to the innovation and trend in the production and processing of raspberry and plum. Training workshops brought together representatives from the key institutions from the Serbian traditional food sector representing SMEs, individual producers, Universities, Clusters, Associations etc.

During these workshops, the valuable information obtained from the participants was generated. Thereafter, the major areas of interventions are classified in four categories, as follows: primary productions of raspberries and plums, processing technology, products and organization of business performance.

Likewise, permanent education of producers, harmonization of legislation with the EU, better transfer of knowledge from any knowledge donor to an SME, better communication with policy makers, and creation of Innovation Strategy for this sector were highlighted by the majority of the participants. Outputs and conclusions obtained during the project will be used for the creation and development of Strategic Research and Innovation Agenda at the national and EU levels, which will be of great interest for fostering innovation in traditional sweet fruits sector in Serbia.

*Keywords: traditional fruits, SRIA, innovation*

*Acknowledgement:* This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 613912.



## AREA

“ADVANCING RESEARCH IN AGRICULTURAL AND  
FOOD SCIENCES AT FACULTY OF AGRICULTURE,  
UNIVERSITY OF BELGRADE”

**Poster presentations**



**WHICH ENVIRONMENTAL FACTORS DETERMINE DEVELOPING  
OF COMMUNITIES OF THE STEPPE GRASS SPECIES  
*Chrysopogon gryllus*?**

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The steppe species *Chrysopogon gryllus* inhabits habitats of different geological bedrocks and soil types, within dry, semi-dry and even mesophilous grasslands. Ecological factors that are crucial for the occurrence and distribution of plant communities with *Chrysopogon gryllus* were analysed.

Relevés were collected from the Database Grassland Vegetation of Serbia (EU-RS-002) and 879 relevés with the presence of *Chrysopogon gryllus* were analyzed. The cluster analysis was made in the program PC-Ord 5. Climatic variables were exported from WorldClim database and categorical data for soil and bedrock type were used. Habitat conditions were described by Pignatti indicator values. The responses of the species were modelled along the coenoclines using the HOF modelling approach.

Cluster analysis indicates the separation of the relevés corresponding to the Balkan alliance *Chrysopogono-Danthonion alpinae* from the large group of relevés of other alliances. This heterogeneous group of relevés was further divided into clusters of steppic grasslands of the *Astragalo-Potentilletalia*, alliances *Festucion valesiaca* and *Festucion rupicola* and Balkan steppes developed on serpentine (*Halacsyetalia sendtneri*).

For indicator values such as moisture, nutrients and temperature, the *Chrysopogon gryllus* exhibits unimodal simetric (model IV) or skewed response (model V). HOF curves for indicator values of continentality and soil reaction (model II) showed a gradually increasing response of the species towards its maximal potential cover, whereas for light response an asymptotic pattern below the potential maximum cover was obtained. Among analysed environmental variables, the most relevant was altitude, followed by climatic variables (precipitation and temperature). Bedrock types were significant in explaining occurrence of *Chrysopogon gryllus* too, especially sand, loess, limestone and serpentine. DCA analysis performed on data set of relevés with the highest cover enabled determination of ecological optimums for tested variables. Results could provide anticipation of responses of this C<sub>4</sub> grass to climate change due to its high ecological plasticity.

*Keywords: ecological analysis, HOF modelling, dry grasslands*

## **THE POSSIBLE ROLE OF TRADITIONAL MAIZE VARIETY TOWARDS TECHNOLOGICAL AND NUTRITIONAL MAIZE GRAIN QUALITY INCREASE**

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The lower yield and weaker response to intensive agricultural technology of Open Pollinated Varieties (OPVs) was the main reason for their replacement by hybrid varieties in commercial maize production. For a long period, maize breeding was focused towards increased and stable yield. Consequently, plant breeding practices, the registration procedures and marketing of new varieties, led to the widespread use of a small number of, often related, the best ranked hybrids in commercial maize production. The final result of this production, maize grain, is usually very uniform in its characteristics and it is not able to respond to specific market requirements. A multi-year study of physicochemical properties of grain maize hybrids in Maize Research Institute "Zemun Polje" has shown that the grain quality varies depending on the variety, growing conditions and seasons. Studies that included local landraces indicated that the evaluated traits in them exhibited a higher range of variations. This certainly makes it interesting, not only in terms of breeding, but also in terms of direct application for specific purposes in human nutrition and industry. By comparing the parameters of grain quality in five white kernel ZP hybrids, with two white and one yellow local landraces, it was observed that landraces have increased protein content and reduced starch content. The protein content in hybrids ranged from 8.39% to 9.22%, and in OPV from 11.25% to 11.77%, respectively. The starch content in hybrids ranged from 66.8 to 71.7%, and in OPV from 64.5 to 66.5% respectively. Therefore, old varieties cannot be only a good source for improving of breeding materials, but can, to a large extent, play a significant role in production of high quality food for human nutrition. Moreover, their grain could be used as a raw material for industrial processing. Both, increased public interest for organic farming and high stability under low-input conditions, emphasize the importance of OPV maize use in this system of agricultural production. Despite the availability of numerous high-yielding hybrids, a distinctive taste of OP varieties stimulates our farmers to maintain them throughout the centuries. Thereby, improvement of selected local landraces through breeding and detailed biochemical characterization could lighten the factors that contribute to more distinctive and improved taste.

*Keywords: genetic diversity, maize, landraces, nutritive value, organic farming*

## SELECTION OF POTENTIAL PLANT GROWTH PROMOTING RHIZOBACTERIA ISOLATED FROM MAIZE RHIZOSPHERE

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In Vojvodina, a northern part of the Republic of Serbia, maize is the dominant field crop, grown on average in the area of 640,000 ha, or about 42% of the total arable land. Application of mineral fertilizers is necessary to obtain higher yields. However, consequences of their indiscriminate use are a significant problem to the environment, leading to resource degradation and negatively affecting several soil ecological functions. Use of biofertilizers and organic amendments could be a reasonable approach to reducing the negative impacts of chemical fertilizers and pesticides without compromising food security. An environmentally friendly method for growth promotion and disease control by biofertilizers containing PGPR (Plant Growth Promoting Rhizobacteria) is a result of complex interrelated processes that include synthesis of some phytohormones (auxins, cytokinins and gibberellins), phosphate solubilization, nitrogen fixation, production of siderophores, antibiotics, hydrogen cyanide, and various lytic enzymes. Therefore, the aim of this study was to examine the effect of selected PGPR isolates from maize rhizosphere on microbial activity in rhizosphere, early growth and N content of maize in greenhouse conditions. An experiment was set up in Mitscherlich pots, in a randomized block design with four replications, two maize hybrids and ten inoculation treatments. Maize seeds used in this study were obtained from the hybrids NS 6010 and NS 6030 (Institute of Field and Vegetable Crops, Novi Sad), with a medium late vegetative cycle (FAO 600). Inoculation was performed with liquid culture ( $\approx 2.5 \times 10^{10}$  CFU ml<sup>-1</sup>) of eight isolates selected on the basis of multiple PGP traits. Tests were conducted on three isolates of *Azotobacter* sp. (A5, A8, A13), two isolates of *Bacillus* sp. (B9, B16) and *Pseudomonas* sp. (P1, P5), one isolate of *Streptomyces* sp. (S6), as well as their mixture, while non-inoculated seeds were designed as control. PGPR treatments had a positive effect on the microbial activity in the rhizosphere, maize growth, and N content in maize plants. Inoculation increased the total number of microorganisms (6–51%), number of azotobacters (4–11%), N-fixing bacteria (11–93%), P-mobilizing bacteria (34–104%), actinomycetes (29–114%), and dehydrogenase activity (2–132%) in the rhizosphere, as well as height (5–15%), dry weight (9–41%), and N content (2–6%) of maize plants. Co-inoculation had an advantage compared to single inoculation, while in case of single strains, better effects were achieved in *Azotobacter* and *Streptomyces* treatments, which were estimated as better producers of IAA and had higher antifungal activity in comparison with isolates of *Bacillus* and *Pseudomonas*. The effect of inoculation was higher in the hybrid NS 6010. This study confirmed that most isolates from maize rhizosphere possess PGPR characteristics and therefore should be tested on soil-plant system, both in greenhouse and field conditions, with the purpose of selection of strains effective for certain environmental conditions and different hybrids.

**Keywords:** *Azotobacter*, *Bacillus*, *Pseudomonas*, *Streptomyces*, maize

## PRESENCE OF SEEDS OF ALLERGENIC WEED SPECIES IN SOIL OF THE URBAN ENVIRONMENTS

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Soil contains the permanent seed bank of different weed species, and 'seed bank' includes only seeds viable for shooting in corresponding circumstances, in soil or on its surface. The seed bank presents a constant source of weeds, enabling their persistence in the area over a longer time period. During 2015, the weed seed bank composition was studied at ruderal sites on the territory of the city of Novi Sad. Along Bulevar Oslobođenja and Subotički Bulevar, five soil samples were taken both from raised divisional islands along Bulevar Oslobođenja and Subotički Bulevar, where soil was loaded during the horticulture arrangement and at five separate locations in Šangaj, where the regular checks of this weed species is carried out, as well as mechanical control measures. A significant quantity of ragweed was separated, and the highest number of weeds was found in soil layer of 0–10 and 10–20cm, with the highest number of 1.856 seeds per m<sup>2</sup> in the layer of 0–10cm. The most numerous, out of all separated seeds, were seeds of weed species *Amaranthus retroflexus* L. During the course of time, in uncultivated soil, annual broad-leaved weeds become dominant with the significant production of tiny seeds, as in the cases of *Amaranthus retroflexus* L. (2,425 seeds/m<sup>2</sup>) and *Stellaria media* (L.) Scop. (2,326 seeds/m<sup>2</sup>), but also annual weeds, as well as in the case of *Plantago lanceolata* L. During processes of the intensive building of objects, a huge amount of soil was removed and transported to other locations, without previous quality control and control of infestation by microorganisms and weed seeds. This soil contains the significant seed bank of weed species, among which are also seeds of invasive, quarantine, dangerous to human health, allergenic weed species such as *Ambrosia artemisiifolia* L., *Artemisia vulgaris* L., and *Daturastramonium* L. The necessary measures for prevention of the spread of allergic and in other ways noxious weeds in cities, should include the control of soil that is delivered from arable land during the construction of facilities and other infrastructure works, namely the testing of the weed seed bank and the implementation of necessary soil sterilization prior to its application, i.e. application to new locations.

*Keywords: weed bank, allergenic weeds, urban environments*

**VEGETATION AND PROVISION OF ECOSYSTEM SERVICES:  
CURRENT HOT TOPICS AND FUTURE PERSPECTIVES  
IN URBAN HORTICULTURE RESEARCH**

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Strategically placed well-chosen vegetation can provide a host of services to the urban environment. These include localised temperature reduction during hot weather, building cooling and insulation, reduction of rainfall runoff and a decrease of localised flooding, removal of air particulate pollutants, to name just a few.

However, the optimal delivery of these services is linked to making the right plant choices. Differences in plant species' structure and function, as well as their management can lead to dramatically different benefits (and costs!) of urban vegetation. Our data show that, for example, a choice of plant species on a green roof (e.g. herbaceous perennial *Salvia* vs a succulent *Sempervivum*) can lead to a 10-fold reduction of a heat flux into a building and a 4-fold reduction in longwave (thermal) radiation into the surrounding urban fabric. Similarly, a choice of roadside urban planting can influence the volume of water runoff; our ongoing experiments show that the use of *Cotoneaster* vs *Photinia* would result in a 30% increase in soil's capacity to receive subsequent rainfall, thus providing an enhanced support for the urban drainage systems.

In this talk, we provide examples from our recently published research of the sets of plant traits that correlate with the greatest numbers of services and point to the future direction of research.

*Keywords: ecosystem services, green infrastructure, plant choice, urban*

## THE INFLUENCE OF AGRICULTURE ON PESTICIDE CONTENT IN THE DANUBE IN URBAN AREAS

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The use of pesticides in agricultural systems is possibly the single most important factor which has contributed to the massive worldwide increase in food production. The pesticide chemical properties such as low water and high fat solubility, stability to photo-oxidation and low vapour pressure are the main elements in their persistence in the environment (soil and water). Pesticides have strongly tended to move off-site depending on chemical properties and persistence of the pesticide, the technique and rate of application, the frequency and timing of rains and irrigation. Incoming pesticides from agricultural areas to urban areas can bring people into a direct contact with toxic chemicals. Therefore, the investigation comprised analyses of the water samples collected from the Danube which flows through the city of Novi Sad, Serbia – as an urban area (45°15'59.0"N 19°51'51.0"E and 45°26'63.85"N 19°86'41.61"E). The pesticide extraction was performed with Waters OASIS HLB cartridges. An extract volume of 10 µL was injected into the liquid chromatography tandem mass spectrometry (LC/MS-MS) system for separation and detection. The carbofuran-D3, atrazine-D5 and isoproturon-D6 were used as internal standards. The obtained results were compared with the MACs according to the Directive 2008/105/EC and Commission Implementing Decision 495/2015/EC. Of a total of 20 detected pesticides, there are 7 herbicides (atrazine, desethyl-atrazine, terbuthylazine-desethyl, metolachlor, terbuthylazine, nicosulfuron and linuron), 8 fungicides (carbendazim, metalaxyl-M, azoxystrobin, cyprodinil, flusilazole, tebuconazole, difenoconazole and pyrimethanil) and 5 insecticides (thiametoxam, imidacloprid, acetamiprid, propoxur and pirimifos-methyl). All the detections were under the MACs.

*Keywords:* pesticide residues, Danube, LC-MS/MS

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## PESTICIDE RESIDUE TESTING OF ORGANIC VEGETABLES

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In the Republic of Serbia, there is a constant boom of organic farming. At the end of 2013, the organic farming covered the area of 7,455 ha, of which the area of 238 ha was involved in vegetable growing. The demand for organic products has significantly increased in recent years due to the rising awareness of health food and the protection of consumers and the environment. That is why the analyses of 63 organically produced vegetables were done in 2014–2015. The validated method based on the QuEChERS sample preparation for more than 200 compounds with determination by GC-MSD and LC-MS/MS were used for the analyses. As a maximum residue level of pesticides we used the orientation value for each pesticide which is 0.01 mg/kg and it applies to the original unprocessed product (EC 178/2002). Also it is very important to know that in organic products no more than a total of two pesticides may be present. No pesticides were found in 63.29% of analysed samples, while 17.27% were with the pesticide detections below the orientation value. Apart from analytical issues, the relationship between the detected and actual usage of pesticides is not that straightforward. For example, long banned organochlorine pesticides were detected in 19.44% of vegetables. The presence of organochlorine pesticides is most probably related to the soil itself considering their persistence. Piretroides were detected in 9.18%. The use of pesticides in organic production is forbidden and the detected residues, in all probability, come from soil, irrigation or drift.

*Keywords: organic products, pesticide residues, LC-MS/MS*

## ANTIBIOTIC RESISTANCE OF *Enterococcus* SPECIES ISOLATED FROM FRESH CHEESES

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The aim of this work was to investigate the prevalence of enterococci and their antimicrobial resistance in samples of fresh cheeses hand made in Serbia. The samples for enumeration of enterococci count were cultured on selective diagnostic Slanetz-Bartley agar. Based on native microscopically preparation (conformation, motility, cleanness of cultures), negative catalase and positive PYR test (PYRAtest, Lachema, Czech Republic), the species of *Enterococcus* spp. were then determined by means of En-coccus test (Lachema, Czech Republic) and one step PCR. Susceptibilities to antimicrobial agents were tested using the disk diffusion method according to the CLSI requirements, using the following antimicrobial disk: vancomycin 30 µg/disk, gentamicin 120 µg/disk, erythromycin 15 µg/disk, ampicillin 10 µg/disk, tetracycline 30 µg/disk, teicoplanin 30 µg/disk. The counts of enterococci were in range of 5.5 to 7.0 log CFU.g<sup>-1</sup>. Of 75 isolates of enterococci, predominant species were *Enterococcus faecalis* (68.6%) and *Enterococcus faecium* (19.8%), followed by *Enterococcus durans* (8.57%) and *Enterococcus casseliflavus* (3.03%). Species *Enterococcus faecalis* and *Enterococcus faecium* identified by En-coccus test were confirmed by one step PCR on 86% and 100% respectively. It was found that 11.4% of isolates were resistant to vancomycin, 14.3% to ampicillin, 9% to tetracycline, 3% to erythromycin and 3% to gentamicin. Higher prevalence of intermediate resistance to erythromycin (74.3%) was found. Isolates of *Enterococcus faecalis*, as a prevalent identified species, were most resistant to ampicillin (14.28%) and to vancomycin (14.28%). Our study suggests that fresh hand-made cheeses play a potential role as a reservoir of enterococci resistant to antibiotics.

**Keywords:** fresh cheeses, enterococci, antibiotic resistance

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## DRYING INFLUENCE ON ACTUAL LOAD OF CHOKEBERRY POLYPHENOLS IN MICROENCAPSULATED ALGINATE OR ALGINATE/INULIN PARTICLES

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Chokeberry represents one of the richest plant sources of phenolics, especially anthocyanins exhibiting strong antioxidant activity. A problem with polyphenols instability can be solved using the microencapsulation technology. The tendency of encapsulation is to improve the stability of extracted compounds during storage and processing. Encapsulation of chokeberry extract within calcium alginate gel with or without inulin as the matrix was employed, and drying influence on obtained particles. Dried forms of microbeads are preferable for utilization in foods and pharmaceuticals, due to their greater stability and mechanical stiffness when compared to hydrogel ones.

An electrostatic extrusion technique was applied for encapsulation of chokeberry extract (2.46 mg GAE/ml total phenolics) in alginate gel beads. Chokeberry extract was prepared from dried chokeberry using 50% ethanol maceration in shaker (170 rpm). Ethanol was evaporated under vacuum and sodium alginate powder was dissolved in the extract. Different types of 1.5% alginate, low and medium viscosity, and combination of alginate/inulin were used to obtain various microbeads. Alginate/extract solution was extruded through a needle (20 gauges).

Encapsulation efficiency was presented as total polyphenolics content encapsulated in beads (TP<sub>e</sub>). Quantification of polyphenolic compounds was performed using Folin-Ciocalteu method, after dissolving beads in a sodium citrate solution (2 g mL<sup>-1</sup>). The beads (0.5g) were homogenised with 3.5 mL sodium citrate for 15 min using a vortex mixer. In order to produce freeze dried beads, hydrogel beads were frozen at -80°C for 1 h before freeze drying, which was carried out at -60°C (0.011 pressure) for 24h and at -75 °C(0.0012 mbar pressure) for an additional hour, in order to remove the capillary water residues (Beta 1-8 Freeze Dryer, Germany). To produce heat dried beads, hydrogel beads were dried at 50°C for 3 h using a classic laboratory drying oven (Instrumentaria, Zagreb).

The difference in the amount of encapsulated polyphenol between different particles prepared with various carriers, with or without inulin was noticed as well as the influence of drying process on the content of polyphenols in the beads. In the case of hydrogel particles, greater polyphenols amount is present in particles made with medium (0.23 mg GAE/g beads) than with low viscosity alginate (0.19 mg GAE/g beads) but it is higher with inulin (0.25 mg GAE/g beads) than without it (0.22 mg GAE/g beads). This trend is also present in the oven and freeze dried particles. The oven dried particles had about 8 times higher amount of polyphenols (1.76–1.98 mg GAE/g beads) compared with the hydrogel particles (0.23–0.25 mg GAE/g beads) of the same type while freeze dried particles had the amount 14–15 times higher (3.24–3.57 mg GAE/g beads). These results show that after drying, alginate or alginate/inulin beads became more concentrated with more total phenolics content. The largest amount of polyphenols was reported in freeze dried particles. Beads prepared by freeze drying had better visual characteristics (low density, high porosity and better throughput) than oven dried beads, which was confirmed by SEM analysis.

**Keywords:** *alginate, inulin, electrostatic extrusion, drying influence, polyphenols*

## APPLICATION OF GENE EXPRESSION ANALYSIS IN IDENTIFYING INTESTINAL RESPONSES OF SALMON TO DIETARY N 3 FATTY ACIDS

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Gene expression analyses can be used as a valuable tool to understand physiological processes in fish and other aquatic animals. Evaluation of genes that play significant roles in different biological processes as embryonic and adult growth, efficiency in nutrient utilization, disease resistance and other are used in many research programs in aquaculture. Gene expression analysis can be used to study the transcriptional regulation of different genes involved in specific metabolic processes in fish to identify active and inactive genes.

The primary focus of this study was to identify the expression of some selected genes in the intestine of salmon (*Salmo salar*) fed different levels of n-3 fatty acids i. e. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) throughout life and further to identify the effects by early feeding and effects triggered by the diet the fish were receiving during the later life stage. Several genes connected to important metabolic processes were analysed. These selected genes are involved in EPA and DHA synthesis, fatty acid oxidation, and synthesis of prostaglandins and immunity.

Preliminary results of this trial showed that different levels of n-3 fatty acids in the diet of fish influenced the expression of some genes. The most up-regulated were genes that regulate elongation of fatty acids and the synthesis of prostaglandins. Genes play a part in prostaglandin synthesis, in particular, seem to be affected by both, diets given at early feeding phases and diets given during the last stages of feeding.

*Keywords: gene expression, aquaculture, n-3 fatty acids, nutritional requirements*

*Acknowledgments:* This study was supported by the FP7 Project AREA (Project number: 316004).

## COMPARATIVE ANALYSIS OF SOYBEAN BREEDING PROGRAMS IN SERBIA AND HEILONGJIANG PROVINCE

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Soybean is grown in Serbia in the last half century on approximately 200,000 ha. On the other hand, in Heilongjiang province in China soybean is grown on 3 million ha, and this province is considered as one of the places of soybean domestication. To fulfill requirements of production in both regions, intense soybean breeding programs were established. The main purpose of breeding program is to provide high yielding, well adapted varieties for each particular region. A comparative analysis of breeding methodology should provide information on similarities and differences between breeding programs, and it aims to improve each breeding methodology.

For the breeding purpose, it is important to consider agronomical practice in each region. In Serbia, soybean is grown on flat plots and at plant density between 400,000 and 600,000 plants per ha. Also, planting is done exclusively by machine. On the other hand, in Heilongjiang province agronomical practice is much more divergent. Actually, two types of soybean production exist and a main difference is planting technique (by hand and by planting machine). There are several subtypes of machine planting of soybean: planting on flat fields, planting on two rows on furrows and planting six rows on furrows. Furrows should provide heat and moisture for plants due to specific climatic conditions. All differences in agronomical practice of soybean production in Serbia and Heilongjiang province have consequences on variety types suitable for particular production. Serbian varieties are adapted on dense plant population, while Heilongjiang varieties give best results under conditions of much lower plant density. Another important difference between varieties is photoperiod sensitivity. Photoperiod sensitivity is controlled by several E genes, among them E1, E3 and E4 genes are considered to be the most important. Heilongjiang varieties are mostly photoperiod insensitive and harbor mainly recessive alleles, while Serbian varieties are photoperiod sensitive with dominant alleles.

As expected, the most common soybean breeding method is single seed descent (SSD) in both regions but here are some differences in breeding methodology. The main difference is during generation advance. Heilongjiang breeders use winter nurseries and can produce up to three generations per year, while Serbian breeders produce only one generation per year. On the other hand, population size is significantly smaller in Heilongjiang breeding programs (approximately 200 plants per population) compared to Serbia (approximately 3,000 plants per population). Faster generation cycling and larger populations have several advantages and drawbacks, but still provide successful generation advance. In both regions, successful soybean breeding programs exist, and apart from differences in agronomical practice and breeding methodology, they provide high yielding varieties for farmers and sustainable soybean production.

*Keywords: soybean, breeding, photoperiod sensitivity*

## APPLICATION OF BISMUTH THIN FILM ELECTRODE FOR IMIDACLOPRID QUANTIFICATION IN RIVER WATER SAMPLES

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In the past several decades, increased use of pesticides in agriculture and the problems related to them have attracted the attention of the scientific community. Neonicotinoid insecticide, imidacloprid is one of the newer classes of synthetic systemic insecticides used worldwide in the last three decades. Because of wide and constantly growing areas of application, high solubility and stability in water, imidacloprid is increasingly present in the environment. Therefore, reliable analytical procedures are needed for a systematic control of its contents in agricultural products, food, soil, and water.

Electrochemical detection has been widely used as a powerful tool in food and environmental analysis. Apart from its relatively simple and inexpensive instrumentation, electroanalysis also offers practically unlimited possibilities for new sensor platform designs, measuring in untreated samples, on-site testing and miniaturization of sensing elements. Choosing of working electrode is a crucial step in development of electrochemical methods for various purposes. Due to the low toxicity of bismuth, this 'green' metal can serve as a substitute electrode material for mercury in electroanalysis. In most respects, bismuth electrodes exhibit similar electroanalytical performance to mercury (with the exception of a more restricted anodic polarization range) while the fact that bismuth is solid at room temperature (in contrast to mercury) imparts great flexibility in the fabrication of bismuth-based sensors.

In this work, bismuth thin film electrode (Bi-TFE) is applied for the fast and sensitive electrochemical detection of the imidacloprid insecticide, with high selectivity and stability, for the first time. The electrochemical behaviour of imidacloprid on Bi-TFE is investigated by using chronopotentiometry, and bismuth is found to have a strong affinity towards imidacloprid electro-reduction. In Briton-Robinson buffer at pH 9, imidacloprid produced a well-defined reduction peak at around  $-0.85$  V (vs. Ag/AgCl,  $3.5$  mol/dm<sup>3</sup> KCl). After optimization of important parameters, the reduction peak varied linearly with the concentration of analyte over the  $1$ – $15$  mg/dm<sup>3</sup> range with a relative standard deviation not exceeding  $4.56\%$ . The achieved limit of detection is  $0.12$  mg/dm<sup>3</sup>, while limit of quantitation is  $0.35$  mg/dm<sup>3</sup> of imidacloprid. Recovery values from  $97.3$  to  $98.1\%$  for spiked river water samples confirmed the accuracy of the proposed method. Finally, the procedure combining chronopotentiometry with the Bi-TFE could be applied for the monitoring of imidacloprid content in various water samples.

*Keywords: imidacloprid, bismuth film electrode, surface water*

**COMPARATIVE EFFECTS OF PARTIAL ROOTZONE DRYING AND DEFICIT IRRIGATION OF 'GRANNY SMITH' APPLE ON FRUIT CHEMICAL COMPOSITION**

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We investigated the effects of partial rootzone drying (PRD), deficit irrigation (DI) and control irrigation (CI) on fruit chemical composition of 'Granny Smith' apple. To establish control over soil water regime, apple trees were planted in plastic containers V=120 l. Plants were irrigated with various amounts of water in order to establish three different treatments: 1) control irrigation (CI) in which the soil moisture amounted to 80% of field water capacity, 2) deficit irrigation (DI), in which 60% of CI water was evenly applied to the whole root system and 3) partial root drying (PRD), where 60% of CI water was applied to one half of the root while the other half was allowed to dry, and the irrigation was shifted when soil water content of the dry side decreased to 15–20%. The experiment continued for 3 years.

DI and PRD treatments both improved fruit quality in the following terms: concentration of soluble solids, total sugar and invert sugar. The effect of treatments on the content of sucrose was not determined. Total acid content and content of ascorbic acid in DI and PRD treatments were found to be lower compared with the control treatment. The effect of treatments on the pH was not determined. Fruits achieved earlier ripening at DI treatment, compared with PRD and CI treatments.

*Keywords: apple, regulated deficit irrigation, PRD, chemical composition*

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## NEW STONE FRUIT CULTIVARS CREATED AT THE FACULTY OF AGRICULTURE FROM BELGRADE

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From the stone fruit breeding program at the Faculty of Agriculture, University of Belgrade, three new cultivars were released in 2014 by the Serbian Ministry of Agriculture and Environmental Protection after several years of testing. Those are apricot (*Prunus armeniaca* L.) cultivar 'RUZA', sweet cherry (*Prunus avium* L.) cultivar 'CANETOVA' and sour cherry (*Prunus cerasus* L.) cultivar 'LENKA'. All three cultivars were chance seedlings selected from local populations. So, the aim of this study was to show the characteristic of these newly created cultivars. Apricot 'RUZA' has late flowering time. It is a self-compatible cultivar. The fruit is large (67 g), ovate. The taste is sweet-acidic, harmonic. It is characterized by a high content of soluble solids (16.3%) and a very strong aroma. Fruits can be kept longer than a month in cold storage. They are suitable both for fresh consumption and for processing. 'CANETOVA' is a new sweet cherry cultivar. It is a self-incompatible cultivar. Regarding the early ripening time, large fruit (8.75 g) and pleasant taste, this cultivar started spreading in the Belgrade surrounding. Sour cherry 'LENKA' is a self-compatible cultivar. Since it has a large fruit (7.6 g), sweet-acidic taste and pleasant aroma, this cultivar could be used both for processing and for fresh consumption.

*Keywords: Prunus, cherry, apricot, chance seedlings*

## BOTANICALS AS POTENTIAL ANTIFEEDING AGENTS FOR GYPSY MOTH (*Lymantria dispar* L.)

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Gypsy moth (*Lymantria dispar* L.) is a polyphagous and economically important pest of forests and orchards, which causes severe damages especially in gradation years. The most efficient control is achieved by integrated approach i.e. by combining all available measures (mechanical, physical, biological and chemical). However, in the past decades, insecticides of botanical origin have become a useful tool in pest management due to their favorable properties. The aim of this study was to assess antifeeding and/or insecticidal potential of ethanol extracts of *Halacsysa sendteneri* Boiss. leaves, *Ailanthus altissima* Mill. bark and leaves (0.5, 1 and 2%) and commercial insecticide Pyros (0.08, 0.04 and 0.02%) on gypsy moth larvae, under the conditions of 'no-choice' test. L<sub>2</sub> and L<sub>3</sub> larvae were used in the test (in ratio 1:1) and distilled water was the control variant. Feeding intensity was recorded after 24 and 48 h and presented as % of consumed leaf area, while the antifeeding effect was expressed in antifeeding Index (AFI) and classified on the scale according to Liu (AFI <20% - antifeeding no effect, 50% > AFI ≥ 20% - slight effect, 70% > AFI ≥ 50% - moderate and AFI ≥ 70% - strong antifeeding effect). The mortality (%), i.e. insecticidal effect was recorded after 24 and 48 h. The results were analyzed in SPSS 17 statistical software, using Duncan's multiple range test for a confidence interval of 95%. The experiment was set up in four replicates. After 24 h, the highest percentage of consumed leaf was in the treatment with the *A. altissima* leaf extract (90.4-96.79%) compared to the control (89.89%), indicating slight attraction. *H. sendteneri* (80.2-90.8%) leaf extract had no effect on the feeding intensity of gypsy moth larvae and was on the same level of significance with the control. However, the lowest consumed area, after 24 h, was recorded in the treatment with *A. altissima* bark extract (2.31-4.7%) and Pyros treatment (0-0.2%). After 48 h, the consumed leaf area in the treatment with *H. sendteneri* ranged from 90.2 to 99.1%, in *A. altissima* leaf extract ranged from 96.1 to 98.9% and in Pyros treatment it remained the same (0-0.2%) due to high larval mortality. *A. altissima* bark extracts decreased the feeding intensity after 48 h (4.4-12.6%) compared to the control (98.3%) i.e. they expressed an antifeeding effect. The larval mortality after 24 hours ranged from 96 to 100% in the treatment with Pyros, while other tested extracts did not have the insecticidal effect regardless of the exposure period (0%). Extracts of leaves of *A. altissima* and *H. sendteneri* did not exhibit the antifeeding effect in any of the tested concentrations and AFI ranged from 0.27 to 3.68 i.e. 0.45-5.72, respectively, after 24 h, and after 48h AFI was 0.29-1.12 i.e. 0.42-4.27, respectively. AFI for *A. altissima* bark was 90.02-94.99 after 24 h, and 77.30-91.35 after 48 h, depending on the concentration. Given indicates a strong antifeeding potential of the extract of *A. altissima* bark.

**Keywords:** *Lymantria dispar* L., botanicals, control, feeding intensity, mortality

**ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS AGAINST  
*Monilinia* spp.**

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*Monilinia* spp. are plant pathogens that cause brown rot of fruit, one of the most important fungal diseases of cherries worldwide. The disease is controlled by preharvest fungicide application, while in most European countries, postharvest application of fungicides is not allowed. To reduce the amount of synthetic pesticides in the environment and their residues in food products, eco-friendly alternatives to synthetic pesticides, such as natural substances, became an object of many studies. Essential oils from aromatic and medicinal plants have shown antifungal properties and therefore may play a role in the control of many plant diseases. Therefore, the aim of this study was to investigate the potential of numerous essential oils to be used against *Monilinia* spp. Cultures of *M. laxa*, *M. fructigena* and *M. fructicola* were exposed to volatile phase of over 50 oils for seven days. Minimum fungistatic and lethal concentrations, as well as minimum exposure duration to achieve the lethal effect, were determined. The growth rate of the tested microorganisms was partially or completely inhibited by the studied oils applied at concentrations of 0.04–0.65 µl/ml air. After seven-day exposure to the concentration of 0.04 µl/ml air only seven oils (five different oregano oils, thyme and lemon grass oils) were lethal to all investigated species. However, after one-day exposure, the same oils showed the lethal effect at 0.08 µl/ml air. These results indicate that some of the tested essential oils could be used for the control of *Monilinia* spp.

**Keywords:** brown rot, essential oils, *in vitro*, lethal effect

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## MOLECULAR CHARACTERIZATION OF CHROMIUM REDUCING BACTERIA ISOLATED FROM HEAVY METALS-POLLUTED SOIL

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Chromium is one of the heavy metals widely dispersed in environments as a result of anthropogenic activities and geogenous processes. Although chromium is an important component for metabolism, its hexavalent form is responsible for environmental pollution. This Cr(VI) form of chromium is one of the most important contamination problems and more soluble and toxic compared to the trivalent form. Similar environmental problems are registered in the factory "Rog" in Ljubljana (Republic of Slovenia), where galvanization process was performed from 1951 to 1994. This process is responsible for environmental problems in this area.

One of the methods proposed for increasing of environmental quality is bioremediation, which uses the microbial population in order to transform the soil pollutants. The aim of this paper is to determine the efficiency of bacterial isolates in chromium reduction, as well as the molecular characterization of these isolates.

Soil sampling was performed in facilities of the factory "Rog" in Ljubljana (Republic of Slovenia). Microbial analyses were performed at the Faculty of Agriculture, Department of Microbial Ecology, University of Belgrade. The abundance of bacteria was analyzed using the 0,1xTSA and LB mediums with addition of different concentrations of  $K_2Cr_2O_7$  used as a source of Cr(VI). The survival of these bacteria was followed using the agarized LB agar supplemented with different  $K_2Cr_2O_7$  concentration. Five isolates with growth on the highest  $K_2Cr_2O_7$  concentrations were used for Cr-removal experiments. Most efficient isolates in chromium reduction were 212/9 and 342/9, which were identified using the 16S rDNA sequencing. Molecular identification was performed using the F984GC and R1378 primers. The PCR mixture (30  $\mu$ l) contained 0,2mM dNTP (Kapa Biosystems, UK), 0,5  $\mu$ M of each primer, 30ng of genomic DNA, and 0.6 U HotStart DNA Polymerase (Kapa Biosystems, UK). Thermal cycling was performed using the Kyratec (Australia) thermocycler programmed for an initial step of keeping 5 min at 95°C, followed by 35 cycles of 60 s at 94°C, 60 s at 55°C, 120 s at 72°C, and extension to the last cycle (10 min at 72°C). The PCR products were recovered by 1.0% low-melting-point agarose gel electrophoresis and then sequenced by ABI 3730XL Sequencer (Macrogen, Inc., Seoul, Korea). Sequences of isolates 212/9 and 342/9 were deposited in NCBI GenBank database under accession numbers KT264836 and KT264838, respectively.

Comparison with database sequences showed the maximal similarity of isolate 212/9 with 16S rDNA sequences of *Bacillus* sp., i.e. with 6 strains of *B. cereus*, 4 strains of *B. thuringiensis* and one strain of *B. toyonensis*. On the other hand, the sequence of 343/9 isolate showed maximal similarity with *Bacillus pumilus* (100% similarity with three *B. pumilus* strains in database).

This research confirms that *Bacillus* strains could find practical application in solving of environmental problems caused by the presence of heavy metals in soil, and indicate the importance of molecular methods in the identification of bacterial strains for this purpose.

**Keywords:** *Bacillus*, bioremediation, chromium, molecular identification

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## SURFACE AND ENDOPHYTIC ROOT COLONIZATION BY PLANT GROWTH PROMOTING BACTERIA

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Rising concerns related to environmental damages caused by conventional agricultural practice pave the way to new, ecologically safe approaches, such as application of plant growth promoting bacteria as biofertilizers or biopesticides. Plant growth promoting bacteria (PGPB) are common inhabitants of soil, rhizosphere and root surface. They use a whole range of versatile in/direct mechanisms that raise nutrients availability, regulate plant hormonal status and help combat the pathogens. Biochemical characterization can help in determining the presence of a particular PGP feature in an isolate. But, the presence of the feature is not the final conformation of the isolate potential. One of the characteristic that affects isolate successfulness is ability to colonize and proliferate on root surface or root inner tissues.

Sterile seeds of mustard, wheat, red clover and sunflower were inoculated with  $10^8$  CFU of *Ensifer adhaerens*, *Pseudomonas putida*, *Serratia liquefaciens* and *Bacillus amyloliquefaciens* separately. After series of biochemical tests, those isolates were characterized as potential PGPB strains. Inoculated seeds were sown in glass tubes filled with sterile Hoagland's solution and grown for two weeks. After this period, plants were taken, roots were separated from shoots and one half of them were used for estimation of isolate presence on the surface. The other half was surface sterilized and used for counting the number of endophytic cells.

*Pseudomonas putida* showed the highest potential to stick and proliferate on root surface of mustard, wheat and sunflower. In case of red clover, cells of *Bacillus amyloliquefaciens* were the most present ones. The lowest number of cells was determined in the case of *Pseudomonas putida* on red clover root surface ( $5 \times 10^5$  CFU g<sup>-1</sup> fresh root). Still, this number was enough to cause positive effects on plant growth.

Counting of cells that penetrated and proliferated in inner root tissues revealed a different situation. The highest number of *Pseudomonas putida* was noted in mustard roots. In case of wheat those were *Serratia liquefaciens* cells, while *Ensifer adhaerens* cells were the most successful in colonizing red clover and sunflower roots. The number of these cells was in the range of  $0.01 \times 10^3$  -  $2.5 \times 10^5$  CFU g<sup>-1</sup> fresh root tissue.

The rhizosphere is a highly competitive habitat and one of the surviving strategies that PGPB use is colonization of inner tissues. This strategy creates more intimate bond between the plant and bacteria and preserves isolates from biotic stresses and competitive microbial communities. Our results suggest that selected isolates have ability to establish themselves inside tested plant species. This ability, together with PGP attributes, qualifies them as potent plant growth promoting isolates.

**Keywords:** beneficial bacteria, *Ensifer adhaerens*, *Pseudomonas putida*, *Serratia liquefaciens*, *Bacillus amyloliquefaciens*, endophyte

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## RHEOLOGY AND TEXTURE ANALYSIS OF YOGHURT AS A TOOL FOR PROCESS CONTROL

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Rheology is a study of the material flow and deformation under applied force. Rheology of yoghurt describes physical properties that are related to texture as a significant part of sensory product quality. It describes the behavior of food and helps to better understand different processes. At the same time, texture analysis is objective instrumental technique useful in maintaining of constant quality.

The aim of this study was to analyze the rheological and textural properties of yoghurt samples produced on different process lines in the dairy plant "Spasojevic", Bajina Basta, Serbia. Rheological properties of the yoghurt samples were monitored using low amplitude strain oscillation (Rheometer, Kinexus Pro, Malvern, UK) by measuring: (i) viscosity as a function of shear rate (ii) tixotropic behavior of yoghurts.

Texture of yoghurt samples was measured by back extrusion test on the Texture Analyzer (Micro Stable System, UK) using a 35 mm diameter disc. Analysis included the measurement of firmness, consistency, cohesiveness and index of viscosity.

Composition including dry matter, proteins and fat content as well as pH of samples was determined. The syneresis of yoghurt was determined by measuring of whey volume expelled from samples after centrifugation.

Results showed that rheological and textural properties of yoghurt are highly dependent on its composition. The significant difference in yoghurt properties produced on different production lines was found. However, it appears that the differences are induced by processing factors and present on a daily basis. Based on current results, it could be concluded that this study has provided valuable information on how to optimize the processing parameters in order to achieve a constant and high product quality.

*Keywords: yoghurt, rheology, texture, quality*

**INDIVIDUAL AND COUPLED EFFECTS OF *Azotobacter* sp.  
AND *Streptomyces* sp. ISOLATES ON ANTIOXIDANT  
PROPERTIES OF MAIZE**

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A three-year field experiment was conducted for testing the effect of selected *Azotobacter* sp. (AB), *Streptomyces* sp. (S) isolates and mixture of them (MIX) on antioxidant properties of maize, *Zea mays* L. (hybrid NS 640). Seeds were inoculated with aqueous inoculums of these plant growth promoting rhizobacteria (PGPR), and grown in the field conditions, without fertilization. Plants were harvested for biochemical and morphological analyses in the silking stage of development (R1). Polyphenolics represent antioxidant compounds, used as biochemical *markers* of stress responses. Activities of phenylalanine ammonia lyase (PAL, EC 4.3.1.5), as the key enzyme in synthesis of polyphenolics, and content of these compounds (total polyphenols, flavonoids, proanthocyanidins) were determined in leaves and roots of inoculated maize plants. Also, as a measure of antioxidant capacity of these plants, DPPH-radical scavenging activity was analysed. Some morphological components of inoculated plants were recorded, as well. There was no difference in PAL activity, total phenolics and flavonoids contents in leaves and roots of control and PGPR-treated plants. Significantly higher (13.4-25.9%) amounts of proanthocyanidins (3.58 mg leucoanthocyanidin g<sup>-1</sup> dry weight) were assessed in roots of AB inoculated plants. Plants showed similar DPPH-scavenging activity (35-43% neutralized DPPH-radicals), which correlated with total polyphenolics content ( $r=0.99$ ,  $r^2=0.98$ ). According to morphological analyses, AB plants had significantly higher aboveground mass and, along with S plants, were significantly higher (15%) in comparison to these from the control and MIX treatment. Obtained results suggest that applied PGPRs did not affect the homeostasis of maize plants; however, AB inoculum stimulated their growth in R1 phase of development.

**Keywords:** *antioxidants, maize, PGPR, polyphenols*

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**THE IMPACT OF EXTRACTION METHOD ON ANTIBACTERIAL AND ANTIOXIDATIVE ACTIVITY OF WILD MUSHROOM *Ganoderma applanatum***

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Since ancient times, selected mushrooms have been widely used as traditional medicinal ingredients for the treatment of various health problems. Among them, *Ganoderma applanatum* (Persoon) Patouillard has ranked a special place due to its exceptional effects on the human body. Having in mind the numerous positive effects on human health, we were focused on determining the antibacterial and antioxidative activity of hot water extract (AN), partially purified polysaccharides (AP) and hot alkali extracted polysaccharides (ANa) obtained from *G. applanatum*.

To evaluate the biological potential of extracts (0.039–20 mg/mL), several methods were employed. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MIC) against selected foodborne pathogens were determined by the broth microdilution method. In order to investigate the antioxidant capacity of extracts (0.1–10 mg/mL), inhibition of lipid peroxidation, DPPH free radical scavenging activity assay, ferric-reducing antioxidant power assay and chelating ability on ferrous ions were applied.

Tested extracts inhibited the growth of all investigated Gram-positive and Gram-negative bacteria; ANa exhibited the highest activity (MIC – 0.156–1.25 mg/mL). MBC was achieved against *Enterococcus faecalis* (5 mg/mL), *Listeria monocytogenes* (10 mg/mL), *Staphylococcus aureus* (10 mg/mL) and *Proteus hauseri* (20 mg/mL). The most evident ability to inhibit lipid peroxidation had AN (EC<sub>50</sub> = 0.9 mg/mL). A significantly high potential of AN, AP and ANa toward DPPH radicals was confirmed by their particularly low EC<sub>50</sub> value (<0.1 mg/mL). Regarding median effective concentrations, AP proved to be a very potent hydrogen-donating naturally-derived mushroom extract (EC<sub>50</sub> = 0.4 mg/mL). Among the three tested extracts, ANa appeared as the best chelator of ferrous ions (EC<sub>50</sub> = 1.1 mg/mL).

In the majority of applied tests, the hot alkali extract proved to be the most effective, especially in the case of the antibacterial activity. The process of hot alkaline extraction results in the formation of a mixture that provides the best biological responses.

These findings could be important in terms of the development of natural, easily accessible sources of antioxidant agents which are able to protect the human body from free radicals and to slow down the progress of many chronic diseases, as well as a way to protect against food pathogens.

**Keywords:** *Ganoderma applanatum*, extraction, antimicrobial, antioxidant

## APPLICATION OF MOLECULAR MARKERS IN FOREGROUND AND BACKGROUND SELECTION OF QUALITY PROTEIN MAIZE

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The use of molecular markers for screening and selecting plants in a breeding program provides several advantages and therefore is very attractive to plant breeders. Marker assisted selection (MAS) has proven to be efficient both for transfer of target genes into a different genetic background in order to improve an existing variety for a specific trait (foreground selection) and for recurrent parent genome (RPG) percent determination (background selection). Marker analysis allows breeders to discard plants without alleles of interest prior to pollination, reducing the size of breeding population. In Quality Protein Maize (QPM) breeding, with molecular markers, breeders can identify heterozygous plants in order to get homozygous recessive plants after selfing, given that the presence of *opaque2* (*o2*) gene in the homozygous recessive state (*o2o2*) is the aim of the selection of QPM genotype. Also, molecular markers can help to reduce the number of generations required to recover a recurrent parent genetic background.

The objectives of this paper were to identify the BC<sub>2</sub> progeny heterozygous for the *opaque2* gene using *o2* specific SSR markers and to determine genetic similarity (GS) between parental line and identified *O2o2* plants. Percentage of heterozygous plants identified in BC<sub>2</sub> generation was approximately 50%, which is in accordance to the expected Mendelian ratio of 1 *O2O2* : 1 *O2o2*. GS values calculated on Dice coefficient were in the range from 0.86 to 0.92, the average being 0.9. Progenies with the highest recovery of the recurrent parent genome were selfed to produce BC<sub>2</sub>F<sub>2</sub> seeds. By selecting the *O2o2* progenies with the highest RPG content number of plants needed to be selfed was reduced. The harvested BC<sub>2</sub>F<sub>2</sub> seeds of all selected plants will be raised and the DNA samples from BC<sub>2</sub>F<sub>2</sub> progenies will be collected and subjected to foreground selection before flowering to identify the progenies that attained homozygosity at *o2* locus.

*Keywords: molecular markers, opaque2 (o2), quality protein maize (QPM)*

## EVALUATION OF THE ANTIOXIDANT PROPERTIES OF SELECTED EDIBLE-CULTIVATED MUSHROOMS

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The level of imbalance between reactive oxygen species (ROS) production and antioxidant defenses defines the degree of oxidative stress. Overproduction of ROS results in often irreversible cellular damages and leads into a range of disorders such as atherosclerosis, hypertension, heart disease, diabetes, chronic inflammation, premature aging and some cancers. Considering the importance of diet in prevention of oxidative stress-related diseases, this study was undertaken to evaluate the *in vitro* antioxidant activities of hot water extracts of selected edible commercially cultivated mushrooms: *Agaricus bisporus*, *Agaricus brasiliensis* and *Lentinula edodes*. Mushrooms have been recognized as important food items since ancient times and their consumption is increasing because of their significant role in human health and nutrition.

Antioxidant capacity was measured using the following assays: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, reducing power and chelating ability on ferrous ions. The results were normalized and expressed as EC<sub>50</sub> (mg/ml) values. Fourier transform infrared (FT-IR) spectroscopy was used for analysis of the chemical composition of hot water extracts.

The FT-IR spectra of hot water extracts of the selected edible mushrooms showed a typical carbohydrate pattern with the presence of polyphenols and small amounts of proteins. With regard to scavenging ability on DPPH radicals, EC<sub>50</sub> values for *A. bisporus*, *A. brasiliensis* and *L. edodes* were 1.88, 0.19 and <0.1 mg/ml, respectively. Ascorbic acid had a good activity as shown by its low EC<sub>50</sub> value (1.64 mg/ml). EC<sub>50</sub> values of the chelating abilities on ferrous ions for *A. bisporus*, *A. brasiliensis* and *L. edodes* extracts were 6.08, 1.98 and 5.16 mg/ml, respectively. For comparison, the chelator EDTA showed a higher activity (EC<sub>50</sub> < 0.1 mg/ml). For reducing power of *A. bisporus*, *A. brasiliensis* and *L. edodes* EC<sub>50</sub> values were found to be 8.83, 1.03 and 2.35 mg/ml, respectively. Ascorbic acid showed an excellent reducing activity (EC<sub>50</sub> < 0.1 mg/ml).

The results of the present study suggest that hot water extracts of edible commercially cultivated mushrooms act as natural antioxidants. Edible mushrooms may be good sources for the development of antioxidant food additives.

**Keywords:** oxidative stress, antioxidant capacity, edible-cultivated mushrooms

## VARIATION IN NUTRITIVE AND ANTIOXIDATIVE PROPERTIES OF GRAIN AMONG LOCAL MAIZE LANDRACES

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Maize Research Institute "Zemun Polje" (MRIZP) gene bank maintains the collection of 2217 local maize landraces, collected from different agro-ecological sites of the former Yugoslavian territories, as well as the collection of 3,589 introduced genotypes (inbred lines, synthetics, composites, and landraces) from 40 countries worldwide. As the original biological material created by the process of natural selection and adapted to local growing conditions, local landraces are considered to be the most important genotypes. Since exploring natural biodiversity, as a source of novel alleles to improve the productivity, adaptation, quality and nutritional value of crops is of prime importance in on-going breeding programs, this research was focused on chemical characteristics contributing to improved nutritional and antioxidative values of grain. For this purpose, sixty-seven local maize landraces from the MRIZP gene bank (at least three landraces from each of the 18 existing agro-ecological groups) were chosen for grain quality analysis. The experiment was conducted in 2010 and 2011, set-up according to a completely randomized block design, in two replications. Landraces were multiplied (*via* pair crossing, i.e. full-sibling) and manually harvested. At least 80 ears per landrace were collected. For each landrace, kernels samples from the ear centre were prepared for grain quality analysis. From a nutritional standpoint, a high level of inorganic P content is considered as desirable. Also, phytates as a primary storage form of phosphorus, are important for seed germination and early seedling establishment; however, low phytate maize offers major environmental and nutritional benefits. In this study, inorganic (P<sub>i</sub>) and phytic phosphorus (P<sub>phy</sub>) contents ranged from 0.09 to 0.46 mg g<sup>-1</sup>, i.e. from 1.58 to 5.72 mg g<sup>-1</sup>, respectively. Analyses of seed antioxidant content revealed significant genetic variability related to total glutathione (GSH) content, ranging from 66.55 to 3153.04 nmol g<sup>-1</sup>, highly pronounced in landraces, previously characterized as drought tolerant. Free soluble phenolics content was in range from 202.06 to 661.99 µg g<sup>-1</sup>. In maize with standard grain quality, tryptophan content is usually under 0.060%. However, in more than a half of landraces evaluated, tryptophan content was high, ranging from 0.061% to 0.086%. Protein content determined by the Kjeldahl method was also high in all the accessions (10.11%–14.70%). A significant and negative correlation between phenolics and total protein contents ( $r=-0.286$ ;  $p\leq 0.05$ ) was observed. Also, correlation analyses revealed a significant and positive correlation between proteins and GSH contents ( $r=0.303$ ;  $p\leq 0.05$ ). In addition, a significant and negative correlation was observed between phytate and protein content ( $r=-0.287$ ). This could indicate that in genotypes with slightly increased protein content, the nutritive value of seeds could be higher, due to lower phytate and increased GSH content.

*Keywords: glutathione, phenolics, phosphorus, tryptophan, Zea mays L*

## **CIDER FERMENTATION WITH SUSPENDED AND YEAST CELLS IMMOBILIZED ON CELLULOSIC MATERIALS**

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In this work, conventional cider fermentation using suspended yeast cells was compared with cider produced by yeast cells immobilized on delignified cellulosic materials (DCM) originated from beech and oak wood. The usage of wood and other cellulosic materials is desirable due to their presence in nature and relatively easy preparation and application. The aims of this research were to study the possibility of yeast cell immobilization on cellulosic materials and to investigate the difference in cider fermentation characteristics between suspended and immobilized yeast cells. Delignification of wood materials was carried out by boiling wood materials in NaOH solution for lignin removal. The immobilization process was performed by mixing yeast cells and DCM for 24 h in a synthetic medium. The presence of immobilized cells was confirmed by Scanning Electron Microscopy (SEM). Adsorption efficiency of immobilization process was studied by colony-forming unit (CFU) method using malt agar. Also, concentrations of free and released yeast cells from carriers during fermentation were noticed and used for method efficiency evaluation. The difference between released yeast populations in samples was reflected in the relative turbidity of the fermented medium.

Three different apple based fermentation media were used for fermentation studies: Apple juice fermented with suspended yeast cells (SUSP); Apple juice fermented with yeast cells immobilized on beech wood (IMBeech); Apple juice fermented with yeast cells immobilized on oak wood (IMOak).

The apple juice fermentation with suspended cells was completed for 120 h, while the fermentations of the samples IMOak and IMBeech were finished earlier due to faster sugar consumption by immobilized yeast cells. The ciders produced by immobilized cells were completely clear, while the sample SUSP was blurry. Obtained results suggest that the cider production process based on immobilized yeast cells is generally preferable to conventional fermentation using suspended cells.

*Keywords: cider fermentation, yeast immobilization, cellulosic materials*

## APPLICATION OF GENOMICS TO TAXONOMY OF AGROBACTERIA

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Bacterial taxonomy currently relies on a polyphasic approach that applies to phenotypic, chemotaxonomic and genotypic properties. In this regard, genotype-based methods are especially relevant in describing novel taxa. Among the genotyping methods, DNA–DNA hybridization (DDH) has been the “gold standard” for bacterial species delineation. However, genomics has started a revolution in the study of species diversity and made a significant impact on taxonomy. Thus, whole-genome average nucleotide identity (ANI) has been proposed as an alternative to DDH for measuring genome similarities when defining prokaryotic species. In the present study, we principally relied on a genomics approach to characterize three atypical strains isolated from fruit plants showing symptoms of crown gall disease.

Two plant-tumorigenic strains KFB 330<sup>T</sup> and KFB 335 were isolated from galls on raspberry (*Rubus idaeus*) in Serbia, while a non-pathogenic strain AL51.1 was recovered from a cherry plum (*Prunus cerasifera*) tumor in Poland. Initial tests showed that they are rod-shaped (0.7–0.9 μm × 1.4–2.6 μm), Gram-negative, motile, oxidase positive and aerobic bacteria. Phylogenetic reconstruction based on 16S rDNA placed them within the genus *Agrobacterium*, with *A. nepotum* as their closest relative. Multilocus sequence analysis (MLSA) based on the partial sequences of *atpD*, *glnA*, *gyrB*, *recA* and *rpoB* housekeeping genes suggested that these three strains represent a novel *Agrobacterium* species, that clustered with type strains of *A. nepotum*, *A. radiobacter*, *A. fabrum* and *A. pusense*. In order to confirm this, we performed whole-genome sequencing of representative strain KFB 330<sup>T</sup> and its closest relative, *A. nepotum* 39/7<sup>T</sup> by using an Illumina HiSeq2500 platform. The resulting draft genome sequences were used for ANI-MUMmer (ANI<sub>m</sub>) and ANI-Blast (ANI<sub>b</sub>) calculations with the JSpecies Web Service. The ANI values between strains KFB 330<sup>T</sup> and *A. nepotum* 39/7<sup>T</sup> were 91.16% (ANI<sub>m</sub>) and 89.12 (ANI<sub>b</sub>), which was below the proposed threshold (94–96%) for the species definition and therefore indicated that they do not belong to the same species. Moreover, the strain KFB 330<sup>T</sup> exhibited the ANI values lower than 91% with related species and genomic species of *A. tumefaciens* complex. The average GC content of draft genome sequence of the strain KFB 330<sup>T</sup> was 58.8%. In addition, we also studied phenotypic and chemotaxonomic characteristics of strains. The major cellular fatty acids of the novel strains were 18:1 w7c (72.8–77.87%) and 16:0 (6.82–8.58%). Furthermore, phenotypic features allowed their differentiation from closely related species. Polyphasic characterization, with special emphasis on genetic and genomic features, showed that the three strains studied represent a novel species of the genus *Agrobacterium*. We proposed the name *Agrobacterium arsenijevicei* sp. nov. (ar.se.ni.je.vi'ci.i N.L. gen. n. arsenijevicei) for the novel species, in honor of Prof. Momčilo Arsenjević, who was a prominent Serbian phyto bacteriologist. The type strain of *A. arsenijevicei* is KFB 330<sup>T</sup>(=CFBP 8308<sup>T</sup>= LMG 28674<sup>T</sup>).

**Keywords:** crown gall disease, genotyping, whole-genome sequence, ANI, *Agrobacterium arsenijevicei*

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## SEEDS OF WHITE CHIA AS THE CONTRIBUTING INGREDIENT OF COOKIES WITH FUNCTIONAL PROPERTIES

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Although well known from ancient times, in the last decade chia was readmitted as high quality food, mainly due to unique composition. Chia is one of the richest plant sources of healthy omega-3-fatty acids, contains a high amount of proteins, fibres and minerals and is abundant in antioxidants. There are several colors of chia seeds, of which black seeds are more available and widespread, while the white ones are rarely found. Nevertheless, white and black seeds are very close in the nutrition value and both belong to *Salvia hispanica* L. varieties.

Although the benefits of chia are many, without a proper vehicle, the healthful attributes of the chia seeds would be lost for consumers. In order to obtain food that consolidates the boons of chia seeds with the indulgence, in this study, cookies containing wholemeal flour, walnut oil and dark chocolate with addition of white chia seeds (originating from Mexico, acquired from Canada) were made. The fact that chia does not lose any nutritional value when it is baked is of great importance for its application in cookies. Wholemeal flour possesses very valuable compounds which originate from husk of wheat seeds and are beneficial for human health. Walnut oil has a favorable composition of fatty acids as well, represents a good source of antioxidants and has a favorable health effect. Besides evoking a range of stimuli that trigger the pleasure centers in the human brain, dark chocolate also contains a superior amount of antioxidants compared to many other foods and beverages, thus contributing to the prevention of cardiovascular disease. Three samples of cookies were prepared: sample 1 (control sample, without addition of chia seeds); sample 2 (with 30 % of chia seeds) and sample 3 (with 60 % of chia seeds). Obtained samples were subjected to the determination of basic chemical composition (mineral, protein, fat, sugar and water content) and sensory evaluation (appearance: colour, surface, shape, damage; texture: structure, doneness, breakage, chewiness; aroma: odour, taste). Based on the results, mineral, protein and fat content increased with the higher proportion of chia seeds in the cookies, while sugar content decreased. These results are in line with expectations, as chia seeds are rich in proteins, fats and minerals and low in sugar, which is reflected in the composition of cookies. Sensory evaluation performed by a panel made up of five selected assessors showed that the cookie samples with the largest amount of chia seeds had the best overall sensory quality, dominating in the assessment of appearance, structure, odour and taste. Other two samples belonged to the category of very good quality, although the differences in the final scores of samples were not remarkable.

This research has shown that the use of chia seeds in the production of cookies increases the nutritional value of the final products, achieves adequate sensory characteristics and presupposed benefits to health, i.e. a new product with functional properties is obtained.

*Keywords: chia seeds, cookies, chemical composition, sensory analysis*

## BEHAVIOUR OF THE NEONICOTINOID INSECTICIDES IN PEPPER GROWN IN GREENHOUSE PRODUCTION

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Pesticide application in accordance with good agricultural practice (GAP) leads to an increase in yield and higher quality of products, while an inappropriate use can cause increased content of residues in agricultural products and environment, and it can also endanger human health. Having in mind that pepper belongs to the group of cultivated plants highly susceptible to a great number of diseases and pests, the growing of pepper in greenhouse conditions requires regular chemical protection. For pepper protection from economically significant pests such as aphids, insecticides from the group of neonicotinoids, acetamiprid and thiacloprid have proved highly efficient. One of the basic requirements for the risk assessment of pesticide residues is the existence of quantitative data on their presence in food.

The main objective of this study was to evaluate the residues and degradation rate of acetamiprid and thiacloprid in pepper fruits grown in greenhouses. The products based on these insecticides, at a recommended concentration of 50 g a.i./ha and 69 g a.i./ha, respectively, were used for treatment of pepper grown in a greenhouse. The trials were set up in accordance with the principles of GAP and OEPP methods for the test design and data processing (EPPO, 2012), as well as for the insecticide efficacy in control of aphids in vegetables (EPPO, 2004). At various time intervals, from treatment to harvest, taking care about pre-harvest intervals (PHI; 14 days for acetamiprid and 7 days for thiacloprid), representative samples of pepper fruits were collected. A sampling was carried out randomly, from more places within the experimental plot, in accordance with FAO (2009) directive with the aim of ensuring a representative sample. Extraction of acetamiprid and thiacloprid from pepper fruits was carried out by QuEChERS method, while HPLC-DAD was applied for measurement of the residue level. The method was validated in accordance with the SANCO/12571/2013 document and was used for real pepper samples. The results of a field trial showed that acetamiprid and thiacloprid dissipated rapidly from 0.47 mg/kg to 0.24 mg/kg and from 1.136 mg/kg to 0.321 mg/kg in the first two days after application. The dissipation of acetamiprid and thiacloprid residues over the time in pepper fruits fitted to the equation  $C_t = 0.443e^{-0.18t}$  and  $C_t = 0.620e^{-0.14t}$ , with the half-lives ( $t_{1/2}$ ) of 3.9 and 4.9 days, respectively. The final residues of acetamiprid and thiacloprid in pepper were 0.08 mg/kg, i.e. 0.198 mg/kg, which is lower than the maximum residue level for these insecticides of 0.3 mg/kg and 1.0 mg/kg at harvest.

*Keywords: pepper, acetamiprid, thiacloprid, residue, half-life*

## THE APPLICATION OF METHOD OF QUALITATIVE MELISSOPALYNOLOGICAL ANALYSIS IN DETERMINING THE BOTANICAL ORIGIN OF HONEY FROM THE RASINA DISTRICT (SERBIA)

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Melissopalynological analysis involved identifying all pollen grains or other microscopic constituents in honey sample originated from the Rasina district (Serbia), in order to determine and control the botanical origin of honey. Pollen analysis, providing information on the plant species foraged by honeybees, was performed by applying the method of qualitative melissopalynological analysis. The microscopic elements were concentrated by centrifuging the honey dissolved in water, whereupon the resulting sediment was examined and evaluated under the light microscope. The sample was subjected to qualitative analysis by counting 500 pollen grains, which were identified and grouped by species or pollen morphological types according to the relevant literature, and using the collection of reference pollen slides and phototheca. For each pollen type, excluding pollen from anemophilous and nectarless plants, the relative frequency as the respective percentage has been calculated with regard to the total number of pollen grains counted.

A total of twenty six pollen types were identified in the honey sample, of which two at the species level, 14 at the generic level and 10 at the family level. The melissopalynological analysis showed that the most frequent pollen in the honey sample was of *Robinia pseudoacacia* (49%). Considering the other frequency classes, the secondary pollen type was *Salix* (17%), the important minor pollen category comprised Asteraceae-type H (5%), Asteraceae-type T (4%), *Prunus*-type (4%), *Malus/Pyrus* (3%), *Rubus*-type (3%), *Polygonum* (3%) and *Trifolium repens*-type (3%), followed by the minor pollen types including Asteraceae-type S (2%), *Rosaceae* (2%), *Medicago* (1%), *Melilotus* (1%) and *Achillea* (1%) and the least represented, individual (<1%) were Caryophyllaceae, Lamiaceae, Ranunculaceae, Apiaceae, *Malva*, *Veronica* and *Geranium*. The honey sample showed the presence of a few honeydew elements: algae and fungal spores and hyphae, as well as pollen grains of wind-pollinated species and plants that do not secrete nectar (*Carex*, *Plantago*, *Quercus*, fam. Pinaceae and Poaceae).

Based on the results of melissopalynological analyses, the examined honey sample has been classified as unifloral acacia (*Robinia pseudoacacia*) honey. In addition to the precise determination of the botanical origin which implies the correct identification of pollen types and interpretation of the pollen spectrum, sensory and physico-chemical data may be used to confirm the results of microscopic analysis.

*Keywords: honey, honeybee, melissopalynological analysis, Robinia pseudoacacia*

## PRESENCE OF 1B/1R TRANSLOCATION AND THEIR INFLUENCE ON WHEAT QUALITY

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The presence of rye translocation was investigated in wheat varieties to estimate possible associations between these proteins and wheat quality. Wheat varieties (*Triticum aestivum* L.) *milenka*, *radika*, *treska*, *babuna*, *kremenka*, *lizinka*, *orovchanka*, *bistra*, *polin*, *skopjanka* and one wheat line *MT 6/2* were analyzed. The high molecular weight composition of glutenin subunits (HMW-GS) was studied using 12% polyacrylamid gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE). The system of designating glutenin loci, alleles and subunits, Glu-1 quality score and a sum of the individual subunits score were calculated. The presence of 1B/1R translocation in wheat genotypes was evaluated on A-PAGE. If the presence of 1B/1R translocation has been observed in wheat cultivars, the Glu-1 score was adjusted and a point was subtracted due to this translocation. The presence of 1BL/1RS translocation is associated with a serious quality defect, including low sedimentation volume, dough stickiness and reduced dough strength, which disqualifies it from breeding programs developing high quality wheat. The presence of rye translocation was observed in 6 genotypes (*milenka*, *radika*, *bistra*, *polin*, *skopjanka* and *MT 6/2*) and their Glu-1 score was adjusted and points were subtracted due to this translocation. The adjusted Glu-1 score in varieties that possess 1B/1R translocation is 3, which is associated with lower bread-making quality. The 1B/1R translocation was not observed in 5 cultivars (*treska*, *babuna*, *lizinka*, *orovchanka* and *kremenka*), that have subunits 2+12 or 2+10 in the Glu-1D locus. In the Glu-A1 locus, only the variety *treska* has 2\* subunit, correlated with good bread making quality. Subunit 7+9 from the Glu-B1 locus was present in all investigated genotypes, suggesting a very low level of HMW-GS variability at this locus. Allele d (subunit 5+10) from the locus Glu-D1 related with the high quality was present in 4 cultivars. Variety *treska* has the highest Glu-1 score, score 9 and the absence of rye translocation. This investigation indicated that cultivar *babuna* has the presence of allele d on the Glu-D1 locus, Glu-1 score 7 and the absence of rye translocation. Taking into account the detrimental effects of the 1B/1R on bread making quality, analyses of rye translocation are an important feature.

**Keywords:** wheat quality, HMW glutenins, 1B/1R translocation, SDS and A-PAGE

**POLYMORPHISM OF GLIADINS IN *Triticum durum* AND *Triticum dicoccum* HYBRIDS ANALYSIS OF *Iva* × *dicoccum red* COMBINATION**

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Wheat quality is determined by the molecular structure of the wheat storage proteins, that controls the pasta and bread-making quality. The composition of prolamins is known to have an important implication on wheat breeding. This information is useful to enable selection of progenies with beneficial genetic combinations by electrophoresis as a rapid biochemical test. Gliadins are generally considered to contribute to the viscosity and extensibility of gluten. In the present study, parental wheat genotypes from *Triticum durum* Desf., variety *Iva* and *Triticum dicoccum* (Schränk ex Schübler), accession *dicoccum red* and their hybrid progeny of F<sub>2</sub> generation were evaluated by acid polyacrylamide gel electrophoresis at low pH (A-PAGE). Gliadins were determined by comparison with the standard varieties *bezostaja-1* and *Chinese spring* that were always placed on the gel, beside the examined parental genotypes and their progeny. Gliadin electrophoretic patterns were determined on the basis of the number, relative mobility (R<sub>m</sub>) and relative intensity (R<sub>i</sub>) of bands. The genetic models of the different gliadin bands in parents were evaluated on the basis of their segregation ratios of the presence or absence of gliadin bands in a hybrid generation. Chi-square tests were used for the fit to different gene hypotheses for gliadin components. The independence of gliadin bands and the presence of recombination were also conducted. Polymorphism analysis of gliadins confirmed 17 gliadin bands in *iva* and 12 gliadin bands in *dicoccum red*. Only 9 mutual bands ( $\omega$ 24,  $\omega$ 27,  $\omega$ 35,  $\omega$ 37,  $\gamma$ 58,  $\beta$ 68,  $\alpha$ 84,  $\alpha$ 89,  $\alpha$ 100) were found. Eleven gliadin bands were different, 8 in *iva* ( $\omega$ 22,  $\omega$ 31,  $\omega$ 39,  $\gamma$ 48,  $\beta$ 63,  $\beta$ 66,  $\beta$ 71,  $\beta$ 75) and 3 in *dicoccum red* ( $\gamma$ 50,  $\gamma$ 52,  $\beta$ 73). Only  $\omega$  and  $\gamma$  regions from the Gli-1 locus and the  $\beta$  region from the Gli-2 locus were analyzed, since, no alterations were detected in the  $\alpha$  region. *Iva* has alleles *m* and *m* and *dicoccum red* has *m* and *s* alleles in the Gli-1 locus and the Gli-2 locus, respectively. Genetic studies indicate that storage protein genes usually exhibit simple co-dominant inheritance. The results from hybrid progeny showed that, among 29 gliadin bands investigated, 5 bands ( $\omega$ 39,  $\gamma$ 48,  $\gamma$ 52,  $\beta$ 73,  $\beta$ 75) were confirmed to be controlled by two genes and they exhibit co-dominant inheritance. The inheritance of 3 bands ( $\omega$ 22,  $\omega$ 31 and  $\gamma$ 50) was coded of two independent dominant genes. It was also found that three pairs of bands ( $\beta$ 63,  $\beta$ 66,  $\beta$ 71) from the same parent are always inherited together, suggesting that each was controlled by closely linked genes. Recombinations of gliadin bands were observed in hybrid progeny. The highest recombination (68,5±6,3%) was observed between gliadin bands  $\omega$ 22 derived from *iva* and  $\gamma$ 50 from *dicoccum red*. The hybrid combination *Iva* × *dicoccum red* has two models of inheritance: co-dominant and coded by two independent dominant genes.

**Keywords:** wheat, gliadins, electrophoresis, A-PAGE, inheritance

## MICROBIOLOGICAL SOIL PROPERTIES AS INDICATORS OF SOIL QUALITY FOR ORGANIC FARMING

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Intensive agricultural production may have a negative impact on microbial processes in the soil. The uncontrolled application of pesticides and fertilizers, low implementation of organic fertilizers and burning of crop residues, reduce diversity and activity of microorganisms and may indicate soil degradation and its low fertility. The objective of this study was to examine the dynamics of microbial activity in different soil types and determine its quality for organic production. The study was conducted in five districts in central Serbia. Soil for microbiological analysis was sampled from two depths: 0–30 cm and 30–60 cm. A total of 160 soil samples were collected: 70 samples of soil under certified organic production, 11 samples in the conversion period (the period of preparations for organic production), 19 samples of soil under conventional agricultural production, and 60 samples of abandoned and uncultivated soil. The number of examined microbial groups was assessed on the basis of the indirect dilution method on appropriate nutritive media, while dehydrogenase enzyme activity (DHA) was measured spectrophotometrically (SRPS EN/ISO 23753-1:2013). The abundance and enzyme activity of microorganisms depended on the location, soil utilization and sampling depths. The total number of microorganisms ( $10^6$ ), number of azotobacters ( $10^1$ ), ammonifiers ( $10^6$ ), oligonitrophyls ( $10^5$ ), fungi ( $10^3$ ), actinomycetes ( $10^3$ ) and DHA, both at the surface and deeper layers, indicated good microbiological properties of the studied soils. The diversity, number and activity of microorganisms on the tested plots were mostly under the influence of physical and chemical soil properties, above all pH reaction. The number of microorganisms and dehydrogenase activity decreased with depth, which are the characteristics of all the tested plots. Although significant differences were not observed in the number of examined groups of microorganisms depending on the soil usage, the differences in DHA were significant. DHA was lower in conventional agricultural production, as well as in the period of conversion. The application of organic fertilizers increased microbial activity in the soil as evidenced by the high DHA in organic farming. The high DHA was also obtained in abandoned and uncultivated soil, which can be explained by the intense activity of the indigenous microbial population in the undisturbed natural environment.

*Keywords: dehydrogenase activity, microbial abundance, organic farming*

## ANALYSIS OF SUGAR PROFILE IN DIFFERENT APRICOT KERNELS

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Apricot is one of the most delicious and commercially most important types of fruit in the world. The fruits of apricot are largely used in the industry; however, large amounts of apricot seeds are discarded during processing. This procedure involves the wasting of potentially precious sources of phytochemicals and it also creates a serious problem of disposal of industrial waste. The aim of this work was to obtain sugar profile of apricot seeds (kernels) of 70 different cultivars, due to the limited information on the sugar content in apricot kernels. Twenty sugars (five mono-, seven di-, four tri- and one tetra, penta, hexa, and heptasaccharide), including two sugar alcohols, were identified and quantified by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC/PAD). According to the obtained average values of all analyzed saccharides, sucrose (37.61%) is the most dominant sugar, followed by glucose (26.5%), fructose (14.53%) and sugar alcohol sorbitol (14.57%), while the rest of carbohydrates make up 6.79%. According to the obtained results, early ripening apricot cultivars stored the highest sugar content. Besides those four most important sugar components which belong to the normal metabolic carbohydrate pathway, numerous minor sugars (arabinose, melezitose, *iso*-maltotriose, trehalose and galactitol), that are in most cases connected with stress conditions, were also found in apricot kernels. Since natural sugars are not associated with health problems and can be used as a substitute for artificial sweeteners, compositional findings from this study may lead to wider use of apricot kernels as very limited knowledge is available on this subject.

*Keywords: sucrose, glucose, fructose, sorbitol, Anion-exchange chromatography*

## OXIDATIVE STRESS PARAMETERS OF AN INVASIVE WEED SPECIES *Ambrosia artemisiifolia* L. ACCORDING TO THE IRRIGATION WATER QUALITY

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The highest percentages of cultivated plants are irrigated using water from canals, and the presence of herbicides and their metabolites may cause damage of the plant population. Damages can be inside or outside the treated area, dismissing pesticide residues, or the movement of water or a stream of sediment. The presence of herbicides and their metabolites in water can contribute to reducing the yield of plants and affect their biological properties, due to the adoption of their residues. The samples for water analyses were taken from the Danube Tisa Danube canal from the localities of Vrbas and Celarevo, away from the zone of a direct influence of waste waters and tributary influx. The water samples were taken according to the guidelines for taking samples of surface water from rivers and streams SRPS ISO 567-6. A multi-residue method was used for the determination of twenty-one herbicides (triazine and urea) and the products of their transformation with isoproturon-d6 and atrazine-d5 as international standards in surface water using liquid chromatography tandem-mass spectrometry (LC-MS/MS) with ESI. The extraction of herbicides from water samples by the method of Milutinovic et al. (2012) was performed on OASIS HLB column, activated with 5 ml of methanol and 5 ml of water. Metamitron, terbuthylazine, atrazine, prometryn and metolachlor as well as terbuthylazine-desethyl (metabolite of terbutylazine) were detected. *Ambrosia artemisiifolia* L., invasive weed species, was grown in the laboratory, controlled conditions (climate chamber was set up on day/night (16h and 27 °C /8h and 12 °C) with 85/54 maximum and minimum relative humidity). For its irrigation, canal water collected during the May, June and July was used. Control was irrigated with distilled water. Measurements of biological parameters were performed before, 5 and 10 days after irrigation. The content of photosynthetic pigments (chlorophylls a, b and carotenoids) (Weltstein, 1957) and phenolic compounds as a response to irrigation with polluted water, was determined. The chromatographic separation for phenolic compounds (ferulic acid, trans-cinnamic acid, 2-hydroxy cinnamic acid, gallic acid, caffeic acid, p-coumaric acid, chlorogenic acid, quercetin and kaempferol) was achieved using the Agilent 1100 (Agilent Technologies, USA) high performance liquid chromatography (HPLC) system with a binary pump and diode array detector – DAD. In the samples, the presence of galic acid, 2-hydroxy cinnamic acid, chlorogenic acid and kaempferol was also determined, which suggests that the environmental impact on the antioxidant capacity is present.

**Keywords:** *Ambrosia artemisiifolia* L., oxidative stress, water quality, herbicides, photosynthetic pigments

## ASSOCIATION ANALYSIS OF FLOWERING TIME IN MAIZE

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The flowering time in maize is one of the key traits for adaptation of this species to diverse agricultural environments. The information of the flowering time for maize inbred lines is of great importance to breeders in order to make timely planting and synchronised crossings of inbred lines in hybrid development. Moreover, the information of hybrid earliness facilitates the proper identification of optimal maize growing regions and reduces the risk of yield losses through frost or drought avoidance. The determination of the flowering time is pretty straightforward, involving the field assessment during a growing season. When time, labour or land resources are limited, the use of molecular markers may help to quickly determine the flowering time of a large number of genotypes. The aim of this study was to identify markers that are closely linked to genetic factors controlling the flowering time in maize using association analysis. To this end, a panel of diverse maize inbred lines from the Institute of Field and Vegetable Crops was genotyped with microsatellites and phenotyped at four environments. The association analysis using the general linear and mixed linear models determined significant correlations between microsatellites and number of days to pollen shedding (male flowering) and number of days to silk emergence (female flowering). Markers *bnlg1253*, on chromosome 3, *bnlg1237*, on chromosome 5 and *bnlg162*, on chromosome 8, had significant associations ( $p < 0.001$ ) with both traits consistent in all tested environments. The proportion of phenotypic variation explained by these three markers varied from 8.2 to 17.2%. For all three markers, the alleles that affect shortening of the flowering time, as well as the alleles that delay flowering were detected. The results suggest that the identified chromosome regions containing QTLs associated with the flowering time consistently across environments could be useful in marker assisted selection.

*Keywords: association analysis, inbred lines, flowering, maize, microsatellites*

## THE INFLUENCE OF FAT CONTENT AND INULIN ON THE RHEOLOGICAL AND TEXTURAL PROPERTIES OF YOGHURT

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Dairy products with dietetic and functional properties, including those with reduced fat content, are very popular on the market. However, products with reduced fat content are often characterized by weak textural properties compared to full fat products.

The aim of this study was to analyze the influence of fat content and inulin addition on the rheological and textural properties of yoghurt.

Yoghurts were made from homogenized and pasteurized cow milk with different fat contents (2.8 and 1.5%) and with addition of 1.5% of inulin (Cosucra, Belgium), by acidification with the starter culture at 43°C until pH 4.6. The rheological and textural properties of gels and yoghurts were analyzed using dynamic low amplitude oscillatory rheology (Rheometer, Kinexus Pro, Malvern, UK) and back extrusion texture analysis (Texture analyzer, Micro Stable system, UK).

Gelation and fermentation time of cow milks with 2.8% MF were longer compared to other samples. The storage moduli ( $G'$ ) and yield stress of reduced fat yoghurt were lower compared to cow milk with 2.8% MF.

Textural properties of reduced fat yoghurts such as firmness, consistency, cohesiveness and index of viscosity were weaker compared to products with higher fat content. However, addition of inulin contributed to improvement of rheological and textural properties of reduced fat yoghurt.

*Keywords: yoghurt, rheological properties, texture, fat, inulin*

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## PROMISING GRAPEVINE HYBRIDS SUITABLE FOR GROWING IN ORGANIC PRODUCTION

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Organic viticulture is defined as the application of the procedures of organic agriculture in view of producing top quality grape and wine. All the aspects of the organic viticulture such as canopy management, soil, diseases and pest control are conducted in view of increasing the quality and health safety of vines. All traditional cultivars originating from the species *Vitis vinifera* L. are susceptible to fungal diseases and pests. Breeders from different countries try to combine the characteristics of resistant cultivars, which originate of species from America and Asia with quality characteristics of traditional European cultivars. Created new cultivars with certain resistance to fungal diseases and pests can be successful growing with low-dose pesticides. In grapevine breeding programs at the Faculty of Agriculture, University of Belgrade, one of the most used methods is interspecies hybridization. The collection of obtained vines is rich in a number of hybrids in terms of yield, grape quality and resistance to diseases and pests. As a starting material for the hybridization and selection of resistant forms, Seyve Villard 12375 is used among others. From the crossing combination Seyve Villard 12375 and new Serbian cultivar Godominka, two hybrids (18374 and 18385) intended for the production of white wines were selected and evaluated in this paper. The characteristics of these hybrids (ripening time, yield components, grape quality and susceptibility to diseases) were compared to the standard cultivar (Godominka). Hybrid 18385 had an earlier ripening time and hybrid 18374 had a later ripening time compared to the standard. Bunch and berry weight and yield in both hybrids were higher than in the Godominka cultivar (161 g; 2.5 g; 3.6 kg/vine). A very significant deviation for bunch weight was obtained in hybrid 18374 (240 g). Both hybrids had a harmonious relationship of sugar and acids in the must (21.9%; 7.3 g/l – hybrid 18374; 21.3%; 6.6 g/l – hybrid 18385). Susceptibility to diseases (*Plasmopara viticola*, *Uncinula necator* and *Botrytis cinerea*) in the hybrids was significantly smaller than in the Godominka cultivar. The hybrids examined in this paper satisfied the aims of selection and they were reported to the Commission for the recognition of new grapevine cultivars in Serbia.

*Keywords: organic viticulture, hybrids, quality, resistant*

**SPOTTED WING DROSOPHILA (*Drosophila suzukii*, Matsumura, 1931)  
AND ITS SIGNIFICANCE FOR THE PRODUCTION  
OF FRUIT IN BOSNIA AND HERZEGOVINA**

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Spotted wing drosophila (*Drosophila suzukii*, Matsumura, 1931) originated in Asia. This pest was noticed for the first time in Japan in 1916. Later, it appeared in China, India, Thailand and Korea. In 1980s, the presence of this species was confirmed in Hawaii, and in late 2008 the pest appeared in the USA. In Europe it was detected in Italy in 2009. During 2010, it was found in France, Russia, Spain and Slovenia. In neighboring Croatia, it was recorded for the first time in Istria in raspberry, peach and grape plantations. In Bosnia and Herzegovina, it has been present since 2013, when it was found for the first time in Herzegovina. Unlike most vinegar flies which infest overripe, rotten or damaged fruit, *D. suzukii* females attack ripening fruit and grape berries, causing significant economic damage by cutting into fruit skin with their saw-like ovipositor in the process of laying eggs. *D. suzukii* adults are yellow-brown in color, with red eyes and a body length of 2-3 mm. On the outer edge of transparent wings, *D. suzukii* males have a typical black-gray spot, unlike females which do not have the spot. Other European vinegar flies do not have such spots on wings, or their spots are different from the one of the spotted wing drosophila in position or shape. Besides, spotted wing drosophila males have two dark bands on the tarsus of each foreleg. *D. suzukii* females have a saw-like ovipositor that allows them to cut into fruit skin. Females do not have the noticeable markings on their forelegs. Eggs are white in color, oval in shape, 0.6 mm in length. Apod larvae develop from eggs. Mature larvae are white or translucent, 3-5 mm in length. The larvae develop through three instars before pupating. The pupae are reddish-brown in color, cylindrical in shape, 2-3 mm in length. Spotted wing drosophila overwinters as adults in sheltered places, although in favorable conditions they can be active throughout the year. In ideal conditions, *D. suzukii* can develop 15 generations per year, and the life cycle can be as short as 10 days, although a typical lifespan of adults is 3-9 weeks. The presence and population dynamics of spotted wing drosophila can be surveyed using olfactory attractants and visual inspection of fruit. The most effective monitoring method in a certain area is by using olfactory or food attractant. In order to establish the presence of the pests in an orchard, it is important to place traps during fruit ripening. Traps can be made of plastic bottles containing apple vinegar as an attractant. A survey of the presence of spotted wing drosophila in Bosnia and Herzegovina was conducted in 2015 in several regions with predominant cultivation of strawberries, raspberries and blackberries, but also other fruit species preferably attacked by spotted wing drosophila. Two types of traps were used for determining the presence of spotted wing drosophila. The traps were placed in different crops during fruit ripening. Traps were checked twice a week and replenished with vinegar where necessary. The highest catch of spotted wing drosophila was recorded in Herzegovina, but its presence was also recorded in the raspberry and blackberry growing regions in Bosnia and Herzegovina, which was of a particular concern.

*Keywords: spotted wing drosophila, Drosophila suzukii, olfactory attractant, distribution*

## AMPELOGRAPHIC AND PRODUCTIVE CHARACTERISTICS OF SOME AROMATIC WHITE WINE VARIETIES OF *Vitis vinifera*

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Differences between some clones of the same variety may have a great influence on the quality of grapes and wine. In terms of selecting most quality clones for planting in domestic agroecological environment, many analyses of introduced clone characteristics were necessary. Tests were performed over a period of two years (2013–2014), at the experimental field of the Faculty of Agriculture - Novi Sad, situated in the Fruška Gora wine region. Resistance to low winter temperature, phenological stages, fertility, sugar and acid contents in the must, resistance to Botrytis, wine quality and some ampelographic characteristics of the following clones: Muscat a petit grain clone R2, Muscat a petit grain clone R1, Muscat Ottonel clone 116, Muscat Ottonel clone 2/1, Muscat Ottonel clone Kt 16, Traminer clone R1, Traminer clone VCR 6, Gewürztraminer clone 47, Traminer Aromatico clone BB48/1, Traminer clone BB47/1, Traminer clone 11GM, were analyzed. Ten vines of each clone were selected for analyses. Comparing the two years, the year of 2014 was colder, with lots of rain in the vegetation period. According to the average data, it can be concluded that all the tested clones had the greatest degree of resistance to low winter temperatures in early winter, and in mid and late winter sensitivity increased. Great differences in phenological observations were noticed between Muscat a petit grain clones, while there were no differences between Traminer and Muscat Ottonel clones. There were some differences in phenological phase duration between tested clones. Almost in all tested clones coefficients of fertility had lower values in the second year. High coefficients of fertility were recorded in all clones. Muscat a petit grain clone R1 achieved the highest yield of 1.15 kg/m<sup>2</sup>, and showed good characteristics in our agroecological conditions. The lowest similarity of the average leaves was noticed between Muscat a petit grain clones.

*Keywords: clone, agroecological conditions, yield, quality*

## ANTIBACTERIAL ACTIVITY OF EXTRACTS OBTAINED FROM INDUSTRIAL GROWN *Pleurotus ostreatus* MUSHROOM

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Today's research is mainly focused on obtaining the different types of extracts from basidiomycetes, since they are recognized as a potential source of bioactive compounds, with the goal to replace the synthetic antibiotics by the natural ones. The aim of this research was to compare the antimicrobial activity of different extracts obtained from Oyster mushroom fruiting body, as well as to identify the organic compounds using FT-IR spectroscopy and finally to summarize available data on the factors affecting their activity.

Hot water (HW), hot alkaline (HA) and methanolic (ME) extracts were prepared from fruiting bodies of the industrial grown fungi, commercial strain *Pleurotus ostreatus* P80. Samples were examined for their antibacterial activity by MIC (minimal inhibitory concentration) test on numerous Gram-positive and Gram-negative bacteria.

The strongest inhibitory and bactericidal activity of HW polysaccharides was noticed against Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, and Gram-negative *Salmonella* Enteritidis ATCC 13076 in concentration of 20 mg mL<sup>-1</sup>. Tested bacteria were even more susceptible to ME extract with the MIC of 10 mg mL<sup>-1</sup> but the MBC was not achieved with the applied concentrations. HA extract was singled out as being the most active against Gram-negative bacteria *Proteus hauseri* ATCC 13315 with the bactericidal concentration of 2.5 mg mL<sup>-1</sup>.

The biological activity of polysaccharide extracts is mostly linked to total polysaccharides, total- and  $\beta$ -glucan content, polyphenols as well as total protein content. Using spectroscopic methods, it was found that HW extracts contained more protein (399 mg g<sup>-1</sup>) and phenolic compounds (6.36 mg g<sup>-1</sup>) compared to HA sample (20 mg g<sup>-1</sup> protein and 4.96 mg g<sup>-1</sup> phenolic compounds). The content of total proteins was the lowest in methanolic extract (12 mg g<sup>-1</sup>), while it was the highest in the phenolic compounds were found in the highest amount (12.4 mg g<sup>-1</sup>). Structural FT-IR analysis also confirmed higher content of  $\beta$ -glucans in HW and ME compared to HA extract. According to obtained results, mushroom extracts have demonstrated promising antibacterial activity.

*Keywords: antibacterial activity, extracts, Pleurotus ostreatus*

## PREPARATION AND CHEMICAL CHARACTERIZATION OF LOW COST ACTIVATED CARBON PRODUCED FROM PLUM KERNELS

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Activated carbons are one of the most widely used adsorbents for the removal of heavy metals, pharmaceuticals, dyes and many other species from the aqueous wastes. These sorbents can be produced from different carbon-containing raw materials and by different activation processes. Today, natural resources play a dominant role in the economic activities and the utilization of lignocellulosic wastes for synthesis of valuable commercial products. In this context, different types of lignocellulosic materials as agricultural waste byproducts were used as potential precursors in the production of low cost activated carbons, contributing to the economic development and preventing environmental pollution.

There are many ways of producing activated carbon, nevertheless all these production methods can be classified into two clearly defined groups: thermal (physical) and chemical activation. Physical activation involved two consecutive steps (carbonization and activation) as opposed to chemical activation with a single carbonization stage. Chemical activation includes impregnating the lignocellulosic raw materials with the chemical agents ( $H_3PO_4$ ,  $H_2SO_4$ , KOH,  $ZnCl_2$ , etc.). The chemical activation, which was used in this study, has two important advantages when compared to the physical activation. The first advantage is the lower temperature at which the process is conducted, and the second one is that the yield of the chemical activation tends to be greater.

In the present study, the primary source of biomass for the production of low-cost adsorbent was lignocellulosic raw material – plump kernels, as agricultural waste byproducts and components of organic solid waste. Activated carbon synthesis was carried out by thermochemical conversion, applying phosphoric acid at 500°C, in the complete absence of nitrogen inert atmosphere.

Chemical characterizations of the prepared activated carbon were analyzed by FTIR spectroscopy. FTIR spectra were recorded with a (Thermo Nicolet Nexus 670 FTIR) spectrometer, from 400 to 4000  $cm^{-1}$  wavenumbers, identifying the functional groups on the surface of the activated carbon. The FTIR spectra of adsorbent showed a broad band at 3381.24  $cm^{-1}$  due to O–H stretching vibration. Peaks between the range of 3700 and 3200  $cm^{-1}$  comprise the overlapping peaks of stretching vibrations of O–H and N–H groups. The special adsorption peak at 1559.62  $cm^{-1}$  could be characterized by primary and secondary amide bands. The sharp bond within 1168.74–991.62  $cm^{-1}$  is attributed to the C–O and P–O groups, and may originate from phosphoric compounds developed due to  $H_3PO_4$  activation and the lignin structure of the activated carbon. According to this, the peak at 1168.74  $cm^{-1}$  is attributed to the stretching vibrations of hydrogen bonded P=O. The shoulder at 1068.35  $cm^{-1}$  can be ascribed to ionized linkage  $P^+-O^-$  in acid phosphate esters and to symmetrical vibration in a chain of P–O–P.

Structural analyses performed in this study have shown that the activated carbon prepared from plum kernels has numerous functional groups on its surface, which can favor the specific interactions and positively affect the adsorption characteristics of the material.

*Keywords: agricultural waste, activated carbon, FTIR,  $H_3PO_4$ , plum kernels*

## THE USE OF RAMAN SPECTROSCOPY FOR RAPID ANALYSIS OF CARBOHYDRATE COMPOSITION OF SERBIAN HONEY “KAČARSKI”

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Honey is essentially a highly concentrated aqueous solution of sugars, predominantly glucose and fructose, followed by small amounts of a large number of complex sugars. In addition to carbohydrates, honey contains a number of enzymes, amino acids, vitamins, minerals, antioxidants, organic acids and other compounds. The chemical composition of honey varies depending on the floral source of the nectar, region, season, etc. Although constituents such as sugars, acids and ash are not considered to have a primary importance for quality control, this very concentrated sugar solution is responsible for the characteristic physical and chemical properties of honey such as high viscosity, “stickiness”, high density, crystallization, hygroscopy and energy value.

The aim of the present study was to detect and confirm the presence of the main sugars (glucose, fructose, sucrose and maltose) in local Serbian honey “Kačarski” using Raman spectroscopy as a rapid method, directly without any preliminary sample preparation. The Raman spectra were recorded in the range 200–1600  $\text{cm}^{-1}$  with XploRA Raman spectrometer from Horiba Jobin Yvon. Raman scattering was excited by a frequency-doubled Nd/YAG laser at a wavelength of 785 nm (maximum output power 20–25 mW) equipped with a 1200 gr/mm grating. Each resulting spectrum per sample is the mean of 10 observations. Complete spectra were analyzed by Origin Pro 8.6. Several observed vibrational bands in the regions 318, 407, 507, 577, 607, 692, 763, 808  $\text{cm}^{-1}$ , followed by 854, 907, 964, 1059 and 1110, 1250, 1447  $\text{cm}^{-1}$  could be identified as fingerprints of the major sugar constituents of honey, fructose and glucose, whereas sucrose and maltose bands have been suppressed. The band at 321  $\text{cm}^{-1}$ , arising from fructose that originated from the d(C–C–C) ring vibration in the pyranoid, overlaps with the band of glucose at 317  $\text{cm}^{-1}$ , thus causing a broadening. The bands 414 and 436  $\text{cm}^{-1}$  in the glucose spectrum and 411  $\text{cm}^{-1}$  in the fructose spectrum overlap also. The strongest band at 507 and 610  $\text{cm}^{-1}$  has been clearly assigned to the glucose spectrum, due to the highest content. The bands at 763 and at 808  $\text{cm}^{-1}$  have been associated with the m(C–C) of fructose. The peak at 856  $\text{cm}^{-1}$  has been observed as being quite weak compared to that in the glucose spectrum. The two bands at 907 and 964  $\text{cm}^{-1}$  in the spectrum of fructose have been assigned to d(COH) and m(C–O) out-of-ring vibrations, and have been shortened according to concentration changes and food matrix. The band at 1059  $\text{cm}^{-1}$  is thought to be originating from the m(C–O) vibration of the glucose ring seen in the glucose spectrum. The shortening of the band centered at 1108  $\text{cm}^{-1}$  could be assigned to the d(C–O–C) of glucose. The band at 1249  $\text{cm}^{-1}$  has been observed as being quite weak compared to their appearances in the glucose spectrum.

*Keywords: honey, glucose, fructose, Raman spectroscopy*

*Acknowledgement:* This study was supported by the EU Commission (FP7 project AREA) and the Serbian Ministry of Education, Science and Technological Development (project TR 31005).

## THE POTENTIAL OF RAMAN SPECTROSCOPY FOR THE ANALYSIS OF CAROTENOIDS IN MAIZE GENOTYPES

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In general, maize is a high-carotenoid food that contains highly differing amounts of individual carotenoids and researchers have always wondered whether it is possible to get health benefits from all of the carotenoids since their concentrations are sometimes so different. In research on carotenoid antioxidants in food, there has been an ongoing debate over the availability of all carotenoids in any particular food if one or two specific carotenoids are present in unusually high amounts, such as lutein and zeaxanthin in maize kernel.

This paper presents the special advantages of Raman spectroscopy for *in situ* studies of carotenoids in maize kernels, such as luteine and zeaxanthin, lycopene and  $\beta$ -carotene. Although these natural pigments occur in plants as minor components at the ppm level, a very sensitive detection can be achieved by Resonance Raman in the visible region, directly in the plant tissue without any preliminary sample preparation.

The Raman spectra in this study were recorded in the range 900-1600  $\text{cm}^{-1}$  with XploRA Raman spectrometer Horiba Jobin Yvon. Raman scattering was excited by a frequency-doubled Nd/YAG laser at a wavelength of 532 nm (maximum output power 20-25mW) equipped with a 1200 gr/mm grating. Each spectrum obtained per different maize genotype is the mean value of minimum fifty obtained spectra. Whole spectra were analyzed by Origin Pro 8.6 and peak analyzer tool for peaks deconvolution using the Voigth function. For each Raman peak, we observed and compared: area and peak intensity, position and ratio between two main peaks in seven maize genotypes with different kernel colors.

Carotenoids in maize kernels exhibit three main Raman peaks in separate spectral regions, 1005, 1100-1220 and 1470-1550  $\text{cm}^{-1}$ , due to the stretching vibrations of the C-CH<sub>3</sub>, C-C and C=C bonds in the polyene chain of carotenoids, respectively. After peak deconvolution, wide Raman peak in spectral region from 1100 to 1220  $\text{cm}^{-1}$  exhibited four peaks (position at 1123, 1154, 1188 and 1210  $\text{cm}^{-1}$ ).

These spectral features were used to identify carotenoids and quantify the relative concentration of carotenoids (luteine, lycopene,  $\beta$ -carotene and zeaxanthin) in various maize genotypes. Significant correlations were observed between area and peak intensity and  $\beta$ -carotene content in the kernel, measured by high-performance liquid chromatography (HPLC).

**Keywords:** maize, carotenoids, Raman spectroscopy, nondestructive method

## CULTIVATION OF *Chironomus riparius* AS FOOD FOR CARP WHICH ARE GROWN IN TANK SYSTEM

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Chironomidae are one of the most abundant benthic macroinvertebrates in fresh waters. Many species can tolerate high loads of pollution. Chironomids in the larval stage represent very important natural food available to the carp in fish ponds. Larvae can be used as food for carp, which grow in the tank systems, so in that way fish get the essential amino acids, vitamins and other nutrients that improve growth, health and physical condition in general. Plant proteins, which make the major part of carp food in intensive systems as the tank system of breeding, often can not fully meet dietary needs of the fish. Anti-nutritive factors represent the additional problem that certifies the need for proteins of animal origin. A water organisms such as chironomids which in nature carp usually uses as food are the right choice for that. The presence of nutrients that are necessary for normal fish development are particularly essential in fostering the younger age category of carp.

In this work the effect of different types of substrates and food on the abundance and density of *Chironomus riparius* larvae was researched. An attempt was made to find a combination of factors that have the most favorable impact on the growth of larvae and completing the life cycle in vitro, so as to ensure successful growth as food for fish.

Positive effect on the number of surviving larvae had a combination of organic unloaded substrate and population's low density. The opposite combination of organic loaded substrate and population's high density manifested a negative effect on the number of surviving larvae. Food for the larvae rich in protein had a positive impact on the growth of the larvae, but increased mortality when used in combination with dense larvae populations and polluted substrate. Chironomidae can tolerate high level of substrate pollution, but only to a certain point when concentration of pollutant becomes the limitation. Thus, the best is using organic unloaded or moderately loaded substrate.

**Keywords:** *Chironomidae, food, common carp, intensive production, breeding*

**Acknowledgement:** The study was supported by the Ministry of Education, Science and Technological Development, the project Improvement of production capacities of carp (*Cyprinus carpio*) through nutrition and selective breeding programs (TR 31075).

## INFLUENCE OF MODERATE WATER STRESS ON THE CAROTENOID CONTENT IN 4 CHERRY TOMATO GENOTYPES

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Carotenoids are the class of hydrocarbons, widespread in nature and important pigments in living organisms. They are important components of fruit quality, because of their nutritional significance and antioxidative activity. Current studies confirmed that drought stress can induce changes in carotenoid synthesis and its final, postharvest concentration. The aim of presenting results was to investigate the effects of moderate water stress on the carotenoid content in 4 cherry tomato genotypes (Criollo, Cervil, Plovdiv and LA1420).

The experiment was conducted in glasshouse conditions. Plants were exposed to drought by withholding irrigation of substrate in the pots (soil water content ca. 25%), while in control conditions, plants received full substrate water holding capacity (soil water content ca. 75%). Carotenoids studied in the fruits included  $\beta$ -carotene, lycopene, lutein and phytoen and they were determined by HPLC.

Results confirmed that the capacity of drought-induced synthesis of carotenoids is highly genotype-dependent. Drought stress did not induce any changes in the amounts of any analyzed carotenoids in the fruits of genotype Plovdiv, in contrast to the fruits of LA1420A where a significant increase was found in all analysed compounds. Results for genotype Criollo showed an increase of 3 analyzed carotenoids ( $\beta$ -carotene, lutein and python), while lycopene amount was not changed, but it was still higher than in other analyzed genotypes. The fruits of Cervil genotype in stress conditions were richer in lutein for more than 45%, and potential of this genotype for increased synthesis of this xanthophyll could be utilized in breeding programs.

These results suggest the potential of moderate water stress conditions for the screening capacity of different tomato genotypes for producing such important fruit nutritional quality compounds as carotenoids are.

*Keywords: moderate drought stress, carotenoids, tomato, fruit quality*

*Acknowledgement:* This study was supported by the EU Commission (FP7 project AREA) and the Serbian Ministry of Education, Science and Technological Development (project TR 31005).

## THE AGE STRUCTURE OF WATER VOLE (*Arvicola terrestris* Linnaeus, 1758) POPULATIONS IN VOJVODINA

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The water vole is a very common rodent species found on the river banks and agricultural fields in the vicinity of the rivers and irrigation canals in Vojvodina. This species usually lives in the smaller family groups, often consisted of one generation of adults and up to two generations of offspring, although it could form larger colonies if ecological factors were optimal. The estimation of the age structure of water vole populations are crucial for the assessment of the potential population outbreaks. The age structure represents the indicator of population growth, as it is correlated with the natality and mortality rates. The body weight is directly proportional to the rodent age and with additional features could be used in the estimation of the maturity and the reproductive potential. The study was conducted from 2011 to 2014, three times per year, at four localities in Vojvodina: Apatin, Bogojevo, Labudnjača and Čelarevo. The localities were selected according to their floristic composition and anthropogenic influence. The total number of trapped animals was 984. Each individual was weighted and the body and tail lengths were measured. According to obtained measurements, specimens were classified in five age groups: pre reproductive, early, middle and late reproductive and post reproductive group. The results were statistically analysed using ANOVA and Fisher's post-hoc test. According to one-way ANOVA, high statistical significances were determined for the influence of localities, seasonal aspects and age groups ( $p_l=0.000000$ ,  $p_{sa}=0.000758$ ,  $p_{ag}=0.000000$  for  $p<0.01$ ), but not for the year as an independent variable. The Fisher's LSD test exposed Bogojevo and Labudnjača as localities with the uniform presence of all age groups and the most stabile water vole populations. Furthermore, autumn months showed high statistical differences in comparison to spring and summer months, as all age groups were present with the proportional frequencies of the specimens. The lowest number of specimens was classified as individuals of the pre reproductive and post reproductive groups, and the most numerous were the individuals of the late reproductive group. The pre reproductive groups had the low number of specimens at all prospected localities in spring due to the period of mating, gravidity and lactation. The individuals of all reproductive stages (early, middle and late) were the most numerous at Bogojevo. The age structure pyramids of water voles at all four localities were stationary or expanding, which indicates the possibility of outbreaks. The water vole populations are affected by a numerous abiotic and biotic factors that interact simultaneously. The stability or potential population outbreaks are determined by the rates of natality and mortality in certain habitats, changing the age structure pyramids. The size and frequencies of particular age groups determine the seasonal fluctuations, which are of the great importance for the prognosis of water vole appearance in nearby agroecosystems, potential plant damage and yield loss.

**Keywords:** water vole, age structure, agroecosystems, *Arvicola terrestris*

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## MOLECULAR BASIS OF OSMOTIC STRESS TOLERANCE IN SEEDLINGS OF *Pisum sativum*

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Water deficit and osmotic stress lead to oxidative stress and creation of a large quantity of reactive oxygen species (ROS). Plants have developed antioxidant defense mechanisms in order to neutralise the damaging effects of ROS. The aim of this research was to determine the molecular basis of osmotic stress tolerance among 7 different cultivars of field pea (*Pisum sativum*) at the early seedling stage. Osmotic stress was stimulated using high molecular osmotic substance Polyethylene Glycol 6000 (PEG 6000) with an osmotic potential level of -0.1MPa. Water with no added PEG 6000 was used as control. Activity of antioxidative enzymes: superoxide dismutase (SOD), ascorbat peroxidase (APx) and glutathione reductase (GR) was measured spectrophotometrically. Abscisic acid (ABA) concentration was measured by the Elisa method. The changes in gene expression of antioxidant enzymes (*Cu/Zn SOD*, *cAPx* and *GR*) and three genes of aldehyde oxidase (*PsAO1*, *PsAO2* and *PsAO3*), which participates in ABA biosynthesis, were analysed. Total RNA was isolated from stressed and non-stressed plant roots and shoots. The gene expression levels of antioxidative enzymes and *PsAO* genes were examined by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) technique. *Arabidopsis* 18S rRNA was used as internal control. Activity of antioxidant enzymes was changed in all pea cultivars under osmotic stress compared to the control. However, different enzymes were activated in the tested cultivars, which might indicate a different degree of tolerance. Thus, the mechanism of eliminating ROS in the tolerant cultivar Trezor is based on the constantly increased activity of all three enzymes. Activity of enzymes was decreased in cultivar Junior, more sensitive to osmotic stress, as well as in the shoot of cultivar Javor in which sensitivity to osmotic stress of shoot was particularly strong. The molecular analysis can explain changes in antioxidant enzyme activity. The results show that, in response to oxidative stress and the accumulation of ROS, which occur in conditions of osmotic stress, antioxidative enzymes, especially APx, were up-regulated in tolerant cultivars (Trezor, Pionir, Mraz), in both shoot and root, i.e. down-regulated in more sensitive cultivars (Junior and Javor). Results also confirmed the expression of three genes (*PsAO1*, *PsAO2* i *PsAO3*) needed for the synthesis of enzyme aldehyde oxidase (AO). Expression of gene *PsAO2* did not indicate significant differences compared to the control, while the expression of genes *PsAO1* and *PsAO3* increased in seedlings of the cultivar which accumulated ABA (Javor), i.e. decreased in the cultivar in which accumulation of ABA was decreased (Trezor). As the expression of gene *PsAO3* had constantly been increased under osmotic stress it could be concluded that the most important gene is *PsAO3*, whose expression is increased in pea plants exposed to stress.

**Keywords:** osmotic stress, field pea, gene expression, antioxidant enzymes, ABA

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**SOYBEAN DISEASES AND PESTS: VOJVODINA (SERBIA) VS.  
HEILONGJIANG (CHINA)**

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Soybean (*Glycine max* (L.) Merr.) is one of the most important crop worldwide. Soybean's homeland is China, where this legume has been grown for more than 5,000 years, while in Serbia, soybean arrived just 40 years ago. Comparing these two regions, it has been noticed that soybean white mold (*Sclerotinia sclerotiorum*), root rot diseases (*Fusarium* sp., *Pythium* sp., *Rhizoctonia solani*), downy mildew (*Peronospora manschurica*) and soybean aphid (*Aphis glycines*) are common diseases and pests for these areas. They have the high economic significance in terms of reduction of the yield in China in relation to Serbia, where soybean genotypes are tolerant to these diseases. Therefore, it is not surprising that the fungicides are applied in the soybean protection in China, while this method of protection is not economically viable in Serbia. In addition to the previous point, the presence of different diseases and pests has also been observed in China and Serbia. For instance, soybean cyst nematode (*Heterodera glycines*), *Phytophthora* root rot, frog-eye leaf spot (*Cercospora sojina*), soybean mosaic virus and soybean pod borer (*Leguminivora glycinivorella*) have a large impact on yield reduction in China, while in Serbia these agents have not been detected. Instead of this, in Serbia, the most frequent are the following diseases: stem canker (*Diaporthe caulivora*), seed decay (*D. longicolla* and *D. sojiae*), charcoal rot (*Macrophomina phaseolina*), bacterial blight (*Pseudomonas syringae*), *Cercospora* leaf blight and purple seed stain (*Cercospora kikuchii*). The differences are obvious and can be used to locate sources of resistance.

*Keywords: soybean, diseases, pests*

## CHEMICAL COMPOSITION AND ANTIMICROBIAL PROPERTIES OF *Mentha piperita* L. ESSENTIAL OIL

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The essential oil of *Mentha piperita* L. was investigated for its chemical composition and antimicrobial properties.

*Mentha piperita* leaves were collected in June 2012, prior to flowering, in the city of Padej (North Banat district), in the Vojvodina province. The essential oil was obtained by hydrodistillation of dried plant material using a Clevenger apparatus. Essential oil composition was determined by GC-FID and GC-MS. Components of the volatile oil were identified based on their retention indices and their mass spectra using the Wiley and NIST Mass Spectral Libraries or literature data.

Twenty-five compounds were characterized in this oil, representing 95.19% of the total oil. L-Menthone (30.51 %), L-menthol (26.29 %) and isomenthone (10.44 %) were predominant components of the volatile oils.

Antimicrobial properties of the *M. piperita* essential oil were tested against common food-borne pathogens, seven G (+) bacteria, eight G (-) bacteria strains and one yeast. The minimum inhibitory concentrations (MIC), as well as minimum lethal concentrations (MLC) of the essential oil, were determined by the broth microdilution assay. Concentrations in the range of 7.50 mg/mL–0.47 mg/mL were tested. The essential oil exhibited stronger antibacterial activity against Gram (+) than Gram (-) bacteria, with MIC and MLC values within the same range, from < 0.47 mg/mL to  $\geq 7.50$  mg/mL. Inhibitory and lethal concentrations against Gram (-) bacteria were in the same range of concentrations, from 3.75 mg/mL to  $\geq 7.50$  mg/mL. *M. piperita* essential oil also showed activity against the yeast *Candida albicans* (MIC= 0.94 mg/mL and MLC = 1.87 mg/mL).

**Keywords:** *Mentha piperita*, essential oils, GC-MS, antimicrobial properties, MIC

## THE EFFECT OF *Asclepias syriaca* ROOT EXTRACT ON GERMINATION AND INITIAL GROWTH OF SUNFLOWER, MAIZE AND SOYBEAN

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Nowadays, weed species *Asclepias syriaca* L., has invaded a great part of Vojvodina in which it has been domesticated completely, predominantly on non-agriculture areas, as well as in orchards. In the last years, its presence and spread have become more frequent in crops such as wheat, rye, sunflower and soybean. For establishing of *A. syriaca* allelopathic properties, the effect of water extract of this species' root was tested for germination and growth of maize, sunflower and soybean. Root samples of *A. syriaca* were taken in the plant fructification phase. The water root extract made from *A. syriaca* was made in the following concentrations: 12.5; 25; 50; 75 and 100 g dry mass of root/l distilled water.

The experiment showed that *A. syriaca* root significantly inhibits germination of sunflower and soybean seeds, while the negative effect on germination of maize seed was not expressed to a significant extent. The occurrence of inhibition in growth of above-ground and underground parts of maize, sunflower and soybean germs was also established. A statistically significant difference in length of the above-ground parts (epicotyls) of soybean germs in control treated by distilled water and those treated by *A. syriaca* root water extract at the highest applied rate of 0,100g/ml was determined by Duncan's test, while for the mentioned parameter, no statistically significant difference was established for soybean germs treated by root water extracts at concentrations of 0.050 and 0.075g/ml. Two highest used concentrations caused a significant reduction in the growth of the underground part of soybean germs, while in others, in comparison to the control, no statistically significant difference was found in the growth. In maize and sunflower germs, solution concentrations of 0.050-0.100g/ml showed the effect on the reduction of their above-ground and underground parts.

*Keywords: Asclepias syriaca, water extract, maize, soybean, sunflower*

**EFFECTS OF *Carvum carvi* L. ESSENTIAL OIL ON THE *TRIBOLIUM Castaneum* (COLEOPTERA, TENEBRIONIDAE) ADULTS**

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Stored pests are the major problem in flour mills, grocery shops and warehouses. *Tribolium castaneum* (Herbst, 1979) is one of the most important pest of stored products and stored grains throughout the world. The adult insects, used for this experiment, were obtained from laboratory cultures maintained in the dark in incubators at 25±1C and 70–80% r.h., reared on wheat and barley bran. Insect species were fed with flour disks containing a known concentration of essential oil of *Carvum carvi* L. The results of insecticidal effect of 9 different concentrations of essential oil were discussed. A mortality rate of adult insects was tested after 24, 48 and 72 hours. The essential oil of *C. carvi* showed strong insecticidal and fumigant activity against adults of *T. castaneum*. The most effective and the highest concentration was 6.32 µl/m, the mortality rate was 45% after 24h and 77.5% after 48h and 72h. Furthermore, it was noticed that the concentrations of 5.75 µl/ml and 5.17 µl/ml caused above 60% of mortality after 48h. The mortality rate, after 72h, at the concentration of 5.75 µl/ml was 72.5%. Concentrations 5.17 µl/ml, 4.6 µl/ml and 4.02 µl/ml also showed insecticidal activity, the mortality rate ranged from 50% to over 60%. Lower concentrations 2.87 µl/ml, 2.3 µl/ml, 1.72 µl/ml, 1.14 µl/ml indicated a weaker toxic effect and mortality was above 50% after 24h, 48h and 72h. According to results of the experiment, the essential oil of *C. carvi*, because of very strong insecticidal activity, could be used as an alternative method for controlling harmful insects.

*Keywords: Tribolium castaneum, essential oil, Carvum carvi, mortality rate*

## **EFFECTS OF HERBAL PHP® IN BROILER CHICKENS NUTRITION ON PRODUCTIVE PERFORMANCES, BLOOD LIPID PROFILE AND ENZYMES ACTIVITY**

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The experiment was conducted in order to investigate the effect of thymol and carvacrol in broiler chicken nutrition on productive performances, blood lipid profile and enzymes activity. The biological experiment was carried out at Poultry research centre of Patent IEC in Crvenka, under production conditions on a total of 1120-day-old Ross 308 strain broilers which were equally distributed into four dietary treatments with eight replicates each. In the control treatment (T1), chickens were fed with commercial diet, while experimental treatments were formed by supplementing the commercial feed with thymol and carvacrol mixture in form of a commercial product Herbal PHP as follows: 0.05% (T2) and 0.1% (T3). In treatment T4, chickens were fed commercial diet supplemented with antibiotics in concentration of 0.1%. At the end of the experiment (42 day of age), chickens in experimental treatment T3 achieved the highest body weight (2095.95 g) with a significant difference ( $p < 0.05$ ) compared to treatments T1 and T2, while the lowest feed conversion ratio was recorded in treatments T2 and T4 with a significant ( $p < 0.05$ ) difference compared to treatment T1. Regarding the European broiler index (EBI), the significant difference between treatments was absent. Also, no significant influence of experimental diets on triglycerides and total cholesterol was observed ( $p > 0.05$ ) during the experimental period. On the other hand, the highest recorded enzyme activities were mainly in treatment T3 with a significant ( $p < 0.05$ ) difference compared to treatment T1. Based on the obtained results, it could be concluded that the addition of Herbal PHP in broilers diet positively affects production performance.

*Keywords: thymol, carvacrol, nutrition, chickens*

**APPLICATION OF RAMAN SPECTROSCOPY TO INVESTIGATE  
PHOTOSYNTHETIC AND NON PHOTOSYNTHETIC TISSUE  
OF LEAF OF GERANIUM**

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Different compounds such as cellulose, proteins, carbohydrates, pigments and other cellular biomolecules have been identified by Raman spectroscopy in the structure of the plant cells. We used Raman spectroscopy for monitoring of leaf chemical characteristics on white and green parts of variegated *Pelargonium zonale*. Raman spectra were observed within 400–2000 cm<sup>-1</sup> wave numbers. The variations in the chemical composition of the leaf could be useful for quick and non-destructive observation which can be effectively used in agricultural and environmental studies.

Our results showed that the position of characteristic bands, in the region from 470 to 1200 cm<sup>-1</sup> originating from polysaccharides that are predominantly associated with cellulose, was pronounced in the green part of the leaf. Raman spectrum of photosynthetic tissue that appears at 1445 cm<sup>-1</sup> can be assigned to the CH stretching vibration of carotenoid, a pigment associated with chlorophyll. The band at 1535 cm<sup>-1</sup> (C=C) that occurred in spectra of non-photosynthetic tissue was most probably due to the presence of terpens (for example, in diadinoxanthin).

*Keywords: Raman spectroscopy, photosynthetic, non-photosynthetic, pigments*

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## LEAF REMOVING AND HARVEST DATE AS TOOLS FOR IMPROVING OF AROMA COMPLEX PROPERTIES IN CABERNET SAUVIGNON WINE

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The optimum harvest date and grape processing often overlap with the concentration of most of aromatic compounds in wine. The general classification of aromas according to their origin implied four main groups: primary aroma (original grape flavor), secondary aroma resulting during primary processing (grape pressing and enzyme activity), fermentation-derived aroma and aging aroma that occurs during wine storing and ageing. Studies should indicate the impact of leaf removing and different harvest dates on aroma complex properties of Cabernet Sauvignon wine. Research was carried out in the vineyard of King Peter I Karadjordjevic-Royal Winery at the Oplenac-Topola municipality. The vineyard is planted in 2006 and covers the area of 3.70 ha, at an altitude of 250 m. The vineyard with Cabernet sauvignon is geographically positioned at GPS coordinates N 44° 14' 35" and E 20° 41' 22". Training system is single Gijot cordon. Leaf removing included control (no defoliation) and treatments with 4 and 8 removed leaves. Tested wine was made from grapes harvested at full maturity and late harvest. Microvinification was performed in the laboratory of the Faculty of Agriculture, Belgrade University. Selected *Saccharomyces cerevisiae* yeasts of strains-R2 Lalemand were used. For determination of aromas, samples were prepared by liquid-liquid extraction, 25 ml of wine is measured in a flask and 5 ml of methylene chloride was added. Extraction was done with magnetic stirring for 1 h in an ice bath. After extraction, the resulting mixture is left in an ultrasonic bath for 5 minutes to get 'bust' obtained emulsion. The organic phase was separated and dried over anhydrous sodium sulfate. GC-MS and GC-FID analyses were done on Agilent 7890A. Aromatic compounds were identified comparing their EI mass spectra with spectra from the library Wiley7 and Nist05 using software NIST MS Search 2.0. as well as comparing the calculated retention index (RI) and retention indices of library Wiley7 and Nist05. Related concentrations were determined on the basis of peak areas in FID chromatogram. Chemical analysis of wine samples is carried out in laboratories of the Department of Instrumental Analysis at the Department of Organic Chemistry, Faculty of Chemistry, University of Belgrade. In wine, the following groups of aromatic compounds were detected: higher alcohols, lactones, organic acids, esters and amides. For most aromatic compounds, higher values of the relative proportion in terms of later harvest were found in treatment with 4 removed leaves.

*Keywords: Cabernet sauvignon, wine, aroma, leaf removing, harvest date*

## RESPONSE SURFACE METHODOLOGY IN THE RECOVERY OPTIMISATION

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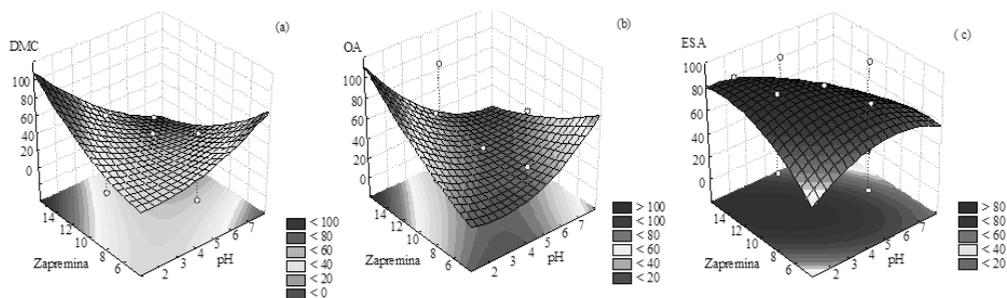
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The paper presents application of the response surface methodology and Box Behnken design with three factors at three levels in optimization of the extraction conditions of the herbicide dimethachlor (DMC) and its metabolites: dimethachlor ethane sulphonic acid (DESA) and dimethachlor oxalamic acid (DOA). For the optimization experiments, combined HLB and Envi-Carb solid phase columns were used. The parameters which were optimized were pH value, elution volumes and quantity of the spiked analytes. The Box Behnken design consisted of 17 experiments.

Agilent HPLC DAD 1220 Infinity LC with automatic liquid sampler, binary pump and diode array detector (DAD) were used for chromatographic separations. Separations were carried out isothermally at room temperature 25°C. The column Phenomenex AQUA 5 mm, C18, 125 A 250 x 2.0 mm, part number: 00G-4299-B0 were used. The mobile phase was a mixture of acetonitrile / water + 1 ml of methanol in the ratio of 70/30 vol%, the injection volume was 20 ml and analysis time 7 min. Peaks were detected at a wavelength of 200 nm. Flow rate was 0.3 ml / min, pressure in the system was on the average 600 bar. The retention times were 4.159 min for DMC, 3.565 min for DESA and for DOA 3.011 min.



The picture shows the recovery of the DMC, DOA and DESA depending on the elution volume and pH value at a constant spike quantity. Graphics are the result of applying a mathematical model to the experimental result developed in the form of a second order polynomial. The experimental results are shown as white circles on the three-dimensional diagram. The optimization goal is to provide conditions for the extraction which will give the maximum analytes recovery.

The results showed that extraction efficiency for dimethachlor, and its metabolites is pH dependent. In order to extract the all compounds with high efficiency, it is necessary to apply very acidic environment. Also, it was observed that the recovery depends on the type and volume of solvent that is required to completely elute the analyte from the combined columns. During the experiments, different solvents were tested as eluants: methanol, dichloromethane, acetone and ammonium acetate, and their mixtures. The best results i.e. good recovery and pure extracts were obtained with 10 mM ammonium acetate.

**Keywords:** RSM, dimetachlor, optimisation, recovery

**EFFECTS OF RIDoFMITE® IN LAYING HENS NUTRITION AS A REPELLENT OF RED MITE (*Dermanyssus gallinae* De Geer, 1778)**

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Red mite (*Dermanyssus gallinae* De Geer, 1778), also known as the red mite, is the hematophagous arthropod which attacks resting laying hens at night. The mites normally feed around the breast and legs of hens, causing pain, irritation, and a decrease in egg production. After feeding, they hide in cracks and crevices away from daylight, where they mate and lay eggs. The mite developmental cycle consists of 5 life stages: egg, larva, protonymph, deutonymph and adult. Under favourable conditions, this life cycle could be completed within seven days, so populations can grow rapidly – causing anaemia in badly affected flocks of poultry. Young birds are the most susceptible. The mites can also affect the health of the birds indirectly, as they may serve as vectors of diseases and pathogens such as salmonellosis, avian spirochaetosis and *Erysipelothrix rhusiopathiae*, while pustules, scabs, hyperpigmentation and feather loss may also be developed. For this reasons, the aim of this research was to investigate the effects of natural dietary red mite repellent RIDoFMITE® based on quantitative and qualitative essential oil blend in laying hens nutrition on red mite populations, egg production, egg mass and hen mortality rate. Investigation was conducted under production conditions on a farm with 2000 laying hens of line Isa Brown with heavy mite infestation. During the experimental period, a statistically significant ( $p < 0.05$ ) reduction in red mite blood feeding of 63% was recorded, so as the reduction in larvae number of 11.72%. Total hens egg production within 14 days of experimental period was increased for 4.5%, egg mass was increased for 15.5%, while mortality was reduced for 60% with statistically significant ( $p < 0.05$ ) differences. Based on the obtained results, it can be concluded that the essential oil blend (RIDoFMITE®) in laying hens nutrition is highly effective against red mite, but having in mind severity of red mite infestation on poultry farms, further investigations are still necessary.

*Keywords: red mite, poultry, nutrition, essential oils, infestation*

## USE OF RAMAN SPECTROSCOPY IN ANALYSING MUCILAGE IN MARSHMALLOW ROOT

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Root of marshmallow (*Althea officinalis* L.) is a well-known drug used in both folk and official medicine. A broad range of physicochemical properties makes this herb excellent demulcent, emollient, expectorant, diuretic and effective anti-inflammatory supplement. The active compounds in marshmallow root include flavonoids, polysaccharides like pectins, certain amino acids and various antiviral, antibacterial and anti-mucilaginous compounds, such as coumarin, kaempferol, phenolic acids, quercetin and tannins.

Since mucilage content in marshmallow roots could vary to a significant extent in different plant populations, affecting the drug quality and its therapeutic efficiency, the aim of this paper was to describe possibilities of using Raman spectroscopy for analysis of mucilage in the particular marshmallow root tissues, and to investigate influence of different growing conditions on their localization and chemical structure.

Roots of marshmallow were hand sectioned, placed on glass microslide and analyzed by Xplora Raman microscope (Horiba). Raman spectra were collected while scanning over a sample, providing the spatial and chemical labeling of various components within the sample simultaneously. Raman spectra from root water infusion, as well as available pure substances, were also performed.

The uses of Raman investigations for fast estimation of marshmallow drug quality and its practical importance were discussed. Raman mapping option offers an insight into a localization and spatial distribution of the main components. Thanks to relatively simple sample preparation which does not require extensive chemical treatment prior to imaging, the technique could be recommended for effective characterization of a drug quality. Therefore, Raman spectroscopy could represent an excellent tool for structural and quantitative analysis. However, some limitations and disadvantages of this method also occur, including considerable difficulty in producing a signal that is distinguishable from noise, lack in referent compounds (the standards), as well as difficulties in analyzing of particular tissues of a sample due to fluorescence impact.

**Keywords:** *Althea officinalis* L., mucilage, root, nondestructive method

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## USE OF POLARISATION AND RAMAN MICROSCOPY FOR IDENTIFICATION AND LOCALISATION OF RESERVE CARBOHYDRATES IN CROP PLANT

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During photosynthesis plants make sugar (glucose and fructose) and convert-excess sugar into storage (nonstructural) carbohydrates. Carbohydrate reserves are usually stored in the lower regions of the stems: stem bases, stolons, corms, and rhizomes, or in fruits/grains, which constitute the major part of edible portion in economically important crops. The most abundant storage carbohydrates in plants are starch, sucrose and fructans. Sucrose is the most common plant disaccharide and is the principal molecule of short-term energy storage and for translocation via phloem. Some plants, including sugarcane and sugar beet, store high concentrations of sucrose. Fructans are polymers of fructose and they are primary reserve carbohydrates in some vegetables (Jerusalem artichoke, garlic, onion, chicory, lettuce), while starch is polysaccharide that is composed of polymer comprised of D-glucose often found in grains (cereals or legume) or in some root and tuber crops (potato, sweet potato).

Raman scattering involves excitation of a molecule by inelastic scattering with a photon (from a laser light source), and can provide information about the molecular vibrations. Functional groups of the molecules can be identified by their unique pattern of light scattering, which could provide information on chemical composition of investigated sample. Raman spectroscopy in combination with microscopy is a technique which enables visualization of the distribution of individual compounds even in live and intact plant tissues. Polarized microscopy includes illumination of the sample by polarized light which allows researchers to obtain information on color absorption, structure, composition and refraction of sample, which can be used to characterize and identify various materials. This technique is especially good for investigations of anisotropic objects, such as starch grain samples.

In this study, we demonstrate the feasibility of Raman and polarisation microscopy for localisation and identification of different types of reserve carbohydrates in each plant tissue sample. The hand sections of plant material were put on a glass slide and analyzed by an Olympus BX50 microscope with accessory for polarization in transmitted and reflected light. Raman spectra were obtained with an Xplora Raman microscope. Raman data were collected from 250 to 3200  $\text{cm}^{-1}$  spectral range using excitation wavelength of 785 nm. Additionally, to obtain exemplary Raman spectra of individual compounds, as well as their polarisation properties, commercially available mono-, oligo- and polysaccharides were studied. Kernels of wheat and maize, raw potato and Jerusalem artichoke tubers, chicory, sweet potato and sugarbeet root have been analysed. Advantages and disadvantages of both techniques were discussed.

*Keywords:* glucose, fructose, sucrose, fructans, starch, spectroscopy

*Acknowledgement:* This study was supported by the EU Commission (FP7 project AREA) and the Serbian Ministry of Education, Science and Technological Development (project TR 31005).

## **APPLICATION OF MOLECULAR MARKERS IN BACKCROSS SELECTION OF MAIZE INBRED LINES**

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The introgression of the favorable alleles from a donor plant into a recipient elite genotype (recurrent parent) in plant breeding is performed through the backcross procedure. The backcrossing process is repeated for as many generations as is needed to create a line that is originally the recurrent parent with the gene of interest from the donor parent. The goal of backcrossing is to obtain a line as identical as possible to the recurrent parent with the addition of the gene of interest that has been added through breeding. Molecular markers can be applied to accelerate the reconstruction of the recurrent parent genotype (background selection).

The objective of the analysis presented in this paper was to identify the BC progeny with the highest proportion of the recurrent parent genome (RPG) using SSR markers evenly distributed throughout the genome. Genetic similarity (GS) values calculated on Dice coefficient between the recurrent parent and BC progenies were in the range from 0.85 to 1. Progenies with the RPG recovery over 95% ( $GS \geq 0.95$ ) were selfed and other progenies ( $GS \leq 0.95$ ) were used for further backcrossing. Application of molecular markers increased the selection efficiency, since the breeding population and the number of generations required to produce the desired genotype were reduced.

*Keywords: backcross selection, molecular markers, genetic similarity*

## IDENTIFICATION OF *Fusarium cf. incarnatum* CAUSING DRY ROT IN *Solanum tuberosum* IN SERBIA

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*Fusarium* dry rot is one of the most important postharvest diseases of potato (*Solanum tuberosum* L.), affecting tubers in storage as well as whole seed and seed pieces after planting. Dry rot is caused by several *Fusarium* species and the most common are *F. sambucinum* and *F. solani*. Seeding dry rot infected tubers can reduce crop establishment because of rotting of potato spouts and decaying of whole plants. Dry rot can greatly affect tuber quality as well and severely reduce its market value. *Fusarium* dry rot is manifested by brown to black internal discoloration of tubers with a dark sunken area on the tuber surface and internal cavities. Seed decay results in reduction of crop establishment and stunted plants.

Samples of potato tubers cv. Kennebec showing typical dry rot symptoms were collected at Medveđa locality (Rasina district) in 2015. Symptomatic tubers with brown, dry decayed tissue and cavities were incubated at room temperature in the moist chamber for 72 h, when mycelial growth was observed. A fragment of mycelia was directly transferred to sterile PDA. After incubation at 25°C under 12 h of fluorescent light for 7 days, five monoconidial isolates were obtained with uniform *Fusarium*-like morphological features. The isolates formed fast growing powdery white to pink colonies. Macroconidia were straight to slightly curved, usually 5-septate, with an average size of 40.35 × 5.75 µm. After 10 days of incubation on Carnation Leaf-Piece Agar (CLA) intercalary chlamydospores were formed, while microconidia were absent. All these morphological characteristics are consistent with a description for *Fusarium cf. incarnatum* (Desm.) Sacc. Pathogenicity of the selected isolate was confirmed by inoculation of potato tubers cv. Kennebec. Whole tubers with 4 mm long sprouts were longitudinally cut and open cut surfaces were sprayed with approximately 1 ml of conidial suspension (1 × 10<sup>4</sup> conidia ml<sup>-1</sup>). Control seed tubers were inoculated with sterile distilled water. Inoculated tubers were incubated in the dark at 18°C and 95% relative humidity for 30 days. All inoculated tubers developed typical *Fusarium* dry rot symptoms, and *F. cf. incarnatum* was successfully reisolated, thus fulfilling Koch's postulates. Morphological identification was confirmed by amplification and sequencing of a portion of the translation elongation factor-1 alpha (TEF-1α) gene. Total DNAs were extracted directly from fungal mycelium with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and PCR amplification was performed with primers EF-1/EF-2. Sequence analysis of TEF-1α region revealed that representative isolate MKF1 (GenBank Accession No. KU923386) shared 99% identity (100% query coverage) with sequences of 14 *F. cf. incarnatum* isolates deposited in the GenBank. Further characterization and research on diversity of pathogens causing *Fusarium* dry rot on potato in Serbia are needed. Possible emergence of new pathogenic isolates and species could lead to a higher incidence of the disease and economically even more significant yield losses.

**Keywords:** *Potato, Dry rot, Fusarium cf. incarnatum, TEF-1α*

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## MORPHOLOGICAL AND MOLECULAR DETERMINATION OF SOME SPECIES OF GENUS *Cuscuta*

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With more than 200 species and over 70 varieties, the genus *Cuscuta* L. (dodders) is one of the most diverse and challenging group of parasitic plants. Parasitism is frequently associated with the extreme reduction or modification of vegetative structures as well as rampant convergence with other parasitic taxa, rendering an assessment of homology with other lineages quite hard. For these reasons, parasitic plants in general, and holoparasites, in particular, have been notoriously difficult to study from a systematic and taxonomic point of view.

To identify species of the genus *Cuscuta* plant material (seed and stem) is collected in the period from 2008 to 2011 at several sites. Scanning electron microscopy was used to study the surface structure of dodder seed, as well as to analyze the parts of the flower: floral sheath (perianthium), pestle (gynaecium) and the stamens (androeceum). Recording was done in the scanning electron microscope (SEM Joel JSM-6390LV). Light microscopy (SEM) was used to study the morphology of field dodder seeds. Visualization was made on a light microscope LEICA DMLS, photographs were made by digital camera LEICA DC 300, and measurement of seed morphological characteristics (length and width) were processed in the LEICA IM 1000 software package.

DNA extraction was performed according to the protocol for the DNeasy Plant Mini Kit (Qiagen, Germany). The success of the extraction was verified by agarose gel electrophoresis. The polymerase chain reaction (PCR) was performed in the laboratory in Toronto, University of Toronto Mississauga, Department of Biology, Canada. Sequences generated in this study were stored in GenBank (accession numbers: KC569803 and KC569804).

Using scanning electron and light microscopy and molecular techniques in 23 examined populations of field dodder (seed, stem, flowers), two species of the genus *Cuscuta* were determined, as follows: *C. campestris* Yunk. and *C. epithymum* (L.) Nath. For the purpose of identification of molecular sequence analysis BLAST (Basic Local Alignment Search Tool) was applied, which showed that the 22 populations of dodder (*C. campestris* Yunk.) collected in the territory of Serbia have nucleotide identity with a maximum of 100% with the sequences contained in the GenBank database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) under the following accession numbers: EF194451, EF194453, EF194454. In contrast to them, BLAST analysis of the query sequence of one population (*C. epithymum* (L.) Nath.) found it to have 99–100% identity with nucleotide sequences contained in the GenBank database under the following accession numbers: AJ430069, AJ430070, AJ430072.

**Keywords:** *Cuscuta campestris*, *Cuscuta epithymum*, molecular determination

## TOTAL PHENOLIC AND FLAVONOID CONTENTS, ANTIOXIDANT AND ANTINEURODEGENERATIVE ACTIVITIES OF FRUIT METHANOL EXTRACTS OF KARAMANKA PEAR

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Karamanka pear is naturalized variety of *Pyrus communis* L., originated from Karaman, Asia Minor. Fruit is medium-sized, pear-shaped, asymmetrical with characteristic pronounced bumps. The flesh is yellowish, melting, juicy and sweet, with characteristic musky smell. Until the WW II Karamanka was the most common pear variety in Serbia, Bosnia and Macedonia. Presently, it is restricted to the valley of the Zapadna Morava, and regions of Vranje, Toplica, Raška and Metohija. For this analysis, pear fruits were collected in Šumadija from organic production. Extraction of flesh, peel and mixed flesh and peel was done with methanol. Total phenolic and flavonoid contents and antioxidant activity (DPPH, ABTS and FRAP assays) of crude extracts (at a concentration of 25 µg/ml), were measured spectrophotometrically. Antineurodegenerative activity was determined against enzymes acetylcholinesterase (AChE) and tyrosinase (TYR) using 96-well plate spectrophotometric methods at concentrations of 25 µg/ml, 50 µg/ml and 100 µg/ml. The peel extract was the richest in phenolics and flavonoids (216 mg GAE/g and 29.13 mg QE/g, respectively), followed by mixed and flesh extracts. The methanol extract of peel showed the strongest antioxidant activity in DPPH test (8.51 µg/ml), ABTS assay (2.53 mg Vit C/g), and FRAP test (1198 µmol Fe(II)/g), compared to mixed and flesh extracts. Inhibition of AChE was concentration-dependent, with the highest values for peel extract, ranged from 30.60 to 36.69%, while positive control galanthamine ranged from 42.38 to 57.11%. Tyrosinase inhibitory activity of extracts was lower compared to standard kojic acid, with the highest value for peel extract at 50 µg/ml (21.28%). Peel extract possessed the highest phenolic and flavonoid contents and the strongest antioxidant and antineurodegenerative activities comparing to flesh and mixed flesh and peel extracts. The presented results revealed that Karamanka pear could be considered as an important source of natural bioactive compounds.

**Keywords:** Karamanka, fruit, phenolics, antioxidant activity, antineurodegenerative activity

## WATER-SOLUBLE CARBOHYDRATE ACCUMULATION IN PEDUNCLE OF WHEAT AND ITS RELATIONSHIP TO MORPHO-ANATOMICAL TRAITS

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Water-soluble carbohydrates (WSC) accumulating in internodes of the wheat stem can be a consequential contributor to grain filling, especially under stress conditions when assimilation is limited. The peduncle located at the first internode directly below the spike, accounts for a considerable proportion of the total stem length in wheat and is thus an important organ for carbohydrate storage. In this study, the WSC content and the WSC specific content (WSSC) were determined in the uppermost internode (peduncle) of the main stem at 10 days after anthesis (10 DAA) across 44 genotypes in two-year field trials. The Random Forest (RF) method was used to define variable importance for water-soluble carbohydrate accumulation (WSC) and its linear density (WSSC). Not a single trait showed particularly high or distinct influence from other traits on WSC or WSSC. Several traits such pith intercellular of peduncle, chlorophyll content and flag leaf area showed to be among the most important traits for WSC in both years. Pith intercellular of peduncle was far the most important for WSC in the first year with 18.39%, but in the second year its impact on WSC was not high, 5.73%. The opposite was true for peduncle extrusion. Its impact on WSC was 7.66% in the first year, but in the second year it was 10.49%. The importance of the chlorophyll content in flag leaf for WSC was 9.84% and 8.92% in both years, respectively, and the importance of the flag leaf area was 8.76% in 2011 and 12.17% in 2012. Biomass per main stem with the importance of 12.77% and 12.12% in both years, area of parenchyma with the importance of 11.91% and 9.06% in 2011 and 2012, respectively and the chlorophyll content with the importance of 10.80% in 2011 and 12.28% in 2012 were the most important traits for WSSC in both seasons. Thus, the chlorophyll content appeared to be the most influential trait for both WSC and WSSC.

*Keywords: wheat, water-soluble carbohydrates, peduncle, grain weight, defoliation*

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## CHEMICAL COMPOSITION AND ANTIMICROBIAL PROPERTIES OF *Salvia officinalis* L. ESSENTIAL OIL

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Essential oil of *Salvia officinalis* L. was investigated for its chemical composition and antimicrobial properties.

*Salvia officinalis* leaves were imported from Montenegro where they were collected at the end of May 2012, during the flowering. The essential oil was obtained by hydrodistillation of dried plant material using a Clevenger apparatus. The essential oil composition was determined by GC-FID and GC-MS. Components of the volatile oil were identified based on their retention indices and their mass spectra using the Wiley and NIST Mass Spectral Libraries or literature data.

Forty-three compounds were characterized in this oil, representing 95.19 % of the total oil.  $\alpha$ -Thujone (22.28 %), camphor (21.72 %) and 1,8-cineole (9.71 %) were predominant components of the volatile oils.

Antimicrobial properties of the *Salvia officinalis* essential oil were tested against common food-borne pathogens, seven G (+) bacteria, eight G (-) bacteria strains and one yeast. The minimum inhibitory concentrations (MIC), as well as minimum lethal concentrations (MLC) of the essential oil, were determined by broth microdilution assay. Concentrations in the range of 7.50 mg/mL-0.47 mg/mL were tested. The essential oil exhibited stronger antibacterial activity against Gram (+) than against Gram (-) bacteria, with MIC values ranging from < 0.47 mg/mL to 3.75 mg/mL and MLC values ranging from < 0.47 mg/mL to  $\geq$  7.50 mg/mL. Inhibitory and lethal concentrations against Gram (-) bacteria were in the same range of concentrations, from 3.75 mg/mL to  $\geq$  7.50 mg/mL. The *Salvia officinalis* essential oil also showed activity against the yeast *Candida albicans* (MIC=1.87 mg/mL and MLC=3.75 mg/mL).

**Keywords:** *Salvia officinalis*, essential oils, GC-MS, antimicrobial properties, MIC

## NEW MOLECULAR METHODS FOR UPGRADING RESEARCH OF PLANT VIRUSES IN SERBIA

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In the frame of AREA project a combined research between two laboratories in Serbia and Italy has been established with the aim to improve research capacity in the most important fields in plant virology and mycology of Microdiag team. Plant pathogens including emerging, re-emerging, and chronic/ endemic, are attracting attention of researchers all over the world for its capability to cause prompt epidemics, and thus serious yield losses. Application of molecular tools is of great importance allowing getting reliable results in detection, identification and characterization of plant viruses, fungi and fungus-like organisms. During our cooperation with colleagues from the University of Aldo Moro, Bari, Italy, we have improved our skills in molecular detection of plant pathogens due to adopting new methods for molecular hybridization and cloning of PCR products. Dot-blot hybridization was used for detection of *Cucumber mosaic virus* (CMV), as well as satRNA of CMV in weed samples using DIG nucleic acid detection kit (Roche, Switzerland). The cloning of coat protein (CP) and movement protein (MP) genes of several Serbian CMV isolates originating from different hosts was carried out using pGEM<sup>®</sup>-T Easy Vector system (Promega GmbH, Mannheim, Germany). Molecular cloning based on separation of a particular DNA fragment from a complex mixture of fragments and producing large numbers of its copies allows identification of number of haplotypes as well as predominant haplotypes existing in natural population of CMV. Furthermore, a computer *program* for analyzing recombination events-RDP3 (Recombination Detection Program) was adopted allowing us to use it for detection of recombinant isolates within the natural population of CMV originating from Serbia. This cooperation also enabled understanding of basic principles of post transcriptional gene silencing (PSTG) and acquiring a very reliable method of measuring gene expression using reverse transcription real-time quantitative PCR (RT-qPCR) technique. RT-qPCRs were performed using the StepOne Real Time PCR System (Applied Biosystems, USA) and 2X Fast SYBR Green PCR Master Mix (Applied Biosystems) as recommended by manufacturer instructions. It is known that PSTG can be triggered by many different factors. The goal of this study was to determine the influence of grafting on virus accumulation and accumulation of RNA silencing associated proteins in grafted plants challenged with *Potato virus Y* (PVY). Also, we wanted to determine if resistant traits against PVY, could be transferred to susceptible cultivars by grafting such cultivars on a resistant rootstock. Local Apulian tomato variety 'Manduria' was used as the resistant rootstock, while UC82 was used as a susceptible scion. Working on the influence of grafting on viral infection, as a possible trigger for PTGS, we were able to demonstrate its potential *usage as a viral disease control* strategy. Beside benefits in acquiring several new methods which improved the expertise of our laboratory for plant virology and mycology, cooperation with Italian colleagues resulted in the publication of one oral presentation at International Working Group of Legume and Vegetable Viruses, in the Netherlands in 2015.

**Keywords:** *PCR cloning, Dot-blot hybridization, RDP3 program, Real-time qRT-PCR, PSTG*

**Acknowledgement:** This research was supported by the project III 43001 funded by the Ministry of Education, Science and Technological Development, Republic of Serbia and the EU Commission project AREA, No. 316004.

## ***Alternaria carotiincultae* - A NEW THREAT FOR CARROT PRODUCTION IN SERBIA**

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World popularity and consumption of carrots (*Daucus carota* var. *sativus* Röhl.) are exhibiting an upward trend. Over 10 million tons of carrot is produced on about 250 000 ha in Europe, which makes it the second largest producer in 2011. Carrot production in Serbia is significant, and in 2014 over 65 000 tons were produced on approximately 7 000 ha. Diseases caused by fungi, especially by species belonging to the genus *Alternaria* are among the most important factors limiting carrot production worldwide. In different carrot growing regions, seven *Alternaria* spp. are described to be pathogens of different importance, while in Serbia, recently, four species have been detected, *A. dauci* (J. G. Kuhn) J. W. Groves & Skolko, *A. radicina* Meir, Drechsler & E. D. Eddy, *A. petroselini* (Neerg.) E. G. Simmons and *A. alternata*, all causing significant yield losses annually in certain years. In order to identify seed-borne species of *Alternaria* causing disease on carrot and to establish their presence in Serbia, an extensive survey was undertaken from 2003 to 2014. Over 60 isolates of *Alternaria* spp. have been obtained from commercial seed samples, necrotic lesions on the leaves and taproots, as well as from infested soil using baiting methods. Pathogenicity of all monosporic isolates was confirmed using spore suspension spraying of carrot seedlings and subsequent development of typical symptoms. Conidia and colony (on PDA and CLA) characteristics, including colony margins and pigment production, crystals and microsclerotia, as well as conidia measurements and catenulation, revealed the presence of previously reported *A. dauci*, *A. radicina* and *A. alternata*, but also indicated the presence of a new species in Serbia, *Alternaria carotiincultae* E. G. Simmons. Closely related *A. radicina* and *A. carotiincultae* are similar, thus particular isolates share morphological and pathogenic features. For further confirmation, selected isolates were molecularly characterized by sequencing various genomic fragments, including ITS and mitochondrial small subunit (mtSSU) rDNA, and major allergen Alt a1, beta-tubulin, and translation elongation factor-1 alpha (EF1a) protein-coding genes. Among selected genomic fragments, sequence analysis of Alt a1 gene proved to be a useful identification tool, and coupled with morphological and pathogenic characteristics allowing successful differentiation between *A. radicina* and *A. carotiincultae*. Comparing to the other detected *Alternaria* spp., Serbian isolates of *A. carotiincultae* were significantly more pathogenic for carrot seedlings, thus representing a serious threat for carrot production.

**Keywords:** *Alternaria carotiincultae*, carrot, pathogenicity, conventional identification, molecular identification

**Acknowledgement:** This research was supported by the project III 43001 funded by the Ministry of Education, Science and Technological Development, Republic of Serbia and the EU Commission project AREA, No. 316004.

## FARMING OF ONE-MONTH-OLD CARP IN MONOCULTURE: SINGLE VS. MIXED AGE GROUPS

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Common carp (*Cyprinus carpio*) is the most commercially important fish species cultured in warm water fish ponds in Serbia. The typical production cycle involves purchasing and stocking one-month-old carp in fishponds where they are farmed until marketable size, subsequently harvested and placed on the market. Younger carps are particularly sensitive compared to older categories; therefore, special attention should be paid during the rearing process. Application of agrotechnical measures, type of feed, pond size, stocking density and type of farming are issues that should be carefully considered prior to the beginning of production. One-month-old carp are usually reared in monoculture as a single age group. Apart from easy control of the production and utilization of only one type of feed, the major drawback to this type of farming is incomplete exploitation of natural and/or supplemental feed. The advantages of culturing mixed age groups of carp together (e.g. one-month-old and yearlings) are in the positive interaction between different age categories due to their different food niches and better utilization of pond space. The aim of this study was to compare the growth rate of one-month-old carp reared in monoculture as single versus mixed age groups.

The experiment was carried out in field conditions at two fish farms “Banatska Dubica” and “Neuzina”. During one production season, from May to October, one-month-old fish of average weight 4.2g were stocked into ponds as single and mixed age groups (one-month-old and yearlings). The results showed that survival of one-month-old fish in ponds as a single age category was from 40% to 70% depending on the size of the pond (1.7–34.3ha). In ponds with mixed age groups, higher survival (<50%) was observed in the smaller pond (64.4ha). Average individual weight of fish depended on the type of rearing system and was higher in ponds with mixed age groups (approximately 130g) than in ponds with one-month-old carps (50g), while survival increased with the decline of average individual weight.

Food conversion rate (FCR) was in the range from 4.4 to 5.4 in all ponds, and did not depend on the survival rate, pond size, type of supplemental feed or type of rearing system. The only exception was pond 1 at fish farm Dubica, where fish were fed with supplemental feed containing 50% of cereals and 50% compound feed, where FCR was 3. The results show that the best survival was obtained in bigger ponds stocked with the single age group. Better survival was also obtained in ponds fed with the mixture of cereals and compound feed compared to ponds fed only cereals, but did not affect the final average individual weight. Higher final average individual weight was found in ponds with mixed age groups that had lower survival and lower stocking density. In conclusion, it could be advised to culture one-month-old carp in mixed age groups, i.e. older fish age groups, in ponds with a surface area of around 30 ha fed compound supplemental feed. This would provide better survival, improved feed utilization and higher final fish weight.

**Keywords:** common carp, survival, growth rate, single age group, mixed age group

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## EVALUATION OF PRODUCTION PERFORMANCE OF CARP YEARLINGS WITH DIFFERENT STOCKING DENSITIES

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Common carp is the most farmed fish species in the world presenting 18% of total production of Cyprinids. This fish species is one of the most important warm water fish in Central and Eastern European countries, and Asia, particularly China.

In Serbia, common carp is mostly reared in semi-intensive production systems based on the utilization of natural food (zooplankton and zoobenthos) in combination with supplemental feed in the form of raw cereals, pelleted and extruded feeds. Nutritional requirements of fish are similar to other farmed animals, but fish have higher needs in proteins. Carp growth is significantly affected by technological measures applied during the production cycle, stocking density, pond size, and quality of stocking material.

In Serbian conditions, carp is usually produced in a three-year cycle with the duration of around 30 months. However, in many other countries a two-year cycle is more common, lasting for 18 months and is characterized with lower stocking densities. This type of farming is economically more feasible, with a higher growth rate of carp, lower amount of supplemental feed and lower weight of marketable carp (<1200 g).

The aim of this study was to investigate the effect of different stocking densities of carp yearlings in field conditions on the body weight gain per surface area unit (BWG/ha), food conversion ratio (FCR) and survival (SC).

The experiment was carried out in field conditions at two fish farms "Banatska Dubica" and "Neuzina". During one production cycle, from April to October, yearlings with an average weight of 60g were stocked at three different stocking densities, 3000, 4000 and 4500 individuals per hectare.

Survival rate ranged from 37 to 83%. BWG/ha was in the range from 965 to 2029 kg/ha, and FCR was from 3 to 4.7. Pond size and stocking densities were inversely proportional to fish survival due to inadequate pond security measures and higher stocking densities, respectively. The BWG/ha was affected by the survival rate and pond size, while FCR was directly influenced by feed quality and stocking density.

The results show that the stocking density affects fish growth and feed utilization. A lower stocking density promotes lower feed and space utilization. A higher stocking density may improve food conversion ratio, but additionally, can degrade the water quality and consequently affect the fish health and survival rate.

Common carp is highly tolerant to variable environment factors and farming conditions. Nevertheless, significant consequences on the production may arise when numerous sub-optimal factors are active at the same time.

*Keywords: common carp, semi-intensive system, stocking density, production performance*

*Acknowledgement:* The study was supported by the Ministry of Education, Science and Technological Development, the project Improvement of production capacities of carp (*Cyprinus carpio*) through nutrition and selective breeding programs (TR 31075).

## NUTRITIVE VALUE OF SOYBEAN - REASON OF PRIORITY IN INDIVIDUAL AND COLLECTIVE NUTRITION

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Soybean has a high nutritional value which, in addition to favorable functional properties and low cost, enhances its wide use in various branches of food industry, mass and individual nutrition, dietetics and pharmaceutical industries. Soya beans contain 30–40% of proteins, 12–24% of oils, 30–34% of carbohydrates, 3–6% of minerals and many vitamins. Soy contains a wide range of bioactive compounds (like isoflavones, trypsin inhibitors, lectins) that exhibit therapeutic and preventive effects in the treatment of cancer, heart and blood vessels diseases, which categorizes soy in the group of “functional foods”.

Soy food has long been a staple of the human diet in Asia. Among various soy foods, soymilk and tofu are becoming more popular as low cost substitutes of traditional dairy products for consumers. Okara and whey are by-products obtained during processing for soymilk and tofu. In addition to these, there are a whole range of nutritive-value products of soybean. In our area, the use of soy protein products for human consumption is relatively recent. Traditionally prepared soymilk and tofu have painty and beany flavors. In our societies, this flavor is unacceptable to most consumers and is the major obstacle to widespread acceptance of almost all soy food products, especially soymilk and tofu. Quality of soy protein products is affected by several factors, such as variety of soybean, soybean growing environment and type of tofu processing. The aim of this work was to promote soybeans in the form of nutritionally-valuable products to local consumers. In our previous work, we produced soy protein products on the pilot-plant scale from the six domestic soybean cultivars (Nena, Krajina, Novosađanka, Lana, Balkan, ZPS-015) by the production method that includes hydrothermal cooking (HTC), and chymosin-pepsin rennet for soymilk coagulation (for tofu preparation). This procedure is significantly different from the traditional ones.

The obtained protein products are characterized by very favorable nutritional characteristics (e.g. as a total protein content – soymilk about 47%, tofu about 53%, okara about 30%, whey about 25%) and with very good sensory qualities (e.g. tofu of soybean genotype Lana was scored with the very good grade for taste (4.9) and smell (4.5) under the commission evaluating it by “point system” – 1 to 5). The results of chemical and sensory analyses showing favorable nutritive characteristics of tofu were complemented by microscopy. Microscopy results showed good textural properties of tofu.

Integrating chemical, microscopy and sensory investigations with state of the art technologies in the field of food sciences – HTC treatment opens the way to a new perspective, especially a more effective use of tofu as more acceptable to most consumers as a plant source of nutritionally valuable components.

*Keywords: soybeans, soy protein products, HTC processing, chymosin-pepsin rennet*

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## ZINC NUTRITIONAL STATUS OF MAIZE HYBRIDS GROWN IN VOJVODINA

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Biofortification of staple crops became a widely accepted approach for increasing a nutritional value of food through agronomic practices, plant breeding or modern biotechnology. In Serbia there is a lack of awareness about nutritional quality of crops and beneficial effects of fertilization with micronutrients. The aim of study was to evaluate zinc (Zn) nutritional status in twelve maize hybrids grown at two sites in Vojvodina (Serbia) in 2014. Fully developed leaves were sampled at growing stage of 4-6 leaves. Leaf Zn concentration ranged from 11.7 to 49.4  $\mu\text{g g}^{-1}$  DW. On average over all hybrids, remarkably higher concentration was recorded in Rimski Šančevi (31.2  $\mu\text{g g}^{-1}$  DW) in comparison with Pančevo (20.7  $\mu\text{g g}^{-1}$  DW). Hybrids NS 4030, NS 3022, Aleksandra NS, NS 5063 and NS 5083 grown in Pančevo had leaf Zn concentration below critical deficiency values (15–20  $\mu\text{g Zn g}^{-1}$  DW). Zn deficiency, decrease in shoot and grain Zn concentration can be induced by high application rates of phosphorus fertilizers. Therefore, the relationship between zinc and phosphorus was evaluated by correlation analyses. A weak positive correlation indicated that lower leaf Zn concentrations in maize hybrids grown in Pančevo were not related to fertilization with phosphorus. Results indicate that more attention should be paid to agronomic practices related to the crop nutritional status in intensive agriculture. Hybrids differing in leaf Zn concentration were used for further evaluation and in the study dealing with methods of biofortification: the effect of seed priming with Zn and Zn foliar application on field performance of maize grown at the same sites.

*Keywords: zinc concentration, maize, biofortification*

## EFFECT OF CROPPING SYSTEM ON MICROBIAL ACTIVITY IN CHERNOZEM SOIL

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Soil microorganisms as a heterogenous group of organisms participate by their presence and enzymatic activity in 60–90% of the total soil metabolic activity. The dominance of certain microbial groups directs the processes to either synthesis or decomposition of organic and inorganic matter which gets into soil, thus determining the quality and fertility of soil. The presence of certain microbial groups and their enzymatic activity is affected by a number of abiotic and biotic factors, above all soil type, soil cultivation and use, application of organic and mineral fertilizers, pesticides, amelioration, etc. Our study consisted of monitoring the abundance of copiotrophic and oligotrophic bacteria and dehydrogenase activity (DHA) in a chernozem soil under different cropping systems. Soil samples were taken from a long-term trial with crop rotations, and an international trial (IOSDV) involving manuring and application of harvest residues and increasing N rates. Trials are located at the Rimski Šančevi Experimental Fields of the Institute of Field and Vegetable Crops in Novi Sad. The soil samples were taken during a 2-year period, after winter wheat harvest from three sampling depths (0–20 cm, 20–40 cm, 40–60 cm). The number of examined microbial groups was assessed by the serial–dilution method followed by plating on different selective media, while dehydrogenase enzyme activity was measured spectrophotometrically. The bacterial groups were selected for the study based on their growth on media characterized by high (copiotrophic) or low (oligotrophic) carbon levels, while dehydrogenase activity is an indicator of oxidation-reaction processes and a sensitive indicator of soil quality/fertility. The number of the tested microbial groups and dehydrogenase enzyme activity varied significantly depending on the cropping system, year of study, and sampling depth. The abundance of copiotrophic and oligotrophic bacteria in the studied soil was high ( $\times 10^5$  per  $g^{-1}$  soil). The highest number of copiotrophic and oligotrophic microorganisms was detected in non-agricultural soils. Microbial abundance was promoted by manuring and mineral fertilization, while incorporation of harvest residues demonstrated less effect in comparison with other cropping systems. Soils under wheat monoculture were more abundant in copiotrophs than soils under wheat grown in crop rotation, which is opposite to the results obtained for oligotrophs. Comparison of cropping systems revealed the largest dehydrogenase activity in non-agricultural soils and soils under wheat grown in monoculture. Among different types of applied fertilizers, the best effect on DHA enzyme activity was obtained by ploughing down harvesting residues, followed by a combination of manure and smaller doses of mineral fertilizers, and the lowest effect was obtained by the sole use of mineral fertilizer or manure. A positive correlation between the enzymatic activity and microbial abundance was not determined. An increase in the soil profile depth resulted in reduced values of the tested parameters of microbial activity.

*Keywords: chernozem, copiotrophic bacteria, cropping systems, oligotrophic bacteria, winter wheat*

**APPLICATION OF RAMAN SPECTROSCOPY IN STUDIES OF ESSENTIAL OIL COMPOSITION: EXAMPLE ON THE HALOPHYTE *Artemisia santonicum* L.**

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Essential oils are products of a plant secondary metabolism and are comprised of many individual components, mainly terpene hydrocarbons (monoterpenes and sesquiterpenes) and different oxygenated compounds (aldehydes, ketones, alcohols, esters, phenols, etc.). They are widely used in cosmetics, pharmaceutical and food industry, as well as in traditional and alternative medicine, such as aromatherapy, due to their antimicrobial, antiviral, anticancer and strong antioxidant activity. Most commonly, the complex chemical composition of essential oils is studied by GC/MS. However, the Raman spectroscopy has been recently reported as a useful method for rapid analysis of the composition and quality of essential oil of a different origin. Since essential oils contain a large variety of compounds, it is expected that dominant molecules will reflect a prominent differences in the Raman signals. Despite the differences in the Raman spectra, there are certain common features related to functional groups in compounds presented in essential oils. At least one band in the vicinity of 1450 cm<sup>-1</sup> attributed to bending modes of CH<sub>3</sub> and CH<sub>2</sub> groups can be expected in the Raman spectra. In order to test the validity of Raman spectroscopy for analysis of essential oils, the samples of the halophytic plant *Artemisia santonicum* were isolated and identified by GC/MS. Samples were obtained from six different populations naturally growing on "saline steppe" habitats in northern Serbia. Tested oils differed much in yield and less in composition, whereas 1,8 cineole, cis-thujone, linalool, chrysanthenone, α-ylangene and sabina ketone were the most present. Raman spectroscopy was performed in the range of 500–3000 cm<sup>-1</sup> with XploRA Raman spectrometer from Horiba Jobin Yvon. Raman scattering was excited by a frequency-doubled Nd/YAG laser at a wavelength of 532 nm (maximum output power 20-25mW) equipped with a 1200 gr/mm grating. Complete spectra were analysed by Origin Pro 8.6. The most important bands in the Raman spectrum of essential oil in *A. santonicum*, appeared at 1672, 1640, 1452, 1378, 1293, 803 and 652 cm<sup>-1</sup>. These bands are similar to those observed in an earlier reported Raman spectrum of linalool. The strong band at 1640 cm<sup>-1</sup> is attributed to C=C stretching modes of alkene functional group in the alcohols, and the band at 1452 cm<sup>-1</sup> appears in the typical region for CH<sub>3</sub> and CH<sub>2</sub> bending modes. There are two CH<sub>3</sub> groups directly attached to a C=C in the chemical structure of linalool, the band at 1378 cm<sup>-1</sup> can be assigned to a CH<sub>3</sub> bending mode. The band at 1293 cm<sup>-1</sup> is assigned to the =CH rocking mode, and the band at 803 cm<sup>-1</sup> seems to be related to the OH group of linalool. The band 652 cm<sup>-1</sup> seems to indicate the presence of 1,8-cineole in this essential oil, since a very strong band in this region is expected for the ring deformation of this compound. Raman spectroscopy can be used in the pharmaceutical industry in order to perform fast quality checks of incoming raw materials.

**Keywords:** essential oil, 1,8-cineole, linalool, Raman spectroscopy

## MICROCONIDIAL STATE OF FOUR *MONILIA* SPECIES ISOLATED FROM APPLE FRUIT

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*Monilinia* spp. like many other fungi have an ability to form a conidial and microconidial state. Conidia have a role in the infection process, while microconidia may lead to apothecial development. These structures have a biological function, but their morphology and size can be useful in identification. There is no data regarding existence and description of the microconidial state of *Monilia polystroma*. The aim of this study was to initiate the formation of microconidia of *M. polystroma* and to compare their morphology with *M. fructigena*, *M. fructicola* and *M. laxa*. Studied isolates originated from apple fruit with brown rot collected in orchards and storage facilities in Serbia. The formation of microconidia was initiated on V8 medium for seven isolates of *M. fructigena*, seven isolates of *M. polystroma*, and three isolates of *M. laxa*, and on PDA medium for two isolates of *M. fructicola*. After three weeks of incubation under 12 h photoperiod at 22±1°C formation and characteristics of microconidia of all isolates were examined. Microscopic features were observed using a Leica DMLS compound light microscope (Leica Microsystems Wetzlar GmbH, Wetzlar, Germany) equipped with a Leica DC 300 digital camera and IM1000 image analysis software. For each isolate, 50 measurements were obtained for microconidial size. For SEM analysis of the microconidial state, mycelial fragments of *M. polystroma* (MPSRB-3) and *M. fructigena* (MFSRB-1) isolates were prepared as described by Soylu et al. (2006) with modifications and observed using a scanning electron microscope (JEOL, JSM 6460, Japan). The microconidial state of *M. fructicola* was apparent macroscopically as black raised areas, while the microconidial state of other species was observed only microscopically. Microconidia of *M. polystroma*, *M. fructigena*, *M. fructicola* and *M. laxa* occurred on individual phialides throughout the colonies or were grouped in microconidial clusters. Isolates of the four species did not differ in morphology of phialides and microconidia, nor in microconidial size. Microconidia were produced on phialides, which were bottle shaped, often asymmetric, having very narrow neck straight to slightly curved, arising from vegetative hyphae, conidiogenous chains or from microconidiophores. Microconidia were unicellular, globose, hyaline, measuring 2.19–3.44 µm for *M. polystroma*, 2.27–3.56 µm for *M. fructigena*, 2.29–3.84 µm for *M. fructicola* and 2.18–4.63 µm for *M. laxa*, while means were 2.73, 2.89, 2.83 and 2.99 µm, respectively. This study has shown that *M. polystroma* is capable of forming the microconidial state with similar morphological features as other *Monilinia* spp. originating from apple fruit.

**Keywords:** *microconidial morphology and size, M. polystroma, M. fructigena, M. laxa, M. fructicola*

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**INFLUENCE OF SANIGOL WATER SOLUTION ON ANTIMICROBIAL  
ACTIVITY AND SURVIVAL RATE OF *E. coli* O 157 UNDER  
*in vitro* CONDITION**

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The diseases of the bacterial etiology present a very important factor in poultry production which could cause infection with a high rate of morbidity and mortality and therefore lead to high economic losses. Zoonotic potential of the same microbial agents like *Salmonella spp.* or *E. coli* presents a special epidemiological problem in poultry farming making a potential risk for human health, mostly to people who work with poultry or to the consumers who eat contaminated poultry meat or eggs. Therefore, the aim of this study was to examine the influence of Sanigol based perchlor vinegar acid water solution on antimicrobial activity and the survival rate of *E. coli* O157 under *in vitro* condition. The test was performed in a volume of 200 µl with final concentrations of Sanigol 50, 25, 15, 10, 5 and 1% in Mueller Hinton Broth (MHB). Plates were incubated at 37°C for 24 hours. The same tests were performed simultaneously for growth control (MHB + test organism) and sterility control (MHB + Sanigol). After 24h of incubation, 10 µl of the resazurin solution was added to each well and the plate was reincubated overnight. A change of color from blue (oxidized) to pink (reduced) indicated the growth of bacteria. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of each drug that prevented this change in color. The result of investigation shows that the Sanigol solution in concentration of 5% leads to MIC of *Listeria monocytogenes* and *Staphylococcus aureus*, followed by 10% solution to MIC of *Salmonella Typhimurium* and *E. coli* O157. Regarding the minimum bactericidal concentration (MBC) the obtained results show that the Sanigol water solution of 15% leads to MBC of *Listeria monocytogenes* and *Staphylococcus aureus*, followed by 25% solution to MBC of *Salmonella Typhimurium* and *E. coli* O157. For determination of bacterial survival rate in 10% solution of Sanigol, suspension of *E. coli* was added in concentration of 10<sup>7</sup> CFU/mL, followed by monitoring of bacterial count reduction in next 30, 60 and 120 min. After 30 min total count of *E. coli* O157 was reduced to 1.66%, after 60 min to 0.05% and after 120 min to 0.00%. Based on the obtained results, it can be concluded the Sanigol can be successfully used as a disinfectant with the high antibacterial action in poultry houses.

**Keywords:** disinfectant, poultry, *Salmonella spp.*, *E. coli*

## VELVETLEAF (*Abutilon theophrasti* MEDIK.) PRODUCTIVITY IN COMPETITIVE CONDITIONS

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*Abutilon theophrasti* Medik. (syn. *Abutilon avicenae* Gaertn.) has been cultivated in China since the beginnings of civilization as a fiber plant. From China it spread through Asia Minor to the Balkan Peninsula as a potential fiber crop plant. Today velvetleaf is a harmful invasive weed on many arable lands in Serbia and is especially problematic where maize, soybean, sugar beet or sunflower are major crops. The successful colonization by velvetleaf can be explained by its biological-ecological set of traits and inadequate weed management on arable and non-arable lands. Weed-management techniques that reduce weed production need to be investigated to provide new approaches to weed management. The first step in that process is determination of weed productivity in different competitive conditions. Field experiments were conducted at an experimental field (Padinska Skela near Belgrade) to quantify growth and seed production of velvetleaf in maize, as well as in velvetleaf monoculture during two years. A density of velvetleaf ranging from 1 to 8 plants m<sup>-1</sup> was artificially created. In a mixture with maize, velvetleaf was sown in crop rows. The growth of velvetleaf was estimated based on plant height, fresh biomass and leaf area index. Velvetleaf fecundity was determined as seed mass plant<sup>-1</sup> and seed mass m<sup>-2</sup>. Differences between years in plant production were very prominent. Generally, velvetleaf productivity in maize depended on its density. Intra-specific competition had a major influence on growth and seed production when velvetleaf density was from 4 to 8 plants m<sup>-1</sup> in maize rows. This information indicates that environmental conditions and weed density can promote/reduce inter- and intra-specific competition and help in the construction of population dynamics models to predict population density, seed bank and competitiveness of weeds and reduce inputs for weed management.

*Keywords: Abutilon theophrasti, maize, competition*

*Acknowledgment:* This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project III 46008) and EU FP7 REGPOT-AREA Project No. 316004.

## MULTIPLEX RT-PCR IN DETECTION OF THREE VIRUSES INFECTING GARLIC IN SERBIA

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Garlic and other species of *Allium* genus are traditionally grown and are very important for the Serbian agriculture. Because of its vegetative propagation, garlic is frequently infected in the same time with several viruses belonging to the genera *Potyvirus* and *Carlavirus*. Because of similar symptoms and certain difficulties in applying serological assays such as enzyme-linked immunosorbent assay (ELISA), garlic viruses may be very difficult to differentiate. For that reason, the fast and reliable method for simultaneous detection of garlic viruses is of great importance and needed for their screening. Reverse transcription-polymerase chain reaction (RT-PCR) contributed to the diagnosis of viruses infecting garlic. To improve the diagnostic capacity, in this research multiplex RT-PCR has been developed as a rapid and convenient screening assay, for simultaneous detection of more than one virus in the same reaction.

Multiplex RT-PCR for detection of mixed infection of three garlic viruses, *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV) and *Garlic common latent virus* (GarCLV) has been developed using previously described virus specific primers: 1-OYDV/2-OYDV, 1-LYSV/2-LYSV and 1-GCLV/2-GCLV, respectively. Specific primers for all selected viruses targeted the coat protein (CP) gene which is one of the most conserved regions. Additionally, primers were selected according to similar melting temperatures and different amplicon sizes, ranged from 409 to 960 bp, which is convenient for separation in the gel electrophoresis. Total RNAs from five selected garlic samples were extracted with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, and subjected to simplex RT-PCRs. RT-PCR was carried out with OneStepRT-PCR Kit (Qiagen) using specific primers to confirm primer specificity and efficiency in detecting of all three viruses. After that, multiplex RT-PCR was carried out with the same samples using the mix of all three primers in order to detect the presence of viruses. Annealing temperature was adjusted to 50°C, as the most suitable. The obtained results of multiplex RT-PCR were in complete agreement with individual RT-PCR results. Amplicons of the predicted sizes were obtained in testing of all samples regardless of single or mixed virus infection. This protocol represents the important contribution to the research on garlic viruses in Serbia allowing simultaneous detection of three garlic viruses and thus reducing testing cost. Furthermore, this protocol could be applied in detection of these three viruses in other host plants belonging to *Allium* species enabling faster detection and raising the capacity of reference laboratories.

**Keywords:** *multiplex RT-PCR, garlic, Onion yellow dwarf virus, Leek yellow stripe virus, Garlic common latent virus*

**Acknowledgement:** This research was supported by the projects TR 31018 and III 43001 funded by the Ministry of Education, Science and Technological Development, Republic of Serbia and the EU Commission project AREA, No. 316004.

## BIOTECHNOLOGICAL NOTE: WHAT CAN BE INFERRED FROM THE MOSS SALT RESISTANCE?

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Bryophytes (i.e. mosses) emerged to the terrestrial ecosystems among the first green plants. This diverse group of plants faced many new problems and developed different mechanisms to cope with those. Here, we present the findings on the resistance to salt stress elaborated in three moss species two of which can be found in nature exclusively on salt soils, namely *Entosthodon hungaricus* (Boros) Loeske and *Henediella heimii* (Hedw.) Hampe. Even though these two moss species are considered as moss-halophytes, experiments show that they are in fact facultative moss halophytes since they developed quite well with no salt addition in cultivation medium. They rather stay attached to salt condition due to low competitive value to other bryophytes and vascular plants. All tested species react differently to salt stress. The cope mechanisms seem to be different in all three species tested. It has been shown that it is connected with the sugar economy. The total sugar contents vary among the species and the newly syntheses of sucrose, fructose and/or glucose appear in a different time frame within the moss cells. Also, the induction and the pathways of sugar syntheses seem to be differently activated in each of tested species. Also, ABA played some role in postponing the response to salt stress induction. Besides, the enzymatic components of anti-oxidative response significantly vary among the species as well as non-enzymatic components such as phenols or amino acids. Here, we give an overview of results we achieved in mosses in comparison with the known mechanisms of higher plants.

*Keywords: abiotic stress, bryophytes, Entosthodon hungaricus, Henediella heimii*

**CONTROL OF *Ostrinia nubilalis* Hbn. AND  
*Helicoverpa armigera* Hbn. IN SEED MAIZE CROP**

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The economic importance of maize is derived from the properties of the plant itself, diversity, its use and production volume. *O. nubilalis* and *H. armigera* regularly occur and cause significant damages in maize crops in Serbia, particularly under global warming conditions. Several measures are applied against these pests (crop rotation, tolerant and resistant hybrids, monitoring, forecast, chemical measures). The trials were conducted in 2015 at two localities (Kula and Nova Kula) on seed maize, hybrids DKC 4608 and DKC 4014, according to standard OEPP methods (PP1/13; 1/152; 1/135). Products on the basis of deltamethrin (100 g a.i./l of product) at rates of 0.075 and 0.125 l/ha and chlorantraniliprole (200 g a.i./l of product) at rate of 0.15 l/ha, were applied. Treatments were conducted with tractor sprayers (high clearance). The plot size was 7500 m<sup>2</sup>. Three assessments were made. The first one prior to treatment (on the 6<sup>th</sup>, i.e. on the 10<sup>th</sup> of August), on 25 randomly selected plants per repetition, and the number of *O. nubilalis* and *H. armigera* egg masses and larvae on silk were registered. In the second assessment (on the 18<sup>th</sup>, i.e. on the 24<sup>th</sup> of August), on 20 randomly selected plants per repetition, the number of damaged plants and ears and the number of vital larvae were found. In the third assessment, prior to harvest (on the 8<sup>th</sup> of September) on 20 randomly selected plants per repetition, the number of damaged plants, plants broken above and below ear (fallen on the ground), damaged ears and vital larvae, was determined. Results are presented as means, efficacy (E%) according to Abbott and significance of differences by LSD test (5%). At the Kula locality, egg masses of *O. nubilalis* were registered on ear silk on 8-14% of plants and larvae on 6-11% and larvae of *H. armigera* on 2-7%. At the N. Kula locality, egg masses of *O. nubilalis* were present on 1-4% of plants and larvae on 9-12%, while *H. armigera* larvae were found on 1% of plants. Eighteen days after treatment at the Kula locality percentage of damaged plants was 6.2–7.5% and at significantly lower level compared to control where 37.5% of damaged plants were recorded. The percentage of damaged ears was 5-15%, and in control 51%. Vital larvae of *O. nubilalis* were present on 3.8-6.3% of plants, depending on the insecticide and application rate, and in control on 27.5%. At the N. Kula locality, 14 days after the end of treatment, the percentage of damaged plants per treatment ranged from 3.7 to 8.7%, and in control 35%, while the percentage of damaged ears was 3.7-10%, and in control 48.7%. The presence of *O. nubilalis* vital larvae was recorded on 2.5-6.3% of plants, and in control on 22.5%. Prior to harvest, deltamethrin efficacy at the Kula locality, depending on application rate and counted parameters, was 80.4-100%, while at the N. Kula locality it was 70.5-100%. Efficacy of chlorantraniliprole, depending on counted parameters, at Kula and at N. Kula localities was 80-90% and 73.5-90.9%, respectively.

**Keywords:** seed maize, *O. nubilalis*, *H. armigera*, deltamethrin, chlorantraniliprole

## OPTIMIZATION OF RT-qPCR ASSAY FOR EVALUATION OF ANTIVIRAL EFFECTS OF MEDICINAL PLANTS EXTRACTS

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In the recent times, human enteric viruses have been increasingly identified as the leading cause of food-borne diseases. This group of viruses of significant public health concern comprises human noroviruses, hepatitis A virus, rotaviruses, sapoviruses, Aichi virus, adenovirus, hepatitis E virus, astroviruses, etc. However, human noroviruses are by far the most prevalent cause of epidemic and sporadic food-borne gastroenteritis in industrialized countries. Other virus of significant concern is hepatitis A virus, which has much lower prevalence, but causes a severe illness with a higher rate of hospitalization and death. According to the 2016 report of the European Food Safety Authority and the European Centre for Disease Prevention and Control, the most food-borne outbreaks, in 32 European countries in 2014, were caused by viruses.

One of the major difficulties in norovirus research is the absence of available animal cell-culture systems for their propagation. In order to evaluate virus inactivation by any process or treatment, cultivable surrogates such as feline calicivirus, murine norovirus, etc. have to be used. However, the latest data suggest that noroviruses are often more resistant to typical food and environmental control measures compared with surrogate viruses. Therefore, in this work we introduce the optimization of an assay to evaluate the antiviral effect of plants extracts on Norovirus GII particles. Norovirus GII suspension was obtained from the previously identified positive human fecal sample with high load of the particles. Number of viral RNA genomes, after treatments, was quantified with reverse transcription – quantitative PCR (RT-qPCR). Kit Ribo Virus (Sacace, Biotechnologies) designed for the rapid preparation of highly pure viral nucleic acids from fluid biological samples has been used for norovirus RNA isolation. Real-time RT-PCR was carried out using PrimerDesign™Ltd advanced kit (genesig) for quantification of norovirus GII, which contains RNA dependent RNA polymerase primer/probe mix and positive control template for Standard curve, including all necessary internal extraction controls.

For the evaluation of antinoroviral properties, twelve plant extracts have been selected (e.g. *Helichrysum plicatum*, *Hypericum perforatum*, *Satureja subspicata*, *Punica granatum*, etc.) with different active compound compositions. Since many of these compounds have an inhibitory effect on RT-qPCR reactions, it was necessary to establish, individually for each extract, the lowest concentration that did not influence the RNA isolation and PCR. The norovirus RNA recovery assay was designed to test the inhibitory effects of plant extracts. If more than 80% (compared with the control) of RNA was recovered, it was considered that extract did not inhibit the assay. Based on the obtained results, a preliminary set of experiments was carried out with different contact times with norovirus particles at two distinct temperatures (25°C and 37°C). The initial results are promising, nevertheless further investigation is required.

Overall, the progress towards the development of nontoxic antiviral agents that can be applied or consumed is of great importance. Especially bearing in mind that currently there are no vaccines available for norovirus and that traditional physical and chemical treatments are not consistently effective and have rather limited applications.

**Keywords:** *Norovirus, antiviral effect, medicinal plants extracts, RT-qPCR*

**Acknowledgement:** Funding for research was provided by Project III 46009 (the Ministry of Education, Science and Technological Development of the Republic of Serbia).

## RESULTS OF POPULATION DYNAMICS SURVEY OF BLOSSOM FEEDER SCARAB (*Epicometis hirta* Poda) AND FLOWER SCARAB (*Oxythyrea funesta* Poda) IN STRAWBERRY FIELDS IN BOSNIA AND HERZEGOVINA

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Blossom feeder scarab *Epicometis hirta* and flower scarab *Oxythyrea funesta* (Coleoptera, Scarabaeidae, Cetoniinae) are thermophilic species widespread in distribution and more numerous in occurrence during certain years or in certain localities. The body of blossom feeder scarab is densely covered with yellowish or white hairs, which makes it clearly different from flower scarab, whose body is with much fewer hairs. Blossom feeder scarab and flower scarab have one generation per year. Adults overwinter in soil. They emerge early in the spring, when they can be found on flowers of different weed plants, particularly frequenting dandelion flowers. They are highly polyphagous insects. In addition to weeds, they attack many cultivated plant varieties, causing significant damage to various fruit crops. They preferably attack species from the family *Rosaceae*. Damage is caused by adults chewing on internal parts of the flower, often chewing through still unopened flower buds in order to reach stamens and pistils. In years of population peaks, blossom feeder scarab and flower scarab can cause significant damage to strawberries. Damage to strawberries is manifested in a way that fully chewed strawberry flowers do not produce fruit, and partially damaged flowers produce deformed fruits. Scarab grubs develop in the soil feeding on decomposing organic matter and do not cause damage. Blossom feeder scarab and flower scarab population size and dynamics were monitored in 2011 at the sites Veljaci–Ljubuški (Western Herzegovina County) and Donja Papratnica–Žepče (Zenica–Doboj County) in two experimental strawberry fields. Both sites were planted with ten different strawberry cultivars (Antea, Arosa, Camarosa, Clery, Galia, Madeleine, Marmolada, Naiad, Siba and Tethis) with 200 plants of each cultivar. Population size and dynamics of blossom feeder scarab and flower scarab were surveyed using *Csalomon*® VARb3k and VARb3z traps. Two VARb3k traps with chemically synthesized floral attractant for blossom feeder scarab and two VARb3z traps with floral attractant for flower scarab were placed at each site. Traps were fixed to wooden poles at the height of 50 cm from the ground, before strawberry flowering began. The traps were checked twice a week. In order to establish the percentage of strawberry flowers damaged by scarab feeding, we visually inspected flowers in periods of full flowering of each cultivar on preselected strawberry bushes. The percentage of damaged strawberry flowers was calculated as the ratio of the total count of damaged flowers to the total count of inspected flowers. We visually inspected all flowers of twenty strawberry bushes of each cultivar. The paper presents the population dynamics of the species *Epicometis hirta* and *Oxythyrea funesta*, their numbers in some localities and extent of damage to strawberry flowers.

**Keywords:** *Epicometis hirta*, *Oxythyrea funesta*, strawberry, population dynamics, damage



# AREA

“ADVANCING RESEARCH IN AGRICULTURAL AND  
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