МАТИЦА СРПСКА
MATICA SRPSKA

СЕДМИ МЕЂУНАРОДНИ НАУЧНИ СКУП
THE 7th INTERNATIONAL SCIENTIFIC MEETING

МИКОЛОГИЈА, МИКОТОКСИКОЛОГИЈА И МИКОЗЕ
MYCOLOGY, MYCOTOXICOLOGY, AND MYCOSES

Књига резимеа
Book of abstracts

2 – 3. ЈУН 2022. / 2 – 3 JUNE 2022
МАТИЦА СРПСКА, НОВИ САД, СРБИЈА / MATICA SRPSKA, NOVI SAD, SERBIA

ГЛАВНИ ОРГАНИЗАТОР СКУПА / MAIN ORGANIZER OF THE MEETING

Матица српска, Нови Сад, Србија
Matica Srpska, Novi Sad, Serbia

СУОРГАНИЗАТОРИ СКУПА / CO-ORGANIZERS OF THE MEETING

Технолошки факултет, Универзитет у Новом Саду, Србија
Faculty of Technology, University of Novi Sad, Serbia

Природно-математички факултет, Универзитет у Новом Саду, Србија
Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

Пољопривредни факултет, Универзитет у Новом Саду, Србија
Faculty of Agriculture, University of Novi Sad, Serbia

Научни институт за ветеринарство Србије, Србија
Scientific Veterinary Institute of Serbia, Serbia

Научни институт за ветеринарство „Нови Сад", Србија
Scientific Veterinary Institute „Novi Sad", Serbia

Институт за ратарство и повртарство у Новом Саду, Србија
Institute of Field and Vegetable Crops in Novi Sad, Serbia

Удружење микробиолога Србије, Србија
Serbian Society for Microbiology, Serbia

Научни одбор Седмог међународног научног скупа:
Микологија, микотоксикологија и микозе
Scientific Committee of the 7th International Scientific Meeting:
Mycology, Mycotoxicology, and Mycoses

Проф. др Милан Матавуљ, председник (СРБИЈА)
Prof. dr Milan Matavulj, President (SERBIA)

Проф. др Ференц Баги (СРБИЈА)
Prof. dr Ferenc Bagi (SERBIA)

Проф. др Александра Булајић (СРБИЈА)
Prof. dr Aleksandra Bulajić (SERBIA)

Проф. др Ђузепе Вентурела (ИТАЛИЈА)
Prof. dr Guiseppe Venturella (ITALY)

Проф. др Гордана Димић (СРБИЈА)
Prof. dr Gordana Dimić (SERBIA)

Др Весна Жупански, научни сарадник (СРБИЈА)
Dr Vesna Županski, research associate (SERBIA)

Др Мануела Задравец, виши научни сарадник (ХРВАТСКА)
Dr Manuela Zadravec, senior research associate (CROATIA)

Проф. др Алесандра Замбонели (ИТАЛИЈА)
Prof. dr Alessandra Zambonelli (ITALY)

Проф. др Георгиос Зервакис (ГРЧКА)
Prof. dr Georgios Zervakis (GREECE)

Проф. др Игор Јајић (СРБИЈА)
Prof. dr Igor Jajić (SERBIA)

Проф. др Бреда Јаковац Страјн (СЛОВЕНИЈА)
Prof. dr Breda Jakovac Strajn (SLOVENIA)

Др Елизабет Јанић Хајнал (СРБИЈА)
Dr Elizabet Janić Hajnal (SERBIA)

Др Марко Јауковић, научни сарадник (СРБИЈА)
Dr Marko Jauković, research associate (SERBIA)

Проф. др Радивоје Јевтић, научни саветник (СРБИЈА)
Prof. dr Radivoje Jevtić, science advisor (SERBIA)

Проф. др Митко Караделев (СЕВЕРНА МАКЕДОНИЈА)
Prof. dr Mitko Karadelev (North Macedonia)

Проф. др Маја Караман (СРБИЈА)
Prof. dr Maja Karaman (SERBIA)

Академик Рудолф Кастори (СРБИЈА)
Akademic Rudolf Kastori (SERBIA)

Проф. др Анита Клаус (СРБИЈА)
Prof. dr Anita Klaus (SERBIA)

Др Александар Кнежевић, виши научни сарадник (СРБИЈА)
Dr Aleksandar Knežević, senior research associate (SERBIA)

Др Јована Кос, виши научни сарадник (СРБИЈА)
Dr Jovana Kos, senior research associate (SERBIA)

Проф. др Костас Коутсоманис (ГРЧКА)
Prof. dr Kostas Koutsoumanis (GREECE)

Доц. др Сунчица Коцић-Танацков (СРБИЈА)
Doc. dr Sunčica Kocić-Tanackov (SERBIA)

Др Весна Крњаја, научни саветник (СРБИЈА)
Dr Vesna Krnjaja, science advisor (SERBIA)

Проф. др Рудолф Крска (АУСТРИЈА)
Prof. dr Rudolf Krska (AUSTRIA)

Проф. др Сомањана Кхатуа (ИНДИЈА)
Prof. dr Somanjana Khatua (INDIA)

Проф. др Милица Љаљевић Грбић (СРБИЈА)
Prof. dr Milica Ljaljević Grbić (SEBRIA)

Проф. др Ксенија Марков (ХРВАТСКА)
Prof. dr Ksenija Markov (CROATIA)

Проф. др Стеван Маширевић (СРБИЈА)
Prof. dr Stevan Maširević (SERBIA)

Др Драгана Милошевић, виши научни сарадник (СРБИЈА)
Dr Dragana Milošević, senior research associate (SERBIA)

Др Армин Мешић, виши научни сарадник (ХРВАТСКА)
Dr Armin Mešić, senior research associate (CROATIA)

Др Антонио Морети (ИТАЛИЈА)
Dr Antonio Moretii (ITALY)

Проф. др Јелена Недељковић-Траиловић (СРБИЈА)
Prof. dr Jelena Nedeljković-Trailović (SERBIA)

Проф. др Виктор Недовић (СРБИЈА)
Prof. dr Viktor Nedović (SERBIA)

Проф. др Худа Нето (МАУРИЦИЈУС)
Prof. dr Hudaa Neetoo (MAURITIUS)

Др Ксенија Нешић, виши научни сарадник (СРБИЈА)
Dr Ksenija Nešić, senior research associate (SERBIA)

Проф. др Драгојло Обрадовић (Србија)
Prof. dr Dragajlo Obradović (SERBIA)

Др Марија Павловић (СРБИЈА)
Dr Marija Pavlović (SERBIA)

Проф. др Хрвоје Павловић (ХРВАТСКА)
Prof. dr Hrvoje Pavlović (CROATIA)

Проф. др Петер Распор (СЛОВЕНИЈА)
Prof. dr Peter Raspor (SLOVENIA)

Проф. др Катерина Русевска (СЕВЕРНА МАКЕДОНИЈА)
Prof. dr Katerina Rusevska (NORTH MACEDONIA)

Проф. др Соња Смоле-Можина (СЛОВЕНИЈА)
Prof. dr Sonja Smole-Možina (SLOVENIA)

Др Славица Станковић, научни саветник (СРБИЈА)
Dr Slavica Stanković, science advisor (SERBIA)

Др Биљана Стојановска Димзоска (СЕВЕРНА МАКЕДОНИЈА)
Dr Biljana Stojanovska Dimzoska (NORTH MACEDONIA)

Проф. др Љиља Торовић (СРБИЈА)
Prof. dr Ljilja Torović (SERBIA)

Др Војислав Тркуља (РЕПУБЛИКА СРПСКА)
Dr Vojislav Trkulja (REPUBLIKA SRPSKA)

Др Игор Уjчић Врховник (СЛОВЕНИЈА)
Dr Igor Ujčić Vrhovnik (SLOVENIA)

Проф. др Зехра Хајрулаи-Мусли (СЕВЕРНА МАКЕДОНИЈА)
Prof. dr Zehra Hajrulai-Musliu (NORTH MACEDONIA)

Доц. др Бојан Шаркањ (ХРВАТСКА)
Doc. dr Bojan Šarkanj (CROATIA)

Проф. др Данијела Сморгровичова (СЛОВАЧКА)
Prof. dr Daniela Šmogrovičová (SLOVAKIA)

Проф. др Ван Абд Ал Кадр Имад Ван Мохтар (МАЛЕЗИЈА)
Prof. dr Wan Abd Al Qadr Imad Wan Mohtar (MALESIA)

Организациони одбор Седмог међународног научног скупа:
Микологија, микотоксикологија и микозе
Organizing Committee of the 7th International Scientific Meeting:
Mycology, Mycotoxicology, and Mycoses

Доц. др Сунчица Коцић-Танацков, председник (СРБИЈА)
Doc. dr Sunčica Kocić-Tanackov, President (SERBIA)

Др Јована Трбојевић Јоцић, секретар (СРБИЈА)
Dr Jovana Trbojević Jocić, Secretary (SERBIA)

Доц. др Саша Деспотовић (СРБИЈА)
Doc. dr Saša Despotović (SERBIA)

Проф. др Симонида Ђурић (СРБИЈА)
Prof. dr Simonida Đurić (SERBIA)

Др Добрила Јакић-Димић, научни саветник (СРБИЈА)
Dr Dobrila Jakić-Dimić, science advisor (SERBIA)

Др Сандра Јакшић, виши научни сарадник (СРБИЈА)
Dr Sandra Jakšić, senior research associate (SERBIA)

Проф. др Маја Караман (СРБИЈА)
Prof. dr Maja Karaman (SERBIA)

Проф. др Јелена Пејин (СРБИЈА)
Prof. dr Јелена Пејин (SERBIA)

СПОНЗОРИ / SPONSORS

Одржавање Седмог међународног научног скупа: *Микологија, микотоксикологија и микозе* помогли су / Contributes of the 7th International Scientific Meeting: *Mycology, Mycotoxicology, and Mycoses* are:

НАУЧНИ ИНСТИТУТ ЗА ВЕТЕРИНАРСТВО СРБИЈЕ
SCIENTIFIC VETERINARY INSTITUTE OF SERBIA

ФАКУЛТЕТ ВЕТЕРИНАРСКЕ МЕДИЦИНЕ, УНИВЕРЗИТЕТ У БЕОГРАДУ
FACULTY OF VETERINARY MEDICINE, UNIVERSITY OF BELGRADE

ТЕХНОЛОШКИ ФАКУЛТЕТ, УНИВЕРЗИТЕТ У НОВОМ САДУ
FACULTY OF TECHNOLOGY, UNIVERSITY OF NOVI SAD

ПРИРОДНО-МАТЕМАТИЧКИ ФАКУЛТЕТ, УНИВЕРЗИТЕТ У НОВОМ САДУ
FACULTY OF SCIENCES, UNIVERSITY OF NOVI SAD

ПОЉОПРИВРЕДНИ ФАКУЛТЕТ, УНИВЕРЗИТЕТ У НОВОМ САДУ
FACULTY OF AGRICULTURE, UNIVERSITY OF NOVI SAD

ИНДУСТРИЈА МЕСА „МАТИЈЕВИЋ“
„MATIJEVIĆ“ MEAT INDUSTRY

КЕФО Д.О.О.
KEFO D.O.O.

БАЦИЛОМИКС Д.О.О.
BACILLOMIX D.O.O.

НАУЧНИ ИНСТИТУТ ЗА ВЕТЕРИНАРСТВО „НОВИ САД”
SCIENTIFIC VETERINARY INSTITUTE „NOVI SAD”

ИНБЕРГ Д.О.О.
INBERG D.O.O.

КОМПАНИЈА „NOACK & CO SOUTH EAST DOO”
NOACK & CO SOUTH EAST DOO NOVI SAD

КОРПОРАЦИЈА „BALL”
BALL CORPORATION

АПАТИНСКА ПИВАРА
APATIN BREWERY

ЈАФА Д.О.О.
JAFFA D.O.O.

ЕУГЕН ЧОКОЛАДЕ
EUGEN CHOCOLATE

КОМПАНИЈА „ANALYSIS”
ANALYSIS D.O.O.

ВИНАРИЈА „CARPE DIEM”
VINARIJA CARPE DIEM

ТУРИСТИЧКА ОРГАНИЗАЦИЈА ВОЈВОДИНЕ
TOURISM ORGANZIATION OF VOJVODINA

Садржај / Content

ПЛЕНАРНО ПРЕДАВАЊЕ / PLENARY LECTURE

TACKLING MYCOTOXINS WORLD-WIDE: MITIGATION THROUGH INNOVATION

Rudolf Krska[1,2]

[1]University of Natural Resources and Life Sciences Vienna (BOKU), Department of Agrobiotechnology (IFA-Tulln), Institute of Bioanalytics and Agro-Metabolomics, Vienna, Austria; [2]Queens University Belfast, School of Biological Sciences, Institute for Global Food Security, Northern Ireland, United Kingdom

Coreponding author: rudolf.krska@boku.ac.at

The Food and Agriculture Organization (FAO) estimated the global food crop contamination with mycotoxins to be 25%. In order to assess the rationale for this figure which dates back to prior 1985, the relevant literature was reviewed and data of around 500 000 analyses from the European Food Safety Authority and large global survey for aflatoxins, fumonisins, deoxynivalenol, T-2 and HT-2 toxins, zearalenone and ochratoxin A in cereals and nuts were examined by M. Eskola et al. 2019. Using different thresholds, i.e., limit of detection, the lower and upper regulatory limits of European Union (EU) legislation and Codex Alimentarius standards, the mycotoxin occurrence was estimated. Current mycotoxin occurrence above the EU and Codex limits appears to confirm the FAO 25% estimate, while this figure greatly underestimates the occurrence above the detectable levels (up to 60-80%). The high occurrence is likely explained by a combination of the improved sensitivity of analytical methods and impact of climate change.

The adaption and integration of existing knowledge with novel findings is essential for the practical implementation of current expertise into tools that can be used along the food and feed chain. That task was taken on by MyToolBox (grant agreement No 678012; www.mytoolbox.eu), a 4 years project funded by the European Commission (EC). The main goal of MyToolBox consisted of the development and merging of various measures to significantly reduce the harm associated with mycotoxin contamination. A combination of pre- and post-harvest measures was initiated by to reduce the losses of crops caused by mycotoxins. This kind of intervention also takes into consideration the type of commodity that is affected. In the end, we have carefully examined the entire chain, from soil-field-crop-food to processing-waste management-alternative energy, to enable food & feed security and safety within a sustainable economic environment. The web-accessible MyToolBox e-platform (mytoolbox.eu) lists the outcomes of the project including novel interventions and provides additional information for all actors along the food chain in an effort to support the decision-making process in mycotoxin management.

ПРЕДАВАЊЕ ПО ПОЗИВУ / INVITED LECTURE

ANALYSIS OF MYCOTOXINS AS CONFIRMATION OF ANIMAL EXPOSURE TO MYCOTOXINS

Sandra Jakšić, Milica Živkov Baloš
Scientific Veterinary Institute „Novi Sad", Novi Sad, Serbia

Corresponding author: sandra@niv.ns.ac.rs

Diagnosing mycotoxicosis in animals is not a simple process. The assumption is usually made on the basis of clinical signs, and then the diagnosis is confirmed by the analysis of feed, since mycotoxins mainly reach the animal's body through feed. However, it often occurs that the presence of legally prescribed mycotoxins is not proven in feed, although all symptoms indicate mycotoxicosis. Therefore, additional analyses are important in order to establish the diagnosis related to feed consumption contaminated with mycotoxins with a greater certainty. That would contribute not only in preserving animal health, but also in avoiding economic damage. This paper presents an overview of analyses that can more reliably confirm the impact of mycotoxins on animal health, and prove intoxication. The review focuses on two topics: pointing out the importance and potentials of modified toxin analysis in feed and analysis of mycotoxins and their metabolites in biological samples.

Biologically modified mycotoxins can be biosynthesized under the impact of some plant, animal or bacterial enzymes. These metabolites of the parent mycotoxin are formed by conjugation with glucose and modified glucose (most extensively studied are deoxynivalenol-3-glucoside and zearalenone-14-glucoside), but also with other groups such as sulphate and glutathione. Besides, two acetylated derivatives of deoxynivalenol (15-acetyldeoxynivalenol, and 3-acetyldeoxynivalenol) are frequently found in naturally infected cereals such as wheat, barley and maize. Derivatives of fumonisins formed by esterification with oleic and linoleic acids have been detected in maize. Modified mycotoxins can be released, hydrolysed, biotransformed and absorbed in the gastrointestinal tract, primarily as the parent compound. Usually, modified mycotoxins remain undetected by analytical methods commonly used to determine mycotoxins, causing underestimation of mycotoxin exposure and risk. Their presence is not prescribed or recommended for control.

Furthermore, the knowledge about mycotoxin metabolism and biomarkers that confirms exposure is important to diagnose animal mycotoxicosis. Analyses of biological material and biochemical parameters as indicators of mycotoxin exposure are of great importance. Evaluation of the most suitable biomarkers for most of mycotoxins and metabolites is significant in plasma, urine and faeces of intoxicated animals. Residues of most mycotoxins and metabolites may be present at different levels in intestinal tissues, bile, kidney and liver.

The role of the laboratory is to provide and apply the methods that can analyze various biological samples and feed not only for the presence of all feed regulated mycotoxins, but also their metabolites and modified forms. Immunochemical methods for mycotoxins depending on the cross-reactivity of antibodies may not detect modified forms. All chromatographic technics for parent mycotoxins could be potentially suitable for their modified forms if they are extractable and have UV absorption or are available for fluorescent detection. The best method for determination of low concentrations and simultaneous analysis of multiple analytes is certainly liquid chromatography–tandem mass

spectrometry, after water/acetonitrile extraction. Based on previous experience and knowledge, it would be important to set toxicological protocols for the analysis of feed and biological material that would prove the mycotoxicosis of the animal.
Key words: mycotoxicosis, modified mycotoxins, biomarkers, metabolites, analyzes.

Acknowledgement
This work was funded by Ministry of Education, Science and Technological development of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2022, Contract No: 451-03-68/2022-14/200031.

ПРЕДАВАЊЕ ПО ПОЗИВУ / INVITED LECTURE

PROCEDURE TO ASSESS EFFICACY OF FEED ADDITIVE FOR AFLATOXIN ADSORPTION

Ksenija Nešić
Serbian Institute of Veterinary Science, Belgrade, Serbia

Corresponding author: ksenija.nesic@gmail.com

Contamination with mycotoxins is a global problem in both food and feed production and represents a serious threat in the entire food chain. Particular attention is drawn to the possible presence of toxin residues in food of animal origin, so many control strategies take place at the level of animal feed, which at the same time positively influence productivity of animals and protect the health of both animals and humans. Facing the mycotoxin problems for decades, many types of struggle have been tried. Various methods have been used to decontaminate mycotoxin affected commodities or to reduce the exposure to mycotoxins, but not all approaches are appropriate for different purposes. From this aspect, aflatoxins are a particularly important class of mycotoxins. The World Health Organization (WHO) - International Agency for Research on Cancer (IARC) evaluated their carcinogenic potential and classified aflatoxin B1 as a Group 1 carcinogen for hepatocellular carcinoma. Climate change in the temperate climate of southern Europe contributes to frequent occurrences of aflatoxins in cereals. Therefore, new products to combat these natural hazards are constantly being developed. However, a number of issues, from safety to efficacy, need to be investigated before such material can be officially put into use.

The procedure starts with adsorption capacity evaluation of the additive in in vitro conditions and it continues with *in vivo* testing. A correlation is usually expected, although it is not always a case and therefore both approaches are necessary. Supplementation of diets with selected adsorbents, especially of the bentonite type, showed to be the most effective against aflatoxicosis in animals. EFSA Scientific Report gives an actual and comprehensive overview on this topic, while an official EU regulation No 1060/2013 prescribes efficiency criteria: bentonite composition, indications for use and the requirement for aflatoxin adsorption. The demand implies a minimum aflatoxin B1 binding capacity of 90%, as well as an analytical method to verify efficacy. Nevertheless, a large number of different methods for determining the capacity of different adsorbents for mycotoxin adsorption have been reported in the literature. The methods differ according to the experimental conditions, temperature and pH value, as well as to the concentrations of adsorbent and toxin used in the experiments. Therefore, it is very difficult to compare the efficiencies of different tested materials, and draw conclusions about their applicability. As a result, feed manufacturers and farmers may be confused and misled about the quality of adsorbents.

Having all the above in mind and the necessity of a comparative purpose, it is important to establish a unique approach to assess efficacy of feed additive and embed it in national regulations.

Key words: adsorbents, food and feed, mycotoxins.

Acknowledgement

This work is supported by the Serbian Ministry of Education, Science and Technological Development (Contract No 451- 03-68/2022- 14/200030).

PREVALENCE OF PATHOGENIC AND MYCOTOXIGENIC FUNGI ON HARVESTED POTATO TUBERS AND MYCOTOXIN DETECTION

Sandhya Devi Takooree[1], Hudaa Neetoo[1], Vijayanti Mala Ranghoo-Sanmukhiya[1], Vivian Vally[2], Aleksandra Bulajić[3], Jacquie van der Waals[4]

[1]University of Mauritius, Faculty of Agriculture, Department of Agricultural & Food Science, Mauritius; [2]Food and Agricultural Research and Extension Institute (FAREI), Reduit, Mauritius; [3]University of Belgrade, Faculty of Agriculture, Belgrade, Serbia; [4]University of Pretoria, Department of Plant and Soil Sciences, Pretoria, South Africa

Corresponding author: sandhyaneha2205@gmail.com

Potato is an economically important food crop for Mauritius. However, potato tubers are susceptible to a wide range of post-harvest fungal diseases which adversely affect their marketable quality. Certain pathogenic fungal species also have the potential to produce mycotoxins, thus representing a food safety concern. The objective of this research was to (i) assess the prevalence of major fungal diseases affecting potato tubers, (ii) identify the etiological agent on diseased tubers and (iii) detect any mycotoxin contaminants on tubers. Briefly, potato tubers displaying symptoms of fungal diseases (n=2,379) were collected, soon after harvest, from packhouses of Mauritius during the period 2019-2021. Disease incidence (DI) for key diseases such as *Fusarium* dry rot (FDR), early blight (EB), charcoal rot (CR), black scurf (BS), black dot (BD) and silver scurf (SS) were assessed by a tuber rating. The etiological agents were identified by microscopy, culturing and molecular methods. Pathogenicity trials were also conducted to confirm the virulence of the detected pathogens on tubers. In addition, for fungal species suspected to produce mycotoxins, up to 10 potato samples collected on the same day and from the same site with identical symptoms were pooled to form composite samples. These were subsequently ground to a paste, mixed with 70% of methanol solvent or distilled water and filtered. The collected filtrate was analysed by ELISA for mycotoxins depending on the microscopic identification of the suspected mycotoxigenic fungus. Mycotoxins tested for were alternariol (ALT), deoxynivalenol (DON), fumonisin (FUM), T-2/HT-2 toxin (T-2/HT-2) or zearalenone (ZEA). Overall, tubers were differentially affected by various fungal agents with FDR being predominant. The diseases could be ranked in the following decreasing order of incidence: FDR (19%) > BS (8%) > EB (7%) > SS (4%) > CR (4%) > BD (2%). Molecular analyses of the isolates identified the causative agent of FDR, EB, CR, BS and BD as *Fusarium oxysporum*, *Alternaria alternata*, *Macrophomina phaseolina*, *Rhizoctonia solani* AG-3 and *Colletotrichum coccodes*, respectively. Representative isolates of all detected pathogenic species successfully fulfiled Koch's postulates. Potato samples were found to be contaminated with a relatively high level of FUM (5,531 µg/kg) while other mycotoxins detected such as ALT (0.02-5.28 µg/kg), DON (0.25-14.4 µg/kg), FUM (18-341 µg/kg), T-2/HT-2 (0-340.5 µg/kg) and ZEA (0.004-14.8 µg/kg) were found to be within the safe limits for human consumption. Overall, we can infer that potato crops in Mauritius are susceptible to infection by pathogenic and mycotoxigenic fungi that can compromise the yield, quality and safety of this important commodity. The findings of this study may assist the different actors in the potato supply chain in the timely diagnosis and management of post-harvest diseases.

Key words: fungi, potato, tubers, mycotoxins, *Fusarium*, fumonisin.

USE OF ZEBRAFISH EMBRYO ASSAY TO EVALUATE THE SAFETY AND TOXICITY OF B-GLUCANS MUSHROOM EXTRACTS FOR FUTURE HEALTH APPLICATIONS

Wan Abd Al Qadr Imad, Wan Mohtar
University Malaya, Faculty of Science, Institute of Biological Sciences, Malaysia

Corresponding author: qadyr@um.edu.my

Mushrooms are heterotrophic organisms. Filamentous fungi such as mushrooms have been utilized medicinally and reverently worldwide since at least 3000 BCE. Many high-value products can be made from mushrooms, including food, medications, cosmetics/detergents, and biofuels. Extracts from mushrooms are also becoming increasingly popular because of the wide range of health benefits they may provide. Mushroom β-glucan is the most studied and used medicinally active component. Numerous studies revealed that β-glucans affect many aspects of metabolism and digestion, including gut microbiome, blood sugar and lipid metabolism, and cholesterol levels, making them a promising candidate for use in the treatment of metabolic syndrome, obesity and diet regulation, digestive disorders, and cardiovascular and diabetes risk reduction. Although β-glucans face considerable obstacles in further clinical testing and translation due to source and extraction technique discrepancies, it is possible. Additionally, these β-glucans must be shown to be safe for use in humans and animals prior to *in vivo* therapy. It is well accepted that zebrafish embryos are excellent models for developmental biology, toxicology, and drug discovery. Zebrafish embryos grow rapidly; they are fully formed five days after fertilization in zebrafish. ZFET assay is unique among vertebrate models for high-throughput chemical screening, which is helpful in pre-clinical drug discovery and toxicology assessment. Therefore, it could be used as a safety screening approach before pre-clinical testing of β-glucans according to national and international standards. As a result, this study utilizes ZFET to evaluate the safety of prominent β-glucan derived extracts from *Ganoderma lucidum*, *Ganoderma applanatum*, and *Lignosus rhinocerus* before their development or commercialization. Critical parameters such as LC_{50}, embryonic hatching delays, teratogenic defects, and heart rate responses have all been discussed in detail in this paper l using high-resolution microscopic images.
Key words: *Ganoderma lucidum*, *Ganoderma applanatum*, Tiger Milk mushroom, zebrafish, toxicology.

AFLATOXINS IN MAIZE FROM SERBIA: A TEN-YEAR REPORT

Jovana Kos[1], Bojana Radić[1], Elizabet Janić Hajnal[1], Radmila Radović[1], Anamarija Mandić[1], Sanja Đekić[2]

[1]University of Novi Sad, Institute of Food Technology in Novi Sad, Serbia; [2]Analysis doo, Serbia

Corresponding author: jovana.kos@fins.uns.ac.rs

Aflatoxins (AFS) are carcinogenic and highly toxic compounds produced by *Aspergillus* species. Food and feed contaminated with AFS pose a high risk to human and animal health. In terms of the Republic of Serbia, the main concern is related to the prevalence of AFS in maize. Serbia represents a leader in maize production and exports in Europe, and it is among the top 10 exporters in the world. In the recent years maize yield as well as quality and safety are strongly affected by weather conditions.

This report aimed to present AFS occurrence in harvested maize samples from Serbia in the ten years (2012-2021). Maize samples were collected from maize growing seasons which were characterized by extreme drought (2012), hot and dry conditions (2013, 2015, 2017, 2021), extreme precipitation (2014), and weather conditions usual for a moderate-continental climate (2016, 2018, 2020). The highest AFS concentrations, as well as contamination frequency of AFS were detected in maize samples originating from the 2012 maize growing season, followed by 2015, 2021, 2013, and 2017. On the other hand, in samples originating from 2014, 2016, 2018, 2019, and 2020, there were no AFS detected. The obtained results indicate that changes in weather conditions, recorded in the period of ten years, had a significant influence on the presence or absence of AFS in maize. They were detected in samples from five among ten investigated years, and in all contaminated samples, from each year, aflatoxin B1 was the most dominant.

In the period of ten investigated years, Serbia was faced with climate changes which had a great overall impact on the prevalence of AFS in maize. The findings of this report as well as climate change prediction for South-East Europe indicate that maize from Serbia may become susceptible to problems concerning AFS and therefore there is a need for Serbia to enhance the control strategy of maize as well as maize management practices which would greatly contribute to improving the quality and safety of food and feed.

Key words: aflatoxins, maize, Serbia, weather conditions, climate changes.

Acknowledgments

This paper is a result of the research funded by The Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/ 20022).

AFLATOXIN LEVELS IN MAIZE, FEED MIXTURES, MILK AND CHEESE IN SERBIA IN 2021

Marko Jauković[1], Nikola Roković[2], Anja Vuksan[3]
[1]Jugoinspekt Beograd, Belgrade, Serbia; [2]Veterinary Scientific Institute of Serbia, Belgrade, Serbia;
[3]Directorate for National Reference Laboratories, Belgrade, Serbia

Corresponding author: jaukovicmarko@gmail.com

Aflatoxins are the most widespread mycotoxins primarily produced by fungi of the genus *Aspergillus* such as *A. flavus* and *A. parasiticus*, infecting grains during storage, spices, nuts, milk and animal feed. Even in low concentrations, they are highly toxic. Major forms of aflatoxins include B1, B2, G1, G2 and M1. Mammals that ingest AFB1 contaminated food eliminate amounts of the main hepatic metabolite known as „milk toxin“ or aflatoxin M1 (AFM1) via milk. The incidence of contamination of aflatoxins in maize, feed mixtures, milk and cheese samples collected from the Serbian producers was investigated by using the competitive enzyme linked immunosorbent assay (ELISA) technique. In this study, a total of 22 samples of maize, 25 samples of feed mixtures, 284 samples of raw, pasteurised and UHT milk and 20 samples of cheese were examined in 2021. AFB1 was quantified in 3 samples (13.6%) of maize, at levels ranging from 2.45 to 48.31 μg/kg and 12 samples (48.0%) of feed mixtures, at levels ranging from 1.04 to 21.48 μg/kg. On the other hand, AFM1 was quantified in 51 samples (17.90%) of milk, at levels ranging from 0.02 to 0.26 μg/kg, and 15 samples (75.0%) of cheeses at levels ranging from 0.15 to 0.46 μg/kg. In Republic of Serbia, maximum limit of AFB1 in maize used for livestock is 30 μg/kg and for feed mixtures for dairy cows is 5 μg/kg. On the other hand, maximum limit of AFM1 in milk is 0.25 μg/L. Maximum limit of AFM1 in cheese is not set. These results suggest the obvious presence of AFB1 in maize and that number of feed mixture, milk and cheese samples contaminated with aflatoxins is not negligible. The occurrence of aflatoxins in raw milk and commercially available milk is one of the most serious problems, as milk is a key source of nutrients for infants and young children. Therefore, continuous monitoring over milk is necessary as well as further research and risk analysis on AFM1 presence in cheese.
Key words: aflatoxins, maize, feed, milk, cheese.

ANALYSES OF AFLATOXIN B1 IN MAIZE IN REPUBLIC OF SRPSKA DURING 2018-2022

Vojislav Trkulja, Jovana Prijić, Bojana Vuković, Bojana Ćurković, Slobodan Đokić, Jelena Mihić Salapura, Gordana Babić

Agricultural Institute of Republic of Srpska, Banja Luka, Bosnia and Herzegovina

Corresponding author: vtrkulja@blic.net

Enhanced interest in fungal contamination raised worldwide from early 1960's due to mycotoxins, as secondary metabolic products from fungi. As one of the most dangerous mycotoxins, Aflatoxin B1 (AFB1) is a common food contaminant all over the world. Produced by *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare on maize (*Zea mays* L.), it is a potent carcinogen that is linked to liver cancer in animals and humans.

Analysis of aflatoxin B1 content in corn samples was done by competitive enzyme immunoassay (ELISA) using a kit from EuroProxima, the Netherlands. During five-year monitoring (2018-2022) 304 samples are analysed for aflatoxin B1 contamination. Samples were taken by the representatives of Republic Administration for Inspection Activities, Republic of Srpska. Approximately 50-100 gram of sample is ground and pulverised into a fine homogenous compound. After that, to 3 gram of ground sample 9 ml of 80% methanol is added and shook thoroughly at room temperature for 10 minutes. Samples are than centrifuged for 10 minutes at 2000 × g. An aliquot of 50 µL of the supernatant obtained after centrifugation is diluted with 150 µL of dilution buffer to obtain a solution containing 20% methanol. According to the obtained results, 13.04% (3 from 23) of samples were positive in 2022, 3.12% (2 from 64) were positive in 2021, 3.89% (3 from 77) in 2020, while in the years 2019 (27 samples) and 2018 (113 samples) the presence of aflatoxin B1 was not detected.

Key words: aflatoxin B1, contamination, ELISA, maize.

CAN WE USE OZONE AND UV LIGHT TO EFFECTIVELY REDUCE DEOXYNIVALENOL, ZEARALENONE AND OCHRATOXIN A IN GROUND MAIZE GRAINS?

Saša Krstović[1], Jelena Krulj[2], Dragan Glamočić[1], Aleksandra Bočarov Stančić[3], Igor Jajić[1]

[1]University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Novi Sad, Serbia; [2]University of Novi Sad, Institute of Food Technology, Novi Sad, Serbia; [3]Institute for Science Application in Agriculture, Belgrade, Serbia

Corresponding author: sasa.krstovic@stocarstvo.edu.rs

Ultraviolet (UV) light and ozone were applied to ground maize samples naturally contaminated with deoxynivalenol (DON), zearalenone (ZEA) and ochratoxin A (OTA). Experiments on the application of UV radiation were done by applying radiation sources at two wavelengths for up to 360 minutes, at different distances of maize from the radiation source. The application of ozone involved the use of three different concentrations of ozone, to which contaminated maize was exposed for up to 180 minutes.

The results showed that long-wave (λ = 368 nm) UV irradiation had the potential for DON degradation, but it was necessary to extend the irradiation process beyond 360 minutes. On the other hand, short-wave (λ = 254 nm) UV irradiation did not achieve statistically significant reduction in the content of any of the tested mycotoxins after 360 minutes. This was supported by the fact that UV irradiation degradation reactions were good fit to the application of a first order kinetic model only in the case of DON when long-wave irradiation was applied.

Degradation of DON, ZEA and OTA using ozone in naturally contaminated ground maize grain proved to be the most effective in the case of OTA (decreased by 70.3%) and ZEA (decreased by 68.1%), while DON was reduced weakly by 42.8%. Moreover, the data showed good fit to the application of a first order kinetic model to DON, ZEA and OTA degradation by ozone.

Key words: degradation, half-life, kinetic model, mycotoxins.

OCCURRENCE OF AFM1 IN VARIOUS TYPES OF CHEESES: AN OVERVIEW

Martina Antunović, Mirela Lučan Čolić
Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Croatia

Corresponding author: martina.antunovic@ptfos.hr

Aflatoxins are secondary metabolites produced by moulds, mostly *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nombus*. The most common are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1) and M2 (AFM2). AFM1 is the main metabolite of the most toxic aflatoxin-AFB1 and it is formed in the animal's liver as a result of consuming AFB1 contaminated feed. Aflatoxins, including AFM1, are known as carcinogenic, mutagenic and teratogenic substances. Contamination of milk and dairy products with aflatoxin M1 is highly present worldwide. Considering the affinity of AFM1 for the casein fraction, it is found that cheeses contain higher levels of AFM1 than the milk from which are produced. Therefore, there is a great concern for human health, especially due to the fact that dairy products are widely used in human diet. Thus, it is necessary to establish strict control measures and mitigation strategies, aiming at reducing the AFM1 in cheeses. The aim of this work is to provide an overview of the studies about the occurrence of AFM1 in various types of cheeses in the last ten years in order to increase food and feed safety.
Key words: aflatoxins, aflatoxin M1, cheese.

OCCURRENCE AND RISK ASSESSMENT OF MYCOTOXINS IN FOOD FROM VOJVODINA

Ljılja Torović
University of Novi Sad, Faculty of Medicine, Institute of Public Health of Vojvodina, Novi Sad, Serbia

Corresponding author: ljilja.torovic@mf.uns.ac.rs

Mycotoxins are secondary metabolites of toxigenic fungi, produced on various food crops in specific climate conditions. From a public health standpoint, mycotoxin accumulation in food could pose a human health risk. Therefore, the paper aimed to present the natural occurrence of mycotoxins and risk assessment in food samples originating from Vojvodina, collected from markets over the last decade.

Mycotoxin analysis was performed on a total of 841 food products: infant formulae (21), milk (80), apple-based infant food (114), fruit juices (242), wine (113), cereal flour (58), cornflour (56), breakfast cereals (136), and grains (21). Multi-year sampling campaigns, spanning from two to five years during the last decade, were conducted in Novi Sad. Analyses were carried out by standard liquid chromatography methods in the authorized and accredited laboratory of the Public Health Institute.

Aflatoxin M1 was found in 76.2% and 4.8% of the samples of milk and infant formulae, respectively, with 20.0% of milk samples exceeding the maximum allowed level (ML). Patulin was detected in 28.1% and 47.9% of apple-based infant food samples and fruit juices, respectively, with 0.4% of the latter above the maximum level (ML). More than half of the wine samples showed the presence of ochratoxin A (52.2%) but in very low concentrations. Cereal flour samples were contaminated with aflatoxins in 5.2% of the cases, 1.7% exceeding the ML, whereas ochratoxin A was found in 29.3% of the samples, in 3.4% even above the ML. Corn flour showed rather high mycotoxin occurrence rates, ranked as follows: fumonisins 96.4%, zearalenone 66.1%, aflatoxins 48.2%, deoxynivalenol 42.9%, ochratoxin A 37.5%. However, the proportion of the samples not in compliance with the regulation was not very high: 1.8%, 3.6%, 7.1%, 1.8% and 1.8%, respectively for the stated mycotoxins. Regarding breakfast cereals, 11.1% were contaminated with aflatoxins and 17.6% with ochratoxin A, with 2.2% exceeding the ML for the ochratoxin A. Grain samples showed the presence of ochratoxin A in 19.0% of the samples, with 4.8% above the ML. Great variability in the presence of mycotoxins was observed throughout the years. For possible adverse health outcomes as a result of dietary exposure to mycotoxins, mean contamination levels are associated with a low level of risk, whereas exceptionally high contamination recorded in some number of individual food products cause serious concern, especially in the case of genotoxic carcinogen aflatoxin B1 and nephrotoxin ochratoxin A. Further concerns are related to cumulative exposure to multiple mycotoxins, often occurring on cereal products.

The overview revealed a rather high occurrence of mycotoxins, but mostly with low concentrations in tested samples. Improvement of the efficiency and coverage of food supply control is of utmost importance for public health, particularly under extreme climate changes.

Key words: mycotoxins, food safety, market, Vojvodina.

MYCOTOXINS AS A GLOBAL PROBLEM OF MODERN SOCIETY

Jelena Živančev, Igor Antić, Maja Buljovčić, Sandra Bulut, Sunčica Kocić-Tanackov
University of Novi Sad, Faculty of Technology Novi Sad, Novi Sad, Serbia

Corresponding author: jelena.zivancev@tf.uns.ac.rs

The impact of climate change on the development of toxigenic fungi and the contamination of agricultural crops and animal products with mycotoxins is a serious problem worldwide, especially in developing countries and transitional nations. Among the emerging issues in food safety, the mycotoxins are the greatest concern, the particularly the so-called principal mycotoxins such as aflatoxins (AFs), ochratoxin A (OTA), trichothecenes (type A: HT-2 and T-2 toxin, and type B: deoxynivalenol (DON)), zearalenone (ZEA), and fumonisins (FBs). Most of the mentioned mycotoxins are regulated and their maximum limits (MLs) are set by the regulations of the European Union, but there are still various types of contaminated agricultural products and foodstuffs that exceed the MLs for these contaminants in the market. Although these mycotoxins have been widely studied in food and feed, very little is known about their occurrence and presence in soil and agro-environmental matrices, which is of major importance. In order to gain knowledge about the fate of mycotoxins in the soil as a medium on which agricultural crops are grown to ensure constant monitoring of the food chain, reliable methods for the quantification of mycotoxins are needed, which rely mainly on mass spectrometry as well as efficient extraction methods that depend on analytes. This is a serious challenge for both developed and developing countries, where awareness of the presence of mycotoxins from the field to the table needs to be raised and strict monitoring and control practices regarding food chain safety introduced. Moreover, scientific attention should be focused on: a) the harmonization of analytical methods; b) improvement of mycotoxin control and monitoring; c) formation of databases on geographical distribution and methods of mycotoxin prevention; d) and development of models for predicting potential mycotoxin contamination of crops in corresponding agricultural regions.
Key words: food and feed, mass spectrometry, mycotoxins, soil and agro-environmental matrices.

Acknowledgments
This work was supported by the Provincial Secretariat for Higher Education and Scientific Research, Republic of Serbia, Autonomous Province of Vojvodina (No. 142-451-2623/2021-01).

SCREENING OF YEAST, MOLDS, AND AFLATOXIN M1 IN RAW SHEPP`S AND GOAT`S MILK FROM AGRICULTURAL FARMS IN SOUTHEASTERN SERBIA

Svetlana Bogdanović, Stevan Mitković, Dobrila Ranđelović
Academy of Professional Studies South Serbia, Department of Agricultural and Food Technology Studies, Prokuplje, Serbia

Corresponding author: celebogdanovic@gmail.com

High content of nutrients in milk, plays an important role in meeting the daily needs in organism. Nutritionally, milk is a very valuable food that has a complex composition. Milk contains water, proteins, fats, carbohydrates, minerals, vitamins, and has great biological and technological value. Milk has a specific composition and is a suitable environment for the development of saprophytic and pathogenic microorganisms. Some types of yeasts and mould tolerate higher concentrations of acid than bacteria and, as aerobes, mainly develop on the surface of milk. Milk aflatoxins are the strongest known carcinogens. The most important aflatoxins in milk are M groups, i.e. M1 and M2. Aflatoxin M1 (AFM1) in raw milk and dairy products is stable and mostly remains unchanged during various technological processes. Microbiological safety of milk is essential for the health of the consumer, and strict quality control is necessary. The aim of the research work was screening of yeast, moulds, and aflatoxin M1 in raw sheep and goat milk from local agricultural farms in four districts of South-eastern Serbia (Toplica, Pirot, Nisava and Jablanica). Microorganisms development depends on the temperature and length of milk storage, so all samples were analyzed immediately after sampling and transporting to the laboratory. In order to examine the frequency of yeasts and mould, microbiological methods were performed in accordance with SRPS EN ISO 21527-1:2011 standard. Detection of afla M1 mycotoxin in milk was performed by Charm MRL Aflatoxin M1 quantitative test. This test involves adding a sample without prior preparation and reading the result with a ROSA Pearl Reader. It is one of the fastest methods for detecting aflatoxins. From 80 samples of sheep's milk in 5 samples the presence of mould and yeast was observed, with the total number of moulds and yeasts ranging from 1.10 * 10 cfu/mL to 1.27 cfu/mL, while from 80 samples of goat's milk in 3 the presence of moulds and yeasts was observed in the sample, the total number of which in all three samples was 1.20 cfu/mL. Aflatoxins presence was not confirmed in any of the samples. Results in this research work have shown us the safe to use sheep and goat milk produced in the agricultural farms in South-eastern Serbia.

Key words: milk, bacteria, aflatoxins, mould, yeast.

PRESENCE OF MOLDS AND OCHRATOXIN A IN DRIED FRUITS AND VEGETABLES

Sandra Bulut[1], Gordana Dimić[1], Sonja Smole Možina[2], Mihael Čargo[2], Irena Rakić[1], Jelena Živančev[1], Igor Antić[1], Jovana Ugarković[1], Sunčica Kocić-Tanackov[1]

[1]University of Novi Sad, Faculty of Technology Novi Sad, Novi Sad, Serbia; [2]University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

Corresponding author: sbulut@uns.ac.rs

Contamination of dried fruits and vegetables is the most common related to the molds. In addition to spoiling food, a much higher problem could represent toxigenic molds, potential producers of mycotoxins affecting a human health. The most common mycotoxins associated with dried fruits and vegetables are patulin, aflatoxins and ochratoxin A.

In this work different dried fruits or vegetables (raspberries, plums, cherries, grapes, pears, apples, blueberries, apricots, cranberries, figs, tomatoes, mixed vegetables), were tested for the presence of molds and ochratoxin A. All samples were purchased at local markets and "healthy food'' stores in Novi Sad, Vojvodina, Serbia. Isolation, determination and enumeration of total number of molds in samples were done based on two different methods, depending on the sample; direct-plating method and dilution method. Both methods included two way each sample's preparation, untreated or treated with 0.35% sodium-hypochlorite. Detection of mycotoxin ochratoxin A presence in analysed samples was performed using Enzyme-Linked Immunosorbent Assay.

Obtained results showed that among all analysed samples, whether they were treated or untreated with sodium-hypochlorite, dried figs were the only ones in which the presence of mold was not detected. The absence of molds was also observed in treated blueberries and untreated apricots. The highest number of molds in untreated samples identified using direct-plating method was in dried grapes (0.62 CFU/g), while the lowest total number of molds was observed in dried blueberries (0.10 CFU/g). The percentage of total number of molds reduction, after treatment with sodium hypochlorite, was up to 100% (dried blueberries). The highest number of molds in untreated samples identified using dilution method was observed in dried apples (170 CFU/g), while the lowest total number of molds was obtained in dried mixed vegetables (10 CFU/g). The reduction of total number of molds, after treatment with sodium hypochlorite, was up to 82% (dried tomatoes).

Species observed in dried fruits were from the genera *Aspergillus*, *Cladosporium*, *Emericella*, *Eupenicillium*, *Eurotium*, *Monilia*, *Mucor*, *Penicillium*, *Rhizopus*, *Talaromyces*, *Trichoderma* and *Xeromyces*, and in dried vegetables were *Aspergillus*, *Penicillium* and *Rhizopus*. Among the most predominant mold genus and species in dried fruits were *Penicillium*, which represented 24.47% of all isolates, *Aspergillus* with share of 22.34% and *Rhizopus* with share of 19.15%, with *P. glabrum*, *A. niger* and *R. oryzae* as the most common species. The most predominant genera and species in dried vegetables were *Penicillium* (6.38%) and *Rhizopus* (5.32%), with *P. glabrum*, *R. microspores* and *R. oligosporus*, as the most common species.

Potential ochratoxin A producers found in this study were *A. niger* and *P. verrucosum*. However, the results of mycotoxicological analysis indicate that the content of ochratoxin A in all samples were lower than 0.1 μg/kg.

Further research should include some other mycotoxins characteristic for dried fruits and vegetables, such as aflatoxins.

Key words: dried fruits, dried vegetables, toxigenic molds, mycotoxins, ochratoxin A

Acknowledgments

This work was supported by the Provincial Secretariat for Higher Education and Scientific Research, Republic of Serbia, Autonomous Province of Vojvodina (Project No. 142-451-2623/2021-01) and by the Project "Mycotoxigenic moulds and mycotoxins in raw materials and products intended for human consumption", supported by Matica Srpska, Novi Sad, Serbia.

FUSARIUM AND DEOXYNIVALENOL CONTAMINATION OF DURUM WHEAT LINES KERNELS

Vesna Krnjaja[1], Slavica Stanković[2], Ana Obradović[1], Milica Nikolić[1], Iva Savić[1], Violeta Mandić[2], Zorica Bijelić[2]

[1]Maize Research Institute „Zemun Polje", Belgrade, Serbia; [2]Institute for Animal Husbandry, Belgrade, Serbia

Corresponding author: vesnakrnjaja.izs@gmail.com

Fusarium infection and deoxynivalenol (DON) contamination on the kernels of seven durum wheat lines (six domestic durum lines ZP 16, ZP 34, ZP 41, ZP 74, ZP 120, ZP DSP 66, and one international durum line Cimmyt 7817) during harvest in two growing seasons (2015-2016) have estimated. Mycological methods were performed to determine the incidence of *Fusarium* spp., while the Enzyme-Linked Immunosorbent Assay (ELISA) was used to quantify the total level of DON. Analysis of data was done by statistical method ANOVA (analysis of variance). Tukey's test was used to compare means at a significance level of 5%. Correlation analyses were performed by Pearson's test.

Based on morphological characteristics, four *Fusarium* species, *F. graminearum*, *F. proliferatum*, *F. sporotrichioides*, and *F. verticillioides*, were identified in 2015. A different structure of the *Fusarium* population, which in addition to *F. graminearum*, *F. sporotrichioides* and *F. verticillioides*, also consisted of *F. poae*, *F. semitectum*, and *F. subglutinans*, was identified in 2016. *F. graminearum* was the predominant species and the most common cause of Fusarium head blight (FHB) and the primary producer of DON. Other *Fusarium* spp. Were isolated sporadically and in a low incidence in the kernels. Fungal species from the genera *Alternaria*, *Aspergillus*, *Chaetomium*, *Epicoccum*, *Nigrospora*, and *Penicillium* have also been isolated.

The incidence of *F. graminearum* and level of DON were significantly affected by the wheat genotypes and investigated years. However, obtained results for these parameters were negatively correlated. Thus, in 2015, the incidence of *F. graminearum* was significantly higher (75.86%) than in 2016 (63.43%), while the level of DON was significantly higher in 2016 (3.636 mg/kg) compared to 2015 (1.126 mg/kg). The highest and the lowest incidence of *F. graminearum* was on the kernels of line ZP DSP 66 (73%) and line ZP 34 (64.50%), respectively. The highest DON level was 3.854 mg/kg (line ZP 120), and the lowest was 1.658 mg/kg (line ZP 41). The mean DON level was 2.381 mg/kg for all tested treatments and was above the maximum limit of 1.750 mg/kg prescribed by the European Regulation 1881/2006/EC for unprocessed durum wheat, while the mean incidence of *F. graminearum* was 69.64%.

Based on obtained results, tested durum wheat lines showed susceptibility to *F. graminearum* and as a consequence higher accumulation of mycotoxin DON. These results indicate the importance of using less susceptible or tolerant lines to the pathogens of FHB and DON accumulation in the selection programs of new durum wheat varieties.

Key words: *Fusarium* spp., deoxynivalenol, durum wheat lines.

OCCURRENCE OF FUMONISINS B1 AND B2 IN MAIZE

Radmila Radović[1], Bojana Radić[1], Jovana Kos[1], Anamarija Mandić[1], Sanja Đekić[2], Elizabet Janić Hajnal[1]

University of Novi Sad[1], Institute of Food Technology in Novi Sad, Serbia; [2]Analysis, Belgrade, Serbia

Corresponding author: radmila.radovic@fins.uns.ac.rs

Maize is one of the most widely used staple foods and animal feeds in the world due to its notable amounts of vitamins, minerals and nutrients, as well as several health benefits for human and animal organisms. In Serbia, approximately 35% of the entire planted area of field crops is covered with maize. During cultivation, maize is exposed to multiple abiotic and biotic stress factors which can trigger maize contamination with numerous fungal secondary metabolites. *Fusarium* presents one of the most common fungal genera with the capability to produce various toxic mycotoxins in maize, including fumonisins. Among all identified fumonisins, B1 and B2 are the most toxic and could induce esophageal cancer. Due to that, there is a need for continuous monitoring and analysis of fumonisins B1 and B2 in food and feed.

This work aimed to examine the occurrence of fumonisins B1 and B2 in maize samples harvested in the Republic of Serbia for four years (2018-2021) and to investigate the impact of weather conditions on the concentration of fumonisins. Determination of the analytes concentrations was conducted using the liquid chromatography-tandem mass spectrometry method (LC-MS/MS).

Fumonisins B1 and B2 were detected in 100% maize samples from the 2018, 2020 and 2021 production years. Furthermore, the maize samples from 2019 contained 92 and 87% of the fumonisins B1 and B2, respectively. The differences in mean concentrations of fumonisins were detected within investigated years, which could be explained by different weather conditions required for their synthesis. Based on these results, it could be concluded, that the weather conditions (especially air temperature and amount of precipitation) in the investigated maize growing seasons have a significant influence on the determined concentrations of fumonisins B1 and B2 in maize. The results of our previous investigations concerning the contamination of maize from the Republic of Serbia with fumonisins B1 and B2, as well as the results of this study show the need for continuous monitoring of fumonisins B1 and B2 in maize.

Key words: maize, fumonisins B1 and B2, weather conditions, LC-MS/MS, Serbia.

Acknowledgments

This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (MPNTR) within grant No. 451-03-68/2022-14/200222.

PRESENCE OF MONILIFORMIN IN MAIZE

Bojana Radić[1], Radmila Radović[1], Elizabet Janić Hajnal[1], Anamarija Mandić[1]; Sanja Đekić[2], Jovana Kos[1]

[1]University of Novi Sad, Institute of Food Technology in Novi Sad, Serbia; [2]Analysis d.o.o., Belgrade, Serbia

Corresponding author: bojana.radic@fins.uns.ac.rs

Moniliformin (MON) is a widespread emerging mycotoxin, produced by a number of plant pathogenic *Fusarium* species (mainly *F. subglutinans, F. avenaceum, F. temperatum, F. verticillioides* and *F. proliferatum*) and one *Penicillium* species (*P. melanoconidium*). Based on the studies conducted in different countries and climatic conditions, MON has mostly been detected in cereal grains and cereal-based food and feed. However, the highest frequency and concentrations of MON were detected in maize and significantly lower concentrations in small-grain cereals (oats, wheat, barley, rye and triticale).

The main objective of this study was to investigate the presence and concentrations of MON in maize samples collected in Northern Serbia during a period of four years (2018-2021), and to analyze the influence of weather conditions during the growing period of maize on MON production. The concentration of MON was determined using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

The prevalence of MON was very high in maize samples during all four examined years. MON was detected in 100% of maize samples collected in the period of three years, from 2019 to 2021, and in 99% of maize samples collected during 2018. Therefore, only one sample of the total of examined samples did not contain MON. Furthermore, the highest MON concentrations were detected in samples collected during 2021, which could be explained by the favorable weather conditions for its synthesis. Taking into account the weather conditions and their impact on MON production, it seems that the weather condition parameters such as monthly average air temperatures and the sum of precipitation did not influence MON presence in maize, but influenced the concentrations of MON.

The obtained results indicate that the incidence of MON in maize from Serbia has been very high and constant in recent years. However, as the maximum levels have not been regulated for MON in food and feed, there is a need for continuously monitoring of MON presence and more toxicity studies due to its potential health hazard such as cardiotoxicity, respiratory distress and haematotoxicity. For a general conclusion about the impact of weather conditions on the presence and concentrations of MON in maize, long-term monitoring studies are also needed.

Key words: moniliformin, maize, LC-MS/MS, Northern Serbia, weather conditions.

PREVALENCE OF *ALTERNARIA* TOXINS IN MAIZE

Elizabet Janić Hajnal[1], Radmila Radović[1], Jovana Kos[1], Bojana Radić[1], Sanja Đekić[2]

[1]University of Novi Sad, Institute of Food Technology in Novi Sad, Serbia; [2]Analysis, Belgrade, Serbia

Corresponding author: elizabet.janich@fins.uns.ac.rs

One of the most important agricultural crops in Serbia is maize (Zea mays), which also represents the most important agricultural product intended for export. In addition to the quantity and quality of the produced maize, the safety aspects must be considered as well, in order to protect human and animal health. As a result of stress factors (biotic and abiotic) during maize cultivation, maize can be contaminated with a large number of different fungal secondary metabolites, i.e. with regulated and non-regulated mycotoxins. *Alternaria* toxins are referred as "emerging" mycotoxins due to their possible harmful effects and consequently they are of concern for public health. The most common *Alternaria* species which are capable to produced toxic secondary metabolites include *A. alternata, A. tenuissima, A radicina, A. arborescens, A. infectoria, A. brassicae* and *A. brassicicola*. Since *Alternaria* species and their toxins are widespread in both semi-arid and humid regions, they have been isolated from a wide range of food and feed, primarily, in cereals (mainly in wheat, sorghum, and barley), fruits, vegetables, oilseeds, beverages, silage, feed, and feed ingredients.

Thus, the present study was undertaken with the aim to determine the presence of three different *Alternaria* metabolites in 400 maize samples collected from the main maize producing regions in Northern Serbia during four years (2018 – 2021). A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used for the detection and quantification of alternariol (AOH), alternariol monomethyl ether (AME) and tentoxin (TEN) in maize samples. In addition, the influence of weather conditions during maize growing seasons was investigated on the frequency and level of *Alternaria* toxins (AOH, AME, and TEN) in maize samples.

As a consequence of the influence of weather conditions in investigated years, the highest percentages of contaminated maize samples by AOH, AME and TEN were 29%, 46% and 21%, respectively in the year 2021, while the lowest percentages of 10, 11 and 1%, respectively were recorded in the year 2019. Similar to the 2019 maize growing season, in the 2018 and 2020 production years, low level of contaminated maize samples by examined *Alternaria* toxins were observed.

Based on the results of this study, as well as, on the results of our previous investigations concerning the contamination of maize from the Republic of Serbia, it could be concluded, that the weather conditions in the investigated maize growing seasons have a significant influence on the frequency and the determined concentrations of investigated *Alternaria* toxins in maize. Besides in small grain cereals, the obtained results indicated the need for continuous monitoring of *Alternaria* metabolites, in maize, as well.

Key words: maize, *Alternaria* toxins, weather conditions, LC-MS/MS, Serbia.

TOXIGENIC POTENTIAL OF *ALTERNARIA* SPECIES FROM CEREALS

Ferenc Bagi[1], Renata Iličić[1], Zagorka Savić[1], Radivoje Jevtić[2], Branka Orbović[2], Michele Suman[3-4], Beáta Tóth[5], Attila Berényi[5], Tatjana Popović[6]

[1]University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia; [2]Institute of Field and Vegetable Crops, Novi Sad, Serbia; [3]Barilla G. E. R. Fratelli Spa Research, Development & Quality, Parma, Italy; [4]Catholic University Sacred Heart, Department for Sustainable Food Process, Piacenza, Italy; [5]Cereal Research Nonprofit Ltd., Szeged, Hungary; [6]Institute for Plant Protection and Environment, Belgrade, Serbia

Corresponding author: ferenc.bagi@polj.edu.rs

Toxigenic potential of *A. alternata* and *A. tenuissima* isolates on durum wheat grains of cultivar Dušan (*Triticum durum* L.) and common wheat cultivar Barbee (*T. vulgare* L.) were tested under laboratory conditions. *A. alternata* and *A. tenuissima* isolates were used for inoculate three different wheat genotype/fungal isolates combination. *Alternaria* toxins alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), tenuazonic acid (TeA) and altenuen (ALT) were determined by LC-MS/MS. Grains of cultivar Barbee proved to be the best substrate for toxin production. Among toxins, AOH, AME and TeA were present in highest concentration. The results underline the possibility of fungal infection and mycotoxin production by *Alternaria* species both in field and storage conditions. Further research is needed for official regulation of acceptable levels of *Alternaria* mycotoxins in food and feed.

Key words: *Alternaria*, toxin production, wheat.

DIRECT AND INDIRECT PHOTOLYSIS OF FUMONISINS IN AQUATIC ENVIRONMENT UNDER SIMULATED SOLAR IRRADIATION

Ivana Jevtić[1], Biljana Abramović[1], Maria Savanović[1], Sandra Jakšić[2]
[1]University of Novi Sad, Faculty of Science, Novi Sad, Serbia; [2]Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia

Corresponding author: ivana.dabic@yahoo.com

The occurrence of fumonisins in the aquatic environment as a consequence of leaching from contaminated fields, and the possibility of their production in water is the current problem of environmental pollution. Since fumonisins are stable in an aqueous solution, it is important to find the most efficient methods for their removal. Advanced oxidation processes have wide application in water purification containing organic pollutants because they mostly lead to their complete mineralization. Therefore, in this study, the degradation efficiency of fumonisins in aqueous media was investigated by direct and indirect photolysis under simulated solar irradiation (SSI). The initial pH value had a significant effect on the kinetics of fumonisin B1 (FB1) degradation, with the highest efficacy observed at pH 4.0 (88%), and the lowest at pH 10.0 (21%) during the 180 min of irradiation. Under these experimental conditions, FB1 photolysis in the first period of degradation follows pseudo-first-order kinetics. Degradation of FB1 and fumonisin B2 (FB2) in the mixture yielded irregularly shaped kinetic curves of degradation, probably due to intermediates that do not separate well chromatographically, resulting from the synergistic effect of these two fumonisins. In comparison to direct photolysis, indirect photolysis using H_2O_2 had an inhibitory effect on the degradation of FB1. Namely, at pH 8.0 24% of FB1 was degraded during 180 min of irradiation, while 74% was degraded by direct photolysis for the same time. In the case of the application of indirect photolysis using $S_2O_8^{2-}$ at pH 4.0, the degradation efficiency of FB1 (91%) is similar as in the case of direct photolysis (88%), at the same pH, and for the same time. Considering the degradation efficiency, it was concluded that in both cases only direct photolysis is performed, probably because SSI does not contain suitable wavelengths for radical ($SO_4^{\bullet-}$) formation. Based on this, we can conclude that direct photolysis at pH 4.0 is the most effective treatment for FB1 removal under SSI, although even at pH 8.0 the efficiency is satisfactory with no need to adjust pH. The obtained results can be significant for the development of technological methods for water purification from fumonisins as possibly carcinogenic contaminants that may be present in the aquatic environment.
Key words: mycotoxins, fumonisins, water, simulated solar irradiation, photodegradation.

Acknowledgement
This work was supported by the Science Fund of the Republic of Serbia (Grant No 7747845, *In situ* pollutants removal from waters by sustainable green nanotechnologies-CleanNanoCatalyze), and the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200125).

INHIBIRORY ACTIVITY OF MICROPARTICLES WITH WINTER SAVORY ESSENTIAL OIL AGAINST TOXIGENIC *ASPERGILLUS FLAVUS*

Sunčica Kocić-Tanackov[1], Sandra Bulut[1], Monika Bračun[1], Darko Lazarević[3], Snežana Kravić[1], Sonja Smole Možina[2]

[1]University of Novi Sad, Faculty of Technology, Novi Sad, Serbia; [2]University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia; [3]Special Nature Reserve "Slano Kopovo", Bečej, Serbia

Corresponding author: suncicat@uns.ac.rs

In recent years there has been a growing need for use of natural antimicrobial compounds, such as aromatic plant products (extracts, essential oils and their components) in food industry. Due to their antimicrobial and antioxidant properties, they can play a significant role in food preservation and can be a good alternative to synthetic additives. However, the negative side of the use of essential oils implies their sensitivity and volatility of the compounds responsible for their activity, such as phenolic compounds. A new approach to food preservation involves the use of various encapsulating substances to obtain micro and nanoparticles, intended to protect the active components.

Therefore, the aim of this study was to determine the antimicrobial potential of selected essential oils (EOs) (wild and Greek oregano, winter savory, French marigold, fennel, immortelle, hyssop and yarrow) and microparticles based on β-cyclodextrin and winter savory EO according to toxigenic species *Aspergillus flavus*.

Essential oils are obtained by steam extraction from plant material produced in organic production. The tested culture of *A. flavus* was isolated from food. Winter savory EO was encapsulated into β-cyclodextrin by co-precipitation method into three different rations (winter savory EO:β-cyclodextrin=10:90; 20:80 and 30:70). Effect of obtained microparticles against *A. flavus* was performed in liquid medium (Sabouraud Maltose Broth).

The obtained results showed that winter savory EO (MIC 1.78 µL/mL and MFC 3.55 µL/mL) had the highest antifungal effect against *A. flavus*, while yarrow and immortelle EOs had the lowest antifungal effect. Microparticles based on β-cyclodextrin and winter savory EO (ration winter savory EO:β-cyclodextrin=30:70) significantly reduced growth of *A. flavus* for 3.62 log cfu ml^{-1}.

The obtained results show a potential application of microparticles with winter savory EO as natural antifungal additives in food industry. Future research will include the mycotoxin inhibition potential of the microparticles, as well as possible applications in different food models.

Key words: *Aspergillus flavus*, microparticles, winter savory, essential oil.

Acknowledgments

This work was supported by the Provincial Secretariat for Higher Education and Scientific Research, Republic of Serbia, Autonomous Province of Vojvodina (No. 142-451-2623/2021-01) and by the Project "New methods for the control of aflatoxigenic molds and aflatoxins in food - current trends and future perspectives", supported by Matica Srpska, Novi Sad, Serbia.

ПЛЕНАРНО ПРЕДАВАЊЕ / PLENARY LECTURE

THE MYSTERIES OF TRUFFLES, THE WORLD'S MOST EXPENSIVE MUSHROOM

Alessandra Zambonelli, Federico Puliga, Simone Graziosi
Department of Agricultural and Food Sciences, Bologna University, Italy

Corresponding author: alessandr.zambonelli@unibo.it

Since humans have dug truffles from the soil, these mushrooms have been one of the most mysterious products of the land. They were known and appreciated from the ancient Greeks and Romans, which advanced fanciful hypotheses on their origin which remained unknown until the nineteenth century.
Truffles are still surrounded by an aura of mystery due their completely underground development and the unique scent that makes them the most expensive mushrooms. Trained dogs are traditionally used to find truffles, for their ability to detect and recognize the odorant volatile organic compounds (VOCs) of the truffle ascomata in the soil. Truffle hunting is a fascinating activity and has a long tradition in Italy and it is now officially inscribed on the UNESCO list of Intangible Cultural Heritage.
Nowadays, the fundamental aspects of truffles biology and ecology are known and most of the precious truffles (*Tuber* spp.) are successfully worldwide cultivated. However, there are still several aspects which remain unsolved. All the truffles are known to form ectomycorrhizal association with the roots of trees and shrubs; however, recently it was shown that they are able also to form other mycorrhizal associations (arbutoid and orchid mycorrhizas) or to live as an endophyte in herbaceous plants. What is the role of these associations in the truffle life cycle? It is now evident that truffles are heterothallic, but with a prevalent haploid lifestyle. Strains forming ectomycorrhizas act as maternal partners, whereas the paternal partners seem to derivate from germinating meiospores. The role of mycophagous animals in spore dispersal is well known. In recent works, it was supposed that they may represent the vectors by which meispores reach their sexual partner, but how fertilization does occur?
The truffle in the soil interact with several other microorganisms, bacteria and fungi, and several of them populate also the truffle ascoma. Bacteria seem to contribute to truffle aroma and nutrition by nitrogen supply and to favourite mycelium development and mycorrhiza formation. However, these beneficial aspects are dependent on the composition of the bacterial communities. There are bacterial species, like *Staphylococcus pasteuri*, that completely inhibit *Tuber* mycelial development. A better understanding of the composition and effects of microbiota thriving inside the truffle ascomata and in mycorrhizospheres could help to improve our knowledge on truffle soil ecology and truffle cultivation strategies.
In conclusion, also from a scientific point of view truffles are ones the most intriguing mushrooms, which are studied by an increasing number of scientists around the world.
Key words: truffles, mysteries**,** mycorrhiza, soil ecology.

ПЛЕНАРНО ПРЕДАВАЊЕ / PLENARY LECTURE

MACEDONIAN RED LIST OF FUNGI (2021)

Mitko Karadelev, Katerina Rusevska, Slavica Tofilovska
Ss. Cyril and Methodius University, Faculty of Natural Science and Mathematics, Department of Biology, Skopje, North Macedonia

Corresponding author: mitkok@pmf.ukim.mk

N. Macedonia as a biodiversity hot-spot area is distinguished by huge fungal diversity. The initial publications on fungal diversity in N. Macedonia chiefly date from the 1930s but in the last three decades' research into diversity and distribution of fungi has notably been intensified. The establishment of the Mycological Laboratory at the Faculty of Natural Science in Skopje was a milestone in beginning comprehensive and systematic study of fungal diversity on the entire country's territory, which resulted in numerous new fungi species. Notwithstanding the fact that species new to the country have constantly been discovered, the current status of fungi (macromycetes) is over 2,000 species.

The latest red-listing efforts were made in collaboration with the Ministry of Environment and Physical Planning. In 2017, an opportunity was created to launch implementation of a national red-listing initiative in N. Macedonia aimed at producing the first official national red lists fully aligned with all IUCN Red List guidelines. The Eastern Europe and Central Asia Regional Office (ECARO) of IUCN, seated in Belgrade, guided and provided training for the roll-out of the national red list process in the country, and it pursued this activity throughout the fungal red list assessment. The European Bank for Reconstruction and Development supported the fungi red-listing via the project entitled "Biodiversity Capacity Building Programme: Promoting Good International Practices in Macedonia", managed by Hardner & Gullison Associates from the USA.

The National Red List of Fungi, including 64 taxa, was generated based on field research results, published and unpublished records on species, exsiccates, research notes and information sourced from other individual fungi collectors. All vital data were assembled for final species assessment compatible with the following IUCN criteria: distributional range, population and trends, habitat and ecology, threats, use and trade, etc. The summary of the threat status is the following: six fungus species have been assigned the category of CR - Critically Endangered (9.3%), nineteen EN – Endangered (29.6%), thirty-four VU – Vulnerable (53.1%), two NT - Near Threatened, two LC - Least Concern, and one species is data deficient. The majority of the taxa – fifty-three - belong to the phylum Basidiomycota whereas ten taxa are affiliated with the phylum Ascomycota. The Critically Endangered species are as follows: *Bovista paludosa* Lév, *Galerina sphagnorum* (Pers.) Kühner, *Galerina tibiicystis* (G.F. Atk.) Kühner, *Hyphoderma etruriae* Bernicchia, *Xeromphalina junipericola* G. Moreno & Heykoop and *Zeus olympius* Minter & Diam.

Key words: IUCN Red List, fungi conservation, North Macedonia.

ПРЕДАВАЊЕ ПО ПОЗИВУ / INVITED LECTURE

AN OVERVIEW OF THE MOST RECENT RESEARCH ON THE GENUS *PLEUROTUS* IN EUROPE

Giuseppe Venturella
University of Palermo, Department of Agricultural, Food and Forest Sciences, Palermo, Italy

Corresponding author: giuseppe.venturella@unipa.it

Mushrooms have a unique nutrient profile, and they are biologically distinct from the plant- and animal-derived foods. The genus *Pleurotus* (Fr.) P. Kumm. comprises ca. 30 species and subspecific taxa with a world-wide distribution. Assessments of *Pleurotus* diversity in Europe revealed the existence of different species, some quite common, others infrequent or very rare, sometimes confused with each other. Europe is the largest market for cultivated mushrooms. Most of *Pleurotus* species are cultivated and its bioactive compounds (mainly polysaccharides) possess antibiotic, antitumor, hypocholesterolemic and, immunomodulation properties. The specificity of the *Pleurotus* species growing in European countries, compared to the species of oyster mushrooms growing in Asiatic countries, lies mainly in their high nutritional value. For this reason, the possibility of combining quality food and medicinal value is the true challenge for the near future for a better enhancement of the European oyster mushrooms. The most recent research on the taxonomy, cultivation, and dietary and medicinal uses of oyster mushrooms in Europe is discussed here.
Key words: *Pleurotus*, *Basidiomycota*, fungi, diversity, Europe.

ПРЕДАВАЊЕ ПО ПОЗИВУ / INVITED LECTURE

MYCODIVERSITY IN MACEDONIA WITH NEW DATA

Katerina Rusevska, Slavica Tofilovska, Mitko Karadelev
Ss. Cyril and Methodius University, Faculty of Natural Sciences and Mathematics, Institute of Biology, Skopje, Macedonia

Corresponding author: krusevska@pmf.ukim.mk

Well known as a biodiversity hot-spot, Macedonia is rich in its mycobiota which is more intensively studied in last two decades. Around 25,000 dry specimens of which have been identified circa 2,500 different taxa are deposited in the Macedonian Collection of Fungi (MCF). The data of these collections is stored in the MACFUNGI database, comprising information on 38,000 collected specimens, also including taxa from different regions in the world. A comprehensive checklist of all up to date registered macromycetes from Macedonia were recently established for the first time, updated with 23 species of basidiomycetes. Therefore, the total number of known fungal taxa is 2044, of which 1789 belong to Basidiomycota and 255 to Ascomycota, where lichens are not included. Recently, from the territory of Macedonia two species as new to science were described: *Astraeus macedonicus* and *Clitopilus abprunulus*.

The aim of this paper is to present the additional new data of 16 taxa recorded for the first time in the country through the ongoing research on mycodiversity in various habitats in the last two years. Ten taxa are basidiomycetes (*Cortinarius bibulus, C. hercynicus, C. porphyropus, Entoloma euchroum, Flammulina elastica, Hortiboletus bubalinus, Hygrophorus piceae, Mycena adscendens, Perenniporia meridionalis* and *Xeromphalina campanella*) and the rest 6 are ascomycetes (*Ciboria amentacea, Neournula pouchetii, Otidea microspora, Rutstroemia luteovirescens, Smardaea planchonis* and *Sowerbyella imperialis*). All listed species are found on single localities, except *C. amentacea* which is known from two sites. The species are collected from 11 localities in different associations, such as: riparian vegetation, deciduous forests – represented by *Betula, Populus, Quercus* or *Platanus* communities, coniferous forests of *Pinus sylvestris*, *Abies* and *Picea*, as well as from pure *Alnus* forest, plantations and city park.

Key words: Macedonian mycodiversity, Ascomycota, Basidiomycota, new diversity data.

ПРЕДАВАЊЕ ПО ПОЗИВУ / INVITED LECTURE

MYCOTOXIGENIC CAPACITY OF *ALTERNARIA* SPP. ISOLATES FROM WHEAT

Dobrila Kovačević[1], Vojislava Bursić[1], Gorica Vuković[2], Dragana Budakov[1]
[1] University of Novi Sad, Faculty of Agriculture, Department of Plant and Environmental Protection, Novi Sad, Serbia; [2]University of Belgrade, Faculty of Agriculture, Belgrade-Zemun, Serbia

Corresponding author: dragana.budakov@polj.edu.rs

Mycotoxins are secondary metabolites produced by some species of fungi and pose a serious problem in wheat production. Once mycotoxins enter food and feed chain, they can seriously harm human and animal health. *Alternaria* spp. causes black spot in wheat. Infection with these fungi, in addition to loss of yield, leads to a decrease in grain quality due to changes in color, loss of nutritional values and mycotoxin contamination. Therefore, the identification and monitoring of *Alternaria* species on wheat grain in addition to investigation of mycotoxigenic potential are of great importance mainly in order to predict if *Alternaria* mycotoxins can be expected in wheat grains and their processed products. In this paper, the composition of species from genus *Alternaria* that have been isolated from wheat samples by using classical phytopathological and molecular techniques will be determined and their ability to produce mycotoxin alternariol, alternariol monomethyl ether and tentoxin on PDB medium and artificially inoculated sterile wheat seed using LC-MS/MS analysis. The potential of isolates from asymptomatic wheat seeds to produce alternariol, alternariol monomethyl ether and tentoxin and their proven toxicity in humans and animals indicates the need for regular monitoring of wheat seeds and other substrates that may be contaminated with *Alternaria* in order to obtain health-safe raw materials and their products.
Key words: *Alternaria* spp., wheat, alternariol, alternariol monomethyl ether, tentoxin.

TWO NOVEL RUSSULOID FUNGI FROM INDIA: MEDICINAL PROSPECTS AND FUTURE DIRECTIONS

Somanjana Khatua
Krishnagar Government College, Department of Botany, West Bengal, India

Corresponding author: somanjana.khatua4@gmail.com

The genus, *Russula*, is considered to be one of the most abundant ectomycorrhizal agaric groups distributed worldwide. Several members are traditionally used as beneficial food and medicine across the globe; despite this, many of them still remain completely unexplored. In this context West Bengal, the exclusive state of India, has a treasury of basidiomycetes that are blessed with diverse agro-climatic zones. In recent years, we have conducted several field surveys to unveil myco-diversity of the state and two morphologically unique Russuloid fungi have been collected in close association with *Shorea robusta*. The local population informed us that the collected specimens traditionally play a key role in eating habits because they are included in the seasonal diet due to their impact on health. However, the taxa are not prized in city markets due to a lack of knowledge that inspires us to conduct thorough research. Fortunately, detailed study based on morphology, anatomy, DNA barcoding and phylogenetic placement revealed novelty of both the species. We designated the mushrooms with red and violet coloured pileus as *Russula alatoreticula* K. Acharya, S. Khatua, A.K. Dutta & S. Paloi (2017) and *Russula pseudocyanoxantha* Paloi, K. Acharya & S. Khatua (2021) respectively. For downstream applications, several fractions were isolated using a quite distinctive extraction protocol and investigated for therapeutic prospects. Among the organic fractions, the ethanolic preparations exhibited strong antimicrobial effect (*R. alatoreticula*> *R. pseudocyanoxantha*) against both Gram positive (*Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Escherichia coli, Salmonella typhimurium* and *Klebsiella pneumoniae*) bacteria. Besides, the extract, particularly from *R. alatoreticula*, showed prominent anticancer potency against the Hep3B liver cancer cell line by imparting morphological changes, interfering cell cycle, depleting mitochondrial membrane potential and alleviating reactive oxygen species (ROS) through Bax, Bcl2 and caspase 9 intrinsic mitochondrial pathway. Alongside, the study also aimed to isolate crude polysaccharides from macrofungi using hot water (HWP), cold alkali and hot alkali solvent systems. All fractions showed effective antioxidant activities, particularly potent was HWP, especially those from *R. alatoreticula* demonstrated better potential in all assays. Subsequently, the preparations revealed a strong immune-boosting property marked by augmentation of murine macrophage viability, phagocytosis, nitric oxide production, ROS generation and filopodia/lamellipodia formation. Thereafter, significant increase in expression of Toll like receptor (TLR)-2, TLR-4, nuclear factor kappa B (NF-κB) were observed that in turn resulted in increased level of cyclooxygenase-2, tumor necrosis factor-α, Iκ-Bα, interferon-γ and inducible nitric oxide synthase explaining mode of action through TLR/NF-κB pathway. Comparatively, HWP fractions again executed better immune-enhancing potential what could be explained by presence of glucose (mainly β-glucan), mannose and galactose in higher extent and organisation of carbohydrate backbone in triple helical conformation. In sum, the studied novel mushrooms could be regarded promising alternatives for use in functional food, nutraceutical and pharmaceutical industries fostering local food-based economy.

Key words: anticancer, β-glucan, DNA barcoding, immune-enhancement, tribal food.

EXPLOITATION OF LOCAL AGRICULTURAL WASTE FOR THE CULTIVATION OF A NEW MUSHROOM VARIETY IN MAURITIUS - THE MILKY MUSHROOM'

Vijayanti Mala Ranghoo-Sanmukhiya
University of Mauritius, Faculty of Agriculture, Department of Agricultural & Food Science, Mauritius

Corresponding author: m.sanmukhiya@uom.ac.mu

Milky mushroom also known as *Calocybe indica* is an edible mushroom that is cultivated worldwide and which is adapted to the tropical climatic conditions. The species *Agaricus* and *Pleurotus* are imported mushrooms that are mostly consumed by the population of Mauritius. In order to reduce our dependence on imported mushroom varieties, this research attempted to explore the performance of a new mushroom variety in Mauritius, using local agricultural wastes, including sugar cane leaves, banana leaves and sugarcane bagasse. This project aimed to develop knowledge in the field of mushroom cultivation that may be translated into business ideas for the hundreds of unemployed graduate scientists and dynamic entrepreneurs. The first part of this project reported the optimization of the spawn material and the optimization of the best agricultural waste that will be used for mushroom growing. It has been determined that potato dextrose agar is the most suitable substrate for laboratory cultivation of this mushroom, while wheat seed is the best for spawning. The most promising substrate for the cultivation of the milky mushroom was found to be bagasse, followed by sugar cane leaves and banana leaves, but a limited yield was observed on sawdust.
Key words*:* agricultural waste, bagasse, mushroom.

POSTHARVEST FUNGAL DISEASES AFFECTING TOMATO CROPS IN MAURITIUS

Nooreen Mamode Ally[1], Hudaa Neetoo[1], Vijayanti Mala Ranghoo-Sanmukhiya[1], Teresa A. Coutinho[2], Aleksandra Bulajić[3]

[1]University of Mauritius, Faculty of Agriculture, Mauritius; [2]University of Pretoria, Centre for Microbial Ecology and Genomics/Forestry and Agricultural Biotechnology Institute, Pretoria; [3]University of Belgrade, Faculty of Agriculture, Belgrade, Serbia

Corresponding author: noorishiny@gmail.com

Tomatoes are important horticultural crops that are widely cultivated for their fleshy fruits. They are rich in various vitamins, minerals and dietary fibre and at the same time impart flavour to food. Production of around 11, 000 tonnes of tomatoes are carried out annually from 750 hectares of land reserved for tomato plantation in Mauritius. Unfortunately, tomato fruits are easily prone to attack by pathogenic fungi during the pre- and post-harvest stages. Postharvest diseases have a negative impact on the yield and quality of the produce and can result in significant economic losses. This project was aimed at conducting a surveillance of fungal diseases affecting open-field and greenhouse-grown tomatoes and identifying the isolates by microscopic examination, culturing, PCR identification and DNA sequence analysis. Samples of infected tomato fruits displaying specific symptoms were collected from fields, greenhouses, markets and packhouses located in different agro-climatic zones of Mauritius. Isolation of fungal agents associated with specific postharvest infections was performed by first surface-disinfecting small cut pieces of the diseased fruit samples using 1% sodium hypochlorite, followed by air-drying and culturing on potato dextrose agar (PDA). The pure fungal cultures obtained were then subjected to macroscopic and microscopic identification. Pathogenicity tests were eventually done to confirm that the isolated fungi were capable of causing diseases on healthy hosts. If Koch's postulates had been fulfilled, the putative agent was identified by PCR and DNA Sequencing. Tomato fruits with various diseases such as Early Blight (EB), Late Blight (LB), Anthracnose (AN), Target spot (TS), Gray mold (GM), *Fusarium* wilt (FW) and Gray leaf spot (GLS) diseases were suspected during the disease survey. The putative etiological agents isolated on PDA produced different colonial characteristics and distinct mycelia after incubation. Molecular analyses of the isolates confirmed the identity of the agents of EB, LB, AN, TS, GM, FW and GLS to be *Alternaria solani*, *Phytophthora infestans*, *Colletotrichum brevisporum*, *Corynespora cassiicola*, *Botrytis cinerea*, *Fusarium equiseti and Stemphylium lycopersici* respectively. The diseases were ranked in the following decreasing order of occurrence: EB (55%) > LB (40%) > GLS (38%) > GM (32%) > FW (25%) > AN (9%) > TS (8%). This research is a stepping stone to future work which will necessitate characterization of the genetic diversity and virulence traits of post-harvest tomato pathogens for improved diagnosis and control.

Key words: tomatoes, crop diseases, fungi, open fields, greenhouses.

THE IMPORTANCE OF RAPID EARLY DETECTION OF *PHYTOPHTHORA INFESTANS* ON POTATOES IN MAURITIUS

Sandhya Devi Takooree[1], Hudaa Neetoo[1], Vijayanti Mala Ranghoo-Sanmukhiya[1], Vivian Vally[2], Aleksandra Bulajić[3], Jacquie van der Waals[4]

[1]University of Mauritius, Faculty of Agriculture, Department of Agricultural & Food Science, Mauritius; [2]Food and Agricultural Research and Extension Institute (FAREI), Reduit, Mauritius; [3]University of Belgrade, Faculty of Agriculture, Belgrade, Serbia; [4]University of Pretoria, Department of Plant and Soil Sciences, Pretoria, South Africa

Corresponding author: sandhyaneha2205@gmail.com

Potatoes are considered as one of the most economically important non-sugar food crops in Mauritius. Unfortunately, the production and yield of potatoes are subjected to considerable fluctuation every season due to the attack of the crop by pathogenic agents. Late blight, a disease caused by oomycota, *Phytophthora infestans*, is a greater threat to the potato crop than any other disease for the island. However, this disease remains the most challenging to manage once the symptoms have appeared, thus requiring rapid detection for effective disease management. The objective of this study was to compare different methods for early detection of the causal agent of potato late blight. Conventional culture-based methods involved the direct isolation of *P. infestans* from infected leaves on Carrot Piece Agar (CPA), Carrot Sucrose Agar (CSA), Commercial Potato Dextrose Agar (CPDA), Fresh Potato Dextrose Agar (FPDA-1 and FPDA-2), Oatmeal Agar (OMA), Pea Sucrose Agar (PSA) and Water Agar (WA), without antibiotic supplementation. Mycelial growth on agar was subsequently identified using molecular techniques. A culture-independent method was also attempted whereby total genomic DNA was directly extracted from symptomatic leaves with mycelial growth followed by PCR amplification with ITS5/ITS4 primers and sequencing. The different media ranked in the following decreasing order of performance: PSA >>> CSA ~ FPDA-1 > CPA ~ CPDA ~ OMA, with growth appearing on PSA within 7 days without contamination. DNA sequencing confirmed the identity of the agent recovered from PSA and from diseased leaves to be *P. infestans*. Findings of this study point to an optimum nutritive medium for recovering and culturing *P. infestans* from leaves with foliar blight without the use of antibiotics. Alternatively, a culture-independent method proved to be as reliable but more suitable for rapid detection and identification during routine disease surveillance.

Key words: late blight, morphology, pea sucrose agar, sequencing.

MORPHOLOGICAL AND GENETIC DIVERSITY IN THE POPULATIONS OF *MYCETINIS ALLIACEUS* AND *GYMNOPUS ANDROSACEUS*

Eleonora Čapelja, Nataša Kočiš Tubić, Ivan Dudaš, Maja Karaman
University of Novi Sad, Faculty of Sciences, Novi Sad, Serbia

Corresponding author: eleonora.capelja@dbe.uns.ac.rs

Population diversity represents their evolutionary potential and is necessary for populations to adapt to constant changes in the environment. Due to the problem of properly defining species, individuals and populations of fungi, the molecular diversity of fungi is relatively poorly studied when compared to other groups of organisms.
In this paper, morphological and genetic diversity was examined in three populations of *Mycetinis alliaceus* (Stara planina, Kopaonik and Biogradska gora) and in two populations of *Gymnopus androsaceus* (Tara and Kopaonik). It was determined that all examined populations of *M. alliaceus* were clearly morphologically and genetically differentiated (Fst 0.31 and 0.32), but the populations from Kopaonik and Biogradska gora showed greater similarity. Populations of *G. androsaceus* showed heterogeneity in the examined morphological characters, while genetic differentiation between them was significant (Fst 0.28). The obtained data represent one of the first data on the population structure of the examined species and can serve as the basis for further research.
Key words: Biogradska gora, diversity, Kopaonik, Stara planina, Tara.

ANTIFUNGAL ACTIVITY OF SURFACTANT IONIC LIQUIDS ON MYCOTOXIGENIC MOLDS

Olivera Popov[1], Strahinja Leovac[2], Teodora Despotovski[2], Maja Karaman[2], Slobodan Gadžurić[2], Eva Pogorelc[3], Eleonora Čapelja[2]

[1]Institute of Field and Vegetable Crops, National Institute of Republic of Serbia, Novi Sad, Serbia; [2]University of Novi Sad, Faculty of Sciences, Novi Sad, Serbia; [3]University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia

Corresponding author: olivera.popov@nsseme.com

Traditionally, mold control usually involves usage of highly toxic compounds, and this practice must be replaced with more environmentally friendly solutions (green chemistry). In this paper, antifungal activity of five newly synthesized ionic liquids (IL) was tested, in vitro. All ionic liquids are based on N-decyl- N, N, N-trimethylammonium chloride which differs only by a single substituent on a quaternary N- atom. The antifungal activity of IL was tested on *Fusarium, Aspergillus, Alternaria, Trichoderma* and *Penicillium* strains using the microdilution method by determining the minimal inhibitory and minimal fungicidal concentrations. Selected genera are major problem in food industry since they are the primary pathogens of agronomically important plants, as well as mycotoxin producers. All five IL used in this study showed antifungal effect in the range of 0.002 mol/dm^3 to 0.036 mol/dm^3. The greatest antifungal activity was observed when strains were treated with (C_2OH)C_{10}DMACI and (C_2OOEt)C_{10}DMACI IL. Results obtained in this study showed that all examined ILs have the potential to be used as effective antifungal agents.

Key words: mycotoxins, green chemistry, food, fungicides.

NEGATIVE FEATURES OF SOME DENTAL PRODUCTS CONTAINING EUCALYPTOL: UPREGULATION OF GENES EXPRESSION ENCODING FOR EFFLUX PUMPS IN *CANDIDA ALBICANS*

Dejan Stojković, Jasmina Glamočlija, Marija Ivanov
University of Belgrade, Institute for Biological Research „Siniša Stanković“, National Institute of Republic of Serbia, Belgrade, Serbia

Corresponding author: dejanbio@ibiss.bg.ac.rs

Eucalyptol is the dominant compound of eucalyptus oil and an ingredient of many products used for teeth whitening and washing. This study aimed to reveal the influence of eucalyptol on *Candida albicans* growth isolated from oral cavity and to test expression of virulence traits in the presence of this compound. In this study, we examined the ability of eucalyptol to inhibit the growth of different *Candida* strains in suspension and biofilm, to block hyphal transition along with their impact on genes encoding for efflux pumps (*CDR1* and *CDR2*) and ergosterol biosynthesis (ERG11). In the case of patients suffering from fungal infections, upregulation of *CDR1* and *CDR2* is an undesirable property for any therapeutic given along the way, while their downregulation is seen as a promising antifungal trait. Eucalyptol showed antifungal activity with a minimal inhibitory concentration of 2–23 mg/mL. The application of eucalyptol reduced the formation of biofilm biomass by more than 50% in three *C. albicans* strains (*C. albicans* ATCC 10231, *C. albicans* 475/15 and *C. albicans* 503/15) at their MIC concentrations. The application of eucalyptol (23 mg/mL) induced a notable reduction in the number of hyphal cells. Treatment with eucalyptol led to the increased expression of *CDR1* and *CDR2* and did not interfere with the *ERG11* expression. Although, eucalyptol showed some antifungal activity against *Candida* strains, its use in cosmeceutical dental products should be with caution. Patients having problems with oral *Candida* infections and using antifungal drugs should not use preparations based on eucalyptol since the compound showed negative features on expression of genes encoding for efflux pumps, possibly leading to lower effectiveness of antifungal drugs.
Key words: eucalyptol, *Candida albicans*, virulence traits, dental products, efflux pumps.

HEMOLYTIC POTENTIAL OF BIOAEROSOL-DERIVED *ASPERGILLUS, PENICILLIUM* AND *TALAROMYCES* ISOLATES

Željko Savković[1], Miloš Stupar[1], Nikola Unković[1], Ana Stančić[2], Jelena Vukojević[1], Milica Ljaljević Grbić[1]

[1]University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac", Belgrade, Serbia; [2]University of Belgrade, Institute for Medical Research, Laboratory for Immunology, Belgrade, Serbia

Corresponding author: zsavkovic@bio.bg.ac.rs

Aspergillus, Penicillium and *Talaromyces* species are frequently cited as contaminants of various substrata and are often associated with indoor environments. The main purpose of this study was to assess the potential pathogenicity of aerosol-derived fungi from *Aspergillus, Penicillium* and *Talaromyces* genera, isolated in the rooms for conservation of cultural heritage artefacts, via estimating hemolytic activity. Hemolysis was detected in 20.58% of tested isolates at 37 ºC (11.76% partial and 8.82% complete) and 64.71% at 25 ºC (38.24% partial and 26.47% complete). The majority of isolates that caused α hemolysis led to the significant oxidation of hemoglobin iron with methemoglobin content in blood agar medium, higher than 80%. *Aspergillus melleus* was the only tested fungi that caused formation of ferrylhemoglobin after the incubation at 25 °C. Obtained I values (index of activity for hemolytic exoenzymes) for α hemolysis were in range of from 0.13 to 0.60 for 37 °C, while for the temperature of 25 °C values were in range of from 0.08 to 0.50. The same values for β hemolysis were in range of from 0.03 to 0.08 (37 °C), i.e. 0.06 to 0.49 (25 °C). Monitoring of pathogenic airborne fungi in indoor environments and estimation of their virulence is essential for the adequate assessment of human health risks.

Key words: fungi, blood agar, hemolysis, pathogens, virulence.

DEGRADATION OF PRETREATED AGRO-FORESTRY RESIDUES BY SELECTED MICROMYCETES

Milica Galić
University of Belgrade, Faculty of Biology, Serbia

Corresponding author: galic.m@bio.bg.ac.rs

Nowadays, there are huge amounts of lignocellulosic materials, consist of cellulose, hemicellulose and lignin, left in agro-forestry practice, which can be transformed into useful products. Biomass exploitation could be aiming not only to replace conventional energy sources but also to preserve biodiversity and natural ecosystems. Five micromycetes were studied with goal to determine their potential to produce active *cellulases* as well as the ability to decompose pretreated wheat straw and oak sawdust after 7 days of solid-state fermentation. Wheat straw was better lignocellulosic than oak sawdust for the production of cellulases in all analyzed micromycetes. Thus, *Penicillium solitum* BEOFB 1190m strain has shown to be the best producer of highly active forms of *xylanases* (7532.36 ± 89.37 U/L). The most active endo- and exocellulases (2299.70 ± 72.17 U/L and 195.66 ± 4.64 U/L, respectively) were produced by *Trichoderma harzianum* BEOFB 1230m, while the maximal value of β-glucosidase activity (215.69 ± 3.13 U/L) was detected after *Fusarium graminearum* BEOFB 820m cultivation. *T. harzianum* also showed high efficiency in wheat straw cellulose and hemicellulose depolymerization (23.90% and 33.00%, respectively), which resulted in the highest dry matter loss (36.25%). The results of the study showed great potential of tested micromycetes to synthesize cellulolytic enzymes and consequently transform abundant, low-cost plant residues such as wheat straw into useful products including biofuel.

Key words: agroforestry residues, cellulolytic enzymes, depolymerization, micromycetes, plant residue.

LIGNOCELLULOSE INDUSTRIAL FOOD WASTE - A VALUABLE SUBSTRATE FOR GROWING EDIBLE AND MEDICINAL MUSHROOMS

Saša Despotović[1], Natalija Velić[2], Darko Velić[2], Hrvoje Pavlović[2], Janez Gorenšek[3], Viktor Nedović[1], Milana Pribić[4], Jelena Pejin[4]

[1]University of Belgrade, Faculty of Agriculture, Belgrade, Serbia; [2]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Osijek, Croatia; [3]IAMB Institute - Institute of Applied Mycology and Biotechnology, Celje, Slovenia; [4]University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

Corresponding author: sdespot@agrif.bg.ac.rs

Promoting the use of waste materials as a resource is one of the fundamental tenets of the circular economy model implemented in the development strategies of many countries. The lignocellulosic waste of the food industry has been primarily disposed of in landfills, posing a danger to the environment. In the context of the circular economy model, this waste is a valuable resource. Thus, its composition can be used as a substrate for growing edible and medicinal mushrooms. The cultivation of edible mushrooms on lignocellulosic waste materials is currently one of the few processes that achieve a dual effect: the production of protein-rich foods and reducing environmental pollution. The edible and medicinal mushroom market shows a steady increase both due to their nutritional properties and being a source of valuable biologically active compounds. Immunomodulatory, cytostatic or cytotoxic effects, control of blood sugar levels, and weight loss are just examples of the action of medicinal mushrooms. After mushroom cultivation, the used substrate lags have the potential for further use as compost, feed, and additive in the production of building materials and as a substrate for biofuels. Mushroom cultivation generally does not require large agricultural areas for cultivation. The equipment used for cultivation is often low-tech; therefore, the cultivation of edible mushrooms on waste materials is an excellent alternative to conventional food production methods, which is especially important for developing countries. However, research is being done to improve and develop equipment and breeding techniques to ensure better process control and productivity.

Key words: food waste, lignocellulose, edible mushrooms, medicinal mushrooms.

LIGNICOLUS MUSHROOM *FOMITOPSIS PINICOLA* AS A POTENT INHIBITOR OF LIPID PEROXIDATION

Maja Kozarski[1], Anita Klaus[1], Jovana Vunduk[2], Vesna Lazić[1], Bojana Spirović Trifunović[1], Srđan Miletić[3], Snezana Spasić[3], Dragica Jakovljević[3]

[1]University of Belgrade, Faculty of Agriculture, Belgrade, Serbia; [2]University of Belgrade, Institute of General and Physical Chemistry, Belgrade, Serbia; [3]University of Belgrade, Institute of Chemistry, Technology and Metallurgy, National Institute, Belgarde, Serbia

Corresponding author: maja@agrif.bg.ac.rs

Excessive production of reactive oxygen species (ROS) often results in irreversible cell damage and leads to a number of disorders such as hypertension, heart disease, diabetes, inflammation, premature aging and cancer. Considering the importance of diet in prevention of diseases associated with oxidative stress, this study was conducted to evaluate antioxidant activity of the methanol extract of wild lignicolus mushroom *Fomitopsis pinicola* as natural source of functional food ingredients. Nowadays functional food products based on the lignicolous mushroom species are increasingly available on the market. Their powders and extracts are consumed as dietary supplements in the form of capsules or tablets and as additives in the formulation of healthier food products.

Antioxidant capacity of *F. pinicola* methanol extract was evaluated *in vitro* by the inhibition of lipid peroxidation (LPx) in the linoleic acid model system. Results were normalized and expressed as EC_{50} values (μg/mL). The Folin-Ciocalteu reaction adapted for a 96-well microplate reader was used to determine the total phenol content (TPC) and result was expressed as mg of gallic acid equivalent (GAE) per g of dry weight (DW) of extract. Phenolic profile of the extract was analyzed by the liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Based on the analysis, TPC of the methanol extract was reported to be 133.11 mg GAE/g of DW. Among hydroxybenzoic acids, the most abundant was gallic acid, represented by 951.12 μg/g of DW. Also, presence of ellagic, protocatechuic, p-hydroxybenzoic and syringic acid was observed. Among hydroxycinnamic acids, chlorogenic acid was the most prevalent, with 0.76 μg/g of DW. With regard to antioxidant activity and inhibition of LPx, EC_{50} value for *F. pinicola* methanol extract was 20.0 μg/mL.

The results of the present study suggest that methanol extract of the wild lignicolus mushroom *F. pinicola* acts as natural antioxidant in the prevention of LPx. This mushroom may be a good source for the development of antioxidant additives.

Key words: lignicolus mushroom, antioxidant potential, lipid peroxidation, phenol profile, functional food.

Acknowledgment

This investigation is the result of research within the "Agreement on the implementation and financing of scientific research work in 2022 between the Faculty of Agriculture in Belgrade and the Ministry of Education, Science and Technological Development of the Republic of Serbia", contract record number: 451-03-68/2022-14/200116, 451-03-68/2022-14/200051 and supported by the Science Fund of the Republic of Serbia, #Grant No: 7748088, "Composite clays as advanced materials in animal nutrition and biomedicine-AniNutBiomedCLAYs".

ANTIFUNGAL AND ANTIBIOFILM ACTIVITY OF PLANT EXTRACTS AGAINST YEAST *CANDIDA ALBICANS, CANDIDA GLABRATA* AND *PICHIA MEMBRANIFACIENS*

Ružica Tomičić[1], Zorica Tomičić[2], Sunčica Kocić-Tanackov[1], Peter Raspor[3]
[1]University of Novi Sad, Faculty of Technology, Novi Sad, Serbia; [2]University of Novi Sad, Institute of Food Technology, Novi Sad, Serbia; [3]University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

Corresponding author: ruzica.tomicic@yahoo.com; ruzica.tomicic@uns.ac.rs

Yeast biofilms pose health risks in clinical environments and food industry. Despite years of effort, new antifungal approaches are needed since it is known that biofilms show much greater resistance to the antifungal agents than their free-living counterparts. Hence, we investigated antifungal activity of plant extracts such as *Humulus lupulus, Alpinia katsumadai* and *Evodia rutaecarpa* against *Candida albicans* ATCC 10261, *Candida glabrata* ZIM 2369 and *Pichia membranifaciens* ZIM 2417. The aim of this study was to investigate whether these plant extracts can interfere with biofilm formation as well as acting on preformed biofilms. The minimal inhibitory concentrations (MICs) of plant extracts were determined using the CLSI M27-A2 broth microdilution method. The method used to assess antibiofilm activity was crystal violet staining. According to the MIC values in this study, all plant extracts were effective in the inhibition of yeast strains. On the other hand, *A. katsumadai, E. rutaecarpa* and *H. lupulus* extracts exhibited the highest antibiofilm activity against *C. albicans* to the stainless steel surface at both tested concentrations (1/2 × MIC and 1 × MIC). *A. katsumadai* was also effective in the initial phase of biofilm formation of *C. glabrata* at 1/2 × MIC and 1 × MIC, while the inhibition of preformed biofilm was more difficult to achieve. Our results clearly demonstrate that biofilms of *C. glabrata* are more resistance to the extracts as compared to *C. albicans*. Regarding *P. membranifaciens*, extracts of *A. katsumadai* and *E. rutaecarpa* were promoted the growth and development of a preformed biofilm.

Key words: biofilm, plant extracts, stainless steel surface, yeast.

Acknowledgements
Ružica Tomičić thank Provincial secretariat for higher education and scientific research, Autonomous Province of Vojvodina, Republic of Serbia (project no. 142-451-2623/2021-01).

ESSENTIAL OILS AS ANTIFUNGAL AND ANTI-ADHESION AGENTS AGAINST *CANDIDA ALBICANS* AND *SACCHAROMYCES CEREVISIAE*

Zorica Tomičić[1], Sunčica Kocić-Tanackov[2], Ivana Čabarkapa[1], Ljubiša Šarić[1], Ružica Tomičić[2]

[1]University of Novi Sad, Institute of Food Technology, Novi Sad, Serbia; [2]University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

Corresponding author: zorica.tomicic@fins.uns.ac.rs; zoricateh@yahoo.com

Multiple drug resistance of food spoilage and human pathogenic microorganisms has been developed in recent years due to inadequate and non-selective use of synthetic antimicrobial agents commonly used in the treatment of infectious diseases and food preservation. The use of natural antimicrobial agents has gained much attention to extend shelf-life, increase the safety of food products in the food industry and inhibit disease-causing microorganisms. The aim of this study was to evaluate antifungal and anti-adhesion activity of fifteen essential oils (EOs) and their compounds against yeast *Candida albicans* ATCC 10231and *Saccharomyces cerevisiae* ATCC 9763. Antifungal activity was determined by testing the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of essential oils and compounds. The essential oils of *Cinnamonum zeylanieum* and *Eugenia caryophyllus* showed the highest antifungal activity with MICs ranging from 0.078 to 1.25 mg/mL and 0.039 to 0.078 mg/mL, respectively. On the other hand, essential oils of *Rosmarinus officinalis* and *Salvia officinalis* had significantly weaker antifungal properties than the other EOs. MIC concentrations were used to assess the inhibition of adhesion of the tested yeasts in a microtiter plate using the crystal violet staining method. Based on the percentage of adhesion inhibition, yeast *S. cerevisiae* showed a high level of resistance to antifungal agents. Among the essential oils examined, *E. cariyophyllus* had the strongest effect with a percentage of inhibition up to 63.5%. The most active anti-adhesion compounds tested were carvacrol and thymol. Considering the role of biofilm in food spoilage and clinical diseases, inhibition of the initial phase of biofilm formation by natural antimicrobial agents may be an alternative to commonly used synthetic ones.

Key words: essential oils, antifungal agents, antiadhesion agents, yeast.

Acknowledgements

Ružica Tomičić thank Provincial secretariat for higher education and scientific research, Autonomous Province of Vojvodina, Republic of Serbia (project no. 142-451-2623/2021-01).

APPLICATION OF ESSENTIAL OILS OF SPICES AND MEDICINAL HERBS AGAINST *CANDIDA ALBICANS*

Maja Vukašinović Sekulić
University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia

Corresponding author: vukasinovic@tmf.bg.ac.rs

Candida albicans is pathogenic yeast species present as a part of the normal microbiota mucosal surface oral and vaginal cavities and digestive tract of warm-blooded animals and humans, without causing any health problems. Intensive growth and infection can occur as a result of excessive use of broad-spectrum antibiotics, corticosteroids, in immunosuppressive individuals, so as under the influence of numerous external factors that weaken the body's defence system. Since antibiotics stimulate the growth of *Candida albicans*, treatment is based on the use of antimycotic drugs and dietary adjustments, when healing process can sometimes take several weeks. In addition, long-term use of antimycotic drugs causes side effects (nausea, vomiting, diarrhea) and their frequent use contributes development of resistant strains of *Candida albicans*, which further complicates curing. In order to alleviate treatment, recent years great interest is focused on finding and application of natural preparations, such as essential oils of spices and medicinal herbs, which possess numerous biological properties, including antifungal activity.

The aim of this study was to evaluate the antifungal activity of essential oils of various spices and medicinal herbs against *Candida albicans* in order to find an alternative treatment for candidiasis.

The antifungal activity of twenty essential oils and herbal tinctures was investigated by agar well diffusion and broth macrodilution methods, and the obtained results were compared with the effect of antimycotic drugs (nystatin) and commercial preparations present in the local marketplace.

Based on the results obtained by the agar well diffusion method, undiluted essential oils and herbal tinctures can be divided into five groups, according to the diameter of inhibition zone they form. Very strong antifungal activity was observed with essential oils of oregano, basil, thyme and cinnamon, with a diameter of the inhibition zones greater than 40mm, while strong activity was also noticed with essential oil of lemon balm (34.5 mm). Essential oils of clove, ginger, mint, bay and lavender have shown moderate antifungal activity (20-30 mm) , while weak activity was registered with essential oils of rosemary, star anise and turmeric (16-20 mm). Very weak antifungal efficacy was observed with sage and immortelle essential oils (12-15 mm), while antifungal activity was not present with essential oils of frankincense and black pepper, as well with herbal tinctures of garlic and wild garlic. The lowest values for minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were achieved with essential oils which had very strong and strong antifungal activity determined by agar well diffusion method, as undiluted and diluted in 96% ethanol.

According to the results obtained in this study, the essential oils of cinnamon, oregano, basil, thyme and lemon balm can be used, instead of nystatin, in infections caused by *Candida albicans* strains.

Key words: essential oils, antifungal activity, *Candida albicans*, candidiasis.

INHIBITORY EFFECTS OF OREGANO AND CINNAMON ESSENTIAL OILS ON THE GROWTH OF *ASPERGILLUS NIGER*

Stefan Kolašinac[1], Jelena Otašević[1], Tanja Žugić-Petrović[2], Zorana Žugić[3]
[1]University of Belgrade, Faculty of Agriculture, Belgrade, Serbia; [2]University of Kragujevac, Faculty of Biology and Ecology, Kragujevac, Serbia; [3]University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

Corresponding author: tanja.zugicpetrovic@yahoo.com

Antimicrobial effects of spice essential oils are increasingly used as an alternative to food preservatives. A number of studies have shown that some compounds isolated from oils display antimicrobial activity. This study examined the effect of oregano and cinnamon essential oils in different concentrations (1, 3, 5, 10, 25 and 50 μL/mL) on the growth rate of *Aspergillus niger* (isolated from sheep meat) at different water activity levels (a_w) (0.99 and 0.95) and different temperatures (15 °C and 30 °C). Chemical analysis of the essential oils of cinnamon and oregano, identified by gas chromatography-mass spectrometry, revealed that cinnamon oils were mainly composed of cinnamaldehyde, 2-methoxycinnamaldehyde and carveol. In oregano oils, two components, carvacrol and thymol were identified. Antifungal effect of essential oils on *A. niger* was determined by measuring the diameter of mycelium on plates with potato dextrose agar. The test results showed that cinnamon essential oil is the most effective because it completely inhibited the growth of mycelium *A. niger* at 1 μL/mL. Oregano oil was less effective, with no observed mycelial growth at 3 μL/mL. The results showed that at a water activity of 0.99, ested essential oils retained an inhibitory effect on the growth rate of *A. niger* on both temperature tests. At 0.95 a_w, the tested essential oils had an inhibitory effect on the growth rate of *A. niger* at the test temperature of 15°C and without inhibitory effect at the temperature of 30 °C, which indicates that the antimycotoxigenic activity of the tested essential oils depends on environmental conditions.
Key words: antimycotoxigenic activity, *Aspergillus niger*, essential oils.

INFLUENCE OF BENZOATE AND SORBATE ON GROWTH OF SELECTED FUNGI OF THE *PENICILLIUM GENERA*

Jelena Šego Božanović, Natalija Velić, Darko Velić, Frane Čačić Kenjerić, Hrvoje Pavlović

Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Osijek, Croatia

Corresponding author: hrvoje.pavlovic@ptfos.hr

The aim of the study was to test antifungal activity of sodium benzoate and potassium sorbate on selected fungal species: *Penicillium aurantiogriseum*, *P. expansum* and *P. italicum* at various pH. Sodium benzoate is a preservative most widely used in carbonated drinks, fruit juices, jams, conserved vegetables, pickles and condiments where, at acidic environment, inhibits fungal growth. Potassium sorbate is primary used as a food preservative. It inhibits molds and yeasts in many food products, such as cheese, wine, yogurt, soft drinks and baked goods. Experimental results suggest increased antifungal activity of tested compounds at lower pH values. Both compounds inhibit fungi at similar concentrations, although lower pH (4.16) enhances inhibitory effect of K-sorbate. Linear inhibition of fungal growth at 50, 100 and 200 ppm of Na-benzoate and K-sorbate at pH 4.4 and 4.16 was tested. Stronger inhibitory potential of K-sorbate in linear colony growth was observed, compared to effect of Na-benzoate.

Key words: Na - benzoate, K - sorbate, antifungal activity, *Penicillium*, pH.

INVESTIGATION OF ANTIFUNGAL POTENTIAL OF AQUEOUS AND ETHANOLIC EXTRACTS OF THE PLANT *COTA TINCTORIA* ON SELECTED ISOLATES OF YEASTS AND DERMATOMYCETES

Dejan Stojković, Marina Kostić, Jovana Petrović, Ana Ćirić
University of Belgrade, Institute for biological research "Siniša Stanković", National institute of Republic of Serbia, Belgrade, Serbia

Corresponding author: dejanbio@ibiss.bg.ac.rs

Fungal infections are a serious health related burden. The increasing and non-selective use of commercial antimicrobial therapeutics, due to numerous infections and diseases, causes an increase in the resistance of pathogenic microorganisms to synthetic antimicrobial drugs. Although a large number of plant species used in traditional medicine, have been well phytochemically and pharmacologically investigated and therefore recognized in modern medicine, large number still remains unexplored.

The aim of this study was to examine the antifungal potential of aqueous and ethanolic extracts of the plant golden marguerite - *Cota tinctoria* on the certain yeasts and dermatomycetes.

The aerial parts of the *Cota tinctoria* plant were used for the extract preparation. The microdilution method was used to examine antifungal activity. The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of the tested extracts were determined. The following fungal species were used: *Candida albicans* (clinical isolate), *Candida albicans* (ATCC 10231), *Candida krusei* (clinical isolate), *Microsporum gypseum* (clinical isolate), *Trichophyton rubrum* (ATCC 28188) and *Trichophyton mentagrophytes* (clinical isolate).

Aqueous extracts, except for the inhibitory effect on *C. albicans*, had no effect on the tested yeasts and dermatomycetes. Ethanol extracts showed different antifungal activity. The most sensitive species were: *T. mentagrophytes* (MIC 0.50 mg/mL, MFC 1.00 mg/mL) and *T. rubrum* (MIC 0.75 mg/mL, MFC 2.00 mg/mL), while the most resistant species was *C. krusei* (MIC 8.00 mg/ml). Commercial antifungals, ketoconazole (MIC 0.003 - 0.05 mg/mL) and fluconazole (MIC 0.006 - 0.05 mg/mL) showed better antifungal activity compared to the tested extracts.

The results of this investigation showed good antifungal characteristics of *C. tinctoria* plant extracts and may have potential application as an adjunct in the treatment of superficial infections caused by certain micromycetes.

Key words: *C. tinctoria*, antifungal potential, yeast, dermatomycetes, superficial infections.

MECHANISMS OF ACTION OF SELECTED FLAVONOIDS TOWARDS SPECIES OF THE GENUS *CANDIDA*

Tamara Carević, Jasmina Glamočlija, Biljana Nikolić, Marija Ivanov, Marina Soković

University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, Belgrade, Serbia

Corresponding author: carevic.tamara5@gmail.com

The frequency of resistance to a large number of antimicrobial agents is increasing, so there is a need to find new ones. A major problem in the treatment of infections caused by *Candida albicans* is a highly resistant biofilm, which is a virulence factor of this species. The mechanisms of action of selected flavonoids (hesperetin, sakuranetin, and taxifolin) on species of the genus *Candida* were explored. The strains that were tested were *C. albicans* 10/15, *C. albicans* 13/15, *C. albicans* ATCC 10231, *C. albicans* 475/15, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750, *C. krusei* H1/16, and *C. glabrata* 4/6/15. The antimicrobial potential of flavonoids was investigated by the microdilution method. Determining the ability of these compounds to disrupt virulence factors, in the model of *Candida albicans*, is based on monitoring the process of disrupting the formation and destruction of previously formed biofilm. The cytotoxic effect on selected human cell lines (lung fibroblasts) was investigated in order to determine whether flavonoids are selectively toxic to species of the genus *Candida*, or cause non-selective toxicity in eukaryotic cells. The laboratory methods used were microdilution method, antibiofilm method (crystal violet assay, measures biofilm biomass), Congo red binding assay (measures the influence of compounds on exopolysaccharide, EPS, production in biofilm), MTT cytotoxicity assay. The most pronounced antifungal potential was observed for sakuranetin with a MIC of 0.041 mg/mL for *C. parapsilosis* ATCC 22019 and a MIC of 0.082 mg/mL for the other strains tested. The antimicrobial potential of hesperetin and taxifolin is the same for all tested strains; MIC value is 0.165 mg/mL. All substances have been shown to be effective in inhibiting biofilm formation, but also in destroying it. The antibiofilm effect of sakuranetin applied in concentration equal to MIC against *C. glabrata* 4/6/15 and *C. krusei* H1/16 is over 85%. These two strains are the most sensitive to the action of sakuranetin. The ability to destroy the previously formed biofilm was pronounced towards *C. albicans* 475/15 (52.80%), and moderate towards *C. albicans* ATCC 10231 (12.22%). The antibiofilm potential of hesperetin is high for *C. glabrata* 4/6/15 (70.4%) and *C. parapsilosis* ATCC 22019 (62.6%), while for other strains the percentage of inhibition is less than 50%, and for the formation of biofilm *C. krusei* H1/16 hesperetin has no effect at all. The distinct effect of this flavonoid on the destruction of previously formed biofilm is emphasized, with significant percentages of destruction, while the percentages of EPS inhibition are low but significant (higher or closer to the effect of ketoconazole). The most pronounced antibiofilm effect of taxifolin is exhibited towards *C. glabrata* 4/6/15 (61.6%), while the percentage of inhibition is not higher than 50% for other strains, which is generally lower effect than for other tested substances. The cytotoxicity test indicates the following range of sensitivity of the examined cell line to flavonoids: sakuranetin>hesperetin>taxifolin. The tested flavonoids have excellent potential to be part of antifungal therapies, especially hesperetin, which has shown a strong antibiofilm effect with pronounced selectivity towards pathogen cells.

Key words: flavonoids, antimicrobial, *Candida*, virulence factors, antibiofilm.

PCR METHODS FOR MYCOTOXIN-PRODUCING FUNGI DETECTION

Marija Pavlović, Aleksandra Tasić, Mihajlo Vićentijević, Ivan Pavlović, Ksenija Nešić
Scientific Veterinary institute of Serbia, Belgrade, Serbia

Corresponding author: majaspavlovic@gmail.com

Fungi can contaminate foods and feeds at different stages of harvesting, processing, handling and storage, whit mycotoxins formation being one of the most significant aspects of food spoilage. Conventional procedures for the detection of fungi are unreliable and time consuming, and may be influenced by environmental conditions, thus number of nucleic acid based methods have been developed. DNA-based methods are independent of the morphological and biochemical characteristics of fungi. These methods have the advantage over conventional cultural and phenotypic methods in more accurate amount of fungi that they can give. Additionally, they are useful when no spores or other characteristic organs develop. On the other hand, number of diagnostic molecular tests are insufficient, for example for *Fusarium* spp. Regarding *Fusarium* species, molecular quantification assays for detecting individual Fusarium species and subgroups exist, but a method for the detection and quantification of the whole Fusarium group is still lacking. Development of PCR method targeting *Fusarium*-specific elongation factor region (EF1α) is underway. Existing PCR assays needs further improvement. On the other hand, the extreme low legal level of tolerance mycotoxins contamination in food and feed matrices nowadays, point out the need to develop the more sensitive, specific, rapid, cost-effective, and safer to use mycotoxigenic fungi detection technologies.

Key words: PCR methods, mycotoxins, fungi.

FROM ON-SITE TO IN-LAB: MICROSCOPIC OBSERVATION OF FUNGAL PROLIFERATION ON 17TH CENTURY MURAL PAINTINGS

Milica Ljaljević Grbić[1], Miloš Stupar[1], Željko Savković[1], Aleksandar Knežević[1], Ivica Dimkić[1], Janez Kosel[2], Črtomir Tavzes[2], Nikola Unković[1]

[1] University of Belgrade, Faculty of Biology, Belgrade, Serbia; [2] Institute for the Protection of Cultural Heritage of Slovenia, Ljubljana, Slovenia

Corresponding author: unkovicn@bio.bg.ac.rs

The fungal community of biodeteriorated 17th century mural paintings within the nave and altar portion of the old Church of the Holy Ascension (Veliki Krčimir, Serbia) has been studied via an array of microscopic analyses in order to detect actively growing fungi and assess their potential damage to the painted layer and mortar. In situ microscopy, performed with portable microscopes, together with optical and scanning electron microscopy, has revealed impairments of the painted layer in the form of cracks and biopitting, along with surface salt deposits and hidden, symptomless fungal growth. Various structures, such as fully developed fruiting bodies and melanized mycelia, clusters of microcolonial fungi and lichen soredia as well as a conidial apparatus and numerous conidia in mass have been observed, all attesting to the presence of actively growing fungal community on the surface of the painted layer and in the interspace between the painted layer and mortar. Based on the observed reproductive structures, the main agents of biodeterioration have been identified as fungi of *Chaetomium* and *Cladosporium* genera. The documented deterioration symptoms are most likely due to hyphal penetration and formation of fruiting bodies and other fungal structures.

Key words: biodeterioration, *Chaetomium*, *Cladosporium*, conservation, fungi, in situ microscopy, mural paintings, SEM, optical microscopy.

ANALYSIS OF CONTAMINATION BY YEASTS AND MOLDS IN HONEY SAMPLES DURING STORAGE

Dragana Ljubojević Pelić[1], Miloš Pelić[1], Suzana Vidaković Knežević[1], Jelena Vranešević[1], Bojana Prunić[1], Nikolina Novakov[2], Milica Živkov Baloš[1]

[1]Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia; [2]University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia

Corresponding author: dragana@niv.ns.ac.rs

Yeasts and molds as well as spore-forming bacteria are the microorganisms of concern in honey. These microorganisms withstand the concentrated sugar, acidity and other antimicrobial properties of honey. Contamination of honey by yeasts and molds possess a challenge to public health. Furthermore, the count of yeasts and molds is used as an important indicator of hygiene levels during honey processing, storage and transportation due to the fact that the development of these organisms affects honey quality and leads to deterioration and spoilage of honey. In the present study, 38 samples of honey collected in various parts of country during 2018 were tested for the number of yeasts and molds. Samples were stored at room temperature and changes in number of yeasts and molds were monitored during storage and the samples were retested in 2019, 2020 and 2021. The aim of this paper was to examine the counts of yeasts and moulds in different honey types during storage. In 42.1% of examined samples the number of yeasts and molds varied from 10 to 560 cfu/g in 2018, from 30 to 980 cfu/g in 2019, from 50 to 980 in 2020 and from 50 to 950 in 2021. The average counts of yeasts and molds were 48, 82, 90 and 105 CFU/g in 2018, 2019, 2020 and in 2021, respectively. In 13.2%, 15.8%, 21.05% and 26.3% of samples the number of yeasts and moulds exceed the limit (100 cfu/g) established by Serbian National Guidelines on microbiological criteria for foodstuffs in 2018, 2019, 2020 and in 2021, respectively. In 57.9% of samples no changes were observed during storage.

Key words: Honey, shelf-life, mycological quality, deterioration.

Acknowledgement

This work was funded by Ministry of Education, Science and Technological development of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2022, Contract No: 451-03-68/2022-14/200031.

MOLD CONTAMINATION IN SMALL-SCALE FACILITIES DURING THE PRODUCTION OF TRADITIONAL DRY-CURED SHEEP HAM

Tanja Žugić-Petrović[1], Zorana Žugić[2], Mirijana Grujović[1], Katarina Mladenović[1], Sunčica Kocić-Tanackov[2]

[1]Faculty of Biology and Ecology, University of Kragujevac, Kragujevac, Serbia; [2]Faculty of Technology, University of Novi Sad, Novi Sad, Serbia

Corresponding author: tanja.zugicpetrovic@yahoo.com

Sjenica sheep ham is an autochthonous product from fermented sheep meat that is traditionally produced in rural households in the municipalities of Sjenica and Tutin. The product is prepared according to a unique recipe and technology. The aim of this study was to define mold strains that grow in the production plant and on dried sheep ham. The observational study collected 128 samples from the production material, indoor and outdoor air of the production facility and from the product. The presence of mold was determined by the parallel use of dichloran 18% glycerol (DG18) agar and sabouraud maltose (SMA) agar. Macroscopic and microscopic morphological characteristics were used in the identification process. Colony color, texture, diameter and production of diffusion pigments. The microflora of the mold was represented by 4 genera (*Aspergillus*, *Eurotium*, *Penicillium* и *Mucor*), which were located both on the surface of the dry ham and in the air of the production premises. Five hundred -twenty-five mold isolates were identified, 304 were collected from the production plant and 221 from the ham samples. In the air, the most represented species was *Penicillium nalgiovense* (62.5%), followed by *Penicillium solitum* (22.5%), *Aspergillus niger* (10%) and *Mucor racemosus* (3%). The predominant surface species found on dried sheep ham were: *P. nalgiovense* (20.81%), *P. solitum* (14.02%) and *Penicillium carneum* (9.95%). The production environment was the main source of spores of associated mycobiota. This stresses the need of implementing Good Manufacturing Practice also in traditional processes. The production environment was the main source of spores of associated mycobiota. This stresses the need of implementing Good Manufacturing Practice also in traditional processes.

Key words: dry-cured sheep ham, mold contamination, dominant species.

ETHNOMICOLOGICAL RESEARCH OF THE VILLAGES SOPOTNICA AND DOJKINCI

Ivan Dudaš, Sofija Divljan, Eleonora Čapelja
University of Novi Sad, Faculty of Sciences, Novi Sad, Serbia

Corresponding author: ivan.dudas@dbe.uns.ac.rs

Ethnomycology is the study of the traditional use of fungi and their sociological impact on the population in an area. This paper presents ethnomicological and ethnobotanical research in the villages of Sopotnica and Dojkinci. The village of Sopotnica is located at 1000 m above sea level on the mountain Jadovnik (southwestern Serbia) within the protected area - Monument of Nature "Sopotnica Falls", and has 148 inhabitants. The village of Dojkinci is located at 800 m above sea level on the mountain Stara planina (South-eastern Serbia) within the protected area of the Nature Park "Stara planina" and has 176 inhabitants. The two studied villages have almost a similar number of inhabitants but differ in structure. The village of Sopotnica is a dispersed village (houses are scattered), while the village of Dokinci is a compact village (houses are densely concentrated). The research was conducted within the scientific research camps of SRSBES "Josif Pančić" in July and August 2018. The research was conducted by interviewing the rural population in order to determine which fungi and plant species they use and for what purposes. A total of 22 people with an average age of 69.5 years were interviewed. The rural population of the investigated villages uses a total of 112 plant and 17 fungal taxa. It was recorded that the rural population in the village of Sopotnica uses 82 plant taxa and 6 taxa of fungi. In the case of the village of Dojkinci, the inhabitants use 95 taxa of plants and 15 taxa of fungi. The results of this research indicate that the inhabitants of both studied villages use plants much more than fungi (13.67 times more plants than fungi in the village of Sopotnica and 6.33 times more plants in the village of Dojkinci). Also, the inhabitants of the village Dojkinci use a considerably higher number of plant and fungal taxa compared to the inhabitants of the village Sopotnica (1.16 times more plants and 2.5 times more fungi). The obtained results are a contribution to ethnomycological knowledge and represent the basis for further research, which is still in its infancy in Serbia.
Key words: ethnomycology, Jadovnik, Stara planina, ethnobotany.

RARE MACROMYCETE SPECIES FROM DELIBLATO SANDS

Boris Ivančević
Natural History Museum, Belgradae, Serbia

Corresponding author: i.boris@beotel.net

Deliblato Sands is a large sand area covering around 300 km^2 in Vojvodina province, in Banat, Serbia. It is the largest sandy terrain in continental Europe, once part of a vast prehistoric desert, having originated from the withdrawal of the Pannonia Sea. The temperate continental climate, the absence of surface watercourses and sandy soils has conditioned the emergence of special habitats and specific living communities. Dominant vegetation is modified steppe grassland plains and steppe forests of anthropogenic origin. The Deliblato Sands is rich in floral diversity; it is home to 900 different plant species. Among them are many endemic, rare or endangered species. Fauna is rich and specific, especially birds. The area was declared a protected special nature reserve.

The fungia (*Mycota*) of this area is very interesting. It is rich in species characteristic of sandy habitats. To date, about a hundred species of macromycetes are known from here, some of which have been recorded in Serbia only in this area. Of particular interest are the fungi from the Gasteromycetes group. Among recorded macromycetes are species that are on the Preliminary red list of fungi of Serbia, on the global IUCN red list, on the Bern Convention list, are in the group of protected and strictly protected species in Serbia, or are potential candidates for that status. The paper highlights several characteristic species of macromycetes recorded in Deliblato Sands, their presence indicates the need to include fungi in the guidelines for the management of this protected area, and measures that will protect and preserve the diversity of macromycetes.

Key words: diversity, fungal conservation, Mycota, sand habitats, Deliblato.

PROMINENT SPECIES OF MACROFUNGI AT THE SITE MITROVAC, NATIONAL PARK TARA, SERBIA

Milana Rakić[1], Eleonora Bošković[1], Miroslav Marković[2], Ivana Jovanović[3], Maja Karaman[1]

[1]University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia; [2]University of Novi Sad, Institute of Lowland Forestry and Environment, Novi Sad, Serbia; [3]Institute for Nature Conservation of Serbia, Belgrade, Serbia

Corresponding author: milana.novakovic@dbe.uns.ac.rs

Tara is very valuable, refugial mountain area in Serbia and yet it is still mycologically insufficiently studied. One of the most important sites, Mitrovac is located in the central part of Mt. Tara. With specific geological, climatic and hydrological characteristics, Mitrovac has preserved numerous sinkholes, a unique post-glacial peat bog and rainforest-type vegetation with one of the few remaining habitats of *Picea omorica*.

Hereby, we represent some of the prominent species of macrofungi that have been observed during multiple surveys conducted in the period 2011-2021, at the site Mitrovac. In addition to edible, poisonous and medicinal species, recorded rare and endangered species are of great importance as well. Some of the observed species are considered as indicators of ecosystem health and may enable adequate decisions regarding the management of their habitats.

In the wider locality of nature reserve Crveni potok (Mitrovac), the following species have been recorded: *Hericium alpestre, Hericium coralloides*, *Ischnoderma benzoinum*, *Mycena laevigata* and *Pycnoporellus fulgens*, which are considered good indicators of valuable old forests. Among them, *Hericium alpestre* and *Hericium coralloides* are on the List of strictly protected wild species of fungi in Serbia. At the same site, the species *Strobilomyces strobilaceus*, which is on the list of 51 rarest and most endangered species of macrofungi in Europe (published in 2015 by the European Council for the Protection of Fungi), has been recorded on several occasions. It is also protected by law in Hungary, Poland, Slovenia and Ukraine, while in Serbia it has the status of a strictly protected wild species. Some of the species from the preliminary Red List of Macrofungi of Yugoslavia (Ivančević, 1998) have also been identified in Mitrovac: *Trametes pubescens*, *Xerocomellus porosporus*, *Pleurocybella porrigens*, *Guepinia helvelloides*, *Pleurotus dryinus*, *Stereum subtomentosum*, *Geastrum triplex*.

A very rare aquatic macrofungus - *Vibrissea truncorum*, has been found in a mixed forest of spruce, fir and beech in the 2nd degree protection regime. Its tiny sporocarps develop on plant material completely or partially submerged in water, along clean mountain streams, springs, in cold rivers and lakes. *Vibrissea truncorum* is on the Red List of Fungi in Slovenia, the Czech Republic and Bavaria. Some more interesting findings are a group of macrofungi specialized in mosses, which are indicators of wet forest habitats: *Galerina hypnorum*, *Rickenella fibula*, *Rickenella swartzii*, *Craterellus tubaeformis*, *Entocybe nitida*, *Entoloma serrulatum*.

In addition to the various forest habitats, there are also potentially important meadow habitats in the Tara National Park. Fungal species of the genus *Hygrocybe* are a good indicators of well preserved meadow habitats (semi-natural pastures and grasslands) which are disappearing in most of Europe. Two such species were found in Mitrovac - *Hygrocybe miniata* and *Hygrocybe acutoconica*.

As an exceptional treasure of the Tara National Park, we should point out over 20 other species recorded in Mitrovac, which are on the lists of rare and endangered species in various European countries (e.g. *Boletopsis leucomelaena*, *Sparassis brevipes*, *Cystolepiota pulverulenta*, *Amanita beckeri*, *Volvariella murinella*, *Flammulaster muricatus*, *Hypsizygus tessulatus*, *Ganoderma carnosum*, *Phellodon tomentosus*, *Calocybe ionides*, *Hydnellum peckii*).

Key words: macrofungi, protected species, Mitrovac, Tara.

EFFECTS OF UV STRESS IN PROMOTING ANTIOXIDANTS ENZYME PRODUCTION IN FUNGAL SPECIES *TRAMETES VERSICOLOR* (L.) LLOYD 1921 AND *FLAMMULINA VELUTIPES* (CURTIS) SINGER 1951

Nenad Krsmanović[1], Milana Rakić[1], Jovana Mišković[1], Maja Karaman[1]
[1]University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia

Corresponding author: nenad.krsmanovic@dbe.uns.ac.rs

UV radiation from the sun has always played important roles in human`s environment affecting nearly all living organisms. At the cellular level, damage can be induced by direct absorption of UV wavelengths, or indirectly, whereby UV is absorbed by intermediate compounds, leading to the production of reactive oxygen species (ROS) that damage other cellular components. Reactive oxygen species, including superoxide anion ($O2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2), represent the most potent free radicals since they can have a destructive effect on various cells and cause oxidative stress.

It is generally accepted that aerobic organisms have developed mechanisms to protect them against O_2 toxicity, such as defensive enzymes, including superoxide dismutase (SOD) and catalase (CAT). The SOD converts superoxide radical into hydrogen peroxide and molecular oxygen, whereas the catalase converts hydrogen peroxide into water and therefore are an essential part of the antioxidant defence system.

This study was designed to provide an overview of the effects of UV radiation on different enzymatic profiles (CAT and SOD) of fungal strains during submerged cultivation and on their major defence mechanisms involved in protection from free radicals induced by UV radiation from UV lamp.

Enzymatic activity was examined from the fresh biomass, after 28 days. Mycelia used in this research were isolated from the fruiting bodies of both species and deposited in fungal culture collection FUNGICULT of the ProFungi laboratory at Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, and referenced under the following numbers: 0071 and 0035, respectively, for *T. versicolor* and *F. velutipes*.

The strain of *F. velutipes* exposed to UV showed almost 4 times higher values of SOD (187.17 U/mL) than *T. versicolor* (48.56 U/mL). *F. velutipes* had similar activity of CAT (8.71 mg/mL and 8.87 mg/mL respectively) with control, while *T. versicolor*, exposed to UV, showed 3 times higher values than control (50.12 mg/mL and 14.37 mg/mL respectively) after 28 days of treatment.

An excessive buildup of free radicals and the oxidative stress consequences may relate to aging and diseases, including cancer, cardiovascular diseases, neurodegenerative disease and diabetes thus, all SODs, and especially MnSODs due to a long half-life and low toxicity, play a crucial role in maintaining health and in the prevention of diseases caused by free radicals.

The obtained results suggest that analyzed fungal strains of *T. versicolor* and *F. velutipes*, could be of potential interest as new sources of natural antioxidants with good antioxidant enzyme capacity and might be used in the treatment of diseases associated with oxidative stress.

Key words: SOD, CAT, Antioxidant, *T. versicolor*, *F. velutipes.*

WHEAT STRAW DELIGNIFICATION BY *BJERKANDERA ADUSTA*: THE EFFECT ON ENZYMATIC HYDROLYSIS

Ivana Đokić, Aleksandar Knežević, Jelena Vukojević
University of Belgrade, Faculty of Biology, Belgrade, Serbia

Corresponding author: b3027_2020@stud.bio.bg.ac.rs

The use of lignocellulosic materials in the production of biofuels and biochemicals holds a huge prospect since wood and agricultural residues represent the most abundant global source of renewable biomass. However, delignification is an inevitable step in lignocellulose pretreatment rendering the cellulose and hemicellulose more exposed to enzymatic saccharification. The aim of this study was to assess the potential of different *Bjerkandera adusta* strains to enhance the efficiency of enzymatic saccharification of wheat straw after solid-state culturing. White-rot fungal strains of *B. adusta* (BEOFB1601, BEOFB1602 and BEOFB1603) were used for partial delignification of wheat straw during solid-state cultivation. Activity of ligninolytic enzymes were measured spectrophotometrically while wheat straw residues were used for determination of hemicelluloses, cellulose and lignin contents. Enzymatic hydrolysis of pretreated wheat straw was conducted using commercial cellulase in loadings of 15, 30 and 60 U g-1 of solid substrate. The content of reducing sugars was measured colorimatrically using 1,4-dinitrosalycilic acid. Enzymes predominantly responsible for lignin degradation by tested fungal strains were peroxidases. The highest rate of lignin degradation was noticed in samples pretreated with the strain BEOFB1601 (42.3 ± 3.7%). The highest reducing sugars yield (8.6 ± 0.3 gGE/L) was achieved after enzymatic saccharification of samples pretreated with the strain BEOFB1601, as the most selective lignin degrader. The obtained results suggest that fungal culturing as a biological pretreatment method can be significantly strain specific. A key mechanism which enhances convertibility of carbohydrates is selective lignin degradation of the biomass.
Key words: *Bjerkandera adusta*, delignification, saccharification, wheat straw.

COMPARATIVE REVIEW OF TOTAL PROTEINS CONTENT IN SUBMERGED CULTURES OF *SCHIZOPHYLLUM COMMUNE* FR. 1815 WITH AND WITHOUT Zn

Jovana Mišković[1], Milena Rašeta[1], Dragica Jakovljević[2], Alessandra Zambonelli[3], Giuseppe Venturella[4], Maja Karaman[1]

[1]University of Novi Sad, Faculty of Sciences, Novi Sad, Serbia; [1]University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Belgrade, Serbia; [3]University of Bologna, Department of Agricultural and Food Sciences, Bologna, Italy; [4]University of Palermo, Department of Agricultural, Food and Forest Sciences, Palermo, Italy

Corresponding author: jovana.maric@dbe.uns.ac.rs

Fungi are considered as an important nutritional food due to high concentration of vitamins, minerals, and proteins. Fungal proteins contain all the essential amino acids which are considered as vegan-protein source. On the other hand, submerged cultivation of filamentous fungi represents a biotechnological system for the production of various metabolites (proteins and secondary metabolites) of great social and economic importance since can be used as sources of functional foods thus can help in prevention of various diseases. Submerged cultivation has many advantages over solid cultivation, such as partial manipulation of metabolite production by changing external factors (substrate composition, pH, temperature, aeration).

A widespread basidiomycete *Schizophyllum commune*, has been reported to contain carbohydrates and proteins that have a positive effect on human`s health and may be used as functional and nutritional food agents. Accordingly, the aim of the study was to examine if the Zn bioaccumulation can affect protein content in filtrate (F) of two *S. commune* strains, originated from Serbia (SRB) and Italy (IT). The submerged cultivation of control (C-without Zn) and test samples (S- with addition of 2.8 g/L Zn) lasted for 28 days while every 3 days the total protein content was measured using Lowry method. Moreover, the F was analyzed using FTIR spectrum. In the IT strain, a higher protein concentration is observed in the first days of cultivation, followed by a slight downward trend until the 14th day, while the lowest concentration was recorded at the 21st day (C, 86.5 ± 2.4 mg ALB/g d.w. and S, 96.3 ± 5.12 mg ALB/g d.w.), followed by a sharp increase in protein by the end of cultivation. In SRB strain, a steady trend was maintained in both the C and the S until the 21st day, when the protein concentration in the S began to increase sharply, opposite to C where a slight drop was observed. When comparing strains, SRB strain showed a higher protein content after the addition of Zn, compared to the IT strain, while both strains showed the highest protein concentration after 28 days of cultivation with Zn (284.14 ± 4.35 mg ALB/g d.w. for SRB strain and 201.76 ± 0.58 mg ALB/g d.w. for IT strain). After 14 days of cultivation, IT strain expressed a higher concentration of protein in C, while the SRB strain showed higher protein content in C after 12 days. This is in accordance with the results of FTIR analysis, where the presence of dominant carbohydrate polymers (α and β glucans) as well as smaller amounts of proteins and polyphenolic compounds were detected in extracts after 14 days of cultivation. In summary, submerged cultures of *S. commune* enriched with Zn have showed statistically significant differences to C in both strains ($p = 0.02$ for IT, and $p = 0.01$ for SRB) and may represent an alternative to a new functional food from non-animal sources, due to positive effect of Zn on its nutritional level.

Key words: proteins, submerged culture, Zn, *Schizophyllum commune.*

ПЛЕНАРНО ПРЕДАВАЊЕ / PLENARY LECTURE

SUPERFICIAL MYCOSES, SIGNIFICANCE IN MODERN MEDICAL MYCOLOGY

Siniša Tasić
University clinical centre Vojvodina, Clinic of skin and venereal diseases, Novi Sad, Serbia

Corresponding author: sinisatas@yahoo.com

Fungi are widespread organisms. They are ubiquitous in nature, being found in the air, in soil, on plants and in water. Of the approximately 1.000.000 different species, only about 100 species can cause diseases in humans. The taxonomy of fungi is complex because the same genus can have both asexual (anamorph) and sexual (teleomorph) stages of reproduction. Fungi, pathogenic to humans and animals belong to the classes; basidiomycetes, ascomycetes, mucormycetes and deuteromycetes. Fungi morphologically can be yeasts, molds and dimorphic (biphasic) fungi. Medical mycology is a distinct discipline of medical microbiology concerned with all aspects of diseases in humans and lower animals caused by pathogenic fungi. Medical mycology is one of the youngest subspecializations.

Fungi cause four basic groups of pathological conditions:

- Mycetism or mushroom poisoning (macromycetes);
- Mycoallergosis or immunopathological conditions due to sensitization of the host to fungal antigens;
- Mycotoxicosis or poisoning by fungal antigens;
- Mycoses or presence and multiplication of the fungi on or in human tissues.

There is a broad spectrum of mycoses ranging from superficial skin diseases to deep-seated, multisystem disseminated diseases. According to the manner of infection, as well as epidemiological characteristics, fungal infectiones (mycoses) are divided into two basic groups, superficial (non-invasive) and deep (invasive) mycoses. Superficial mycoses are infections caused by fungi that affect the most superficial layers of the epidermis and lead to the destruction of keratin in the skin, hair and nails. The basic groups of superficial (non-invasive) mycoses are: dermatophytosis, candidiasis of the skin and mucous membranes and pityriasis versicolor.

Dermatophytosis is a fungal disease of the skin and skin appendages caused by a group of hyaline molds-dermatophytes specialized to grow on the nonliving keratinized cells of the outer epidermis (stratum corneum), hair and nails of humans and lower animals.

Candidiasis includes a broad spectrum of diseases from cutaneous, mucosal, systemic or mutisystem disseminated diseases caused by the yeast *Candida* albicans or other *Candida* species. Candidiasis as a superficil mycosis affects the skin, nails, oral and genital mucous membranes.

Pityriasis versicolor is a superficial, chronic, recurrent fungal infection caused by lipophilic yeast of the genus *Malasezzia*.

Key words: fungi, medical mycology, fungal infections, superficial mycoses, dermatophytosis, candidiasis, *Pityriasis versicolor*.

ПЛЕНАРНА ПРЕДАВАЊА / PLENARY LECTURES

SUPERFICIAL MYCOSES IN DOGS AND CATS

Natalija Milčić Matić
University of Belgrade, Faculty of veterinary medicine, Belgrade, Serbia

Corresponding author: natalijamm@gmail.com

Dermatophytosis and Malassezia dermatitis, represent the superficial mycoses of greatest significance in dogs and cats.

Dermatophytosis in dogs and cats is a skin disease caused by a superficial fungal infection of keratinized skin structures by zoophilic, geophilic or anthropophilic fungal organisms, most commonly *Microsporum canis*, *M. gypseum* and *Trichophyton mentagrophytes.*

The infective form of dermatophytes is the arthrospore which is formed by segmentation and fragmentation of fungal hyphae. These can be transmitted by direct contact between an infected and uninfected animal or by fomite transmission, which can include grooming appliances, bedding, collars, ectoparasites and exposure to a contaminated environment. Arthrospores adhere strongly to keratin and can germinate within 6 hours after contact with the skin. Fungal hyphae invade the ostium of hair follicles, proliferate on the hair surface, and migrate proximally to the hair bulb, during which time the fungus produces its own keratinolytic enzymes (keratinase, elastase, and collagenase) that allow penetration of the hair cuticle and grow in multiple directions. . Within 7 days of incubation, hyphae begin to form arthroconidia, completing the fungal life cycle. The clinical lesion appearance typically occurs one to three weeks after exposure.

The most consistent clinical sign is single or multifocal circular patches of alopecia with variable scaling and pruritus. Some patients may experience the classic ring lesion with central healing and fine follicular papules and crusts at the periphery. Onychomycosis is rare, and may present as an asymmetric paronychia or onychodystrophy.

Dermatophytosis is diagnosed by utilizing a number of complementary diagnostic tests, including Wood's lamp examination, microscopic examination of plucked hairs and scraped scales, dermatophyte culture and biopsy.

Dermatophytosis in healthy dogs and shorthaired cats often undergoes spontaneous remission within 3 months. Animals with generalized dermatophytosis, require aggressive systemic antifungal therapy (itraconazole, terbinafine) usually in combination with topical therapy. Dogs and cats with dermatophytosis require long-term treatment (4 to 20 weeks) to eliminate the infection and minimize the risk of its reoccurrence or spread to other animals or humans.

Malassezia pachydermatis is a normal commensals and occasional pathogens of canine and feline skin and mucosae. *M. pachydermatis* expresses a variety of protein and glycoprotein adhesion molecules that bind to carbohydrate ligands on canine corneocytes and secreted different substances. These substances contribute to the pathogenesis of the disease, through proteolysis, lipolysis, alteration of local pH, eicosanoid release, and complement activation. Malassezia dermatitis in dogs is usually secondary to an ongoing skin disorder, predominantly allergic diseases, keratinization disorders, recurrent bacterial pyodermas, and endocrine diseases.

Clinical signs include erythematous, greasy or waxy, scaly and crusty skin lesion. Pruritus is intense, and in chronic cases marked lichenification and hyperpigmentation is present. They often have an offensive rancid or yeasty odour.
The diagnosis of Malassezia dermatitis is based on clinical signs, presence of elevated numbers of yeast organisms in lesioned skin. Because Malassezia is located within the stratum corneum, topical therapy alone may be sufficient to resolve the clinical signs of infection (chlorhexidine, miconazole or ketoconazole shampoo). When topical therapy is impractical or ineffective, oral azoles can be used.
Key words: dog, cat, dermatophyte, Malassezia.

ПРЕДАВАЊЕ ПО ПОЗИВУ / INVITED LECTURE

DERMATOPHYTIC INFECTION IN SERBIA WITH TERAPEUTICMANAGMENT AND CONSUMPTION OF ANTIFUNGAL DRUGS

Dunja Vesković[1,2], Tatjana Roš[1,2], Milana Vuković[3], Nemanja Todorović[3], Mladena Lalić- Popović[3], Dejan Ogorelica[1,2]

[1]University Clinical center of Vojvodina, Clinic of Dermatovenereology Diseases, Novi Sad, Serbia; [2]University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; [3]University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia

Corresponding author: munja93@gmail.com; dunja.veskovic@mf.uns.ac.rs

Dermatophytosis have a worldwide distribution with high prevalence (20-25%) in most developing countries. Despite regional characteristics and predispositions for dermatophyte infections, superficial skin infections show a low tendency to self-limitation, and absence of, or poor medical care further increases the epidemic spread of skin mycoses. Choosing the right treatment is not always simple because of the availability of adequate medicine, possibility of drug interactions and side effects. This study analyses a practical approach to the most commonly-used topical (azoles, triazoles and terbinafine) and systemic medicines (polyene macrolides, azoles and allylamines), referring also to their dosage and duration of use.

The objective of this study was to present the current therapeutic options for superficial fungal skin infections in Serbia with the evaluation of the consumption and characteristics of antifungal medicines during period of two years.

PubMed and Google Scholar databases were searched in order to identify current guidelines for the treatment of dermatophyte infections in Europe. Data on drug consumption in the Republic of Serbia were collected from the publications of the Medicines and Medical Devices Agency of Serbia (ALIMS) on the trade and consumption of drugs for human use, for each year during the observed period, from 2017 to 2019.

In Serbia, there were registered seven topical antifungals for dermatological use and six for systemic use. The most consumed topical antifungals for dermatological use (ATC:D01A) were clotrimazole (D01AC01) and miconazole (D01AC02), for each observed year. In 2019 number of dispensed topical antifungal preparations of clotrimazole was 420991, and 258165 for miconazole. Regarding antifungals for systemic use (ATC:D01B), the most consumed were itraconazole (J02AC02) and fluconazole (J02AC01). The consumption of itraconazole was 0.0904 DDD (Defined Daily Dose)/1000 inhabitants/day, and fluconazole 0.0744 DDD/1000 inhabitants/day.

There are fewer possible therapeutic options in Serbia compared to developed European countries in terms of variety of topical and systemic antifungal medicines. Since the most common dermatophytosis, tinea pedis, guidelines recommend the use of imidazole derivatives for the treatment, the highest consumption of topical antifungals, clotrimazole and miconazole was expected. Oral terbinafine and itraconazole are strongly recommended for the treatment of different dermatophytosis. High consumption of itraconazole is in line with the guidelines, while high consumption of fluconazole is explained by its use for other indications. Topical antifungal-corticosteroid fixed combinations are recommended by

international guidelines for the treatment of various types of inflammatory dermatomycoses. In Serbia, there is only one topical corticosteroid-antibiotic-antimycotic
combination, but there are no authorized topical antifungal-corticosteroid fixed combinations. The type of treatment of dermatomycosis depends on the type of tinea infection, the severity of the infection, and each patient's characteristics and preferences. In Serbia, there are no national guidelines for treating dermatophytosis. Therefore, general practitioners, as well as specialists that treat patients with fungal diseases, make the decision on which medicine to prescribe usually by their own experience, or by the influence of local pharmaceutical marketing.
Key words: fungus, tinea, dermatophyte, candidiasis, superficial mycosis.

ПРЕДАВАЊЕ ПО ПОЗИВУ / INVITED LECTURE

DIFFERENTIAL DIAGNOSIS OF *NOSEMOSE* BEES - PROCEDURE AND SIGNIFICANCE

Igor Stojanov, Radomir Ratajac, Ivan Pušič
Scientific veterinary institute "Novi Sad", Novi Sad, Serbia

Corresponding author: igor@niv.ns.ac.rs

For the living world, in the existing ecosystem, for the reproduction, production and spread of plant species, bees are one of the most important parts of nature. In the pollination of certain field and fruit-vegetable plant species, bees participate with almost 100%. Any factor that contributes to reducing the presence of bees in nature, directly affects the fertility of the plant world, and indirectly affects the rest of the entire living population. *Nosemosis* is a microorganism that has a great impact on the health of bees. In the previous period, *Nosema* sp. is classified as a single-celled parasite, a protozoan, but today it is classified as a fungus (Microsporidia). There are about 30 different species of *Nosema* in nature. For the bee population, especially when it regards to the European honey bee (*Apis millifera*), two species of *Nosema* are very important, *Nosema apis* and *Nosema ceranae*. The correct confirmation of the type *Nosema* provides a better understanding of the outcomes and consequences for the apiary in which the clinical picture of nosemosis has been occurred. The phenotypic diagnostic method, despite the present morphological differences, does not provide the possibility of reliable confirmation of the *Nosema* species. For these reasons, in order to make a differential diagnosis, it is necessary to determine which type of Nosema is present by molecular methods. In our work, by molecular method (PCR), we analyzed bees sampled from two administrative areas. The examination showed that *Nosema* ceranae was found in the two examined areas, while the presence of *Nosema apis* was not confirmed. These results may indicate that *Nosema ceranae* is predominant in the study area and has completely replaced *Nosema apis*.
Key words: differential diagnosis, *Nosemosis*, bees.

ПРЕДАВАЊЕ ПО ПОЗИВУ / INVITED LECTURE

BIOAGENTS AS POWERFUL CONTROL TOOLS AGAINST TOXIGENIC FUNGI IN AGRICULTURAL PRODUCTION

Mila Grahovac[1], Jovana Grahovac[2], Vanja Vlajkov[1], Marta Loc[1]
[1]University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia; [2]University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

Corresponding author: mila.grahovac@polj.edu.rs

Toxigenic fungi present significant problem in food and feed production. Not only that these organisms can cause yield loss, but they are also pathogens of high risk due to their toxigenic potential. Genera *Penicillium*, *Aspergillus* and *Fusarium* are well known among the most significant toxigenic fungi present in plant production. They can contaminate various plant products destined for human and animal consumption with various mycotoxins (patulin, aflatoxin, zearalenone, deoxinivalenol etc.). Due to the risks that these pathogens pose, their control is of high importance for food and feed safety. Chemical control possibilities are either unavailable or ineffective, or not aligned with sustainability goals due to resistance and residue issues. Therefore, biological control tools are the only currently available option that could provide both, effective control of phytopathogenic fungi, including toxigenic ones, while ensuring environmentally friendly effect and preventing resistance development in target organism. Many recent studies worldwide point to biocontrol agents as powerful tools for control of toxigenic fungal plant pathogens. In our recent *in vitro* studies, a number of different biocontrol agents (essential oils, hydrolates and *Bacillus* spp.) proved to have significant antimicrobal activity against toxigenic fungi such as *Fusarium avenaceum*, *F. graminearum* and *Aspergillus flavus*. Microbial control of *A. flavus* is particularly interesting due to the fact that besides beneficial bacteria, atoxigenic strains of the same species may exhibit significant levels of control.

Our recent molecular studies suggest that in *A. flavus* population in corn fields of Vojvodina province, atoxigenic strains are present (in a very small share) and may be exploited as a source of biocontrol agents against toxigenic strains of the population. Moreover, our results of antimicrobial activity of a bioagent *Bacillus amyloliquefaciens* against highly toxigenic strain of *A. flavus* obtained from contaminated corn samples are particularly promising. Namely, *B. amyloliquefaciens* strain was selected among other *Bacillus* strains as the one with the most pronounced antifungal activity. It was identified as *B. amyloliquefaciens* based on 16S rRNA sequence analysis and screened for the presence of genes (srfAA, ituD, ituA, bacD and bacA) that regulate production of the most significant antimicrobal metabolites. Presence of all tested genes was detected in *B. amyloliquefaciens* strain. Cultivation conditions (aeration, mixing, cultivation broth composition) significantly affected level of registered antimicrobial activity of the strain. Considering that antimicrobial activity of *B. amyloliquefaciens* against highly toxigenic *A. flavus* strain varied depending on cultivation conditions of the bioagent, the activity of genes that regulate production of antimicrobal metabolites under different cultivation conditions should be examined in further studies. Also, antimicrobal activity of *B. amyloliquefaciens* and, consequently, aflatoxin contamination reduction, are still to be proven in *in vivo* trials in corn fields.

In general, *in vivo* confirmation of registered antimicrobial activity *in vitro* for any bioagent active against any toxigenic fungi is a necessary step in further studies directed towards commercialization of a bioagent.

Key words: toxigenic fungi, biocontrol agents, metabolites.

PLANT PATHOGENIC SPECIES OF THE GENUS *FUSARIUM* - A SOURCE OF CONTAMINATION OF APPLE FRUITS

Mladen Petreš[1], Marta Loc[1], Mila Grahovac[1], Dragana Budakov[1], Vera Stojšin[1], Emir Šahinović[2], Tatjana Dudaš[1]

[1]University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia; [2]University of Sarajevo, Faculty of Agriculture and Food Sciences, Sarajevo, Bosnia and Herzegovina

Corresponding author: mladen.petres@polj.uns.ac.rs

Apple is a highly nutritious fruit, widely used in human nutrition and due to its possibility of long-term storage, it is available on the market throughout the year. Storage losses are most often caused by plant pathogenic fungi. *Fusarium* species are pathogens of a wide-host range, and *F. avenaceum, F. culmorum, F. lateritium, F. equiseti, F. proliferatum* and *F. solani* are the most important causal agents of apple fruit rot. The incidence of *Fusarium spp*. on rotten apple fruit varies from year to year and the average incidence for the three years (2016 - 2018) was around 11% in the Republic of Serbia. To date, *F. avenaceum* has been detected as a causal agent of apple rot in Slovenia, Croatia, Italy, the USA and the Netherlands, while in Serbia it was found together with the species *F. graminearum*. The losses that occur during the storage period may exceed the losses that occur in orchards. In addition to the direct damage (quality deterioration of stored fruits), these species have the capability to produce secondary metabolites, including mycotoxins, so there is a risk of contamination of fruits with these compounds. *F. graminearum* produces zearalenone, deoxynivalenol and nivalenol, while *F. avenaceum* produces moniliformin, enniatins, fusarin C, antibiotic Y, 2-amino-14,16-dimethyloctadecan-3-ol (2-AOD-3-ol), chlamydosporol, etc. In our previous study, it was found that *F. avenaceum* strain KA13 and *F. graminearum* strain TaB10 are able to produce mycotoxins in infected apple fruits. Also, we predicted a significant number of secondary metabolite biosynthetic gene clusters in genomes of these strains using bioinformatics tools (39 and 27 gene clusters, respectively), which may indicate a high potential for the production of a wide range of secondary metabolites. Also, the results of our ongoing research indicate that it is very likely that other *Fusarium* species will be found as causal agents of apple fruit rot, which could result in expanding the range of mycotoxins that can contaminate apple fruit in storage.

Key words: *Fusarium avenaceum, Fusarium graminearum,* apple, secondary metabolites, mycotoxins.

OCCURENCE OF *FUSARIUM EQUISETI CORDA* (SACCARDO) AS CAUSAL AGENT OF SEED ROT OF *HYSSOPUS OFFICINALIS L.*

Maja Ignjatov[1], Dragana Milošević[1], Milica Aćimović[1], Slađana Medić-Pap[1], Žarko Ivanović[2]

[1]Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia; [2]Institute for Plant Protection and Environment, Belgrade, Serbia

Corresponding author: maja.ignjatov@ifvcns.ns.ac.rs

Hyssop (*Hyssopus officinalis L.*) is a perennial polymorphous plant species with essential oil mainly accumulated in the flowers and leaves. It is grown in Serbia for the needs of pharmaceutical companies and tea production, because of its quality and chemical composition. During a routine quality control of hyssop seeds collected from Rumenka (Vojvodina Province), in 2018, fungal infection followed by seed rot was noticed on an average of 22%. Infected seeds were covered with white mycelium followed with violet pigmentation occurring under the seeds. The presence of *Fusarium* spp. was confirmed with microscopic observation. Isolation was done aseptically by arranging infected seeds onto surface of potato dextrose agar (PDA), and incubated at 25 °C with a 12-h photoperiod (Mathur and Kongsdall, 2003). After seven days, 12 *Fusarium* spp. isolates were designated as JBL 4003/1 - 4003/12. Pathogenicity test was performed in vitro using a modified agar slant method in the test tube with PDA amended. After 10 days, fungal mycelia of tested isolates caused seed rot and seedling decay, like naturally infected hyssop seeds. All isolates were re-isolated and sub-cultured on Potato Dextrose Agar (PDA) and Carnation Leaf Agar (CLA) using a hyphal tip transfer technique, fulfilling Koch's postulates. Isolate JBL 4003/1 was distinguished based on pathogenicity and cultural characteristics. It caused seed rot after four days, on PDA colony was fast growing reaching 6-8 cm in diam. in five days, forming abundant, whitish to peach aerial mycelium followed with beige to light brown pigmentation in agar. Isolate formed relatively long and narrow macroconidia (24 to 54 × 3.2 to 4.5 μm) with a tapered and elongated apical cell and prominent foot-shaped basal cell, with four to six septate, with no microconidia. Chlamydospores were solitary and intercalary. Based cultural and morphological characteristics indicated that the isolate belong to species *Fusarium equiseti Corda* (Saccardo). To obtain a DNA sequence-based identification, total DNA was extracted directly from the mycelium. Following DNA extraction, the translation elongation factor 1-alpha region was amplified by PCR using the primer pair EF1 and EF2. The amplified and purified DNA fragment of chosen isolate JBL4003/1 was sequenced in both directions and deposited in the GeneBank under Accession Number MK061540.1. BLAST analysis revealed that the Serbian isolate MK061540 showed the highest nucleotide identity of 100% with *F. equiseti* isolates from United States (MG826890), Canada (KU587617), Turkey (KT286761), and Serbia (JQ412101). Based on morphological and pathogenic properties, as well as the sequence analysis, to our knowledge, this is the first case of *F. equiseti Corda* (Saccardo) as the causal agent of *Hyssopus officinalis (L.)* seed rot in Serbia. Considering the importance of H. Officinalis in pharmaceutical industries, knowledge of the composition of populations of *Fusarium* species transmitted by hyssop seeds is of great importance for the establishment of appropriate measures for protection.

Key words: *Fusarium equiseti*, seed rot, hyssop, *Hyssopus officinalis.*

SEEDBORNE FUNGI ON STORED ONION SEEDS

Slađana Medić-Pap[1], Sonja Tančić-Živanov[1], Dario Danojević[1], Maja Ignjatov[1], Aleksandra Ilić[1], Jelica Gvozdanović Varga[1]

[1]Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia

Corresponding author: sladjana.medicpap@ifvcns.ns.ac.rs

Seed as a highly-valuable resource is preserved in collections for many years. Although the seed is kept under optimal conditions, monitoring of germination and the presence of fungi during seed preservation is of great importance. Therefore, the aim of this paper was to examine the seed health status and germination of forty-three onion accessions kept in the timespan of 15 years in the Institute of Field and Vegetable Crops collection. Germination of seed samples varied from 7-93%. The presence of fungi in the collection was determined on thirty-three tested samples. Fungi from the genera *Acremonium, Alternaria, Aspergillus, Cladosporium, Epicoccum, Fusarium* and *Penicillium* were developed. The following Fusarium species identified on the seeds were *F. proliferatum*, *F. graminearum*, *F. sporotrichoides, F. solani*, *F. pseudograminearum* and *F. equiseti*. Based on factor analysis, *Fusarium* and *Penicillium* were the species which affected germination, while the occurrence of *Alternaria* species on onion seed is connected to the year of harvest.

Key words: onion, seed, fungi, germination, collection.

REACTION OF SUGAR BEET GENOTYPES TO *FUSARIUM* ROOT ROT

Milica Meseldžija, Dragana Budakov, Tatjana Dudaš, Marta Loc, Teodora Mihaljfi, Dobrila Kovačević, Vera Stojšin
University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia

Corresponding author: vera.stojsin@polj.edu.rs

Fusarium spp. pathogenic to sugar beet root is present in our soils and depending on environmental conditions may cause damages and economic loss in cultivated sugar beet, especially in dry years. There is no ideal solution to prevent this soilborne disease, however selection of sugar beet genotypes tolerant to *Fusarium* root rot would be a step forward to the solution. In this research, reactions of 28 sugar beet genotypes were tested by artificial inoculation with a pathogenic *Fusarium* isolate. Identification of *Fusarium* spp. was performed by polymerase chain reaction (PCR), using primers ITS1 and ITS4. *Fusarium equiseti* was determined by comparing the sequence with the sequences in NCBI database. Reaction of plants to the attack of the pathogen was evaluated 21 days after inoculation, on a scale from 0 (healthy root) to 4 (necrosis affected more than 50% of root tissue). Significant differences were observed among all tested genotypes. Necrosis appeared around the inoculation site on the root neck and from that site spread to other parts of the root. Wilting, leaf chlorosis and necrosis have also been spotted in some plants. The average score of plants of the most tolerant genotype was 1.30, while the average score of plants of the most sensitive genotype was 3.36. Symptoms of *Fusarium* root rot on young sugar beet plants were manifested in varying intensity depending on the genotype. This research shows the potential to improve the protection of sugar beet from *Fusarium* root rot, indicating the great importance of genotype selection in sugar beet production.

Key words: *Fusarium* root rot, *Fusarium equiseti*, sugar beet.

SPECIFICITY IN REACTION OF WINTER WHEAT VARIETIES SIMONIDA AND ZVEZDANA TO FUSARIUM HEAD BLIGHT CAUSED BY *FUSARIUM GRAMINEARUM* SPECIES COMPLEX

Vesna Župunski, Radivoje Jevtić, Branka Orbović
Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia

Corresponding author: vesna.zupunski@ifvcns.ns.ac.rs

Important tool for the integrated control of Fusarium head blight (FHB) is monitoring changes in the prevalence of *Fusarium* species and toxin production. The knowledge of factors influencing FHB infection is usually related to climatic factors, tillage practice and the level of resistance/susceptibility of wheat varieties. However, little is known on differences in reactions of varieties on members of *Fusarium* species complexes. In this study, *F. graminearum* species complex (FGSC) was monitored on two winter wheat varieties Simonida and Zvezdana, created in Institute of Field and Vegetable Crops, Novi Sad. We analyzed 83 FGSC isolates collected from 21 locations in 2019. The study included four regions in Serbia (Vojvodina, Belgrade, Southern and Eastern Serbia and Šumadija and Western Serbia). Monitoring of FGSC populations were made using species- and trichothecene-specific primers. Species specific primers were Fg16F/R and Fgr-F/Fgc-R. Trichothecene genotyping was performed using the primer sets (Tri303F/R, Tri315F/R, and Tri5F/R) for the sequences of the Tri3, and Tri5 genes. Tri-5-specific PCR assay was used to assess the genetic potential of *F. graminearum* isolates for mycotoxin production. We used multiple correspondence analysis (MCA), to investigate associations between Fusarium-damaged kernels (FDK), location, variety, members of the FGSC, and their predisposition for mycotoxin production. On average, the FDK of Simonida was 11.8% while it was 22.1% in Zvezdana. We found that the FGSC population structure infecting Simonida and Zvezdana was not the same. Isolates that were identified as *F. graminearum* s. stricto, using Fg16F/R species-specific primer pair took 79.7% of the FGSC population isolated from Zvezdana, while in Simonida these took only 29.4%. Isolates that were not identified with Fg16F/R, but were with Fgr-F/Fgc-R primer pair contributed to 10.1% and 17.6% of FGSC population infecting Zvezdana and Simonida, respectively. Species-specific primer pairs did not identify 10.2% of FGSC population infecting Zvezdana, and 53% of FGSC population infecting Simonida. All isolates that were not identified using species specific primers previously showed morphological characteristics specific to FGSC. After sequencing of translation elongation factor 1 (TEF) gene and comparison with isolates from NCBI and Fusarium-ID database, TEF sequences of non-identified isolates overlapped with the isolates *of F. graminearum* only in the range from 97 to 99%, which was not enough to confirm their identity. The dominant trichothecene genotype (70 out of 83 isolates), was 15-AcDON with respect toTri315F/R, and produced a PCR product for the Tri5 gene. Among isolates infecting Zvezdana, 83.5% had high genetic potential for mycotoxin production, while only 35.3% from this category was isolated from Simonida. Multiple regression analysis indicated that *Alternaria* spp (P=0.037) and variety (P=0.004) were the most influencing factors on the FDK of Simonida and Zvezdana, indicating the necessity for further investigation on their impact on the pathogenesis of the *F. graminearum* clade and mechanisms affecting difference in response of winter wheat varieties to FHB caused by *Fusarium gramnearum* species complex.

Key words: Fusarium head blight, wheat, molecular identification, DON, *F. graminearum* species complex.

SCREENING OF *BACILLUS* SPP. AS POTENTIAL BIOCONTROL AGENTS AGAINST SUNFLOWER PATHOGENS

Dragana Miljaković[1], Dragana Milošević[1], Maja Ignjatov[1], Jelena Marinković[1], Gordana Tamindžić[1], Branislava Tintor[1], Zorica Nikolić[1]
[1]Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia

Corresponding author: dragana.milosevic@ifvcns.ns.ac.rs

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops worldwide. Numerous pathogens can affect sunflower cultivation and production, causing serious yield losses. The control of sunflower pathogens is conditioned by the limited management strategies, and it is focused on genetic resistance and chemical treatments. However, the excessive usage of agrochemicals leads to the loss of soil fertility and gradual decrease of microbial diversity, which is subsequently reflected in reduced growth and yield. For as much, the use of *Bacillus* spp. is becoming more frequent in modern agriculture as an environmentally sustainable and safe alternative to synthetic pesticides and fertilizers. Spore-forming *Bacillus* spp. are well known to protect plants from seed or soil-borne pathogens by the synthesis of various metabolites with antifungal activity, such as hydrolytic enzymes and antibiotics. These bacteria are also reported to enhance plant growth through the phytohormone production and nutrient acquisition. This study aimed to select the most effective *Bacillus* spp. from a group of ten antagonistic strains by antifungal activity assay. *Bacillus* spp. were primarily isolated from the soil and identified as *B. safensis* (B2), *B. pumilus* (B3, B11, B21, B22, B23) and *B. subtilis* (B5, B7, B13, B32) by 16S rDNA sequencing. The four analyzed fungi: *Macrophomina phaseolina, Alternaria alternata, Cladosporium cladosporoides*, and *Sclerotinia sclerotiorum*, were obtained from sunflower seeds and identified using PCR analysis and primers specific for ITS region. The antifungal activity of bacterial strains was examined in a dual plate assay, which implies the simultaneous cultivation of bacterial and fungal culture on potato dextrose agar (PDA). *Bacillus* spp. demonstrated the highest antagonism against *S. sclerotiorum*, followed by *C. cladosporoides*, *M. phaseolina*, and *A. alternata*, with an average percent of growth inhibition (PGI) of 77%, 70%, 64% and 59%, respectively. Overall, *Bacillus* spp. included in this study showed very strong biocontrol potential, although the effect of particular strain varied depending on the tested fungi. The highest antagonistic effect toward *M. phaseolina* and *A. alternata* was exhibited by *B. safensis* B2 and *B. pumilus* B3. Strains *B. pumilus* B11 and *B. subtilis* B32 were the most efficient against *C. cladosporoides*, whereas *B. pumilus* B3 and *B. subtilis* B7 had the highest antifungal activity versus *S. sclerotiorum*. Findings point to the fact that the most effective *Bacillus* spp. could be used as potential biocontrol agents for improving plant health and productivity. Further evaluation of effective *Bacillus* strains in greenhouse and field experiments is needed to determine their effectiveness in disease supression and growth promotion of sunflower.
Key words: antifungal activity, *Bacillus*, biocontrol, sunflower.

Acknowledgments
This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-68/2022-14/ 200032.

BIOLOGICAL CONTROL OF *SCLEROTINIA SCLEROTIORUM* BY TRICHODERMA ATROVIRIDE IN LETTUCE

Snježana Topolovec-Pintarić[1], Martina Stvorić[1], Ana-Maria Kovaček,[1] Borna Grubišić,[1] Ana Pošta[2], Lucia Pole[2], Armin Mešić[2]

[1]University of Zagreb, Faculty of Agriculture, Zagreb, Croatia; [2]Ruđer Bošković Institute, Zagreb, Croatia

Corresponding author: tpintaric@agr.hr

The fungus *Sclerotinia sclerotiorum* (Helotiales) is the causal of white rot, economically important disease of various crops in temperate climates. Sclerotium is the most important *S. sclerotiorum* resting body in nature because it remains viable and dormant for many years in the soil. Control measures such as solarization and fallow land are partially successful, but they are not feasible in many breeding conditions. The soil application of fungicides is environmentally unacceptable and limited to foliar application in vegetation. Therefore, the development of biological control is of great importance. Species of fungal genus Trichoderma are among the most commonly studied biocontrol microbes and are presently marketed as bio-control agents or active ingredients of biopesticides, biofertilizers, plant growth enhancers, and stimulants of natural resistance. The most represented species *are T. harzianum* and *T. viride*, and they are combined in 55% of commercial biofungicides on the international market. We tested antagonism of Croatian indigenous isolate *T. atroviride* against *S. sclerotiorum*, under laboratory and protected environments. In vitro assays showed excellent antagonisms to *S. sclerotiorum* evidencing hyperparasitic activity. The sclerotia were completely degraded after 2 months. In the greenhouse, the disease caused by *S. sclerotiorum* in lettuce was reduced by treating seedlings with a *T.atroviride* spore suspension. The lettuce infected with *S. sclerotiorum* and treated with *T. atroviride* were healthy and in better condition than untreated infected lettuce and also, than control, untreated uninfected lettuce.

Key words: antagonism, beneficial fungi, fungi–fungi interaction, hyperparasitism.

ANTIMICROBIAL ACTIVITY ESSENTIAL OIL OF *ORIGANUM HERACLEOTICUN* AGAINST *PROTOTHECA BOVIS*

Ivana Čabarkapa, Ana Varga, Dragana Plavšić, Ljubiša Šarić, Zorica Tomičić, Olja Todorić

University of Novi Sad, Institute of Food Technology, Novi Sad, Serbia

Corresponding author: ivana.cabarkapa@fins.uns.ac.rs

Prototheca is a unicellular, achlorophyllous yeast-like microalga. The most important animal disease caused by *Prototheca* is bovine mastitis. The outbreaks of bovine mastitis caused by *Prototheca bovis* (formerly *P. zopfii* gen. 2) have been reported in several countries including Serbia. Bovine protothecosis is a serious therapeutic problem given that *Prototheca* has proven highly resistant to common drugs used for the treatment of mastitis. Therefore, an outbreak of protothecosis in dairy cows may lead to very significant economic losses.

The objective of this study was to determine the antimicrobial activity of *O. heracleoticum* essential oil against five mastitis-associated strains of *P. bovis*.

In addition, this study determined antimycotic and antibiotic susceptibility patterns of *P. bovis.* The following antibiotics and antifungal agents were tested: ampicillin (AMP10), gentamicin (GEN10), streptomycin, (STR10), clindamycin (DA2), fluconazole (FLU25), econazole (ECN10), clotrimazole (CLO50), miconazole (MCL10), itraconazole (ITC50), ketoconazole (KCA10), voriconasole (VO10), nystatin (NY100IU), and amphotericin B (AMB20).

Essential oil of *O. heracleoticum* inhibited the activity of *P. bovis* strains, with minimal inhibition concentration (MIC) of 0.156 µL/mL and with minimal cidal concentration (MCC) of 0.625 µL/mL. Strains of *P. bovis* were found to be highly resistant to most of the recommended antimicrobials used against bacteria and fungi. From a total of 12 antimicrobials only nystatin, amphotericin B, gentamicin, and streptomycin were found to be effective against all *P. bovis* strains. Obtained results revealed that the tested EO of *O. heracleoticum* possesses remarkable antimicrobial activities and could be used in the development of pharmaceutical formulation as an alternative to conventional antibiotic-antimycotic therapy.

Key words: *Prototheca bovis*, antimicrobial activity, essential oil, mastitis.

ETIOLOGY OF VINE DIE-BACK OF GRAPEVINE IN SERBIA

Miljan Grkinić, Mira Vojvodić, Aleksandra Bulajić
University of Belgrade, Faculty of Agriculture, Belgrade, Serbia

Corresponding author: bulajic_aleksandra@yahoo.com

Grapevine is a very important cash crop in Serbia with more than 54.000 hectares of vineyards with enlarging areas each year. Growers frequently report plant die-back due to unknown grapevine trunk diseases which etiology is poorly or not yet studied in Serbia. At the locality of Zabari grapevine plants with chlorotic and necrotic leaves, reduced growth and internal vine necrosis were sampled. Several single spore isolates with uniform appearance were obtained and maintained on PDA. Pathogenicity of one selected isolate (10G-21) was confirmed by wound-inoculating healthy rooted vines which 15 days-post-inoculation (dpi) developed external and internal black necrotic zones. Control plants remained symptomless. Successful re-isolations from all symptomatic plants complied with Koch's postulates. All obtained isolates formed light grey colonies on PDA which gradually become dark grey to black after 5 dpi. Formation of pycnidia occurred after 18 dpi on pine needle nutrition media incubated in 12h light regime. Morphological features resembled to those of *Diplodia seriata* which was further confirmed by ITS rDNA sequencing and molecular characterization. BLAST analysis of sequence of isolate 10G-21 (≈550 bp) showed 100% similarity with more than 100 sequences of *Diplodia seriata* isolated from grapevine and different host plants all over the world. Our results confirmed *Diplodia seriata* as causal agent of vine die-back of grapevine and revealed the need for faster but accurate identification enabling efficient disease control management.
Key words: *Diplodia seriata*, morphology, pathogenicity, molecular identification.

Acknowledgement
This paper is the result of projects 451-03-68/2022-14/200116 funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

ENTOMOPATHOGENIC POTENTIAL OF *TRICHODERMA ASPERELLUM* FUNGAL ISOLATE

Teodora Mihaljfi[1], Miloš Petrović[1], Mihaela Kavran[1], Aleksandra Konjević[1], Marta Loc[1], Mila Grahovac[1], Dragana Budakov[1]

[1] University of Novi Sad, Faculty of Agriculture, Department of Plant and Environmental Protection, Novi Sad, Serbia

Corresponding author: dragana.budakov@polj.edu.rs

Species of the genus *Trichoderma* are known as plant growth stimulators but also as biological agents that can be used in pest biocontrol. Pest biocontrol include the application of formulations which contain effective strains of microorganisms or their synthesized products of metabolism. In this experiment, the entomopathogenic potential of one *Trichoderma* spp. fungal isolate was investigated on larvae and adults of *Zophobas morio* (giant mealworm) and *Tenebrio molitor* (mealworm beetle), as well as on larvae of *Culex pipiens* (common house mosquito). Identification of *Trichoderma* spp. fungal isolate was performed by polymerase chain reaction (PCR), using primers ITS1 and ITS4 and consecutive sequencing determined *Trichoderma asperellum* by comparison with the sequences in the NCBI database. The tested individuals of *Zophobas morio* and *Tenebrio molitor* were not susceptible to the presence of the fungal isolate in digestive tract. *C. pipiens* larvae (L2-L3 stage) were exposed to this fungus by growing in conidial suspension of different concentrations. Susceptibility of mosquito larvae to entomopathogenic *T. asperellum* was registered in suspensions with 10^7 and 10^8 spores/mL. After 48 hours of exposure, larvae mortality was 97% in suspension containing 10^8 spores/mL, and 57% in suspension containing 10^7 spores/mL.

Key words: *Alternaria*, toxin production, wheat.

POLYPHASIC IDENTIFICATION OF POSTHARVEST DECAY AGENTS OF LEMON FRUITS IN SERBIA

Stefan Stošić[1], Dušica Delić[2], Svetlana Živković[1]
[1] Institute for Plant Protecion and Environment, Belgrade, Serbia; [2] Institute of Soil Science, Belgrade, Serbia
Corresponding author: stefan.stosic@gmail.com

Lemon fruits are an important source of vitamin C, potassium, folate, carotenoids, polyphenols, flavonoids, coumarins and terpenes. These lemon compounds have antioxidant and anti-inflammatory properties which have beneficial effects on human health. The annual import of this crop in 2021 was 30,366 tons and it has an increasing trend in the last ten years according to the data of the Serbian Statistical Office. This research aimed to elucidate the etiology of blue and green molds detected on lemon fruits in Serbia. Samples of lemon fruits with *Penicillium*-like symptoms were collected from supermarkets and open markets as part of a broader survey, during 2015-2020. Isolation of the fungi was achieved following standard phytopathological procedures and the integrative approach was employed in their identification. Colony growth and morphology were examined on Czapek yeast autolysate agar (CYA), Malt extract agar (MEA) and Creatine sucrose agar (CREA), and on CYA at two additional incubation temperatures (5 and 37 °C). For molecular identification, ITS and partial β-tubulin (*BenA*) genes were sequenced. A pathogenicity test was carried out and the possible difference in pathogenicity among isolates was assessed with analysis of variance (ANOVA) and subsequent Tukey's test. Using the polyphasic approach, four species were identified: *Penicillium expansum*, *Penicillium digitatum*, *Penicillium polonicum* and *Talaromyces rugulosus*. All isolated species exhibited moderate to intensive growth on CYA and MEA, except *T. rugulosus* which had weak growth on these media. Radial segmentation was noticed in *P. polonicum* and *P. expansum* on CYA, whereas *P. digitatum* and *T. rugulosus* had compact colonies. Velutinous cultures were observed in all species excluding *P. expansum* where the variation of the textures was observed – from fasciculate to synnematous. Conidiophores' branching was: terverticillate in *P. expansum* and *P. polonicum*, biverticillate in *T. rugulosus* and irregular in *P. digitatum*. Acid production on CREA was present in cultures of *P. expansum* and *P. polonicum* and lacked in the other two species. At 5 °C only *P. expansum* and *P. polonicum* formed colonies, while the absence of growth was observed at 37 °C for all species. The phenotypic appearance of the isolates and growth on tested media and temperatures were in agreement with species descriptions in the literature. Multilocus phylogenetic analyses revealed clustering of our isolates with other isolates of the corresponding species. All four species proved to be pathogenic on lemon fruits, producing symptoms similar to those observed on naturally infected fruits. Statistically significant differences in virulence have been determined between species – *P. digitatum* was the most virulent, *P. polonicum* and *T. rugulosus* were the least virulent. Fulfilment of Koch's postulates was completed by isolating the MEA cultures which resembled the cultures recovered from the originating hosts. The results of this study are the first records of the beforementioned *Penicillium*/*Talaromyces* species as postharvest pathogens on lemon fruits in Serbia.

Key word: *Penicillium*, *Talaromyces*, mold, pathogen, *Citrus limon.*

Acknowledgement
This research was financially supported by Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/ 200010).

PATHOGENICITY ASSAY OF *PENICILLIUM* SPP. ON APPLE FRUITS

Tatjana Dudaš, Dragana Budakov, Marta Loc, Jelena Vukotić, Mladen Petreš, Mila Grahovac, Vera Stojšin

University of Novi Sad, Faculty of Agriculture, Department of Plant and Environmental Protection, Novi Sad, Serbia

Corresponding author: tatjana.dudas@polj.edu.rs

Several *Penicillium* species are causal agents of apple blue mold, resulting in huge economic losses and posing threat to human health due to mycotoxin production. In this research, a pathogenicity test on apple fruits was performed for twenty isolates of *Penicillium expansum*, three isolates of *Penicillium crustosum*, and one isolate of *Penicillium solitum*. Apple fruits uniform in size, color, and shape and without physical injuries were disinfected in 2% sodium hypochlorite solution for 2 minutes, rinsed for 1 minute under running tap water, and left to dry. Two wounds (3×3 mm) were made with sterile nail on the opposite sides of each apple fruit in the equatorial part. The conidial suspension of the pathogen was prepared in 0.05% Tween 20 from 7 days old colonies. Each wound was inoculated with 10 µL of conidial suspension (5×10^6 conidia/mL) using a pipette. Control apple fruits were inoculated with 0.05% Tween 20 in sterile distilled water. Four apple fruits were inoculated per treatment, incubated for 10 days at 20 °C and lesion diameters were measured. All isolates were pathogenic on apple fruits. Statistically significant differences were observed among isolates of different *Penicillium* species: *P. solitum* developed the smallest lesions (4.2 mm), while the *P. expansum* isolate P8 showed the strongest virulence (52.3 mm). There were no statistically significant differences among the three isolates of *P. crustosum* with an average lesion size of 25.3 mm, while some differences appeared among 20 isolates of *P. expansum*, with lesion sizes ranging from 43.9 mm to 52.3 mm.

Key words: Apple blue mold, *Penicillium expansum*, *Penicillium crustosum*, *Penicillium solitum*, pathogenicity.

CLADOSPORIUM CLADOSPOROIDES, PATHOGEN OF SUNFLOWER SEED

Dragana Milošević[1], Maja Ignjatov[1], Zorica Nikolić[1], Vladimir Miklič[1], Gordana Tamindžić[1], Dragana Miljaković[1], Maja Karaman[2]
[1]Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia;
[2]University of Novi Sad, Faculty of Sciences, Novi Sad, Serbia

Corresponding author: dragana.milosevic@ifvcns.ns.ac.rs

The sunflower (*Helianthus annuus* L.) is one of the most important oil crops in the world and the most important cultivated oil crop in Serbia, where it is grown on about 160,000 to 210,000 ha and the seed yield ranging from 1.7 to 2.3 t/ha. Several seed-borne fungi including species *of Alternaria, Aspergillus, Cladosporium, Drechslera, Epicoccum, Fusarium* and *Penicillium*, have been reported from sunflower seeds and could cause different levels of losses in its production. During the routine seed quality control and seed health testing analysis of sunflower, according to ISTA Rules, *Cladosporium* spp. infection was observed on an average of 5%. The aim of this study was the isolation and identification of *Cladosporium* spp. based on their morphological characteristics and molecular analyses. Isolation of the pathogen was carried out by transferring infected seeds onto a potato dextrose agar (PDA), and incubated for 5 days at 25 °C. *Cladosporium*-like colonies were transferred onto fresh PDA and water agar (WA, 17 g agar and 1 liter of distilled H_2O) to obtain monosporial isolates. Seven days later, five isolates formed grey-greyish brown, velvet-like colony with apically and laterally branched conidiophores. Margin of the colony was white to grey-olivaceous. Conidias were mostly globose to subglobose, 3-4.5 µm in diameter, mild to dark olivaceous brown. Based on morphological characteristics all isolates belong to *C. cladosporoides* species. To confirm pathogenicity, the sunflower plants grown in pots were inoculated with the previously obtained isolate of *C. cladosporoides*, all plants were inoculated with a suspension of conidia (1×10^3 conidia/mL) of 7-day-old fungal culture on PDA. The pathogenicity test showed that all five isolates of *C. cladosporoides* caused development of prominent symptoms on inoculated sunflower seedlings, confirming their pathogenicity. In all symptomatic sunflower seedlings, the presence of *C. cladosporoides* was confirmed by re-isolation and morphological comparison with a respective isolate. Identification of representative isolate 54Sun was confirmed by molecular analyses. Total DNA was extracted directly from fungal mycelium with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Molecular detection utilizing PCR and primers specific for ITS region successfully amplified one clear band of the predicted size between 500-600 bp in 54Sun isolates, as well as the positive control. No amplification was obtained in the negative control (PCR mix with RNase-free water). After purification with a QIAquick PCR Purification Kit (Qiagen), the amplified DNK product was sequenced directly in both directions and deposited in GenBank (Accession No. MH496035). Obtained sequence of the Serbian isolate was compared with the previously reported isolates available in the GenBank (http://www.ncbi.nlm.nih.gov/BLAST/), using the ClustalW program and MEGA7 software. The ITS sequence showed 100% homology with the *C. cladosporoides* isolates MH474491, MH474488 and MH474410 from USA, and the isolate MG719633 from Pakistan. *C. cladosporoides* is known as one of the predominant seed-borne pathogens. According to our

results, the presence of *C. cladosporoides* has no significant influence on the quality of sunflower seed or seed germination.

Key words: sunflower, *C. cladosporoides,* PCR, sequencing.

Acknowledgments

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-68/2022-14/ 200032.

CIP - Каталогизација у публикацији
Библиотеке Матице српске, Нови Сад

582.28(048.3)

МЕЂУНАРОДНИ научни скуп "Микологија, микотоксикологија и микозе" (7 ; 2022 ; Нови Сад)
Књига резимеа [Електронски извор] / Седми међународни научни скуп "Микологија, микотоксикологија и микозе", 2–3. јун 2022, Нови Сад = Book of abstracts / The 7th international scientific meeting "Mycology, mycotoxicology, and mycoses", 2–3 June 2022, Novi Sad. - Novi Sad : Matica srpska, 2022. - 1 elektronski optički disk (CD-ROM) : tekst ; 12 cm

Nasl. sa naslovnog ekrana.

ISBN 978-86-7946-387-6

a) Микологија - Апстракти

COBISS.SR-ID 67647241