



**THE 6th INTERNATIONAL SCIENTIFIC MEETING
*MYCOLOGY, MYCOTOXICOLOGY, AND MYCOSES***

**ШЕСТИ МЕЂУНАРОДНИ НАУЧНИ СКУП
*МИКОЛОГИЈА, МИКОТОСКИКОЛОГИЈА И МИКОЗЕ***

- Book of Abstracts -
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**27 – 29 SEPTEMBER, 2017
27 – 29. СЕПТЕМБАР 2017.**

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Скуп је медијски подржан и од стране часописа *WORLD MYCOTOXIN JOURNAL*.
The Meeting was supported through media by *WORLD MYCOTOXIN JOURNAL*.



ПЛЕНАРНА ПРЕДАВАЊА / PLENARY LECTURES

MYCOTOXIN INTEGRATED MANAGEMENT AND MYCOKEY ACTIONS

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The management of good agricultural practices in the pre-harvest is a key issue for minimizing the risk of mycotoxin accumulation in the crops before the harvest. Such practices can involve crop rotation, tillage, proper fertilization and fungicide or biological control distribution, variety selection, timely planting and harvests and the control of the insects which often act as vectors of toxigenic fungi spores. On the other hand, the reduction of mycotoxins along the agro-food chains is also highly depending from a correct post-harvest management that must aim firstly at the separation of the infected crop products from the healthy material. Therefore, the use of different tools such as manual sorting or optical sensors is also a crucial point for reducing the level of mycotoxin contamination of a given crop. Moreover, it is extremely important to prevent post-harvest contamination during the storage by obtaining low temperature and humidity conditions, in order to limit the development of toxigenic fungal genera. An update review of an integrated management of pre-and post-harvest practices aiming at the minimizing the risk of mycotoxin contamination of the main crops of agro-food importance and main effective solutions proposed by EU project MycoKey (<http://www.mycokkey.eu/>) will be provided in the presentation. MycoKey is a funded by European Commission (5 M euro) under Horizon 2020 program. It aims to deliver in 2019 the first integrated ICT based solutions to address mycotoxin contamination along the food and feed chain, by using an holistic and sustainable approach. In the past, several actions have been developed to counteract mycotoxins effects and risks in the single stages of the value chain, often difficult to be applied by common farmers and SMEs. MycoKey will integrate innovative key actions into an user friendly and cheap application, able to provide real-time information and suggestions for mycotoxin management to several stakeholders. Running studies are investigating new methods to prevent the contamination in the field, during processing and storage. 32 partners from Europe, China, Nigeria, and Argentina, including research institutions, SMEs, industries and associations are working together for 4 years, focusing on aflatoxins, deoxynivalenol, zearalenone, ochratoxin A, fumonisins. They will also operate together to strengthen the global knowledge on mycotoxins, feed an effective cooperation with China, and to create interaction with other projects and initiatives, giving recommendations to regulators.

Keywords: toxigenic fungi, good agricultural practices, sorting, storage

Acknowledgments

This presentation has been supported by the EU Project MycoKey N. 678781

AN INTEGRATED TOOLBOX FOR MYCOTOXIN MANAGEMENT FOR SAFER FOOD AND FEED: THE MYTOOLBOX APPROACH

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Due to its significant implications for food and feed safety, food security and international trade, the occurrence of fungal and, subsequently, mycotoxin contamination in various crops is of major concern. Integrating different approaches to reduce moulds and mycotoxins along the whole food and feed chain is the goal of the EU project MyToolBox. During its four years of implementation, the €5 million project will develop a series of integrated measures to achieve significant reduction in losses of crops due to fungal and mycotoxin contamination. About 40% of the consortium consists of industry partners, of which five partners are end-users from the farming community. While the majority of the eleven consortium members originate from EU member states, three partners are from China, and five others are from Serbia, the Ukraine and Turkey, demonstrating a clear interest in promoting solutions beyond the European Union. Besides pursuing a field-to-fork approach, the MyToolBox project will also consider safe use options of mycotoxin contaminated batches such as microbial energy conversion to efficiently produce biofuels. Ensuring food and feed security and safety within a sustainable economic environment throughout the entire production chain is a major motivation behind MyToolBox.

The mycotoxin commodity combinations that will be addressed are the most prevalent *Fusarium* mycotoxins (Deoxynivalenol, T-2/HT-2 toxins, zearalenone and fumonisins) in wheat, oats, maize and animal feed chains, ochratoxin A in wheat, and aflatoxins in maize, peanuts and dried fruit (figs). To achieve mycotoxin reductions of up to 20-90%, depending on the commodity and toxin, an ergonomic, secured web-based and mobile-friendly MyToolBox platform will be developed to assist the end-user at each level of the chain. This MyToolBox platform will guide the end-user to the most effective measure(s) to reduce biological contamination in crops, and will provide the necessary intelligence to ensure these measures take into account the prevailing conditions such as geographical location, meteorological conditions, land-use, crop management, storage and intended end use with relevance to specific crops. In cooperation with the Chinese partners, MyToolBox also aims to develop a sound scientific basis for standard-setting of authorisation of mycotoxin detoxifying feed additives in China, and consequently improving market access of relevant EU products.

HUMAN MYCOTOXIN BIOMARKERS AND OCCURRENCE OF MYCOTOXINS IN CROATIAN CEREALS (CROMYCOSCREEN DATA)

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To be able to properly estimate the exposure to mycotoxins, the two approaches can be used: the biomarker approach and the recalculation base on the mycotoxin occurrence data. Both approaches have its own advantages and drawbacks. There is more data and methods available for mycotoxin detection in raw materials (mainly cereals as the main source of mycotoxins), but for exposure assessment additional data is needed. The consumption data is different for every individual, and approximations based on the big databases can be used. The other problem is the food preparation techniques, since most of the cereals are heavily processed before eaten, and there is big data gap compared to raw cereals data, and the mycotoxin reduction and/or modification during processing. On the other hand, usage of human biomarkers is usually limited due to low concentrations found, less available methods for detection, and different metabolites of original mycotoxins. Since most xenobiotics are metabolised to become more polar, and excreted via urine, it is most often used for mycotoxin biomarker studies. In Croatia, both approaches were tested. The urine biomarker screening of the 40 pregnant women revealed that they were exposed to low levels of ochratoxin A, but high levels of deoxynivalenol. On the other side the CroMycoScreen project collected the data on the occurrence of both regulated and unregulated mycotoxins in cereals from all Croatian counties, and revealed that there were 128 different fungal, bacterial and plant metabolites present, including the emergent mycotoxins with special interest for EFSA. Out of all samples, 10 % were not suitable for human consumption, and 0.5 % were not suitable as animal feed. One of the project outcomes was to set up the mycotoxin heat map of Croatia, showing the more problematic regions for different mycotoxins, enabling the planning in the crop rotation policies and smart usage of contaminated cereals for biofuel production or as feed for more resistant animals.

Keywords: mycotoxis, biomarker, occurrence, LC-MS/MS

AFLATOXIN M₁ IN SERBIA: SYSTEMATIC REVIEW OF OCCURRENCE AND EXPOSURE ASSESSMENT-AN UPDATE

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Aflatoxin M₁ (AFM₁) contamination of milk and milk products is a serious problem in last years, because of its toxicity and consequently an important impact on public health and economy. In Serbia, recent drought and then flooding confirmed that AFTs are one of the foodborne hazards most susceptible to climate change. Since 2012, high AFTM₁ concentrations in milk and dairy products occurred in Serbia. Thus the aim of this systematic review is to collect data on the occurrence of AFTM₁ in raw milk and dairy products during last decade in Serbia. Estimation of dietary exposure (EDI) and Hazard Indices (HI) for different age groups of the population was also carried out, based on the AFTM₁ content of the milk samples and on available food consumption data in Serbia. EDI and HI were calculated for different age groups of the population. On the basis of the obtained results, from 2015 to 2016, 218 (15.5%) and 232 (6.4%) samples, respectively, were contaminated with levels above the maximum permitted level in Serbia (>0.25 µg/L). It could be concluded that the safety of milk in Serbia has improved in recent years. However, according to the European Union regulation, a large percentage of milk (30.1 - 31%) was contaminated with levels above the maximum permitted level in EU (0.05 µg/L). Therefore, in order to protect consumer health, and to reduce economic losses, it is extremely important to control the level of AFs in milk and should be considered as a high priority for risk management actions.

Keywords: aflatoxin m₁, estimated daily intakes, hazard indices, public health

MYCOTOXINS IN MAIZE: ANNUAL VARIATIONS AND THE IMPACT OF CLIMATE CHANGE

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The presence of aflatoxins (AFs), ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON), and fumonisins (FUMs) was examined in maize samples from the Republic of Serbia. The maize samples were collected during the period 2012–2016, and analyzed every year after harvest using validated Enzyme Linked Immunosorbent Assay (ELISA) method. The obtained results were considered regarding the weather conditions parameters recorded during the investigated maize growing seasons. Significant differences in weather conditions parameters recorded in the five-year period resulted in different mycotoxin profiles between the investigated years. Obtained results indicate that the presence of ZEA and DON in maize is characteristic of years with abundant precipitation, while AFs and OTA mainly occur in maize during hot and dry years. Furthermore, FUMs were detected with different contamination frequency in maize samples from every year. Based on the findings obtained in this study, as well as on noted changes in weather conditions in the recent years it could be assumed that maize from Serbia may become susceptible to problems concerning mycotoxins. Therefore, there is a necessity for monitoring and research related to the mycotoxins occurrence in maize from Serbia.

Keywords: climate changes, maize, mycotoxins

**EFFECT OF ACTIVATED CLINOPTILOLITE IN AFLATOXIN B1
CONTAMINATED LAYING HEN DIETS ON AFLATOXIN B1 RESIDUES
AND QUALITY OF EGGS**

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This study was carried out to determine the effect of a high level of aflatoxin B1 in laying hen diets, supplemented with deactivated and activated clinoptilolite, on inner and outer quality, and aflatoxin B1 residues in eggs. Two experimental groups were formed and fed high aflatoxin B1 diets (965 ppb) containing deactivated and activated (450 °C for 60 minutes) clinoptilolite (2% of diet) for 49 days. In the experiment, a total of 960 55-week-old Lohmann LSL (white) laying hens were used. Each group had 8 replicates and 480 hens. Egg weight, inner and outer egg quality parameters and egg aflatoxin B1 levels were determined in a total of 90 eggs collected on the 15th, 30th and 49th days of the experiment. Diets containing deactivated or activated clinoptilolite decreased aflatoxin B1 production in laying hen diets after incubation period of 15 days. Activation of clinoptilolite by heat treatment significantly reduced aflatoxin B1 level in eggs ($p<0.05$). In addition, the use of clinoptilolite as an antifungal agent in the presence of high aflatoxin B1 level in layer hen diets significantly increased the weight of eggs and significantly reduced the ratio of broken-cracked and dirty eggs ($p<0.05$). Chicken blood albumin, creatinine and calcium levels were higher in hens fed diet containing activated clinoptilolite ($p<0.05$). However, triglyceride and VLDL levels decreased significantly in the blood of these animals ($p<0.05$). In conclusion, the supplementation of hen diets containing high aflatoxin B1 with activated clinoptilolite improves production performance, egg quality and decreases aflatoxin B1 residue in the egg.

Keywords: aflatoxin B1, clinoptilolite, egg, laying hens

Acknowledgments

TUBITAK 1507-SME R&D (Research & Development) Initial Support Program Project No: 7120527

DETERMINATION OF THE PRESENCE OF AFLATOXIN B1 IN FOOD AND FEED IN REPUBLIC OF SRPSKA (Bosnia and Herzegovina) IN THE PERIOD BETWEEN 2014 AND 2016

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Aflatoxins are a mixture of related chemical compounds of bisfokumarine type. They are synthesized in a variety of agricultural and food products such as oilseeds, spices, cereals and other products. In the period of 2014–2016, 418 samples were analyzed for the presence of aflatoxin B1, using the ELISA method. The analysis included samples of silage (75 samples), concentrated feed (272 samples), dry fruits (16 samples), nuts (15 samples), flours (5 samples) and other types of samples (35 samples). The presence of aflatoxin B1 was determined in all of the analyzed samples. The highest detected concentration of aflatoxin B1 was 4 µg/kg in the silage samples, 30 µg/kg in the concentrated feed samples, 0.40 µg/kg in samples of dried fruit, 0.81 µg/kg in nuts, 0.5 µg/kg in flour samples and 0.5 µg/kg in the other analyzed samples. Higher concentration of aflatoxin B1 from maximum residual level specified in Regulation was detected in samples of concentrated feed (2.57%).

Keywords: Aflatoxin B1, ELISA, food, feed

OCCURRENCE OF MULTIPLE MYCOTOXINS IN DISTILLER'S DRIED GRAINS WITH SOLUBLES DETERMINED BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY

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Livestock production is an important part of national economies of the most countries worldwide and has a significant role in providing high quality products to consumers. Possible contamination of feedingstuffs with molds and mycotoxins may therefore negatively affect the health of both animals and humans. Nowadays, the legislation of numerous countries requires the use of oxygenated fuels to reduce air pollution. Further significant increase of its production is expected due to the worldwide increasing demand for ethanol production. Distiller's dried grains with solubles (DDGS), the main by-product of the ethanol production, are frequently used as feeding supplement that significantly increases a nutrient value of the feed. Considerable matrix pre-concentration of processed cereals to DDGS due to the depletion of starch and relative stability of mycotoxins predetermines this feedingstuff supplement to contain high levels of mycotoxins. Thorough monitoring of mycotoxins prior the use of high quantities of DDGS as supplement should be conducted to prevent from outbreak of mycotoxicoses.

Within this study, an ISO 17025 accredited method based on QuEChERS-like extraction and ultra-high performance liquid chromatography coupled to tandem mass spectrometry (U-HPLC–MS/MS) analysis was utilized for determination of 57 mycotoxins of the genera *Fusarium*, *Alternaria*, *Aspergillus*, *Penicillium*, *Claviceps* and *Phomopsis* in a wide set of 132 corn and 27 wheat DDGS originating from Europe, Asia and Northern America. Generally, high incidence and content of mycotoxins in the analyzed material was observed. Out of the 57 mycotoxins analyzed, 36 analytes were detected. The mycotoxins detected in the majority of the samples were primarily *Fusarium* mycotoxins – deoxynivalenol, zearalenon and their metabolites, then zearalenon, fumonisins and emerging enniatins and beauvericin. When comparing the contamination of DDGS according to the cereal matrix, corn-based DDGS showed significantly higher contamination contrary to the wheat-based ones.

Keywords: mycotoxins, distiller's dried grains with solubles, feedingstuffs, ultra-high performance liquid chromatography, tandem mass spectrometry

Acknowledgments

This work was supported by the Operational Programme Prague – Competitiveness (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and by the “National Program of Sustainability I” - NPU I (LO1601 - No.: MSMT-43760/2015).

The work was also supported by the project DS-2016-0059 of the Ministry of Education, Youth and Sports of the Czech Republic.

THE FREQUENCY OF PRESENCE OF AFLATOXIN B1 IN FOODSTUFFS OF VEGETABLE ORIGIN

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Cereals, nuts and spices are foods that are used in the daily human diet. According to FAO the average consumption of foods of vegetable origin in people's diet is increasing. Due to inadequate conditions during storage of foods of vegetable origin, there is possibility of contamination by mold that produces mycotoxins. Since the intake of these products in organism has been increased, there is a risk of exposure to mycotoxins and their harmful effect on the consumers' health. The aim of this study was to determine the presence of aflatoxin B1 in products of vegetable origin (cereals, nuts and spices). Aflatoxin B1 was determined by enzyme-immunochemical method (*ELISA*), using commercial kit. 38 samples were tested. In 25 analyzed samples, the content of aflatoxin B1 was higher than 1 µg/kg (1 µg/kg is limit of detection). Out of the total number of tested samples, in 18 samples the content of aflatoxin B1 was determined higher than the allowed amount for this product group by the current regulations (2 µg/kg for cereals, 2 µg/kg for nuts and 5 µg/kg for spices).

Keywords: aflatoxin B1, foodstuffs of vegetable origin, *ELISA*, safety

DEOXYNIVALENOL OCCURRENCE IN SERBIAN MAIZE UNDER DIFFERENT WEATHER CONDITIONS

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The aim of this paper was to investigate deoxynivalenol (DON) occurrence in maize samples originating from two harvest seasons in Serbia. The key differences between harvest seasons were weather conditions, specifically the humidity. The samples were analyzed using high performance liquid chromatography with DAD detection, after clean-up on SPE columns. In samples from 2014, DON was found in 82 (100.0%) samples with the average content of 2.517 mg/kg (ranged from 0.368 to 11.343 mg/kg). Two samples exceeded maximum level permitted by EU regulations. However, analyzing larger number of samples (163) from 2015 harvest season, DON was present in 51 (31.3%) samples in significantly lower concentrations (average of 0.662 mg/kg, ranged from 0.106 to 2.628 mg/kg). None of the samples from 2015 exceeded maximum level permitted by EU regulations. The data on DON presence in Serbian maize were in relation to the different weather conditions that prevailed during the two harvest seasons.

Keywords: deoxynivalenol, HPLC, maize, Serbia, weather

APPLICATION OF ATR-FTIR ANALYSIS FOR DETERMINATION OF FUMONISINS IN CORN

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In order to develop rapid, inexpensive and, at the same time, reliable method for the analysis of molds of the genus *Fusarium* as an indicator of the presence of fumonisins in corn samples, possible application of Fourier transform infrared spectroscopy (FTIR) with attenuated total reflection (ATR) technique was examined. The content of fumonisins in contaminated corn samples had previously been quantified by ELISA method. At the spectrum of the sample contaminated with a high concentration of mycotoxins, there was a lack of the peak at $1,743\text{ cm}^{-1}$, but the peak was observed at 1709 cm^{-1} . To the purpose of result classification the principal component analysis (PCA) and cluster analysis were applied. Conclusions of the two methods were similar both when applying ATR technique in the whole region of the spectrum ($1,150\text{--}1,770\text{ cm}^{-1}$) and when the whole spectrum was divided into two regions: $1,150\text{--}1,450$ and $1,450\text{--}1,770\text{ cm}^{-1}$. However, classification of samples was somewhat better in the ranges $1,150\text{--}1,770$ and $1,450\text{--}1,770\text{ cm}^{-1}$. Of the 16 analyzed corn samples, only very contaminated corn sample with 190 mg/kg was correctly classified as compared to the other samples with the content of less than 10 mg/kg . Also, it was found that evaluation of fumonisins in corn by this technique requires further investigation encompassing recording of spectra of contaminated corn of the same genotype in order to avoid the possible impact of different hybrids on the spectrum.

Keywords: ATR-FTIR, fumonisins, corn

Acknowledgments

This investigation was financially supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Project No. OI 172042.

***Alternaria* TOXINS IN SERBIAN WHEAT: OCCURRENCE AND MITIGATION PROCESSES**

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The need for more complete information about the contamination of crops with *Alternaria* toxins recently gained in importance. The toxicity of metabolites produced by certain species from *Alternaria* genus in the whole supply chain resulted in the area of mycotoxicological research in increased interest for *Alternaria* toxins and fungi which producing them. It should be noted that scarce information is available worldwide about the occurrence of *Alternaria* toxins, especially about behaviour of *Alternaria* toxins in food and feed during the processing. Therefore, the aim of the present study was to examine the presence of three *Alternaria* toxins in wheat samples harvested during three years (2011-2013), as well as to investigate the possibilities of reduction of alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA) content by using of wheat cleaning procedure, and dry milling process, as well as during extrusion process. Therefore, a total of 92 wheat samples (*Triticum aestivum*) from North Bačka, South Bačka, West Bačka, North Banat, South Banat and Srem (districts of Autonomous Province of Vojvodina, Serbia) were collected after harvest in the period from June to July during 2011–2013. In order to determine the level of reduction of the present *Alternaria* toxins content during wheat grain cleaning and milling processes, as well as during extrusion process, experiment was carried out on the commercial wheat sample, wheat treated by fungicide and wheat inoculated by *Alternaria tenuissima*, while non-treated wheat was used as a control. The content of TeA, AOH and AME in wheat samples, in samples of wheat chaff, kernels without chaff, cleaned grain, impurities, milling fractions and extruded products were analysed by HPLC with electrospray ionization triple quadrupole mass spectrometry (LC-ESI-MS/MS). Among 92 analyzed wheat samples, 63 (68.5%) were contaminated with TeA, 11 (12.0%) with AOH and 6 (6.5%) with AME. Only 20 (21.7%) samples were without *Alternaria* toxins. The cleaning of wheat grain in examined wheat samples led to the reduction of TeA by 39.5 - 53.6%. AOH and AME were quantified in removed impurities from all wheat samples, although in most uncleaned wheat samples AOH and AME were not detected. After dry milling process, TeA was quantified in almost all milling fractions in all wheat sample, while AOH and AME were detectable mostly in last break and milling flows and by-products. Since, *Alternaria* toxins are concentrated mainly in the peripheral parts of the kernel; a special attention should be given to its concentration in whole wheat flour, in dark flours and especially in milling by-products, while white flour can be considered as relatively safe product. In case of application of single screw extruder optimal effects of extrusion process are achieved in the case of high raw material moisture (w=24%), high capacity (q=25 kg/h) and medium speed of extruder screw rotation (v=390 rpm) when reduction of TeA=60-65%, AOH=90% and AME=95% was achieved.

Keywords: alternariol, alternariol monomethyl ether, distribution, LC-MS/MS, reduction, tenuazonic acid, wheat

Acknowledgments: This paper is a result of the research within the projects III 46001 and TR 31007 financed by the Ministry of Education, Science and Technological Development, Republic of Serbia.

PRESENCE OF DEOXYNIVALENOL IN WHEAT MILLING PRODUCTS IN SERBIA DURING 2016–2017

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Deoxynivalenol (DON) is one of several mycotoxins produced by certain *Fusarium* species that frequently infect wheat, corn, rice, oats, barley and other grains in the field or during storage. DON affects animal and human health causing vomiting, acute temporary nausea, diarrhea, abdominal pain, headache, dizziness and fever. The objective of this study was to evaluate the natural occurrence of deoxynivalenol (DON) in white wheat flour, whole wheat flour and wheat bran. In this study, a total of 75 white wheat flour, whole wheat flour and wheat bran samples were collected in the period of 2016–2017. All samples were analyzed for DON by enzyme-linked immunosorbent assay. DON was detected in 23 out of 45 white wheat flour samples (51.11%), at levels ranging from 99 µg/kg to 440 µg/kg. Out of 15 whole wheat flour samples, 14 were contaminated by DON (93.33%), at levels ranging from 98 µg/kg to 479 µg/kg. The maximum contamination level of DON (2790 µg/kg) in this study was found in wheat bran. Presence of DON was detected in all 15 samples of wheat bran (100%). These results suggest a high percentage of contaminated samples, especially among wheat bran samples, which raises a risk for consumers of wheat bran and the need to monitor final products before consumption.

Keywords: Deoxynivalenol, wheat flour, wheat bran, ELISA

MYCOTOXINS IN ALCOHOLIC AND NON-ALCOHOLIC BEERS FROM THE CZECH MARKET

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After water and tea, beer is the third most popular beverage worldwide. In some countries, annual consumption is more than 100 L per capita. Therefore, the dietary exposure to natural toxins via beer might be of relevance for a high percentage of consumers. Brewed from malted cereal grains, beer is known to be often contaminated with mycotoxins. Research in this field is mainly focused on the *Fusarium* mycotoxins deoxynivalenol (DON) and its metabolites deoxynivalenol-3-glucoside (D3G) and 3-acetyl-DON (3-ADON), which are known to frequently contaminate brewing grains.

The objective of the presented study was to analyze 57 mycotoxins of the genera *Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus*, *Claviceps* and *Phomopsis* in beer (n = 37) from the Czech market. Both alcoholic (n = 18) and non-alcoholic (n = 19) beers were investigated. For determination of analytes, ultra-high-performance liquid chromatography hyphenated with high-resolution tandem mass spectrometry (U-HPLC–HRMS/MS) was used. Four mycotoxins (DON, D3G, T-2 toxin and mycophenolic acid) were detected in analyzed samples. All alcoholic beers were contaminated with at least one toxin. The majority of samples (94 %) were positive for D3G (3 - 31 µg/L). DON was detected in 89 % of the samples (1 - 16 µg/L). The two remaining mycotoxins were detected in several samples at levels close to their quantification limits. Non-alcoholic beers were contaminated only with DON and D3G. DON was detected in 32 % (3 - 11 µg/L) and D3G in 16 % of the samples (7 - 14 µg/L). The tolerable daily intake (TDI) for DON would be fulfilled from approximately 25 % after consumption of the most contaminated beer when not taking into account the DON contained D3G.

Keywords: mycotoxins, deoxynivalenol, deoxynivalenol-3-glucoside, beer, non-alcoholic beer, ultra-high performance liquid chromatography, high resolution tandem mass spectrometry

Acknowledgments

This work was supported by the “Operational Programme Prague – Competitiveness” (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the “National Programme of Sustainability I” - NPU I (LO1601 - No.: MSMT-43760/2015).

The work was also supported by the project DS-2016-0059 of the Ministry of Education, Youth and Sports of the Czech Republic.

CARRYOVER OF DEOXYNIVALENOL-3A-SULFATE INTO CHICKEN EGGS

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Carryover of mycotoxins into animal tissues has significance for human and animal health. The European Union has regulated maximum levels of specific mycotoxins in milk and its products. However, regulation of mycotoxins in eggs has not yet been established because of scarce occurrence data. We developed and validated an analytical method for the determination of deoxynivalenol (DON), de-epoxy-deoxynivalenol, deoxynivalenol-3 α -sulfate (D3S) and de-epoxy-deoxynivalenol-3 α -sulfate in freeze-dried eggs of laying hens. Sample extraction was carried out with acetonitrile/water/acetic acid 79/20/1 (v/v/v), followed by SPE clean-up with phospholipid removal columns and LC-ESI-MS/MS determination. The method was validated and applied to eggs collected during a 10-week animal trial conducted at the Department of Poultry Science, Texas A&M University. Laying hens were assigned into three dietary treatment groups: 1) control; 2) ~3-4 mg of DON/kg of diet; 3) ~7-8 mg of DON/kg of diet. The only metabolite found in concentrations above the limit of quantification (2.9 μ g/kg) was deoxynivalenol-3 α -sulfate. Its average content in the samples collected on three sampling occasions from group 2 was 17.1 ± 1.1 μ g/kg, 22.7 ± 1.1 μ g/kg and 25.1 ± 1.7 μ g/kg. Average contents in samples from group 3 were 30.9 ± 4.9 μ g/kg, 34.9 ± 2.5 μ g/kg and 38.1 ± 3.3 μ g/kg. Hence, we discovered a significant carryover of D3S into eggs, which is in line with literature reporting that D3S is the main metabolite of DON in chicken.

Keywords: carryover, deoxynivalenol-3 α -sulfate, chicken eggs

DETERMINATION OF OCHRATOXIN A IN WINE USING ELISA

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Method for the determination of ochratoxin A (OTA) in wine using ELISA is validated and verified externally by participating in the three rounds of PT scheme. Validation of the method was determined precision and accuracy ($RSDr \leq 20$, recovery $\pm 15\%$). The detection limit (LOD) of OTA in both red and white wine was 0,2 µg/L. Satisfactory results PT scheme at different concentration levels OTA are externally verify the quality of test results ($|z| \leq 1$). These results demonstrate the reliability of the determination of the content of mycotoxins in the wine using ELISA. During 2016 the OTA content was tested 132 wine samples of small winery in the Autonomous province of Vojvodina. Sample preparation was based on the extraction with organic solvent dichloromethane. The concentration of the OTA in most of the samples of wine was below the detection limit, while in the case of eleven analyzed wine content of mycotoxins has ranged between 0.2 and 0.9 µg /L. Test results indicate that OTA in wine does not represent a significant source of human exposure to this mycotoxin in our country.

Keywords: Ochratoxin A, wine, ELISA test, precision, accuracy

INHIBITORY ACTIVITY OF LACTIC ACID ON AFLATOXIGENIC FUNGI GROWTH AND AFLATOXIN BIOSYNTHESIS

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Food contamination by aflatoxigenic fungi and aflatoxins presents problem for food safety even today. Since lactic acid has “Generally Recognized as Safe – GRAS” status, the aim of this research was to determine its potential in food protection against aflatoxin-producing fungi and aflatoxins.

Antifungal activity of the lactic acid was tested against two aflatoxigenic species (*Aspergillus flavus* – four isolates and *A. parasiticus* – one isolate) using the method of agar plates. Aflatoxigenic species isolated from peant, maize flour and popcorn.

In this study lactic acid showed inhibitory effect on the growth of investigated fungi and on biosynthesis of aflatoxins. At lactic acid concentration of 50µL/mL growth of all fungiwere completely inhibited. The largest lactic acid concentration (100.0µg/mL) inhibited of aflatoxin biosynthesis by 73.57%.

Macroscopic and microscopic investigations showed that the application of lactic acid caused changes in macro-morphology and micro-morphology of the investigated fungi.

The obtained results showed that lactic acid could be efficient in food protection from aflatoxin-producing fungi and aflatoxins.

Keywords: aflatoxigenic fungi, aflatoxins, lactic acid

Acknowledgments

The work is the part of the investigations realised with the scope of the Project No. TR-31017 financially supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia.

FUMONISINS PRODUCTION POTENTIAL OF *Fusarium verticillioides* ISOLATED FROM SERBIAN MAIZE AND WHEAT KERNELS

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The production of fumonisins by potentially toxigenic *Fusarium verticillioides* isolates originating from Serbian maize and wheat kernels was tested *in vitro*. A total of six *F. verticillioides* isolates were incubated on yeast extract sucrose medium (YESA) for 4 weeks at 25 °C in the dark. Their toxin production potential was tested by applying a modified HPLC method for determination of fumonisins in cereals, since the TLC method gave no results. Analyses were performed on a HPLC-FLD system after sample extraction from YESA and extract clean-up on a SPE column.

Although the isolates were tested for fumonisin B₁, B₂ and B₃, only fumonisin B₁ was detected. The results showed that all tested isolates had toxigenic potential for fumonisin B₁ production. The average fumonisin B₁ production of the isolates ranged from 7 to 289 µg/kg, thus indicating a highly variable toxigenic potential among the isolates. Isolate 1282 expressed the highest toxigenic potential for fumonisin B₁ production (289 µg/kg), while isolate 2533/A showed a questionable potential for fumonisin production (7 µg/kg).

Keywords: fumonisin, *Fusarium verticillioides*, cereals, toxigenic potential

COMPARISON OF ELISA AND HPLC METHODS FOR THE DETECTION OF MYCOTOXINS BY ANALYSING PROFICIENCY TEST RESULTS

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Different analytical techniques for the detection of mycotoxins have been developed in order to control the levels of mycotoxins in food and feed. Conventional analytical methods for mycotoxin determination are involving techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and gas chromatography (GC). Also, rapid methods for mycotoxin analysis have become increasingly important. Enzyme-linked immuno-sorbent assay (ELISA) is one of the most common rapid methods for determination of these natural contaminants. The aim of this study was to provide a comparison between two different methods of analysis (HPLC and ELISA) for the detection of different mycotoxins using data that originate from commercial proficiency tests. Based on the statistical evaluation of the results for both methods, in three proficiency tests for various mycotoxins (aflatoxins, ochratoxin and zearalenone), it could be concluded that both techniques can equally be used, although ELISA is considered to be the screening one.

Keywords: ELISA, HPLC, food and feed, mycotoxins

COMPARISON OF METHODS FOR DETERMINATION OF THE TOXIGENIC POTENTIAL OF *Aspergillus parasiticus* SPEARE AND *Aspergillus flavus* Link ISOLATED FROM MAIZE

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Maize is considered one of the most susceptible crops to mycotoxins worldwide. Compared to other mycotoxins, the greatest attention has been paid to aflatoxins, due to their potential carcinogenicity and due to significant and longstanding problems they can cause in humans and animals. *A. flavus* and *A. parasiticus* produce aflatoxins in many economically significant crops in both fields and storages. Because of the potential aflatoxin contamination of maize grain, the toxigenic potential of *A. flavus* and *A. parasiticus* isolates, originating from Serbia, was tested in the present study. Furthermore, various applied methods for detection of these mycotoxins were compared in the study.

Cultural, serological and analytical methods for the detection of mycotoxins were compared in the course of the experiment by the direct extraction of aflatoxins from the nutrient medium. The cultural methods for the detection of aflatoxin production were applied to 20 isolates of *A. flavus* (MRIZP Af18-20) and *A. parasiticus* (MRIZP Ap1-17). These methods are based on the yellow pigment formation in mycelia and nutrition media, occurrence of fluorescence on PDA (potato dextrose agar), agar containing β -cyclodextrine (CD-PDA), as well as on the red pigment formation after adding ammonium hydroxide to the existing medium. The ELISA was used to check quantitative and qualitative analyses of total aflatoxins (B1, B2, G1, G2) while the HPLC method was applied to establish ability of isolates to synthesize aflatoxins B1, B2, G1, G2.

The yellow pigment formation, fluorescence and colony colour changes of isolates into red, as a proof of toxigenicity of isolates, were confirmed in all cases by ELISA. A high potential of total aflatoxin production was determined in the majority of observed isolates. The ability of *A. parasiticus* isolates to synthesize aflatoxins G1 and G2 was confirmed by the HPLC method. This was essential for a better understanding of the key role of the suitability of cultural methods for preliminary evaluation of a large number of isolates. Our goal was to employ rapid biochemical approaches to prevent aflatoxin contamination of crops, and to reduce human and animal exposure to foodborne mycotoxins.

Keywords: *A. flavus*, *A. parasiticus*, maize, toxigenic potential

TOXIC EFFECTS OF CO-EXPOSURE TO MYCOTOXINS

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Animals and humans are continuously exposed to a variety of mycotoxins produced by food-contaminating molds. Despite the efforts of scientists to understand the mechanisms of mycotoxin toxicity, an insight has been gained for only 10 of the 400 known mycotoxins. Only a few human and animal diseases have been associated with mycotoxin exposure for certain. Research started with aflatoxins and continued with ochratoxins, trichotecenes, and fumonisins, but all these compounds were analyzed separately. Recently, some already known mycotoxins that had not been considered important, such as citrinin and sterigmatocystin, have returned in the focus of mycotoxin research because they seem to increase the toxic effect if combined with other mycotoxins. With highly sophisticated technology that can measure many mycotoxins in food at the same time, we have become aware of combined exposure but still do not understand the toxicological significance of extremely low concentrations of dozens of mycotoxins in food. So many mycotoxins in one place are expected to have additive effects in mammals, but there have been reports on antagonistic interaction between mycotoxins, which probably explains while we are still alive. Unfortunately, the requirement of the EU to switch research from *in vivo* to *in vitro* studies may result with unrealistic ideas about combined toxicity because cell cultures have different metabolism from mammals. Other difficulties involve a very high cost of mycotoxins and equipment, disagreement between scientists about the research end-points, and high variation in experimental designs. The last two issues stymie the comparison of the toxic effects of mycotoxin co-exposure.

Keywords: ochratoxin, citrinin, sterigmatocystin, trichotecenes, fumonisins

DETECTION OF MYCOTOXINS THROUGH DIFFERENT ANALYTICAL METHODS

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Mycotoxins are secondary metabolites produced by fungi which can affect variety feedstuffs. These compounds elicit toxicological effects which represent risk for both humans and animals. Their toxicity occurs at very low concentrations, therefore there is a need of sensitive and reliable methods for their detection. With this review our aim is to evaluate classical and emerging methods for the analysis of mycotoxins in concerning their advantages and disadvantages. Currently are commercially available several sensitive methods based on chromatographic or immunochemical techniques. Especially widely are used different chromatographic methods for quantitative determination of mycotoxins, including gas-chromatography (GC) and high-performance liquid chromatography (HPLC) coupled with ultraviolet, fluorescence or MS detectors. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is used as a promising technique for screening, identification and quantitative determination of a large number of mycotoxins. Immunometric assays, such as enzyme-linked immunosorbent assays (ELISA), are frequently used for screening purposes. On the other hand, a variety of emerging methods have been proposed. They are based on novel technologies, including immunochromatography (i.e. lateral flow devices), fluorescence polarization immunoassays (FPIA), infrared spectroscopy (FT-NIR), molecularly imprinted polymers (MIPs) and optical biosensors. In addition, during the last years, the highlight was put on nanoscale materials included in biosensors, which are some of the smart devices used for determination of mycotoxins.

Keywords: Biosensors, ELISA, FT-NIR, GC, HPLC, LC-MS/MS, molecularly imprinted polymers

IMPACT OF *Fusarium* MYCOTOXINS ON SWINE HEALTH – FIELD OBSERVATIONS

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Mycotoxins are structurally diverse fungal metabolites that can contaminate a variety of dietary components consumed by animals and humans. The aim of this paper was to present the field observations of clinical and pathological consequences on swine health in the cases when *Fusarium* mycotoxins were detected in swine feed. The material for research included the samples from swine farms located in the region of Vojvodina, where health disorders resembling intestinal problems in different swine categories were detected. The applied research methods included: epidemiological and clinical evaluation, gross pathology examination, bacteriological tissue testing originating from diseased dead animals. The presence of deoxynivalenol (DON), T-2 toxin and zearalenone (ZEA) in thirteen complete swine feed mixtures were analyzed by enzyme-linked immunosorbent assay methods, using Ridascreen® FAST DON, Ridascreen®FAST T2, and Ridascreen®FAST Zearalenon test kits (R-Biopharm, Germany). By clinical and pathological examination, the lesions predominantly located in digestive tract were observed in different swine categories. The problem of persistent enteric infections in suckling piglets and alteration of growth performance were notified in weaners and fatteners. In adult categories, reduced feed consumption, sometimes distinct feed refusal and vomiting were observed. In all examined samples of complete feed mixtures for different swine categories the concentration of DON exceeded the maximum permitted levels, but also the presence of other *Fusarium* mycotoxins was detected. The obtained results indicate the existence of feed mixtures contamination with low levels of *Fusarium* mycotoxins and their possible positive interaction with etiological agents present in swine farms.

Keywords: swine health, *Fusarium* mycotoxins, Vojvodina

CO-EXPOSURE AND TRANSPORT OF OCHRATOXIN A AND CITRININ IN KIDNEY AND LIVER OF RATS

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Ochratoxin A (OTA) and citrinin (CTN) are nephrotoxic and hepatotoxic mycotoxins commonly found together in grain. There is the EU legal obligation to monitor OTA in various commodities but there is no such an obligation for CTN. Recent research on the presence of CTN concentration in grains showed high contamination (50-75 % samples) with a considerably large concentration range (0 – 400 µg kg⁻¹). Target organs of OTA and CTN toxicity are kidney and liver of rats. The aim of this work was to see the fate of OTA and CTN in kidney and liver of rats co-treated with both mycotoxins. Adult male Wistar rats were treated with OTA (0.125 and 0.250 mg kg⁻¹ b.m.) daily for three weeks and with CTN (20 mg kg⁻¹ b.m.) for two last days of experiment. In addition to the control group, we also had a group receiving OTA alone and CTN alone (in the same doses as above). OTA showed a dose-dependent increase in both organs compared to control. In animals receiving combined treatment, liver and kidney OTA was significantly lower than in animals receiving OTA alone. In contrast, CTN liver and kidney concentrations in the OTA + CTN-treated animals were three to six times higher than in animals given CTN alone. These findings could be explained by higher affinity of the organic anion transporter 1 (OAT1) and OAT3 for CTN than for OTA, which led to lower OTA accumulation in the kidney. This is the first animal study of combined exposure to OTA and CTN to confirm earlier *in vitro* findings that CTN decreases OTA accumulation. It also suggests that mycotoxins when combined may compete for the same binding site and change each other's accumulation in target organs for better or for worse.

Keywords: ochratoxin A, citrinin, organic anion transporters, kidney, liver

BABY FOOD SAFETY FROM THE PERSPECTIVE OF MYCOTOXIN OCCURRENCE

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In the past, price of agricultural products and products thereof was decisive for the majority of consumers. Currently, due to the influence of public experts as well as media, the consumer has much higher demands on food in general and they call for thorough monitoring of the possible presence of undesirable substances. One of many factors influencing food safety is the incidence of microscopic fungi and their toxic secondary metabolites – mycotoxins. Particularly sensitive to the effects of toxic substances including mycotoxins are small children. Their detoxification system is not yet sufficiently developed and when compared to adults, they receive more food per unit of weight. Strict legislation established for some mycotoxins (patulin, zearalenone, aflatoxin, etc.) for infant and baby foods should lead manufacturers to careful selection of raw materials, strict observance of technological procedures and proper laboratory control of the final products. Within presented study, numerous different commodities intended for infants and young children were tested on the mycotoxins content. Specifically, fruit and vegetable baby foods, cereal-based foods (mash, cookies, biscuits, and crunchies) and children's herbal teas were tested. The samples were purchased during 2015-2017. A total of 107 samples were analysed predominantly from Czech (n=77), as well as from Polish (n=15) and from Hungarian (n=15) market. For determination of 57 mycotoxins, modern ultra-high performance liquid chromatography coupled with tandem mass spectrometry (U-HPLC–MS/MS) was used. From the results of the analysis performed in 2015 and 2016, several conclusions are implied. In the cereal products, rather trace levels of primarily *Fusarium* mycotoxins (enniatiins B, B1, A1, zearalenone, etc.) regardless of the location at which the products were acquired, were detected. Legislative regulations set by the European Commission were not exceeded. In two samples (corn crunchies, apple biscuits), the indicative level for the sum of HT-2 and T-2 toxin set by the Commission Recommendation No 165/2013 were exceeded. The largest number of mycotoxins (n=8) was observed in a sample of corn crunchies and therefore in 2017, a special attention was paid to corn-based extruded products. Results are in details discussed at the poster. As regards fruit-based foods (primarily from apples), predominant presence of *Fusarium* (enniatiins B1, B, A1, A, beauvericin, etc.) and *Alternaria* mycotoxins (alternariol, alternariol-methylether) was observed. Frequent contaminant of apple matrix patulin was not detected in the investigated samples. In accordance to the results of our earlier studies, it was also found that herbal teas were rather rich matrices from the view of mycotoxins contamination. The majority (15 out of 19 samples) of dry teas were contaminated by mycotoxins. In two samples, total content exceeding 1,200 µg kg⁻¹ was observed. However, the subsequent experiments evaluating their transfer into tea infusions revealed that only minority of mycotoxins is extracted and consumed. From the original content of mycotoxins present in the samples of dry teas, only alternariol and mycophenolic acid were transferred to the aqueous extracts (maximum of 42%).

Keywords: baby food, cereals, vegetables, fruit, mycotoxins, ultra-high performance liquid chromatography, tandem mass spectrometry

Acknowledgments: This work was supported by the “Operational Programme Prague – Competitiveness” (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the “National Programme of Sustainability I” - NPU I (LO1601 - No.: MSMT-43760/2015). The work was also supported by the project DS-2016-0059 of the Ministry of Education, Youth and Sports of the Czech Republic.

APPLICATION OF LC-ESI-MS/MS BASED MULTI-CLASS METHODS FOR COMPLEX FEED MATRICES

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Due to several pre- and post-harvest conditions like climate impacts, processing or storage, a variety of contaminants as well as residues can enter the food chain. The treatment of animals with veterinary drugs against animal diseases and crops with pesticides against pests has steadily increased the last decades. This circumstance may leave residues in comestible goods of plant or animal origin. Additionally to the anthropogenic application of chemicals, natural occurring contaminants like plant toxins or mycotoxins may infest feed and food products, which endanger human and animal welfare.

In this work, a liquid chromatography-electrospray ionization tandem mass spectrometric method was developed to allow a simultaneous quantification of about 700 fungal metabolites, 500 pesticides, 100 veterinary drugs, 40 bacterial toxins and 30 plant toxins.

For chromatographic separation a reversed phase HPLC column (C18, 150 x 4.6 mm, 5 µm) was used with a binary gradient elution. The operation of the mass spectrometer was conducted in positive and negative ionization, using a scheduled selected reaction monitoring (sSRM) mode, containing two parents to fragment ion transitions for each analyte with optimized fragmentation and ion source parameters. A survey on animal feed products was processed to prove the methods applicability and give first insights about analytical identification data in this complex matrix model. Based on data deriving from spiking experiments of both, the compound feed formula as well as the individual ingredients, this work aims to discuss the applicability of the LC-ESI-MS/MS based dilute and shoot approach for the analysis of animal feed.

Keywords: LC-MS/MS, mycotoxin, pesticide, veterinary drug, feed

LABORATORY COMPETENCE EVALUATION THROUGH PROFICIENCY TESTING – MYCOTOXINS IN FOOD

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Laboratory for analysis of mycotoxins in food at the Institute of Public Health of Vojvodina (Novi Sad, Serbia) participated in 15 proficiency testing schemes in period 2012–2016, comprising 22 determinations of regulated mycotoxins: aflatoxins B1, B2, G1, G2 and M1, ochratoxin A, deoxynivalenone, zearalenone, fumonisins and patulin, in different food commodities: wheat, corn, barley, breakfast cereals, infant food, milk, wine and fruit juice. Analyses were carried out by high performance liquid chromatography with ultraviolet (patulin, deoxynivalenol) or fluorescence detection (aflatoxin M1, ochratoxin A, zearalenone) using *o*-phthalaldehyde precolumn derivatization (fumonisins) or UV postcolumn derivatization (aflatoxins B1, B2, G1, G2), following clean-up on immunoaffinity columns with specific antibodies, except in case of patulin when solvent extraction and solid-phase C-18 clean-up were used. Laboratory performance assessed in terms of *z* scores showed all satisfactory results. In depth evaluation revealed following distribution of *z* scores (absolute values): 59.1% up to 0.5, 36.4% between 0.5 and 1.0, and 4.5% above 1.0. Analysis of trends performed for multiple determinations of individual mycotoxins showed several changes of *Z* score to better or worse rank. Overall assessment of the performance in proficiency testing demonstrated laboratory competence for analysis of mycotoxins in food.

Keywords: quality assurance, proficiency testing, food, mycotoxins

MYCOTOXIN OCCURRENCE IN SPICES ORIGINATING FROM THAILAND

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Different herbal spices are often used as aroma and flavour enhancers, colorants or preservatives in cuisine anywhere in the world. Additionally, large quantities of spices are used in traditional medicine due to the high content of various health-promoting substances such as e.g. antioxidants. However, positive effects of spices may be overshadowed by the presence of various contaminants such as mycotoxins. Mycotoxins can indicate utilization of poor quality raw material. In the EU, specific maximum limits (ML) for aflatoxins and ochratoxin A have been established only for a few spice raw materials, namely turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), liquorice (*Glycyrrhiza glabra*), nutmeg (*Myristica fragrans*) and capsicum species ((EU) No 165/2010 of 26 February 2010 and (EU) No 594/2012 of 5 July 2012 amending Regulation (EC) No 1881/2006). Interestingly, mycotoxin regulation is considerably varying throughout the world. For example, the EU ML for sum of aflatoxins in spices is 10 µg/kg; the Thailand's ML is 20 µg/kg in food (Anukul M. et al., 2013). Due to the limited knowledge regarding mycotoxin contamination of spices, quality control of various kinds of spices from the viewpoint of mycotoxin contamination is important to determine. The objective of this study was to analyze a wide set of 10 types of spices originating from Thailand for mycotoxin presence and evaluate their safety. Namely, black and white pepper, cardamom, coriander seeds, cinnamon, cumin, chilli powder, nutmeg, star anise and turmeric powder were tested. A total of 300 samples (30 of each kind of spices) provided by our institutional partner (Department of veterinary Pharmacology, Kasetsart University, Bangkok, Thailand) were investigated for the presence of 57 mycotoxins (*Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus*, *Claviceps*). The used multi-mycotoxin method was based on QuEChERS-like procedure. For identification and quantification of target analytes, ultra-high performance liquid chromatography coupled with high-resolution tandem mass spectrometry (UHPLC–HRMS/MS) was used employing Q-Exactive Plus (Thermo Scientific). As resulted from our study, altogether 70% of the examined samples were positive for mycotoxin presence. The most abundant substances were *Alternaria* and *Fusarium* toxins, however, no specific ML have been set by EU for these mycotoxins in spices. The mycotoxins produced by the genera *Aspergillus* and *Penicillium* were also detected. The concentration range was relatively broad ranging from units to hundreds of µg/kg. The highest average total content of mycotoxins was determined in star anise matrix - 447 µg/kg (27 positive samples; maximum 2,167µg/kg). The average total content of mycotoxins in the remaining matrices was as follows: 425 µg/kg (cardamom; 24 positive samples), turmeric powder (286 µg/kg; n=1), 163 µg/kg (black pepper; n=30), 144 µg/kg (nutmeg; n=22), 126 µg/kg (chilli powder; n=16), 74 µg/kg (cumin; n=18), 72 µg/kg (white pepper; n = 23), 63 µg/kg (cinnamon; n=30), 25 µg/kg (coriander seeds; n=19). Legislative regulations set by the European Commission were exceeded for aflatoxin B1 (ML=5 µg/kg) and sum of aflatoxins (ML=10 µg/kg) in case chilli powder (2 - 10 times) and nutmeg (1.2 - 28 times). ML for sum of aflatoxins (20 µg/kg in Thailand) was exceeded for the same samples.

Keywords: mycotoxins, spices, Thailand, ultra-high performance liquid chromatography, high resolution tandem mass spectrometry

Acknowledgments: This work was supported by the “Operational Programme Prague – Competitiveness” (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the “National Programme of Sustainability I” - NPU I (LO1601 - No.: MSM-T-43760/2015). The work was also supported by the project DS-2016-0059 of the Ministry of Education, Youth and Sports of the Czech Republic.

DETERMINATION OF MULTIPLE MYCOTOXINS IN MAIZE USING QuEChERS SAMPLE PREPARATION AND LC-MS/MS DETECTION

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Mycotoxins are toxic secondary metabolites that are naturally produced by several species of fungi on agricultural products, particularly grain-based products. Due to the ubiquitous presence of mycotoxins in both food and feed supply chains, and their association with various toxicological risks in both humans and animals, they have become a major economic and health concern. A reliable and easy method has been used for the multiple mycotoxins determination of aflatoxins (AFs), deoxynivalenol (DON), zearalenone (ZEA) and fumonisins (FBs) in maize samples. Liquid chromatography coupled mass spectrometry (LC-MS/MS) was used. Mycotoxins have been extracted from maize using a QuEChERS (Quick Easy Cheap Effective Rugged Safe) - based extraction procedure. All validation parameters were in accordance with Reg. (EC) No 401/2006. The analyses of eight maize samples showed the AFs, DON, ZEA and FBs contamination with the values below the state limit standards.

Keywords: AFs, DON, ZEA, FBs, QuEChERS, maize, LC-MS/MS

DATA ACQUISITION OF TRIPLE QUADRUPOLE LC/MS FOR THE CITRININ DETERMINATION

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The analysis of citrinin - CIN is challenging as it is need to be detected in low concentrations in complex sample matrices. Citrinin is a polyketide mycotoxin with the molecular formula C₁₃H₁₄O₅, (IUPAC: (3*R*,4*S*)-4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3*H*-2-benzopyran-7-carboxylic acid). Citrinin was first isolated as a pure compound from a culture of *Penicillium citrinum* by Hetherington and Raistrick in 1931. Toxicity studies indicated that citrinin had cytotoxic, genotoxic, mutagenic, and immunotoxic effects to humans and animals, and the most susceptible organ is kidney. Before the CIN quantification the acquisition data of LC-MS/MS must be established, which includes the determination of ion monitoring reaction (SRM), find fragmentation energy (Frag.) and collision cell energies (CE) at which CIN response will be the highest for the given conditions. The best CIN response is obtained at Frag. of 66 V and CE of 17 and 29 V.

Keywords: CIN, data acquisition, LC-MS/MS

ПЛЕНАРНА ПРЕДАВАЊА / PLENARY LECTURES

***Candida glabrata*: NOVEL VIEW ON TREATING YEAST INFECTIONS**

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The relation of yeast towards the humans is changing. Yeast interference with human health is steadily growing in last couple of decades. The main reason behind this seems to be connected with a growing number of immunocompromised persons the way we treat yeast infections and new medical practices like cancer treatments and organ transplantations. Consequently, understanding of yeast pathogenic traits has grown, but sadly also their resistance to several antifungal drugs too. We entered this area of research with new methodological approaches to evaluate their pathogenic nature and to fight their ever increasing drug resistance. The lecture will take us through our work in this field and will address crucial questions, which are highly relevant for the current time. 1. Relation of yeast vs. human: Which yeasts can people tolerate, which not and why? 2. How to assess yeast impact on humans and how to assess pathogenic nature of yeast? 3. How to utilise *Saccharomyces boulardii* probiotics to fight *Candida glabrata* infections? 4. In the case of *C. glabrata* and *Saccharomyces cerevisiae* versus several immunomodulatory drugs that are regularly used with antifungals we also explored questions on alternative approaches in drug research via drug combinations and possible dangers that come with this. 5. And end with some thoughts on what should be done to develop better antifungal drugs, since resistance is a complex net of responses and therefore impossible to predict/control.

MYCOREMEDIATION - FUNGI DO THE EARTH RESCUE

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Microorganisms, tiny mostly microscopic organisms play many roles in our life and global ecosystem on Earth. One of them is global recycling of matter and the result is release of energy, growth and releasing many elements important in sustaining life. Detrimental effect on environment started parallel as the result of growth of human population. Now, in 21st century, we are aware that resources are very limited and we have to find the solutions for many environmental issues. Fungi are very diverse group of organisms ubiquitously present in natural ecosystems and they are conceived as the major decomposers of complex biomaterials. They are capable of thriving in a different habitats from surface waters to terrestrial environments where they are dispersed by their propagules. As heterotrophic organisms, they assimilate nutrients by absorption from the extracellular environment. Fungi secrete many hydrolytic enzymes and organic acids and transport digested substances inside their cell wall. Fungi are evolved into very efficient organisms in degrading complex polymers like ligno-cellulose and chitin, as well as other compounds and play key roles in a number of biogeochemical processes, especially where growth of bacteria is retarded. This metabolic diversity makes fungi very interesting organisms in microbial bioremediation, destruction or removal or reduction of hazardous elements or compounds below toxic levels. Mycoremediation has been coined by Paul Stamets is a process where fungal mycelia or their enzymes are used in bioremediation. Single fungal species can be used in microbial bioremediation as well as group of fungi (or consortium) or fungi together with bacteria in detoxification or remediation. One of the natural bioremediation processes is decay of wood where complex and resistant macromolecules (like lignin) are degraded and mineralized. Enzymes that fungi produce for degradation of cellulose and hemicellulose were found to be active in degrading a vast range of aromatic pollutants. *Phanerochaete chrysosporium*, white rot fungus, can besides degrading wood, degrade many organopollutants like PCBs, dioxins, DDT or benzo[a]pyrene. Fungi are important organisms in bioremediation of synthetic dyes and pigment. Due to their usage in textile industry, dyes and pigments are resistant to degradation. Additionally, they are often toxic to many organisms. Decolourization of effluents of textile industry is one of the goals of water purification processes and is critical to dye-containing wastewaters. Biodegradative ability of many white rot fungi (like species of the genus *Phanerochaetae*, *Trametes* or *Ganoderma*) is used for decolourization of Orange II, Tropaeolin O or Congo Red and many others. Human activities are the main cause of the rising environmental pollution. One of the way to minimize waste and environmental pollution is to develop environmentally friendly methods to eliminate toxic substances from our surroundings. Due to their high metabolic activity and low nutrient request, fungi are important allies in bioremediation processes.

Keywords: bioremediation, mycoremediation, fungi, environmental pollution, biodegradative ability

FUNGI BEETWEN SCIENCE AND ART

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During the last two decades, multidisciplinary comprehensive research into biodeterioration phenomenon affecting cultural heritage objects in Serbia, notably in regard to the role of fungi, has led to accumulation of knowledge and better understanding of this complex issue. Fungi as known biodeteriogens and health hazard agents were during this period studied on a number of stone monuments and masonry, and variety of works of art encompassing frescoes, oil paintings, wooden and gypsum sculptures, textile artifacts, paper heritage, etc. Via application of traditional and implementation of novel methods aimed at detection and assesment of fungal deterioration potential, we gained insight into diversity and adverse effects of fungi colonizing these peculiar and very susceptible substrata. In the interdisciplinary approach to the study of biodeterioration of cultural heritage objects, information regarding mycobiota, such as primary sources of contamination, inner hot spots, contamination ‘pathways’ and presence of pathogenic and toxigenic fungi are necessary first step for developing efficient long-term plan for systematic conservation of these valuable works of art and human health safeguard.

***Ganoderma lucidum* – FROM TRADITION TO MODERN MEDICINE**

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Ganoderma lucidum has a long tradition of use in folk medicine of the Far East, which is documented in the oldest Chinese pharmacopoeia, written in the first century B.C., declaring it as a superior medicine. The healing properties of *G. lucidum* reflected on folk names such as: Reishi, Mannentake, Ling Zhi etc., which mean “herb of spiritual power”, “mushroom of immortality” or “10 000 years mushroom”, respectively. It is known, for thousands of years, that this species extends life span, increases youthful vigour and vitality and it was used in the treatments of hepatitis, kidneys' disease, hypertension, arthritis, asthma, bronchitis, arteriosclerosis, ulcers and various types of cancer. However, Western civilisation did not discover its healing properties until the 20th century. Modern scientific researches and numerous clinical trails, conducted in recent decades, have confirmed the ancient knowledge of Eastern nations and given them a scientific basis. These studies have demonstrated many biological activities of *G. lucidum* extracts and compounds, including: immunomodulating, antioxidative, cytotoxic, hypoglycaemic, anti-inflammatory, antiallergic, antimicrobial, etc. It has been reported that its extracts play important role in detoxification of the body and protection of the liver, as well as in reducing cardiovascular problems, stress and anxiety. However, its most important effect is undoubtedly immunostimulating one as it is the basis of many other positive effects. The Japanese government introduced *G. lucidum* on the official list of auxiliary agents for the treatments of various cancers, Alzheimer's disease, diabetes and chronic bronchitis. Many chemical components have been isolated from *G. lucidum*, but polysaccharides and terpenoids are the main carriers of its bioactivities.

Keywords: *Ganoderma lucidum*, traditional usage, modern studies, bioactivities

**SPATIAL DISTRIBUTION OF GENETS IN POPULATION OF
SAPROTROPHIC FUNGI *Marasmius rotula* ON MT. STARA PLANINA**

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This study was conducted to determine the size and spatial distribution of mycelial individuals of *Marasmius rotula* at one locality on Mt. Stara planina in the Republic of Serbia. Total of 12 sporocarps were collected from investigated locality (Vidlič, Stara planina). Sporocarps were distributed in four groups and distances between them were approximately 10–30 meters. Genomic DNA was extracted from each sporocarp and used for inter-simple sequence repeat (ISSR) polymorphism analysis using (GTG)₅ and (GACA)₄ primers. Both primers showed reproducible band patterns on agarose gels and sporocarps with identical band patterns were considered to belong to the same individual (genet) and were grouped accordingly. Grouping with both primers determined that 12 analyzed sporocarps belong to 4 distinct genets (A, B, C, D). Approximate genet diameters were 2 m for two genets (A, B) and 15 m for one genet (C) while diameter of one genet (D) was not possible to determine since it was represented only by one sporocarp. The results presented here are the first data about size and spatial distribution of genets of *M. rotula*. To determine whether the obtained genet sizes are general trait of an analyzed species or a special trait appeared as an effect of environmental conditions, more information on the genet distribution of other *M. rotula* populations is needed.

Keywords: genet distribution, ISSR, *Marasmius rotula*, population

FIRST RECORD OF RARE SPECIES OF *Fomitopsis iberica* IN SERBIA

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Fomitopsis iberica Melo & Ryvarden is thermophilic, Mediterranean fungal species of fungi found in many European countries, but its sporocarps are appearing rarely. Fruit bodies of this species are annual, broadly sessile, growing in imbricate clusters and in some places overgrown around thin stems of herbaceous plant. In this study we present the first record of this rare species of fungi for the Republic of Serbia. Sporocarp of *F. iberica* was collected from a dead stump of white poplar (*Populus alba*) in natural reserve “Begečka jama”, located on the left bank of river Danube near village of Begeč. The specimen was collected by members of Mycological society of Novi Sad during the field investigation for annual summer mushroom fair, which was held in June 2015 in Novi Sad, Serbia. Identification of analyzed species was based on macroscopic and microscopic morphological characters of collected sporocarp. This finding is an important contribution to the knowledge of the distribution of *F. iberica* in Europe. Results presented here also emphasize the importance of amateur mycological societies in expanding our knowledge about distribution of fungal species and their biodiversity.

Keywords: Begečka jama, brown rot, *Fomitopsis iberica*, Fomitopsidaceae, mediterranean species

EVALUATION OF FITO-PREVENT EFFICACY IN PREVENTION OF ORANGE FRUIT INFECTION WITH *Penicillium expansum*

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Attempts to find alternatives to chemical control have been ongoing for some time, and indeed many fungi have become resistant to commonly used fungicides. In this study we evaluated the ability of FITO-PREVENT (mixture of different essential oils) as bio-control agent against plant pathogenic filamentous fungi *Penicillium expansum* on inoculated oranges.

To determine the infection capacity of *P. expansum*, oranges were divided into four groups (I - blank without treatment - uninfected, II - with treatment – uninfected, III - with treatment - infected and IV - control without treatment – infected). Fruits were inoculated with 20µL suspension concentrations 10⁶ spores mL⁻¹ by piercing the skin with 0.5-mm-diameter needles to a depth of 1.5 mm at three sites center of the fruit. The development of infection and change in oranges weight were monitored for 7, 14, and 21 days. The development of the infection was monitored visually and by determining the number of mold at cut pieces (1cm²) of peel taken around infected fields. Conditions during fungal growth were set at 25°C and 60% relative humidity. First signs of infection were visualized after 14 days in infected control group without treatment (group IV). After 21 days signs of infection were not observed in any other tested group (group I, II and III).

Based on obtained results it can be concluded that application of FITO-PREVENT has led to the complete absence of disease in infected oranges.

Keywords: *Penicillium expansum*, infection, oranges

INDIGENOUS LIGNOCELLULOSE DEGRADING FUNGI: NEW ISOLATES FOR TECHNOLOGICAL AND ENVIRONMENTAL USE

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Potential for technological and environmental use of Serbian autochthonous fungal species is insufficiently explored. Therefore, two Serbian autochthonous fungi from subphylum Basidiomycota (*Trametes hirsuta* F13 and *Stereum gausapatum* F28), and one from subphylum Ascomycotina (*Myrmaecium fulvopruinatum* F14) with highly active lignocellulose degrading enzymes are presented. *T. hirsuta* F13 and *S. gausapatum* F28 poses very high laccase activity, which was measured by oxidation of guaiacol. The highest values of $175 \pm 0.9 \text{ U l}^{-1}$ (F13) and $58 \pm 0.9 \text{ U l}^{-1}$ (F28) were measured in solid state fermentation (SSF) on beech wood sawdust (BWS) supplemented with mineral medium for ligninolytic enzyme production. When BWS was not supplemented with the additional minerals, only dH₂O was used, maximal activities were $110 \pm 0.7 \text{ U l}^{-1}$ (F13) and $19 \pm 0.1 \text{ U l}^{-1}$ (F28). *M. fulvopruinatum* F14 showed the best activities for cellulase ($5682 \pm 327 \text{ U l}^{-1}$) and xylanase ($7721 \pm 293 \text{ U l}^{-1}$) enzymes, which were the highest values of hydrolytic enzymes obtained among the isolates. Cellulases and xylanases were produced on BWS supplemented with Mandels mineral salt medium.

To use lignocellulosic waste in fermentation processes it is necessary to remove lignin to facilitate enzymatic hydrolysis of holocellulose. Since commercial enzymes are expensive, production could become more cost effective with the application of locally produced enzyme extracts. Analyses of lignin removal by *T. hirsuta* F13 and *S. gausapatum* F28 from lignocellulosic substrate, and cellulosic waste hydrolysis by enzyme extracts obtained from *M. fulvopruinatum* F14 have shown that these three isolates have potential for application in biotechnological processes on renewable resources.

The aim of presentation of this study was to draw attention of a wider audience to the research field that can help solving many environmental problems and to try to animate more people to get involve in research and development of methods for use of autochthonous fungi in various industrial processes.

Keywords: *Trametes hirsuta*, *Myrmaecium fulvopruinatum*, *Stereum gausapatum*, enzymes, indigenous fungi.

ANTIOXIDATIVE ACTIVITY OF *Lenzites warnieri* BASIDIOCARPS

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Considering that mushrooms synthesize different kinds of compounds with antioxidative activity and that search for natural antioxidants is a topical study area, testing of unstudied species is fully justified. The aim of the study was to evaluate antioxidative capacity of *Lenzites warnieri* basidiocarps using different solvents. Antioxidative potential of 96% ethanolic, 70% ethanolic and methanolic extracts was evaluated by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) bleaching assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. Additionally, total content of phenols and flavonoids in extracts were determined as galic acid equivalent (GAE) and quercetin equivalent (QE), respectively. Presented as EC₅₀, 70% ethanolic extracts showed the highest antioxidative capacity by DPPH assay (3.08 ± 0.49 mg/mL) and 96% ethanolic extract by ABTS assay (3.08 ± 0.24 mg/mL). Methanolic extract exhibited the lowest antioxidative activity in both assays (6.02 ± 0.99 mg/mL and 4.92 ± 0.38 mg/mL, respectively). Results showed that antioxidative capacity of extracts depended on solvents and assay used, indicating that ethanolic extracts were with higher capacity in free radicals neutralization. The highest content of total phenols was detected in 70% ethanolic extract (37.45 ± 0.36 µg GAE/mg of dried extract) while the lowest amount was noted in methanolic extract (22.73 ± 0.05 µg GAE/mg of dried extract). Total flavonoid contents were negligible and ranged between 1.91 ± 0.10 and 2.24 ± 0.13 µg QE/mg of dried extract. The obtained results indicate that *Lenzites warnieri* possess significant antioxidative capacity which is mainly correlated to phenols present in the extracts.

Keywords: *Lenzites warnieri*, antioxidative activity, basidiocarps, extracts

***Lactarius piperatus* AS A PROMINENT ANTIBACTERIAL AND ANTIBIOFILM AGENT**

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As one of the most common causatives of nosocomial infections, bacterial strain *Pseudomonas aeruginosa* (Schröter) Migula, prone to development of resistance to commercially available antimicrobial agents, is of great clinical importance. The aim of this study was to determine the antibacterial and antibiofilm potential of extract of wild *Lactarius piperatus* (L.) Pers. Methanolic extract evaluated against a very resilient opportunistic pathogen *P. aeruginosa*, using different methods. Bacterial strain *P. aeruginosa* (ATCC 27853), obtained from the Laboratory culture collection, and modified microdilution method was used to evaluate extract's antibacterial potential. In addition, extract was evaluated as a biofilm formation antagonist, using the following concentrations: 0.5, 0.25 and 0.125 of the obtained MIC value using microdilution method. Commercially available antimicrobial agents streptomycin and ampicillin (Sigma Aldrich, Germany) were used as controls. Antibacterial activity of the extract was 3.15 ± 1.08 mg/mL for inhibitory activity (MIC), and 6.25 ± 2.17 mg/mL for bactericidal activity (MBC), while for the antibiotics streptomycin and ampicillin MIC and MBC were in range of 0.01-0.03 mg/mL and 0.02-0.05 mg/mL, respectively. The methanolic extract reduced biofilm formation in a dose dependent manner in range of 81-49.50%, with 0.5 MIC concentration as the most prominent. Antibiofilm activity of the controls was comparable with one obtained from the mushroom extract. This makes the tested peppery milk cap mushroom a significant antibiofilm agent. Even though antibiotics proved to be more effective against the tested microorganism than the methanolic extract, considering their side effects and misuse, data indicating potent natural sourced activity seem to be of great importance.

Keywords: *Lactarius piperatus*, *Pseudomonas aeruginosa*, antibacterial activity, antibiofilm activity

INFLUENCE OF *Saccharomyces boulardii* PROBIOTIC YEAST ON FERMENTATION KINETICS OF WINE

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This study followed the fermentation kinetics and Plovdivina grape must and Plovdivina grape with the addition of locust bean and ground rosemary leaves in an amount of 2% by must weight. At the beginning of the fermentation selected *Saccharomyces cerevisiae* yeast was added in an amount of 0.25 g/L. The fermentation lasted for ten days at a temperature of 22°C, after which the wine was filtered and added a selected *S. boulardii* yeast in an amount of 0.25 g/L. Fermentation kinetics in wine samples was monitored by following the changes in the number of yeast cells of *S. boulardii* using the spectrophotometric method and control at $\lambda = 620$ nm, and by following the change of the amount of sugar in the wine. Titratable acidity and sugars were analyzed every three days after planting the yeast *S. boulardii*. Must sugar content was 17.1%, and a total acid content 4.2 g/L. Titratable acidity of Plovdivina wine (A1) after plating the yeast *S. boulardii* amounted to 6.54 g/L l (calculated in relation to tartaric acid), Plovdivina wine with the addition of carob (A2) 6.24 g/L, while in Plovdivina wine with rosemary addition (A3) it amounted to 5.70 g/L. Further analysis of the wine showed no change in acidity. Sugar analysis by rapid French method found that there was a change of the ammount of sugar in a wine sample A1 from 0.68 g/ further analysis of the wine showed no change in acidity. Sugar analysis by rapid to 0.39 g/L, wine sample A2 from 1.54 g/L to 0.72 g/L, in the wine sample A3 from 1.01 to 0.67 g/L.

ANTIFUNGAL EFFECT OF SELECTED COMPOUNDS ON SOME SPECIES OF THE GENUS *Candida*

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Antifungal effect of sodium bicarbonate, hydrogen peroxide, tea tree essential oil and boric acid on selected *Candida* species (*C. albicans*, *C. famata* and *C. krusei*) was tested. The most susceptible species on tested antifungal compounds was *C. famata*, following *C. albicans* while *C. krusei* was the most resistant yeast. The effect of tested compounds is proportional to the concentration; higher concentrations applied have more potent fungicidal effect to tested species. At 24th hour of incubation, fungicidal effect of tested compounds was especially pronounced. Cell counts were reduced from 10⁴ to 10 or 10² cells per mL of suspension, depending on yeast susceptibility and applied concentration of antifungal compound.

Keywords: *Candida*, antifungal effect, NaHCO₃, H₂O₂, tea tree oil, H₃BO₃

ANTIOXIDANT PROPERTIES OF SOYBEAN SEEDLINGS INOCULATED WITH

Trichoderma asperellum

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This study was conducted in order to assess the effect of inoculation of soybean (*Glycine max* L.) seeds with *Trichoderma asperellum*, followed by mites (*Tetranychus urticae*) exposure on lipid peroxidation (LP) process and the activity of antioxidant enzymes. *T. urticae* is an occasional pest of soybean that causes biotic stress. Biotic stress leads to overproduction of reactive oxygen species (ROS) which may cause damage to vital biomolecules. Enzymatic antioxidant defense systems protect plants against oxidative stress. *T. asperellum* is commonly used as biocontrol agent against plant pathogens. It has been suggested that previous inoculation of seeds with *T. asperellum* may cause induced resistance against biotic stress. The aim of this study was to determine LP intensity and antioxidant enzymes activity in inoculated and non-inoculated soybean seedlings with and without exposure to mites. Noticeably higher LP intensity was detected in non-inoculated group treated with mites compared to control group. Inoculated soybean seedlings treated with mites had lower LP intensity compared to non-inoculated group. Also, it has been noticed that inoculation with *Trichoderma asperellum* itself, produced mild stress in plants. In addition, positive correlation between enzymes activity and LP was noticed. The level of oxidative stress in plants was followed by the change of LP intensity. According to results obtained, it was concluded that the greatest oxidative stress occurred in non-inoculated group treated with mites and that inoculation successfully reduced oxidative stress. The results indicate that inoculation of soybean seeds with *T. asperellum* improves resistance of soybean seedlings against mites attack.

Keywords: antioxidant systems, *Glycine max* L., oxidative stress, *Tetranychus urticae*, three-way-interaction, *Trichoderma asperellum*

INFLUENCE OF CLIMATE FACTORS ON THE OCCURANCE OF MACROFUNGI WITHIN SELECTED FOREST HABITAT ON TARA MT.

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This research represents results of the longterm study of macrofungal occurence influenced by the air temperature and the soil moisture. Investigation was conducted in the period of four years (2010-2013), within selected experimental areas situated in the forest stand of spruce and fir, on Tara Mt (locality Mitrovac). The climate factors were measured on the experimental plots as well, with the air temperature measured throughout the year and the soil humidity only on the days when monitoring was carried out. Total of 137 macrofungal species, belonging to 68 genera, were identified. It was noted that the observed meteorological parameters have important influence on the occurrence of macrofungi - air temperature during the winter months, while the soil moisture in the vegetation period.

Keywords: Macrofungi, climate factors, Tara mountain

IN VITRO DEGRADATION OF POLY [(R)-3HYDROXYBUTYRATE] AND BIOPOL™ BY FUNGI ISOLATED FROM TERRESTRIAL AND FRESH WATER ENVIRONMENTS

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Since terrestrial environments and freshwater bodies today unfortunately serve as primary waste dumps, and since fungi are an important part of the degrading microbiota, in order to prove their participation in the degradation process, a simple degradation test suitable for fungi and for terrestrial conditions had to be developed. Several solid media, differing in the content of non-alkanoate organics and supplemented with 0.1% PHA (or BIOPOL™) as a main source of carbon have been tested. The testing principle consists in clearing the turbid medium in test tube or plates, the turbidity of which is caused by suspended particles of PHA substrate.

All media tested supported the growth of tested fungi. For the discrete and transparent clearing of zones a mineral medium with 0.01% peptone, 0.01% yeast extract, and 0.1% PHB or BIOPOL™ was chosen finally where the fine and evenly distributed turbidity is accomplished by a specific procedure. This method allows the investigation of the degradability of PHA-based plastic materials as well as screening for fungal ability to depolymerise pure PHA homopolymers. Using this medium, 143 strains of terrestrial mycelial fungi belonging to different systematic and ecological groups were tested for their ability to degrade PHAs. All of the fungal strains tested were able to grow on BIOPOL™ as a sole carbon source, but only 50% were able to depolymerize PHA according to the method applied. This indicates that all or at least one of organic compounds in BIOPOL™ (PHB, PHV, triacetin) could serve as the carbon source.

The number of strains tested percentage of active strains and the species showing highest BIOPOL™-degradation activity are given for the following groups of fungi investigated: Deuteromycotina: 19 strains, 58%; *Penicillium simplicissimum*, *P. atrovenetum*, and *Trichoderma polysporum*. Basidiomycotina: 65 strains, 54%; *Pleurotus ostreatus*, *Collybia peronata*, *Lentinus edodes*, and *Serpula lacrimans*. Ascomycotina: 19 strains, 42%; *Nectria episphaeria*. Zygomycotina: 4 strains, 50%, *Mucor hiemalis*, *Mucor* sp. All of tested chytridiomycets (*Phlyctochytrium africanum*, *P. palustre*) and Myxomycets (*Dictyostelium discoideum*, *Physarum polycephalum*) proved to be active.

Keywords: PHA, BHB, BIOPOL, terrestrial fungi, degradation, screening method

MUSHROOM MYCELIA AS A COHESIVE COMPONENT OF NEW ECOLOGICAL FRIENDLY PACKAGING

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The dependence of modern man from chemo-synthetic plastic is a major problem considering ecology, environmental protection and sustainable development in general. Chemo-synthetic plastic materials are difficult to degrade, causing the load of the natural environment, filling landfills and oceans faster than we can get rid of them. With regard to products from non-renewable resources (oil and gas), under growing public pressure, researchers designed a viable, sustainable alternative to petro-chemical plastics, bio-degradable plastic material of which is the latest and perhaps the most promising one that is made from natural waste materials and – fungal mycelia. Due to their specific mycelial growth, filamentous fungi may invade various waste materials (dry leaves, wood shavings, sawdust, bark, straw, shredded paper and cardboard and other ligno-cellulosic materials, especially by-products of agricultural and wood industry) degrading them and simultaneously merging them into a compact mass similar to cork. Pre-treated (crushed, washed, blended), waste materials are then molded into designed molds of projected form. The porous biomass is then inoculated with mycelium of selected species of mushrooms and incubated under optimized conditions of humidity, temperature, aeration, light, pH values, etc. After a few days, ligno-cellulosic waste is being invaded by hyphae of fungi that form whitish mycelium similar to that invading ligno-cellulosic substrate while growing oyster mushrooms. When the mass becomes mostly white, designed molds of packaging are dried in an oven at temperatures above 60°C, which devitalize mycelia (preventing sporulation) pasteurize packaging and solidify to strength that can support and preserve the object to be packaged. The possibilities of designing more flexible packaging materials based on fungal mycelium as a cohesive factor have been investigated, which could in the future replace the expanded polystyrene and similar packaging and building materials. The advantage of these materials, in addition to their excellent construction and packaging performances, is that are produced from renewable resources, which require significantly less energy to produce, which are CO₂ sinks, which are non-toxic, bio-degradable, recyclable, if are so designed even edible for livestock, and sustainable in every aspect of the development.

Keywords: Biodegradable packaging, Lignocellulosic waste, Fungi, Sustainable development.

EFFECT OF NITROGEN SOURCES ON THE PRODUCTION OF ANTIFUNGAL METABOLITES BY *Streptomyces hygroscopicus*

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Biotechnologically produced antifungal compounds for control of plant diseases caused by phytopathogenic fungi represent a promising alternative to synthetic pesticides. Fungi from the genera *Alternaria* and *Fusarium* are listed among important storage pathogens of apple fruits. *A. alternata* causes significant annual losses of apple fruit. Also, *F. avenaceum* is one of the most commonly encountered *Fusarium* species identified as the causal agent of a wet apple core rot. Species of the *Streptomyces* genera are soil bacteria that produce significant quantities of bioactive compounds in appropriate media. Defining the culture medium composition is the basis of bioprocesses development. Nitrogen source is critical component of cultivation medium and also the most useful tool for stimulation of the antifungal metabolites production. The aim of this study was to select the best nitrogen sources in medium for the production of antifungal compounds effective against *A. alternata* and *F. avenaceum* by *S. hygroscopicus*. Activity of the cultivation liquids on *A. alternata* and *F. avenaceum* isolates was tested *in vitro* using well diffusion method. The results indicate that maximum inhibition zone was reached in medium with soybean meal as nitrogen source with inhibition diameter more than 35 mm for both tested fungi.

Keywords: *Alternaria alternata*, antifungal metabolites, *Fusarium avenaceum*, nitrogen source, *Streptomyces hygroscopicus*

BACTERICIDAL ACTIVITIES OF SELECTED MACROFUNGUS EXTRACTS AGAINST *Staphylococcus aureus*

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The increasing of the antibiotic resistance exhibited by pathogenic microorganisms has resulted in research directed toward evaluation of novel sources of antimicrobial compounds. Previous studies have indicated that macrofungi, as a specific response to the natural hostile environment, produce secondary metabolites with antimicrobial properties. In this study, antimicrobial activities of the extracts from six wild mushrooms: *Amanita echinocephala*, *Russula medulata*, *Cerena unicolor*, *Hericium erinaceus*, *Ishnoderma benzoinum*, and *Laetiporus sulphureus* were evaluated against Gram-positive bacterium *Staphylococcus aureus*. The antimicrobial potential of the methanolic mushroom extracts was investigated by the microdilution method. Antimicrobial activity was observed in all species included in the study. All the extracts that demonstrated inhibitory activities were further tested for bactericidal activity and minimum bactericidal concentration (MBC) values were determined. The tested microorganism was most sensitive to the examined extracts from the polypore fungi *C. unicolor* and *H. erinaceus*. The highest bactericidal activity was obtained in the extracts from the species *C. unicolor* (MBC=1.563 mg/mL).

The experimental results revealed that the methanolic extract of *C. unicolor* possessed significant bactericidal activity. The findings suggest the potential use of this wild mushroom as antimicrobial agent.

Keywords: mushroom, antimicrobial activity, microdilution method, minimum bactericidal concentration (MBC)

THE FIRST REPORT OF *Calvatia fragilis* IN SERBIA

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Genus *Calvatia* is a cosmopolitan genus of puffballs containing more than 50 species. Recent genetic studies showed it to be polyphyletic. There is insufficient data about its distribution in Serbia, with *Calvatia gigantea* being the only well-known and widespread species since *C. utriformis* and *C. excipuliformis* were transferred to the separate genus *Handkea*.

Calvatia fragilis (Quél.)Morgan is one of several puffball species with striking purple mature gleba. Although still considered as a smaller form of *C. cyathiformis* by some authors, recent molecular studies separated it as a species of its own. *C. fragilis* is widespread in North America and is reported from Africa, central Asia, Australia, New Zealand and Southern Europe. The first record of the species in Serbia was reported from two relatively close locations in the Bor District (Eastern Serbia) in autumn 2014. One location is situated approximately 0.5 km to the Northeast of Brestovac (340m, 44°02'49'', 22°04'50'') and the other 1.3km to the West of Brestovačka banja (430m, 44°03'33'', 22°01'35''). These locations are characterized by patchwork of dry oak woodland, intersected by traditionally cultivated land. Both collections were found at the forest margin, in the grass near various shrubberies. The identification was based on the macroscopic and microscopic analysis of the fruiting bodies. Following features, which clearly point to *C. fragilis* and separate it from other similar species with purple gleba (*C. cyathiformis* and *C. leiospora*) were identified in the specimens: relatively small, subglobose to turbinate gasterocarps (30-70mm wide and 40-50mm high), thin peridia prone to breaking, rudimentary subgleba composed of compact cells, presence of a thick rhizomorph, globose verrucose spores $6.8 \pm 0.5 \mu\text{m}$ in diameter and extremely fragile capillitium.

Keywords: *Calvatia fragilis*, puffball, Serbia

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF CHEMICALLY MODIFIED CHAGA MUSHROOM POLYSACCHARIDES

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Inonotus obliquus (Fr.) Pilát or "chaga" is a parasitic fungi that grows on birch. Its sclerotium is used in traditional medicine in Eastern Europe, especially in Russia; recent studies confirmed that it has numerous physiological and pharmacological activities, including immuno-modulating, anticancer and hypoglycemic. Among other metabolites, chaga's complex polysaccharides were identified as its main pharamacological principles. The aim of this study was to evaluate and compare antimicrobial activity of chaga polysaccharide fraction before and after chemical modifications. Powdered sclerotium was first extracted with 80% EtOH to remove small and/or lipophilic molecules. Residue was used to make hot water extract which was then subjected to dialysis and enzymatic deproteinization. Purified polysaccharide fraction (PP) was liophilized and was further used for chemical modifications – partial acetylation with Ac₂O (AcPP) and methylation with MeI (MePP). Modifications were conirmed by FT-IR spectra of the samples. Both modified samples and the pure polysaccharide fraction were tested against 7 bacterial strains (*Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Salmonella enteritidis* and *Yersinia enterocolitica*) and *Candida albicans* using microdilution method (CLSI 2005). While PP showed only average inhibitory activity against bacteria (1.25-20 mg/mL), AcPP showed 2 to 4 times better activity than PP in most cases. However both samples showed bactericidal activity only towards one bacterial strain – PP against *Y. enterocolitica* (20 mg/mL) and AcPP against *B. cereus* (20 mg/mL). MePP was superior to both PP and AcPP, with inhibitory activity being several to twenty times greater in some cases, with the lowest minimum inhibitory activity being 0.078 mg/mL against *Y. enterocolitica*. MePP also showed prominent bactericidal activity against all tested strains, with the lowest being 0.625 mg/mL against *Bacillus cereus*. PP and AcPP showed only inhibitory activity against *C. albicans*, at the highest tested concentration (20 mg/mL) while MePP showed both inhibitory and fungicidal activity against the yeast (0.3125 and 2.5 mg/mL, respectively).

Keywords: Chaga, polysaccharides, chemical modification, antibacterial, antifungal

ANTIFUNGAL ACTIVITY OF *Mentha piperita* AND *Carum carvi* ESSENTIAL OILS

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Aromatic plants are one of the most important sources of biologically active secondary metabolites, which possess various antimicrobial characteristics. The aim of this work was to examine the effect of antifungal activities of mint and caraway essential oils against the selected fungi. Eight species of molds were selected for antifungal testing: *Alternaria alternata*, *Aspegillus flavus*, *A. niger*, *A. versicolor*, *Eurotium herbariorum*, *Penicillium aurantiogriseum*, *P. chrysogenum*, and *P. expansum*. Testing of essential oils antifungal activity against the selected species was conducted using the disc diffusion method by adding mint and caraway essential oils (0.5, 1, 5, and 10 µl per disc). Antifungal activity of essential oils was expressed by the diameter of inhibition zone (mm). The most powerful effect of mint essential oil was recorded against *E. herbariorum*, as its growth was completely inhibited by the quantity of 5 µl. The weakest inhibitory effect was observed against *P. chrysogenum* (inhibition zone 13.67 mm) by the quantity of 10 µl. The most powerful antifungal activity of caraway was observed against *E. herbariorum* as growth was completely inhibited by the quantity of 10 µl. The weakest inhibitory effect was observed against *A. niger* (inhibition zone 28 mm) by the quantity of 10 µl.

Keywords: antifungal activity, mint, caraway, essential oils

ANTIFUNGAL ACTIVITY OF THE SERBIA AND MONTENEGRO AUTOCHTHONOUS WINES AND EVALUATION OF TOTAL PHENOLIC, FLAVONOID AND PROANTHOCYANIDIN CONTENTS

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In this paper, it were showed the antifungal activity and phytochemical content of the six red wines originating from autochthonous grapes (Crnogorski Vranac (Montenegro), Vranac Pro Corde (Montenegro), Pecelj Vranac (Serbia) Prokupac (winery Ivanovic, Serbia) Prokupac (Toplički vineyards Prokuplje, Serbia), Filigran crna tamjanika (winery monasteries Bukovo Negotin, Serbia). Three samples were tested (1-wine extract, 2-partially evaporated wine and 3-original wine).

Antifungal activity was investigated using microdilution method (minimal inhibitory concentrations MIC and minimal fungicidal concentration MFC were determined) at four yeast (*Saccharomyces boulardii*, *Rhodothorula mucilaginosa*, *Candida albicans* ATCC 10231 and *C. albicans*) and three filamentous fungi (*Penicillium italicum*, *Trichoderma viride* ATCC 13233 and *Aspergillus flavus* ATCC 9170). The strongest activity showed extracts of wines Crnogorski Vranac, Vranac Pro Corde and Pecelj Vranac with MIC values ranged from 2.5 mg/mL to 5 mg/mL for the *R. mucilaginosa*. Partially evaporated and original wine, were showed a limited and selective antifungal activity.

The total phenolics, flavonoids and proanthocyanidins, were determined by Folin-Ciocalteu, aluminum chloride and butanol-HCl method. The highest concentration of total phenolic content was 92.19 mgGA/g in wine extract Vranac Montenegro, and the highest concentration of proanthocyanidins was in partly evaporated wine Montenegro Vranac, 62.34 mg CCHE/mL. The partially evaporated and the original wine Vranac Pro Corde has the highest concentration of total phenolics (196.78 - 179.82 mgGA/mL). When comparing the wines, the highest concentration of total flavonoids (all samples) was noted in the wine Vranac Pro Corde, which also was emphasized by the highest concentration of proanthocyanidins (extract and originally wine). It can be concluded that the antifungal properties of wine related with the amount of total phenols and proanthocyanidins in wine.

Keywords: Autochthonous wines, antifungal activity, phytochemical content, phenolics, flavonoids, proanthocyanidins

COMPARISON OF ANTIOXIDANT CAPACITIES OF TWO *Ganoderma lucidum* STRAINS OF DIFFERENT GEOGRAPHICAL ORIGINS

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Ganoderma lucidum, used in a traditional Chinese medicine, represents one of the most important medicinal mushrooms in the world, whose fruiting bodies and spores have been traditionally used because of a wide spectrum of biological activities such as antidiabetic, antioxidative, antiproliferative, cardioprotective, etc. Its ethnomedicinal importance in some parts of the Balkan region (Serbia and Croatia) is almost totally unknown and there should be more scientific investigations carried out. The aim of this work was to make a comparative study of antioxidative activities and total phenolic content of ethanol and hot water extracts of *G. lucidum*, collected from forests in Serbia (Morovičke šume, Fruška Gora) and Croatia (Donji Lapac, Plješevica). The present study was carried out to evaluate antioxidant potential of examined extracts via scavenging potential on ABTS, DPPH, OH[•] and NO[•] radicals, as well as of chelating effects via FRAP assay, together with determination of their total phenolic content. Results showed that both GLS extracts possessed better antiradical activities ($IC_{50}=0.23\pm0.01$ for H₂O and 2.75 ± 0.01 μ g/mL for EtOH for OH[•] and DPPH assay, respectively) than in the ABTS assay (151.40 ± 1.07 mg TE/g d.w. for EtOH), while the phenolic content was generally equal in extracts of Serbian and Croatian samples (60.74 ± 0.57 mg GAE/g d.w. for EtOH and 77.10 ± 0.27 mg GAE/g d.w. for H₂O, respectively). Therefore, these extracts could be considered as a good source of natural antioxidants. These results showed that examined *G. lucidum* extracts (especially H₂O) contain high amount of phenolic content which could significantly enhance the antiradical potential and reduce potential on iron ions. This is the first study reporting the comparison of antioxidant activities and phenolic contents of two different extracts between two *G. lucidum* strains from two different geographical origins from the Balkan region.

Keywords: antioxidant capacity, *Ganoderma lucidum*, phenols, reducing power, radical reduction

THE INFLUENCE OF HEAVY METALS ON THE BIOMASS PRODUCTION AND CATALASE ACTIVITY IN *Pleurotus ostreatus* AND *Bjerkandera adusta*

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Heavy metals are potent inhibitors of the enzymatic reactions, while catalase is an extremely important enzyme in protecting the cells of aerobic organisms from oxidative damage by reactive oxygen species (ROS). This study analyzes the impact of heavy metals, mercury (Hg) and lead (Pb), on the biomass production and catalase activity in a submerged culture of selected white-rot fungi (*Bjerkandera adusta* and *Pleurotus ostreatus*). Cultivation of mycelia was carried out in a modified Melin-Norkrans (MMN) medium (control groups - CG), with addition of three different concentrations of heavy metals: 10 µM, 100 µM and 500µM (test groups - TG).

For both fungal species, the increasing concentration of analyzed metals was followed by the reduction of biomass (*B. adusta*: CG: 83.8 mg, TG_{Pb}: 72.6 - 55.9 mg, TG_{Hg}: 90.7 - 28.5 mg; *P. ostreatus*: CG: 91 mg, TG_{Pb}: 101.3 - 54.5 mg, TG_{Hg}: 70.4 - 33.2 mg), wherein Hg had stronger inhibitory effect than Pb. The minimum activity of catalase was observed in the control groups (*B. adusta*: 180.01 U/mg; *P. ostreatus*: 1931.79 U/mg), growing without the influence of stress factors. In the presence of heavy metals, increased catalase activity was recorded compared to the control groups (*B. adusta*: 10 µM Pb: 532.26 U/mg CAT, 100 µM Pb: 394.55 U/mg CAT, 500 µM Pb: 190.18 U/mg CAT, 10 µM Hg: 1118.12 U/mg CAT, 100 µM Hg: 189.94 U/mg CAT; *P. ostreatus*: 10 µM Pb: 4505.24 U/mg CAT, 100 µM Pb: 3008.96 U/mg CAT, 500 µM Pb: 2128.73 U/mg CAT, 10 µM Hg: 2079.34 U/mg CAT) as a response to oxidative stress and increased number of ROS. Mercury exhibited extremely detrimental effect - higher concentrations of this metal (500µM for *B. adusta*; 100 and 500 µM for *P. ostreatus*) resulted in complete inactivation of catalase.

The results obtained indicate a higher resistance of *P. ostreatus* than *B. adusta*.

Keywords: lead, mercury, *Pleurotus ostreatus*, *Bjerkandera adusta*, catalase

ANTIFUNGAL ACTIVITY OF MACROFUNGI EXTRACTS ON PHYTOPATHOGENIC FUNGAL STRAINS OF GENERA *Fusarium* SP. AND *Alternaria* SP.

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During the last decades, intensive application of synthetic fungicides in the agricultural crop protection practice caused growing concern for the existence of toxic chemical residues in food as well as in the whole environment. Instead of using synthetic fungicides, it is suggested that crop protection be carried out by using preparations based on compounds of natural origin (secondary metabolites of plants or microorganisms, including macrofungi from Basidiomycota) as biological control agents. The potential of macrofungal species as biocontrol agents was analyzed in this investigation for eight autochthonous species from different locations in Serbia. Both the terricolous species: *Coprinus comatus*, *Coprinellus truncorum*, *Amanita strobiliformis*, *Hydnum repandum* and the lignicolous species: *Flammulina velutipes*, *Stereum subtomentosum*, *Trametes versicolor* and *Bjerkandera adusta* were examined, with an aim to detect some novel sources of antifungal agents. This study surveyed antifungal activity of selected macrofungal extracts (MeOH, EtOH and CHCl₃) against phytopathogenic *Fusarium* and *Alternaria* strains isolated from garlic, soybean and rice: *F. proliferatum*, *F. verticillioides*, *F. proliferatum*, *F. graminearum* and *A. padwickii*. Microdilution method in 96 well micro-plates was applied for the estimation of antifungal effects of macrofungi extracts in the range from 24.75 to 198.00 mg/ml and determination of minimal inhibitory (MIC) and minimal fungicidal concentration (MFC). EtOH extract of mychorhizal species *H. repandum* showed antifungal activity against all analyzed phytopathogenic strains, with the strongest effect on *Fusarium* strains (MIC 24.75 mg/ml; MFC 24.75 mg/ml). Among others, MeOH extracts of *S. subtomentosum* and *C. micaceus* showed similar effects while only *B. adusta* showed slight effect on *Fusarium* strains (MIC 24.75–99.00 mg/ml; MFC 24.75–99.00 mg/ml) and none effect on *A. padwickii*. The obtained results indicate the possibility of using examined extracts as efficient antifungal agents and provide the basis for the new formulations for biocontrol agents against phytopathogenic fungi in the future.

Keywords: Biocontrol, antifungal activity, *Fusarium*, *Alternaria*, *Hydnum repandum*

ANTIFUNGAL ACTIVITY OF NEWLY SYNTHESIZED IONIC LIQUIDS ON PHYTOPATHOGENIC FUNGAL SPECIES OF GENERA *Fusarium* AND *Alternaria*

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Green Chemistry as a scientific field that combines the chemical and engineering research is mainly focused on the design of products and processes that minimize the usage and the formation of hazardous substances in the environment. A modern sustainable approach in agricultural practice would be a replacement of synthetic and toxic substances in treatment of crop with benign and environmentally friendly compounds. As one of the possible directions could be using of ionic liquids, which are well known to manifest antimicrobial activity, hence can be used in the development of new antimicrobial agents such as antiseptics, biocides and antifungals. This study examines antifungal activity of newly synthesized six different ionic liquids: 1-butyl-3-methylimidazolium salicylate [bmim][Sal], 1-(4-hydroxy-2-oxobutyl)-3-methylimidazolium salicylate [OHC₂OC₂mim][Sal], 1-(2-oxobutyl)-3-methylimidazolium salicylate [C₂OC₂mim][Sal], 1-(3-hydroxypropyl)-3-methylimidazolium salicylate [OHC₃mim][Sal], imidazolium salicylate [Im][Sal] and cholinium taurate [Chol][Tau] against phytopathogenic *Fusarium* and *Alternaria* strains isolated from three plants – garlic (*Allium sativum*): *F. proliferatum*, *F. verticillioides*, *F. proliferatum*; soybean (*Glycine max*): *F. graminearum* and rice (*Oryza sativa*): *A. padwickii*. Antifungal effect was estimated by micro-plate microdilution method for establishing minimum inhibitory (MIC) and minimum fungicidal concentration (MFC). [bmim][Sal] showed the highest antifungal effect on all analyzed phytopathogenic strains (MIC 19.80 mg/mL; MFC 19.80 mg/mL). All ionic liquids showed strain specific but good antifungal activity on *A. padwickii*, with MIC detected at the range from 18.97 mg/mL to 37.59 mg/mL, and MFC from 19.80 mg/mL to 51.48 mg/mL. The obtained results indicate the possibility of using new ionic liquids in biocontrol, representing their application in crop protection.

Keywords: Ionic liquids, antifungal activity, phytopathogenic fungi, *Fusarium*, *Alternaria*

MORPHO-ANATOMICAL CHARACTERIZATION OF *Tuber macrosporum*/*Corylus avellana* MYCORRHIZAS FROM CULTIVATED SEEDLINGS: CASE REPORT

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This study investigated the presence of mycorrhizae on seedlings from part of ten-year-old truffles plantation (about 3,000 m²) located in Eastern Serbia. This study is observation of the presence of ectomycorrhizal fungus from genus *Tuber* during its symbiotic stage on the roots of *Corylus avellana* L. Ten root samples were collected (randomly) and observed macroscopically and microscopically analyzed. There were changes in morphology and anatomy of the infected roots of *C. avellana*. Mantle was clearly observed to cover the roots and the mycelia formed the Harting net. Among arbitrary selected seedlings, there were found mature fruiting bodies on the surface of the soil. The truffles, identified as *Tuber macrosporum* Vittad, were found in the immediate vicinity of the hazelnut trees. There has been no previous information of mycorrhizae *Tuber macrosporum*/*Corylus avellana* on artificially created truffles plantation in Serbia.

Keywords: *Tuber* spp., *Corylus avellana* L, ectomycorrhizae, morpho-anatomical characters, ascocarp, ascospore

ADHESION OF *Candida* SPP. TO VARIOUS SURFACES IS INFLUENCED BY YEAST CELL HYDROPHOBICITY

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Invasive fungal infections, such as candidiasis, represent a public health problem of major importance. *Candida* species are commensal micro-organisms that become pathogenic when the defence mechanisms of the host are weakened, and these organisms then have the ability to cause a variety of superficial and systemic infections. In the past twenty years, *Candida* species are responsible for around 80% of fungal infections in the hospital environment. Although most cases of candidiasis have been attributed to *Candida albicans*, more recently, non-*albicans Candida* species, such as *C. krusei*, *C. glabrata* and *C. parapsilosis* have been identified as frequent human pathogens. The first event in *Candida* infection is its adherence of the organism to host and/or medical-device surfaces, often leading to the formation of biofilm. The aim of our study was to assess the potential of *Candida* species to adhere to stainless steel (AISI 304) material with different degrees of surface roughness ($R_a = 25.20 - 961.90$ nm) and polystyrene as most frequently used contact materials. The influence of cell surfaces hydrophobicity and surface roughness of stainless steel on the degree of yeasts adhesion was investigated. Adhesion of yeast strains to polystyrene and stainless steel was assessed by crystal violet staining. Our results indicated that all *Candida* strains were able to adhere to polystyrene and stainless steel surfaces in a species- and strain-dependent manner. *C. albicans* showed a higher ability to adhere to both surfaces compared with non-*albicans Candida* species. No correlation was found between cell surfaces hydrophobicity and adhesion of yeast cells to polystyrene and stainless steel surfaces. Adhesion of majority *Candida* was significantly influenced by the roughness of stainless steel surface.

Keywords: adhesion, yeast, polystyrene, stainless steel, cell surfaces hydrophobicity

ADHESION OF *Candida glabrata* TO POLYSTYRENE SURFACE IS EFFECTED BY PROBIOTIC YEAST

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Following the widespread use of immunosuppressive therapy together with broad-spectrum antimycotic therapy, the frequency of mucosal and systemic infections caused by *Candida glabrata* has increased significantly. *C. glabrata* is considered as the second most frequently isolated pathogenic yeast after *Candida albicans*. Due to increasing resistance of *C. glabrata* to existing drugs, it is very important to look for new strategies helping the treatment of such fungal diseases. An increasing number of potential health benefits are being attributed to probiotic treatments. They include various bacterial probiotics, while among yeast only *Saccharomyces boulardii* (nom. nud.) is used extensively as a probiotic and often marketed as a dietary supplement. *S. boulardii* is very efficient as a biotherapeutic agent for the prevention and treatment of intestinal diseases, mainly diarrhea. The beneficial effect of *S. boulardii* in the case of *C. glabrata* infections have not been studied yet. Also, the influence of *S. boulardii* presence on the efficiency of *C. glabrata* virulence traits, like adhesion and antimycotic resistance, is not known. Therefore, we tested the adhesion of 48 *C. glabrata* strains in a co-culture with *S. boulardii* to polystyrene surface at different temperatures, pH values and in the presence of three clinically important antifungal drugs, namely fluconazole, itraconazole and amphotericin B. We show a significant repressive effects of *S. boulardii* on *C. glabrata* adhesion, resulting in lower effective concentration of antimycotics in our tests, but we also show that these effects, including a possible stimulative effect on *C. glabrata* adhesion, is highly dependent on specific conditions, antimycotic concentrations and strains involved.

Keywords: adhesion, *Candida glabrata*, *Saccharomyces boulardii*, antimycotics.

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AND FRUITS SUPPLEMENT IN REDUCED NITRITE SALTS CONDITION

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Because of the growing negative perception of consumers related to the use of meat products produced by conventional curing methods, organic and natural products are increasingly accepted by consumers. Such products contain a large number of natural products derived from plants, spices, as well as their derivatives in form of essential oils, extracts, concentrates, and so on. These derivatives contain large number of active substances which are known to inhibit the metabolic processes of bacteria, yeasts and molds. Therefore, the goal of this paper was to investigate the synergistic antimicrobial activity of the models with a reduced presence of nitrite salt in aqueous solution, emulsions of essential oils in varying concentrations *in vivo* via antibiogram tests on pathogenic microorganisms.

The effect of the six model groups was analyzed. Two groups were fruit powder solutions in concentrations of 0.2% to 1.2% (Acerola powder and fruit powder mix), while the other four groups were models of aqueous emulsion of essential oil in concentrations ranging from 0.05% to 1.2% (tea tree, clove, oregano, and cinnamon essential oils). In all models reduced amount of the sodium salt of 1.80%, 0.0075% nitrite salt and the liquid derivative as a natural source of the nitrate salt of 3% were used. Antibiogram tests were performed on five pathogenic bacteria (*C. perfringens*, *E. coli*, *S. enterica*, *L. monocytogenes*, and *S. aureus*). All antibiogram tests were performed according to Kirby-Bauer disk diffusion protocol.

Results of antibiograms showed that without the presence of additional antimicrobial agents, in model systems with reduced content of salts, inhibition zones were not detected. Additionally, models with essential oils of tea tree oil and oregano had the widest inhibition zone diameters, ranging from 17.76±0.48mm for *E. coli* up to 42.50±0.13mm for *S. aureus*.

Keywords: antibiograms, essential oils, food pathogens, natural antimicrobials, organic meat products

ASSESSMENT OF DIESEL FUEL UPTAKE BY FUNGI ISOLATED FROM PETROLEUM CONTAMINATED SOIL

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The aim of this research was to isolate and identify the fungi from petroleum-contaminated soil, and to test their ability to grow in the presence of diesel fuel and uptake in solid and broth media. Among 16 isolated and identified filamentous fungi, six were tested to determine their growth on media enriched with diesel fuel as a sole carbon source. All tested isolates showed different degree of diesel fuel utilization. Colony diameter was measured, where the highest colony growth dynamics ratio was demonstrated for *Penicillium* sp. (74.16%) and *Cladosporium* sp. (71.43%), and the lowest for *Absidia spinosa* (23.15%). Results of dry mass measurements demonstrated the highest potential of *A. strictum* (71.43%) to utilize diesel fuel in broth medium. All other tested isolates showed insignificant dry mass production ratio values. The highest utilization degree was documented for *A. strictum* (60.28 %) and the lowest for *Penicillium* sp. (25.18%).

Keywords: diesel fuel utilization, fungi, soil

EFFECT OF WATER ACTIVITY ON THE RADIAL GROWTH OF FUNGI ISOLATED FROM DRY-CURED SHEEP HAM, *IN VITRO*

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In the Western Balkans, traditional dry-cured sheep ham called Pastrma or Stelja is produced. Dry-cured sheep ham or Sjenica (Western Serbia) sheep prosciutto is produced in a very complex manner, and a prerequisite for the production of meat is the sanitary safety of raw materials which meet veterinary and sanitary conditions of production.

Isolation and preliminary categorization was carried out in this study, as well as "*in vitro*" testing of the effects of water activity (a_w) on the growth of fungi from dried meat of sheep carcass (sheep prosciutto). The study used 9 samples of dry-cured sheep meat taken from three households in two productive years (2015 and 2016 yr.) in the area of Sjenica. Seven different types of fungi were isolated and identified: *Penicillium corylophilum*, *P. carneum*, *P. patulum*, *Aspergillus nidulans*, *A. niger*, *Eurotium herbariorum* and *Mucor racemosus*. Water activity was tested on a of MY50GF agar from the series of malt extract yeast extract glucose fructose agar. Water activity was set to values of 0.87, 0.89, and 0.97. The results of the research showed that the speed of the growth rate of colonies is under the direct influence of water activity. Fungi grew the fastest ar water activity 0.97 a_w , wherein from all the tested species the highest growth was recorded between 3 (*A. niger*), 7 (*P. patulum*) and 10 days (*A. nidulans*). At lower a_w values the lesser decline in the growth rate was observed, while the germination of the spores of *A. niger* was not detected the first three days. *E. herbariorum* showed the best micellar growth and sporulation on all a_w values.

Keywords: sheep prosciutto, fungi, water activity, growth speed

ПЛЕНАРНА ПРЕДАВАЊА / PLENARY LECTURES

**PHOMOPSIS STEM CANCKER (*Diaphorte/Phomopsis* SPP. COMPLEX) ON
SUNFLOWER 37 YEARS LATER**

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Currently, Phomopsis stem canker is one of the most damaging sunflower diseases in the world. The disease was first described in Yugoslavia in 1980 with *Phomopsis helianthi* (syn. *Diaporthe helianthi*) as the causal agent, after which it was reported causing disease, first in Europe, then in the United States and Argentina. Due to the premature dying of plants, more than 40 percent of yield losses have been reported to occur in affected fields.

In Australia, *Diaporthe* species besides *D. helianthi*, which includes *D. gulyae*, *D. kongii*, *D. masirevici* and *D. novem*. have been identified causing Phomopsis stem canker of sunflower. Recent findings indicate that *D. gulyae* is prevalent in the United States and Argentina.

Today, 37 years later, Phomopsis stem canker can hardly be found in regions in the former Yugoslavia where the disease outbreak occurred for the first time. However, the disease continues to be a threat in the United States, Australia and Argentina.

DERMATOMYCOSES DUE TO DERMATOPHYTES

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Dermatophytoses, vs. Tinea, are frequent skin infections caused by dermatophytes, fungi that require keratin for their growth and belong to one of the three genera: *Microsporum*, *Trichophyton* and *Epidermophyton*. Regarding the source of infection dermatophytes can be divided into anthropophilic, zoophilic and geophilic species. Species of dermatophytes cause superficial infections of the skin, hair and nails. Therefore, dermatophytoses can be divided into Tinea capitis, Tinea of the glabrous skin and Tinea of the nails. Clinical presentation of the dermatophytoses is defined by the site of infection and by the species which causes infection. Besides the clinical picture and history of the disease, for the final diagnosis of dermatophytosis, KOH, fungal culture, Woods and histological examinations are very important.

There are a lot of treatment options for dermatophytoses, and the final decision mainly depends on the site of infection and the cause of infection. While Tinea capitis, Tinea barbae and Tinea unguium require systemic antifungal treatment, other dermatophytoses can be successfully treated by topical therapy.

Keywords: Dermatophytoses, skin, hair, nails, treatment

FUNGAL INFECTION IN OUR DERMATOLOGICAL PRACTICE

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The purpose of this presentation is to briefly inform about coincidence of fungal infections in our dermatological practice.

In the last decades, the pet population significantly increased as well the interest in having animals as a pets. However, nowadays not only cats and dogs are popular pets, the other domestic and exotic mammals (e.g. rabbits, rodents, ferrets, hedge hogs) are also common and they can be a potential source of zoonotic infections transmissible to their owners. In addition, in our dermatological clinic we often exam owners if we or human dermatologist assumed that disease may be a zoonotic dermatosis. According to generally accepted estimates, about 5% of all human dermatological cases are zoonotic dermatological disorders and about 2% are ringworm infections. Considering the close contact between pets and their owners, especially between children and pets, understanding of epidemiology, pathogenesis, diagnosis, treatment and control of dermatophytes infection in pets is very important. Pets are important source and often asymptomatic carriers of the infection. The most commonly isolated dermatophytes in our practice are *Microsporum canis* (especially in cats), *M. gypseum* and *Trichophyton mentagrophytes*. The nonlipid-dependent yeast *Malassezia pachydermatis* is normal inhabitant of the skin and ear canal, and occasional pathogen. Abnormal overgrowth of this yeast is usually seen in some pruritic dermatoses and otitis externa, mostly in dogs, but occasionally in other animal species. Zoonotic potential of this yeast is not completely clear.

Keywords: cat, dog, exotic pet mammals, dermatophytosis, zoonotic dermatoses

***Trichoderma* SP.: PLANT GROWTH PROMOTING FUNGUS (PGPF) AS
MICROBIAL FERTILIZER WITH BENEFICIAL EFFECT ON SOIL**

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Trichoderma spp. are fungi present in all soils and other diverse habitats. In soil, they are the most prevalent culturable fungi. They are favored by the presence of high levels of plant roots, which they colonize readily. Some strains are highly rhizosphere competent, able to colonize and grow on roots as they develop. The most strongly rhizosphere competent strains can be added to soil or seeds as biofertilizers.

In addition to colonizing roots, *Trichoderma* spp. attack, parasitize and gain nutrition from other fungi. Since *Trichoderma* spp. grow and proliferate best when there are abundant healthy roots, they have evolved numerous mechanisms for both attack of other fungi and for enhancing plant and root growth. it is now clear that there must be hundreds of separate genes and gene products that can be used as biocontrol agents with mechanisms likes mycoparasitism, antibiosis,.competition for nutrients or space, tolerance to stress through enhanced root and plant development, induced resistance and inactivation of the pathogen's enzymes

Strains from *Trichoderma* spp. are promising fungi toward the development of sustainable agriculture.

ANTIFUNGAL ACTIVITY OF INDIGENOUS *Bacillus* spp. ISOLATED FROM SOIL

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Biocontrol using plant growth-promoting rhizobacteria (PGPR) represents an alternative approach to disease management, since PGPR are known to promote growth and reduce diseases in various crops. Among the different PGPR, members of the genus *Bacillus* are preferred for most biotechnological uses due to their capability to form extremely resistant spores and produce a wide variety of metabolites with antimicrobial activity. The objective of this research was to identify antagonistic bacteria for management of the plant diseases. Eleven isolates of *Bacillus* spp. were obtained from the soil samples collected from different localities in the Province of Vojvodina. The antifungal activity of bacterial isolates against five fungal species was examined using a dual plate assay. *Bacillus* isolates exhibited the highest antifungal activity against *Fusarium proliferatum*, *Fusarium oxysporum* f. sp. *cepa*, and *Alternaria padwickii*, while they had the least antagonistic effect on *Fusarium verticillioides* and *Fusarium graminearum*. Molecular identification showed that effective bacterial isolates were identified as *Bacillus safensis* (B2), *Bacillus pumilus* (B3, B11), *Bacillus subtilis* (B5, B7), and *Bacillus megaterium* (B8, B9). The highest antagonistic activity was exhibited by isolates B5 (from 39% to 62% reduction in fungal growth) and B7 (from 40% to 71% reduction in fungal growth). These isolates of *B. subtilis* could be used as potential biocontrol agents of plant diseases.

Keywords: *Bacillus*, biocontrol, *Fusarium*, *Alternaria*, antifungal activity, isolation, soil

MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF *Fusarium tricinatum* AND *Fusarium acuminatum* AS CAUSAL AGENTS OF GARLIC BULBS ROT IN SERBIA

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Garlic (*Allium sativum* L.) is considered to be one of the oldest crops in the world. During 2016, infected garlic bulbs occurred in storage in several localities of Vojvodina Province. Symptomatic cloves showed typical rot symptoms such as softened and spongy areas covered with white fungal growth with deep lesions formed on the cloves which became dry over time. A total of 36 isolates of *Fusarium* species were obtained from diseased cloves of garlic. Colony morphology and microscopic properties of isolated *Fusarium* species were recorded from the cultures grown on PDA and CLA, respectively. Identification of two chosen isolates was performed by sequencing the EF-1 α gene. The TEF sequence of isolate JBL12 showed 100% similarity with several *F. tricinatum* sequences and sequence of JBL539 showed 99% identity with several *F. acuminatum* sequences and they were deposited in the NCBI GenBank. Based on the results of the morphological and molecular identification, isolates JBL12 and JBL539 were identified as *F. tricinatum* and *F. acuminatum*, respectively, as new causal agents of garlic bulbs rot in Serbia. Specific primers were designed for the PCR identification of the *F. tricinatum*.

Keywords: garlic (*Allium sativum*), bulb and clove rot, *Fusarium* spp., EF-1 α gene

DARK-FIELD MICROSCOPE STOOL ANALYSIS – ITS ROLE IN DIAGNOSIS OF YEAST OVERGROWTH IN GUT

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Not long after birth, yeast, predominantly *Candida albicans* colonizes the epithelium of oral cavity and the whole gastrointestinal tract. *C. albicans* lives in yeast, a non-harming form, as a commensal member of the microbial flora, but may turn into pathogen infective form under certain conditions that encourage its overgrowth. In this phase, it may damage the intestinal wall and enter the blood stream, causing invasive candidiasis with high mortality rate. It is essential to recognize candidaemia and start the lifesaving therapy on time. Recognizing the risk factors which allow candida to overgrow is the most important step in preventing candida's overgrowth and chronic candidiasis, the previous status of invasive candidiasis. If this recognition is missed, and the overgrowth advances, a question remains how to discover and treat it and in which phase it should be done. A stool culture requires time and proves the presence of live yeast cells only. If the live yeast cells are not present in the stool, the result of the culture will be negative. In this paper, the author presents her experience of stool analysis under dark-field microscope, as a rapid, easy to carry out method for detecting the presence of live or dead yeast cells and yeast overgrowth.

Keywords: yeast infection, candida infection, candida overgrowth, chronic candidiasis, stool analysis, dark-field microscope

POTENTIAL OF CAMPHOR TO INTERFERE WITH *Candida albicans* PATHOGENICITY

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Candida albicans is common commensal but under certain conditions it can cause serious diseases. Its pathogenicity is associated with transition from yeast to hyphal form and potential for biofilm formation. Camphor was tested for its potential to inhibit the growth of *C. albicans*, as well as to interfere with morphogenic transition from its yeast to pathogenic hyphal form. Four strains of *C. albicans* were isolated from oral cavities of patients and its susceptibility was tested in microdilution method. Cell morphology of *C. albicans* was monitored after 24 h incubation of strains in medium enriched with fetal bovine serum, with the addition of minimum inhibitory concentration. Camphor was tested for its potential to inhibit formation of biofilm. Amphotericin B was used as a positive control. Results showed MIC value of 0.125 mg mL⁻¹, and MFC value 0.25 mg mL⁻¹ for camphor, while amphotericin B showed better activity with MIC 0.63 x 10⁻³ mg mL⁻¹ and MFC 1.25 x 10⁻³ mg mL⁻¹, indicating amphotericin B as much better antifungal compound. Microscopic observation showed that cells treated with camphor were in yeast form, whereas cells treated with amphotericin B were in hyphal form. Formation of biofilm in cells treated with camphor was reduced by 64.15%, which is of great importance, since amphotericin B did not inhibit biofilm formation in tested concentrations. Although camphor shows lower antifungal potential it had profound inhibitory effect on biofilm formation and transition to pathogenic hyphal form. These multiple effects may be of practical significance.

Keywords: *Candida albicans*, pathogenicity, biofilm, hyphae

EPIDEMIOLOGY OF FUNGAL NAIL INFECTION: A ONE-YEAR RETROSPECTIVE STUDY

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Fungal nail infections represent around 50% of all nail disorders. The aim of this retrospective study was to examine epidemiological characteristics of fungal fingernail and toenail infection at the Institute of Dermatovenereology, Belgrade in one-year period.

Data were obtained from the protocol of mycological laboratory in which samples of all patients of the Institute of Dermatovenereology, Belgrade in 2016 with clinically suspected fungal nail infection were examined by potassium hydroxide (KOH) preparation, and fungal cultures.

In this study, 1896 patients with suspected fungal infection of fingernail or toenail were included. Fungal fingernail infection was diagnosed in 332 patients (17.51%; 34.6% males and 65.4% females), from which positive KOH preparation had 327 patients (98.5%), while fungal culture was positive in 156 patients (46.99%). The most frequently was isolated *Candida* spp. (91.03%), followed by *Aspergillus* spp. (4.48%), *Trichophyton interdigitale* (1.92%), *Trichophyton rubrum* (1.28%), and *Trichophyton species* (1.28%). Fungal toenail infection was confirmed in 974 patients (51.37%; 33.1% males and 66.9% females), from which 971 patients (99.7%) had positive toenail KOH preparation, and 407 patients (41.8%) had positive fungal toenail culture. *Candida* spp. (79.36%) was the most frequent cause of toenail fungal infection, followed by *Trichophyton interdigitale* (8.59%), *Trichophyton rubrum* (7.86%), *Trichophyton species* (1.97%), *Aspergillus* spp. (1.97%) and *Trichophyton violaceum* (0.25%). KOH preparation and fungal culture of fingernail or toenail, were the most frequently positive in the age-group 60-69 years (30.6%, 31.4%, 29.4% and 28.5% respectively). Positive KOH preparation in both, fingernail and toenail, were found in 124 patients (6.5%; 34.7% males and 65.3% females), while positive fungal culture in 49 patients (2.6%; 40.8% males and 59.2% females). In all these patients the cause of infection was the same in fingernail and toenail (*Candida* spp. – 89.8%, *Aspergillus* spp. – 6.12%, *Tr. interdigitale* – 4.08%).

These results showed that 17.51% of examined patients with suspected fungal nail infection at the Institute of Dermatovenereology, Belgrade had fungal fingernail infection, and 51.37% had fungal toenail infection. Females, and persons from 60 to 69 years old were more frequently affected, and the most common cause of fingernail or/and toenail fungal infection was *Candida* spp.

Keywords: fungal infection, fingernail, toenail

FARM WATER AS A POSSIBLE SOURCE OF FUNGAL INFECTIONS

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The quality of drinking water depends on the water sources, but also on the quality of the water distribution system which supplies the water on to the final user. In addition, the possibility of contamination of water used for watering animals in the farm buildings depends on the hygienic conditions on farms. Microbiological quality of water on farms in Serbia has not been one the main focuses of animal breeders, although according to the Food Safety Law water is considered as food. As feed safety for the animals, which includes microbiological analyses, is an important concern of breeder farmers, it is also important to control the water safety in order not to become a cause of the animal health problems. Change of the water quality is not important only from the sanitary epidemiological point of view, but the presence of different microorganisms, especially fungi, can cause changes in taste and smell, as organoleptic properties of water. According to legal regulations, there is no difference between the quality requirements for drinking water relative to the water supply intended for animals. For the aforementioned reasons, the subject of this study is microbiological control of water samples from the drinkers for animals at farms. The aim of the work is to examine which fungi are possibly present in the water and what is their number. In total, 35 samples of water from pig and poultry farms were tested. The method of direct seeding and filtration was used. The presence of different types of mold (*Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., *Mucor* sp. and *Rhizopus* sp., and *Candida* sp.) was determined. The results indicate the necessity of microbiological control of water for watering of farm animals, which implies the analysis for the presence of molds.

Keywords: water for watering animals, molds

FIRST CASE REPORT ON PATHOGENIC FUNGUS *Fonsecaea* sp. Negroni FROM SKIN OF *Pelophylax* kl. *esculentus* L. IN SERBIA

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Non-harmful adhesive tape method was applied directly on the skin surface of edible frog (*Pelophylax* kl. *esculentus*), captured in vernal ponds on the locality “Stevanove ravnice” within the Special Nature Reserve „Deliblatska peščara“, in order to detect fungal dwellers of frogs’ skin. Light microscopy analyses of LactophenolCottonBlue-mounted adhesive tape samples taken from frog's ventrum revealed the presence of melanized septate hyphae, branched conidiophores with chains of single-celled ovoid conidia, arising directly from the skin, which corresponds to morphological features of dematiaceous hyphomycete - *Fonsecaea* sp. Since members of genus *Fonsecaea* are frequently cited as causative agents of chromomycosis in amphibians, as well as human phaeohyphomycosis, worldwide, it is of great significance to study the presence of this fungal pathogen on amphibians in Serbia in order to make the basic reference data of the incidence of these pathogens in this region.

Keywords: adhesive tape method, chromomycosis, dematiaceous fungi, *Fonsecaea* sp., frogs, pathogen

EFFECT OF NOZZLE TYPE ON THE FUNGICIDE EFFICACY FOR FUSARIUM HEAD BLIGHT SUPPRESSION ON WHEAT

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Effect of fungicide treatments on Fusarium head blight (FHB) and grain yield of wheat depending on application technique i.e. use of different nozzle types, was evaluated in the study. Nozzles types TJ 11004, Albuz ATR 8004 and Arag TFA 11004 were used for application of systemic fungicide Duett Ultra (0.5 l/ha). FHB intensity (%) was determined on the basis of a visual assessment of the number of infected heads and the perecentage of the disease symptoms on the individual head.

Differences in grain yield between the treated variants, as well as between the treated and untreated variants, were determined after hand threshing. The lowest percentage of FHB development in wheat and the highest yield was recorded in variants where fungicides were applied by nozzle type ATR 8004. Application technique directly affects the reduction of fusarium head blight in wheat and indirectly it also reduces yield loss.

Keywords: application technique, fungicide, fusarium head blight, wheat, yield

OCCURRENCE OF POT MARIGOLD (*Calendula officinalis* L.) RUST IN AGROECOLOGICAL CONDITIONS OF SERBIA

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Pot marigold (*Calendula officinalis* L.) is an important medicinal plant species in Serbia. Only a few pot marigold pathogens have been described so far. In 2015 and 2016, the orange-yellowish pustules, slightly arising above the tissue surface, were observed on pot marigold leaves at flowering, on three locations (Kovilj, Titel and Backi Petrovac). Number of infected plants varied between locations, ranging from 46% (B. Petrovac), 58% (Titel) to 62% (Kovilj). Microscopy showed rounded, unicellular uredospores 14-19 x 10-15 µm in size. Nearby the pot marigold production field, a weed plant species *Senecio vulgaris* L. was found. On leaves and stem of *S. vulgaris*, uredosoruses, with the same morphological characteristics as those from pot marigold were recorded. Number and coverage of uredosori on plant surface were significantly higher on *S. vulgaris* than in pot marigold. Uredospores of *S. vulgaris* were unicellular, orange, 15-19 x 12.5-17 µm in diameter. By analyzing the uredospore morphology and according to literature data available, we believe that causative agent of pot marigold rust is *Puccinia lagenophorae* (Cooke). This pathogen is widespread worldwide, with around 110 host species of 16 genera of the *Asteraceae* family. It is stated that *P. lagenophorae* that originates from the Australian continent has been present in Europe since 1960. In the agroecological conditions of Serbia, only uredospores on these two plant species have been observed so far.

Keywords: *Calendula officinalis*, rust, *Senecio vulgaris*, *Puccinia lagenophorae*

FUNGUS *Schizophyllum commune* - DOMINANT CAUSE OF WOOD DECAY OF THE NOVI SAD CITY ALLEYS, BOULEVARDS AND PARKS

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In the frame of investigation of the main cause of dying trees of the alleys and parks in the City of Novi Sad, the investigation of the presence and the diversity of macrofungi have been undertaken in the period 2013-2016. The relative poor generic diversity of lignicolous (pathogenic or potentially pathogenic and saprotrophic) macrofungi with only 24 species representing this group (19 basidiomycets: *Schizophyllum commune*, *Fomes fomentarius*, *Agrocybe aegerita*, *Funalia trogii*, *Lentinus strigosus*, *Stereum hirsutum*, *S. rugosum*, *Coriolus versicolor*, *Trametes hirsuta*, *Flammulina velutipes*, *Pseudotrametes gibbosa*, *Ganoderma applanatum*, *G. Resinaceum*, *G. lucidum*, *G. adspersum*, *Polyporus squamosus*, *Piptoporus betulinus*, *Panellus stipticus*, *Pholliota squarosa*, *Meripilus giganteus*, *Laetiporus sulphureus*, *Auricularia auricula-judae*, and 5 ascomycets: *Rhytisma acerinum*, *Bulgaria inquinans*, *Nectria cinnabarina*, *Xylaria hypoxylon*, *X. polymorpha*) have been recorded. Such a poor qualitative composition of this very important fungal group could be explained by reducing arbored walks and alleys to the small number of plant species, but also to the reduced number of fungi resistant to the heavy air pollution caused by very nearby (1-5m) fuel combustion in engines.

Even though being preliminary, our results point to the necessity of conservation and protection of the most beautiful features of The Novi Sad City, its alleys and arbored walks and parks, undertaking a measures of curing damaged trees and treating by fungicides in order to wipe out the epiphytia caused in more than 95% of cases, by Split-gill (*S. commune*), present on dead wood but also on damaged trees of: *Tilia cordata*, *T. platyphyllos*, *T. argentea*, *Acer negundo*, *A. platanoides*, *Aesculus hyppocastaneum*, *Platanus acerifolia*, *Fraxinus ornus*, *Betula pendula*, *Robinia pseudoacacia*, *Celtis australis*, *C. occidentalis*, *Catalpa* sp., *Corylus avelana*, *Prunus* spp., *Salix alba*, *Carpinus betulus pyramidalis*, etc. Altogether, during last decade, around 200 trees have colapsed or been sanitary cut in the Novi Sad arbored walks, alleys and parks due to the damage caused primarily by Split-gill fungus, and the restauration of arbored walks nearby the streets characterized by much polluted air, caused by the engine fuel combustion is becoming almost impossible mission. By analyzing the trees along the streets of Novi Sad could be concluded that, besides of appearance of suffocation of plants, due to wide asphalt surfaces that are located very next to tree-trunks and heavy air pollution, by fungi caused illnesses are the dominant cause of the decay of trees. With its great adaptation to arid climate, and being resistant to the air pollution, *S. commune* turned out to be the most agresive and succesful universal fungal invader of trees from old alleys. This fungus also becomes an important factor of threatening for immunocompromised persons because there were recorded cases in the Region of allergies, abscesses and inflammatory conditions caused by this basidiomycetous fungus among individuals of the human population.

Keywords: *Schizophyllum commune*, Novi Sad, alleys, parks, *Lignicolous* fungi

RESISTANCE OF EARLY MATURITY MAIZE GENOTYPES TO SOUTHERN CORN LEAF BLIGHT

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In order to study the resistance of early maize genotypes to Southern Corn Leaf Blight (SCLB) or Maydis Leaf Blight (MLB), RCBD experiments with 20 inbred lines and hybrids in 2014 and 16 genotypes in 2015 were conducted at Karaj and Sari Stations. Inoculation was carried out with spore suspension using syringe (3ml/each whorl) firstly at 3–4 leaf stage of maize, and then, sorghum grain inoculated with fungus was applied at 6–8 leaf stage in whorl of each plant using the bazooka technique. Evaluation was done on the base on disease progress at pollination stage and two weeks after pollination stage with scale 0–5. The results of variance analysis and mean comparison showed that there are different reactions among genotypes to disease. In this study, in 2014, 15% genotypes categorized into resistant group, and these were genotypes: KE 77003/10 × KE 75039, KE 72012/12 × K1263/1(KSC 400), and K 2331 × KE 75039. Among 16 genotypes in 2015, three hybrids – No. 16 with pedigree (KE 76009/311× K 1264/5-1), No. 10 with pedigree (K 2331 × KE 75039) and No. 9 with pedigree KSC 400 (KE 72012/12 × K1263/1) – were identified as resistant hybrids that can be a good source of resistance to SCLB.

Keywords: Maize, resistance, leaf disease, *Bipolaris maydis*

***Colletotrichum orbiculare* ON WATERMELON: IDENTIFICATION AND *IN VITRO* INHIBITION BY ANTAGONISTIC FUNGI**

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Anthrachnose caused by the fungus *Colletotrichum orbiculare*, is one of the most significant diseases of *Cucurbitaceae*. In Serbia watermelon fruits with typical anthracnose lesions were collected during the year of 2015. Affected fruits showed sunken, dark brown to black lesions with orange conidial masses produced in black acervuli. In an attempt to identify the causal organism, small pieces of necrotic tissue were surface sterilized and placed on potato dextrose agar (PDA). Macroscopic and microscopic morphology characteristics of three isolates were observed after growth on PDA for 7 days at 25 °C under a 12 h light/dark cycle. Fungal colonies developed white, grey to black dense aerial mycelium. Conidia were hyaline, aseptate, straight and cylindrical to clavate, 9–12.5 µm × 4–5.5 µm. Fungal isolates were also characterized by sequencing of the internal transcribed spacer (ITS) rDNA region using ITS1F/ITS4 primers and β-tubuline 2 gene using T1/Bt2b primers. The nucleotide sequences were deposited in GenBank (ITS Acc. No. KT454386, KT454387 and KT454388; β-tubuline 2 gene Acc. No. KT581236, KT581237 and KT581238). BLAST analysis of ITS and β-tubuline 2 gene sequences showed that our isolates were 100% identical to other *C. orbiculare* in NCBI GenBank. Pathogenicity test was conducted on symptomless, detached watermelon fruits. All tested isolates caused anthracnose lesions on watermelon fruits after 10 days of incubation. *Trichoderma harzianum* (DSM 63059) and *Gliocladium roseum* (DSM 62726) were evaluated *in vitro* for their antagonistic potential against *C. orbiculare*. The results of this study identify *T. harzianum* and *G. roseum* as promising biological control agents (BCAs) for further testing against anthracnose disease on watermelon fruits.

Keywords: *Colletotrichum orbiculare*, watermelon, identification, antagonistic fungi

Acknowledgments

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Projects TR 31018 and OI 173026.

CIP - Каталогизација у публикацији
Библиотека Матице српске, Нови Сад

582.28(048.3)

INTERNATIONAL Scientific Meeting Mycology, Mycotoxicology, and Mycoses (6 ; 2017 ; Novi Sad)

Book of abstracts [Elektronski izvor] / The 6th International Scientific Meeting Mycology, Mycotoxicology, and Mycoses, 27-29 September, 2017, Novi Sad, Serbia = Књига резимеа / Шести међународни научни скуп микологија, микотоксикологија и микозе, 27-29. септембар 2017., Нови Сад, Србија. - Novi Sad : Matica srpska, 2017. - 1 elektronski optički disk (CD-ROM) : tekst ; 12 cm

Nasl. sa naslovnog ekrana.

ISBN 978-86-7946-194-0

а) Микологија - Апстракти
COBISS.SR-ID 317145607