Abstracts of the international symposium Power of Fungi and Mycotoxins in the Midst of Climate Change (PoFMy), held on 16-17 September 2022 at the University North, Koprivnica; Croatia.

PoFMy is the fifth Symposium about fungi and mycotoxins that was organised in Croatia. The first two were national symposia with international participation, both organised as one-day meetings in Zagreb in 2004 and 2008, at the initiative of Stjepan Pepeljnjak (Faculty of Pharmacy and Biochemistry, University of Zagreb). The third (Primošten, 2011) and fourth (Šibenik, 2015) PoFMy was organised by the Croatian Microbiological Society. Finally, the fifth PoFMy expanded the "mycelial network" and joint forces from the Croatian Microbiological Society, Croatian Society of Toxicology, Institute for Medical Research and Occupational Health (Zagreb), and University North (Koprivnica) organised the meeting. As the COVID pandemic forced us all to use online tools, PoFMY, like many other symposia these days, was organised in hybrid form.

The Organising Committee comprised: Bojan Šarkanj as president, Dubravka Rašić as secretary, and members Ivana Dodlek Šarkanj, Daniela Jakšić, Marija Kovač Tomas, and Manuela Zadravec. The International Program Committee members were: Maja Šegvić Klarić as president, Daniela Jakšić, Jovana Kos (Serbia), Tihomir Kovač, Maja Peraica, Dubravka Rašić, Massimo Reverberi (Italy), Alberto Rittieni (Italy), Gianfranco Romanazzi (Italy), Michael Sulyok (Austria), Bojan Šarkanj, Valentina Španić, Manulea Zadravec, and Slaven Zlajić as members, and honorary members Stjepan Pepeljnjak and Bogdan Cvjetković.

Reports from the second and third Symposium were published in a thematic issue of the *Archives*, and this time we bring you abstracts of the 5th PoFMy, including 14 invited lectures, 8 oral presentations, and 9 poster presentations.

Maja Šegvić Klarić and Dubravka Rašić

PoFMy Abstracts – invited lecturers
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Multimycotoxin analysis – the sky is the limit

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Over the past decades, the sensitivity and robustness of LC-MS/MS instruments has continuously improved. Whereas it was not feasible to obtain accurate results down to the µg/kg range without any sample clean-up and analyte enrichment initially, the latest generation of instruments now tolerate the injection of diluted crude extracts. This is a pre-requisite for multi-analyte methods covering different compound classes with a wide range of physicochemical properties, as any type of clean-up may lead to the loss of certain compounds. In addition, dedicated data acquisition modes such as scheduled MRM (multiple reaction monitoring) mode enable one to acquire large analyte lists, e.g. for pesticide residue analysis, where methods covering some 200 compounds have become fairly routine. We have developed a method covering 1000 (mostly) fungal metabolites based on a single extraction and subsequent analysis of the diluted extract. The aim of this presentation is to discuss the obstacles that have to be overcome in the development and validation of such a method. As considers the latter, most of the guidelines available on proper method validation have been designed for assays targeting only one or very few analytes and following these guidelines without any modification is impractical in case of multi-analyte analysis. Finally, the option of extending the method to other contaminant classes will be given.

KEY WORDS: clean-up procedures; LC-MS/MS; method development; MRM; mycotoxins

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Ten years after the "aflatoxin crisis" in Serbia: where are we now?

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Ten years ago, in late 2012, the public in the Republic of Serbia was informed through different media about aflatoxins (AFS). The first information was related to the presence of AFS in maize, while soon after that contamination of milk with aflatoxin M1 (AFM1) additionally increased confusion among producers and consumers. Public concern grew rapidly following opposing information in various media. On the other hand, scientists agreed that the Republic of Serbia was faced at the time with a big challenge due to the appearance of one of the most toxic natural contaminants in the food chain, AFS. They highlighted extreme drought conditions, recorded during the summer months in 2012, as the factor with the strongest influence on the high prevalence of AFS in maize, and consequently, contamination of maize-derived products, milk, and dairy products. During that period, AFS's appearance attracted the attention of the public and politicians, and Serbia was faced with an "AFS crisis" followed by a protest of agricultural workers, appointment of a new Minister of Agriculture, several changes in regulations related to the maximum level of AFM1 and aflatoxin B1, confusion between consumers, and decrease in the purchase of milk and dairy products. Furthermore, the "AFS crisis" resulted in a significant economic loss (about a hundred million dollars), as Serbia is a leader in maize production, and among the top ten maize exporting countries in the world. Unfortunately, the weather conditions marked by increasing temperatures and lack of precipitation were dominant during the maize growing seasons in Serbia that followed. AFS contamination of maize and consequently of milk was again detected in 2013, 2015, 2017, and 2021. The presence of AFS in Serbia, in five of the ten most recent years, represents great concern regarding human exposure to AFS, due to the fact that milk and maize are one of the main foodstuffs in the human diet in Serbia, especially in children. Climate change predictions for this part of Europe indicate that the warming trend, favourable for *Aspergillus* species and AFS synthesis will continue in the future. Therefore, there is a great need for Serbia to enhance its control strategy, which should be based on continuous monitoring, increasing investments, as well as multidisciplinary integration and education of all participants in the food chain, with the main aim of minimizing the presence of AFS in the food chain.

KEY WORDS: carcinogenic mycotoxin; climate changes; maize; milk; weather conditions

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

Sterigmatocystin – an extraordinary mycotoxin

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Sterigmatocystin (STC) is a polyketide-derived mycotoxin structurally related to aflatoxin B1 (AFB1). Various research pointed to the notable differences in their toxic potencies, biotransformation, and mode of action. Our recent study showed that STC forms a stable aggregate in aqueous solutions in a concentration-dependent manner. Interestingly, none of the sibling molecules, including AFB1 or 5-methoxysterigmatocystin (MET-STC), do not share this property. In addition, the circular dichroism spectrum of exceptionally high intensity yielded from the STC aqueous aggregate had never been documented for any other small molecule. In the presented research it will be demonstrated how this phenomenon can be implemented in the research of STC and why it absolutely must be considered. Special emphasis will be put on the interactions of STC with different biomacromolecules.

KEY WORDS: aggregate; hydrophobic interactions; mycotoxins; noncovalent interactions; spectroscopy

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Mycotoxins: a world yet to be discovered

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Mycotoxins can undoubtedly be considered one of the most underestimated dangers of the food chain by consumers. Unfortunately, the same observation can be made for certain official institutions or government agencies responsible for the surveillance and control of their levels in food and feeds. Mycotoxins were discovered in the 1960s and many efforts have been made to understand their biological synthesis, toxicity, prevalence, and presence globally. All these scientific reports, documents, tests, analyzes and protocols to eliminate mycotoxins have generated few regulations and procedures regarding mycotoxins. The weak points of all these pathways are their focus, which is restricted to merely a few mycotoxins, mainly aflatoxins, ochratoxins, trichotecenes, patulin, a few matrices and a few toxicological aspects. The enemies are masked mycotoxins, emergent mycotoxins, the mutual synergic effects of several mycotoxins or those with other contaminants or virus or bacteria. The goal is a more holistic approach considering the multiple contamination, the additive or the synergic effects on human health, new matrixes like functional foods, food integrators or novel foods. The future is to reconsider the toxicity of foods or ingredients as a complex enemy and not as a sum of single dangers. In this presentation, I will focus on beauvericin and enniatins and their occurrence in milk, baby food, biological human fluids. Many colleagues like to introduce these compounds as emergent dangers for humans, but this definition is now obsolete. At the moment, results about the risk evaluation of these compounds, often co-occurrence with antibiotics or other toxic substances, are limited and no rules or limits are given to farmers, producers, or food transformers to follow. The efforts must focus on drawing attention from governments, food agencies, surveillance centers, etc. in order to reduce the exposure risks to mycotoxins as much as possible.

KEY WORDS: beauvericin; enniatins; food safety; multiple contamination; mycotoxins detection

The impact of seasonal weather variations on mycotoxin occurrence in fermented meat products

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Croatian traditional dry-cured meat products encompass various types of dry-fermented sausages and dry-cured meats, such as prosciutto, pancetta, dry-neck, etc. In the traditional production process, in addition to differences in recipes followed by producing households, there are also significant differences in hygienic and environmental production conditions which affect the specificity of microflora and yield differences in the quality and safety of the final products. One of the safety aspects that raises concern is contamination with toxigenic moulds and their secondary metabolites mycotoxins. In view of the already recognized weather impact on the occurrence of toxicogenic moulds and mycotoxins, the aim of this study was to relate data on seasonal weather variations during the products' maturation to mycotoxin concentrations determined in TMPs (n = 250) collected in five Croatian regions (eastern, northern, central, western, and southern) during two sampling years (2020 and 2021). Aflatoxin B_1 (AFB_1), ochratoxin A (OTA), sterigmatocystin (STC), citrinin (CIT), and cyclopiazonic acid (CPA) were identified using LC-MS/MS (liquid chromatography-tandem mass spectrometry). Mycotoxin contamination was related to regional weather conditions (average temperatures and precipitation amount) witnessed during production, which can generally be characterized as warm and rainy, therefore increasing the risk of mould growth and mycotoxin production. Despite the absence of a statistically significant difference in the concentration of individual mycotoxins across the production regions, a difference in mycotoxin occurrence was found. The eastern (39 %) and the western (33 %) region, having a moderate climate, delivered the largest number of contaminated samples, while the southern region (9%), often compared to subtropics, delivered the smallest number of such samples, so that the determined mycotoxins were probably mainly produced by the *Penicillium* rather than the *Aspergillus* species. Due to the interaction of various factors that may affect mycotoxin biosynthesis during production, the detected concentrations cannot be solely related to the weather, indicating the necessity of further research on potential sources and mechanisms of contamination during all stages of TMP production and storage, which may lead to the occurrence of mycotoxins.

KEY WORDS: dry-cured meats; influence; mycotoxin contamination; regional weather; sausages

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Occurrence of *Aspergillus* series *Versicolores* in the built environment across USA – their growing significance as emerging pathogens

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Members of *Aspergillus* series *Versicolores* have been isolated from various environments and substrates, e.g., soil, indoor environments, various food and feeds, caves, hypersaline water, and associated with many health issues of humans and animals (e.g., onychomycosis, endophthalmitis, ear infection, invasive pulmonary infections, aspergilloma, homograft valve infection, endodontic infection, vaginitis). The majority members of this series have the potential to produce the mycotoxin sterigmatocystin, a carcinogenic and mutagenic precursor to aflatoxin B1. They are also believed to be contributors in some cases of Sick Building Syndrome. An increasing interest in the medical and environmental ramifications of this group is due to their common occurrence in the indoor environment. In this study, fungal samples were collected via settle plates from 17,673 homes (only one sample per home) across the USA for a period of 4 years (2015 to 2018). The main goal was to measure the frequency of *Aspergillus* species from *Aspergillus* series *Versicolores* in the built environment. The country (USA) was divided into 4 artificial regions: northwest (NW), northeast (NE), southwest (SW) and southeast (SE), to determine any differences in geographical distribution of fungi. It was discovered that depending on the year and region, the frequency of recovered *Aspergillus* species series *Versicolores* fluctuated from 17 to 38 %. On average, nearly one out of every four samples (25 %) taken from the West Coast of the United States have at least one or more members of *Aspergillus* series *Versicolores*, and one out of every three (30 %) samples taken from the East Coast. In contrast to other fungi, the distribution of *Aspergillus* series *Versicolores* showed very little fluctuation over the course of a year, averaging around 20 to 30 %.

KEY WORDS: carcinogenicity; human health issues; indoor fungi; mycotoxins; sterigmatocystin

Impact of global megatrends on the spread of microscopic fungi in the Pannonian Biogeographic Region

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Global megatrends are slow processes that are hardly noticeable initially, but later cause long-term global effects. The European Environmental Agency has set 11 global megatrends. Some of them – globalization, risk of pandemic, technological development and climate change – have major impacts on microfungi in a vulnerable region of Europe, the Pannonian Biogeographic Region (PBR). A total of 340 fungal isolates were collected in connection with the megatrends in the PBR. Based on our sampling and literature review, it has been shown that a significant amount of fungal strains enters this region through global trade, on imported products from subtropical and tropical regions, primarily fruits, spices and packaging materials. A total of 62.5 % of the sampled exotic fruits were infected with microscopic fungi. Most of the identified strains belonged to the genera *Fusarium* (51.9 %) and *Aspergillus* (7.4%). Countries that are currently similar to the future climate of a recipient country might pose the greatest invasion risk in the future. Spores originating from a different climate can survive in microenvironments with a climate similar to that of the source country. Several examples of these microenvironments were also investigated, e.g., greenhouses, inner space of household devices (washing machines, bottled water dispensers, air conditioners, etc.). Due to technological development, these fungi gain new habitats in the immediate surroundings of humans. The effects of predicted climate scenarios were also tested on fungi, being endemic or unintentionally introduced by global trade from regions of warm temperate climate. Common fungal species were selected for the study and exposed to heat waves during 7 days according to two climate scenarios: one moderately (RCP 4.5, T_{avg} = 27 °C, T_{max} = 35 °C) and one strongly pessimistic (RCP 8.5, T_{avg} = 30 °C, T_{max} = 40 °C) that include predictions for the Central Hungarian Region for July 2050. According to our results, *Aspergillus flavus*, *A. niger*, *A. tubingensis* and *Fusarium* strains introduced from tropical regions tolerated heat waves, unlike *Penicillium* and *Talaromyces* spp. and endemic *Cladosporium* spp. which were unable to grow under the RCP 8.5 treatment. The effects of global megatrends on fungi raise new issues of human, animal and plant diseases, also having economic and environmental perspectives. In this presentation, some intervention possibilities are presented.

KEY WORDS: climate change; dispersal of microscopic fungi; globalization; imported fungal strains; technological development

PoFMy Abstracts – invited lecturers
Arh Hig Rada Toksikol 2022;73:A24-A32

Distribution of mycotoxigenic moulds isolated from traditional meat products in different Croatian climate regions and their ability to produce mycotoxins

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Mycobiota grow on the surface of traditional meat products (TMP), which gives them a specific texture, flavour, and colour. However, in unfavourable conditions moulds produce mycotoxins if they possess the genes involved in their biosynthesis. The most toxic mycotoxins in TMP products are aflatoxin B1 (AFB) and ochratoxin A (OTA), while the incidence of cyclopiazonic (CPA) acid and citrinin (CIT) on meat products has until now not been researched. The aim of this study was to identify moulds species capable of producing the studied mycotoxins by proving the existence of the genes involved in their biosynthesis. This study analysed 250 TMPs sampled during 2020 and 2021 from five different climate regions of the Republic of Croatia (eastern, northern, central, western, and southern). The tested genes involved in the AFB1 biosynthesis are: *aflD* encoding norsolorinic acid reductase, *aflM* encoding versicolorin A dehydrogenase and *aflP* encoding sterigmatocystin-Omethyltransferase, OTA *otanpsPN* encoding OTA nonribosomal peptide synthetase and *otapks* encoding OTA polyketide synthase; CIT *pksCT* gene encoding CIT polyketide synthase, and CPA *dmaT* encoding dimethylalyl tryptophan synthase. A total of 181 isolates of 9 different potentially toxic mould species were identified, out of which 50 % of the identified isolates of potential AFB1 producers (*A. flavus*), 58 % isolates of potential OTA producers (*P. nordicum, A. niger, A. westerdijkiae, A. welwitschie, A. tubingensis*), 71 % of isolates of potential CIT producers (*P. citrinum*) and 48 % of isolates of potential CPA producers (*P. commune, A. flavus, P. polonicum*) all had the investigative genes involved in mycotoxin biosynthesis. In 21 % of the samples contaminated with some of the detected mycotoxins (OTA or CPA), their producers (*P. nordicum, P.commune* and *A. flavus*) were determined, while contamination of the samples in which the producers were not identified can be explained either by some previous contamination of TMP ingredients or by the possibility that the producers had been covered and became overgrown by other mould species at the end of the ripening stage and therefore avoided detection. The incidence of the number and identified mould species has been linked to data on the weather conditions relating to the various Croatian regions. Therefore, the western region had the highest number of isolated toxic mould species, followed by the northern region, while the greatest diversity of toxic mould species was in the western and eastern regions.

KEY WORDS: *Aspergillus*; dry-cured meat; mycotoxin biosynthesis; *Penicillium*; regional weather conditions

The challenge of studying the toxicity of mixtures: the example of mycotoxins

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Food is contaminated by multiple contaminants, mycotoxins being the most frequently occuring natural ones. Mycotoxins are secondary fungal metabolites produced mainly by *Aspergillus*, *Penicillium*, and *Fusarium*. Mycotoxin co-contamination is confirmed by the co-occurrence of these toxins in food and feed stuff and by co-exposure monitoring surveys. The co-occurrence of mycotoxins in food is explained by three different reasons: (i) most fungi are able to simultaneously produce several mycotoxins, (ii) commodities can be contaminated by several fungi simultaneously or in quick succession, and (iii) the complete diet comprises different commodities. In practice, the co-occurrence of mycotoxins represents the rule and not the exception. Besides mycotoxins, food can be contaminated with other contaminant such as heavy metals. Unfortunately, the toxicity of contaminant combinations cannot always be predicted based upon their individual toxicities. The data on the combined toxic effects of mycotoxins are limited and therefore the health risk from the exposure to a combination of mycotoxins is incomplete. Most studies concerning the toxicological effect of contaminants have been carried out taking into account only one compound. A synergistic effect between mycotoxins of the trichothecene familly mycotoxins was observed both for intestinal cytotoxicity and inflammatory response and the synergy was already seen at low doses. The combined exposure to the mycotoxin deoxynivalenol and Cadmium was also studied in several human cell lines and the interactions were specific to the target organ. The interaction between deoxynivalenol and genotoxins, present either as food contaminants or as toxins from our microbiota, was also investigated. We demonstrated that deoxynivalenol exacerbated the intestinal DNA damages induced by these toxins, suggesting a role in colorectal cancer. Altogether, these data demonstrated that mycotoxin cocktails can lead to synergistic interactions and that mycotoxin contamination should be taken into consideration in the global context of all food contaminants and the host intestinal microbiota.

KEY WORDS: co-exposure; cytotoxicity; food safety; heavy metals; monitoring

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Ochratoxin A and citrinin – partners in crime and cross-border traveling

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Ochratoxin A (OTA) and citrinin (CTN) are nephrotoxic mycotoxins commonly found together in grain. EU legislation regulates monitoring of OTA in various commodities; however, a similar monitoring obligation does not exist for CTN. With the aim of studying the effects of these mycotoxins on mammalian kidneys, adult male Wistar rats were subchronically (21 days) treated with individual and combined oral doses of OTA and CTN. Using spectrophotometry and HPLC methods, the oxidative stress parameters (malondialdehyde – MDA, glutathione – GSH and enzymes – superoxide dismutase, glutathione peroxidase and catalase) and concentrations of these mycotoxins were measured in rat kidneys. The results showed that concentrations of CTN in the kidneys of OTA + CTN treated animals were three up to six times higher in animals given CTN alone. Concentration of OTA were 1-3 μg/g tissue. Rats were also treated with the antioxidant resveratrol (RSV) in order to reduce oxidative stress but its prooxidative effects in some organs was noticed. This encouraged us to study the effects of OTA and CTN on proteins that facilitate their transport across basolateral and brush-border membranes of renal epithelial cells. The immunochemical analyses of rat organic anion and cation transporters (rOats and rOcts) revealed mycotoxin- and transporter-related changes, as well as differences between the individual and combined effects of the studied mycotoxins on protein expression. Physiologically relevant proteins such as sodium-glucose transporters 1 and 2, actin and Na^+/K^+-ATPase were generally not affected by the comparable mycotoxin doses. Finally, the combination of the powerful antioxidant resveratrol, OTA and CTN had a strong downregulating effect on almost all of the studied organic anion and cation transporters, exceeding the effects of individual and/or combined OTA and CTN high doses by even affecting the physiologically relevant cytoskeletal protein actin.

KEY WORDS: mycotoxins, organic anions; organic cations; oxidative stress; transporters

PoFMy Abstracts – invited lecturers
Arh Hig Rada Toksikol 2022;73:A24-A32

Anti-mycotoxigenic activity of South African medicinal plants

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Over time, phytopathogenic fungi have developed resistance to synthetic fungicides, which has led to the increased contamination of food commodities with mycotoxins. Additionally, synthetic fungicides have negative toxic effects to human being and animals. As a result, there is a need to search for and develop alternative inexpensive non-toxic biofungicides. Acetone extracts of 25 South African plants were prepared from leaves and tested for antioxidant, antifungal activity, and mycelial growth inhibition (MGI) effects against toxigenic strains of *Fusarium verticillioides, Aspergillus flavus,* and *Aspergillus ochraceous*. The extracts had varying degrees of anti-mycotoxigenic effects. *Markhamia obtusifolia* (Baker) Sprague had the lowest minimum inhibitory concentration (MIC) values as low as 0.08 mg/mL against *Aspergillus flavus* and *Furasium verticilloides* at both 24 and 48 hours incubation period, respectively, while *Curtisia dentata* exhibited similar MIC values against *Aspergillus ochraceous*. In the mycelial growth inhibition (MGI) evaluation, *Fusarium verticilloides* was more sensitive to plants extracts, which yielded inhibition percentages ranging from 37.29 to 47.17 %, while *Kirkia wilmsii* exhibited highest MGI of 50.08 % against *Fusarium verticilloides* at the 6th day of incubation. Based on antifungal activity and availability of plant material: *Curtisia dentata, Markhamia obtusifolia, Solanum aculeastrum, Strychnos mitis,* and *Zanthoxylum capense* extracts were subjected to gas chromatography mass spectrophotometry (GC-MS) analysis. *Curtisia dentata* revealed the presence of triterpenoinds such as β-amyrin (53.30 %), α-amyrin (6.42 %), β-sitosterol (2.47 %), and vitamin E (4.99 %), while *Markhamia obtusifolia* yielded the presence of neophytadiene (4.38 %), palmitic acid (3.61 %), and 4-(1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol (2.04 %). The results suggest that natural products from plants may be used as possible substitutes for synthetic fungicides. Given the antifungal and antioxidant potential of the selected plants, they may have potential as possible leads for the development of biofungicides that may prevent growth of mycotoxigenic fungi and oxidation related food spoilage.

KEY WORDS: antifungal; antioxidant activity; mycelial growth inhibition; mycotoxins; gas chromatography mass spectrophotometry

The possibility of modulation of patulin production by polyphenols from traditional Croatian traditional apple varieties within the project HRZZ-UIP-2020-02-8461

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The effects of climate change, which play an important role in shaping environmental factors and intensifying the secondary metabolism of agriculturally important/foodborne fungal species, have led to an unexpected risk of contamination of food and feed with mycotoxins, which have enormous and complex implications for food safety and, consequently, for human health and the global economy. Several fundamental issues are at stake, but HRZZ-UIP-2020-02-8461 is attempting to come at least one step closer to solving the overall problem of avoiding modulation of fungicide efficacy due to changing climatic factors and stress the adaptation mechanisms of fungal cells, primarily for *Penicillum expansum* growth and the consequential patulin production. Apples are among the most popular fruits, but they are the most frequently infested by *P. expansum* and contaminated with its secondary metabolites. Despite this, there are currently no satisfactory strategies to reduce patulin concentrations in apples and apple products. However, there are traditional apple varieties that contain a higher number of polyphenolic compounds compared to commercial apple varieties. These compounds are an active component of plant defence against stress conditions. In addition, traditional varieties have higher antioxidant activity and a gene profile that makes them more resistant to climatic conditions, plant diseases, and other forms of abiotic stress. This has led to the idea of developing polyphenol-based strategies to reduce patulin content in apples and apple products, both for the ongoing HRZZ-UIP-2020-02-8461 and future projects. This presentation will provide an opportunity to discuss our idea, present initial published results, and identify potential new ways to achieve the overall goal of diminishing negative impact of mycotoxins from a food and feed safety perspective.

KEY WORDS: oxidative status of the fungal cell; patulin; *Penicillium expansum*; polyphenols; traditional Croatian apple varieties

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Alternaria alternata, a source for harmful food contaminants or promising pharmaceutical scaffolds?

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Black molds of the genus *Alternaria* occur ubiquitously and are able to grow under varying temperature and moisture conditions, as well as on a large diversity of substrates. *Alternaria alternata* is known to generate a broad spectrum of secondary metabolites. Among these, alternariol (AOH), its monomethyl ether (AME), tentoxin, and tenuazonic acid represent the most studied ones, which is not least due to the commercial availability of the test compounds. AOH and AME possess genotoxic and mutagenic properties. However, in complex mixtures of *Alternaria* toxins, genotoxicity is often steered by the pattern of co-occurring altertoxins. Depending on the proportion of the different *Alternaria* toxins in naturally occurring mixtures, an intriguing overlay of biological effects are observed, ranging from immunomodulation and endocrine disruption to geno- and cytotoxicity. Today, *Alternaria* toxins are still seen as "emerging mycotoxins", but the first indicative values for AOH, AME, and TeA have recently been recommended by the European Commission. With respect to molecular mechanisms, secondary metabolites formed by *Alternaria alternata* might represent novel scaffolds for drug development. Cellular activities of certain promise for structural optimisation comprise, among others, interference with topoisomerases, inhibition of casein kinase, and suppression of inflammatory responses. Taken together, further studies are urgently needed to clarify the relevance of *Alternaria* mycotoxins for food safety, but some secondary metabolites formed by *Alternaria alternata* provide novel scaffolds which might be of interest for drug development.

KEY WORDS: alternariol; altertoxin; casein kinase; endocrine disruption; topoisomerase inhibition

PoFMy Abstracts – invited lecturers
Arh Hig Rada Toksikol 2022;73:A24-A32

The impact of climate change on the development of molds, mycotoxins, and quality of cereals with proposed measures – preliminary project results

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Since there is no systematic monitoring of the most important cereal crops in the Republic of Croatia, the Andrija Štampar Teaching Institute for Public Health, the Ministry of Environmental Protection and Energy, and the Fund for Environmental Protection and Energy Efficiency signed an agreement on the allocation of grants for the financing of the project "Investigation of the impact of climate change on the development of moulds, mycotoxins, and grain quality with proposed measures" (KK.05.1.1.02.0023), co-financed by the European Union from the European Fund for Regional Development. Partners in the project are the Faculty of Pharmacy and Biochemistry of the University of Zagreb and the Faculty of Food and Biotechnology of the University of Zagreb. In accordance with the project plan, during the two years of research, six rounds of sampling were conducted on three types of cereals (corn, barley, and wheat) from three different areas of Croatia: northern, eastern, and central Croatia. Sampling was carried out during growth in the field, immediately after harvest and after storage in warehouses and domestic conditions. All of the samples were analysed for the presence and characterization of moulds, mycotoxins (aflatoxin B1, total aflatoxins, OTA, ZON, DON, citrinin, patulin, fumonisins, and T-2, and HT-2), and selected quality parameters of cereal grains: nutritional parameters (energy value, water, ash, fats, saturated atty acids, carbohydrates, sugars, raw fibres, proteins, and salt), and selected minerals and vitamins, with important nutritional value from this source: Mg (magnesium), Zn (zinc), Fe (iron) and P (phosphorus), and well as vitamin B3 and vitamin B5. The preliminary results indicate that mould is present on all cereals, regardless of the area and time of sampling. It was found that the following types of moulds prevail: *Fusarium* spp.*, Alternaria* spp.*, Mucor* spp.*, Rhizopus* spp.*, Penicillium* spp.*, Cladosporium* spp.*, Trichoderma sp.*, as well as yeasts. In samples of corn, barley and wheat, the presence of a certain type of mycotoxin occurs sporadically. The results of this project will provide insight into the food safety of cereals and to establish an algorithm to assess the impact of climate change on the occurrence of moulds and mycotoxins, but also a possible decline in grain quality and their nutritional composition. Finally, we expect to find proper variety selection for individual climatic areas, as one of the essential factors for successful yield and safe production, just like possible pre- harvest, harvest, and storage measures. In conclusion, given that the EU recently established the latest Regulation Commission Regulation (EU) 2022/1370 of 5 August 2022 amending Regulation (EC) No 1881/2006 as regards the maximum levels of ochratoxin A in certain foodstuffs, the obtained results will be commented in the light of the new requirements of the cited Regulation.

KEY WORDS: crops; EU regulation; food safety; mycotoxins monitoring; storage.

Acknowledgement: The paper was made as part of the project funded by the European Regional Development Fund: MZOE – OPKK – KK.05.1.1.02.0023 - Research on the impact of climate change on the development of molds, mycotoxins and grain quality with proposed measures, led by Andrija Štampar Teaching Institute of Public Health, Zagreb, Croatia

Microfiltration affects mycotoxin recovery

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Frequent multi-mycotoxin occurrences in various foods, alongside adverse health effects and legislative requirements, demand constant and continuous monitoring to properly assess the level of mycotoxin contamination. Sensitive and unambiguous quantification of multiple analytes requires *state-of-the-art* analytics, such as liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). To protect the instrument from small particles while reducing the matrix effect, many LC-MS/MS methods employ membrane filtration during sample preparation. However, when membrane filtration is used, possible adsorptive effects at membrane filters should also be considered, as they could prevent the accurate determination of mycotoxin (co-)occurrence and the real risk assessment for public health. In this study, five different material types of syringe filters, i.e. nylon (NY), polytetrafluoroethylene (PTFE), polyethersulfone (PES), mixed cellulose ester (MCE), and cellulose acetate (CA), were used to assess the impact of microfiltration on mycotoxin recovery. All 11 of the legislatively regulated mycotoxins were selected for the investigation, including aflatoxins B1, B2, G1 and G2, deoxynivalenol, fumonisins B1 and B2, zearalenone, T-2 and HT-2 toxins, and ochratoxin A. The combined mycotoxin standard solution was prepared in concentrations 0.25-50 ng/mL using acetonitrile/water/formic acid (49.5/49.5/1, v/v/v). This multi-mycotoxin solution was filtrated using different syringe filters where the first three filtrate drops and the following filtrate were collected separately into vials and injected in triplicates into a LC-MS/MS system and analysed using a validated instrumental method. The recovery for each mycotoxin was calculated comparing the average area of the filtered and unfiltered standard solution and expressed in percentage. The results revealed that PTFE filters have the least impact on mycotoxin loss during filtration, while commonly used NY filters could affect the recoveries of certain mycotoxins if not properly used. A decrease in the recoveries of almost all mycotoxins was observed in the first three nylon filter drops, especially for aflatoxin B1 and zearalenone (up to 30 %), while the following filtrate contained a satisfying amount of all of the tested mycotoxins, when compared to the unfiltered solution. Therefore, for accurate mycotoxin determination, the first few filtrate drops should be discarded, as they saturate membrane filter materials, once again pointing to the importance of a proper method development procedure.

KEY WORDS: liquid chromatography-tandem mass spectrometry; multi-mycotoxins; public health; risk assessment; sample preparation

Moniliformin in maize: is there a non-contaminated sample?

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Emerging *Fusarium* mycotoxins are gaining increasing scientific interest due to their frequent contamination of food and feed, although toxicity and toxicokinetics data are limited. Moniliformin (MON) is an emerging mycotoxin produced by many *Fusarium* species (mainly F. *subglutinans, F. avenaceum, F. temperatum, F. proliferatum,* and *F. verticillioides*). In addition, one *Penicillium* species (*P. melanoconidium*) is also able to produce MON. It is characterized by worldwide distribution and has mainly been detected in cereals, such as maize, wheat, barley and oats, as well as in their derived products. The potential health hazards of MON are cardiotoxicity, haematotoxicity, and respiratory distress, but legal maximum levels have not been regulated for it in food and feed. However, in 2018, the European Food Safety Authority (EFSA) published a scientific opinion on the risks to human and animal health related to the presence of MON in food and feed, but the lack of relevant toxicity data prevented a risk assessment. Therefore, the main aim of this study was based on one of the EFSA recommendations, which is to collect more occurrence data on MON in food and feed. Given that maize is an important crop in the Republic of Serbia due to its agricultural and economic contributions, and that the literature data indicates that the highest frequency and concentrations of MON were detected in maize compared to other crops, maize was chosen as the matrix. The analysis of samples collected from the main maize production areas in the Republic of Serbia during the decade showed that it was difficult to find a sample without MON. Furthermore, the results showed that the detected concentration levels of MON in maize samples were influenced by different weather conditions observed during the investigated years. Based on the above stated, it can be concluded that MON is a dominant, non-regulated emerging mycotoxin in maize from the Republic of Serbia. On the other hand, there are limited data from neighbouring countries related to the occurrence of MON in maize collected from different years. Therefore, for a better estimate of the agronomic and weather conditions suitable for MON production, more data on its occurrence in maize grown in European countries is needed. Additional studies are also needed in order to obtain more information on its toxicity and thus enable a comprehensive risk assessment for humans and animals and the justification for the introduction of MON into the Regulation.

KEY WORDS: emerging mycotoxins; feed contamination; Fusarium species; Republic of Serbia; weather conditions.

Acknowledgement: This paper is a result of the research funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/200222).

Chronic dietary exposure assessment to ergot alkaloids in Croatian adults

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Ergot alkaloids (EAs) are mycotoxins produced by several species of fungi in the genus *Claviceps*. It commonly affects cereals such as rye, wheat, triticale, barley, millets, and oats. The toxicity of EAs is well known and has been characterised. A group tolerable daily intake (TDI) of 0.6 μg/kg bw per day was derived for the sum of the EAs. The food samples from monitoring program collected between 2015 and 2019 were available for chronic dietary exposure assessment. The analytical results included the concentrations sum of 12 main EAs: ergometrine, ergometrinine, ergosine, ergosinine, ergocornine, ergocorninine, ergotamine, ergotaminine, ergocristine, ergocristinine, ergocryptine (α- and β-isomers), and ergocryptinine (α- and β-isomers). Samples were divided into food groups according to the EFSA FoodEx classification. For each food group, results were taken only for samples for which there was data in the food consumption database. Data on consumption were obtained from the National Survey on Food Consumption of the Adult Population in Croatia, conducted in 2011-2012, on a representative sample of 2002 respondents. From total number of respondents who participated in the study, 1,996 consumers with reported consumption of cereal grain products were taken into account for the purpose of exposure assessment to EAs. For adults, the mean lower bound (LB) exposure to EAs was 0.0059 μg/kg bw per day, mean middle bound (MB) exposure was 0.0131 μg/kg bw per day, and the mean upper bound (UB) exposure was 0.0203 μg/kg bw per day. The LB P95 exposure to EAs was 0.013 μg/kg bw per day, MB P95 exposure was 0.027 μg/kg bw per day, and UB P95 exposure to EAs was 0.041 μg/kg bw per day. Overall, the „Grains and grain-based products" food category is the only contributor to the MB mean chronic dietary exposure to EAs. The main subcategories contributed of given food category were „Bread and rolls" with 75.4 % and „Grain milling products" with 18.1 % followed by „Breakfast cereals" (4.1 %) and „Grains for human consumption" (2.5 %). Neither the average chronic dietary exposure nor the chronic dietary exposure to the sum of EAs at the 95th percentile exceeded the established TDI value in the LB, MB, and UB approach.

KEY WORDS: *Claviceps* genus; food safety; monitoring; mycotoxins; TDI

A bet is open: who would win a fight between phytophatogenic fungi and plant-promoting bacteria?

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The increasing occurrence of extreme events has made climate change one of the most challenging problems in today's world with great economic consequences for the fragile agricultural sector. The agricultural sector, and thus crop productivity, quality, and price stability, are threatened by warming temperatures, floods, intense heat, drought, wildfires, and storms. As a result, researchers and policy makers are focusing on developing novel approaches to adapt to a rapidly changing climate. In the project entitled "Potential of the rhizosphere microbiome in the adaptation of agriculture to climate change (PERSPIRE)", funded by the EU Regional Development Fund, we focused our research on the effects of flooding events (water retention after heavy rains) as a result of climate change on the development of plants and the response of their rhizospheric microbiome, on whose "health" plants are tightly dependent. The experiment was conducted in a controlled plant-growth chamber (16 hours day/8 hours night; 25 °C per day/20 °C per night; 60-70 % relative humidity), using cabbage *(Brassica oleracea* var. *capitata* f. *alba*) as a model plant. In the experiment, that lasted 57 days from sowing, plants were exposed to either one (72 h duration) or two short-term floods (72 h duration, 10 days recovery between floods) at different stages of development. At different time points (day 0, after flooding, and after the recovery period), all of the soil was removed from the pots, thoroughly mixed, and subsamples were collected for culture-based isolation and characterization of PGPB (plant growth-promoting bacteria). PGPB were isolated by inoculation on non-selective nutrient agar and incubation at 37 °C (5 days). Approximately 20 morphologically distinct bacterial colonies were selected from each of the sampling points, purified and analyzed for various PGP characteristics using culturable methods on different selective media. A total of 48 bacterial isolates (*Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Peribacillus*, *Enterobacter* etc.) exhibiting most PGP properties, were further selected for testing antagonistic/synergistic activity against the mycelia of three selected phytopathogenic fungi (*Fusarium oxysporum*, *Fusarium verticilloides,* and *Sclerotinia sclerotiorum*). Sequencing of 16S rRNA and ITS2 marker genes was used to determine the identity of both bacteria and fungi used in the experiment. The bacterial suspension (adjusted to a concentration of 1.5 McFarland) was inoculated onto paper discs placed on the rim of the Petri dish containing potato dextrose agar. Bacterial cultures were grown for 72 hours, then a mycelial agar plug was added to the center of the agar plate and the plates were incubated at 27 °C for 5 days. The growth of both bacteria and mycelia was recorded each day. Different bacterial isolates showed variable percent inhibition of fungal mycelia. However, a total of six bacterial isolates isolated exclusively from flooded samples showed strong antagonistic activity against mycelia. We believe these bacteria adapted to flooding conditions and could be potential bioinoculums used in the biocontrol of pathogenic fungi in crops affected by flooding as a consequence of climate change.

KEY WORDS: climate change; floods; fungi; PGPB; phytophatogens

Mycotoxins and their impact on male infertility

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Epidemiological studies have shown that there is a global decline in male fertility primarily as a result of poor sperm quality, which could be attributed to exposure to endocrine disrupting chemicals present in the environment, food, and pharmaceutical products, including mycotoxins and pesticides. Fungi are eukaryotic microorganisms that cause major spoilage of fresh food and feedstuffs. Mycotoxins are chemical contaminants produced by toxigenic fungi. They are capable of causing severely acute or chronic effects to humans. Studies using animal and cell models indicate that zearalenone, deoxynivalenol, ochratoxin A, and aflatoxin B1 can adversely affect fertility. They may also induce oxidative stress resulting in sperm DNA damage and, subsequently, reducing fertilisation rates and lower embryo quality. Due to their chemical stability, mycotoxins may be present in food when fungi are no longer present. From a food control point of view, physical decontamination methods are considered to be preferable; however, chemical techniques have also been adopted. Electrolyzed oxidizing water (EOW) is a chemical product that has three main physiochemical properties: available chlorine concentration, oxidation-reduction potential, and pH. It offers many advantages over other conventional chemical methods, including less adverse chemical residues, safe-handling, secure, energy-saving, cost-effective, and environmentally-friendly methods. It could be used for the development of safer and more socially acceptable methods for fungi decontamination and mycotoxin detoxification. Extensive research on the development of novel packaging technologies can be applied to control the growth of fungi and mycotoxin in food products. Hermetic storage packaging technology help in the mitigation of fungi and mycotoxin in agricultural products. Active food packaging ensures food safety by preventing contamination and proliferation of fungi. Antimicrobial packaging is more effective than the direct application of antimicrobial chemicals to food. The antimicrobial effect of essential oil, polypeptides, chitosan, natamycin, and the nanoparticle is extensively exploited in developing antifungal film and coating. Plant-derived components to prevent mycotoxin contamination are a viable alternative that has been thoroughly researched. Nanotechnology can provide green and environmental-friendly alternatives to manage fungal and mycotoxin contamination.

KEY WORDS: antimicrobial effects; endocrine disruptor; fungi; poor sperm quality; sperm DNA damage

Lactic acid bacteria in biological detoxification of mycotoxins in flours and bread

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Flours and bread are products highly prone to mycotoxin contamination since mycotoxin producing filamentous fungi may contaminate them during all stages of their production. Aflatoxins (AF), fumonisins (FUM), ochratoxin A (OTA), zerealenone (ZEN), deoxynivalenon (DON), ergot, T2, and HT2 are mycotoxins that most commonly occur in this ecological niche. Food outbreaks related to mycotoxin contamination of cereals and their products are well familiar in the past. Their presence in food is related to serious health problems such as cancer and mycotoxicosis. Therefore, mycotoxin free food is of utmost importance for public health. A strong societal demand of less processed, more natural, and safe food imposes the use of biodetoxification as safer and reliable method of the chemical preservation. Numerous studies have shown the potential of lactic acid bacteria (LAB) such as *Lacticaseibacillus rhamnosus, L. plantarum, L. fermentum*, and the yeast *Saccharomyces cerevisiae* as aflatoxin decontaminating agents. They possess high mycotoxin binding abilities and can be used as part of the starter cultures in the fermentation of food or as additives in small quantities without compromising the characteristics of the final product. The review summarizes the occurrence of mycotoxins in flours and bread and detoxification activities of lactic acid bacteria (LAB), their mechanisms of mycotoxin detoxification, and the inherent and environmental factors affecting the detoxifying properties of LAB.

KEY WORDS: cereals; decontamination; filamentous fungi; food safety; *Saccharomyces cerevisiae*

Mycoviruses: A novel approach for controlling mushroom pathogenic fungi

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The global production of button mushrooms (*Agaricus bisporus*) is severely affected by dry bubbles, caused by the filamentous fungus *Lecanicillium fungicola* (Cordycipitaceae, Hypocreales, Ascomycota). The number of agrochemicals approved in mushroom cultivation for disease control purposes is highly limited; however, numerous mycoviruses are known to induce hypovirulence in the range of plant pathogenic fungi. Therefore, the aim of our study is to examine viruses in isolates of *L. fungicola*, focusing on the possibilities of biological control. Based on their ITS (internal transcribed spacer) sequences, the majority of strains, originating from diseased mushrooms in different countries, were confirmed as *L. fungicola*. In addition, strains representing different *Akanthomyces* and *Simplicillium* species were also detected, which are mainly known as insect and nematode-pathogens. These species are genetically closely related to fungi possessing the ability to produce different mycotoxins, therefore, despite the lack of data, their toxin-producing potential cannot be ruled out. Out of 60 mushroom pathogenic strains, a total of 10 *Lecanicillium* and *Akanthomyces* spp. isolates were shown by cellulose column chromatography to harbour double stranded (ds) RNA elements with different banding patterns, indicating RNA virus infection. The results of fruit body infection and dual plate assays support the hypothesis of a potential association between the presence of mycoviruses and moderate virulence of the pathogens. According to preliminary next-generation sequencing (NGS) data, in agreement with the results of dsRNA gel analysis, viruses detected in a group of dsRNA-carrying fungal strains were found to be similar to the recently described chrysovirus with a tetra-segmented dsRNA genome from the insect pathogenic fungus *Isaria javanica*, also belonging to the family Cordycipitaceae. Our findings suggest that (1) certain insect pathogenic fungi can also cause dry bubble symptoms in mushroom cultivation, and (2) mycoviruses might be promising candidates for studies aiming at biological or integrated pest management.

KEY WORDS: dry bubble disease; *Lecanicillium fungicola*; mushroom cultivation; mycotoxins

Acknowledgement: Lorant Hatvani has received funding from the Research Leaders 2025 programme co-funded by Teagasc and the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement number 754380.

PoFMy Abstracts – oral presentations
Arh Hig Rada Toksikol 2022;73:A33-A38

Mycotoxin contamination in the Arab world: Highlighting the main knowledge gaps and discussing the proposed legislations to enhance food safety

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Mycotoxins are among the most alarming food and feed contaminants threatening human and animal health. Since the discovery of aflatoxins in the 1960s, our knowledge about mycotoxins increased dramatically: biological syntheses have been unravelled; myriad analytical detection methods were developed; surveys in different matrices were conducted; investigations into toxicities took place; several methods for control have been tested; and legislative debates to protect the public from their toxic effects continue. Based on that, hundreds of review articles were published to summarize different areas in the mycotoxin field, including their contamination per country or per region. However, mycotoxin contamination in the Arab world, which includes 22 countries in Africa and Asia, have not been specifically reviewed. To this end, the present work has been conducted to review the contamination of mycotoxins in the Arab world to identify the main knowledge gaps needed to enhance the safety of food and feed. Our search, using several keywords, covered all the (non-)indexed publications written in English or Arabic or French. To the best of our knowledge, a total number of 306 papers were published between 1977 and 2021, focusing on the natural occurrence of mycotoxins in different matrices, which fall under one of 14 different categories (animal feed; animal products; baby food; cereals; cereal by-products; dairy products; legumes and pulses; nuts; spices; fruits and vegetables; juices and drinks; biomarkers; oils; and others). The relevant information (e.g., detected mycotoxins; number of samples; concentrations; method of detection) were extracted, processed and visualized using Microsoft Excel, R, GraphPad Prism, and RAWGraphs. The main results are presented as follows: i) research on mycotoxins has increased over the years, however the accumulated data on their occurrences are scarce to non-existent in some countries and regions; ii) state-of-the-art technologies on mycotoxin detection are not well implemented; neither are contemporary multi-mycotoxin detection strategies in the Arab world, showing the need for capacity-building initiatives; iii) mycotoxin profiles differ among food and feed categories, as well as between human biofluids. An overview will be presented relying on visualization for an easy take home message delivery. Furthermore, the current legislations in the Arab countries will be discussed in relation to the occurrence of mycotoxins in the Arab world.

KEY WORDS: analytical detection methods; concentration; exposure; food and feed contamination; toxicity.

Acknowledgement: The authors thank the Egypt Scholars organization, which is an independent, non-profit organization founded on the principle of volunteerism.

Employment of the QuEChERS/dSPE extraction procedure for sterigmatocystin (STC) and 5-methoxysterigmatocystin (5-M-STC) from beer and their detection by TLC

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Sterigmatocystin (STC) and 5-methoxysterigmatocystin (5-M-STC) are structurally related mycotoxins with cytotoxic and genotoxic properties. They are predominantly produced by fungi of the genus *Aspergillus*, series *Versicolores*. The presence of these mycotoxins was investigated in indoor environments, while the data regarding food products remains insufficient, especially for 5-M-STC. Given the high consumption of beer in Croatia and the world, the presence of STC and 5-M-STC in beer may negatively affect the health of exposed individuals. Thus, 58 different samples of beer were collected from the Croatian market, extracted by combining the QuEChERS (Quick Easy Cheap Effective Rugged Safe) extraction procedure and dispersive solid phase extraction (dSPE). The prepared extracts were analysed by thin-layer chromatography (TLC) using silica gel 60 as a stationary phase and toulen:ethyl acetate:formic acid 90 vol. % (5:4:1) as a mobile phase. Following chromatographic separation, to enhance the detection of the fluorescent spots assigned to STC and 5-M-STC, the TLC plates were dried and heated for 10 minutes in an oven at 140 °C. Additionally, the TLC plates were sprayed with aluminium chloride solution prepared in ethanol 60 vol. %. The limit of detection (LOD) was 1.3 µg/mL for STC and 3.2 µg/mL for 5-M-STC. While STC was not detected in any of the samples, 5-M-STC was detected in 46.55% of the samples. The presence of 5-M-STC was confirmed by UV-Vis spectroscopy following the extraction of the spot of interest from the TLC plate. Further analysis of the same extracts by application of more sensitive chromatographic techniques will allow for a more accurate interpretation of these observations, allow the detection of STC and 5-M-STC in the concentrations below the LOD, and enable us to quantify both metabolites.

KEY WORDS: chromatographic separation; exposure; health effects; mycotoxins; toxicity

Fumonisins B1 and B2 in maize from the Republic of Serbia over ten years

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Fumonisin (FUMs) mycotoxins present a group of hydrophilic secondary metabolites of *Fusarium* and *Aspergillus* fungal species. Food and feed contamination by FUMs is an unavoidable issue worldwide, due to their toxic effect on human and animal health. Epidemiological research has revealed that in high-exposure populations, FUMs are associated with oesophageal cancer, primary liver cancer, neural tube defects, and cardiovascular diseases. FUMs can be divided into four major groups: fumonisin A, B, C and P series. In terms of toxicity and occurrence frequency, the most abundant and toxic FUMs analogue is fumonisin B1 (FB1), which contributes to approximately 70 % of FUMs and is one of the most common mycotoxins contaminating feed and food. According to the International Agency for Research on Cancer (IARC), FB1 has been classified as a Group 2B potential carcinogenic to humans. According to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), FUMs most often occur in maize and its products. Therefore, although maize contains quality nutrients such as vitamins A, B, and E, and many minerals, which contribute significant health benefits to the human body, there is a need for continuous monitoring and analysis of FUMs in maize and its products. Maize presents one of the most prevalent cereal crops in the world and forms a staple food in many countries, including Serbia, therefore it should be completely health-safety. In Serbia, maize covers about 35 % of the total planted area of field crops. The occurrence of FB1 and fumonisin B2 (FB2) was investigated in Serbian maize samples collected during ten years. Furthermore, the second aim was to examine the influence of weather conditions on their concentration in the investigated maize samples. The obtained results showed that a high occurrence of FB1 and FB2 was detected in maize samples from each of the examined years, regardless of the different weather conditions recorded in the examined ten years. With regard to this issue, it could be considered that the Republic of Serbia may become susceptible to problems concerning FUMs in maize, which is why there is a constant need for monitoring and determination of FUMs in maize.

KEY WORDS: Fusarium species; human and animal exposure; monitoring; mycotoxins contamination; weather conditions.

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

Natural early and mid-senescence and agronomical traits in wheat at two locations

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Wheat is one of the main cereal crops for ensuring food security for the 21st century. Thus, the increase of grain yield is a major target for breeders, where insights into wheat productivity can be achieved by studying the activity of components of the photosynthetic apparatus, such as chlorophyll fluorescence kinetics. The present study was conducted in a set of three winter wheat varieties (*Triticum aestivum* L.) (Tika Taka, El Nino, and Vulkan) at two experimental sites in the vegetation season 2019/2020. Chlorophyll fluorescence measurements were used to evaluate the onset and rate of the flag leaf and head senescence every few days, beginning at the heading stage (0 day after heading, DAH) until mid-senescence stage (26 DAH) on field-grown varieties with similar grain yield capacity and maturation time. The objectives of this study were to evaluate the agronomical performance of wheat varieties and to test photosynthetic efficiency across different environments. Consequently, this research focused on how *Alternaria* and *Fusarium* infections affected chlorophyll *a* parameters along with grain yield. The analysis of variance demonstrated significant effects of locations on grain yield and its components**.** Tika Taka exhibited the highest yield reduction (27.1 %) at Tovarnik, compared to Osijek, followed by El Nino (20.5 %) and Vulkan (18.7 %). Apart from significant differences for locations, 1000 kernel weight showed significant differences for varieties. Although there were not any significant differences in the grain yield between varieties, higher reductions over locations could have been due to earlier senescence and contracted grain fill duration at location Tovarnik due to prolonged wet conditions, which provoked natural infection by *Fusarium* or *Alternaria* species at the grain filling stage. This is supported by the evidence that at Tovarnik there were increased amounts of tentoxin, deoxynivalenol (DON), don-3-glucoside (D3G), 3-acetyldeoxynivalenol (3-ADON), zearelenone (ZEN), culmorin and 15-hydroxyculmorin and therefore the energy fluxes ratios ABS/RC, TR_0/RC, ET_0/RC, DI_0/RC increased in effects of dual-stress. Opposite to that, at location Osijek, 'staygreen' traits positively affected grain size, and thus overall grain yield, through grain fill maintenance.

KEY WORDS: *Alternaria*; chlorophyll; *Fusarium*; grain yield; photosynthetic efficiency

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Overview on mycotoxin contamination in Iranian foods

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Mycotoxins are fungal secondary metabolites produced by numerous fungal species (e.g., *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* species). The inevitable mycotoxin contamination in food and feed has been recognized as a serious global issue for human and animal health. Human and animal exposure to mycotoxin in developing countries, like Iran, is found to be more vulnerable as regulatory implementations are not being perfectly followed due to several factors such as the ignorance of manufacturers and high expense of food laboratory control. In the current work, a literature review has been conducted on mycotoxin contamination in Iranian food, feed, and human biological fluids covering the last 20 years (2002 to 2022). The collected data included commodity type, mycotoxin type, number of samples, region/province, method of detection, and the detected concentrations. These data were processed and analysed to provide a summary of the incidence and contamination levels of foodborne mycotoxins in Iran. Although data on aflatoxin M1 (in dairy products such as milk), aflatoxin B1, and total aflatoxins (in nuts, dried fruits, and cereals), ochratoxin A (in dried fruits), and patulin (in juices) exist, data on other main mycotoxins are scarce to non-existent. For emerging mycotoxins, there is still a need to conduct survey studies to profile their presence in Iranian food and feed. The impact of climate change on toxigenic fungi and mycotoxins must also be investigated to endure the safety of exported as well as local marketed food in Iran.

KEY WORDS: aflatoxins; climate change; mycotoxins exposure; food safety; ochratoxin A

Mycotoxin levels in maize grown on different conservation soil tillage systems

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As climate change (CC) is recognised as one of the main threats to food and feed security and safety, each concerned and interested party is working on finding the most elegant solution. The expected impact of CC on the presence of mycotoxins in food and feed is of great concern. One of the most adaptable and applicable platforms to combat climate change on a global level is conservation agriculture (CA). In 2021, an experiment at two different locations (eastern and western Croatia) was set up with different conservation tillage treatments (as part of the three main pillars of CA). One of the main goals of this project was to determine the influence of different conservation soil tillage treatments (in comparison with conventional) on the occurrence intensity of different types of mycotoxins (maize in 2021 year). Soil and maize were analysed and mycotoxin profiles were obtained. As expected, certain regulated mycotoxins were prevalent in maize, while emerging mycotoxins, *Aspergillus* and *Alternaria* metabolites had higher occurrence and concentrations in soil. The effect of tillage treatment showed differences in the concentrations of mycotoxins in both soil and maize, where conservation soil tillage treatment showed reduced mycotoxins concentrations. Only one sample exceeded the concentrations regulated for fumonisins, while other samples had all mycotoxin levels within legal limits.

KEY WORDS: *Alternaria; Aspergillus*; climate change; food and feed safety; mycotoxin detection.

Acknowledgement: This work has been fully supported by the Croatian Science Foundation under project "Assessment of conservation soil tillage as advanced methods for crop production and prevention of soil degradation" (IP-2020-02-2647).

Oxidative stress markers measured in the kidney of male Wistar rats treated with sterigmatocystin

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Sterigmatocystin is a mycotoxin structurally similar to aflatoxin B1 and a precursor to its biosynthesis. It is synthesized by 55 different species of mould that belong to phylogenetically and ecologically diverse genera. Sterygmatocistin can be found in various foodstuffs, even after processing, as well as in indoor humid spaces and dust. The LD_{50} for oral exposure is 160 mg/kg b.w. The mechanism of action is associated with the formation of exo-epoxides and the formation of DNA adducts. Therefore, the assumed mechanism of action is related to the occurrence of oxidative stress, which can have a great impact on health. This study investigates the role of sterigmatocystin in the generation of oxidative stress in the kidneys of male Wistar rats. Rats were treated once with steigmatocystin at three different doses: 1/4, 1/8, and 1/16 LD_{50} (40, 20, and 10 mg/kg b.w.). Parameters of oxidative stress that have been measured are activities of the enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), the concentration of reduced glutathione (GSH) and malondialdehyde (MDA). Controls included solvent control corn oil and water. When compared to the controls, a statistically significant difference was observed in MDA concentration and SOD activity. The difference was observed for all three concentrations in MDA and 1/16 and 1/4 treatments in SOD. A significant difference in the activity of CAT and GPx and concentration of GSH was not noted in kidneys, although CAT showed an increasing and GPx decreasing trend. The results showed that there was a high occurrence of oxidative processes (SOD) and tissue damage (MDA) in the kidneys and that they were sensitive to the occurrence of oxidative stress caused by sterigmatocystin. However, further studies are needed to discover the mechanism of sterigmatocystin toxicity.

KEY WORDS: antioxidative enzymes; *in vivo* study; malondialdehyde; mycotoxins; nephrotoxicity

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Arh Hig Rada Toksikol 2022;73:A39-A44

Biodiversity of Fusaria on crops from different regions of Croatia

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Mycotoxin-producing fungi and opportunistic pathogens endanger food safety, as 25–50 % of crops harvested worldwide are contaminated with mycotoxins. Thus, the purpose of this study was to explore the biodiversity of moulds in crops (corn, wheat, and barley) collected during the vegetation period (July of 2020) in northern, central, and eastern Croatia. From each of the samples (n = 45), the moulds were isolated on Dichloran Rose Bengal Chloramphenicol (DRBC) agar and Dichloran-glycerol (18 %) (DG18) agar following the peptone broth dilution method. Mean mould concentration (CFU/g) was obtained based on the number of colonies grown after 5 days at 25 °C, and identification to the genus level was based on their macro- and micromorphology. Because the fungi from the genus *Fusarium* are prevailing plant pathogens and mycotoxins-producing moulds, the specific goal of this study was to identify the isolates assigned to the genus *Fusarium* to the species level by comparing their partial sequences of internal transcribed spacer (ITS) and elongation factor alpha (*TEF-1a*) with those in the FUSARIOID-ID database (available at: https://www.fusarium.org/). Higher concentrations of moulds (CFU/g) were found in samples from eastern (1.17×10^5 on DG18 and 2.11×10^5 on DRBC agar) and central part of Croatia (1.11×10^5 on DG18 and 9.78×10^4 on DRBC agar) in comparison to northern Croatia (5.53×10^4 on DG18 and 7.46×10^4 on DRBC agar). Moulds from the genus *Cladosporium* prevailed in all crops and the *Fusarium* species were the second most frequently detected in barley collected at all of the sampling locations. Other moulds belonged to *Alternaria*, *Phoma, Penicillium*, *Mucor,* and *Epicoccum*. The collected *Fusarium* isolates (n = 15) were resolved into nine different species: *F. aethiopicum* (1/15), *F. annulatum* (4/15), *F. cerealis* (1/15), *F. graminearum* (2/15), *F. proliferatum* (1/15), *F. secorum* (3/15), *F. sporotrichioides* (1/15), *F. subglutinans* (1/15), and *F. verticilioides* (1/15). Majority of detected Fusaria (*F. annulatum, F. proliferatum, F. subglutinans, F. secorum* and *F. verticillioides*) belong to the *F. fujikuroi* species complex and were dominant in cereals from Eastern Croatia. Detecting and identifying different moulds can be of great importance in tracking the health of a plant population, as well as for controlling the safety of feed and food and ultimately in monitoring and preserving the health of our whole eco-system.

KEY WORDS: CFU; ITS; monitoring; moulds in crops; TEF-1α.

Acknowledgements: This work was partially financially supported by the European Fund for Regional Development under project "Investigation of the impact of climate change on the development of molds, mycotoxins and grain quality with proposed measures" (KK.05.1.1.02.0023).

Determination of ethanol concentration during cultivation of selected wine yeast strains in the presence of ochratoxin A

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In the mid-19th century, the role of yeast in alcoholic fermentation and thus in converting grapes into wine was proven. The quantity and good condition of yeasts for wine production is a matter of great importance, which can be disrupted by the presence of certain compounds such as mycotoxins. These compounds, e.g. ochratoxin A (OTA), can affect the fermentation process and physiological state of the yeast. The aim of this work was to examine the influence of two concentrations of OTA (2 and 4 μg/mL) on the ethanol production ability of selected wine yeast strains (*Saccharomyces cerevisiae* 5, *S. uvarum*, *S. bayanus*, *S. cerevisiae* DSMZ, *Kluyveromyces marxianus*, *Hanseniaspora uvarum,* and *Pichia guilliermondii*). To determine ethanol, ultra-high performance liquid chromatography (UPLC) with an RID detector was used and the data were processed using the response surface methodology (RSM). After 12 hours of cultivation, significant concentrations of ethanol were detected only in samples with yeasts *S. bayanus* (4.375-4.525 mg/mL) and *S. cerevisiae* DSMZ (9.150-9.210 mg/mL). Although it was observed that both of the investigated concentrations of OTA affected the fermentation activity of yeasts, the addition of OTA had no significant effect on ethanol production. However, the results suggested that the addition of 2 or 4 μg/mL of OTA in samples with *S. uvarum* had a positive effect on ethanol production, as an ethanol concentration of only 0.288 mg/mL was detected in the control sample, and in the samples with added OTA 0.62 and 0.58 mg/mL, respectively. After 24 hours of cultivation, as expected, higher concentrations of ethanol were detected. The obtained results clearly indicated that yeast strain and cultivation time had a significant influence on the synthesis of ethanol independently of the addition of OTA. The highest ethanol concentrations were measured in samples with the yeasts *S. bayanus* (6.87-7.05 mg/mL), *S. cerevisiae* DSMZ (6.275-6.715 mg/mL), and *H. uvarum* (7.365-7.49 mg/mL). In the tested samples, except for the samples with *P. guilliermondii*, there was no significant difference in the measured ethanol concentration between the control sample and the samples with added OTA. Therefore, the obtained results indicate that the tested yeasts were able to adapt to the presence of OTA and carry out metabolic processes relatively undisturbed.

KEY WORDS: alcoholic fermentation; mycotoxins; RSM; UPLC, wine production

PoFMy Abstracts – poster presentations
Arh Hig Rada Toksikol 2022;73:A39-A44

Climatic effects on aflatoxin-producing fungi and aflatoxin contamination

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Aflatoxin-type strong mycotoxins impair development, the immune system, and cause cancer. Due to regulations designed to protect human exposure, aflatoxin contamination of crops causes significant economic loss for growers, marketers, and processors of various sensitive commodities. When specific strains of the Aspergillus fungus infect crops, aflatoxin contamination occurs. Many industries who routinely deal with aflatoxin contamination are aware that climate variations have an effect on the level of contamination based on personal experience and anecdotal evidence. The fungi that produce contamination are directly impacted by climate, which in turn effects contamination to an extent. As the climate changes, so do the complex communities of mushrooms that produce aflatoxins. The composition of the microbial community and the number of organisms that produce aflatoxins in the environment are both subject to change. Because it affects how crops grow and how insects inflict wounds where aflatoxin-producing organisms can grow, climate change also impacts how susceptible hosts are to contamination. Aflatoxin contamination can occur in both sweltering, irrigated deserts and warm, humid regions. In temperate regions, pollution can reach dangerous levels during droughts. In most cases, the contamination process consists of two stages, the first of which affects the crop while it is still in the development stage and the second of which impacts the crop once it has reached maturity. With warm, wet conditions favoring the second phase and dry, hot conditions favoring the first, rain and temperature have different effects on the stages. Contamination is impacted by climate both regionally and temporally. Geostatistics and multiple regression analysis have been used to discuss how the weather affects pollution. Geostatistical investigations have linked environmental traits to recurrent contamination patterns. The procedure pinpoints the elements of the environment that have the greatest impact on pollution. Similar results can be obtained via multiple regression analyses, which let you rank environmental factors based on how much of an impact they have on pollution. Understanding the effects of the climate may enable the development of better management strategies, the more efficient use of monitoring resources, and the modification of agronomic practices in advance of global climate change.

KEY WORDS: biomonitoring; carcinogenic mycotoxin; climate change; crops; pollution