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Mycology
Mycotoxicology
Mycoses



BOOK of ABSTRACTS

Matica Srpska · 8–9 October 2026 · Novi Sad · Serbia
Hybrid Conference · In-person & Online via Zoom

8th International Conference
Mycology
Mycotoxicology
Mycoses

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MYCOLOGY, MYCOTOXICOLOGY, AND MYCOSES

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ORAL PRESENTATION

MYCOTOXICOLOGY

PLENARY LECTURE

MYCOTOXINS AND BEYOND: *IN VITRO* HIGH-THROUGHPUT TOXICITY ASSESSMENT OF MICROBIAL TOXINS AND MICRO-AND NANOPLASTICS

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ABSTRACT

Mycotoxins remain among the most relevant natural contaminants of food and feed, while human exposure increasingly occurs in a complex environment involving mixtures of toxins and emerging contaminants. Fumonisin B1 (FB1) and aflatoxins are prominent examples, particularly in maize and maize-based commodities, and their toxicity extends beyond conventional cytotoxic endpoints to subtle disturbances of cellular metabolism and mitochondrial function. Studies using intestinal Caco-2 and hepatic HepG2 cells demonstrated that FB1 and aflatoxin B1 (AFB1) can alter mitochondrial respiration, membrane potential and reactive oxygen species production, including subcytotoxic concentrations. These findings highlight mitochondrial bioenergetics as a sensitive mechanistic endpoint for investigating low-dose mycotoxin toxicity and combined exposures.

Such interactions become particularly relevant when considering co-exposure with other natural food contaminants. Combined exposure of HepG2 cells to aflatoxin M1 (AFM1) and the cyanotoxin microcystin-LR at subcytotoxic concentrations resulted in altered mitochondrial membrane potential

and inhibition of basal respiration, ATP-linked respiration and spare respiratory capacity, illustrating that effects of contaminant mixtures may become apparent at the bioenergetic level even when conventional cytotoxicity endpoints provide limited information.

An emerging dimension of this multi-contaminant paradigm is the interaction between toxins and micro- and nanoplastics (MNPs). MNPs are increasingly detected throughout the food chain and may interact with both chemical and biological hazards. MPs provide surfaces for microbial attachment and biofilm formation, potentially facilitating persistence and transmission of foodborne pathogens such as Campylobacter. Their interactions with foodborne toxins may likewise modify toxin transport, bioavailability and biological effects. We therefore investigated FB1, alongside the bacterial toxin cereulide, as model toxins for binding to polyethylene terephthalate (PET) and polypropylene (PP) microplastics. Toxin-preincubated MPs were evaluated using 3D HepG2 spheroids, IncuCyte live-cell analysis and Seahorse extracellular flux analysis. Differences in cellular and bioenergetic responses between toxin–MP combinations and toxins or MPs alone indicate that biological responses may provide a functional proxy for investigating toxin–plastic interactions.

Together, these studies support moving beyond the assessment of mycotoxins as isolated hazards towards realistic multi-hazard and multi-contaminant exposure scenarios, in which mycotoxins coexist with other microbial toxins, foodborne pathogens and particulate contaminants that may influence their transport, bioavailability and biological effects.

KEY WORDS:

Mycotoxins; fumonisin B1; microplastics; mitochondrial dysfunction; co-exposure.

ACKNOWLEDGMENTS

This research was supported by the European Union's Horizon 2020 research and innovation programme through the IMPTOX project (grant agreement No. 965173), the Research Foundation Flanders (FWO; grants 1506419N and G000123N), Ghent University Special Research Fund (BOF20/BAS/120 and BOF01P03220), the Federal Public Service Health, Food Chain Safety and Environment (RT 21/5 CYANTIR), and the Research Foundation Flanders through FWO–ARIS Weave programme.

PLENARY LECTURE

REASSESSING THE CYCLOPEPTIDE CONTENT OF EUROPEAN *AMANITA* SECT. *PHALLOIDEAE*

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ABSTRACT

The deadly *Amanita* mushrooms have long fascinated natural product chemists, owing in particular to their highly potent cyclopeptide toxins. Although the major families of amatoxins, phallotoxins and virotoxins have been investigated for decades, our recent work shows that this apparently well-explored chemical space still conceals substantial structural and biosynthetic diversity.

Our research has progressively combined classical natural product chemistry with modern mass spectrometry, molecular networking and genome mining to revisit cyclopeptides from *Amanita* species. Re-examination of historical toxin chemistry has allowed us to refine the chemical diversity of phallotoxins and virotoxins across *Amanita* section *Phalloideae*, while placing these metabolites in the context of the recent phylogenetic reassessment of the group. This work has also established MS/MS reference resources for these iconic toxins, together with comprehensive MS/MS profiles of whole-mushroom extracts from DNA-barcoded specimens, providing a robust foundation for their systematic detection and exploration across *Amanita* section *Phalloideae*.

Beyond known toxins, tandem mass spectrometry has become a powerful tool not only for dereplication, but also for anticipating structural features prior to isolation. MS/MS-guided molecular networking enabled us to detect and prioritize previously uncharacterized cyclopeptides. Remarkably, this strategy led to the identification of a new phallotoxin series, a long-sought precursor to viroidin-type virotoxins, and the new cycloamanide viduamanide in the holotype of the rare species *Amanita vidua*. This illustrates a shift from discovery by chance toward the prediction and targeted isolation of previously uncharacterized metabolites. Viduamanide is also of particular biosynthetic interest, as it represents the first cycloamanide lacking proline, challenging the long-standing assumption that this residue is essential for cycloamanide cyclization.

Genome mining has subsequently added an additional dimension to MS/MS-based dereplication, allowing the observed metabolome to be linked to its underlying biosynthetic potential. By combining metabolomic features with predicted ribosomally synthesized peptide precursor sequences, we have been able to link observed cyclopeptides to their biosynthetic genes and prioritize candidates for targeted isolation and subsequent NMR-based structure elucidation. This integrated strategy led to the discovery of amerivirosamanides A–E from the North American destroying angel *Amanita amerivirosa*, , a species that has undergone an invasive range expansion in Western Europe over the past two decades, revealing an unexpected level of structural diversity within the cycloamanide family. These compounds also raise intriguing questions concerning their biosynthesis. In particular, the occurrence of non-proteinogenic amino acids and unusual residues—including a dehydrotryptophan-containing cyclopeptide-illustrates the remarkable chemical plasticity of ribosomally synthesized and post-translationally modified peptides in *Amanita*.

Taken together, these results illustrate how revisiting a classical natural product family through the combined lenses of structural chemistry, MS/MS, molecular networking and genome mining can transform a supposedly well-known toxin repertoire into a dynamic and still largely unexplored landscape of chemical and biosynthetic diversity. More broadly, they suggest that the integration of metabolomics and genomics can progressively shift the exploration of *Amanita* cyclopeptides from discovery by chance toward the prediction and targeted isolation of new metabolites.

KEY WORDS

Amanita; *Phalloideae*; cyclopeptides; molecular networking; genome mining.

ACKNOWLEDGMENTS

This study was supported by a Ph.D. grant from the French Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation through Doctoral School "Innovation thérapeutique: du fondamental à l'appliqué" and sponsorship from Université Paris-Saclay and CNRS. This work has been supported as part of France 2030 programme "ANR-11-IDEX-0003". My warmest thanks to my long-standing collaborator, Mehdi Beniddir, for many years of fruitful collaboration.

INVITED LECTURE

ADVANCED OXIDATION PROCESSES FOR MYCOTOXIN DEGRADATION IN WATER: A CRITICAL REVIEW OF RECENT EXPERIMENTAL STUDIES

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ABSTRACT

Mycotoxins are a diverse group of low-molecular-weight compounds produced as secondary metabolites by several toxigenic fungal genera, primarily *Aspergillus*, *Alternaria*, *Claviceps*, *Penicillium,* and *Fusarium*. Although more than 500 mycotoxins have been identified, scientific research has mainly focused on aflatoxins, ochratoxins, fumonisins (FBs), zearalenone (ZEA), patulin, and trichothecenes. Traditionally, studies have concentrated on the occurrence of mycotoxins in cereals and cereal-based products. However, recent investigations have demonstrated their presence in aquatic environments. Even at low concentrations, continuous exposure may pose chronic risks to human and animal health.

The detection of mycotoxins in water has stimulated the development of advanced treatment technologies. Among them, advanced oxidation processes (AOPs) have emerged as promising alternatives to conventional physical, chemical, and biological methods. AOPs rely on the generate highly reactive species or exploit high-energy irradiation capable of transforming persis-

tent contaminants. Various AOPs have been investigated for mycotoxin removal, including direct photolysis, photocatalysis, simulated solar and UV irradiation, pulsed-light and γ-irradiation photolysis, ozonation, and electrochemical oxidation. Their efficiency depends on the mycotoxin structure, water composition, pH, and operating conditions.

Among the evaluated technologies, UV/H_2O_2 and UV/persulfate treatments have demonstrated particularly high efficiencies, achieving extensive degradation within 20–90 min. TiO_2-based photocatalysis enabled up to 100% degradation of ZEA, FB_1, and FB_2 within 30 min. Ozonation proved to be the fastest method, removing 96% of deoxynivalenol within 15 s without detectable undesirable by-products under the reported experimental conditions. γ-Irradiation also showed excellent performance, completely degrading FB_1 at a dose of only 0.5 kGy, although the potential formation of toxic radiolytic products requires careful dose optimization. Pulsed-light treatment and electrochemical oxidation achieved mycotoxin removal efficiencies of 93–98%, but remain less extensively studied. Importantly, degradation efficiency alone does not establish detoxification, as transformation products may retain or acquire biological activity.

Our recent experimental studies indicate that fumonisins can be efficiently removed from various water matrices using AOP technologies making them one of the most extensively investigated mycotoxin group in aquatic environments. Direct UV photolysis achieved complete FB_1 degradation in ultrapure water within 120 min, while UV/H_2O_2 and UV/persulfate treatments enabled complete degradation within 60 min and 20–60 min, respectively. TiO_2 Wackherr photocatalysis achieved approximately 99% degradation of FB_1 and FB_3 in only 30 min.

The application of AOP technologies in real water matrices and under practical water treatment conditions remains challenging due to matrix complexity, high energy and oxidant

consumption, and the possible formation of toxic by-products. Future research should focus on integrating AOPs with biological treatments, membrane technologies, and nanomaterials to improve process efficiency, sustainability, and safety, thereby facilitating large-scale industrial applications.

KEY WORDS

Myctoxins, fumonisins, AOPs, photolysis, aquatic environments.

INVITED LECTURE

MICROPARTICLES IN MYCOTOXICOLOGY: NOVEL APPROACHES TO THE CONTROL OF TOXIGENIC FUNGI AND MYCOTOXINS

Sandra Bulut[1*], Monika Bračun[2], Maja Stojkov[3], Sonja Smole Možina[2], Igor Antić[1], Jelena Živančev[1], Branislava Nikolovski[1], Jelena Bajac[1], Milana Pribić[1], Sunčica Kocić-Tanackov[1]

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ABSTRACT

Toxigenic fungi and the mycotoxins represent a persistent challenge to food and feed safety worldwide. Among the most relevant mycotoxins, aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, zearalenone, and patulin are predominantly associated with species belonging to the genera *Aspergillus*, *Fusarium*, and *Penicillium*. Growing concerns regarding conventional antifungal treatments have stimulated the search for safer and more sustainable alternatives, with particular attention being directed towards essential oils and other plant-derived bioactive compounds. Although these natural agents exhibit broad antifungal and antimycotoxigenic potential, their practical application is frequently constrained by high volatility, poor water solubility, chemical instability, and sensitivity to environmental conditions.

Microencapsulation offers a promising strategy to overcome these limitations by protecting bioactive compounds, improving their stability and dispersibility, and modulating

their release. By controlling the availability of active compounds, microparticle-based systems may prolong their interaction with fungal cells and enhance their effectiveness. Following release from the carrier, natural antifungal compounds can affect multiple cellular targets, including cell wall and membrane integrity, ergosterol homeostasis, mitochondrial function, cellular redox balance, and energy metabolism. These disturbances may influence not only fungal growth and sporulation but also secondary metabolism and the expression of genes involved in mycotoxin biosynthesis. Importantly, inhibition of fungal growth and suppression of mycotoxin production do not necessarily occur in parallel, emphasizing the need to evaluate both endpoints when assessing new antifungal formulations. Studies have demonstrated the potential of encapsulated natural compounds to control toxigenic *Aspergillus*, *Fusarium*, and *Penicillium* species and to reduce the associated risk of contamination with major mycotoxins. In our research, microparticle formulations loaded with *Satureja montana* essential oil demonstrated antifungal activity against toxigenic *Aspergillus* and *Penicillium* species in model food systems, as well as promising effects on aflatoxin production. Although microparticle-based systems show considerable potential, their effectiveness depends on complex interactions among the carrier, active compound, fungal species, and food matrix.

A better understanding of these interactions, particularly their effects on mycotoxin biosynthetic pathways, release behaviour, sensory properties, safety, and performance under realistic storage conditions, will be essential for translating microparticle-based systems into practical strategies for mycotoxin management.

KEY WORDS

Microparticles; mycotoxins; toxigenic fungi; essential oils; food safety.

ACKNOWLEDGMENTS

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INVITED LECTURE

LONG TERM RETROSPECTIVE STUDY OF WILD MUSHROOM POISONING IN ISRAEL

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ABSTRACT

Despite the semiarid climate of Israel, its mycobiota is very diverse. The estimated number of macrofungal species is over 850. Of these species, 65 are poisonous or suspected as poisonous, and three are hallucinogenic. Three species containing cyclopeptides are proven to be deadly. Picking fruit bodies of wild macrofungi became very popular in Israel in the last recent years. The mushrooms picking season in Israel usually starts with the first rain, during late November to mid-March. Nevertheless, during the summertime poisonous wild mushrooms can also appear in Israel within irrigated areas such as orchards, grassland and near water sources. We conducted a long-term (2010–2021) retrospective study to monitor the frequency, severity, seasonal character, species composition, and geographical distribution of mushroom exposure and poisoning in Israel.

Data collected by the Israel Poison Information Center in Rambam Health Care Campus, Haifa.

We report that only 4% of cases exposures due to biologic agents were caused by macrofungi. Males were significantly ($P < 0.004$) more affected than females. Most cases involved either above 18 years of age (41%) or below the age of 6 years (39%). Most of the children under 6 years of age did not develop signs or symptoms of toxicity. During 2017–2021, 128 cases (82.5%) involved the consumption of raw mushrooms, mostly by children under 6 years of age. The most common season of mushroom exposure was rainy winters ($P < 0.05$). Ingestion of *Chlorophyllum molybdites* was the leading of mushroom ingestion in summer and that of *Inocybe* species in winter. Furthermore, since we published the data we continued tracking the poisoning cases.

In the last three years, *C. molybdites* is still leading in human ingestion in the summers and *Inocybe* spp. in the winters. Moreover, we see an increased exposures at home due to fungi that grows in pots. In the recent years we see more poisoning cases of *Amanita proxima*, which is primary nephrotoxic. Thus, we are focusing now to identify the toxic compounds of *A. proxima*. Our study contributes to improved awareness of mushroom exposure and poisoning patterns among the Israeli population.

KEY WORDS

Amanita proxima; Chlorophyllum molybdites; Inocybe; wild macrofungi

PLENARY LECTURE

MYCOTIC AND OHER BRAIN INFECTIONS IN IMMUNO COMPROMISED PATIENTS: NEURORADIOLOGIC PATTERN

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ABSTRACT

Fungal infections of the central nervous system – CNS, are typically associated with immunocompromised patients, organ transplants, chemotherapy, and human immunodeficiency virus infections. Radiological characteristics are often nonspecific, and may present with meningitis, cerebritis, abscess formation and vasculitis. Typical patterns are best identified through magnetic resonance imaging – MRI. A rapid diagnosis and the administration of the appropriate therapy are crucial for favourable outcome.

KEY WORDS:

Central nervous system mycoses; magnetic resonance imaging; brain abscess; meningitis; vasculitis

PLENARY LECTURE

THE ROLE OF THE LUNG MICROBIOTA IN THE DEVELOPMENT OF PULMONARY ASPERGILLOSIS: FROM SYMBIOSIS TO INFECTION

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ABSTRACT

Pulmonary aspergillosis represents a spectrum of diseases caused by *Aspergillus* spp., ranging from colonization to invasive infection, primarily affecting patients with structural lung disease or impaired immunity. Traditionally, the pathogenesis of pulmonary aspergillosis has been attributed mainly to host immune dysfunction and environmental exposure to fungal spores. However, recent advances in metagenomic sequencing have revealed that the lung harbors a dynamic microbial ecosystem composed of bacteria, fungi, and viruses that interact with the host immune system and influence susceptibility to infection.

Emerging evidence suggests that alterations in the lung microbiota, often referred to as dysbiosis, may play a key role in the transition from commensal colonization to pathogenic fungal invasion. Changes in bacterial communities, reduced microbial diversity, and the disruption of microbial interactions have been associated with increased airway inflammation and impaired antifungal immune responses. In particular, interactions between bacterial genera such as *Pseudomonas*, *Streptococcus*,

and *Haemophilu* and fungal organisms, including *Aspergillus fumigatus*, may modulate fungal growth, biofilm formation, and host immune signaling. Furthermore, chronic lung diseases such as COPD, bronchiectasis, and cystic fibrosis are characterized by profound alterations in airway microbiota, which may facilitate fungal persistence and disease progression.

Understanding the complex interactions between the lung microbiota, fungal pathogens, and host immunity provides new insights into the pathogenesis of pulmonary aspergillosis. These findings suggest that pulmonary aspergillosis should be considered not only as a consequence of host immunosuppression but also as a manifestation of microbial ecosystem imbalance. Future research focusing on microbiota-host-fungal interactions may open new perspectives for diagnostics, risk stratification, and innovative therapeutic approaches targeting microbial communities.

KEYWORDS

Lung microbiota; pulmonary aspergillosis; dysbiosis; airway mycobiome; host–microbe interactions.

INVITED LECTURE

STARSHIPS, GIANT TRANSPOSABLE ELEMENTS DRIVING FUNGAL GENOME EVOLUTION AND ADAPTATION

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ABSTRACT

Transposable elements (TEs) were traditionally viewed as selfish, parasitic DNA with little to no benefit to their hosts. Recently, a more comprehensive view has emerged, where TEs are seen as essential components of genomes, playing a key role in generating evolutionary novelty and driving adaptation. A newly described superfamily of giant mobile elements, called *Starships*, exemplifies this role as genomic innovators. These elements not only encode genes required for their own movement, but also carry "cargo" genes that can provide beneficial traits to their fungal hosts, including secondary metabolite gene clusters and resistance to environmental stressors. *Starships* are mobilized by a tyrosine recombinase, called Captain, encoded as the first gene within each element, which catalyzes excision and reinsertion into the genome. Recent studies have demonstrated that *Starships* are able to transfer between fungal species as evolutionarily divergent as the distance separating humans from kangaroos, highlighting their importance as drivers of fungal genome evolution and adaptation.

Aspergillus fumigatus is an opportunistic human pathogen responsible for more than two million cases of invasive asper-

gillosis annually. At least 20 Starships have been found to be actively mobilizing within populations of *A. fumigatus*, including in clinical isolates. One such element, *Nebuchadnezzar*, carries a gene cluster important for biofilm formation and associated with increased mortality in infection models, highlighting the potential clinical relevance of *Starship* cargo.

Still, the exact molecular mechanisms of *Starship* mobility remain poorly understood. We are developing *Nebuchadnezzar* in *Aspergillus fumigatus* as a model system for studying *Starship* biology in a clinically relevant human fungal pathogen, using molecular tools to track its mobilization within and between strains under different environmental conditions. This approach will help us better understand how *Starships* contribute to genome plasticity, adaptation and virulence in *A. fumigatus*.

KEY WORDS

Starships, transposable elements, *Aspergillus fumigatus*, tyrosine recombinase, genome evolution and adaptation.

INVITED LECTURE

TIME FOR A PARADIGM SHIFT? UNMASKING THE PATHOGENIC POTENTIAL OF *CANDIDA ALBICANS* IN CYSTIC FIBROSIS

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ABSTRACT

Cystic fibrosis (CF) is a hereditary multisystem disease characterized by chronic respiratory infections and progressive lung damage. Research and clinical attention have traditionally focused on bacterial pathogens, yet increasing evidence suggests that fungi, particularly *Candida* species, may also contribute to disease progression. Although *Candida albicans* is the most frequently isolated fungal species from CF sputum, the clinical significance and pathogenic potential of Candida in CF remain insufficiently understood.

For decades, the detection of *Candida* species in expectorated specimens from CF patients was considered clinically irrelevant and often attributed to oral contamination during sputum collection rather than genuine lower-airway colonization or infection. Consequently, the contribution of these fungi to CF pathogenesis has been largely underestimated.

We investigated the antifungal susceptibility of a broad collection of clinical *Candida* isolates recovered from CF respiratory specimens, encompassing multiple *Candida* species. Under CF-mimicking conditions, these isolates exhibited reduced susceptibility to antifungal agents compared with standard

EUCAST testing conditions, while oxygen limitation further diminished antifungal efficacy.

In parallel, we performed an in-depth characterization of clinical *C. albicans* isolates. These strains displayed pronounced filamentation and biofilm formation in expectorated sputa and artificial CF medium that reproduces sputum chemistry, with morphogenesis being strongly influenced by normoxia, elevated CO_2, and hypoxia. Using CFTR-deficient zebrafish embryos as a preclinical CF infection model, we demonstrated that clinical *C. albicans* isolates are pathogenic, causing strain-dependent epithelial barrier damage and lethal infection. CFTR deficiency markedly increased host susceptibility to C. albicans infection, highlighting the protective role of CFTR against fungal pathogenesis. Although antifungal treatment partially improved infection outcomes, it did not fully restore host resistance. Furthermore, isolates engineered to express GFP under the *ECE1* promoter revealed candidalysin expression under *in vitro, ex vivo,* and *in vivo* conditions (particularly in CFTR-deficient hosts).

Together, these findings demonstrate that *Candida spp.* has reduced antifungal susceptibility in CF-like environments, and that *C. albicans* CF strains possess considerable virulence potential, supporting a paradigm shift in our understanding and management of fungal colonization in cystic fibrosis lung disease.

KEY WORDS

Cystic fibrosis; Candida albicans; antifungal susceptibility; fungal virulence; CFTR deficiency; biofilm formation; candidalysin.

INVITED LECTURE

THE IMPORTANCE OF CONVENTIONAL AND MOLECULAR METHODS FOR THE IDENTIFICATION OF FUNGAL PATHOGENS CAUSING SKIN, HAIR, AND NAIL INFECTIONS

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ABSTRACT

Dermatophytes are keratinophilic fungi, classified as anthropophilic, zoophilic, or geophilic according to their natural reservoir, that cause superficial infections of the skin, hair, and nails, with onychomycosis as their most persistent clinical manifestation. Onychomycosis affects an estimated 4–17% of the general population, and because systemic antifungal therapy carries a recognized risk of hepatotoxicity while clinical inspection alone misclassifies up to 40% of nail disease, laboratory confirmation of the causative organism is considered a prerequisite for treatment rather than an optional step. This narrative review aimed to compare the diagnostic performance, workflow, and practical trade-offs of the conventional and molecular

methods currently used to identify dermatophytes, including correct specimen collection, and to outline a rational diagnostic pathway.

Dermatophyte epidemiology varies geographically and over time: *Trichophyton rubrum* remains the predominant species in Europe and North America, followed by *T. interdigitale*, while zoophilic species such as *Microsporum canis* and geophilic species are recovered less often and are more frequently associated with inflammatory tinea of the skin and scalp. Reliable diagnosis depends on correct specimen collection: nail material should be obtained from the proximal margin by filing, skin scrapings from the active, scaling border of a lesion, and hair samples by epilation to include the root, with prior antifungal treatment discontinued for at least four weeks before sampling.

Direct microscopy with potassium hydroxide preparation proved rapid but poorly specific (sensitivity 80–82%, specificity 16–40%) and unable to identify genus or species. Fungal culture, despite remaining the historical reference standard, showed comparatively low sensitivity (37.5–57.4%) and required one to six weeks for a result, hindered by contaminant overgrowth and dependence on organism viability. MALDI-TOF MS, applied to a cultured isolate under an optimized ethanol/formic-acid extraction protocol, achieved 100% correct identification and 65% species-level resolution. Real-time and multiplex PCR assays consistently outperformed culture, reaching 90–100% sensitivity and reducing turnaround from weeks to 4–6 hours, while detecting up to 50,9 percentage points more dermatophyte-positive samples than culture across the compiled literature, at the cost of being unable, on its own, to confirm organism viability.

No single method reviewed combined the speed, sensitivity, and organism-level specificity required for confident diagnosis. The evidence instead supports a layered diagnostic strategy, combining rapid microscopic triage, culture and molecular

testing performed in parallel, and MALDI-TOF MS applied to any resulting isolate, consistent with current guideline recommendations, in order to provide accurate and timely confirmation of dermatophyte infection before systemic antifungal therapy is initiated.

KEY WORDS

Dermatophytes; onychomycosis; diagnosis; PCR; MALDI-TOF MS.

INVITED LECTURE

FUNGAL RHINOSINUSITIS: THE IMPORTANCE OF MYCOLOGICAL DIAGNOSIS IN SURGICAL MENAGMENT AND THE DEVELOPMENT OF FUTURE THERAPEUTIC STRATEGIES

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ABSTRACT

Fungal rhinosinusitis (FRS) encompasses a heterogeneous group of disorders ranging from non-invasive colonization to potentially life-threatening invasive disease. The invasive forms are acute fulminant, chronic, and granulomatous (indolent) invasive fungal sinusitis. The noninvasive forms are fungus infestation, fungal ball ("sinus mycetoma") and allergic fungal sinusitis (AFS). Mycological diagnosis, together with histopathological, endoscopic, and radiological assessment, plays an important role in establishing the diagnosis and characterizing the causative fungal species.

To emphasize the importance of systematic mycological evaluation in patients with suspected FRS, with particular focus on its role in surgical decision-making and its potential contribution to the development of individualized treatment strategies.

Clinical, endoscopic, radiological, microbiological, and histopathological findings in patients with different forms of FRS were evaluated. Particular attention was given to the correlation between mycological findings, intraoperative appearance, and final histopathological diagnosis, as well as to the potential clinical relevance of fungal species identification and antifungal susceptibility testing.

AFS is the most common form of fungal rhinosinusitis. Patients with AFS are atopic to aeroallergens including the involved fungal organism, immunocompetent, have nasal polyps and chronic allergic rhinosinusitis and may occasionally present with proptosis from orbital extension of disease. Sinus CT shows sinus mucosal hypertrophy and often hyperattenuation of sinus contents. Diagnosis is made from endoscopic sinus surgery (ESS) that is also main treatment of disease. Histopathology material is obtained during surgery with or without an associated positive surgical sinus fungal culture. The histopathology shows extramucosal allergic mucin that stains positive for scattered fungal hyphae and eosinophilic-lymphocytic sinus mucosal inflammation. The common species seen in AFRS isolates were *Cladosporium* spp. (38.5% of isolates) and dematiaceous molds in the same percentage, while the remainder of the cultures were hyaline moulds. The fungus identification rate varied between entities and was 72.2% in AFRS and 33.3% in fungal ball specimens. Chronic granulomatous invasive fungal rhinosinusitis is very rare in our country due to its continental climate, but imported cases can be seen from time to time. In one case the first operation revealed a noninvasive fungal form of disease without identification of fungus and 2 months after the first operation a reoperation was performed due to disease relapse, and chronic granulomatous invasive fungal rhinosinusitis was diagnosed. This case also shows possibility of the disease to change from a noninvasive to invasive form.

Mycological diagnosis represents an important component of a multidisciplinary approach to FRS. Integration of microbiological, histopathological, endoscopic, and radiological data may facilitate more precise disease classification and individualized treatment. Future research should focus on rapid molecular identification of fungal pathogens, antifungal susceptibility profiling, and the development of targeted therapeutic strategies, with the potential to improve treatment outcomes and reduce recurrence.

KEY WORDS

Chronic rhinosinusitis, endoscopic sinus surgery, fungal rhinosinusitis, fungus ball, mycotic infection.

INVITED LECTURE

APPLICATION OF MALDI-TOF MS IN THE IDENTIFICATION AND CHARACTERIZATION OF FUNGI ISOLATED FROM THE RESPIRATORY TRACT OF PATIENTS WITH CYSTIC FIBROSIS

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ABSTRACT

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become the first-line method for microbial identification, providing species-level results within minutes of growth and resolving cryptic species. In cystic fibrosis (CF), where *Candida albicans* and *Aspergillus fumigatus* are the fungi most frequently recovered from the airways, and their contribution to lung function decline remains debated, rapid and informative laboratory characterization of isolates is particularly relevant. Beyond identification, peak intensities and whole-spectrum similarity carry phenotypic information that can be extracted from the same spectrum

acquired for routine identification. We therefore evaluated two applications: detection of biofilm-producing *C. albicans* and rapid assessment of itraconazole activity against *A. fumigatus*.

Seventeen *C. albicans* isolates from the sputum of 13 CF patients were tested for biofilm formation by the safranin microtiter method, with the cut-off defined as three standard deviations above the mean absorbance of the negative control at 490 nm. Spectra of isolates identified with a log score of at least 2.0 were analysed in flexAnalysis and MBT Explorer software, and peak intensities were correlated with the biofilm index using Pearson's test. Separately, a spore suspension of an *A. fumigatus* isolate from a CF patient was incubated for 12 h with two-fold itraconazole dilutions (64–0.5 µg/mL) and a drug-free control. Composite correlation indices were calculated in MBT Compass Explorer to determine the minimal profile change concentration (MPCC), which was compared with the minimum inhibitory concentration (MIC) obtained by gradient strip diffusion after 48 h.

Biofilm was produced by 13 of 17 isolates (76%): two were strong, three moderate and eight weak producers. All spectra shared ten peptide peaks, which differed between groups in intensity rather than in presence. The intensity of the 6238 *m/z* peak correlated with biofilm-forming capacity (r = 0.65), as did, moreweakly, the 6200 *m/z* peak (r = 0.55); intensities above 6848 and 9977 arbitrary units (a.u.) distinguished biofilm producers and strong producers, respectively. For *A. fumigatus*, the 16873 *m/z* peak decreased and the 14165 *m/z* peak increased with rising itraconazole concentrations. The MPCC was 2 µg/mL, within one two-fold dilution of the MIC of 4 µg/mL, and available 36 h earlier.

MALDI-TOF MS can therefore characterize CF respiratory isolates beyond identification, indicating biofilm production and antifungal activity without additional assays. Both

findings derive from single-centre sets of limited size and require multicentre validation before clinical application.

KEY WORDS

MALDI-TOF mass spectrometry; cystic fibrosis; *Candida albicans*; biofilm; antifungal susceptibility testing.

INVITED LECTURE

ACTUALITY IN THE TREATMENT OF FUNGAL INFECTIONS IN IMMUNOCOMPROMISED PERSONS

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ABSTRACT

Invasive fungal infections (IFIs) present a growing public health threat with high morbidity and mortality. Historically linked to prolonged neutropenia in hematologic malignancies, the epidemiological landscape of medical immunosuppression has shifted drastically. An expanding cohort of non-hematologic immunocompromised hosts now faces significant IFI risks. This vulnerable population includes solid organ transplant (SOT) recipients, advanced HIV/AIDS patients, individuals undergoing targeted biologic therapies for autoimmune disorders, and critically ill patients experiencing severe post-viral lung injuries in the intensive care unit (ICU).

Managing IFIs in non-hematologic patients involves distinct diagnostic and therapeutic challenges. SOT recipients face organ-specific threats, such as high Aspergillus risk in lung transplants or Candida vulnerabilities following complex abdominal surgeries. Concurrently, the rise of monoclonal antibodies and small-molecule kinase inhibitors has transformed autoimmune care but unmasked complex cell-mediated immunodeficiencies, increasing susceptibility to opportunistic fungi.

Because standard cultures suffer from low sensitivity and tissue biopsies often carry prohibitive risks in unstable patients, immediate clinical action frequently relies on a pragmatic, risk-factor-driven empiric initiation of antifungal therapy.

The primary clinical dilemma in this cohort centers around severe drug-drug interactions (DDIs). Standard triazoles (e.g., voriconazole, posaconazole) are potent CYP3A4 inhibitors. When introduced alongside calcineurin inhibitors (like tacrolimus) to protect an organ graft, they trigger dangerous spikes in immunosuppressant levels, risking severe nephrotoxicity or graft failure. Consequently, careful management via immediate empirical dose reductions and aggressive therapeutic drug monitoring (TDM) is vital. While next-generation global therapeutics (e.g., rezafungin, olorofim) aim to completely bypass these metabolic pathways, local clinical practice must optimize traditional pipelines. Success hinges on strict antifungal stewardship, strategic class deployment, and localized safety standardization to safely balance host organ protection against invasive mycoses.

KEY WORDS

Invasive fungal infections; immunocompromised patients; antifungal therapy; drug–drug interactions; antifungal stewardship.

INVITED LECTURE

FUNGAL INFECTIONS IN HEMATOLOGICAL PATIENTS

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ABSTRACT

Invasive fungal infections (IFIs) remain an important cause of morbidity and mortality in patients with hematological malignancies, particularly those receiving intensive chemotherapy or undergoing allogeneic hematopoietic stem cell transplantation. Their incidence varies between 3.4% and more than 10%, depending on the underlying disease, treatment modality, and degree of immunosuppression. Invasive aspergillosis represents the most common mold infection in high-risk hematological patients, while invasive candidiasis remains clinically relevant. Recent epidemiological changes include an increasing proportion of non-albicans Candida species, azole-resistant Aspergillus, and emerging non-Aspergillus molds. Major risk factors include prolonged neutropenia, corticosteroid exposure, impaired immune function, and environmental fungal exposure. Early diagnosis remains challenging and requires integration of clinical findings, radiological imaging (CT scan) and fungal biomarkers, especially galactomannan and beta D glucan test. Persistent febrile neutropenia should raise suspicion of IFI, particularly in high-risk patients. Invasive pulmonary aspergillosis is the most common clinical presentation of IFI in hematological patients. Management strategies should be individualized according to risk stratification of patients and suspected fungal

pathogens. Antifungal prophylaxis, empirical treatment, and diagnostic-driven approaches represent complementary strategies. Posaconazole prophylaxis is recommended in selected high-risk hematological patients, while voriconazole, isavuconazole, echinocandins, and lipid formulations of amphotericin B play important roles in targeted treatment. Early recognition, timely initiation of appropriate antifungal therapy, and risk-adapted management are essential to improving clinical outcomes and reducing the substantial healthcare burden associated with IFIs.

KEY WORDS

Invasive fungal infections; hematological malignancies; aspergillosis; candidiasis; antifungal prophylaxis; neutropenia.

INVITED LECTURE

FUNGAL INFECTIONS IN GYNECOLOGY – FROM DIAGNOSTIC CHALLENGES TO MODERN THERAPY

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ABSTRACT

Vulvovaginal candidiasis (VVC) is one of the most common infections of the female genital tract and represents a particular diagnostic and therapeutic challenge during pregnancy. Although *Candida* spp. may frequently be detected in the vaginal microbiota, the presence of the microorganism does not necessarily indicate clinically relevant infection. Symptoms such as pruritus, burning, erythema, edema, dyspareunia and altered vaginal discharge are not specific for VVC and may occur in a range of infectious and non-infectious conditions. Therefore, accurate diagnosis requires correlation of the clinical presentation with appropriate laboratory findings.

Recurrent VVC represents an additional challenge, particularly when non-*albicans Candida* species, previous antifungal exposure or host-related factors are involved. Species identification becomes especially relevant in persistent or recurrent cases, as some non-*albicans Candida* species may be more difficult to detect by conventional microscopy and may respond differently to standard azole therapy.

Pregnancy represents a distinct biological context in which hormonal changes, alterations of the vaginal microbiome,

epithelial function and local immune responses may influence the interaction between the microorganism and the host. Importantly, the detection of *Candida* during pregnancy should not be automatically interpreted as a cause of adverse pregnancy outcomes. Current evidence does not establish vaginal candidiasis as an independent proven cause of preterm birth, preeclampsia, gestational diabetes mellitus or recurrent pregnancy loss.

Therapeutic decision-making during pregnancy must consider not only antifungal efficacy but also gestational age, *Candida* species, disease severity and fetal safety. Consequently, the management of VVC in pregnancy should follow a clinically integrated approach, combining gynecological assessment, microbiological confirmation and species identification in complicated cases, evaluation of relevant host factors, and individualized selection of pregnancy-compatible therapy.

A modern approach to vaginal candidiasis therefore moves beyond the simple model of "symptom–*Candida*–antifungal treatment" toward clinically actionable microbiology and individualized management. Greater collaboration between gynecologists, microbiologists, infectious disease specialists and other relevant clinicians may contribute to more accurate diagnosis and more rational treatment of VVC, particularly in pregnant women and patients with recurrent or complicated disease.

KEY WORDS

Vulvovaginal candidiasis; *Candida*.

INVITED LECTURE

DERMATOPHYTOSES AND ANTIFUNGALS: CLINICAL FORMS, TREATMENT PROTOCOLS AND THE CHALLENGE OF RESISTANCE

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ABSTRACT

Dermatophytoses are the most common fungal infections in humans, affecting an estimated 20–25% of the world population. Globally the leading agent is *Trichophyton rubrum*, but the local spectrum differs. Among 1,411 culture isolates from our clinic's mycology laboratory (2018–2023), 790 (56%) were dermatophytes. The leading species was the zoophilic *Microsporum canis* (46.7%), followed by *T. mentagrophytes* var. *interdigitale* (29.4%). The share of *M. canis* declined over the period, and in 2023 *T. interdigitale* overtook it.

The lecture reviews the ecology of dermatophytes following the 2017 taxonomic revision and the clinical forms by anatomical site. It gives particular attention to diagnostic pitfalls: tinea incognito caused by topical corticosteroids or calcineurin inhibitors, and Majocchi granuloma and deep dermatophytosis, which require biopsy and systemic therapy. Reliable diagnosis depends on close cooperation between clinician and laboratory. The methods covered are direct microscopy, culture, MALDI-TOF, and molecular identification, including ITS sequencing.

Therapeutic options are presented by mechanism of action and clinical indication. Topical allylamines and azoles are suf-

ficient for limited tinea corporis, cruris and pedis. Systemic therapy is required for tinea capitis, extensive or recalcitrant disease, follicular involvement and onychomycosis with matrix involvement. Standard adult regimens of terbinafine, itraconazole and fluconazole are summarised, together with their key adverse effects and drug interactions.

The most important recent development is the emergence of terbinafine-resistant *Trichophyton indotineae*. Its resistance is mediated by point mutations in the squalene epoxidase (*SQLE*) gene. Spreading from South Asia, it has been documented in more than 60 European cases, including autochthonous and sexual transmission. In extensive or treatment-refractory tinea, species identification and antifungal susceptibility testing should precede escalation of therapy, and itraconazole is the usual alternative.

KEY WORDS

dermatophytosis; antifungal agents; terbinafine resistance; *Trichophyton indotineae*; *Microsporum canis*

PLENARY LECTURE

THE ROLE OF SYSTEMATICS IN THE STUDY OF MEDICINAL MUSHROOMS: FROM TAXONOMIC FOUNDATIONS TO PHARMACOLOGICAL VALIDATION

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ABSTRACT

This contribution reviews the role of integrative fungal systematics in medicinal mushroom research, product authentication, and bioprospecting. The study of fungal systematics is fundamental for both the scientific research and the industrial application of medicinal mushrooms. Accurate classification and identification of medicinal mushrooms is essential to ensure that reported therapeutic properties are correctly attributed to the species investigated in clinical and pharmacological trials as well as to those used in mushroom-based products. The shift from classical taxonomy—which was based exclusively on morphology—to integrative molecular systematics including phylogenetics and phylogenomic approaches has significantly improved the identification of medicinal mushrooms. Molecular markers enable the differentiation of cryptic species and the resolution of species complexes, while also helping to correct nomenclatural and identification errors that have persisted for years in scientific publications and commercial mushroom-based products labelling. Such errors may compromise the reproducibility and comparability of pharmacological studies.

Taxonomy also represents an important quality-control tool. That's way another aspect of fundamental importance is the use of taxonomy in accurate identification and classification as a prerequisite for the standardization of extracts, consumer safety, traceability of biological materials, and regulatory approval of fungal derived compounds in the nutraceutical and pharmaceutical sectors. Furthermore, the integration of phylogenetic information with metabolomic profiles through chemosystematics approaches can reveal relationships between fungal lineages with and their bioactive compounds. Modern fungal systematics therefore not only clarifies evolutionary relationships but also supports targeted bioprospecting and the discovery of previously unexplored fungal taxa with potential therapeutic applications.

KEY WORDS

Fungal systematics; Medicinal mushrooms; Molecular taxonomy; Quality control; Bioprospecting.

PLENARY LECTURE

SOIL ECOLOGY AND MICROBIAL INTERACTIONS: IMPACTS ON TRUFFLE CULTIVATION

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ABSTRACT

Truffles are hypogeous ectomycorrhizal fungi that complete their entire life cycle in the soil, where they interact with a complex community of organisms, including plants, fungi, animals, bacteria, and even viruses. These interactions, strongly influenced by soil physicochemical properties and environmental conditions, play a crucial role in truffle development, reproduction, and productivity. Besides establishing ectomycorrhizal associations with their host plants, truffles can colonize the roots of non-host herbaceous plants as endophytes, potentially contributing to mycelial persistence and genetic diversity. Interactions with other ectomycorrhizal fungi may influence truffle establishment through competition for host roots and soil resources, while animals, including insects, play important roles in spore dispersal and fertilization.

Particular attention is given to truffle-associated bacteria, which can positively or negatively influence ectomycorrhiza formation, mycelial growth, fruiting body development, and truffle quality. Recent experimental studies have demonstrated that selected bacterial strains can enhance Tuber melanosporum

mycorrhization under greenhouse conditions. Furthermore, recent investigations have revealed a close association between Tuber magnatum mycelium and bacteria belonging to the genus Bradyrhizobium, which have facilitated, for the first time, the establishment of stable mycelial cultures under laboratory conditions. These findings open up new possibilities for developing innovative inoculation strategies based on selected T. magnatum mycelial strains as an alternative to traditional spore-based inoculation.

Overall, truffle cultivation should be approached from an ecosystem perspective, considering not only the fungus and its host plant but also the complex network of microbial interactions occurring in the soil. A deeper understanding of these relationships is essential for improving truffle cultivation and developing more sustainable and reliable production systems, while opening new opportunities for the biotechnological exploitation of beneficial truffle-associated microorganisms.

KEY WORDS:

Tuber spp.; *ectomycorrhizae; soil organisms; bacterial interactions; truffle cultivation.*

MYCOLOGY

PLENARY LECTURE

ENVIRONMENTAL DRIVERS OF SOIL FUNGAL DIVERSITY IN MEDITERRANEAN FORESTS

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ABSTRACT

The European Mediterranean region is recognised as one of the most significant global climate change hotspots, driven by accelerated warming, more frequent heatwaves, and declining precipitation. Extreme weather events, including wildfires, storms and floods, have increased in frequency and intensity in recent decades. A long history of human influence in the region, combined with pronounced climatic and geographical contrasts, has shaped its terrestrial ecosystems into a distinctive mosaic of forests, shrublands, grasslands, agricultural land, and urban areas.

Despite the decline in forest area in the Mediterranean during the 20th century, forests now cover around 10% of the region's total land surface. However, the decline of traditional agriculture and livestock grazing in recent decades has led to natural forest expansion on abandoned agricultural land. Mediterranean forests are adapted to scarce, rocky, shallow, calcareous soils that are nutrient-poor and low in organic matter. They support exceptionally rich, unique and endemic biodiversity, which is increasingly threatened by land conversion, urbanization and overtourism. As the dominant eukaryotes in forest

soil biomass, fungi play key roles in tree growth, biogeochemical cycling and carbon sequestration. In this lecture, the project Environmental drivers of fungal community composition in the Mediterranean region of Croatia (FunMed), funded by the Croatian Science Foundation, will be presented and preliminary results discussed in the context of existing knowledge.

The main project objective is to determine the influence of environmental factors (soil physicochemical properties and microclimatic parameters such as temperature and humidity) on spatial and temporal changes in biomass, diversity, and functional composition of soil and litter fungal communities in forest habitats in the Mediterranean region of Croatia. Soil samples for physicochemical analyses and eDNA metabarcoding of fungal communities have been collected in two habitat types in Croatia: 1) forest stands dominated by holm oak (Quercus ilex) and 2) elevational transects along mountains near the Adriatic Sea. The main hypotheses to be tested in the project are: (1) fungal community diversity decreases with increasing forest ecosystem management intensity and elevation; (2) the proportion of ectomycorrhizal species relative to other functional fungal groups in the studied forests increases with stand age and decreasing temperature and humidity; (3) total fungal biomass in the soil decreases with lower soil moisture content (drier conditions); and (4) fungal community diversity increases with higher soil pH.

KEY WORDS

Biodiversity; climate change; ectomycorrhiza; eDNA metabarcoding; soil fungi.

PLENARY LECTURE

DIVERSITY OF ECTOMYCORRHIZAL FUNGI ASSOCIATED WITH FOREST TREE SPECIES IN SERBIA

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ABSTRACT

Ectomycorrhizal (ECM) fungi are essential for the functioning, stability, and adaptability of forest ecosystems. They form a mutually beneficial association with the roots of tree species, facilitating water and nutrient uptake and protecting trees against various environmental stresses. Although the study of ECM fungi in Serbia has traditionally relied on monitoring of their aboveground fruit bodies, these studies often fail to reflect the actual composition and structure of ECM communities belowground.

This lecture provides a comprehensive overview of research focused exclusively on belowground diversity, namely the direct analysis of ECM root tips of key forest tree species in Serbia (genera *Populus*, *Fagus*, *Picea*, *Quercus*, and *Abies*). Vital ECM root tips were categorized into different morphotypes based on their morphological and anatomical characteristics. By combining morpho-anatomical characterization and molecular identification of ECM morphotypes (via PCR and ITS rDNA sequencing), the diversity of ECM fungi was successfully mapped across different forest stands in the Republic of Serbia.

Fungal diversity was investigated across a variety of distinct habitats. In white poplar (*Populus alba*) stands, 30 ECM fungi

were recorded in a mature plantation, 20 in an autochthonous stand within the Kovilj-Petrovaradin marsh, 4 at an extreme site near the Timok River, and 15 in a Euramerican poplar (*Populus* x *euramerican*a) stand within the Gornje Podunavlje Special Nature Reserve. In beech (*Fagus sylvatica*) stands across Serbian mountains, 27 different ECM taxa were documented, distributed across Fruška Gora (7), Čemernik-Ostrozub (7), East Boranja (7), and the Homolje Mountains (9). Furthermore, pedunculate oak (*Quercus robur*) stands yielded 21 ECM taxa in a mature stand and 18 in a young stand in Morović, alongside 17 taxa identified in the Gornje Podunavlje Special Nature Reserve. The sessile oak (*Quercus petraea*) on Fruška Gora hosted 26 different ECM taxa, found at the Info Center (17) and Brankovac (12) sites. At the southernmost natural distribution range of Norway spruce (*Picea abies*), 29 different ectomycorrhizas were observed across Stara Planina (9), Kopaonik (13), and Tara (12). Furthermore, an investigation in three differently declined spruce stands on Kopaonik revealed 19 ECM taxa, whereas silver fir (*Abies alba*) trees at the same three sites hosted a visibly higher richness of 33 ECM fungal taxa.

Many of the recorded ECM fungi were described for the first time as ECM morphotypes on the studied tree species, both in the Republic of Serbia and worldwide.

KEY WORDS

Ectomycorrhizal root tips; belowground diversity; forest ecosystems; morpho-anatomical characterization; molecular identification.

ACKNOWLEDGMENTS

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MYCOLOGY

PLENARY LECTURE

THE YEAST AS FIL ROUGE FOR MODERN LIFE SCIENCES: 30 YEARS AFTER THE YEAST GENOME BECAME KNOWN AND 60 YEARS AFTER FIRST YEAST SPECIALIZED SYMPOSIA

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ABSTRACT

Yeasts occupy a unique position in mycology, bridging fundamental fungal biology, biotechnology and human health. Among them, *Saccharomyces cerevisiae* has become one of the most influential model organisms in modern life sciences. Thirty years after the publication of its complete genome sequence in 1996—the first complete genome of a eukaryotic organism—yeast continues to provide a *fil rouge* connecting major advances in genetics, molecular biology, genomics, systems biology and synthetic biology.

The study of yeast has contributed fundamentally to our understanding of processes that are central to fungal and eukaryotic life, including cell-cycle regulation, DNA replication and repair, intracellular trafficking, mitochondrial function, stress responses, protein homeostasis, ageing and cellular adaptation. Importantly, many of the molecular mechanisms uncovered in *S. cerevisiae* are conserved among fungi and across eukaryotes, making yeast an invaluable system for understanding both basic fungal biology and processes relevant to human health and disease.

The genomic era transformed yeast research from the analysis of individual genes to genome-wide exploration of gene function, genetic interactions, metabolic networks and cellular phenotypes. More recently, advances in genome engineering and synthetic biology have enabled researchers not only to understand yeast genomes, but also to redesign them, opening new perspectives for studying the fundamental principles of fungal life.

This lecture will use yeast as a continuous thread through these scientific developments, highlighting how a traditional organism of fermentation became a central experimental platform for modern biology. It will also consider the future role of yeast in fungal biotechnology, systems biology, genome engineering and the emerging effort to understand and engineer living systems.

KEY WORDS

Saccharomyces cerevisiae; yeast genomics; molecular biology; systems biology; synthetic biology

MYCOLOGY

INVITED LECTURE

MAPPING A CENTURY OF MACEDONIAN MYCOLOGY

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ABSTRACT

The Balkan Peninsula represents a biogeographic crossroads characterised by complex geology, geomorphology, climate, and hydrology, together with exceptionally diverse vegetation shaped by centuries of human activity, including land use, agriculture, forestry, and pastoralism. These factors have contributed to one of the richest concentrations of biodiversity in Europe, and the region is widely recognised as an important biodiversity hotspot. Mycological research in the Balkans dates back to the early twentieth century. However, unlike the more extensively studied regions of Central and Western Europe, knowledge of the region's fungi remains fragmented and scattered across publications in multiple languages. The aim of this lecture is to provide an initial overview of mycological research (focusing primarily on macrofungi) in Macedonia, serving as a starting point for a more detailed and comprehensive study in the Balkan.

Selected case studies, partly based on fieldwork and participation in regional research projects and collaborative teams, are used to illustrate the practical and methodological challenges of developing mycological knowledge in Macedonia with uneven traditions of fungal collecting, limited taxonomic capacity,

and variable institutional support. These examples include the compilation of the first comprehensive checklists (focused mainly on Ascomycota and Basidiomycota), the development of preliminary and official fungal Red Lists, and ongoing efforts to document species distributions through targeted field surveys, fungarium studies, and citizen-science reported records.

Together, these examples demonstrate the importance of long-term regional and cross-border collaboration in advancing mycological research and knowledge. The lecture also considers the uneven state of knowledge regarding fungal diversity, conservation, and Red List assessments across the Balkans, reflecting differences in the history, intensity and institutional development of national mycological research, without attempting an exhaustive country-by-country account.

Rather than presenting a comprehensive species inventory, this overview offers a historical and methodological perspective on how mycological knowledge in Macedonia has been developed and continues to evolve. It also highlights the need for continued collaboration, coordinated inventories, digitisation of fungarium collections, molecular verification of records, taxonomic training, and harmonised regional approaches to fungal conservation.

KEY WORDS

Macedonian mycology; mycological history; fungal Red Lists; macrofungi checklists; conservation.

INVITED LECTURE

WHY FUNGI MATTER: FROM FOREST ECOSYSTEMS TO FUTURE INNOVATIONS

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ABSTRACT

Fungal biodiversity is increasingly recognized not only as a fundamental component of forest ecosystems but also as the foundation of a biodiversity-driven innovation ecosystem. The development of fungal-based solutions begins long before laboratory research – it starts with the exploration of natural ecosystems, where the discovery, identification and documentation of fungal diversity create the knowledge and biological resources required for future scientific and technological advances. Transforming biodiversity into innovation therefore requires an integrated and long-term strategy linking biodiversity research, interdisciplinary science and collaboration with industry.

The first stage of this process involves field exploration, taxonomy and molecular identification, supported by research infrastructures including fungaria, fungal culture collections and fungal extract libraries. These infrastructures preserve biological resources, ensure their long-term accessibility and provide reproducible material for future scientific and industrial research. The establishment and maintenance of such infrastructures increasingly rely on long-term strategic programmes that recognize biodiversity as a resource for future innovation rather than solely as an object of conservation. Investment in these research infrastructures should therefore be recognized

as a strategic prerequisite for future innovation rather than only as a contribution to biodiversity conservation.

The resulting biological resources become the foundation for systematic and hypothesis-driven screening and the development of multiple research pathways. Depending on the identified potential, these may include the discovery of novel bioactive metabolites for human and animal health, the development of cultivation technologies for promising fungal species, or the creation of fungal-based biomaterials and other sustainable biotechnological solutions. Equally important is the exploration of underinvestigated and taxonomically challenging fungal groups, whose extraordinary diversity remains largely untapped despite their considerable potential for future discoveries.

Scientific discovery alone, however, is insufficient to generate societal impact. Intellectual property protection, strategic project development and long-term partnerships with industry are essential components of an effective innovation ecosystem, enabling research outcomes to evolve into technologies, commercial products and practical applications. The integration of expertise spanning mycology, chemistry, biotechnology, engineering and entrepreneurship creates an environment in which biodiversity can be efficiently translated into innovation while strengthening regional and international innovation capacity.

Future fungal innovations will depend not only on scientific creativity but also on our ability to build integrated innovation ecosystems connecting forest exploration, biological collections, interdisciplinary research and industrial collaboration. In this perspective, fungal biodiversity should be regarded not as the final objective of research but as the starting point of an innovation ecosystem transforming biological resources into knowledge, technologies and sustainable solutions for society.

KEY WORDS

Fungal biodiversity, innovation ecosystem, biotechnology, bioeconomy.

ACKNOWLEDGMENTS

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MYCOLOGY

INVITED LECTURE

GANODERMA MBREKOBENUM: AN EMERGING MEDICINAL MUSHROOM

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ABSTRACT

Ganoderma mbrekobenum is an emerging medicinal mushroom from tropical Africa with demonstrated bioactive potential. However, commercial cultivation remains limited because of a lack of appropriate strains and large-scale cultivation technology. This study investigates the growth and yield performance of 21 G. mbrekobenum isolates under indoor and outdoor cultivation systems. We sterilized, inoculated, and incubated substrates composed of hardwood pellets and soybean hulls (1:1) under controlled conditions. We measured growth parameters, including mycelial colonization rate, time to pinning, fruit body formation, fresh weight, and biological efficiency (BE). Under outdoor cultivation, isolates 10 and 18 produced the highest average yields of 142.28 ± 25.31 g (BE = 10.82%) and 142.50 ± 12.34 g (BE = 10.84%), respectively, exceeding the parent strain yield of 133.50 ± 17.54 g (BE = 10.15%). In indoor cultivation, isolate 6 achieved the highest yield (225.75 ± 6.10 g; BE = 17.21%), followed by isolate 15 (216.67 ± 14.78 g; BE = 16.51%), both outperforming the parent (166.42 ± 8.47 g; BE = 12.68%). These results surpass previous reports on G. mbrekobenum productivity and are comparable to yields of other Ganoderma

species. Collectively, the findings highlight the potential of G. mbrekobenum as a viable candidate for commercial cultivation, particularly in tropical regions.

KEY WORDS

Ganoderma mbrekobenum, Outdoor, Indoor, Progenies, Cultivation, medicinal mushroom.

MYCOLOGY

INVITED LECTURE

MORE THAN A MYTH: MYCOTHERAPY UNVEILED THROUGH MODERN SCIENCE

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ABSTRACT

Medicinal mushrooms have been used in traditional medicine for centuries, yet many of their therapeutic claims have remained scientifically unverified. Our research has focused on the investigation of mushroom-derived metabolites as a source of bioactive compounds with potential pharmaceutical, biomedical, and food applications. Obtained results of studies on *Agaricus blazei, Laetiporus sulphureus*, and *Trametes versicolor*, together with other medicinal and wild mushroom species, illustrate how multidisciplinary research integrating natural products chemistry, microbiology, pharmacology, and food science has revealed the considerable biotechnological and biomedical potential of mushroom-derived metabolites.

Our research combines the characterization of phenolic compounds, terpenoids, polysaccharides, and other secondary metabolites with microbiological, biochemical, and cell-based assays. Antimicrobial studies demonstrated that *A. blazei* extracts exhibited anti-quorum sensing activity against *Pseudomonas aeruginosa* at sub-inhibitory concentrations (0.025–0.1 mg/mL), reducing biofilm formation and virulence without affecting bacterial growth. Several mushroom extracts also displayed

pronounced antimicrobial and antibiofilm activities against clinically relevant pathogens.

Another major research focus addresses wound-healing and metabolic disorders. In an *in vivo* rat wound model, treatment with *A. blazei* extracts resulted in up to 30% faster wound closure compared with untreated controls, supporting their potential for further preclinical development. In parallel, antioxidant activity and inhibition of α-amylase and α-glucosidase were evaluated as indicators of antidiabetic potential, with several extracts showing strong antioxidant capacity and concentration-dependent enzyme inhibition.

Beyond biomedical applications, mushroom-derived metabolites have also been explored as natural food preservatives because of their combined antimicrobial and antioxidant properties, with the potential to improve food quality, microbiological safety, and shelf life.

Collectively, our findings provide compelling evidence that mushrooms are far more than subjects of traditional folklore. Their secondary metabolites represent a valuable source of novel bioactive compounds with considerable potential for the development of future therapeutics, functional foods, and natural preservation systems.

KEY WORDS

Medicinal mushrooms, Fungal metabolites, Antimicrobial activity, Wound healing, Antidiabetic potential.

ACKNOWLEDGMENTS

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INVITED LECTURE

FROM DIVERSITY TO DIALOGUE: ECTOMYCORRHIZAL SYMBIOSES IN BRAZIL AND THE MOLECULAR LANGUAGE OF FUNGAL-PLANT INTERACTIONS

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ABSTRACT

Ectomycorrhizal symbioses are among the most important plant–fungal interactions in terrestrial ecosystems, playing key roles in plant nutrition, community dynamics, and ecosystem functioning. Despite their ecological relevance, ectomycorrhizal associations in tropical and subtropical South America remain considerably less explored than those of temperate regions. The main focus of this research is ectomycorrhizal diversity in Brazilian ecosystems, with particular emphasis on studies conducted in coastal sandy environments, restinga, in Southern Brazil. Integrating field observations, fungal identification, and morpho-anatomical characterization of ectomycorrhizas, this work revealed a diverse assemblage of ectomycorrhizal fungi, including representatives of Boletales and other major ectomycorrhizal lineages. Roots and soil were sampled in three restinga areas on Santa Catarina Island; fungal and plant partners were identified by ITS rDNA sequencing of ectomycorrhizas and basidiomata, and roots and leaves, respectively. A survey of available records documented 74 ectomycorrhizal fungal taxa in Brazilian restinga. In the field study, 354 roots bearing ecto-

mycorrhizal mantles were collected and 73 morphotypes were differentiated; 19 yielded high-quality ITS sequences, 12 associated with *Guapira opposita*, and /tomentella-thelephora represented 11 of the 19 sequenced morphotypes. Among these associations, the investigation led to the recognition and description of a previously unreported ectomycorrhizal subtype, guapirioid, highlighting how much of the structural and functional diversity of tropical ectomycorrhizas remains to be uncovered. This subtype is characterized by a conspicuous mantle but no Hartig net or fungal penetration into the root cortex; determinate root tips also lack a typical root cap and protoderm. Beyond documenting this diversity, understanding ectomycorrhizal symbiosis also requires investigating the molecular mechanisms that allow fungi and plants to recognize, communicate with, and accommodate each other. These ecological and morphological observations are connected with the ongoing research on mycorrhiza-induced small secreted proteins (MiSSPs), fungal proteins implicated in the molecular dialogue between ectomycorrhizal partners and in the establishment of symbiosis. Structural modelling and interaction analyses with *Laccaria bicolor* indicated that MiSSP13 and MiSSP16.5 interact with papain- and pepsin-like plant proteases at regions compatible with inhibitory activity, supporting possible inhibition of cysteine and aspartate proteases during symbiosis establishment. By moving from fungal diversity and ectomycorrhizal morphology to molecular signalling, this talk aims to illustrate the multiple biological scales through which ectomycorrhizal interactions can be investigated and to emphasize the importance of integrating biodiversity research with functional approaches to better understand these complex plant–fungal associations.

KEYWORDS

Ectomycorrhiza; restinga; *Guapira opposita*; root determinacy; MiSSPs.

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MYCOLOGY

INVITED LECTURE

VIABILITY AND DIVERSITY OF YEASTS IN KOMBUCHA BEVERAGE

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ABSTRACT

Kombucha is a traditional fermented beverage with a history of several thousand years in the East and yet is quite popular today in the West. Kombucha is traditionaly prepared by fermenting black tea, sweetened with sugar, with what is popularly known as a "tea fungus" (symbiotic consortium of yeasts and acetic acid bacteria). The spatial distribution, quantitative kinetics, and phylogenetic diversity of the yeast community directly dictate the structure of metabolites of the beverage by modulating reducing sugar availability and precursors for downstream bacterial organic acid synthesis. The main factors that cause diversity among kombucha yeasts are: environmental factors such as geographical and climatic conditions, influence of fermentation substrate, incubation conditions, quality and quantity of inoculum etc. Kombucha yeasts can be divided into four groups: acidotolerant non-saccharomyces yeasts, traditional highly-fermentative genera, oxidative and film-forming genera and osmotolerant genera. From different kombucha beverages the following yeast genera were isolated and identified: *Zygosaccharomyces, Candida, Kloeckera, Hanseniaspora, Torulaspora, Pichia, Brettanomyces, Saccharomyces, Lachancea, Saccharomycoides, Schizosaccharomyces*, *Kluyveromyces, Mycotorula, Mycoderma, Torula* etc. Methods for the isolation and identification of yeasts from kombucha are: conventional methods based on

morphological and physiological tests, rapid identification tests like API or Vitek2 Compact System and molecular methods (analyses of 26S RNA region or metagenomics analyses). As for the number of yeasts in kombucha it supports a typical growth curve for yeast. An initial population of yeast depends on used inoculum and is about 5-6 log units, after that an exponential phase follows in the next 24 hours and reach the peak at about 7 log units/mL. This is followed by a stationary phase which lasts almost until the end of fermentation. After that, the death phase appears. In kombucha beverage a spetial distribution assimetry of yeasts is present. The number of yeasts are 1-2 log units higher in cellulose pellicle than in liquid phase, because of enhanced physical protection and high localized nutrient density. In conclusion, future strategies wich lead to kombucha standardization are: regulation of yeasts fermentation to avoid over production of ethanol, isolation of low-ethanol strains, create starter cultures (syntetic communities) from specific yeasts and acetic acid bacteria depends on beverage we want to obtain, manipulation with inoculum ratio, improvement of nutritional value of beverage, adding encapsulated probiotic bacteria etc.

KEY WORDS:

kombucha; yeasts; fermenataion; diversity; viability

ACKNOWLEDGMENTS

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BOLETALES WORKSHOP: DIVERSITY, TAXONOMY AND MOLECULAR IDENTIFICATION

LECTURE

THE ORDER *BOLETALES* IN THE NEOTROPICS: THE CASE OF GUATEMALA AND ITS PARTICULAR DIVERSITY

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ABSTRACT

Historically, certain fungal taxa were assumed to exhibit cosmopolitan distributions, however current molecular evidence confirms high continental endemism, with exceptional few species such as *Amanita muscaria* and *Boletus edulis*, that are distributed in Asia, Europe and North America.

The neotropical realm, spanning from the Southern United States to the Southern Cone, harbors high level of endemism in Boletales, including unique genera such as *Singeroporus*, *Phylloporopsis*, *Phylloboletellus* or *Garcileccinum*. Despite this diversity, generic richness exhibits a latitudinal gradient, decreasing from 65 genera in North America to 26 in South America (including introduced taxa). This pattern is heavily influenced by distinct vegetational and orographic variations that dictate fungal survival, dissemination and mycorrhizal symbioses.

Situated in Northern Central America, Guatemala represents one of the most thoroughly documented regions for *Boletales* on the continent. It flanked by two oceans and characterized by

a volcanic chain at the south and, at the northwest and east — with mountain chains reaching more than 3000 meters a.sl.; this has created ecological refugia for fungi of North American affinity. Guatemala serves as the southern distribution limit for several northern taxa as *Boletus edulis* s.s. Conversely, lowlands and mid-elevation habitats harbor a distinct diversity shared with other countries in the isthmus or the Caribbean, including *Amoenoboletus*, *Aureoboletus*, *Boletellus*, *Heimioporus*, *Neoboletus*, *Phylloporopsis*, and *Rubroboletus* genera. To date, 52 genera of *Boletales* have been documented in Guatemala. The most species-rich lineages include *Boletus*, *Hemileccinum*, *Neoboletus*, *Phylloporus*, *Porphyrellus*/*Neoporphyrellus*, *Tylopilus*, *Suillus*, and *Xerocomus*. Recent taxonomic additions further expand this diversity within *Veloporphyrellus*, *Alloboletus*, *Aureoboletus*, *Imleria*, *Lanmaoa*, *Neoboletus*, and *Tylopilus*.

Some genera represent a challenge for correct identification at first sight: this is the case of some reticulated *Neoboletus* or the reddish *Rubroboletus* and *Imperator*. A particular challenge in identification is local *Boletus* s.str. group related to the European *B. reticulatus*, which has thinner stipe and different grades of reticulation and have more than 6% of difference in complete ITS region. It is assumed that there are at least five or six new species in this group that could be distributed only in Mesoamerica, according to phylogenetic analyses.

BOLETALES WORKSHOP: DIVERSITY, TAXONOMY AND MOLECULAR IDENTIFICATION

LECTURE

THE MOST COMPREHENSIVE PAN-EUROPEAN STUDY OF THE GENUS *HORTIBOLETUS* (*BOLETACEAE*)

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ABSTRACT

Hortiboletus is one of the most taxonomically difficult genera for species identification in the family *Boletaceae*. We conducted a detailed morphological and molecular re-assessment of European and Levantine species of *Hortiboletus*, based on more than 240 collections from almost the entire Europe and Israel. A total of 133 collections were sequenced. *Hortiboletus hershenzoniae* is a newly described species from Israel and also found in Lebanon. It is sister to *H. engelii* and associated with the evergreen oak *Quercus calliprinos* and potentially with *Q. ithaburensis*. Accurate morphological descriptions, type studies, biogeography, macro- and microphotos and a historical overview on the nomenclatural issues surrounding *H. rubellus, H. bubalinus, H. engelii*, and *H. hershenzoniae* were given. An epitype collection is designated for *H. rubellus*. *Xerocomus erubescens* is confirmed to be a synonym of *H. engelii*, based on DNA barcoding of its isotype. We studied type specimens of a few American taxa and proposed a taxonomic combination *Hortiboletus flavorubellus*, which came out to be conspecific with *Boletus rubellus* var. *flammeus*. *Boletus harrisonii* proven to be conspecific with *H. campestris*. Our multilocus phylogenetic analysis of four markers (ITS, LSU, tef1-α, and rpb2) reaffirmed that *Hortiboletus* is a sister genus to *Xerocomellus*. Using the Genealogical Concordance Phylogenetic Species Recognition method, at least 19 phylogenetic species and eight putative phylogenetic species of the genus *Hortiboletus* can be delimited.

Based on multilocus analysis, it contains from 24 to 25 species-level clades worldwide, 17 out of which represent known species, one newly described and potentially six to seven undescribed species. After Biketova et al. (2025) publication, one more species has been described – *H. swaticus* (Ullah et al. 2025). However, based on our analysis it clusters in *H. indorubellus* clade. Tandem repeats within the ITS region were reported for the first time in the genus *Hortiboletus* and in the entire subfamily *Boletoideae*. AC-reach microsatellite inserts were detected in the ITS1 region of some species (including *H. rubellus, H. bubalinus*), with intraspecific and intrageneric difference in length up to 12 bp. The ITS2 region of ten *Hortiboletus* species has long, minisatellite-like tandem repeat insertion ranging from (103) 133 to 253 bp with 2.0–3.2(–5.3) copy number of repeats. Due to frequent intragenomic polymorphisms especially within these insertions, the ITS region of both *H. rubellus* and *H. bubalinus* has been difficult to sequence with the classical Sanger method and cloning has been performed to obtain full ITS sequences of some collections.

KEYWORDS

Boletales taxonomy; biogeography; diversity; phylogeny; tandem repeats; xerocomoid fungi.

BOLETALES WORKSHOP: DIVERSITY, TAXONOMY AND MOLECULAR IDENTIFICATION

LECTURE

UNCOVERING FUNGAL DIVERSITY FOR CONSERVATION: DNA BARCODING OF *BOLETALES* IN THREATENED HIMALAYAN PINE ECOSYSTEMS OF PAKISTAN

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ABSTRACT

Boletales are keystone ectomycorrhizal fungi that form symbiotic associations with pines, enhancing nutrient uptake, soil stability, carbon cycling, and forest resilience under climate stress. Despite their ecological importance, their diversity in Pakistan's Himalayan pine ecosystems remains poorly documented, limiting conservation planning in these threatened habitats. This study integrates extensive field surveys, morpho-anatomical characterization, and ITS-based DNA barcoding, complemented by nrLSU barcoding (where necessary for phylogenetic analysis) to assess pileate-stipitate *Boletales* diversity across ecological and altitudinal gradients of northern Pakistan. Many specimens were collected from biodiversity-rich northern areas of Pakistan. Taxonomic and phylogenetic analyses revealed 50 species distributed across different genera, including *Aureoboletus*, *Austroboletus*, *Baorangia*, *Boletinellus*, *Boletus*, *Caloboletus*, *Gyroporus, Hortiboletus*, *Leccinum*, *Neoboletus*, *Porphyrellus*, *Rheubarbariboletus*, *Rubroboletus*, *Suillus, Veloporphyrellus*, *Xanthoconium*, and *Xerocomellus*, with *Bole-*

taceae emerging as the most dominant family. By documenting taxonomic novelties, new distributional records, and molecular insights, this study significantly enriches the mycological inventory of Pakistan. Importantly, it underscores the role of *Boletales* as ecological stabilizers and bioindicators of forest health, providing a baseline framework for advancing fungal systematics, guiding sustainable forest management, and integrating fungal diversity into conservation strategies for threatened Himalayan pine ecosystems.

KEY WORDS

Biodiversity hotspot, ECM, Species conservation, boletoid fungi, South Asia.

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BOLETALES WORKSHOP: DIVERSITY, TAXONOMY AND MOLECULAR IDENTIFICATION

LECTURE

SPORE MORPHOMETRY IN *BOLETALES*: HOW HIDDEN TRAITS MAY SEPARATE CRYPTIC SPECIES

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ABSTRACT

Fungal taxonomy increasingly recognises that many species complexes, once treated as a single taxon, are morphologically cryptic under classical macro and microscopic analyses. Yet the absence of an obvious difference does not mean no structural difference exists. Across disciplines, from human muscle lipid droplets to fibroblast mitochondrial networks to the internal order of engineered materials, patterns invisible to casual observation become measurable once the right analytical lens is applied. We propose that spore morphology in *Boletales* holds comparable hidden structure, and that quantifying it can raise taxonomic resolution for separating cryptic species that field and molecular characters alone struggle to resolve.

Our objective was twofold: first, to demonstrate that segmented and measured spore traits can discriminate closely related *Boletales* taxa; second, to establish a pipeline extending this approach to the taxonomically difficult family *Sclerodermataceae*.

We tested this using *Cyanoboletus pulverulentus* and *Cyanoboletus mediterraneensis*, a pair long separated with diffi-

culty using conventional characters, recently addressed in our published work. Basidiospores were imaged by light microscopy, individually segmented, and measured for dimensions and convex hull in order to evaluate suprahilar depression. These traits were compared statistically between species. In parallel, we are developing standardised image acquisition protocols, including consistent focal-plane sampling across multiple imaging depths, and beginning to apply supervised (U-Net, YOLO) and unsupervised (DINO) deep learning models to extract features beyond what manual measurement captures, with initial application planned for *Scleroderma* and *Pisolithus* within *Sclerodermataceae*.

Quantified spore traits reliably separated *Cyanoboletus pulverulentus* from *Cyanoboletus mediterraneensis*, confirming that real, measurable morphological differences underlie what field mycologists often treat as a difficult or inseparable pair. Critically, this separation did not require machine learning: careful segmentation and measurement of already visible characters proved sufficient on its own. Deep learning methods are being layered on as a complementary, not essential, tool, expected to reveal additional and less obvious features in more morphologically homogeneous groups such as *Scleroderma* and *Pisolithus*, where diagnostic characters may be harder to define by eye.

These results support spore morphometry as a valid and scalable axis of taxonomic evidence for *Boletales*, standing alongside molecular data rather than beneath it. As we extend this framework to *Sclerodermataceae*, we expect morphometric characters, whether extracted manually or through AI assisted segmentation, to contribute meaningfully to resolving cryptic diversity in groups where DNA alone has struggled to draw clean boundaries, strengthening the taxonomic and conservation value of fungal collections worldwide.

KEYWORDS

Spore morphometry; cryptic species; *Boletales*; image segmentation; *Sclerodermataceae*.

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BOLETALES WORKSHOP: DIVERSITY, TAXONOMY AND MOLECULAR IDENTIFICATION

LECTURE

STRANDED ON A LARGE ISLAND: ECOLOGICAL NOTES ON *BOLETACEAE* IN SARDINIA

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ABSTRACT

The family *Boletaceae* (*Boletales*, *Basidiomycota*) represents an important component of Mediterranean fungal communities, with several species playing key ecological roles through ectomycorrhizal associations with woody plants. Sardinia, a large Italian island characterized by a heterogeneous landscape, a predominantly Mediterranean climate, and a remarkable diversity of sclerophyllous and deciduous plant communities, provides a variety of habitats suitable for boletes. Nevertheless, ecological information on the distribution, habitat preferences, and host associations of Sardinian *Boletaceae* remains relatively fragmented. Our ongoing study provides ecological observations on selected *Boletaceae* occurring in Sardinia, focusing on their habitat characteristics, associated vegetation, putative ectomycorrhizal hosts, phenology, and substrate preferences. Field observations indicate that *Boletaceae* are primarily associated with mature or well-established woodland

and shrubland communities, particularly those dominated by *Quercus* spp., *Arbutus unedo*, *Cistus* spp., and other Mediterranean woody hosts. These ecological associations highlight the importance of conserving structurally complex Mediterranean forests, woodlands, and shrublands as reservoirs of fungal diversity. Particular attention should be given to habitats supporting rare or geographically restricted taxa, whose persistence may be threatened by habitat fragmentation, recurrent fires, changes in land management, and increasing climatic variability. Further systematic surveys integrating fungal identification, vegetation analysis, soil characterization, and molecular approaches to mycorrhizal associations are needed to clarify the ecology and conservation status of Sardinian *Boletaceae*. Such information would contribute to a better understanding of Mediterranean fungal diversity and support evidence-based strategies for fungal conservation in Sardinia.

KEYWORDS

Boletaceae; Sardinia; Mediterranean ecosystems; ectomycorrhizal associations; fungal diversity

BOLETALES WORKSHOP: DIVERSITY, TAXONOMY AND MOLECULAR IDENTIFICATION

LECTURE

THE *BOLETALES* DIVERSITY AND THEIR ECTOMYCORRHIZAE FROM PURE OAK FORESTS OF SWAT, PAKISTAN

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ABSTRACT

Boletales are strongly implicated as ectomycorrhizal (ECM) symbionts with an array of ectotrophic plants, including members of *Pinaceae*, *Fagaceae*, *Myrtaceae*, *Fabaceae*, and *Phyllanthaceae*. These fungi are essential for plant nutrition and water uptake, which contributes to the overall productivity and biodiversity of forest ecosystems. *Quercus* is the most diverse genus of ECM host plants; it is distributed in the Northern and Southern Hemispheres, from temperate to tropical regions. However, fungal community structure of oaks was poorly studied not only in Pakistan but in entire South Asia in comparison to other parts of the world.

Previous studies of oak-associated fungal diversity in our country have been based solely on sporocarp collections. However, to obtain a complete picture of the fungal community, it is important to analyze environmental samples: plant root tips and soil samples. This information is important to integrate oak forests into global biodiversity conservation strategies and to understand what actions are required to conserve them and their biological components, including fungi, which are known

for their exceptionally high diversity levels. For soil and ECM root samples, we used Sanger sequencing for basidiome and morphotypes and Illumina high throughput sequence analyses for soil of the ITS region. These findings exhibit diverse and rich ECM communities associated with oaks. After morpho-anatomical characterization of sporocarps, followed by multilocus (ITS, LSU, *rpb1*, *tef1-α*) phylogenetic analyses, different species of *Boletales* have been detected. The species diversity detected in ECM roots matched with those obtained in above-ground survey, which confirms the ECM nature of these fungi and their hosts. The data obtained by Illumina sequencing unveil hidden diversity of *Boletales* associated with oaks. The generated data can be used in forest management especially in afforestation and reforestation, as well as nursery management.

KEY WORDS

Boletoid fungi; ectomycorrhizal fungi; below-ground survey; environmental sampling; next generation sequencing.

POSTER PRESENTATION

AFLATOXINS B1 AND M1 IN THE ESTIMATES OF FOODBORNE DISEASE BURDEN

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ABSTRACT

Aflatoxins are major foodborne mycotoxins with well-established carcinogenic potential, particularly in relation to hepatocellular carcinoma (HCC). The 2026 update of the World Health Organization (WHO) estimates of the global burden of foodborne disease provides revised burden estimates for aflatoxin B1 (AFB1) and, for the first time, incorporates aflatoxin M1 (AFM1). This study examines both the resulting burden estimates and the methodological framework underlying their derivation.

AFB1 exposure was estimated from contamination of maize and peanuts, whereas AFM1 exposure was assessed through milk. For both toxins, population-attributable fractions for HCC were derived from country-specific dietary exposure and carcinogenic potency factors using a multiplicative model accounting for the interaction between aflatoxin exposure and hepatitis B virus infection.

In 2021, AFB1 was estimated to cause 14,000 incident HCC cases (95% uncertainty interval [UI]: 5,920–28,000), 13,200 deaths (5,570–26,400), and 412,000 disability-adjusted life-years (DALYs; 179,000–813,000) globally. AFB1 represented the largest disease burden among the assessed non-metal chemical hazards, with the highest regional DALY rates in South-East Asia (8.81

per 100,000 population) and Africa (8.04 per 100,000). Among the nine chemical hazards evaluated, AFB1 was the third largest contributor to mortality, accounting for approximately 1.2% of attributable deaths. In contrast, AFM1 accounted for only 29 HCC cases (16–48), 27 deaths (15–45), and 895 DALYs (487–1,570), corresponding to a global DALY rate of 0.011 per 100,000. Global and regional trajectories indicated declining AFB1- and AFM1-attributable DALY rates between 2000 and 2021. However, the estimates likely underestimate the overall health burden of aflatoxins because AFB1 exposure was restricted to maize and peanuts, AFM1 exposure to milk, and the quantified disease burden to HCC. Acute aflatoxicosis, AFB1-associated impaired growth, exposure through other cereals, co-occurring mycotoxins, and combined effects of chemical mixtures were not incorporated.

The estimates reinforce the substantial global public-health importance of AFB1 while indicating a comparatively negligible contribution of AFM1 to HCC burden and highlighting important methodological constraints that should inform interpretation of these estimates.

KEY WORDS

Food; Mycotoxin; Hepatocellular carcinoma; DALY; Public health.

ACKNOWLEDGMENTS

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POSTER PRESENTATION

THE CAPACITY OF CONTROL BODIES AND LABORATORIES IN SERBIA FOR SAMPLING AND TESTING MYCOTOXINS

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ABSTRACT

Widespread and frequent occurence of mycotoxins is significant problem in Serbia that pose serious health hazardous effects in humans and animals. All preventive measures and strategies for control of mycotoxins include engagement of control bodies and testing laboratories. Representativeness and validity of data proved to be of high importance in tackling the problem. In order to obtain representative and valid data various regulation regarding capacity of accredited organization must be fulfilled. By applying the methodology (the Accreditation Body of Serbia's website was searched), it was determined that 32 laboratories in Serbia have accredited methods for testing at least one of the regulated mycotoxins. Only 6 of them have methods necessary to test all mycotoxins in all commodities which are regulated by the Rule book on maximum concentration of certain contaminants in food (Official Gazette no. 73/2024, 90/2024, 47/2025, 61/2025 and 103/2025). Five of them are coming from private sector and just one is public. All of the six laboratories use standard methods. On the other hand, it was determined that only 5 control bodies have accredited methods for sampling in order to test mycotoxins. All of them are coming from the

private sector. Unfortunately, none of them have accredited the latest regulated method for sampling which is defined by the Rule book on methods of sampling and testing food for determination of presence and levels of certain contaminants (Official Gazette no. 103/2024). It was determined that the capacity of the laboratories, in both the private and the public sector, for testing mycotoxins in Serbia were sufficient. However, the capacity of control bodies for sampling needs further improvements.

KEY WORDS

Mycotoxins, laboratories, control bodies.

CLIMATE-DRIVEN AFLATOXIN TRENDS ALONG THE MAIZE–MILK FOOD AND FEED CHAIN IN SERBIA

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ABSTRACT

Climate change has fundamentally reshaped the pattern of aflatoxin occurrence in Serbia, shifting from years with no detectable contamination to sporadic contamination and, ultimately, to recurrent contamination affecting the entire maize–milk food and feed chain. As one of Europe's leading maize producers and exporters, Serbia has experienced recurrent aflatoxin contamination for more than a decade, posing significant challenges to food and feed safety and security, animal production, public health, and agricultural sustainability.

This study aimed to evaluate long-term climate-driven trends in aflatoxin contamination along the Serbian maize–milk food and feed chain and to assess their implications for food and feed safety. Multi-year monitoring data on aflatoxins in maize (2009–2025) were integrated with long-term monitoring data on aflatoxin M1 (AFM1) occurrence in milk and dairy products. Contamination trends were interpreted in the context of climatic conditions, with particular emphasis on drought episodes and elevated summer temperatures.

The results demonstrated pronounced year-to-year variability in aflatoxin occurrence, ranging from years with no

detectable aflatoxins or contamination frequencies below 20% to contamination frequencies exceeding 80% during seasons characterized by severe drought and heat stress. Since 2021, aflatoxins have been consistently detected in maize, with contamination frequencies above 50% and individual concentrations exceeding 100 μg/kg in the most affected seasons. The long-term data showed that years with elevated aflatoxin B1 contamination of maize were consistently associated with the occurrence of AFM1 in milk and dairy products, demonstrating the carry-over of contamination through the maize–milk food and feed chain.

In addition to its implications for food and feed safety, recurrent aflatoxin contamination poses a substantial economic challenge for Serbia, affecting maize exports, livestock production, and the dairy sector.

The long-term trends observed in this study indicate that climate change is transforming aflatoxin contamination from an episodic event into a recurring challenge, underscoring the need for integrated monitoring and climate-informed risk management across the entire maize–milk food and feed chain.

KEY WORDS

Aflatoxins, maize, milk, Serbia, climate change.

ACKNOWLEDGMENTS

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A TWO-YEAR SURVEY OF AFLATOXIN B1 OCCURRENCE IN MAIZE AND MAIZE SILAGE IN SERBIA

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ABSTRACT

Aflatoxins are naturally occurring harmful substances produced as secondary metabolites mostly by Aspergillus spp. Among them, aflatoxin B1 (AFB1) is considered one of the most toxic and carcinogenic aflatoxins, with a particular concern due to its hepatotoxic effects and the possible transfer of its metabolite, aflatoxin M1, into milk. Maize and maize silage are frequently used as important feed materials in animal nutrition and may represent a potential source of AFB1 exposure. The aim of this study was to determine the content of AFB1 in maize and maize silage samples collected from diverse agricultural farms in Serbia, mostly from the territory of the AP Vojvodina as the most important agricultural area, during two harvesting years. The samples were analysed using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. A total of 148 samples were analysed, including 47 maize and 37 maize silage samples in 2024, while 28 maize and 36 maize silage samples were collected and analysed in 2025. In the first sampling year AFB1 was detected in 23 (48.94%) maize samples, with an average concentration of 54.22 μg/kg (ranging from 3 to 184 μg/kg). In the

second year AFB1 was present in 17 (60.71%) maize samples, with an average concentration of 53.76 μg/kg (range from 8 to 141 μg/kg). Regarding maize silage samples, AFB1 was detected in 20 (54.05%) samples, with an average level of 12.82 μg/kg (ranging from 2 to 42 μg/kg) for 2024, while in 2025 there were 8 (22.22%) positive samples ranging from 2 to 6 μg/kg with 4.38 μg/kg as average. When comparing found results with maximum permitted levels established by Serbian regulations, it is apparent that in harvest years of 2024 and 2025 had 29.79% and 42.86% of total maize samples exceed those limits, however, when compared with EU regulation, these percentages rise to 34.04% and 46.43%, respectively. Among the maize silage samples analysed in 2024 only 2.7% (1) of total samples exceeded Serbian regulation, whereas 13.51% (5) exceeded EU regulation, while none of the samples analysed in 2025 exceeded regulations. These findings emphasize the importance of regular monitoring of AFB1 contamination in maize and maize silage used for animal feed, together with the adoption of good agricultural and storage practices to uphold feed safety standards and safeguard animal health.

KEY WORDS

AFB1, animal feed, ELISA, Serbian regulation, EU regulation.

ACKNOWLEDGMENTS

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MYCOTOXIN OCCURENCE IN NUTRIENT--DENSE FOODS IN NORTH MACEDONIA: A SIX-YEAR SURVEILLANCE STUDY

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ABSTRACT

The increasing consumer preference for nutrient-dense foods—such as whole-grain cereal bars, dried fruits, nuts, spices, and breakfast cereals—is driven by their rich nutritional and functional profiles, which include essential dietary fibers, vitamins, minerals, antioxidants, and anti-inflammatory bioactive compounds. However, these products are inherently vulnerable to contamination by toxigenic fungi. This food safety risk is further exacerbated by shifting environmental dynamics and climate change, which actively alters fungal ecology and accelerates the geographic spread of mycotoxin proliferation. To address these emerging challenges, a comprehensive and systematic six-year longitudinal surveillance program was conducted between 2020 and 2025 to evaluate the occurrence, concentration, and potential co-occurrence of major regulated mycotoxins, including aflatoxins (AFs), ochratoxin A (OTA), zearalenone (ZEN), and deoxynivalenol (DON), across 978 samples collected from retail markets. Following rigorous sample extraction and clean-up using specific immunoaffinity columns, the mycotoxins

were quantified using high-performance liquid chromatography with fluorescence detection (HPLC-FLD), while DON concentrations were determined using an enzyme-linked immunosorbent assay (ELISA). Both analytical methodologies were strictly validated and accredited according to ISO/IEC 17025 standards, and the resulting dataset was assessed against the Macedonian Regulation on Maximum Levels of Certain Contaminants in Food, which is fully harmonized with European Union legislation via Commission Regulation (EU) 2024/1022. The surveillance findings demonstrated a reassuringly high level of regulatory compliance, with most samples containing mycotoxin concentrations well below the established maximum levels (MLs). Specifically, DON was detected in only 7.9% of granola breakfast cereal samples at levels ranging from 21.75 to 208.05 μg/kg, safely below the 500 μg/kg ML. Among 37 analyzed spice samples, two *Capsicum* spp. samples were positive for aflatoxins (12.35 to 42.44 μg/kg), while OTA was detected in several ginger samples, at concentrations ranging from 0.37 to 8.85 μg/kg. No presence of ZEN was detected in any of the tested samples. Dried fruits exhibited low levels of aflatoxins (0.73 to 1.27 μg/kg) and OTA (0.20 to 1.27 μg/kg), and notably, no co-occurrence of multiple mycotoxins was observed in any sample. Ultimately, while these results confirm overall market safety, the localized detection of these regulated toxins highlights the critical need for continuous, long-term monitoring programs to proactively safeguard public health against climate-driven food safety threats.

KEY WORDS

Mycotoxin surveillance; nutrient-dense food; food safety.

MYCOTOXINS IN PET FOOD: A RECENT OUTBREAK IN DOGS

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ABSTRACT

The harmful effects of mycotoxins in food on the health and productivity of domestic animals and humans are well recognized and are addressed through regulations, research, prevention, and control measures. In contrast, mycotoxicosis in companion animals has received considerably less attention. This is particularly concerning in dogs, which share a close relationship with humans and are increasingly regarded as family members. Despite this unique status, the risks associated with mycotoxin exposure in dogs remain insufficiently recognized and investigated. Commercial dry dog food is primarily manufactured from animal by-products but also contains plant-derived ingredients, particularly soy products and cereal grains such as corn, wheat, rice, barley, and sorghum. Although dogs do not have a strict nutritional requirement for carbohydrates, these ingredients are widely incorporated into commercial diets because they are inexpensive, nutritionally valuable, and have little effect on palatability or digestibility. However, cereals and other plant ingredients are also common sources of mycotoxin contamination. Among the most frequently detected mycotoxins in pet food are aflatoxins (AFs), deoxynivalenol (DON), fumonisins (FUM), ochratoxin A (OTA), and zearalenone (ZEA). Despite their frequent occurrence, information on their prev-

alence, toxicological significance, and long-term health effects in dogs remains limited. Compared with food-producing animals, dogs may be particularly susceptible to chronic mycotoxin exposure because of their relatively long lifespan and prolonged consumption of the same commercial diets. Nevertheless, studies evaluating the effects of mycotoxins on canine health, metabolism, immune function, and overall well-being remain scarce. Published clinical reports, together with a recent outbreak of aflatoxicosis in Serbia, emphasize the need for greater attention to this issue. The Serbian outbreak occurred in three dog shelters and resulted in the deaths of 19 mixed-breed dogs within days of clinical onset. Affected animals developed jaundice, anorexia, polydipsia, apathy, abdominal distension, hemorrhagic diarrhea, and melena. Blood biochemistry revealed markedly elevated liver enzyme activities, while postmortem examination demonstrated icterus, ascites, extensive hemorrhages, hepatic fibrosis, and liver necrosis. Analysis of the commercial dry dog food fed for more than two months detected an average aflatoxin concentration of 150 μg/kg, confirming contaminated feed as the likely source of intoxication. This paper aims to increase awareness of canine susceptibility to mycotoxins and emphasize the importance of effective mycotoxin monitoring and control in pet food through the combined efforts of manufacturers, regulatory authorities, veterinarians, and dog owners, particularly in the absence of specific regulatory limits for mycotoxins in pet food.

KEY WORDS

Dog food, mycotoxicosis, pet animals.

ACKNOWLEDGMENTS

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DIETARY PROBIOTICS AS A SUSTAINABLE STRATEGY TO PRESERVE GUT BARRIER INTEGRITY AND MITIGATE HEPATOTOXICITY DURING MYCOTOXICOSIS IN BROILERS

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ABSTRACT

Mycotoxin contamination of poultry feed, particularly by cytotoxic trichothecenes like T-2 toxin, compromises avian welfare, drives economic losses, and challenges global food safety. Because conventional mycotoxin binders often exhibit variable binding affinities, alternative, sustainable strategies are urgently required to combat mycotoxicosis by reinforcing natural physiological defenses. This study evaluated the efficacy of dietary probiotic supplementation utilizing *Enterococcus faecium* as a functional intervention to preserve gut barrier integrity and protect hepatic health during a mycotoxin challenge. Broiler chickens were allocated into experimental groups receiving a standard basal diet, a T-2 toxin-challenged diet, probiotic supplementation alone, or a combined toxin-probiotic regimen.

Histopathological evaluation confirmed that the liver and gastrointestinal tract are primary target organs during mycotoxicosis, demonstrating a clear tissue-specific response. In toxin-exposed cohorts, T-2 toxin provoked acute, dose-dependent

hepatotoxicity, characterized by a profound loss of classical hepatic lobular architecture, localized zones of parenchymal necrosis, prominent sinusoidal dilation, and interlobular mononuclear cell infiltration. Widespread cytoplasmic fatty degeneration was also recorded across all toxin-only birds. Conversely, the duodenal mucosa exhibited remarkable structural resistance to direct trichothecene-induced necrosis or villus atrophy. However, mycotoxin exposure traditionally threatens the gut barrier by downregulating crucial mucosal components.

Notably, dietary inclusion of the probiotic served a dual protective role, effectively sustaining both liver health and gut barrier homeostasis. In the combined toxin-probiotic groups, severe hepatic tissue necrosis and parenchymal destruction were significantly minimized, transitioning instead to a stable macrovesicular lipid profile within hepatocytes. Simultaneously, within the duodenum, probiotic administration actively reinforced the first line of host mucosal defense, inducing a marked, visible proliferation of secretory goblet cells within the intestinal epithelium. This upregulation of goblet cells counteracts toxin-induced barrier vulnerability by enhancing protective mucus secretion and shielding underlying enterocytes. These findings demonstrate that utilizing probiotics represents a highly effective, alternative biocontrol strategy during mycotoxicosis. Probiotics not only attenuate acute structural liver damage but also strategically enhance gut barrier integrity, offering a sustainable solution to mitigate the systemic toxicological impacts of food-borne mycotoxins in the poultry industry.

KEY WORDS

T-2 toxin, probiotics, broilers, gut barrier integrity, hepatotoxicity.

IDENTIFICATION OF THE AFLATOXIGENIC POTENTIAL OF *ASPERGILLUS FLAVUS* ISOLATES

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ABSTRACT

Maize is one of the main cereals in the world and an important component of human and animal nutrition. In recent years, corn production in the Republic of Serbia has been experiencing very hot and dry summers, which lead to the attack of phytopathogenic fungi, the production of mycotoxins and large economic losses. One of the most common fungi that attack corn are species from the genus Aspergillus, especially Aspergillus flavus, which in favorable agroclimatic conditions can produce aflatoxin as one of the extremely toxic and carcinogenic secondary metabolites of phytopathogenic fungi. The aim of this study was to isolate A. flavus from corn harvested in Serbia and assess the presence of key genes for aflatoxin biosynthesis in A. flavus isolates. A total of 18 samples were collected during the 2024 harvest from the Bačka, Banat, and Srem (Autonomous Province of Vojvodina – APV) regions (six samples per region). Five representative samples were subsequently selected for further mycological analysis. The samples were ground, and fungal isolation was performed using the serial dilution method on DG18 medium and incubation at 25 °C for five days. Based on morphological characteristics, all samples

were contaminated with fungi from the genus Aspergillus. Total DNA was extracted from pure cultures using the genomic DNA isolation kit (Qiagen DNeasy Plant Mini Kit). PCR products were resolved by electrophoresis on a 1.5% agarose gel and visualized under UV illumination after staining with Midori Green Advance. Species identity was confirmed by amplification and sequencing of the ITS region, and all 5 isolates showed 100% homology to Aspergillus flavus. Using molecular identification based on the NOR1, hypB, aflR and aflT genes, the aflatoxigenic potential of the isolates was investigated and it was determined that 3 out of 5 isolates showed aflatoxigenic capability. A. flavus strains AF01 (atoxigenic) and K1 (toxigenic) were used as controls. These findings indicate the presence of both aflatoxigenic and atoxigenic isolates of Aspergillus flavus in corn from Serbia, indicating potential risks of aflatoxin contamination in agricultural production and highlighting the need for continuous monitoring and improved control strategies.

KEY WORDS

Maize; *Aspergillus flavus*; aflatoxins; ITS.

RAPID ANTIGEN EXTRACTION FROM *ASPERGILLUS FLAVUS* FOR FIELD-DEPLOYABLE LATERAL-FLOW IMMUNODIAGNOSTICS

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ABSTRACT

Aspergillus flavus is an important fungal contaminant of agricultural commodities and a major producer of aflatoxins, among the most significant mycotoxins affecting food and feed safety. Because fungal growth and mycotoxin accumulation may develop before contamination is readily recognized, rapid methods capable of detecting *A. flavus* at an early stage may provide valuable support for crop protection and mycotoxin risk assessment. However, conventional laboratory-based approaches for fungal detection often require extended sample processing and specialized equipment. Here, we investigated a rapid mechanical sample-preparation strategy for recovery of immunoreactive *A. flavus* antigens from crude fungal biomass and evaluated its compatibility with lateral-flow immunodiagnostics. *A. flavus* biomass was disrupted using the handheld

MonoLyser HyperLysis system with Lysing Matrix Z. To minimize potential interference with antibody-based detection, distilled water was used as the extraction medium. Different short lysis conditions were considered (5, 10, 30, 60, 120, 180, 300, 600, 900, and 1200 seconds), with particular emphasis on rapid processing suitable for field applications. Each lysis condition was analyzed in triplicate, resulting in 30 replicates in total. A 30-s treatment at 50% device power was selected to generate crude lysates for immunoassay analysis. Following brief settling of particulate material, the supernatants were applied directly to a commercial prototype the whole *A. Flavus* lysate lateral-flow assay and visually assessed after 15 min. The assay detects target fungal antigens through antibody interactions, generating a visible test line, while the control line confirms proper sample migration and assay performance. The short mechanical treatment generated lysates containing readily detectable immunoreactive *Aspergillus* antigens. Clear test and control lines were observed following direct application of the crude extract, indicating that rapid bead-beating efficiently released target antigens while retaining epitopes recognized by the assay antibodies. The absence of apparent matrix-related suppression of the test signal further demonstrated the suitability of the crude lysate for direct lateral-flow analysis without an additional purification step. These findings establish a practical sample-preparation window for rapid immunological screening of *A. flavus*. Although antigen detection does not directly quantify aflatoxin contamination, rapid recognition of the fungal source may contribute to earlier identification of materials requiring further mycotoxin assessment. Future studies should investigate different *A. flavus* strains, agricultural matrices, and the relationships among antigen abundance, fungal biomass, and aflatoxin production. Combining rapid antigen screening with direct mycotoxin detection could support early monitoring of *A. flavus* and associated risks.

KEY WORDS

Lateral-flow immunoassay; rapid diagnostics; sample preparation; *Aspergillus flavus*.

ACKNOWLEDGMENTS

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DEGRADATION OF *FUSARIUM* MYCOTOXINS IN WATER BY ECO-FRIENDLY PHYSICOCHEMICAL METHODS

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ABSTRACT

Fusarium mycotoxins are primarily associated with contaminated agricultural crops, but their relevance extends beyond food and feed safety. In the field, before or after harvest, these stable secondary metabolites may be mobilized from crops and agricultural soils and transferred to surface waters through leaching, runoff and drainage. The reported occurrence of mycotoxins in water indicates that aquatic systems may represent an additional environmental route of exposure. Since conventional processing and remediation approaches are often insufficient for complete mycotoxin elimination, sustainable treatment strategies are needed. Nanotechnology provides several complementary approaches to combating mycotoxins, including the development of nanoparticles capable of toxin degradation. In this context, green synthesis of ZnO-based nanophotocatalysts using plant-derived extracts represents a promising route for environmentally acceptable, solar-driven water remediation. This study investigated the degradation of zearalenone (ZEA)

and deoxynivalenol (DON), in water using photolysis and photocatalysis under simulated solar irradiation (SSI).

Photodegradation experiments were performed in a commercial batch photoreactor with xenon lamp using aqueous mycotoxin solutions, with non-adjusted pH. In photocatalytic experiments, newly synthesized green ZnO-based nanophotocatalysts obtained using plant-derived extracts, including green tea (Camellia sinensis) and banana peel, were applied. The catalyst loading was 0.5 mg/cm^3, while initial toxin concentrations were 0.5 μg/cm^3 for ZEA and 5.0 μg/cm^3 for DON. The reaction time was 60 min. Analytical determination was performed by liquid chromatography with fluorescence detection for ZEA and UV/DAD detection for DON.

The results demonstrated high susceptibility of ZEA to irradiation and photocatalytic treatment. While only 24% of ZEA was degraded after 120 min in the manually assembled halogen photoreactor, almost complete degradation was achieved within 60 min in the commercial photoreactor under SSI. Green ZnO-based nanophotocatalysts showed high photocatalytic activity, with 95% ZEA removal. DON was more resistant to direct photolysis, with 30% degradation after 60 min, but photocatalysis substantially improved its removal. Commercial ZnO completely degraded DON, while green tea- and banana peel-based ZnO nanophotocatalysts degraded 93% and 58%, respectively. In Danube River water, the photocatalytic efficiency was reduced, likely due to competition from dissolved organic matter and inorganic ions for catalytic active sites.

Overall, green ZnO-based photocatalysis appears to be a promising eco-friendly method for the degradation of *Fusarium* mycotoxins under simulated solar irradiation, highlighting their potential as sustainable materials for water treatment.

KEY WORDS

Zearalenone; deoxynivalenol; photolysis; photocatalysis; nanotechnology.

ACKNOWLEDGMENTS

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COMPARATIVE EVALUATION OF AFLATOXIN M1 REDUCTION IN MILK BY UV AND SIMULATED SOLAR IRRADIATION

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ABSTRACT

Milk is a nutritionally valuable food and an important source of proteins, vitamins, and minerals necessary for human growth and development. Therefore, particular attention is devoted to assessing its safety and quality. Mycotoxins are toxic secondary metabolites produced by certain fungal species. Among them, aflatoxin M1 (AFM1), a hydroxylated metabolite of aflatoxin B1 (AFB1) formed in dairy animals following ingestion of contaminated feed, is of particular concern in milk due to its occurrence and potential adverse effects on public health. Ruminants have a limited capacity to inactivate AFB1, as approximately 10% of the ingested toxin undergoes microbial degradation in the rumen, while the remaining fraction is transported to the liver, where it is converted to AFM1 by hydroxylation. Due to their high toxicity, recent studies have focused on adsorption, physical removal, chemical, and biological degradation of AFB1 and AFM1 with varying degrees of effectiveness.

The aim of this study was to investigate the reduction of AFM1 in pasteurized cow's milk by exposure to UV and sim-

ulated solar irradiation (SSI). Naturally contaminated milk was used, and the presence of AFM1 (c_0 = 0.094 μg/kg) was confirmed by both ELISA and HPLC-FLD techniques. Milk samples were treated in a TOPT-V batch photoreactor (Toption, China), equipped with high-pressure 300 W mercury and xenon lamps as UV and SSI sources, respectively. The mercury lamp provides UV irradiation at 250-365 nm, whereas the xenon lamp provides visible irradiation at 400-780 nm. Using immunoaffinity columns, AFM1 was purified from the photodegradation samples, taken after 30, 60, and 120 min of irradiation. The obtained acetonitrile extract was evaporated to dryness, the residue was reconstituted in hexane, and derivatization was performed using trifluoroacetic acid. Following incubation and a second evaporation step, the samples were reconstituted in the mobile phase and analyzed by HPLC-FLD.

After 120 min of UV treatment, AFM1 concentration was reduced by 42%, while SSI treatment resulted in a 77% AFM1 reduction in the same time span. AFM1 concentration progressively decreased with irradiation time in both experiments, with the most pronounced reduction occurring during the first 30 min of SSI treatment. These findings indicate the potential of light-based treatments for reducing AFM1 levels in milk and provide a basis for further optimization of the process. The particular significance of this study lies in the possibility of developing an efficient, relatively simple, and sustainable approach to milk decontamination with minimal disruption, provided that the degradation products, safety, nutritional quality, and sensory properties of the treated product are thoroughly evaluated before practical application.

KEY WORDS

Aflatoxin M1; Food safety; Milk; Photodegradation; HPLC-FLD.

ACKNOWLEDGMENTS

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MICROENCAPSULATED *SATUREJA MONTANA* L. ESSENTIAL OIL AS A NATURAL BIOPRESERVATIVE AGAINST TOXIGENIC *ASPERGILLUS OCHRACEUS*, *IN VITRO* AND IN FOOD SYSTEMS

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ABSTRACT

Molds represent a significant group of food contaminants that cause spoilage, with certain toxigenic species capable of synthesizing hazardous mycotoxins. Among them, ochratoxin A (OTA) is of particular concern due to its frequent occurrence in food commodities and itspronounced toxicity, highlighting the need for effective control measures. In recent years, essential oils have gained substantial attention as natural alternatives to synthetic preservatives. However, their direct application in food systems is often limited by poor water solubility, chemical instability, and strong sensory attributes – limitations that can be overcome by microencapsulation to protect bioactive compounds and achieve controlled release. This study aimed to investigate the effect of *Satureja montana* L. essential oil microcapsules on the growth of the toxigenic species *Aspergillus ochraceus* and the biosynthesis of ochratoxin A, both in vitro and in a food model.

The antifungal activity of free *S. montana* essential oil and its emulsion was initially determined using the microdilution

method. The minimum inhibitory (MIC) and minimum fungicidal concentration (MFC) of the essential oil against A. ochraceus were 7.10 and 56.82 µl/ml, respectively, whereas the emulsion exhibited enhanced activity (MIC 3.55 µl/ml; MFC 14.20 µl/ml). Microcapsules containing S. montana essential oil significantly suppressed mold growth; the highest tested concentration (2.67 mg/ml) yielded 94.5% growth inhibition *in vitro*, accompanied by visible alterations in macro- and micromorphological characteristics. In the tomato juice model, microcapsules at a concentration of 15 mg/l completely inhibited the growthof *A. ochraceus* over 10 days of incubation at 25 °C. Furthermore, microcapsules applied at 17 mg/ml achieved complete inhibition of OTA synthesis in both YES broth and tomato juice over 14 days of incubation at 25 °C. These findings demonstrate the high efficacy of microencapsulated *S. montana* essential oil in controlling fungal proliferation and preventing ochratoxin A production, highlighting its potential as a natural biopreservative in the food industry.

KEY WORDS

Aspergillus ochraceus; ochratoxin A; *Satureja montana* L.; essential oil; microencapsulation; antifungal activity; biopreservation.

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BIOACTIVE POTENTIAL OF AIR-DRIED *LAETIPORUS SULPHUREUS* FRUITING BODIES: SOLVENT-DEPENDENT ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES

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ABSTRACT

Laetiporus sulphureus (Bull.) Murrill 1920 is an edible and medicinal mushroom with reported antioxidant and other biological activities; however, data on the solvent-dependent bioactive properties of specimens from the Balkan region remain limited. In this study, fruiting bodies of *L. sulphureus* collected in Vrelo Bosne, Sarajevo, Bosnia and Herzegovina, were subjected to solvent extraction using hot water (H_2O), 70% ethanol (70% EtOH), chloroform ($CHCl_3$), and 80% methanol (80% MeOH), and the resulting extracts were evaluated for *in vitro* antioxidant and antiproliferative activities. Antioxidant potential was assessed using radical-scavenging capacity (RSC) assays, including DPPH and ABTS, as well as lipid peroxidation (LP) and ferric reducing antioxidant power (FRAP) assays, while total phenolic content (TPC) was determinedexpressed as an estimate

of the overall measure of phenolic contentstituents and expressed as mg gallic acid equivalents (GAE) per g dry weight (d.w.). The 70% EtOH extract showed the strongest RSC (DPPH: IC_{50} = 71.79 ± 1.35 μg/mL; ABTS: 76.86 ± 1.73 mg Trolox equivalents (TE)/g d.w.), as well as the highest reducing potential (FRAP: 151.47 ± 0.21 mg ascorbic acid equivalents (AAE)/g d.w.), and TPC (124.95 ± 1.00 mg GAEgallic acid equivalents (GAE)/g d.w.). The $CHCl_3$ extract exhibited the strongest inhibitory activity against LP (IC_{50} = 154.35 ± 4.26 μg/mL).

Antiproliferative activity was evaluated by the MTT assay against human breast cancer cell lines MCF-7, T47D, and MDA-MB-231, as well as ovarian A2780 and cervical HeLa and SiHa cell lines, with all extracts tested at concentrations of 50 and 100 μg/mL. The $CHCl_3$ extract exhibited the most pronounced growth-inhibitory activity, particularly at 100 μg/mL, reaching 99.85 ± 0.13% inhibition against SiHa, 98.92 ± 0.20% against A2780, 98.19 ± 0.29% against HeLa, 94.70 ± 1.83% against T47D, and 91.88 ± 1.03% against MCF-7 cells. The 70% EtOH extract also showed substantial growth-inhibitory activity, whereas the 80% MeOH extract generally exhibited lower activity.

Overall, the results demonstrate solvent-dependent antioxidant and antiproliferative activities of *L. sulphureus*, with the 70% EtOH extract showing the most pronounced antioxidant profile and the $CHCl_3$ extract exhibiting strong growth-inhibitory effects across several cancer cell lines. These findings support further investigation of *L. sulphureus* as a source of bioactive compounds with potential applications in functional food and medicinal research.

KEY WORDS

Laetiporus sulphureus; solvent extraction; antioxidant activity; antiproliferative activity; MTT assay.

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FUNGAL DISEASES IN SERBIAN AQUACULTURE: CURRENT STATUS, DIAGNOSTIC CHALLENGES, AND FUTURE PERSPECTIVES

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ABSTRACT

Fungal and fungus-like pathogens represent an important but often underestimated cause of disease in freshwater aquaculture, contributing to substantial economic losses through increased mortality, impaired fish welfare, reduced growth performance, and decreased product quality. In Serbia, common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) are the principal aquaculture species, and mycotic diseases occur both as primary infections and, more frequently, as opportunistic infections associated with environmental stress, handling, transportation, spawning, or concurrent bacterial, parasitic, and viral diseases. Although fungal pathogens are regularly encountered in routine diagnostics, comprehensive data on their occurrence and significance in Serbian aquaculture remain limited.

The study provides an overview of the occurrence, diagnosis, and epidemiological significance of fungal and fungus-like diseases in Serbian aquaculture, while identifying current diagnostic challenges and priorities for surveillance, prevention, and disease control.

The analysis synthesized laboratory records from the National Reference Laboratory for Fish Diseases, published scientific literature, and routine diagnostic investigations conducted in Serbian fish farms during the past decade. Clinical, pathological, microbiological, histopathological, and molecular findings were reviewed to evaluate the diversity, distribution, and diagnostic significance of fungal pathogens affecting cultured fish. Particular emphasis was placed on oomycete infections caused by *Saprolegnia* spp., the principal etiological agents of saprolegniosis, and on *Ichthyophonus hoferi*, an emerging mesomycetozoean pathogen recently identified for the first time in farmed rainbow trout in Serbia.

The analysis demonstrated that saprolegniosis remains the most frequently diagnosed fungal-like disease in Serbian freshwater aquaculture, predominantly affecting fish eggs, fry, broodstock, and fish exposed to handling stress, skin injuries, or unfavorable environmental conditions. Outbreaks were most commonly associated with reduced water quality and low water temperatures, which facilitate the development of opportunistic infections. Accurate diagnosis requires the integration of clinical examination with microscopic evaluation, histopathology, and molecular methods, while fungal isolation may provide additional confirmation where feasible. The recent laboratory confirmation of *Ichthyophonus hoferi* in Serbian rainbow trout represents an important milestone in the understanding of emerging aquatic mycoses and highlights the necessity of expanding diagnostic capacity and strengthening epidemiological surveillance.

Fungal and fungus-like diseases remain a persistent health challenge for Serbian freshwater aquaculture despite receiving less attention than bacterial and viral diseases. Because therapeutic options have become increasingly limited following the withdrawal of several traditional antifungal compounds, prevention through biosecurity, optimization of environmental

conditions, reduction of handling stress, and early disease detection has become the cornerstone of disease management.

KEY WORDS

Aquaculture; Saprolegnia; Ichthyophonus hoferi; Fish mycoses; Serbia.

ACKNOWLEDGMENTS

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CRAYFISH PLAGUE: AN EMERGING THREAT TO NATIVE CRAYFISH POPULATIONS IN SERBIA

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ABSTRACT

Crayfish plague, caused by the oomycete *Aphanomyces astaci*, is one of the most devastating diseases affecting freshwater crayfish worldwide. Since its introduction to Europe from North America during the nineteenth century, the pathogen has caused repeated mass mortality events and dramatic declines in indigenous crayfish populations. While North American crayfish species serve as natural reservoirs and exhibit high resistance to infection, European crayfish species are highly susceptible and often experience mortality rates approaching 100%. In Serbia, native crayfish species, including the noble crayfish (*Astacus astacus*) and the narrow-clawed crayfish (*Pontastacus leptodactylus*), represent ecologically important and economically valuable components of freshwater ecosystems. Their populations are increasingly threatened by habitat degradation, climate change, and the expansion of invasive alien crayfish species that can introduce and maintain *A. astaci*.

The objective of this review was to summarize the current knowledge on crayfish plague, assess the risk of pathogen introduction into Serbia, and highlight available diagnostic

approaches alongside priorities for surveillance, prevention, and biosecurity.

The review synthesized published scientific literature, current international recommendations, and available epidemiological information on the occurrence and spread of *A. astaci* in Europe. Diagnostic approaches considered include species-specific real-time PCR as the reference method for pathogen detection, supported by histopathological examination, microscopic identification of characteristic hyphal structures, and molecular haplotyping for epidemiological investigations.

To date, no laboratory-confirmed cases of crayfish plague have been reported in Serbia. However, isolated mortality events in native crayfish populations have raised clinical suspicion of the disease, although the presence of *A. astaci* has not been confirmed by laboratory testing. At the same time, the documented spread of invasive North American crayfish species and confirmed outbreaks of crayfish plague in neighboring countries increase the likelihood of pathogen introduction into Serbian freshwater ecosystems. These circumstances highlight the need to establish active surveillance, strengthen laboratory diagnostic capacity, implement molecular monitoring programs, regulate crayfish translocations, and promote biosecurity measures for both wild populations and aquaculture.

Although crayfish plague has not yet been laboratory-confirmed in Serbia, it represents a significant emerging threat to native crayfish populations and freshwater biodiversity. Early detection through coordinated surveillance, improved diagnostic preparedness, and close collaboration among veterinary institutions, environmental authorities, fisheries managers, and researchers will be essential to prevent pathogen establishment and protecting native crayfish.

KEY WORDS

Aphanomyces astaci; crayfish plague; native crayfish; Serbia.

ACKNOWLEDGMENTS

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THE FINDING OF YEASTS ON THE MUCOUS MEMBRANES OF DOGS AND THEIR SIGNIFICANCE

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ABSTRACT

The most clinically significant yeasts found on the mucosa and/or skin of dogs, belonging to the kingdom *Fungi*, are *Malassezia pachydermatis* (class *Malasseziomycetes*) and yeasts of the class *Saccharomycetes*: *Candida sp.* and *Saccharomyces cerevisiae*. These species may be found on the mucosa and/or skin of dogs as part of the ubiquitous microbiota, but also act as potential causative agents of various pathological processes under conditions of impaired immune status. As part of the ubiquitous microbiota, yeasts can be isolated from the ocular, oral, and nasal mucosa, the external ear canal, the urogenital tract, feces, and milk.

Their presence as part of the normal flora typically does not indicate disruption of the mucosal or gastrointestinal bacterial microbiome, and they are rarely isolated on routine microbiological examination of submitted samples. However, an increased yeast burden in examined materials may reflect yeast dominance resulting from the direct or indirect influence of biotic or abiotic factors. Inappropriate or prolonged antibiotic use is most commonly implicated in increased yeast presence. Treatment of bacterial infections without prior antibiogram testing can disrupt the mucosal microbiome: alongside the intended

suppression of pathogenic bacteria, the saprophytic bacterial flora which normally limits excessive yeast proliferation through competitive exclusion is also unintentionally affected. Non-selective antibiotic pressure thus creates conditions favorable for yeast overgrowth and dominance. Besides antibiotic use, increased yeast numbers on canine mucosa may also result from impaired mucosal surface integrity, allergic reactions, the presence of skin folds, increased moisture, and immunosuppression.

In this study, we examined various sample types mycologically: vaginal swabs, nasal swabs, ocular swabs, oral swabs, milk, preputial swabs, wound swabs, urine, semen, and feces from dogs. Over the course of one year, 221 samples were examined in total. The most frequently represented sample types (approximately 20% each) were ear swabs, vaginal swabs, and urine. Sabouraud dextrose agar was used for yeast isolation, and identification of isolates was performed by direct microscopic examination and biochemical testing. The presence of *Candida albicans*, *Malassezia pachydermatis*, and *Saccharomyces cerevisiae* was confirmed. Analysis of ear swabs (a total of 54 samples of ear swabs were examined) revealed *Malassezia pachydermatis* in 8 samples (14.81%). Among vaginal swabs (a total of 69 samples of vaginals swabs were examined), 2 samples were positive (2.89%, *Candida albicans*); among fecal samples (a total of 29 samples of feces were examined), there was 1 finding each of *Candida albicans* and *Saccharomyces cerevisiae* (6.89%); milk yielded (a total of 5 samples of milk were examined) 1 finding of *Candida albicans* (20%); wound swabs (a total of 8 samples of wound swabs were examined) yielded 1 finding of *Malassezia pachydermatis* (12.5%); and ocular swabs (a total of 14 samples of eyes swab were examined) yielded 1 finding of *Malassezia pachydermatis* (7.14%). Overall, yeasts were not detected in high numbers across the examined samples, regardless of sample type. Since yeasts represent part of the ubiquitous microbiota of the skin and mucosa in healthy dogs where they are most

often not detectable their detection in this study may indicate pathogenic potential for the host.

KEY WORDS

Yeasts, microbiota, mucosa, dogs.

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CANDIDA BIOFILMS ON STAINLESS STEEL SURFACES: ENVIRONMENTAL DETERMINANTS AND NATURAL ANTIBIOFILM STRATEGIES

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ABSTRACT

Yeast biofilms pose a significant challenge in clinical and industrial settings due to their enhanced tolerance to antifungal agents and ability to persist on abiotic surfaces, including medical devices. Adhesion is the initial and critical step in biofilm development, influenced by yeast species, environmental conditions, and surface properties. Understanding the factors governing yeast adhesion and identifying effective natural antibiofilm agents is essential for developing strategies to prevent and control biofilm-associated infections. This study aimed to investigate the effects of growth medium, temperature, and stainlesssteel surface roughness on the adhesion of selected *Candida* species, and to evaluate the antifungal and antibiofilm activity of selected plant extracts against *Candida* biofilms. The adhesion of *Candida albicans*, *Candida glabrata*, *Candida par-*

apsilosis, and *Candida krusei* to AISI 304 stainless steel discs with different surface roughness values (Ra = 25.20–961.90 nm) was investigated. Yeast cells were grown in Malt Extract Broth (MEB) or Yeast Peptone Dextrose (YPD) broth at 7, 37, and 43 °C, and adhesion was quantified using crystal violet staining. The antifungal activity of *Humulus lupulus*, *Alpinia katsumadai*, and *Evodia rutaecarpa* extracts was evaluated against *C. albicans* and *C. glabrata*. Minimum inhibitory concentrations (MICs) were determined using the CLSI M07-A10 broth microdilution method. Inhibition of biofilm formation and activity against preformed biofilms were assessed at 1/2 × MIC and 1× MIC using crystal violet staining. Growth medium significantly affected yeast adhesion to stainless steel. *C. albicans* and *C. glabrata* exhibited higher adhesion in MEB, whereas *C. parapsilosis* and *C. krusei* adhered more extensively following growth in YPD broth. Temperature and surface roughness also significantly influenced adhesion of all tested yeasts. All plant extracts exhibited antifungal activity against the investigated strains. *A. katsumadai*, *E. rutaecarpa*, and *H. lupulus* showed the highest antibiofilm activity against *C. albicans* on stainless steel at both tested concentrations. *A. katsumadai* effectively inhibited the initial biofilm formation of *C. glabrata*, whereas preformed biofilms were more difficult to eradicate. *C. glabrata* biofilms demonstrated greater resistance to the extracts than *C. albicans*. In conclusion, yeast adhesion and biofilm development on stainless steel are strongly influenced by environmental conditions and species-specific characteristics. Selected plant extracts show promising potential for controlling *Candida* biofilms, although their efficacy depends on the yeast species and biofilm developmental stage. These findings highlight the importance of considering both surface-associated adhesion mechanisms and natural antibiofilm agents in the development of strategies for preventing yeast biofilm-associated infections.

KEY WORDS

Yeast, biofilm, stainless steel surface, plant extracts.

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PROBIOTIC YEAST *SACCHAROMYCES BOULARDII* MODULATES *CANDIDA GLABRATA* ADHESION AND ANTIFUNGAL SUSCEPTIBILITY

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ABSTRACT

The increasing clinical importance of infections caused by the pathogenic yeast *Candida glabrata*, together with its growing resistance to commonly used antifungal agents, highlights the need for alternative or adjunctive approaches to antifungal therapy. Probiotic microorganisms have attracted considerable attention due to their potential to modulate the growth and virulence-associated properties of pathogenic microorganisms. Among probiotic yeasts, *Saccharomyces boulardii* (nom. nud.) is widely used as a biotherapeutic agent, particularly for the prevention and treatment of gastrointestinal disorders. However, the effects of *S. boulardii* on the adhesion and antifungal susceptibility of *C. glabrata* remain insufficiently characterized. This study aimed to investigate the influence of *S. boulardii* on *C. glabrata* adhesion under different environmental conditions

and in the presence of antifungal agents. The adhesion of 48 *C. glabrata* strains was evaluated in monoculture and co-culture with *S. boulardii* on polystyrene surfaces using crystal violet staining. Experiments were performed under different temperature (28 °C, 37 °C, 39°C, and 42 °C) and pH (4.0, 5.5, 7.0, and 8.5) conditions and in the presence of three clinically relevant antifungal agents: fluconazole, itraconazole, and amphotericin B. Despite its non-adhesive properties, the presence of *S. boulardii* significantly altered *C. glabrata* adhesion. The effect was strongly strain-dependent, with both antagonistic and synergistic interactions observed. Under selected conditions, *S. boulardii* reduced *C. glabrata* adhesion and enhanced the inhibitory effects of antifungal agents, as reflected by lower effective antifungal concentrations. Amphotericin B exhibited the strongest inhibitory activity against the *C. glabrata* isolates. Temperature did not significantly affect the modulatory activity of *S. boulardii*, whereas alkaline pH and increased antifungal concentrations reduced its ability to suppress *C. glabrata* adhesion. Overall, *S. boulardii* can modulate *C. glabrata* adhesion and its response to antifungal treatment, although these effects are dependent on the strain and environmental conditions. These findings support further investigation of probiotic yeasts as potential complementary agents for controlling *C. glabrata* infections.

KEY WORDS

Adhesion, *Candida glabrata*, *Saccharomyces boulardii*, antimycotics, growth conditions.

ACKNOWLEDGMENTS

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EVALUATION OF BACTERIOGENIC FE-NANOPARTICLES AS ALTERNATIVE THERAPEUTIC AGENTS AGAINST *CANDIDA* SPECIES

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ABSTRACT

Fungal infections remain a major global healthcare challenge despite substantial advances in antifungal drug discovery. Candidiasis is currently the most frequent fungal disease affecting humans worldwide. In recent years, the epidemiological and clinical importance of fungal infections caused by *Candida* species has attracted considerable interest, with increasing reports of intrinsic and acquired resistance among these species. Thus, the development of novel and effective therapies for *Candida* infection remains a critical challenge in modern medicine. Nanoparticles, particularly iron-based nanoparticles, have attracted interest as potential antifungal agents due to their reported antifungal activity and favorable biocompatibility in some studies compared with certain other metal nanoparticles. Among these, biosynthesised Fe-nanoparticles produced by bacteria represent a promising approach that may offer antifungal activity while potentially providing a more environmentally compatible

synthesis route. In this study, we investigated bacteriogenic Fe-nanoparticles (FeNPs) produced by two bacterial strains, *Bacillus cereus* (RDJA_1) and *Lysinibacillus* sp. (CB_1.2), as targeted antifungal agents. Their antifungal activity was evaluated against a broad range of pathogenic *Candida* species using minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) assays. The bacteriogenic Fe nanoparticles were tested against drug-resistant *Candida auris*, which is resistant to the majority of conventional antifungal agents, as well as against *C. albicans, C. krusei, C. tropicalis, C. parapsilosis, C. dubliniensis, C. kefyr* and *C. utilis*. Notably, Fe nanoparticles from CB_1.2 exhibited broader activity, showing both inhibitory and fungicidal effects (expressed as volumes of Fe-nanoparticle suspension applied) against *C. kefyr* (MIC 22.5 µl; MFC 45 µl), *C. dubliniensis* (MIC 45 µl; MFC 90 µl) and *C. tropicalis* (MIC 60 µl; MFC 80 µl). CB_1.2, also demonstrated only inhibitory activity against *C. auris, C. krusei, C. parapsilosis* (MIC 80 µl) and *C. albicans* (MIC 90 µl), using the microdilution method in 96-well microtitre plates. FeNPs from RDJA_1 possessed inhibitory potential against only two species, *C. albicans* and *C. utilis* (MIC 90 µl). These findings demonstrate that bacteriogenic Fe-nanoparticles, especially those biosynthesised by CB_1.2, inhibited the growth of all tested *Candida* species except *C. utilis*. Biosynthesised Fe-nanoparticles therefore represent a green, sustainable and promising strategy for controlling pathogens, offering targeted antifungal activity while reducing reliance on conventional antifungal agents. It is becoming clear that nanoparticles could form the next generation of antifungal agents. Together, these findings emphasise the importance of sustainable, natural product-based approaches to addressing *Candida* infections.

KEY WORDS

Bacteriogenic Fe-nanoparticles; antifungal activity; *Candida* species; drug-resistant *Candida auris*.

FUSARIUM VERTICILLIOIDES – A CAUSAL AGENT OF POSTHARVEST ROT OF BROCCOLI (*BRASSICA OLERACEA* VAR. *ITALICA*)

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ABSTRACT

Broccoli (*Brassica oleracea* var. *italica*) is highly perishable vegetable, consumed after minimal processing, which makes postharvest fungal decay both an economic and a food safety concern. *Alternaria* species are well known pathogens of *Brassica* crops, whereas *Fusarium* species are rarely reported on broccoli, although many of them can produce mycotoxins in colonised tissue. The aim of this study was to identify the fungi associated with rot of retail broccoli heads and to identify pathogenic isolates.

In spring 2026, broccoli heads purchased at a retail store in Novi Sad showed symptoms: a dark brown to black, floret tips, and rot with a grey to white mycelial coating with softening of the underlying tissue. Microscopic examination of the dark floret tips revealed abundant *Alternaria* sp. conidia. For the isolation purposes, tissue fragments from the lesion margin were disinfected in 1% sodium hypochlorite for 5 min and plated on potato dextrose agar (PDA) and incubated at 25±0.5 °C. Two isolates were obtained, one morphologically consistent with the genus *Alternaria*, and the other to genus *Fusarium*. The isolates

were purified by single-hyphal-tip transfer. DNA was extracted with the DNeasy Plant Mini Kit (Qiagen); ITS and partial *RPB2* were amplified from both isolates (ITS1/ITS4, 5F2/7cR), and partial *TEF1-α* (EF1/EF2) from the isolate corresponding to *Fusarium* sp. For both isolates, pathogenicity was tested on fresh broccoli florets wounded with a sterile needle and inoculated with mycelial plugs, with sterile PDA plugs as the negative control. Five florets were used per treatment, incubated at 25±0.5 °C for two weeks.

Obtained isolates sequences were compared with GenBank reference sequences using BLASTn, identifying the isolates as *Alternaria brassicicola* and *Fusarium verticillioides*; sequences are currently being deposited. The *F. verticillioides* isolate formed white, floccose mycelium with a violet reverse on PDA and did not sporulate under the conditions applied. In the pathogenicity test it induced a grey to white coating and tissue softening around the inoculation points, corresponding to the symptoms on the original material, and was consistently re-isolated, fulfilling Koch's postulates; control remained symptomless. The *A. brassicicola* isolate did not induce symptoms under the same experimental conditions, suggesting that it occurred secondarily as a saprophyte.

The results confirm *F. verticillioides* as a causal agent of rot on broccoli obtained from the retail market. As this species is a well documented producer of fumonisins, and mycotoxin contamination of *Brassica* vegetables is neither regulated or routinely monitored, further research should focus on the mycotoxigenic potential of the isolate and quantification of fumonisins in colonised tissue.

KEY WORDS

Postharvest rot; *Brassica oleracea var. italica*; *Alternaria brassicicola; Fusarium verticillioides*; fumonisins.

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ANTIFUNGAL ACTIVITY OF CITRAL, THYMOL, AND CINNAMALDEHYDE AGAINST PHYTOPATHOGENIC MOLDS: INDIVIDUAL AND COMBINED EFFECTS

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ABSTRACT

Phytopathogenic fungi are major contributors to crop losses worldwide, causing diseases that reduce yield, quality, and storage stability of agricultural products. Their persistence and resistance to conventional fungicides necessitate the exploration of natural alternatives. Plant-derived bioactive compounds have emerged as promising antifungal agents. This study investigates the antifungal activity of three pure natural compounds, Citral, Thymol, and Cinnamaldehyde, against five phytopathogenic molds of agronomic importance: *Aspergillus niger* (black mold, associated with post-harvest spoilage), *Penicillium* sp. (blue mold decay in fruits), *Fusarium oxysporum* (vascular wilt in vegetables and ornamentals), *Alternaria alternata* (leaf spots and fruit rot), and *Botrytis cinerea* (gray mold in grapes and strawberries).

The broth microdilution method was employed to determine minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The compounds were dissolved in ethyl alcohol and isopropyl alcohol to ensure solubility and

uniform distribution. Results revealed strong antifungal activity, with Thymol exhibiting MIC values between 64–128 µg/mL and MFC values of 128–256 µg/mL, particularly effective against *Alternaria alternata* and *Botrytis cinerea*. Cinnamaldehyde showed potent inhibition of *Fusarium oxysporum* with MIC at 32 µg/mL and MFC at 64 µg/mL. Citral demonstrated moderate but consistent activity across all tested molds, with MIC values ranging from 128–256 µg/mL

In addition to individual effects, combined treatments were evaluated. Binary combinations (Thymol + Citral, Thymol + Cinnamaldehyde, Citral + Cinnamaldehyde) displayed synergistic interactions, reducing MIC values by 2–4 fold compared to single agents. For example, Thymol + Cinnamaldehyde inhibited *Botrytis cinerea* at 32 µg/mL, compared to 128 µg/mL when applied individually. The triple combination of Citral, Thymol, and Cinnamaldehyde demonstrated the strongest antifungal effect, achieving complete inhibition of *Fusarium oxysporum* and *Alternaria alternata* at concentrations as low as 16 µg/mL.

These findings highlight the potential of these bioactive compounds, both individually and in combination, as eco-friendly antifungal agents for sustainable crop protection. Their natural origin, reduced toxicity, and synergistic activity make them attractive candidates for integration into biocontrol programs. Future research should focus on field-level validation, formulation stability, and optimization of combined treatments to maximize efficacy against phytopathogenic molds.

KEY WORDS

Antifungal activity; phytopathogenic molds; microdilution method; MIC/MFC.

GUM ARABIC NANOENCAPSULATED THYME ESSENTIAL OIL COATINGS FOR POSTHARVEST FUNGAL CONTROL AND QUALITY PRESERVATION OF GARDEN EGG (*SOLANUM MELONGENA* L.)

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ABSTRACT

Garden egg (*Solanum melongena* L.) is an economically important vegetable crop in Nigeria whose commercial value is constrained by a short postharvest shelf life, largely due to fungal decay. This study investigated gum Arabic-based nanoencapsulation of thyme (Thymus vulgaris) essential oil, prepared via solvent displacement (NPGAEO) and nanoelaboration (NEGAEO) methods, as a coating strategy to control postharvest fungal deterioration and preserve fruit quality. Gas chromatography mass spectrometry identified 16 phytoconstituents in the thyme oil, dominated by camphene (10.64%), α-terpineol (9.60%), and caryophyllene (6.07%). Dynamic light scattering revealed average particle sizes ranging from 273 to 461 nm, with NEGAEO exhibiting the most negative zeta potential (–19.3 mV), indicating superior colloidal stability relative to the other formulations. Fungal isolates recovered from decayed fruits, comprising *Fusarium oxysporum, Fusarium equiseti, Penicillium citrinum, Aspergillus clavatus, Aspergillus flavus*, and *Syncephalas-*

trum racemosum, were confirmed through morphological and molecular identification. Both, in vitro and in vivo, antifungal assays demonstrated that the thyme oil loaded nanocoatings, NPGAEO and NEGAEO, significantly suppressed mycelial growth and reduced disease incidence and severity across all tested pathogens relative to the untreated control and the oil free formulations (NPGA and NEGA), with NPGAEO consistently providing the strongest protection. Beyond disease control, the thyme essential oil treatments improved postharvest fruit quality, exerting favourable effects on pH, total soluble solids, water activity, and firmness retention throughout storage, while also enhancing Phenylalanine Ammonia Lyase (PAL) activity, an indicator of induced host defense response. These findings demonstrate that gum Arabic encapsulated thyme essential oil, particularly the solvent displacement formulation, offers a sustainable, biodegradable, and effective alternative to synthetic fungicides for extending the postharvest life and maintaining the quality of garden egg fruits, with promising implications for smallholder growers and value chain stakeholders in Nigeria.

KEY WORDS

Nanoencapsulation; thyme essential oil; gum Arabic; fungal decay; garden egg.

FIVE YEARS OF MACROFUNGAL DIVERSITY RESEARCH IN TARA NATIONAL PARK (SERBIA)

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ABSTRACT

Tara National Park, located in western Serbia, represents one of the country's most important biodiversity hotspots, characterized by old-growth and well-preserved forest ecosystems. Despite its recognized ecological significance, the fungal diversity of this protected area remained insufficiently documented until the initiation of systematic mycological surveys in 2020. The present study summarizes the results of a five-year monitoring program (2021–2025) aimed at providing the first comprehensive assessment of macrofungal diversity within Tara National Park. This research was initiated through the collaboration between Tara National Park, the Mushroom Society of Novi Sad, and the ProFungi Laboratory of Faculty of Sciences, University of Novi Sad.

Field investigations were conducted during fourteen expeditions, predominantly in autumn, when macrofungal fruiting reaches its seasonal maximum. Surveys covered the principal habitats and localities across the park, including Crni Vrh, Mitrovac, Kaluđerske Bare, Zaovine, Jagoštica, Rača, Rastište, and Bilješke Stene, encompassing both managed and strictly pro-

tected forest stands. Species identification was based on standard morphological and taxonomic approaches, while nomenclature followed currently accepted taxonomic concepts.

During the five-year survey, a total of 559 macrofungal species were recorded. The majority belonged to the phylum Basidiomycota (537 species, 187 genera, 82 families), whereas Ascomycota was represented by 29 species distributed among 22 genera and 16 families. The families Russulaceae (61 species), Mycenaceae (31 species), Cortinariaceae (27 species), and Tricholomataceae (26 species) were the most species-rich. Compared with the first published inventory from the study area, which reported 320 taxa after the initial two years of research, continued monitoring increased the known macrofungal diversity by more than 70%, highlighting the importance of long-term surveys for documenting fungal biodiversity.

The recorded mycobiota includes 15 protected and 5 strictly protected species under Serbian legislation, 49 indicator species of old-growth and well-preserved forests, and 84 species included in national Red Lists of European countries, emphasizing the exceptional conservation value of Tara National Park. The high proportion of habitat-specialist and conservation-priority fungi confirms the outstanding ecological integrity of the park's forest ecosystems.

The generated dataset provides a valuable baseline for future biodiversity monitoring, conservation planning, forest management, and the long-term assessment of ecological changes in protected forest ecosystems. This research was crowned with the publication of the book "Mushrooms of the Tara National Park", in which the most important and interesting recorded species of fungi are presented.

KEYWORDS

Macrofungi, Biodiversity monitoring, Tara National Park.

FUNGI AND CYANOBACTERIA CO-INOCULATION PROMOTES STABILIZATION AND BIOCRUST FORMATION ON QUARTZ SAND MINE TAILINGS

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ABSTRACT

The induction of biological soil crusts (biocrusts) has gained increasing attention as a strategy for stabilizing mine tailings; however, the microbial interactions underlying biocrust development, particularly those involving cyanobacteria and fungi, are still poorly understood. In this study, five cyanobacterial strains (*Nostoc* sp., *Trichormus* sp., *Tolypothrix* sp., and two *Chroococcidiopsis* sp.) and five fungal strains (*Penicillium oxalicum*, *Purpureocillium lilacinum*, *Aspergillus clavatus*, *Penicillium brasilianum*, and *Penicillium concavorugulosum*) were evaluated both individually and in all possible cyanobacteria–fungi co-inoculation combinations on quartz sand mine tailings. Following a 90-day incubation period, biocrust development was characterized using SEM imaging, measurements of chlorophyll-a and extracellular

polymeric substances (EPS), fungal abundance, water repellency (WDPT), biocrust thickness, and sediment penetration resistance. SEM observations confirmed successful microbial establishment, efficient binding of sediment particles, and EPS-mediated surface stabilization. The effects of co-inoculation varied considerably depending on the specific cyanobacterial–fungal pairing. Some combinations significantly increased chlorophyll-a content, EPS production, water repellency, biocrust thickness, and sediment stability compared with cyanobacterial inoculation alone. In contrast, other pairings produced minimal effects or even reduced these parameters, highlighting the importance of microbial compatibility in determining co-inoculation outcomes. Correlation analyses revealed positive relationships between chlorophyll-a, loosely bound EPS (LB-EPS), and WDPT, whereas biocrust thickness was negatively associated with WDPT, chlorophyll-a concentration, and sediment penetration resistance, suggesting differing responses among key biocrust functional properties. Overall, selected cyanobacteria–fungi consortia exhibited synergistic interactions that substantially improved biocrust formation and enhanced sediment stabilization. These findings demonstrate that carefully designed cyanobacterial–fungal partnerships can effectively establish biocrusts on degraded mine tailings, offering a promising nature-based approach for restoring and rehabilitating disturbed substrates.

KEY WORDS

Biological soil crusts; cyanobacteria–fungi consortia; mine tailings; extracellular polymeric substances (EPS); sediment stabilization.

ACKNOWLEDGMENTS

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CARPOSPHERE AND PHYLLOSPHERE MYCOBIOME OF APPLE CULTIVARS IDARED AND GOLDEN DELICIOUS

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ABSTRACT

Bearing in mind the limited knowledge about the mycobiome of economically important apple cultivars, such as Idared and Golden Delicious, and considering the significance of fruit crops affected by fungal pathogens, the aim of this research was to examine the postharvest carposphere and phyllosphere mycobiome of two apple varieties. Fruit and leaf samples of both cultivars were obtained from the orchard of the Ivanov agricultural estate in Zrenjanin. The stored apples of the Idared and Golden Delicious varieties exhibited symptoms of rot without visible mould growth, while numerous necrotic spots of various shapes and sizes were observed on both the upper and lower surfaces of the leaves. Identification of the total and culturable mycobiome was performed via metabarcoding and cultivation-based analyses, respectively. It was found that fungi from the phylum Ascomycota dominated the mycobiome of both cultivars. Fruits of the Idared variety showed greater resistance to phytopathogenic fungi compared to those of the Golden Delicious variety, as did the leaves compared to the fruits. In the carposphere of the Idared cultivar, the most prevalent genera

were *Candida* (38,75%,) *Starmerella* (31,45%), and *Pichia* (12,99%), whereas in the Golden Delicious cultivar, the dominant genera were *Alternaria* (28,12%), *Cladosporium* (8,28%), and *Aureobasidium* (7,42%). The phyllosphere of Golden Delicious was characterized by a high prevalence of the genus *Monilinia*, while the phyllosphere of Idared showed the highest relative abundance of the genera *Aureobasidium* and *Cladosporium*. Analysis of the culturable mycobiome revealed 12 species belonging to 9 genera, among which *Aspergillus* and *Pichia* displayed the greatest diversity. Greater diversity of the culturable mycobiome was recorded in the carposphere and phyllosphere of apples of the Golden Delicious variety, with small overlaps between varieties, up to 17%. This strong variation in fungal communities demonstrates the extent to which the cultivar shapes the postharvest mycobiome, which is crucial for adequate protection against pathogens.

KEY WORDS

Apple cultivars; fungal pathogens; mycobiota; postharvest; storage.

ACKNOWLEDGMENTS

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AIRBORNE FUNGAL DIVERSITY OF THE SEMI-HYPOGEAN ENVIRONMENT OF THE MAMMOTH PARK AT THE ARCHAEOLOGICAL SITE OF VIMINACIUM (SERBIA)

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ABSTRACT

Airborne fungal propagules present an important component of the atmospheric microbiome and may influence both the biodeterioration of cultural heritage and human health. The archaeological site of Viminacium is one of the largest Roman settlements in Serbia and Southeastern Europe. In 2021, newly excavated monoxyle from the 1st century BC and a shipwreck dated to the 15th-17th century AD were temporarily stored in the distinct semi-hypogean space of Mammoth Park within the archeological site. The aim of this study was to characterize the culturable airborne fungi in Mammoth Park at Viminacium and to assess their biodeterioration potential. Air sampling was conducted during the summer of 2021 at five locations using a SAS Super DUO 360 volumetric air sampler. Airborne propagules were collected on potato dextrose agar (PDA) and, after seven days of incubation at 25 °C, counted for quantitative assessment of fungal load. Isolates were identified by Sanger sequencing of the internal transcribed spacer (ITS) region using ITS1/ITS4 primers. Production of extracellular enzymes, pigments, and

acids was estimated via cultivation and subsequent color change on differential media: 1. Czapek agar with phenol red (CzPR) for manganese peroxidase (MnP) production, 2. 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) agar (ABTSA) for laccases, 3. xylan agar (XA) for hemicelulases, 4. creatine sucrose agar (CREA) for extracellular acids, and 4. minimal Czapek agar (mCZA) for extracellular pigments. Very high concentrations of airborne fungal loads were documented in all five monitoring points (>13,000 CFU m^{-3}), with *Penicillium* and *Cladosporium* isolates dominating most sampling sites. A total of 30 fungal taxa were identified, belonging predominantly to the genera *Penicillium*, *Cladosporium*, *Fusarium*, *Epicoccum*, *Alternaria*, *Pseudopithomyces*, *Mucor*, *Circinella*, and *Absidia*. Lingninolytic potential was documented for most of the isolates (14 out of 24), which were predominantly laccase producers. Furthermore, extracellular acid production was observed in ten, hemicellulases in nine, and pigment production in one isolate, suggesting a high biodeterioration potential and a possible threat to wooden artifacts. The data contribute to an understanding of fungal diversity associated with cultural heritage environments, environmental risk assessment, and establish a baseline for future conservation studies at this internationally significant archaeological site.

KEY WORDS

Airborne fungi; Biodeterioration potential; Cultural heritage; Viminacium; Wooden artifacts.

ACKNOWLEDGMENTS

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FUNGAL PRINT IN THE AIR: LINKING EXPOSURE TO TESLA'S LEGACY AND OCCUPATIONAL HAZARD

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ABSTRACT

The indoor fungal print of museums plays an important role in safeguarding of cultural heritage for future generations and in ensuring visitors and staff' comfort and health. For this reason a multidisciplinary project "Air quality analyses in the Nikola Tesla Museum, with special emphasis on depots, exhibition space, and occupational hazard." was instigated in 2024. A total of 72 samples, i.e. 30 air samples collected using Super SAS DUO 360 air sampler, 23 and 19 samples of surface deposits collected from air condition filters and ventilation outlets with sterile swabs and adhesive tape method, respectively, were analyzed in order to qualitative and quantitative asses the degree of fungal contamination in depots, exhibition area and offices of Nikola Tesla Museum in Belgrade. Air fungal contamination higher than the upper limit of 150 CFU/m3, as recommended by WHO and MIBAC, was documented in 22 out of 30 analyzed premises with the highest contamination documented being 390 CFU/m3. Toxigenic molds Aspergillus flavus and A. niger were detected very rarely and in low numbers, and the fungi from other genera were not recorded at concentrations

higher than 50 CFU/m3 (*Cladosporium, Alternaria and Penicillium*). Deposit samples collected by sterile swabs revealed expected composition of mycobiota originating from soil and plants from outdoor environments, with exception of A. flavus and A. niger with small relative frequency and abundance. Microscopic analysis of adhesive tape samples showed actively growing fungal reproductive structures: melanized conidia, conidiophores, chlamydospores, and mycelia networks, as well as non-fungal structures serving as nutrient source: plant trichomes, pollen grains and different plant fibers. Obtained results were the driving force behind the initiative to replace filters in timely fashion. From a mycological perspective, the air quality in the analyzed rooms of the Museum is not hazardous to the health of employees and visitors, nor to the artifacts. The air quality control project at the Nikola Tesla Museum represents an important step toward achieving optimal environmental conditions for museum collections, as well as in the domain of occupational health. At the same time, it serves as a unique example of good museum practice in the field of conservation, which can act as a reference point for other heritage protection institutions in Serbia.

KEY WORDS

Air quality, heritage, Nikola Tesla, molds, museum.

FT-IR CHARACTERIZATION AND GLUCAN PROFILE OF *MORCHELLA CONICA* MYCELIUM OBTAINED BY OPTIMIZED SUBMERGED LIQUID FERMENTATION

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ABSTRACT

Submerged liquid fermentation (SLF) has attracted considerable attention due to its ability to produce high mycelial biomass yields within a relatively short cultivation period. Efficient SLF systems can be integrated into sustainable biomass production and food biotechnology, enabling the production of valuable bioactive compounds from fungal mycelia. Glucans are among the most important bioactive polysaccharides in edible and medicinal mushrooms, while Fourier-transform infrared (FT-IR) spectroscopy provides a rapid approach for characterizing their structural features. In this study, *Morchella conica* mycelium was cultivated under previously optimized SLF conditions and analyzed using Fourier-transform infrared (FT-IR) spectroscopy and beta glucans quantification with the Megazyme beta-Glucan assay kit. FT-IR spectra recorded over the range of 4000-400 cm-1 revealed characteristic absorption bands corresponding to hydroxyl groups (3269cm-1), aliphatic C-H stretching (2928 cm-1), amide I and II regions (1636-1545 cm-1), and carbohydrate-associated C-O-C vibrations (1021 cm-1).

Glucan analysis showed that the dry mycelial biomass contained 11.55% beta-glucan, while the total glucan content was 22.17%. These findings demonstrate the potential of optimized SLF for producing glucan-rich *M. conica* mycelium.

KEY WORDS

Morchella conica, mycelium, glucans, FT-IR spectroscopy.

ACKNOWLEDGMENTS

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PHYSICOCHEMICAL AND STRUCTURAL CHARACTERIZATION OF EXO- AND ENDOPOLYSACCHARIDES FROM *SCHIZOPHYLLUM COMMUNE* STRAINS CULTIVATED IN SUBMERGED CULTURE

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ABSTRACT

This study presents a comprehensive physicochemical characterization of exo- and endopolysaccharides obtained from submerged cultures of two *Schizophyllum commune* strains (Italy, ITA, and Serbia, SRB) cultivated for 14 days, with the aim of elucidating structural differences relevant to their biological properties. PSHs were isolated from both the extracellular medium (exo-PSHs) and mycelial biomass (endo-PSHs) and analyzed using the Congo red assay, scanning electron microscopy (SEM), Raman spectroscopy, ATR-FTIR spectroscopy, and thermogravimetric analysis (TGA).

The Congo red assay indicated distinct conformational behavior between the strains. PSHs from the ITA strain exhibited

a characteristic bathochromic shift under mild alkaline conditions, consistent with the presence of a triple-helical structure typical of β-glucans. In contrast, PSHs from the SRB strain showed no significant shift in the maximum absorption wavelength, suggesting a predominance of random coil or linear heteropolysaccharide structures and the absence of a stable triple-helical conformation. SEM analysis revealed that the morphology of exo-PSHs was strongly influenced by both strain origin and drying method. Air-dried samples displayed compact and rough surfaces, whereas lyophilized samples formed smoother, porous network-like structures. Notable microstructural differences between ITA and SRB exo-PSHs further suggested strain-dependent biosynthetic and compositional variability. Raman spectroscopy confirmed that exo-PSHs from both strains are predominantly β-(1→3)/(1→6)-D-glucans, consistent with schizophyllan-type structures, with characteristic bands corresponding to C–H, C–O–C, and glycosidic vibrations. Minor spectral variations indicated subtle differences in supramolecular organization and possible variations in triple-helix stability. ATR-FTIR spectra further supported the predominance of β-glucan structures in all samples, while additional signals indicated the presence of hydrogen-bonded water, residual ethanol from extraction, and minor amounts of proteins, lipids, and phenolic compounds. Differences in carbonyl-associated bands suggested a higher content of co-extracted biomolecules in ITA-derived PSHs compared to SRB samples. TGA analysis revealed higher thermal stability in ITA exo-PSHs, with delayed decomposition and greater structural robustness compared to SRB samples.

Overall, the results demonstrate that the origin of the *S. commune* strain significantly influences PSH conformation, purity, and physicochemical properties, which may ultimately affect their biological functionality.

KEY WORDS

Schizophyllum commune; polysaccharides; Raman spectroscopy.

IMPROVED METHOD FOR ISOLATION OF TRITERPENOID FROM *GANODERMA RESINACEUM* BOUD

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ABSTRACT

Genus *Ganoderma* has enjoyed a great deal of scientific attention for more than 20 years, as well as a great deal of financial success as highly sought medicinal mushrooms. However, there is a discrepancy between the most popular species, *Ganoderma lucidum*, and less extensively studied species, such as *Ganodema resinaceum*. On the MESFAST (*Medicinal Fungi Secondary Metabolites And Therapeutics*) database, genus *Ganoderma* has 665 secondary metabolites, only 29 of which are derived from *G. resinaceum*. Recently, this number has been increased. Just within the triterpenoid class, 43 compounds have been discovered. The need for research of novel compounds is at all times high; however, optimization of separation methods is often overlooked. In this work, we present an improved method for the isolation of the less reported compound, ergosta-6, 22-dien-3β, 5α, 8α-triol, a sterol previously reported to exhibit anti-inflammatory activity. This sterol has been reported in *G. resinaceum* previously, although in very small

quantity. Liquid–liquid extraction of the crude ethanolic extract with ethyl acetate, followed by silica gel column chromatography and reverse-phase preparative HPLC, afforded a 138-fold higher yield (relative to the mass of raw material used), than that reported in the initial study. Starting with approximately 570 g of raw material, 6 mg of high-purity compound was isolated. Structural characterization was performed by 1D and 2D NMR methods, enabling complete resonance assignment. This work highlights the necessity of carefully optimizing conditions to obtain the maximum yield of high-purity compounds needed for further research. By obtaining high-quality compound libraries, it is possible to further understand the function of such metabolites as well as derive better protocols for quality control of products from medicinal mushrooms. It also serves as a case study for alternative sources of bioactive compounds within the genus *Ganoderma*.

KEY WORDS

Ganoderma resinaceum, Medicinal mushrooms, Triterpenoids, Structural elucidation, NMR spectroscopy.

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CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF SUBCRITICAL WATER EXTRACTS FROM THE MUSHROOM *INONOTUS OBLIQUUS*

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ABSTRACT

Inonotus obliquus, commonly known as Chaga, is a medicinal mushroom that primarily grows on birch trees in cold temperate and boreal regions of the Northern Hemisphere. Due to its rich composition of biologically active compounds, Chaga has been used for centuries in traditional medicine, particularly in Russia, Siberia, the Baltic countries, China, and Korea. Traditional applications of *I. obliquus* have been associated with the treatment and prevention of various disorders, which has stimulated increasing scientific interest in its pharmacological properties. The investigations so far have revealed that *I. obliguus* produces a wide range of secondary metabolites, including phenolic compounds, melanins, carbohydrates, proteins and triterpenes.The aim of this research was to determine the chemical composition (total content of proteins, carbohydrates, phenols, total, α- and β-Glucans), extraction yield, as well as antimicrobial and antioxidant activity of subcritical water extracts obtained from pseudosclerotium *I. obliguus* originating from Mongolia (IM) and from Vlasina mountain, Serbia (IS). Supercritical water

extractions (SWE) were conducted at temperatures of 120 °C and 200 °C. The highest amount of carbohydrates, total, α- and β-gucans was determined in the samples obtained at 200 °C. However, the total glucan content differed significantly in the extracts obtained at 120 and 200 °C, in IS and IM. Total protein content was high regardless of temperature. The yields were determined as follows: IM SWE 120 °C (33.7 ± 1.10 g/100 g), IM SWE200 °C (21.27 ± 2.46 g/100 g), IS SWE 120 °C (20.52 ± 0.79 g/100 g) and SWE 200 °C (18.93 ± 0.23 g/100 g), respectively. The antimicrobial potential of subcritical water extracts was examined by the microdilution method using Gram-positive bacteria *Enterococcus faecalis* (ATCC 29219) and *Staphylococcus aureus* (ATCC 25923), as well as Gram-negative *Escherichia coli* (ATCC 25922) and *Actinetobacteria baumannii* (ATCC 19606) as test microorganisms. Gram-negative bacteria appeared to be more resistant in the presence of both tested subcritical water extracts (MBC – 20 mg/mL). According to the obtained minimum inhibitory concentration (MIC) obtained for *E. faecalis* and *S. aureus* (MIC -1.25 mg/mL), as well as for *E. coli* and *A. baumannii* (MIC -2.5 mg/mL) both extracts were shown to have the same effect on the tested bacteria. Chaga, known for its strong antioxidant effect, is primarily associated with phenolic compounds, which are key molecules capable of scavenging free radicals. The DPPH• free radical scavenging activity assay was used to measure the antioxidant capacity of the extracts *in vitro*. Extracts were tested in the range from 0.156 to 10 mg/mL, the ability to reduce free DPPH• radicals up to 80% was achieved at 0.625 mg/mL.

KEY WORDS

Chaga; subcritical water extraction; antimicrobial activity; antioxidant activity.

ACKNOWLEDGMENTS

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SELENIUM-ENRICHED PROTEINS FROM *PLEUROTUS DJAMOR*: ACCUMULATION CAPACITY AND SDS-PAGE CHARACTERIZATION

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ABSTRACT

Edible mushrooms of the genus *Pleurotus* are highly valued for their nutritional properties and their ability to accumulate essential trace elements. Selenium (Se) is an important micronutrient with antioxidant and health-promoting functions, making selenium-enriched mushrooms attractive candidates for the development of functional foods. This study investigated selenium accumulation and protein-associated selenium in fruiting bodies of *Pleurotus djamor* cultivated on substrates supplemented with selenium yeast. Total selenium content in mushroom samples and isolated protein fractions was determined by ICP-OES. Water-soluble proteins were isolated by ammonium sulfate precipitation and characterized by SDS-PAGE electrophoresis.

Selenium concentration increased from 1.06 ± 0.5 μg/g dry weight to 197.93 ± 8.45 μg/g dry weight in mushrooms cultivated on substrate supplemented with 100 mg Se/kg. Fructification was delayed by 3–5 days in treatments containing more than 50 mg Se/kg. The yield of water-soluble proteins was 18.6 ± 2.2 mg/100 mg of dry weight. SDS-PAGE analysis revealed a complex protein pattern comprising more than ten bands with molecular masses ranging from 9.5 to 110 kDa, with the most intense bands detected at approximately 36 and 17 kDa. Selenium concentration in the isolated protein fraction reached 134.6 ± 5.5 μg/g of the protein dry weight. However, increased selenium supplementation was associated with a reduction in the yield of water-soluble proteins compared with control samples. The results demonstrate that *P. djamor* efficiently accumulates selenium from selenium-enriched substrates and incorporates a considerable proportion of the element into protein fractions. These findings demonstrate the feasibility of producing selenium-enriched *P. djamor* and highlight its potential as a source of selenium-containing proteins for functional food applications.

KEY WORDS

Pleurotus djamor; selenium; bioaccumulation; proteins; SDS-PAGE; functional foods.

ACKNOWLEDGMENTS

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INTEGRATIVE ANALYSIS OF MINERAL COMPOSITION AND ANTIOXIDANT-RELATED TRAITS IN COMMERCIALLY AVAILABLE EDIBLE MUSHROOMS

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ABSTRACT

Edible mushrooms are increasingly recognized as valuable functional foods due to their nutritional profile and abundance of bioactive compounds. However, their mineral composition and antioxidant properties can vary considerably among species and are further influenced by environmental and cultivation conditions. Understanding this variability is important for evaluating their nutritional quality and potential contribution to a balanced diet. Moreover, integrating mineral composition with biochemical and antioxidant characteristics can provide a more comprehensive assessment of species-specific functional properties than considering individual nutritional parameters separately. This study aimed to characterize the mineral profiles of four commercially available fruiting bodies of four edible mushroom species, *Lentinula edodes*, *Agaricus bisporus*, *Pleurotus*

ostreatus, and *Hericium erinaceus*, and to explore their relationships with antioxidant-related traits using correlation and principal component analyses.

Potassium (K), calcium (Ca), sodium (Na), and phosphorus (P) contents were determined in the fruiting bodies and integrated with previously obtained data on DPPH, ABTS, and FRAP antioxidant assays, total phenolic content (TPC), and total protein content. We observed significant interspecific differences in mineral composition. *A. bisporus* had the highest K content; *L. edodes* was characterized by the highest Ca content, while *H. erinaceus* showed the highest Na content. P content was highest in *A. bisporus* and *P. ostreatus*. Exploratory Pearson correlation analysis revealed distinct correlation between mineral and antioxidant-related traits. Sodium was strongly negatively correlated with total protein content ($r = -0.90$), FRAP ($r = -0.85$), and TPC ($r = -0.82$), whereas P was positively correlated with protein content ($r = 0.71$), FRAP ($r = 0.62$), and TPC ($r = 0.61$). TPC, protein content, and FRAP also showed strong positive associations ($r = 0.99$). Principal component analysis explained 88.8% of the total variation, with PC1 and PC2 accounting for 62.3% and 26.5%, respectively, and revealed clear differentiation among the four analyzed species based on their integrated mineral and antioxidant-related profiles. Differing cultivation requirements, particularly for *A. bisporus*, may have contributed to variation in mineral profiles and their associations with antioxidant-related traits. The distinct positioning of the investigated species in the PCA further emphasized that no single nutritional or antioxidant parameter was sufficient to characterize their overall functional profile. Instead, combinations of mineral and biochemical traits contributed to species differentiation. Overall, the multivariate approach demonstrated pronounced species-specific nutritional profiles and revealed associations between mineral composition and antioxidant-related

characteristics, supporting an integrated assessment of the nutritional and functional properties of edible mushrooms.

KEY WORDS

Edible mushrooms; Mineral composition; Nutritional profile; Pearson correlation; Principal component analysis.

ACKNOWLEDGMENTS

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THE IMPACT OF *CORDYCEPS SINENSIS* ON THE BEER FERMENTATION PROCESS

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ABSTRACT

Cordyceps sinensis, a well-known entomopathogenic fungus used for centuries in traditional Chinese medicine, is recognized for a broad range of bioactive compounds, including cordycepin, polysaccharides, and adenosine derivatives, which exhibit antioxidant, immunomodulatory, anti-inflammatory, and anti-tumor properties, and which are also associated with improved physical strength and endurance. Beyond its established medicinal applications, growing scientific interest has been directed toward exploring the potential of Cordyceps extracts to influence microbial metabolism, particularly within fermentation-based food and beverage production. However, the precise mechanisms by which these extracts interact with living yeast cells during fermentation remain poorly understood and warrant systematic investigation. This study aimed to evaluate the impact of *Cordyceps sinensis* extract on the growth dynamics of brewing yeast and on the overall course of the beer fermentation process, including resulting changes in physicochemical and sensory characteristics of the finished beverage. Extract of *Cordyceps sinensis* were prepared and incorporated into wort

at several concentrations prior to inoculation with a standard brewing yeast strain. Fermentation trials were conducted under controlled laboratory conditions, with control batches fermented without extract addition. Yeast viability were monitored at regular intervals throughout fermentation, while pH, specific gravity, apparent attenuation, and ethanol content were also measured at defined time points. Addition of *Cordyceps sinensis* extract led to a measurable increase in yeast cell viability and biomass during the early stages of fermentation compared with control samples. Fermentation kinetics were accelerated at moderate extract concentrations, whereas higher concentrations slightly delayed attenuation, suggesting a dose-dependent relationship. The findings indicate that *Cordyceps sinensis* extract can modulate yeast growth and fermentation kinetics in a concentration-dependent manner, while also contributing potentially beneficial bioactive compounds to the resulting beverage. These results support further investigation into *Cordyceps sinensis* as a functional supplement in brewing, with promising applications in the development of novel functional beers possessing enhanced health-promoting properties and unique sensory qualities.

KEY WORDS

Cordyceps sinensis; fungus; beer; yeast; fermentation.

ACCELERATING BIOLOGICAL ACIDIFICATION BY *LACHANCEA THERMOTOLERANS* THROUGH MIXED-CULTURE FERMENTATION FOR SOUR BEER PRODUCTION

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ABSTRACT

Non-*Saccharomyces* yeasts have attracted increasing attention as emerging microbial platforms for industrial biotechnology due to their diverse metabolic capabilities and ecological adaptability. Among these species, *Lachancea thermotolerans* has gained particular interest in the brewing industry because of its ability to biologically acidify fermentation systems through lactic acid production during alcoholic fermentation. This characteristic offers an alternative to conventional bacterial acidification strategies in sour beer production, reducing contamination risks while increasing process flexibility and enabling controlled acidification across diverse fermentation applications.

Despite its promising industrial potential, broader implementation of *L. thermotolerans* remains constrained by relatively slow fermentation kinetics and prolonged processing times. In addition, the impact of interspecies yeast interactions on acidification performance and fermentation outcomes remains insufficiently explored. The present study investigated

the fermentation behavior and acidification capacity of *L. thermotolerans* in pure and mixed-culture fermentations for sour beer production and evaluated whether controlled yeast interactions could improve process efficiency while maintaining desirable sensory properties. Experimental optimization focused on fermentation temperature (20 and 25 °C), inoculation ratio, co-inoculation strategy, and glucose supplementation. Pure fermentations of *L. thermotolerans* achieved strong biological acidification, consistently reaching final pH values near 3.5 with increased titratable acidity; however, fermentation required up to 14 days for completion. Mixed fermentations with *Saccharomyces cerevisiae* (US-05) significantly accelerated fermentation while maintaining low final pH values (~3.6–3.7), although acidification remained less pronounced than in pure *L. thermotolerans* cultures. Supplementation with glucose (1 °P) further enhanced acidification, lowering final pH values by up to approximately 5%. Although pure *L. thermotolerans* fermentations at 25 °C progressed more rapidly without significant sensory defects, fermentations involving conventional brewing ale strains (*S. cerevisiae*) at 25 °C developed undesirable off-flavors.

Replacing conventional brewing ale strains in mixed fermentations at 25 °C with thermotolerant Kveik strains reduced fermentation duration by approximately 30–40% without generating undesirable sensory characteristics. Under optimized conditions, including elevated *L. thermotolerans* inoculation ratios and glucose supplementation (1 °P), fermentations reached completion within approximately 8 days and achieved final pH values as low as 3.49. Mixed cultures containing higher *L. thermotolerans* proportions (1:40 and 1:80) produced flavor profiles comparable to pure cultures and resulted in balanced acidity and enhanced drinkability.

These findings demonstrate that controlled interspecies yeast interactions can strategically modulate fermentation kinetics, acidification dynamics, and sensory outcomes while

potentially reducing cooling energy requirements, highlighting the broader biotechnological potential of *L. thermotolerans* and mixed-culture fermentation systems.

KEY WORDS

Lachancea thermotolerans; non-*Saccharomyces* yeasts; mixed-culture fermentation; biological acidification; sour beer.

ASSESSING YEAST PRODUCTIVITY ON AGRO-INDUSTRIAL BY-PRODUCTS: BREWER'S SPENT GRAIN AND BUCKWHEAT HULL

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ABSTRACT

Brewer's spent grain and buckwheat hulls are by-products of beer industry and buckwheat processing which, in the spirit of the circular economy, should not be treated as waste but as secondary raw materials with multiple uses. Their transformation contributes to environmental protection by reducing the large amount of waste sent to landfill and creating new, sustainable products. In this paper, the effect of brewer's spent grain (unwashed and washed with hot distilled water to remove impurities and achieve pH neutral) and buckwheat hulls on the productivity of four types of yeast (*Saccharomyces boulardii*, *S. cerevisiae*, *Candida albicans* and *C. utilis*) on potato–glucose agar was investigated. A filtered, sterilised suspension of brewer's spent grain and buckwheat hulls (20 g/350 mL) was used instead of demineralised water. Productivity (PR) was calculated as the ratio of CFU on test media to control, with PR ≥ 0.7 considered acceptable and statistical analysis (two-way ANOVA, Tukey's HSD, Shapiro-Wilk test) was performed. For all yeast species (except *S. boulardii*) productivity was greater than 0.7 compared to the control, and no statistically significant differ-

ence between treatments was observed. In contrast, for *S. boulardii*, unwashed brewer's spent grain statistically significantly reduced productivity (PR = 0.66), while at the same time buckwheat hulls (H1–Poland) performed statistically significantly better (PR = 1.06) than the control. Colonies grown on PDA with washed brewer's spent grain were smaller and darker compared to the control. The disadvantage of buckwheat hulls is strong influence on media colour (from opalescent, light amber in control to opaque and brown). Buckwheat hulls extract (H1 Poland) represents a superior and sustainable alternative to brewer's spent grain for yeast cultivation, but strain-specific responses must be considered when selecting growth supplements.

KEY WORDS

Brewers' spent grain, buckwheat husks, by-products utilisation, productivity, yeasts, circular economy.

ACKNOWLEDGMENTS

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THE POTENTIAL OF *TRICHODERMA* SPP. DIFFUSIBLE AND VOLATILE METABOLITES FOR BIOCONTROL OF PHYTOPATHOGENIC FUNGI AND CORN GROWTH PROMOTION

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ABSTRACT

The genus Trichoderma is classified among the most exploited microbial biocontrol agents employing the portfolio of pathogen suppression mechanisms, with antibiosis mediated by diffusible extracellular metabolites as the most abundant one. This study has investigated the potential of four Trichoderma isolates (T1, K4, G2 and G5) from substrates used in production of edible mushrooms to suppress growth of corn fungal pathogen in vitro (Aspergillus flavus, with confirmed mycotoxigenic potential). Several investigation routes were followed: 1) dual culture assays, investigating competition mechanisms, 2) diffussion methods, investigating the biocontrol effects of diffusible metabolites from Trichoderma culture supernatants after 7-days cultivation, and 3) double plate method, investigating the biocontrol potential of volatile Trichoderma metabolites. Furthermore, the effects of Trichoderma volatile metabolites to seed germination and growth promotion of corn in the initial growth phases were investigated using the double plate method.

Dual cultivation has resulted in percentual inhibition of Aspergillus flavus growth of 67.77%, 46.66%, 66.66% and 62.22% for the isolates T1, K4, G2 and G5, respectively. The diffusion methods resulted in biocontrol efficacy of 55.56% for the isolates T1, G2, and G5, and 53.33% for the isolate K4. Slightly lower inihibition percentage of Aspergillus flavus was observed in double plate experiments employing volatile metabolites for the isolates T1, G2 and G5: 59.26%, 61.73% and 50.62%, respectively, while the isolate K4 has exhibited a higher inhibition percentage of 67.90%, indicating the dominance of volatile metabolites in the G4 antifungal metabolome. Investigation of volatile metabolites' effects to corn growth has resulted in seed germination and growth inhibition by the isolates T1, T4 and G5. On the other hand, the volatile metabolites of the isolate G2 have significantly contributed to corn growth improvement, resulting in 173% increase in root length and 504% increase in shoot length. Future research will be aimed at identification of the Trichoderma isolate G2 to the species level and scale up of the experiments involving fungal biomass for production of plant-beneficial volatile metabolites.

KEY WORDS

Volatile organic compounds, antibiosis, Aspergillus, seed germination, biological control.

ACKNOWLEDGMENTS

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DETECTION OF THE *FUM1* GENE ASSOCIATED WITH FUMONISIN B1 BIOSYNTHESIS IN *FUSARIUM PROLIFERATUM* ISOLATED FROM SOYBEAN SEEDS

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ABSTRACT

Soybean (*Glycine max* (L.) Merr.) is one of the most important sources of plant protein worldwide, serving as a valuable resource for both human nutrition and animal feed. In the Republic of Serbia, soybean is cultivated on approximately 230,000 hectares annually. During the 2023/24 soybean seed health assessment, conducted on samples collected from different regions of Serbia, symptoms of seed rot were observed. A total of 43 fungal isolates were recovered from infected soybean seed samples, of which *Fusarium proliferatum* was identified as the predominant species (20 isolates). The identity of these isolates was confirmed by morphological characteristics and further validated by PCR using species-specific primers. PCR analysis also confirmed the presence of the *FUM1* gene (780 bp) in the investigated isolates. The detection of this gene, indicates their potential for fumonisin B1 production and highlights the possible risk of mycotoxin contamination in soybean seeds. These

findings emphasize the importance of monitoring mycotoxigenic *Fusarium* species associated with soybean seeds to ensure seed quality and feed safety.

KEY WORDS

Fusarium proliferatum, soybean, fumonisin B1, seed rot.

ACKNOWLEDGMENTS

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BRIDGING CLINICAL TOXICOLOGY AND MYCOLOGICAL EXPERTISE: CASE-BASED INSIGHTS INTO POISONOUS MUSHROOMS AND MUSHROOM POISONING IN MACEDONIA

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ABSTRACT

Wild mushroom foraging has become increasingly popular in Macedonia over the past two decades, particularly during the summer and autumn months, following rainfall, when conditions favour the development of fungal sporocarps. Cases of mushroom poisoning are reported annually. Approximately 120 potentially poisonous macrofungal species have been recorded in the country, mainly belonging to the genera *Amanita*, *Lepiota*, *Entoloma*, *Cortinarius*, and *Inocybe*, several of which may cause severe or fatal intoxications. Accurate and timely identification of the ingested species is essential for appropriate clinical management; but this is often difficult because physical specimens are rarely available at the time of hospital admission. Rapid assessment of suspected mushroom poisoning is essential for appropriate clinical management.

The University Clinic of Toxicology and the Mycological Laboratory at the Institute of Biology, Faculty of Natural Sciences

and Mathematics, have established a formal Memorandum of Cooperation that enables real-time interdisciplinary consultation in toxicological emergencies. This study reports a family cluster of mushroom poisoning involving a 44-year-old father, a 44-year-old mother, and their 10-year-old son from Demir Hisar, Macedonia. After consuming self-collected wild mushrooms believed to be edible, the child developed impaired consciousness approximately one hour after ingestion, and required emergency medical evaluation. Several hours later, both parents experienced dizziness, confusion, visual disturbances, and hallucinations, a clinical presentation compatible with ibotenic acid-muscimol neurotoxic syndrome.

During consultation with the Poison Information Centre, photographs of the consumed mushrooms stored on the father's smartphone were transmitted via the Viber messaging application to a mycologist from the Mycological Laboratory. Expert visual assessment of the images was performed within the established toxicology-mycology consultation framework. However, the moist and altered appearance of the specimens, together with the absence of habitat collection-site and ecological data prevented definitive taxonomic identification. No physical specimen was available for direct morphological or molecular taxonomic examination. Despite these limitations, the photographic assessment, exposure history and observed clinical manifestations, supported recognition of a neuropsychiatric mushroom poisoning syndrome, facilitated risk stratification, and contributed to appropriate supportive treatment. No significant organ toxicity developed, and all three patients recovered without complications.

This case demonstrates the value of formal collaboration between clinical toxicologists and mycologists, supported by digital communication technologies. When direct examination of mushroom specimens is not possible, smartphone-transmitted photographs may provide clinically useful information for

presumptive identification and syndrome recognition, although they cannot replace direct taxonomic examination. The successful Institutional cooperation between toxicology and mycology services established through formal cooperation between the University Clinic of Toxicology and the Mycological Laboratory at the Institute of Biology demonstrates how institutional partnerships can enhance diagnostic capacity and strengthen the management of mushroom poisoning in Macedonia.

KEY WORDS

Toxicology; mycology; mushrooms; poisoning; neurotoxicity syndrome; interinstitutional partnership, telemedicine.

OCCURRENCE OF YEAST AND MOULDS IN RAW DONKEY MILK

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ABSTRACT

Donkey milk has attracted increasing attention due to its nutritional properties and potential use as an alternative food source for sensitive consumer groups. However, information regarding fungal contamination of donkey milk remains limited. The occurrence of yeasts and moulds in this dairy product has been investigated far less frequently than bacterial contaminants, despite their importance for product quality, shelf life, and food safety.Therefore, the aim of this study was to evaluate the occurrence of yeasts and moulds in raw donkey milk. A total of 51 milk samples were obtained by manual milking from clinically healthy donkeys between May 2023 and October 2024. Samples were transported under refrigerated conditions and analyzed using standard microbiological methods for the enumeration of yeasts and moulds. In selected positive samples, fungal colonies were further examined by light microscopy to assess their morphological characteristics.Yeasts and moulds were detected in most samples, with counts ranging from 2 to 300 CFU/mL. Most samples exhibited relatively low fungal counts, while only a small number of samples showed values above 100 CFU/mL. Moulds were detected in four samples only, with counts of 2, 4, 4, and 12 CFU/mL, indicating a low prevalence (7.8%)

and low contamination level. Microscopic examination of mould isolates revealed structures morphologically resembling *Aspergillus* spp.; however, species-level identification was not performed. The generally low mould counts observed in this study suggest that raw donkey milk exhibited satisfactory fungal quality. Nevertheless, the occasional detection of *Aspergillus*-like isolates highlights the importance of monitoring fungal contaminants in raw milk, as some members of this genus are recognized as spoilage organisms and may represent a potential food safety concern under favourable conditions.These findings contribute to the limited body of knowledge regarding the fungal microbiota of donkey milk and provide a basis for future studies involving species identification, mycotoxin-producing potential, and risk assessment associated with fungal contamination of this increasingly valued dairy product.

KEYWORDS

Donkey milk; yeasts; moulds; *Aspergillus* spp.; food safety; fungal contamination.

THE MYCOLOGICAL SIGNIFICANCE OF HONEY: FUNGAL MICROBIOTA, ANTIFUNGAL PROPERTIES, AND IMPLICATIONS FOR FOOD SAFETY AND HEALTH

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ABSTRACT

Honey is a complex natural product that serves not only as a nutrient-rich food but also as a unique ecological niche for diverse fungal communities and a valuable source of naturally occurring antifungal compounds. Growing interest in the interactions between honey, fungi, and bee-associated microbiota has expanded current understanding of fungal ecology, food safety, and the development of alternative antifungal strategies. This review provides a comprehensive overview of the mycological significance of honey by integrating current knowledge on fungal diversity, ecological interactions, contamination pathways, antifungal mechanisms, and potential biomedical applications.

Honey naturally harbors a variety of osmophilic yeasts and filamentous fungi originating from floral nectar, pollen, bees, environmental sources, and post-harvest handling. While many of these microorganisms constitute part of the normal honey microbiota, inappropriate harvesting, processing, or storage

conditions may facilitate the proliferation of spoilage fungi and opportunistic mycotoxigenic species, particularly members of the genera *Aspergillus* and *Penicillium*, with potential implications for food quality and consumer safety. Consequently, fungal monitoring and microbiological quality assessment represent essential components of honey quality assurance.

Conversely, honey exhibits broad-spectrum antifungal activity attributed to the synergistic action of multiple bioactive constituents, including hydrogen peroxide, methylglyoxal, bee defensin-1, antimicrobial peptides, phenolic acids, flavonoids, organic acids, and high osmotic pressure. Experimental studies have demonstrated inhibitory effects against clinically and veterinary important fungal pathogens, including *Candida albicans*, *Candida glabrata*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, and dermatophytes, highlighting the therapeutic potential of honey as a complementary natural antifungal agent. Particular attention is also given to the beneficial bee-associated microbiota, especially *Bombella apis* and lactic acid bacteria, which contribute to colony health by suppressing fungal pathogens and maintaining microbial homeostasis.

Recent advances in molecular microbiology, metagenomics, and metabolomics have considerably improved our understanding of the honey mycobiome and its ecological complexity. Nevertheless, significant knowledge gaps remain regarding fungal biodiversity across different botanical and geographical origins, interactions between fungal communities and bee microbiota, mechanisms underlying antifungal activity, and the standardization of bioactive compounds responsible for therapeutic efficacy. Within the One Health framework, honey represents an important intersection of environmental, animal, and human health, offering promising opportunities for developing sustainable antifungal interventions. Future multidisciplinary research integrating mycology, food microbiology, apiculture, natural product chemistry, and clinical sciences will be essential for

translating these findings into evidence-based applications in medicine, veterinary practice, food preservation, and functional food development.

KEYWORDS

Honey; mycology; fungal microbiota; osmophilic yeasts; antifungal activity; *Candida; Aspergillus*; bee microbiota; food safety; One Health.

POTENTIAL MECHANISMS OF ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF NATIVE THAI MUSHROOMS ASSESSED VIA BIOREPORTER SCREENING

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ABSTRACT

The rising global antimicrobial resistance (AR) has become as important as climate change. However, the existing antibiotics and the development of novel ones cannot keep up with the constantly evolving AR mechanisms of pathogenic and biofilm-forming bacteria. Thus, novel solutions require multiple mechanisms of action, known as polypharmacology. Currently, 60% of all existing antimicrobial compounds are derived from natural sources, including fungi. Species from

Basidiomycota class possess particularly high potential for antimicrobial compounds with more than one mode of action. Interestingly, Thailand's mycoflora is under-researched, with as high as 96% of species with the potential to be novel. This presents a vast pool for novel molecules with potent antimicrobial/antibiofilm activity. In our study, we tested common species highly present on Thai markets, including *Termitomyces clypeatus* R. Heim, *Volvariella volvacea, Schizophyllum commune, Lentinus squarrosulus*, and *Thaeogyroporus portentosus*. Varying concentrations of mushrooms' ethanolic extracts (0.156-20 mg/mL) were assessed against *Pseudomonas aeruginosa* ATCC 35032, *Staphyloccocus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29219, *and Salmonella* Enteritidis ATCC 22076 using the Minimal Inhibitory Concentration (MIC) Assay. On the other hand, antibiofilm activity was screened with a crystal violet staining assay. Further elucidation of potential antimicrobial mechanisms was examined via Bioluminescent Whole-Cell Bioreporter Panel based on six genetically modified *E. coli* bioluminescent reporter strains with respective promoter sensitivities: K802NR (promoter *lasI* for QS disruption), TV1061 (promoter *grpE* for heat shock and protein damage), DPD2794 and DPD1718 (promoter *recA* for genotoxicity and DNA damage), DPD2544 (promoter *fabA* for fatty acid biosynthesis inhibition), and DPD2511 (promoter *katG* for oxidative stress). All extracts demonstrated low antimicrobial efficacy, while the antibiofilm activity was significant. *T. clypeatus* R. Heim showed 100% inhibition of *E. coli* and *P. aeruginosa* biofilm. *L. squarrosulus* exhibited strong antibiofilm properties and moderate direct antimicrobial activity against *S. aureus* and *P. aeruginosa*. This efficacy might be associated with quorum-sensing disruption and potential genotoxic stress induction. *S. commune* displayed moderate effects, while *V. volvacea* and *T. portentosus* exhibited high-dose cytotoxicity linked to severe oxidative stress. Finally, *T. clypeatus* R. Heim and

L. squarrosulus have emerged as promising candidates that warrant further investigation for potential antibiofilm agents.

KEYWORDS

Thai mushrooms; antimicrobial activity; antibiofilm activity; bioreporter whole-cell bacterial panel; ethanol extract

ACKNOWLEDGMENTS

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WATER ACTIVITY AS AN INDICATOR OF THE STABILITY OF PROCESSED ANIMAL PROTEINS AND FINISHED PET FOOD

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ABSTRACT

Processed animal proteins, including fish meal, poultry meal, pork meal and lamb meal, represent important raw materials used in the production of pet food. The stability of these products during storage largely depends on the availability of free water, expressed as water activity (aw), which is considered one of the most important indicators of the microbiological stability of dry products. Low water activity limits the growth of microorganisms, including moulds, thereby contributing to the preservation of product quality and safety.

The aim of this study was to evaluate the microbiological stability of processed animal proteins and finished pet food by determining water activity, moisture content, and mould count.

A total of 50 samples were analysed, including 30 samples of processed animal proteins (fish meal, poultry meal, pork meal and lamb meal) and 20 samples of finished pet food. Water activity and moisture content were determined in all samples, while mould counts were determined by cultivation on DG18 agar.

Water activity values ranged from 0.1799 to 0.4220, with a mean value of 0.276 ± 0.066. Moisture content ranged from 2.28% to 7.52% (4.15 ± 1.48%) in finished pet food and from 1.81% to 5.02% (3.78 ± 0.86%) in processed animal proteins. In all analysed samples, the mould count was below the limit of detection.

The obtained results indicate that the analysed products exhibited very low water activity and low moisture content, accompanied by the absence of mould growth. These findings support the importance of water activity as a useful parameter for assessing the microbiological stability of processed animal proteins and finished pet food, supporting its use as a routine quality control parameter for dry products. Furthermore, the determination of water activity may serve as a useful tool for assessing storage conditions and maintaining product stability throughout shelf life. Continuous monitoring of water activity, together with moisture content and microbiological parameters, may contribute to improving quality control systems and ensuring the safety of pet food products. The results also highlight the value of considering water activity together with moisture content when evaluating the stability of dry animal-derived products. Such measurements can provide complementary information for routine monitoring of product quality during storage and production.

KEYWORDS

Pet food; water activity; moisture content; processed animal proteins; moulds.

ACKNOWLEDGMENTS

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NANOTECHNOLOGY-BASED STRATEGIES FOR MOULD CONTROL AND MYCOTOXIN MITIGATION

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ABSTRACT

Moulds are considered a public health hazard, as certain species produce mycotoxins and release spores into the environment. The prevention and control of mould contamination is developing with great difficulty due to fungicidal resistance and the high toxicity of fungicides ingredients. The frequent occurrence of fungicidal resistance is a consequence of the repeated use of agrochemicals, as well as shifts in mould populations, which have favored the selection of resistant mutants. The application of nanoparticles (NPs), and particularly engineered nanoparticles (ENPs), is a new technology and a starting point in preventing mould contamination. This study describes the extant knowledge concerning antifungal and anti-mycotoxin potential of different NPs in food sector.

The efficacy of ENPs depends on their chemical composition, particle size, and concentration. Nanotechnological solutions follow three core strategic directions: direct mould inhibition, mycotoxin adsorption, and the development of toxin-eliminating nanomaterials incorporated into food packaging. The mechanisms of action exhibited by NPs are diverse and

fundamentally dictated by their specific physicochemical types. Basically, the primary nano-fungal interaction begins with physical adherence to the cell wall. Mould spores are more resistant to mechanical damage than bacterial cells, since the most important component of the spore cell wall, chitin, is more resistant than the peptidoglycans that build the bacterial cell wall. A possible model of nano-fungal interaction is that ENPs after entering the mold cell are localized in the endosome, where they interact with endosomal proteins associated with mycotoxin biosynthesis and mold virulence. A principal mechanism of action involves the intracellular generation of reactive oxygen species (ROS) within the endosome. Oxidative stress triggers the cell's antioxidant defence mechanism, effectively delaying the transcription pathways responsible for mycotoxins production.

Metal NPs (MNPs) are synthesised from inorganic elements or their compounds (oxides). Silver NPs are the most used MNPs, characterised by low volatility and high-temperature stability. They can be added to various matrices, polymers, and stabilizers. Ag NPs exhibit simultaneous and synergistic antifungal action through multiple mechanisms including ROS production, cell membrane disruption, which increases membrane permeability, and interaction of Ag ions with proteins, enzymes and nucleic acids. Because of this type of action, suppression of fungal growth and metabolism occurs.

Carbon nanotubes (CNTs) are used in various industry segments. CNTs are nano-sized hollow cylindrical forms of carbon that have demonstrated antifungal activity. CNTs have the capacity to bind both polar and non-polar compounds, due to their unique size, structure and large specific area. According to recent studies, the physical interaction of CNTs with microorganisms, rather than oxidative stress, is the primary antimicrobial activity of these nanostructures. The efficacy of CNTs increases with the addition of Ag, ZnO, TiO_2 NPs, or functional chemical groups.

Essential oils (EOs) belong to the class of volatile secondary metabolites and consist of a complex mixture of low-molecular-weight organic components (phenols, terpenoids, chitosan, sulphur compounds and others). The EOs, due to their highly lipophilic nature and low molecular weight, can disrupt the cell membrane, causing cell death or inhibiting the sporulation and germination of fungi. High volatility, thermal instability, and poor dispersibility are limitations of using EOs in industry and agriculture. The solution is in nanotechnology and nano-micro encapsulation procedures, aimed at limiting oxidation processes and extending the shelf life of the oils.

Nanocomposites are new generation of ENPs. They have become promising, customizable solutions by combining multiple materials into a single system that integrates adsorption, antimicrobial action, stability, and controlled release to simultaneously inhibit moulds and bind mycotoxins.

The increasing number of resistant mould isolates worldwide poses a challenge for the development of effective fungicides that are affordable and low in toxicity. Despite this potential, additional research is required to assess the impact of nanomaterials on human and animal health, in accordance with existing guidelines and legal frameworks.

KEYWORDS

Moulds; fungicidal resistance; engineered nanoparticles (ENPs); green chemistry.

ACKNOWLEDGMENTS

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SCHIZOPHYLLUM COMMUNE AS A BIOLOGICAL BINDER FOR LIGNOCELLULOSIC BIOCOMPOSITES: MULTIFUNCTIONAL MATERIAL CHARACTERISATION

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ABSTRACT

The transition towards renewable and biodegradable materials has increased interest in fungal mycelium as a biological platform for converting lignocellulosic residues into functional biocomposites. Filamentous fungal growth enables the formation of interconnected hyphal networks capable of binding particulate substrates without synthetic adhesives, providing a promising route towards low-density materials derived from renewable feedstocks and agricultural waste. In this study, the white-rot fungus *Schizophyllum commune* was employed as a natural biological binder for the production of mycelium-based lignocellulosic composites and their mechanical, thermal and electrical properties were characterised.

Two substrate formulations were investigated: a reference material based on 100% straw and a modified formulation containing 70% straw and 30% sawdust. Substrates were inoculated with *S. commune*, conditioned at 59.6 -72.5% moisture content and incubated under controlled conditions at 21.5- 23.4 °C until colonisation. Following fungal growth, the composites were dried at 40-60 °C to terminate biological activity and stabilise their properties. No additional synthetic binder was used. Mechanical behaviour was evaluated by compression testing and differential modulus analysis, thermal conductivity and diffusivity were determined using the dynamic plane source method, and frequency-dependent electrical behaviour was examined by impedance spectroscopy. Both mycelium-bound materials exhibited the strain-dependent mechanical response characteristic of porous bio-based cellular structures. Modification of the lignocellulosic substrate markedly affected mechanical performance: the straw-sawdust formulation exhibited substantially higher differential modulus values, reaching 1,541.40 kPa at 10-30% deformation and showing an approximately 3.65-fold overall improvement relative to the reference formulation. Despite differences in substrate composition and mechanical behaviour, both composites maintained very low thermal conductivity, with mean values of 0.0477 and 0.0480 W m^{-1} K^{-1}, respectively, and no significant difference between formulations. Thermal diffusivity was similarly stable at approximately 0.128-0.130 × 10^{-6} m^2 s^{-1}. Electrical characterisation additionally revealed formulation-dependent, frequency-responsive behaviour, including an inductive-to-capacitive transition in the modified composite near 95 MHz.

These findings demonstrate that *S. commune* can function as an effective biological binder in lignocellulosic biocomposites while substrate composition provides a means of modulating material performance. The combination of enhanced mechanical properties, low thermal conductivity and frequency-dependent

electrical characteristics highlights the potential of fungal biotechnology for transforming agricultural and wood-derived residues into multifunctional, fully bio-based materials for insulation, packaging and other sustainable engineering applications.

KEYWORDS

Schizophyllum commune; mycelium biocomposites; fungal biotechnology; lignocellulosic residues; sustainable biomaterials.

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