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A Critical Review of Microfungal Contamination in Cultivated Edible Mushrooms: Sources, Pathogenicity, and Integrated Management

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ABSTRACT

Mushroom farming has become important globally due to its nutritional, economic and environmental value. The economic threat to productivity and food safety caused by contamination of filamentous microfungi is not effectively dealt with for the long term. This essay presents a useful, thematic synthesis of the kinds of microfungal contaminants, where do these come from, how are they infecting the cultivated edible mushrooms and how they could be controlled sustainably especially post-harvest and integrated management. The authors performed a methodological narrative literature review based on thematic analysis framework using main scientific databases on fungal contaminants, their contamination pathways and respective management methods. The results were thematically categorized into systems of cultivation, diversity of pathogen and pathogenic mechanisms, detection methods and controls. The results show that Trichoderma, Aspergillus, Penicillium and Cladobotryum are common economic contaminants. Contamination at key points can occur during spawns, casing, and after-harvest handling and storage due to poor hygiene, insufficient sterilization as well as environmental condition. These fungi slow the growth of mushroom mycelia by growing at a faster rate, enzyme degradation and/or mycotoxin production, which is an important risk for food safety and human health which isn't very much thought-of actively. The research points out that there has been no objective quantification of risk from mycotoxins and no harmonized, in situ detection of contaminated mushrooms. An Integrated Disease Management (IDM) approach comprising of preventive, physical, biological and minimal chemical interventions is recommended for effective and sustainable management of disease-causing insect pest. Future studies should place special emphasis on creating real-time molecular diagnostics, effective and environmentally safe biocontrol agents, and eco-friendly environmental control systems to protect the safety and sustainability of global mushroom production.

1-Introduction

Mushrooms are recognized globally for their nutritional, medical and economic significance for food purposes. The high-quality proteins, dietary fiber, vitamins particularly B-compelx and bioactive compounds with antioxidant and immunomodulatory properties are rich sources of [1, 2]. Therefore, an increased demand for functional food and sustainable protein has propelled the mushroom industry towards rapid growth, resulting in a global production of more than 34 million metric tonnes [3]. Mushroom production supports food security and livelihoods in low- and middle-income countries (LMIC) with a high prevalence of smallholder farming [4,7]. They can further be used for sustainability in agriculture by converting agricultural waste into biomass. While the industry has grown rapidly, certain microfungi contaminants we know of, cause we adopt pathogen by pathogen action. Mushroom growing systems, whether it is a traditional one or climate controlled have several steps to undergo such as substrate preparation, sterilisation, spawning, incubation, casing, fruiting and postharvest handling which are all susceptible to contamination [12] Numerous studies have characterized fungal pathogens but no single report critically links pre-harvest contamination events with postharvest losses and food safety threats (especially production of mycotoxins) and integrated and future-proof management strategies. To address this shortcoming, we provide a systematic and analytical framework; this review is more than a descriptive collection. Mushroom crop contaminants are predominantly of the genera Trichoderma, Aspergillus, Penicillium and Cladobotryum; they compete for space and nutrients, they directly pathogenize the mushroom mycelia or they produce toxic secondary metabolites[13,17]. The economic impact is very high, since for instance a green mould outbreak (Trichoderma aggressivum) can cause a yield loss of 100%[18]. Apart from losing the product itself, contamination also raises production costs due to the need for more cleaning and disposal. Most importantly, they reduce the marketability of products and heighten under-appreciated food safety concerns regarding mycotoxins in fruiting bodies [17, 19].the objective of this study is to prepare a critical and organized picture of the stored knowledge on microfungal contamination of cultivated edible mushrooms. The aim of our project is to collate the diversity, source and infection mechanisms of major contaminants, assess the ecological and environmental drivers of contamination from cultivation to postharvest, critically evaluate current detection and management methods, and identify key gaps in knowledge and propose an integrated framework for sustainable disease management in future. This assessment specifically enlarges the characterization of usually neglected mycotoxin risk and postharvest spoilage.

2- Methodology

A systematic review has been conducted critically on microfungal contamination of cultivated mushrooms as reported in the literature. The methodology aimed to elaborate a thematic synthesis of the factors causing contamination, principal pathogens and the IMs applied from cultivation to postharvest.

1.1 Literature Search Strategy

Scientific databases, in which Scopus, Web of Science, PubMed, ScienceDirect are the major, encompassing systematic search of the literature was performed. A predefined set of keywords guided the search including ‘mushroom contamination’, ‘fungal pathogens’, ‘cultivated mushroom’, ‘*Trichoderma* green mould’, ‘*Cladobotryum* cobweb’, ‘*Lecanicillium* dry bubble’, ‘*Mycogone* wet bubble’, ‘*mycotoxin*’, ‘postharvest spoilage’, ‘integrated disease management’, and ‘biological control’. The aim was to find peer-reviewed articles, reviews, and book chapters providing primary data or authoritative syntheses relating to those topics on widely cultivated species such as *Agaricus bisporus*, *Pleurotus* spp. and *Lentinula edodes*.

1.2 Inclusion and Exclusion Criteria

Studies were selected based on key themes, namely fungal genera with pathogenic mechanisms, source of contamination, symptoms of diseases, methods of detection, control methods that include physical, chemical, biological, and postharvest aspects. The articles assessed through comprehensive critical syntheses or strong experimental design were prioritized. The developed exclusion criteria were used to remove studies which were not directly related to the topic in question (e.g. studies relating to bacterial contamination, strictly wild mushroom ecology, unrelated plant pathology) as well as non-peer-reviewed reports lacking methodological detail.

1.3 Data Extraction and Thematic Synthesis

Thematic analysis framework was used to synthesise and critically evaluate data, rather than simply to give a descriptive summary of studies. After spotting the trends and determining common themes, contradictions or inconsistencies were noted in the studies. The primary themes from the extracted data are as follows:

a) From spawning to postharvest storage – the systems and critical control points in cultivation.

b) The Potential of Microfungal Contaminants as Pathogens and Toxins.

c) Mechanisms of disease establishment and competition

d) Methods for detecting and identifying: ancient methods that are now advanced using molecular techniques.

e) Use of preventive, physical, chemical, and biological measures.

f) Environmental and Ecological Forces of Contaminate

2.4 Quality Assessment

All studies were subjected to critical appraisal as to their rigor. This is a simple scoring approach. We gave preference to studies with good experimental designs, adequate controls and clear reporting of data. Weighting was given to seminal works and high citation impact peer-reviewed journal articles in constructing the conceptual framework for the synthesis. Some low impact studies in some specialised areas were descriptive and of low impact and were likewise included because they provided useful context.

1.4 Limitations

There are many limitations to this review First, it was limited to English-language publications which may have led to the omission of relevant studies in other languages. Secondly, the review concentrated on major genera being commercially cultivated and, thus, may not reflect the contamination dynamics of niche or regional species. One limitation was that the literature selection, while systematic in approach, was not a fully systematic review that followed a registered protocol and quantitative meta-analysis. We recognise this limitation and detail presented is an effort at generous and balanced synthesis.

3- Findings and Critical Synthesis

3.1 Mushroom Cultivation Systems as Determinants of Contamination Risk

The fundamental susceptibility of cultivated mushroom species to contamination is influenced by their physiological and ecological requirements. Button mushroom and oyster mushrooms. Substrates of rich nutrition like composted manure, straw (oyster mushroom) and *Lentinula edodes* (shiitake) is best suited to grow fungi and other opportunistic micro fungi [24,32]. Species with long incubation periods, like *Lentinula edodes*, are naturally more vulnerable since the long period provides an opportunity for contaminants to establish[31,32].

This review claims that the foundation of all other management practices to mitigate risk is species-specific vulnerability based on growth rate and substrate ecology.

3.1.1 Substrates, Environmental Conditions, and Postharvest Continuity

The makeup of the substrate is key. Failure to fully pasteurize or sterilize nutrient-rich substances is the major source of contamination for the thermotolerant fungi Trichoderma and Aspergillus [13, 33, 34]. The same conditions that favour mushroom production also lead to the excessive production of contaminants,¾that is, high humidity (80 to 95 per cent), moderate temperature and poor circulation of air. Managers should consider the environmental management controls used in the growing room as continuous with the postharvest storage controls. When stored under high temperature and humidity conditions, the growth of quiescent infections or Cladobotryum surface contaminants takes place rapidly. This is directly linked to the conditions of cultivation. The cause of spoilage at market level and accumulation of toxin mycotoxins can be attributed to that[36].

3.1.2 Critical Points of Contamination: From Spawning to Market

a) The use of contaminated spawn or aseptic lapses during inoculation introduces fast-growing competitors like *Aspergillus* and *Penicillium* at the most vulnerable phase [12].

b) Casing refers to the layer essential for fruiting of *Agaricus*. If casing is not properly pasteurized, it acts as a reservoir for *Trichoderma* and *Cladobotryum* [19]. Further, the high moisture casing accelerates their spread.

c) Harvesting, handling, and storage represents a serious, yet often neglected, vulnerability. Postharvest pathogens like the Cladobotryum dendroides can enter and grow on fruits due to physical injury, unhygienic instruments, as well as uncontrolled storage conditions. The occurrence of latent infection before harvesting and the manifestation of symptoms after harvesting are two occurrences that have been little studied, although useful. Nevertheless, they have certain practical consequences, especially on the safety and durability of food products [36].

3.2 Microfungal Contaminants: From Ecology to Mycotoxin Risk

Mushroom production is spoiled mainly by filamentous micro fungi (moulds) because of their vigorous and rapid mycelial growth. The main contaminants may be classified according to their ecological strategy and risk.

- Primary Colonizers/Mycoparasites (e.g., *Trichoderma* spp.): Aggressive competitors that attack mushroom mycelia.

- Secondary invaders/ saprophytes (*Penicillium* spp., *Aspergillus* spp.) significant for poor sanitation or existing substrate degradation nonetheless represent a large food safety risk.

- Fruiting body pathogens can damage the marketable product causing crop loss. For example, *Cladobotryum* spp. and *Lecanicillium fungicola* cause alarming damage in white button mushrooms (Agaricus bisporus).

3.2.1 Sources and Their Management Implications

Airborne spores are everywhere, entering via air conduits and man activity [41,44]. The major vectors are untreated water, poorly pasteurized substrate, residual organic matter on equipment and personnel [13, 45]. A key finding that emerges from the synthesis is that any event of contamination is unlikely to be a single fault or failure. In fact, failures are occurring at the level of the biosecurity chain at multiple points. Therefore, it is important to take a systems-wide view of biosecurity contamination rather than just in isolation.

3.2.2 Common Genera and Their Integrated Impacts

- ***Trichoderma* spp.:** This green mould is caused by *T. aggressivum* is known to cause a yield loss that transpires very rapidly through colonization[13, 18, 48].
- ***Penicillium* and *Aspergillus* spp.:** De spite often being termed secondary saprophytes, their presence is an indicator of poor hygiene. Significantly, **their capacity to produce a strong mycotoxins (*aflatoxins* by *A. flavus*, ochratoxins by *Penicillium* species) makes them an important food safety risk. The review identifies what is likely an under-quantified risk of consumers being exposed to carcinogenic compounds through contaminated mushrooms and the need for investigation. [17].**
- ***Cladobotryum* spp.:** This pathogen, the cause of the disease cobweb, has a tendency to multiply rapidly in the fruiting bodies. It occurs during the cool and humid cropping and the postharvest stages. Hence, controlling the disease at these two stages is important. Moreover, the use of proper climate control in the whole life cycle of the product is also vital. [36, 46].
- ***Fusarium*, *Mucor*, and *Rhizopus* spp.:** Fast-growing secondary invaders are often associated with tissue damage or inferior substrates that lead to spoilage and production of a range of mycotoxins. [17, 27].

3.3 Major Microfungal Diseases as Model Systems

The four major diseases are prototypes of different pathogenicity strategies.:

- **Green Mould (*Trichoderma*):** Competes aggressively with substrate and parasites, which thrive due to enzyme versatility [18].
- **Cobweb Disease (*Cladobotryum*):** Research predicts fast posthumous spread, environmental opportunism flourish in humid air[36].
- **Dry Bubble (*Lecanicillium fungicola*):** Models redirect disruptions in tissue differentiation on fruiting bodies, resulting in excessive mycelial overgrowth and consequential poor quality. [19].
- **Wet Bubble (*Mycogone perniciosa*):** Tissue is broken down and it is infected at early primordia [19]. When looking at these diseases, it becomes evident that nothing works all the time. A countermeasure can be designed to counter each pathogen using an integrated strategy in which the pathogenic strategy (competitor vs. tissue disrupter) and stage of infection (substrate colonization vs. fruiting body) are most decisive.

3.4 Mechanisms of Pathogenicity and Competition: Converged toolbox.

Contaminating microfungi have a convergent set of mechanisms that explain their success and strategies to control them.:

a) **Enzymatic Degradation:** Mushroom hyphae produce cellulases, hemicellulases and chitinases that enables rapid colonization of substrate and decomposition. [48].

b) **Mycoparasitism:** A mechanism of direct attack that happens in *Trichoderma*, with hyphal coiling and lytic enzyme secretion. [49].

c) **Secondary Metabolites:** The ability of a *Trichoderma* fungus to produce antifungal named as peptaibols or an *Aspergillus* and *Fusarium* fungus to produce a mycotoxin gives them a competitive advantage. It is a type of chemical warfare, which suppresses competitors and raises serious food safety concerns[17, 50].

d) **Rapid Growth & Spore Resilience:** Due to their higher metabolic rate and the ability to produce many resistant spores, contaminants outcompete fungi for space and remain in the environment.

The convergence of these mechanisms suggests a pathogenic “arms race” whereby contaminants preadapt to survive in rich, microbial environments; simple hygiene is inadequate without multipronged control.

3.5 Detection and Identification: A Serious Void in Anticipatory Oversight.

In today's world, detection methods are inadequate for fast on-site decision making.

- **Conventional Methods:** Techniques based on morphology and culture are foundational, slow, and often lack species-level resolution for cryptic taxa. [19].
- **Molecular Approaches:** At high sensitivity and specificities, PCR, qPCR and ITS sequencing can realize early detection of pathogens like *T. aggressivum* [18, 48]. But they are mostly restricted to high-quality laboratories which creates a significant translational gap for routine diagnostics at the farm level.
- **Advanced and Future Tools:** Using metagenomics to gain a global insight into the cultivating microbiome could allow the earlier identification of dysbiosis prior to disease onset [51]. Biosensors mark the next frontier for the detection of fungal volatiles or DNA in real-time [36]. A major gap identified in this review is the need for field-deployable, cost-effective diagnostic tools such as LAMP assays and paper-based sensors that shift the paradigm from reactive fungicide spraying to pro-active, data-driven management.

3.6 Integrated Control and Management: From Reactive to Proactive

To intelligently control, existing isolated and reactive approaches must evolve into a fully integrated and proactive approach. The following hierarchy is suggested based on assessment and synthesis of the literature.

1. Prevention and Exclusion Foundation:The primary measures that address nearly all contamination sources are Good Agricultural Practices (GAPs), stringent hygiene (HACCP-like protocols) and certified pathogen-free spawn and substrate [19].

2. Environmental control as a strategy: Real-time adjustment of temperature, humidity and CO2 are actively managed to favour the mushroom mycelium at the expense of its pathogens and this logic, extends into storage and transport [27, 36].

3. The biological control of targeted species: The application of antagonistic microorganisms such as *Bacillus* spp. or non-pathogenic *Trichoderma* strains can be a sustainable and environmentally friendly method of suppression. Efforts should be directed to developing resilient shelf-stable formulations for mushroom growing systems [50].

4. Final Option: Restricted Use of Chemicals:Fungicides should be viewed as a tactical tool used for time-limited rescue treatments in the context of an IDM program with strict attention to residue management, safety to workers and resistance risk [17, 19]. According to case studies, a combination of heat-treated substrates, strict sanitation, and biocontrol agents can sustainably suppress green mould in European agaricus farms [27]. The application of this multi-layered IDM model will work not as a magic bullet but through synergetic, layered reduction of disease pressure.

3.7 Challenges, Knowledge Gaps, and Future Research Directions

- **Resistance and Climate Change:** Growing resistance to fungicides of crops and climate change effects on the pathogenic range necessitate adaptive and resilient management. [13, 51].
- **Knowledge Gaps:** Three main gaps have been identified, which are:(1) the quantitative link between pre-harvest contamination and postharvest mycotoxin accumulation in edible mushrooms,(2) the absence of practical real-time diagnostic tools suitable for on-farm studies and (3) the ecological interactions in cultivation microbiome which provide disease suppression.
- **Future Directions:** Future research should are on: (a) Metabolomic and metagenomic studies to track the risk pathways of mycotoxins, (b) Development of low-cost, field-ready molecular diagnostics (eg LAMP) and biosensors as well as (c) Breeding for host resistance and engineering optimized synthetic microbial communities for biocontrol.Fungal diversity mirrors the diversity of plants and animals and is impacted by the environment and climate of the fungus. Many different factors such as rainfall, temperature, diversity of habitat, soil characteristics, humidity, etc play a very important role in determining the composition, abundance, and distribution of fungal communities. [52,53,54].

4- Conclusion

This critical review brings together evidence that microfungal contamination is still an important constraint because of various factors on the sustainability of mushroom production. *Trichoderma*, *Penicillium*, *Aspergillus* and *Cladobotryum* are defined as the most important threatening fungi that deploy aggressive competitive, enzymatic, and mycotoxigenic strategies. The thematic linkage of hazards from cultivation to postharvest shows that effective management is not limited to the farm gate. It is not only advisable but also essential to adopt a hierarchical framework for Integrated Disease Management (IDM) which emphasizes prevention and environmental control, incorporation of biological tools, and reduction in chemical Inputs. Future success will depend on the translation of molecular diagnostics to the farm, quantification and mitigation of risk due to mycotoxins, and developing biocontrol strategies that are resilient to climate change. These advances will deliver safety, productivity and profitability for the global mushroom industry.

Ethical Approval

This study did not involve human participants or animals. All experimental procedures were conducted in accordance with institutional and laboratory biosafety guidelines.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Data Availability

All data supporting the findings of this study are available within the manuscript.

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