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Research Article

Fungal Contaminants in Oyster Mushroom Cultivation and Their Integrated Management Strategies

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Abstract

Oyster mushroom (*Pleurotus* spp.) cultivation has occurred as an economically important agricultural activity due to its nutritional, medicinal, and environmental benefits. Oyster mushrooms can grow on a wide range of lignocellulosic agricultural wastes, making their cultivation biologically sustainable and economically achievable for small- and large-scale producers. However, fungal contamination remains one of the most significant challenges in oyster mushroom production systems. Contaminant fungi resist with mushroom mycelium for nutrients and space, resulting in poor substrate colonization, reduced yield, inferior mushroom quality, and severe economic losses. The present study examines major fungal contaminants associated with oyster mushroom cultivation, including *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., and *Cladobotryum* spp. The paper converses their occurrence, symptoms, sources of contamination, and impact on mushroom production. Furthermore, various management approaches such as substrate pasteurization, hygienic handling practices, environmental control, biological management, and integrated contamination management approaches are critically analysed. The study highlights the importance of preventive measures and early contamination detection for sustainable mushroom production. Integrated management practices that combine sanitation, environmental regulation, and biological approaches are considered the most effective strategies for reducing contamination risks in oyster mushroom cultivation systems. Future research should focus on advanced molecular detection techniques and environmentally friendly biological control methods to improve sustainable mushroom farming practices.

INTRODUCTION

Oyster mushroom (*Pleurotus* spp.) is one of the most widely cultivated edible mushrooms globally because of its high nutritional value, medicinal properties, adaptability to diverse environmental conditions, and ability to grow on inexpensive agricultural residues (Chang & Miles, 2004). Oyster mushrooms are rich in proteins, vitamins, minerals, essential amino acids, and bioactive compounds that contribute significantly to human nutrition and health (Sanchez, 2010). Due to increasing consumer awareness regarding healthy foods and sustainable agricultural practices, the commercial cultivation of oyster mushrooms has expanded rapidly across many countries.

The cultivation of oyster mushrooms utilizes agricultural by-products such as wheat straw, rice straw, sugarcane bagasse, corn stalks, and sawdust as growth substrates (Royse et al., 2017). This characteristic makes oyster mushroom cultivation environmentally beneficial because it helps recycle agricultural waste into valuable food products

(Jarial, et al., 2024). Furthermore, oyster mushroom farming requires relatively low investment and simple cultivation techniques, making it suitable for small-scale growers and rural entrepreneurs.

Despite these advantages, contamination by undesirable microorganisms remains a major encounter in mushroom cultivation systems. Among the various contaminants, fungal contaminants are particularly injurious because they aggressively compete with mushroom mycelium for nutrients and environmental resources (Fletcher & Gaze, 2008). The humid and nutrient-rich environments necessary for mushroom cultivation also create favourable environments for contaminant fungi. These contaminants can significantly reduce mushroom yield, delay mycelial colonization, and cause complete crop failure in severe cases. Several fungal species including *Trichoderma*, *Aspergillus*, *Penicillium*, and *Cladobotryum* have been frequently reported as major contaminants in oyster mushroom cultivation systems (Sharma, Kumar, & Annepu, 2017). These fungi may enter the cultivation environment through contaminated substrates, poor sanitation, infected spawn, airborne spores, or improper handling practices. Effective contamination management is therefore essential to maintain high productivity and ensure sustainable mushroom production. Fungal contamination significantly reduced oyster mushroom yield and biological efficiency under Indian cultivation conditions. Green mold contamination caused by *Trichoderma spp.* was identified as the most destructive contaminant affecting substrate colonization and fruiting performance.

This research aims to provide a comprehensive overview of major fungal contaminants affecting oyster mushroom cultivation and to discuss effective management strategies for minimizing contamination risks in commercial mushroom production systems.

MATERIALS AND METHODS

Study area and experimental design of study was conducted under controlled laboratory at Prof. Ramkrishna More Art, Commerce and Science College, Akurdi, Pune, Maharashtra and mushroom cultivation unit conditions to investigate the occurrence of fungal contaminants in oyster mushroom (*Pleurotus oyster*) cultivation and evaluate different contamination management strategies. The experiment followed a completely randomized design (CRD) with different treatment groups for contamination management practices. Each treatment was replicated three times to ensure experimental reliability and accuracy of observations (Royse et al., 2017).

Collection and Preparation of Substrate: Wheat straw was used as the primary substrate for oyster mushroom cultivation due to its widespread availability and suitability for *Pleurotus* species cultivation. The substrate was chopped into small pieces measuring approximately 3–5 cm in length and soaked in clean water for 12 -16 hours to achieve proper moisture content (Chang & Miles, 2004). After soaking, excess water was drained, and the substrate moisture level was maintained at approximately 65–70%, which is considered optimal for oyster mushroom mycelial growth (Sanchez, 2010).

Substrate Pasteurization: The prepared substrate was pasteurized using the hot water treatment method to reduce microbial contamination. The substrate was immersed in hot water maintained at 80–85°C for 1 hour. After pasteurization, the substrate was allowed to cool under hygienic conditions before spawning (Fletcher & Gaze, 2008). Proper pasteurization was carried out to eliminate harmful microorganisms while preserving beneficial microbial populations that support mushroom growth (Chang & Miles, 2004).

Spawn Inoculation: Certified pure spawn of *Pleurotus oyster* was obtained from an authorized mushroom research laboratory. Spawning was performed under aseptic conditions to minimize contamination risks. Approximately 5% spawn (wet weight basis) was mixed uniformly with the pasteurized substrate inside sterilized polyethylene cultivation bags (Royse et al., 2017). The bags weight was 3 kg (wet weight) and they were tightly packed and kept perforated to facilitate aeration during mycelial colonization.

Incubation Conditions: The inoculated bags were incubated in a dark cultivation room maintained at a temperature of 24–28°C with relative humidity levels between 80–90%. Environmental conditions were monitored regularly to support optimal mycelial growth and minimize contamination development (Sanchez, 2010). Proper ventilation was maintained throughout the incubation period to reduce excess carbon dioxide accumulation and airborne contamination.

Isolation and Identification of Fungal Contaminants: Contaminated substrate samples showing abnormal fungal growth were collected aseptically from cultivation bags. The samples were cultured on Potato Dextrose Agar (PDA) medium and incubated at 27°C for fungal growth and isolation (Sharma et al., 2017). The isolated fungal contaminants were identified based on colony morphology, spore characteristics, colour, and microscopic examination using standard fungal identification keys. Major contaminants identified included *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., and *Cladobotryum* spp. (Fletcher & Gaze, 2008).

Contamination Management Treatments: Different contamination management practices were evaluated during the study. The treatments included as T1: Control (without contamination management), T2: Proper substrate pasteurization, T3: Hygienic handling and sanitation practices, T4: Environmental control (humidity and ventilation regulation), T5: Integrated contamination management approach. The integrated management treatment combined sanitation, substrate pasteurization, environmental regulation, and hygienic handling practices for effective contamination reduction (Savoie & Mata, 2003).

Data Collection: Observations were recorded regularly throughout the cultivation period. The following parameters were evaluated by percentage of contaminated bags, mycelial colonization rate, Time required for pinhead formation, number of contaminated spots, mushroom yield (kg).

Statistical Analysis: The collected experimental data were analysed statistically using analysis of variance (ANOVA) to determine significant differences among treatment groups. Mean values were compared at a 5% significance level to evaluate the effectiveness of contamination management strategies (Gomez & Gomez, 1984).

RESULTS AND DISCUSION

Major Fungal Contaminants in Oyster Mushroom Cultivation

Green Mold Disease Caused by *Trichoderma* spaces: Green Mold caused by *Trichoderma* species is considered one of the most destructive fungal contaminants in mushroom cultivation worldwide (Savoie & Mata, 2003). Species such as *Trichoderma harzianum* and *Trichoderma viride* rapidly colonize mushroom substrates and competed aggressively with mushroom mycelium. Initially, white mycelial growth appears on the substrate surface, which later develops green spore's characteristic of *Trichoderma* contamination. The rapid growth rate of this fungus suppresses the development of oyster mushroom mycelium and reduces substrate colonization efficiency (Sharma et al., 2017). Symptoms are green patches on substrate surfaces, inhibition of mushroom mycelial growth, reduced mushroom yield, delayed fruiting body formation. Sources of Infection improper substrate pasteurization, Contaminated spawn materials, excess substrate moisture, Poor hygienic conditions. Research indicates that *Trichoderma* species produce extracellular enzymes and antifungal metabolites that inhibit mushroom growth and enhance competitive colonization (Savoie & Mata, 2003).

Black Mold Caused by *Aspergillus* spaces: *Aspergillus* species are common airborne fungi frequently associated with mushroom substrate contamination. These fungi grow rapidly under warm and humid environmental conditions commonly found in mushroom cultivation rooms (Fletcher & Gaze, 2008). The contamination is characterized by black or dark-coloured powdery spores that spread easily through air movement. Certain *Aspergillus* species may also produce harmful mycotoxins that affect product safety. Symptoms are black spore formation on substrate, reduced mycelial colonization, development of unpleasant odour. Sources are airborne fungal spores, Poor ventilation systems, inadequate sterilization procedures

Blue-Green Mold Caused by *Penicillium* spaces: *Penicillium* species are commonly observed during spawn preparation and storage stages. These fungi produce blue-green colonies and compete with mushroom mycelium for nutrients and substrate resources (Sharma et al., 2017). Symptoms are blue-green fungal patches, reduced spawn viability, slow substrate colonization. Improper storage conditions and contaminated equipment often contribute to *Penicillium* contamination in mushroom cultivation systems.

Cobweb Disease Caused by *Cladobotryum* spaces: Cobweb disease caused by *Cladobotryum* species is another important fungal disease affecting mushroom cultivation. The disease spreads rapidly under high humidity conditions and appears as grey cotton-like fungal growth over mushroom beds and fruiting bodies (Fletcher & Gaze, 2008). Symptoms are Cottony fungal growth on mushrooms, soft rot symptoms, rapid spread across cultivation beds. Poor air circulation and excessive humidity are major factors contributing to disease development.

Sources of Fungal Contamination: Fungal contamination can occur at multiple stages of oyster mushroom cultivation. One of the primary sources of contamination is inadequate substrate sterilization or pasteurization. If harmful fungal spores survive substrate treatment, they can rapidly colonize the substrate during incubation (Chang & Miles, 2004). Contaminated spawn is another major source of fungal infection. Spawn prepared under non-sterile laboratory conditions may carry contaminant fungi that spread throughout the cultivation substrate. Additionally, airborne spores present in cultivation environments contribute significantly to contamination problems. Improper hygiene practices by workers also increase contamination risks. Unclean equipment, contaminated tools, and poor sanitation conditions facilitate fungal spread within cultivation rooms (Royse et al., 2017). Environmental factors such as excessive humidity, poor ventilation, and temperature fluctuations further encourage contaminant growth. High moisture levels particularly Favor the development of Molds such as *Trichoderma* and *Cladobotryum*.

Impact of Fungal Contamination on Oyster Mushroom Production: Fungal contamination negatively affects mushroom production both economically and biologically. Contaminant fungi compete directly with mushroom mycelium for nutrients and space, thereby reducing biological efficiency and mushroom yield (Sharma et al., 2017). Contamination often delays substrate colonization and fruiting body formation. In severe cases, complete crop failure may occur due to extensive substrate colonization by contaminant fungi. Furthermore, contaminated mushrooms may exhibit poor texture, discoloration, and reduced market value. Some contaminant fungi also produce secondary metabolites and mycotoxins that may pose health risks to consumers (Fletcher & Gaze, 2008). Consequently, contamination not only reduces productivity but also affects food safety and commercial profitability (Evageliou et al., 2019).

Management Strategies for Fungal Contaminants

Preventive Sanitation: Prevention remains the most effective strategy for contamination management in mushroom cultivation systems. Maintaining strict hygiene standards significantly reduces contamination risks. Important sanitation measures include are regular cleaning of cultivation rooms, disinfection of equipment and tools, use of clean protective clothing, restricted access to production areas (Fletcher et al., 2008), proper sanitation practices can substantially minimize airborne fungal contamination.

Proper Substrate Pasteurization: Effective substrate pasteurization eliminates harmful microorganisms while preserving beneficial microbial populations necessary for mushroom growth (Chang & Miles, 2004). Common pasteurization methods include as steam pasteurization, hot water treatment, lime-based chemical pasteurization. Proper temperature and exposure duration are essential to ensure effective substrate treatment.

Use of High-Quality Spawn: Using certified contamination-free spawn significantly reduces infection risks. Spawn should be produced under sterile laboratory conditions and stored appropriately before use (Royse et al., 2017).

Environmental Management: Environmental regulation plays an important role in preventing fungal contamination. Proper humidity, temperature, and ventilation management reduce favourable conditions for contaminant fungi (Curvetto et al., 2002). We will recommended practices include as a maintaining balanced humidity level, ensuring proper air circulation, preventing excessive condensation. (Bernas et al., 2006).

Biological Control Approaches: Biological control methods involve the use of beneficial microorganisms that suppress contaminant fungi through competitive exclusion or antagonistic interactions (Savoie & Mata, 2003). Biological approaches are considered environmentally friendly alternatives to chemical fungicides and contribute to sustainable mushroom production systems.

Table 1: Criteria used for identification of fungal contaminants

Identification Parameter	Observation Method	Purpose
Colony morphology	Visual examination on culture media	To observe texture, growth pattern and colony appearance
Colony Colour	Macroscopic Observation	To differentiate fungal species based on pigmentation
Spore characteristics	Microscopic examination	To identify shape, size and arrangement of spores
Hyphal structure	Compound microscope observation	To determine septation and branching pattern
Sporulation pattern	Microscopic and visual observation	To confirm fungal genus /species identification

Table 2: Fungal contaminants and identification:

Sr. No	Fungal Contaminant	Colony Morphology	Colony Colour	Microscopic Characteristics	Common effect on Oyster Mushroom Cultivation
1	Trichoderma spp.	Fast-growing, Cottony to granular colonies	Green with margins	Branched Conidiophores with Clustered conidia	Competes for nutrients and suppresses mushroom mycelial growth
2	Aspergillus Spp.	Powdery or Velvety Colonies	Yellow, Black or green depending on species	Septate hyphae with globose conidial heads	Causes substrate contamination and reduces yield
3	Penicillium spp.	Velvety, spreading colonies	Blue- green to green	Brush- like conidiophores with chains of conidia	Competes with mushroom mycelium for nutrients
4	Cladobotryum spp.	Cotton, fluffy colonies	White to pale pink	Septate hyphae with elongated conidia	Causes cobweb disease and affects fruits body development

Table 3: Treatment and Observation:

Treatment No.	Contamination Management Practice	Contamination (%)	Reduction over Control (%)	Oyster mushroom Yield	Observation
T1	Control	48.50±1.20	0.00	52.30±1.45	Heavy fungal contamination observation with poor mycelia growth
T2	Proper Substrate Pasteurization	28.40±0.95	41.44	68.70±1.32	Reduced contamination due to elimination of competing fungi
T3	Hygienic Handling and Sanitation Practices	24.80±1.10	48.87	72.10±1.28	Improved hygiene reduced secondary contamination during handling
T4	Environment Control	20.60±0.88	57.53	76.50±1.36	Controlled humidity and ventilation suppressed fungal spread
T5	Integrated Contamination management Approach	9.30±0.65	80.82	91.80±1.52	Minimum contamination and highest yield achieved through integrated management practices

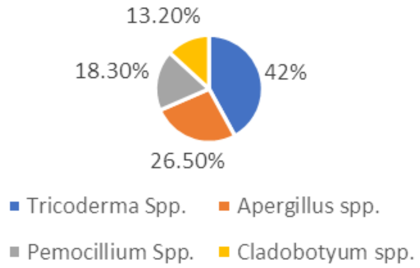

Fig. 1: Distribution of Major Fungal contaminants

Integrated Contamination Management: Integrated contamination management combines sanitation, environmental control, biological methods, and preventive strategies to achieve effective contamination reduction (Mamiro, D. P., & Mamiro, P. S. 2011). Integrated approaches provide long-term sustainable solutions for contamination management and improve overall mushroom productivity.

Future Perspectives: Future research should focus on advanced contamination detection technologies and sustainable biological management approaches. Molecular diagnostic tools may enable rapid identification of contaminant fungi before severe outbreaks occur. The development of resistant mushroom strains and eco-friendly bio-control agents may further improve contamination management in commercial mushroom cultivation systems. Artificial intelligence-based monitoring systems could also enhance environmental regulation and contamination prediction.

Conclusion: Fungal contamination remains one of the most significant challenges in oyster mushroom cultivation systems. Contaminants such as *Trichoderma*, *Aspergillus*, *Penicillium*, and *Cladobotryum* can significantly reduce mushroom yield, quality, and profitability. Poor sanitation, contaminated spawn, improper substrate treatment, and unfavourable environmental conditions contribute to contamination outbreaks.

The results indicated that the integrated contamination management method (T5) was the most effective treatment in reducing fungal contamination in oyster mushroom cultivation. The treatment significantly lowered contamination incidence (9.30%) and produced the highest mushroom yield compared to the control treatment (T1), which recorded the highest contamination incidence (48.50%). Among the isolated fungal contaminants, *Trichoderma* spp. showed the highest frequency of occurrence and caused severe suppression of oyster mushroom mycelial growth. The study demonstrated that the combined application of substrate pasteurization, sanitation practices, hygienic handling, and environmental regulation effectively minimized fungal contamination and improved oyster mushroom production. (Mangan. A. 2008).

Preventive approaches combined with early contamination detection provide the most sustainable solution for maintaining healthy mushroom cultivation systems. Future technological advancements in contamination detection and biological management may further improve the sustainability and productivity of oyster mushroom farming.

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