**Comprehensive Study on Supplements significance and Their Impact on Contamination, Growth and Yield of Oyster Mushrooms**

**Abstract**

This study examines the effects of substrate supplements on contamination, growth, and yield in oyster mushroom (*Pleurotus* spp.) cultivation, along with their economic feasibility. Mushroom cultivation is an eco-friendly agribusiness that requires minimal space and utilizes lignocellulosic agricultural residues. Traditionally, agricultural wastes such as wheat straw, rice straw, and sawdust have served as common substrates. However, the productivity and quality of mushroom crops can be significantly enhanced through supplementation with various organic and inorganic additives. The addition of substrate supplements can significantly influence the contamination dynamics in mushroom cultivation. While supplements enrich the substrate with essential nutrients that promote vigorous mycelial growth and higher yields, they also create a nutrient-dense environment that may favor the proliferation of undesirable microorganisms, such as molds, bacteria, and competitor fungi. High-nutrient supplements like soybean meal, though beneficial for yield improvement, require sterilization to mitigate contamination risks, thereby increasing production costs. The choice of supplementation strategy must balance productivity, economic viability, and contamination control to ensure sustainable and efficient mushroom cultivation. Future research should focus on optimizing supplement combinations, refining sterilization techniques, and identifying novel, cost-effective additives to further enhance the profitability and sustainability of oyster mushroom production.

**Keywords:** Oyster mushroom, High-nutrient supplements, agricultural residues, vermicompost

**1. Introduction**

Mushrooms are diverse organisms that have been valued for their nutritional and medicinal benefits since ancient times (Martínez-Ibarra et al., 2019). They belong to Basidiomycetes and grow on organic substrates, offering a rich source of protein, vitamins, and minerals (Ahmed et al., 2009; Miah et al., 2017). Due to their high protein content, the FAO recommends mushrooms as a food source to address malnutrition (WB, 2004).

Mushroom cultivation is an eco-friendly agribusiness that requires minimal space and utilizes lignocellulosic agricultural residues (Neupane et al., 2018; McGrath, 2003). However, raw substrates often lack essential nutrients like nitrogen, phosphorus, and potassium, necessitating substrate supplementation to enhance growth and yield (Xing et al., 2006; Naraian et al., 2014). Organic and inorganic supplements, such as compost, vermicompost, and oil cakes, improve mycelial colonization and mushroom productivity (Estrada et al., 2009). Successful oyster mushroom production largely depends on the choice of substrate, as it provides essential nutrients for fungal growth and fruiting. Traditionally, agricultural wastes such as wheat straw, rice straw, and sawdust have served as common substrates. However, the productivity and quality of mushroom crops can be significantly enhanced through supplementation with various organic and inorganic additives. Substrate supplements not only enrich the nutrient profile but also influence critical aspects of mushroom cultivation, including mycelial colonization rate, fruiting body development, biological efficiency, and overall yield. At the same time, inappropriate supplementation may increase the risk of contamination by competing microorganisms, which can severely affect both crop health and economic returns. Therefore, a balanced understanding of supplementation strategies is crucial to optimize oyster mushroom cultivation (Pardo-Giménez et al., 2016).

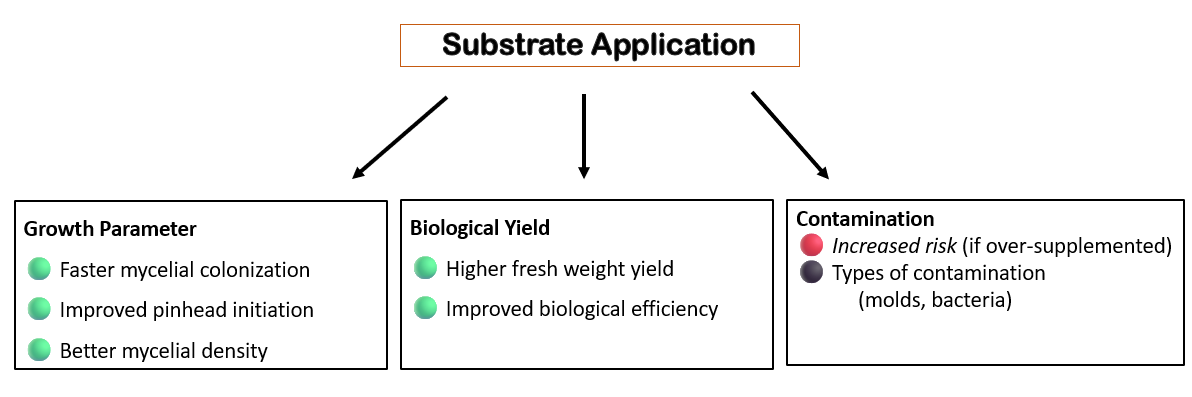
While supplementation boosts yield, excessive use may increase contamination risks and reduce productivity (Yildiz et al., 2002; Fanadzo et al., 2010). This study examines the effects of substrate supplements on contamination, growth, and yield in oyster mushroom (*Pleurotus spp.*) cultivation, along with their economic feasibility.

**2. Definition of Substrate Supplements**

In oyster mushroom cultivation, substrate supplements play a critical role in accelerating mycelial colonization, promoting robust fruiting body formation, and increasing biological efficiency. However, their use must be carefully managed, as excessive supplementation can alter substrate composition in a way that favors the growth of contaminating microorganisms. Thus, the selection, type, and quantity of substrate supplements are key factors influencing both the productivity and health of the mushroom crop. Substrate supplementation is a widely used technique to enhance mushroom yield and quality. Zied et al. (2018) emphasized its significance in industrial mushroom production, as it provides essential nutrients that improve biological efficiency. Most commercially available supplements are nitrogen-rich, but their effectiveness compared to carbon-based alternatives like cellulose and hemicellulose remains unclear (Pardo-Giménez et al., 2016). While nitrogen-based supplements improve yield and mushroom quality, carbohydrate-rich supplements derived from agricultural and commercial waste offer a cheaper and locally available alternative, potentially benefiting small-scale farmers.

Recent studies suggest that integrating protein-based and carbon-based supplements may optimize mushroom growth by balancing nitrogen supply with structural carbohydrate components (Zied et al., 2021; Wang et al., 2023). Additionally, spent mushroom substrate (SMS) can be recycled as a supplement to create a circular economy, improving sustainability and reducing waste (Pardo-Giménez et al., 2016).

The timing of supplementation also influences mushroom development. Naraian et al. (2009) reported that slow-release nutrient supplements, applied before spawning, enhance mycelial colonization and vegetative growth. Recent findings indicate that stage-specific supplementation can improve not only yield but also mushroom size, texture, and shelf life (Liu et al., 2022). These advancements highlight the potential for refined supplementation strategies to enhance both productivity and sustainability in oyster mushroom cultivation.



**Fig.1- Impact of substrate application of Mushroom**

**3. Effect of Substrate Supplements on Contamination**

The addition of substrate supplements can significantly influence the contamination dynamics in mushroom cultivation. While supplements enrich the substrate with essential nutrients that promote vigorous mycelial growth and higher yields, they also create a nutrient-dense environment that may favor the proliferation of undesirable microorganisms, such as molds, bacteria, and competitor fungi. The increased availability of easily accessible nutrients can accelerate the growth of contaminants, particularly if environmental conditions such as temperature, humidity, and sanitation are not properly managed. In other words we can say that, while supplementation enhances mushroom yield, it also increases the risk of contamination by competitive fungi. Bhatta and Bist (2017) observed that Trichoderma contamination was highest in oyster mushroom bags supplemented with gram flour, likely due to the high availability of soluble nutrients favouring saprophyte growth. In contrast, wheat bran and maize cob supplementation resulted in minimal contamination, likely due to faster mycelial colonization, which outcompetes contaminants.

Several studies have identified common contaminants in oyster mushroom cultivation, including Aspergillus, Coprinus, Penicillium, and Rhizopus species (Biswas & Kuiry, 2013; Jaivel & Marimuthu, 2010). *Trichoderma harzianum*, in particular, is a major threat, as it aggressively competes with Pleurotus spp., reducing fruiting surface by 30–50% (Castle et al., 1998). Improper pasteurization of the substrate can further increase contamination risks (Spilman, 2002).

Recent research suggests that bio-based antifungal treatments and modified substrate sterilization techniques can help control contamination (Wang et al., 2022). Substrate inoculation with beneficial microbes, such as *Bacillus* and *Pseudomonas* *spp*., has shown promise in suppressing Trichoderma and other contaminants (Singh et al., 2023). Additionally, optimized supplementation strategies, such as controlled nutrient release formulas, can reduce contamination risks while maintaining high yields (Kananen et al., 2000).

Thus, while substrate supplementation boosts oyster mushroom productivity, careful selection of additives, proper pasteurization, and biological control measures are essential to minimize contamination and maximize yield.

**4. Effect of Substrate Supplements on Growth Parameters**

**Mycelial colonization rate-** The rate and completion time of mycelial growth are crucial factors influencing the overall production efficiency of mushrooms. Various studies indicate that substrate composition and supplementation significantly impact mycelial running rates and spawn colonization. Hasan et al. (2015) found that increasing wheat bran supplementation (up to 50%) in sugarcane bagasse significantly accelerated the mycelial running rate in *Pleurotus djamor*. Similarly, Nuruddin et al. (2010) observed the highest mycelial running rate (0.70 cm/day) in *P. ostreatus* when supplemented with 10% cow dung, suggesting that controlled organic supplementation can enhance mycelial colonization. Regarding completion time of mycelium running, Seephueak et al. (2019) reported that *Ganoderma lucidum* exhibited the fastest mycelial colonization when 15% palm oil sludge was added to rubber sawdust, completing growth in 42.85 days. Shalahuddin et al. (2018) demonstrated that chemical supplementation (NPK 4g/10kg straw) significantly reduced spawn run time in oyster mushrooms from 20.40 days (control) to 16.20 days. Likewise, Mahjabin et al. (2011) noted that chemical nutrients reduced the mycelial running duration to 13.25 days, whereas organic supplements like NPK (5g/10kg straw) extended it to 31.75 days. Recent studies have further explored innovative bio-based supplements. For instance, fermented soybean meal has been found to accelerate mycelial running while improving substrate digestibility (Zhang et al., 2022). Additionally, chitin-based amendments from crustacean shells have shown potential in enhancing mycelial vigor and disease resistance (Kim et al., 2023). These findings suggest that careful selection of organic and chemical supplements can optimize mycelial growth, reduce colonization time, and enhance yield, making substrate management a key aspect of efficient mushroom production.

Primordia initiation and fruiting body development- The initiation of primordia, marking the transition from vegetative growth to reproductive development in mushrooms, is strongly influenced by substrate composition and supplementation. Various studies indicate that organic and synthetic supplements can significantly reduce the time required for primordia emergence and enhance biological efficiency. Arsia et al. (2018) observed that among different flour and bran supplements, *Pleurotus flabellatus* exhibited the fastest primordia initiation (18 days) with bajra flour, whereas gram powder and control treatments required the longest period (21 days). Similarly, Hasan et al. (2015) found that supplementing sugarcane bagasse with wheat bran (30%) delayed primordia formation (5.50 days) compared to lower supplementation levels. Alam et al. (2010) reported that in *Calocybe indica*, the shortest primordia initiation time (13.5 days) occurred with a 40% rice bran supplement, followed by 20% wheat bran (14.8 days). The longest period (19.3 days) was recorded in the unsupplemented control, indicating that nutrient-rich substrates accelerate fruiting body development.

Nuruddin et al. (2010) demonstrated that cow dung supplementation (15-20%) with rice straw reduced the stimulation-to-primordia initiation period to 6.03 days, compared to 7.23 days in the control. Ali (2009) also reported a delayed initiation period (11.5 days) when sugarcane bagasse was supplemented with 10% wheat bran. Recent studies suggest that bioactive amendments, such as fermented soybean meal and chitin-based supplements, not only accelerate primordia formation but also enhance disease resistance and fruiting efficiency (Zhang et al., 2022; Kim et al., 2023). Additionally, seaweed extracts and microbial inoculants have been explored for their role in improving pinning uniformity and primordia survival (Singh et al., 2024). These findings highlight that optimal supplementation strategies can enhance early primordia initiation, improve biological efficiency, and boost overall mushroom yield, making substrate selection a key factor in commercial mushroom cultivation.

**Effect of Substrate Supplements on Biological Yield-** Biological yield, defined as the total fresh weight of harvested fruiting bodies in relation to the dry substrate weight, is a critical determinant of mushroom productivity. Various supplementation strategies, including organic, chemical, and microbial amendments, have been extensively explored to enhance yield and biological efficiency (B.E.).

Sanjel et al. (2021) reported that rice bran supplementation resulted in the highest total fresh mushroom yield (793.04 g/bag) and biological efficiency (137.92%), closely followed by the control (780.59 g/bag, 135.75%), molasses supplementation (763.21 g/bag, 132.73%), and wheat bran supplementation (721.9 g/bag, 125.54%). Mustard oilseed cake supplementation produced the lowest yield (521.84 g/bag, 90.75% B.E.), potentially due to its high fat content, which inhibits mushroom growth (Krupodorova & Barshteyn, 2015).

Seephueak et al. (2019) investigated the supplementation of rubber sawdust with palm oil sludge and found that the highest yield (74.82 g/bag, 22.01% B.E.) was obtained with 5% supplementation, with a decline in yield observed at higher supplementation levels. Similarly, Shalahuddin et al. (2018) evaluated the effect of different chemical nutrient formulations on the production of *Pleurotus ostreatus* and reported the highest biological yield (282.36 g) with the application of 4 g NPK per 10 kg straw substrate.

The role of carbon sources in spawn production and subsequent yield improvement has also been explored. Satpal et al. (2017) demonstrated that glucose supplementation in spawn resulted in the highest yield (613.33 g/kg dry substrate, 61.33% B.E.), followed by starch, sucrose, and maltose. In contrast, control spawn (without sugar supplementation) yielded significantly lower biological efficiency (40 g/kg, 40% B.E.).

Further studies have highlighted the benefits of incorporating protein-rich agricultural by-products. Pardo-Giménez et al. (2018) reported that supplementation with defatted pistachio and almond meal significantly improved the yield and quality of *Agaricus bisporus* and *Pleurotus ostreatus*, with an increase of more than 30% compared to non-supplemented substrates. Sharma (2009) also observed that supplementation with 2% wheat bran and 2% rice bran resulted in a 48.1% and 48.3% increase in yield, respectively, over the control. However, excessive supplementation beyond 2-3% has been reported to negatively impact yield due to nutrient imbalances and overheating of compost (Viziteu, 2004; Gupta & Vijay, 1991).

Recent advancements in microbial and biofertilizer applications have provided new insights into yield enhancement. Kim et al. (2023) found that chitin-based biofertilizers improved oyster mushroom yield by 20%, while Singh et al. (2024) demonstrated that seaweed extracts and beneficial microbes significantly enhanced primordia survival rates and uniformity in yield. These findings suggest that integrating biostimulants with traditional supplementation strategies may offer an effective approach to optimizing biological efficiency in commercial mushroom cultivation.

An appropriately supplemented substrate ensures a better carbon-to-nitrogen (C:N) ratio, which is critical for the metabolic processes involved in mushroom growth (Colla et al. 2023). Improved nutrient availability can lead to earlier pinning, larger fruiting bodies, and multiple flushes of mushrooms, thereby significantly enhancing biological efficiency. However, the effect of supplementation on yield is influenced by the type of supplement, its concentration, the mushroom species, and environmental conditions during cultivation.

Overall, the reviewed studies underscore the significance of selecting appropriate supplementation strategies tailored to specific mushroom species and substrate compositions. While organic and chemical supplements such as rice bran, wheat bran, and molasses have demonstrated yield-enhancing effects, emerging trends in microbial inoculants and biofertilizers hold promise for sustainable and efficient mushroom production.

**5. Effect of Substrate Supplements on Yield**

Economic yield, an essential parameter in mushroom production, is influenced by substrate composition, supplementation, and nutrient management. Several studies have explored different supplementation strategies to enhance economic returns in mushroom farming.

Shalahuddin et al. (2018) investigated the impact of different levels of chemical nutrients (NPK) on the economic yield of *Pleurotus ostreatus*. The study comprised four treatments with varying NPK concentrations: T1 (only 10 kg straw), T2 (2 g NPK in 10 kg straw), T3 (4 g NPK in 10 kg straw), and T4 (6 g NPK in 10 kg straw), maintaining a 2:1:1 ratio of NPK. The highest economic yield (267.38 g) was observed in T3, while the lowest (208.11 g) was recorded in T1, highlighting the beneficial effects of moderate NPK supplementation on yield enhancement.

Zied et al. (2018) demonstrated that the incorporation of agro-industrial waste materials such as peanut and acerola juice residues, along with noble grains like soybean, corn, and cotton bran, significantly improved industrial mushroom yield. The presence of sulfur (S), copper (Cu), and manganese (Mn) in these materials contributed to improved substrate quality and enhanced economic returns.

Moonmoon et al. (2011) examined the effects of sawdust supplementation with wheat bran, rice bran, and maize powder on the yield and quality of *Lentinula edodes*. The study revealed that supplementation with 25% wheat bran resulted in the highest economic yield, whereas 40% wheat bran supplementation produced the best quality mushrooms, indicating the importance of optimized supplement ratios in commercial production.

Kalmis et al. (2008) reported that supplementation of wheat-based substrates with 25% olive mill effluent led to improved economic yield, emphasizing the potential of olive mill byproducts as cost-effective supplements for sustainable mushroom farming.

Recent studies have also explored the potential of alternative organic supplements in mushroom cultivation. For instance, supplementation with defatted pistachio and almond meals has been found to significantly improve the quality and yield of *Agaricus bisporus* and *Pleurotus ostreatus* (Pardo-Giménez et al., 2018). Additionally, organic byproducts such as brewery waste and spent coffee grounds have been identified as effective supplements for enhancing both yield and economic viability in commercial mushroom production (Singh et al., 2022).

Overall, strategic supplementation with agro-industrial residues, organic amendments, and optimized nutrient management plays a crucial role in maximizing economic yield in mushroom cultivation. Future research should focus on refining supplement compositions and exploring novel organic waste materials to further enhance profitability and sustainability in mushroom farming.

### **Benefits of Cotton Seed Cake in Oyster Mushroom Cultivation**

#### **1**. **Enhanced Yield and Biological Efficiency**

Research indicates that CSC can significantly improve mushroom yield and biological efficiency (BE):

The utilization of cotton seed cake (CSC) as a substrate or supplement in oyster mushroom *Pleurotus ostreatus* cultivation has been explored by various researchers. Notably, a study conducted by Nasir Ahmad Khan and colleagues in 2019 investigated the effects of combining CSC with wheat straw and rice bran on mushroom yield and growth parameters. Their findings indicated that while wheat straw alone produced the highest yield (407 g), the combination of wheat straw, CSC, and rice bran (50:25:25) resulted in the most rapid mycelial growth.

Additionally, research by Tadesse Teklu in 2024 examined the cultivation of oyster mushrooms using substrates composed of cotton seed, khat leftovers, and spent coffee grounds. The study concluded that cotton seed-based substrates significantly enhanced both biological and economic yields compared to other substrates.

These studies suggest that while CSC can be beneficial in improving certain growth parameters, its effectiveness may vary depending on the specific combination and proportion with other substrates.

#### **2**. **Improved Mycelial Growth and Faster Fruiting**

CSC has been associated with accelerated mycelial colonization and earlier fruiting:

* Mycelial growth was faster on cotton seed and paper waste (14 days) compared to wheat straw (15.67 days) and sawdust (19.67 days). Pin-head formation occurred quickest in cotton seed (17 days).

**3.** **Reduction of Anti-Nutritional Factors**

Cottonseed contains gossypol, a toxic compound limiting its use. However, solid-state fermentation (SSF) with *Pleurotus ostreatus* has been shown to reduce gossypol content, enhancing the nutritional profile of the substrate.

**6. Conclusion**

The use of substrate supplements in oyster mushroom cultivation significantly influences contamination rates, growth parameters, and overall yield. Cost-effective supplements such as wheat bran and rice bran enhance substrate quality, accelerate primordia initiation, and improve biological efficiency while maintaining economic feasibility. However, high-nutrient supplements like soybean meal, though beneficial for yield improvement, require sterilization to mitigate contamination risks, thereby increasing production costs. The choice of supplementation strategy must balance productivity, economic viability, and contamination control to ensure sustainable and efficient mushroom cultivation. Future research should focus on optimizing supplement combinations, refining sterilization techniques, and identifying novel, cost-effective additives to further enhance the profitability and sustainability of oyster mushroom production.

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