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EVALUATION OF PLANT-DERIVED BIO-ADDITIVES ON MORPHOLOGICAL TRAITS, YIELD AND BIOLOGICAL EFFICIENCY OF PLEUROTUS OSTREATUS (JACQ.) P. KUMM.

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ABSTRACT

The cultivation of oyster mushroom (*Pleurotus ostreatus*) offers a sustainable avenue for nutritional, medicinal and economic development. Enhancing its morphological and yield parameters through natural substrate enrichment is a promising approach to improve both quality and productivity. The present study evaluated the effects of selected botanicals Allium sativum, Solanum aethiopicum, Azadirachta indica, Allium chinense, Zingiber officinale, Allium hookeri, Ocimum sanctum, Mentha sp., and Curcuma longa on the growth and yield of P. ostreatus. The experiment was conducted for two consecutive years (2021-2023) at the Mushroom Crop Room and Research Laboratory, SHUATS, Prayagraj, using a completely randomized design with ten replications. Morphological traits, including pileus width and stipe length and vield attributes such as number of fruiting bodies, total vield and biological efficiency, were recorded and statistically analyzed. Results revealed that Allium chinense consistently produced the widest pileus (7.46 cm), longest stipe (8.05 cm), highest number of fruiting bodies (120.30), maximum yield (204.50 g) and biological efficiency (87.37%), followed by Azadirachta indica and Allium sativum. The observed improvement in growth and yield attributes may be attributed to the bioactive metabolites in these botanicals, which enhance enzymatic activity and suppress microbial competition within the substrate. The study concludes that botanical enrichment, particularly with A. chinense, can serve as an effective and eco-friendly strategy for improving the morphology, yield and biological efficiency of oyster mushrooms, thereby supporting sustainable and profitable mushroom cultivation practices.

Keywords: Allium chinense, biological efficiency, botanical supplementation, morphological traits, *Pleurotus ostreatus*, yield.

Introduction

Mushroom cultivation is recognized as an ecofriendly and economically viable agro-enterprise due to its low input requirements, high nutritional value and potential for sustainable income generation (Royse et al., 2017). Among edible species, the oyster mushroom (Pleurotus ostreatus) stands out for its adaptability to diverse substrates, rapid growth and excellent nutritional and medicinal properties (El Enshasy et al., 2020). It is a rich source of proteins, vitamins, minerals and dietary fiber and contains bioactive compounds such as polysaccharides and phenolics that exhibit immune-modulating antioxidant and effects (Jayachandran et al., 2017; Wachtel-Galor et al.,

2011). However, productivity of *P. ostreatus* is often reduced by microbial contamination and poor substrate utilization, affecting morphological parameters (Chang and Miles, 2004). Conventional chemical additives, though effective, raise quality concerns. environmental. and Hence. researchers are exploring sustainable alternatives such as botanical bio-additives derived from plants with antimicrobial and growth-promoting properties (Singh et al., 2023; Sharma and Sharma, 2024). Botanicals like Allium sativum, Azadirachta indica, Zingiber officinale, Ocimum sanctum and Curcuma longa contain flavonoids, alkaloids, and sulfur compounds that suppress competing microflora, enhance enzyme activity, and stimulate fruit body development

(Borlinghaus *et al.*, 2014; Islas *et al.*, 2020; Bellettini *et al.*, 2019). Supplementation with such extracts has been shown to improve biological efficiency and yield by enhancing ligninolytic enzyme production and nutrient mobilization (Chukwu *et al.*, 2022; Toppo *et al.*, 2021). In this context, the present study evaluates the influence of selected botanicals, particularly native species from northeastern India, on the morphological traits, yield, and biological efficiency of *Pleurotus ostreatus*, aiming to identify effective eco-friendly bioadditives for sustainable mushroom production.

Materials and Methods

Experimental sites: The study was conducted in the Mushroom Crop Room and Research Laboratory, located within the Department of Plant Pathology at SHUATS, Prayagraj (U.P.), during 2021–2022 and 2022–2023.

Experimental materials: Spawn of *Pleurotus ostreatus* was procured from Directorate of Mushroom

Research (DMR), Solan, Himachal Pradesh (Strain – DMRP – 254).

Collection of botanicals: Botanical specimens were sourced from multiple local and commercial markets across Kohima, the capital of Nagaland. These marketplaces are known for their wide assortment of plant products such as aromatic herbs, leafy greens, roots, and traditional spices integral to local diets and folk medicine. During the sampling process, emphasis was placed on selecting fresh, disease-free, and representative plant materials frequently used by indigenous communities. Valuable ethnobotanical insights, including local names and traditional applications, were gathered through interactions with vendors and shopkeepers. The collected botanicals were subsequently air-dried under shade, properly packed, and transported with care to preserve their quality and suitability for subsequent experimental analysis.

Table 1: Details of selected botanicals

Scientific Name	Common name	Family	Plant part used
Allium sativum	Garlic	Amaryllidaceae	Cloves
Solanum aethiopicum	Wild bitter tomato (Likok)	Solanaceae	Fruit
Azadirachta indica	Neem	Meliaceae	Leaves
Allium chinense	Chinese scallion (Khuve)	Amaryllidaceae	Bulb
Zingiber officinale	Ginger	Zingiberaceae	Rhizome
Allium hookeri	Hooker chives (Repji)	Amaryllidaceae	Leaves
Ocimum sanctum	Tulsi	Lamiaceae	Leaves
Mentha sp.	Mint	Lamiaceae	Leaves
Curcuma longa	Turmeric	Zingiberaceae	Rhizome

In-vivo evaluation

The cultivation of Pleurotus ostreatus (oyster mushroom) was carried out under controlled in vivo conditions using wheat straw as the primary substrate. To ensure aseptic conditions, the cultivation room was thoroughly cleaned and whitewashed to minimize the risk of contamination. Both the crop room and the base substrate were fumigated and sterilized following the standard sterilization procedure described by Sohi (1987). Fresh plant materials, including leaves, bulbs, rhizomes, and stems, were dried, finely powdered, sieved, and sterilized in an autoclave at 121°C under 15 psi pressure for 15-20 minutes. The sterilized botanical powders were then cooled and stored in airtight, labeled containers for later use in substrate supplementation. For spawning, 40 g of mushroom spawn was inoculated per kilogram of moist substrate, and botanical powders were incorporated at a concentration of 2.5% based on substrate weight. The

treated substrate was uniformly mixed and packed into perforated polythene bags, each having 8-10 small holes to facilitate aeration. The bags were arranged at 20-25 cm spacing to ensure proper ventilation, while control sets containing only wheat straw were maintained under identical conditions. The spawn run phase was conducted in a dark room at 20-25°C with 70-85% relative humidity, maintained through periodic misting and ventilation. During incubation, temperature and humidity were stabilized around 25°C and 85-90%, respectively. After complete mycelial colonization, the bags were slit open to initiate fruiting. Light watering (3–4 times daily) and gentle ventilation (1-2 hours daily) were provided. Fruiting bodies typically appeared within 3-5 days of opening, and mature mushrooms were carefully harvested by hand twisting before watering to prevent substrate damage. Each fruiting body was immediately weighed and data were recorded for each flush. This process continued for successive flushes to determine the total yield. The

Biological Efficiency (BE) of each treatment was calculated using the formula provided by Gupta and Sharma (1994):

Biological Efficiency (BE) was then calculated using the formula:

$$BE(\%) = \frac{Total\ weight\ of\ fresh\ mushroom\ harvested}{Dry\ weight\ of\ substrate} \times 100$$

Results

The influence of various botanical treatments on the growth, yield, and biological efficiency of *Pleurotus ostreatus* was found to be statistically significant, as reflected by the morphological and yield parameters recorded during the experimental period. The pooled means demonstrated consistent treatment effects, indicating that the incorporation of botanical additives in the substrate substantially enhanced mushroom growth and productivity compared to the untreated control.

Pileus width (cm): The maximum pileus diameter was obtained from *Allium chinense* (7.46 cm), which was significantly higher than all other treatments. This was followed by *Azadirachta indica* (6.49 cm) and *Allium hookeri* (6.13 cm). Moderate pileus expansion was noted in *Allium sativum* (5.98 cm), *Zingiber officinale* (5.71 cm), and *Ocimum sanctum* (5.49 cm). Comparatively smaller pileus sizes were recorded in *Mentha* sp. (5.29 cm), *Curcuma longa* (5.07 cm), and *Solanum aethiopicum* (4.96 cm), whereas the control (4.47 cm) consistently exhibited the lowest pileus width.

Stipe length (cm): The longest stipe length was recorded in *A. chinense* (8.05 cm), significantly exceeding the untreated control. *A. sativum* (7.08 cm) and *A. hookeri* (7.01 cm) also promoted substantial

stipe elongation. Moderate stipe lengths were observed in *Z. officinale* (6.82 cm), *A. indica* (6.46 cm), and *Mentha* sp. (6.36 cm), while shorter stipes were found in *O. sanctum* (6.03 cm), *C. longa* (5.69 cm), and *S. aethiopicum* (5.73 cm). The minimum stipe length was recorded in the control (5.19 cm).

Number of mature fruiting bodies: A considerable variation was observed in the number of fruiting bodies among treatments. The highest number was produced in *A. chinense* (120.30), followed by *A. sativum* (115.30) and *A. indica* (105.05). Moderate fruiting was obtained from *A. hookeri* (97.95), *Z. officinale* (86.65), *O. sanctum* (83.85), and *Mentha* sp. (68.35). Fewer fruiting bodies were observed in *C. longa* (77.25) and *S. aethiopicum* (64.40), while the control exhibited the minimum count (56.95).

Yield (g): Substantial differences in yield were recorded across treatments. The highest yield was achieved in *A. chinense* (204.50 g), followed by *A. indica* (188.42 g) and *A. sativum* (166.62 g). *A. hookeri* (164.63 g) also produced a higher yield compared to most treatments. Moderate yields were recorded in *Z. officinale* (148.72 g), *O. sanctum* (129.05 g), and *Mentha* sp. (128.57 g). Lower yields were observed in *C. longa* (122.78 g) and *S. aethiopicum* (106.45 g), while the control (98.62 g) registered the lowest yield.

Biological Efficiency (BE%): Biological efficiency followed a similar trend as yield. The maximum BE was recorded in *A. chinense* (87.37%), followed by *A. indica* (84.78%) and *A. sativum* (80.14%). *A. hookeri* (79.81%) also demonstrated high efficiency. Moderate BE values were observed in *Z. officinale* (74.35%), *S. aethiopicum* (70.67%), and *O. sanctum* (70.58%), while lower efficiencies were recorded for *C. longa* (68.97%) and *Mentha* sp. (68.49%). The untreated control showed the lowest BE (63.05%).

Table 2: Effect of different treatments on the morphological traits of *Pleurotus ostreatus*

Treatments	Pileus width (cm)	Stipe length (cm)	
T_0 – Control	4.47	5.19	
T ₁ - Allium sativum	5.98	7.08	
T ₂ - Solanum aethiopicum	4.96	5.73	
T ₃ - Azadirachta indica	6.49	6.46	
T ₄ - Allium chinense	7.46	8.05	
T ₅ - Zingiber officinale	5.71	6.82	
T ₆ - Allium hookeri	6.13	7.01	
T ₇ - Ocimum sanctum	5.49	6.03	
T ₈ - Mentha sp.	5.29	6.36	
T ₉ - Curcuma longa	5.07	5.69	
F- test	S	S	
S. Ed. (±)	0.21	0.48	
CD (5%)	0.48	1.08	

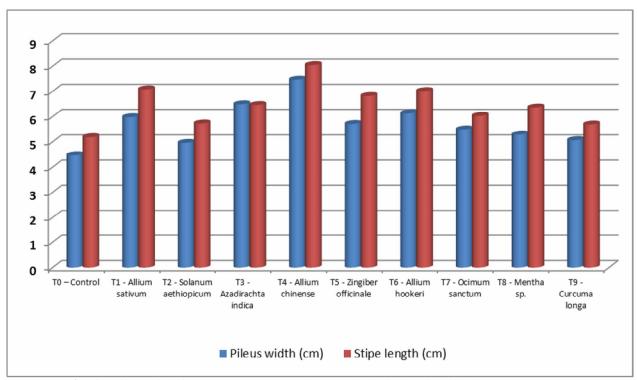


Fig. 1: Effect of different treatments on the morphological traits of *Pleurotus ostreatus*



Plate 1: Measuring of pileus width (cm)



Plate 2: Measuring of stipe length (cm)

Table 3: Effect of different	treatments on the v	ield attributes of	Pleurotus ostreatus

Treatments	Number of fruiting bodies	Yield (g)	BE (%)
T_0 – Control	56.95	98.62	63.05
T ₁ - Allium sativum	115.3	166.62	80.14
T ₂ - Solanum aethiopicum	64.4	106.45	70.67
T ₃ - Azadirachta indica	105.05	188.42	84.78
T ₄ - Allium chinense	120.3	204.5	87.37
T ₅ - Zingiber officinale	86.65	148.72	74.35
T ₆ - Allium hookeri	97.95	164.63	79.81
T ₇ - Ocimum sanctum	83.85	129.05	70.58
T_8 - Mentha sp.	68.35	128.57	68.49
T ₉ - Curcuma longa	77.25	122.78	68.97
F- test	S	S	S
S. Ed. (±)	4.22	2.19	1.54
CD (5%)	2.98	4.95	3.48

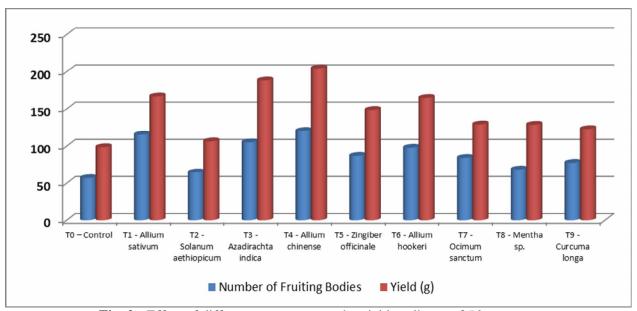


Fig. 2: Effect of different treatments on the yield attributes of *Pleurotus ostreatus*



Plate 3: Fruiting bodies of *Pleurotus ostreatus*

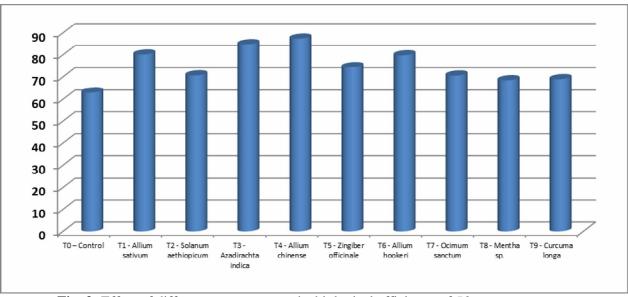


Fig. 3: Effect of different treatments on the biological efficiency of *Pleurotus ostreatus*

Discussion

The observed variations in the morphological and yield parameters of *Pleurotus ostreatus* across different botanical treatments can be attributed to the biochemical influence of plant-derived compounds on substrate microflora, enzymatic activity and nutrient dynamics. The superior pileus width (cm) recorded in Allium spp. and Azadirachta indica treatments is likely a result of their antimicrobial and bioactive constituents, such as allicin and azadirachtin, which suppress competitive microorganisms and enhance substrate selectivity, thereby facilitating improved nutrient availability and uptake for mushroom growth (Pandey et al., 2021). This may have improved nutrient assimilation which may have supported the enhanced metabolic activity and greater allocation of assimilates to reproductive structures, resulting in larger fruiting bodies. Similar findings were reported by Chukwu et al. (2022) and Toppo et al. (2021), who demonstrated that substrate conditioning and neem-based treatments significantly improved morphological traits, including pileus dimensions, in *Pleurotus* species. The superior performance of A. chinense may be further explained by its sulfur-based phytochemicals, which act both as antimicrobials natural and stimulants as lignocellulolytic enzyme activity, thus enhancing substrate degradation and nutrient release. Increased stipe length (cm) observed in Allium and neem treatments also reflects the dual effects of antimicrobial activity and enzymatic stimulation. Sulfur compounds like allicin are known to disrupt microbial cell integrity, thereby reducing substrate competition, while phenolic and flavonoid compounds present in these

botanicals induce ligninolytic enzymes such as laccase and manganese peroxidase in P. ostreatus (An et al., 2021). This enzymatic induction accelerates lignocellulose decomposition, leading to more uniform colonization and better substrate aeration, which favor vertical mycelial growth and stipe elongation. The enhanced number of mature fruiting bodies under these treatments further indicates that suppression of competing molds and bacteria created a cleaner substrate environment, allowing a higher percentage of primordia to develop into fully matured fruiting bodies. Bellettini et al. (2019) and Kortei et al. (2018) similarly reported that reduced contamination and improved substrate hygiene directly correlated with fruiting body greater proliferation mushrooms.

The substantial improvement in yield and biological efficiency (BE) achieved with Allium and A. indica treatments can be primarily attributed to their antimicrobial metabolites, including azadirachtin, and nimbin, which inhibit the growth of competitive molds such as Trichoderma spp. and bacterial contaminants (Borlinghaus et al., 2014; Islas et al., 2020). The suppression of these competitors enables the mushroom mycelium to colonize the substrate more effectively, forming dense and uniform networks that ensure efficient nutrient uptake and distribution. This clean colonization enhances the conversion of substrate biomass into fruiting bodies, reflected in the higher BE values. Similar mechanisms were described by Jha et al. (2023) and Drobnjaković et al. (2023), who found that garlic and neem extracts effectively reduced green mold incidence

improved BE in *Pleurotus* cultivation. Additionally, the bioactive compounds in these botanicals may act as enzymatic inducers, stimulating laccase and peroxidase activity that accelerates lignin degradation and nutrient mobilization, further contributing to increased yield (Zhao *et al.*, 2017).

Overall, the synergistic effects of microbial suppression and enzymatic enhancement under Allium and A. indica treatments explain the superior performance observed in morphological and yield attributes of *Pleurotus ostreatus*. These findings align with the conclusions of Carrasco et al. (2018), who emphasized that substrate supplementation and sanitation significantly improve yield and biological efficiency by optimizing nutrient availability and minimizing microbial interference. Therefore, incorporating botanicals such as A. chinense, A. sativum and A. indica into substrates represents an effective and eco-friendly strategy for enhancing the growth and productivity of oyster mushrooms, ensuring both higher yield and improved biological efficiency under controlled cultivation conditions.

Conclusion

The present study demonstrated that incorporation of botanical powders into wheat straw substrate markedly enhanced the growth, yield, and biological efficiency of Pleurotus ostreatus under controlled cultivation conditions. Among the tested botanicals, Allium chinense exhibited the most pronounced effect, followed by Azadirachta indica and Allium sativum, indicating their strong potential as natural bio-stimulants in mushroom production. The improvement in morphological traits such as pileus width and stipe length, along with increased yield and biological efficiency, can be attributed to the synergistic effects of antimicrobial and enzymatic activities induced by the bioactive compounds present in these botanicals. Sulfur-based metabolites in Allium species and triterpenoids in neem (A. indica) not only suppressed competing microflora but also promoted enzymatic degradation of lignocellulosic substrates, thereby improving nutrient availability and substrate selectivity. The findings reaffirm that maintaining a clean and nutritionally balanced substrate environment is critical for achieving higher mushroom productivity. Treatments that reduced microbial contamination consistently showed better mycelial colonization, higher fruiting body numbers, and superior yield performance. These results are consistent with previous reports emphasizing that substrate supplementation and sanitation play vital roles in optimizing mushroom growth and biological efficiency. Overall, the study highlights the potential of *Allium* and *Azadirachta*-based botanicals as eco-friendly and cost-effective substrate supplements that can replace or reduce reliance on synthetic additives in mushroom cultivation. Their use not only enhances yield and quality but also contributes to sustainable production practices by utilizing natural plant resources with antimicrobial and enzymatic benefits. Future research should focus on optimizing dosage levels, exploring synergistic combinations of botanicals, and assessing the biochemical and nutritional qualities of the harvested mushrooms to fully harness the benefits of these natural supplements in commercial oyster mushroom production.

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