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RESEARCH PAPER

Influence of botanicals and biocontrol agents on the nutritive value of cultivated oyster mushrooms

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Abstract: The present investigation was undertaken to evaluate the influence of selected botanicals and microbial biocontrol agents on the nutritive profile of three commercially cultivated oyster mushroom species, *Pleurotus ostreatus*, *P. eous* and *P. membranaceus*. The experiment was conducted using a Completely Randomized Design with eight treatments comprising neem cake, eucalyptus and thulasi leaf extracts, neem formulation (3000 ppm) and three entomopathogenic fungi *viz*., *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii*—along with an untreated control. Nutritional parameters including protein, ash, moisture and carbohydrate contents were quantified following standard AOAC protocols. Results revealed that neem cake was the most effective treatment across all three species, producing significantly higher protein, ash and carbohydrate contents compared to other botanicals and the control. *Beauveria bassiana* and *Verticillium lecanii* also improved nutritional values, indicating their supplementary role in enhancing substrate decomposition and reducing pest-mediated nutrient depletion. In contrast, eucalyptus and thulasi leaf extracts primarily influenced moisture content but did not significantly enhance protein or mineral accumulation. Species-wise comparison indicated that *P. eous* exhibited the highest protein enrichment under neem cake, while *P. membranaceus* displayed greater moisture retention across treatments. The overall findings emphasize that substrate-based amendments, particularly neem cake, offer dual benefits of improving nutritive quality while supporting sustainable pest management. The integration of plant-derived materials and compatible biocontrol agents can therefore enhance the nutritional value and market quality of oyster mushrooms without compromising on safety or environmental considerations. This study highlights the potential of eco-friendly approaches for strengthening mushroom production systems under Indian cultivation conditions.

Key words: Botanicals, Biocontrol agents, Nutritional quality, Oyster mushrooms, *Pleurotus* spp

Introduction

Oyster mushrooms (*Pleurotus* spp.) are widely recognized as highly nutritious edible macrofungi valued for their rich protein content, essential minerals, dietary fibres, vitamins, low caloric value, and the presence of immune modulatory bioactive compounds (Chang and Miles, 2004; Correa *et al*., 2016, FAO, 2004). Their ability to grow rapidly on an array of lignocellulosic agricultural residues such as wheat straw, rice straw, soybean straw and sawdust makes them one of the most economically viable mushrooms for commercial cultivation (Cruz, 2007). Owing to their nutritive and medicinal value, oyster mushrooms have gained importance in both domestic and export markets, contributing significantly to the mushroom sector in India (Sharma *et al*., 2017; Anon., 2018). Nutritional attributes of oyster mushrooms—including protein, carbohydrate, ash, moisture and mineral composition—are influenced by several biotic and abiotic factors, among which substrate quality and pest incidence are critical. Pest infestation, particularly by sciarid and phorid flies, is a major constraint in mushroom production, causing direct feeding damage on mycelium and fruiting bodies and indirectly reducing yield, quality and nutrient accumulation in harvested mushrooms (Bhattacharyya *et al*., 1993; Deepthi *et al*., 2004). Since mushrooms are consumed fresh and have a very short crop duration, the use of synthetic insecticides is discouraged due to residue hazards and the absence of permissible waiting periods (Noble, 2005). To address this challenge, botanicals and microbial biocontrol agents have emerged as safer and eco-friendly alternatives for pest management in mushroom cultivation. Neem-based products and plant extracts have demonstrated promising insecticidal properties due to the presence of compounds such as azadirachtin, salannin and meliacins (Gahukar, 1995; Bhat *et al*., 1998; Erler *et al*., 2008; Gagan *et al*., 2013). Likewise, entomopathogenic fungi such as *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii* have been reported effective against dipteran pests associated with mushrooms (Zimmermann, 1993; Andreadis *et al*., 2016; Andreadis *et al*., 2021).In addition to suppressing insect pests, botanicals and biocontrol agents influence substrate decomposition, microbial activity and nutrient mobilization, which may directly affect the biochemical composition of mushroom fruiting bodies. Previous studies have shown that integrating oil cakes or biological agents into mushroom substrates can enhance protein, ash, moisture and carbohydrate levels by improving substrate degradability and nutrient uptake (Bonatti *et al*., 2004; Alam *et al*., 2007; Patil *et al*., 2010; Zied *et al*., 2011; Singh *et al*., 2021). However, responses vary across mushroom species and treatment combinations, highlighting the need for species-specific evaluation.Despite the importance of nutritive enhancement in oyster mushrooms, limited research exists on how pest-management strategies—particularly botanicals and microbial biocontrol agents interact with substrate decomposition and influence the nutritional quality of *Pleurotus* spp. under Indian conditions. Therefore, understanding the dual impact of these treatments on pest suppression and nutrient enrichment is essential for developing holistic, residue-free mushroom production technologies.The present study examines the influence of selected botanicals and biocontrol agents on the nutritive profile of three commercially cultivated oyster mushroom species *viz*., *Pleurotus ostreatus*, *Pleurotuseous* and *Pleurotus membranaceus* with a focus on major quality parameters such as protein, ash, moisture and carbohydrate content.

Material and methods

The study was conducted during November–December 2020 at the Mushroom Production House, Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh. Nutritional analyses of oyster mushrooms were carried out in the Department of Food Science and Technology and insect-related observations were supported by the Department of Entomology.Three species of oyster mushrooms—*Pleurotus ostreatus*, *Pleurotus eous* and *Pleurotus membranaceus*—were evaluated. Spawn was procured from the Mushroom Research Laboratory. A mixture of wheat straw and soybean straw (1:1) served as the cultivation substrate. Substrates were steeped in a solution of 500 ppm formaldehyde and 75 ppm carbendazim for 16 hours as per Vijay and Sohi (1987), followed by draining to maintain 65–70% moisture. The experiment was conducted using a Completely Randomized Design (CRD) comprising eight treatments with three replications. The treatments included the incorporation of entomopathogenic fungi *Metarhizium anisopliae* (T_1), *Beauveria bassiana* (T_2) and *Verticillium lecanii* (T_3) at 3 ml L^{-1} into the substrate and neem cake (T_4) mixed into the substrate at 5% (w/w). Botanical sprays consisted of eucalyptus leaf extract (T_5) and thulasi leaf extract (T_6), each applied at 5%, while Neem 3000 ppm (0.3%) was sprayed at 1 ml L^{-1} (T_7). The control (T_8) received only water spray. Fresh leaves of eucalyptus and thulasi were washed, shade-dried, and homogenized with an equal volume of water. The slurry was filtered through muslin cloth and refiltered through cotton to obtain a clear extract for spraying at 5% concentration. Neem cake was thoroughly mixed with the substrate during spawning.The treated substrate was filled (1 kg per bag) into polythene bags (12" × 18", 150 gauge), spawned and perforated for aeration. Bags were incubated for spawn run and subsequently hung for cropping. Water was sprayed three times daily to maintain humidity until harvesting. Three flushes were harvested for each treatment. Fresh fruiting bodies from each treatment and replication were harvested at maturity and immediately weighed. A representative sample from each treatment was shade-dried and powdered for nutritional analysis of Protein (%), Ash (%), Moisture (%) and Carbohydrate (%). Nutritional analysis of oyster mushroom samples included the estimation of protein, ash, moisture and carbohydrate content. Protein was determined using the micro-Kjeldahl method as described by AOAC (1935), wherein the nitrogen percentage obtained after digestion and distillation was multiplied by 6.25 to calculate crude protein. Ash content was estimated by incinerating 5 g of dried mushroom sample in a muffle furnace at 520°C for 5 hours following AOAC (1984) procedures, and the ash percentage was computed based on the final residue weight. Moisture content was measured by oven-drying 5g of fresh samples at 60°C for 6 hours and expressing the weight loss as percentage moisture. Total carbohydrate content was quantified through acid hydrolysis using Fehling's solution, followed by titration against standard dextrose to calculate carbohydrate percentage. Nutritional parameters were analyzed using CRD. Treatment means were compared using Critical Difference (CD) at a 5% probability level according to Gomez and Gomez (1984).

Results and discussion

The results of the investigation demonstrated that botanicals and biocontrol agents significantly influenced the nutritional characteristics of *Pleurotus ostreatus*, *P. eous* and *P. membranaceus*, as reflected through variations in protein, ash, moisture and carbohydrate composition across treatments. Neem cake consistently produced the highest protein content in all three species, indicating that substrate enrichment strongly favours nitrogen assimilation and amino acid synthesis, a principle earlier supported by the nutritive behaviour of mushrooms grown on enriched substrates as described by Bonnati *et al*. (2004). In *P. ostreatus*, protein levels reached 30.79% under neem cake, followed closely by *Beauveria bassiana*, *Verticillium lecanii* and *Metarhizium anisopliae*, while considerably lower values in thulasi and eucalyptus leaf extracts remained statistically similar to the untreated control, indicating that simple aqueous botanicals may not contribute to enhanced nitrogen mobilization. Comparable protein patterns in *P. eous* and *P. membranaceus* reaffirm the positive influence of substrate-based amendments, consistent with Alam *et al*. (2007), who reported nutrient increments in mushrooms cultivated on chemically and biologically modified substrates. The moderate protein rise under fungal biocontrol agents in the present study may be due to indirect improvements in substrate decomposition, aligning with the general understanding of fungal interactions described by Patil *et al*. (2010), wherein substrate–microbe dynamics play a decisive role in nutrient expression. Similarly, ash content, which represents the mineral fraction of mushrooms, was highest in neem cake across all species, followed by the entomopathogens, showing that nutrient-dense or microbially active substrates facilitate release and uptake of mineral elements essential for fruit body development. These findings resonate with the mineral augmentation observed from oil cake amendments reported by Zied *et al*. (2011), suggesting that neem cake functions as both a nutrient source and a substrate conditioner. In contrast, eucalyptus and thulasi extracts failed to enhance ash content, implying negligible contribution to mineral solubilisation, a trend that resembles the limited mineral changes observed under certain leaf extracts in earlier reports such as Hoa *et al*. (2015).The protein and ash contents of the three oyster mushroom species under different botanical and biocontrol treatments are presented in Table 1.

Moisture content exhibited the highest variability among all nutritional parameters, with eucalyptus leaf extract consistently producing maximum moisture levels in all three species. This suggests that essential oil–rich botanicals may alter the physical properties of the substrate, influencing its water-holding capacity, a phenomenon that aligns with substrate-moisture interactions observed by Soni (2002) under different cultural conditions. *P. membranaceus* inherently showed higher moisture values compared to *P. ostreatus* and *P. eous*, indicating species-specific physiological adaptations to moisture availability. Neem cake also maintained high moisture, likely due to its organic buffering capacity, which improves substrate porosity, similar to the effects of organic supplements emphasized by Cruz (2007) and Chiranjeevi (2020). Meanwhile, treatments such as thulasi extract and the control maintained lower moisture levels, indicating limited substrate modification. Excessively high moisture under eucalyptus, although favourable for early mushroom development, may not translate into enhanced nutrient accumulation, reflecting the nutrient dilution effect described by Roupas *et al*. (2012), wherein water content and biochemical composition may inversely correlate. The moisture and carbohydrate contents of the three oyster mushroom species under different botanical and biocontrol treatments are presented in Table 2.

Carbohydrate content displayed a consistent upward trend in substrate-based treatments, with neem cake producing the highest values across all species (56.40% in *P. ostreatus*, 52.40% in *P. eous* and high values across *P. membranaceus*), followed by neem oil and *V. lecanii*. These observations correspond with the carbohydrate enhancement patterns discussed by Fan *et al*. (2017), who highlighted the role of metabolic efficiency during fungal interactions and substrate degradation. Carbohydrates, being major structural and storage components of mushroom fruit bodies, are highly responsive to substrate quality; hence the superior carbohydrate accumulation in neem cake–treated bags indicates optimized enzymatic degradation of lignocellulosic substrates, a concept earlier reinforced by Freire *et al*. (2007) in the context of substrate–fungus synergy. In contrast, carbohydrate values in botanical sprays remained closer to control, implying that aqueous leaf extracts do not contribute substantially to polysaccharide synthesis, which concurs with the limited nutrient enhancement seen in biologically weakened substrates described by Deepthi *et al*. (2004). The combined nutritional response of the three oyster mushroom species to botanical treatments is illustrated in Fig 1, showing clear variations in protein, ash, moisture and carbohydrate contents across *P. ostreatus*, *P. eous* and *P. membranaceus*.

Table 1. Protein (%) and ash (%) content of three oyster mushroom species under botanicals and biocontrol treatments

Treatment	Protein (%)			Ash (%)		
	P. ostreatus	*P. eous*	*P. membranaceus*	*P. ostreatus*	*P. eous*	*P.membranaceus*
T1	29.80	27.90	24.76	11.13	12.00	12.83
T2	30.53	30.47	25.93	13.23	12.45	14.10
T3	30.15	28.67	25.47	12.50	12.27	13.44
T4	30.79	32.22	26.47	14.47	13.05	14.53
T5	27.16	27.31	22.89	10.55	10.70	11.65
T6	26.96	27.84	22.45	10.25	10.15	11.05
T7	28.80	26.94	23.41	11.05	11.80	12.32
T8	25.04	25.87	21.30	9.23	9.83	10.14

Table 2. Moisture (%) and Carbohydrate (%) content of three oyster mushroom species under botanicals and biocontrol treatments

Treatments	Moisture (%)			Carbohydrate (%)		
	P. ostreatus	*P. eous*	*P. membranaceus*	*P. ostreatus*	*P. eous*	*P.membranaceus*
T1	85.23	82.50	90.32	51.55	48.10	48.63
T2	87.60	83.50	91.10	52.23	48.35	49.25
T3	88.30	84.31	92.50	53.10	49.37	51.05
T4	89.60	85.64	93.00	56.40	52.40	52.24
T5	91.13	85.73	93.87	52.83	48.73	48.55
T6	84.40	80.98	87.84	49.00	46.12	44.35
T7	85.20	82.00	90.87	54.37	50.35	52.00
T8	83.40	80.20	87.00	48.36	45.50	43.53

The overall pattern across species demonstrates that substrate-integrated amendments, especially neem cake, provide significantly enhanced nutritional benefits when compared to surface-applied botanicals. This superiority is attributable to neem cake's dual role as an organic nutrient source and as an amendment that improves substrate structure, enabling sustained release of nitrogen, minerals and organic compounds, as well as enhanced enzymatic hydrolysis, a relationship consistent with the substrate nutrition principles discussed by Duhan *et al*. (2017). The entomopathogens *B. bassiana*, *V. lecanii* and *M. anisopliae* also improved nutritive quality, likely due to their suppression of larval feeding stages, reducing nutrient depletion otherwise caused by sciarid and phorid flies; this aligns with reports by Andreadis *et al.* (2021), who demonstrated that entomopathogenic fungi reduce pest-mediated damage in mushroom crops. Further, the enhanced nutrient preservation under entomopathogens may also be attributed to their compatibility with mushroom substrates, as

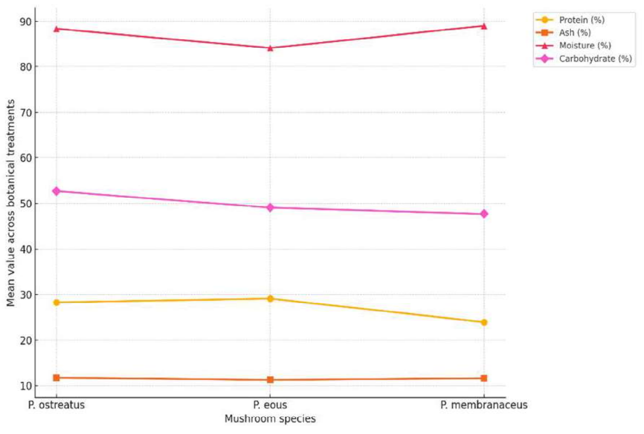

Fig 1. Mean protein, ash, moisture and carbohydrate contents of three *Pleurotus* species subjected to botanical treatments

noted by Ajvad *et al.* (2020), who showed that fungal biocontrol agents integrate well within mushroom production systems. The negligible impact of eucalyptus and thulasi leaf extracts on nutrient enhancement confirms that botanical sprays may lack the biochemical complexity required to meaningfully influence substrate microbial dynamics or nutrient release, a conclusion supported by the limited efficacy of certain botanicals highlighted by Erler *et al.* (2009).

Conclusion

The present study demonstrated that botanicals and biocontrol agents significantly influenced the nutritive quality of cultivated oyster mushrooms. Among the treatments, neem cake consistently enhanced protein, ash and carbohydrate contents across all *Pleurotus* species, indicating its superior role as a substrate-based amendment. Entomopathogenic fungi such as *Beauveria bassiana* and *Verticillium lecanii* also improved nutritional attributes, likely through improved substrate decomposition and reduced pest interference. Botanical leaf extracts primarily affected moisture content but contributed minimally to nutritional enhancement. Overall, integrating nutrient-rich substrate amendments with compatible biocontrol agents offers an effective strategy for producing high-quality, nutritionally superior oyster mushrooms in a sustainable manner.

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