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ORIGINAL RESEARCH ARTICLE

Effects of different botanicals for substrate sterilization in oyster (*Pleurotus ostreatus*) mushroom production

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ABSTRACT

Effective sterilization of substrate is a crucial step for successful oyster mushroom production, yet the use of eco-friendly options to energy-intensive and chemical sterilization methods remains limited in Nepal. Therefore, this experiment was carried out from November 2024 to February 2025 at Prithu Technical College, Deukhuri, Dang, Nepal to evaluate the effectiveness of a few selected plant extracts as sterilizing agents in *Pleurotus ostreatus* production. This research aimed to identify a suitable plant-based alternative that could improve yield parameters. Six treatments: Neem (*Azadirachta indica*), Lantana (*Lantana camara*), Papaya (*Carica papaya*), Fenugreek (*Trigonella foenum-graecum*), and Turmeric (*Curcuma longa*) at 5% concentration were evaluated in a completely randomized design (CRD) with three replications. Data were recorded up to the third flush for phenological, growth, and yield parameters. The result showed significant variation among the assigned treatments. Neem-treated substrate performed best, exhibiting the shortest cropping duration (70 days), the shortest time for pinhead formation (29 days) and fruiting body formation (37 days), the highest total yield (593 g per bag), and the highest biological efficiency (118.6%). While fenugreek and turmeric treatments delayed developmental processes and resulted in decreased yield. There were no significant differences in the number of fruiting bodies and leaves per fruiting body among the treatments. Overall, Neem extract was the most effective botanical for substrate sterilization, offering a sustainable, eco-friendly alternative to conventional methods, improving yield and growth efficiency in oyster mushroom production.

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INTRODUCTION

Mushrooms are macro-fungi, which are large enough to be seen with the naked eye and harvested by hand, with distinct fruiting bodies that are above ground (epigeous) or below ground (hypogeous) (Miles and Chang, 1997; Wiafe-Kwagyan, 2023; Kumar *et al*., 2024). The oyster mushroom is a saprophytic macro-fungus that produces high-value protein for human consumption by utilizing polysaccharides (cellulose, hemicelluloses) typically found in a variety of lignocelluloses (Frimpong-Manso *et al*., 2011). It is one of the most widely grown types of mushrooms in the world, after the white button mushroom (Nongthombam *et al*., 2021; Jarial *et al*., 2024). Commercially grown oyster mushrooms include *Pleurotus ostreatus*, *Pleurotus eryngii*, *Pleurotus cornucopiae*, *Pleurotus citrinopileatus*, *Pleurotus djamor*, *Pleurotus pulmonarius*, and *P. cystidiosus* (Qu *et al*., 2016). The edible oyster mushroom (*Pleurotus ostreatus*) is distinguished by its peculiar flavour as well as texture (Vera *et al*., 2022). Its biotechnological applications, such as bio-bleaching, catalyzing complex chemical reactions in the paper industry, decolorizing textile dyes, and detoxifying environmental pollutants, have attracted significant attention (Park *et al*., 2007). Oyster mushrooms are highly val-

ued for their nutritional benefits. They have high protein content while being low in fat and carbohydrates. Additionally, they contain low levels of sodium and are rich in dietary fiber, vitamin B12, and folic acid, all of which are relatively uncommon in vegetables (Vera *et al*., 2022). Mushrooms lack chlorophyll and rely on plant material as a feeding source. They are simply known as saprophytic, parasitic, or symbiotic in nature. The nutritional requirements of mushrooms are carbon, nitrogen, and inorganic matter; cellulose, hemicelluloses, and lignin serve as the primary carbon sources. Growing media provides essential nutrients for mycelium development, which is a crucial component of mushroom production. It has been suggested that *P. ostreatus* prospers by utilizing a variety of agricultural and agro-industrial waste (Vendrusco *et al*., 2008). Rice straw, sawdust, sugarcane bagasse, cornstalks, leftover cotton, banana leaves, and other sterile substrates are used in the commercial cultivation of *P. ostreatus* (Thongklang & Luagham, 2016; Chang & Miles, 2004).

In order to prevent competing microorganisms from hindering the growth and yield of the targeted mushroom mycelium, substrate sterilization is a vital step in the cultivation of oyster mushrooms (*Pleurotus spp.*) (Rathod *et al*., 2023; Grimm *et al*., 2024). While these substrates are rich in nutrients beneficial to mushrooms, but they also naturally harbor a wide variety of moulds, bacteria, and other fungi (Vajna *et al*., 2012; Wei *et al*., 2024). These microbial competitors can have a negative impact on mushroom cultivation by depleting essential nutrients, producing inhibitory secondary metabolites, or directly attacking the mushroom mycelium (Malik *et al*., 2025; Wiafe-Kwagyan, 2023). The production of oyster mushrooms is often hindered by fungal diseases like cobweb disease, wet bubble, and dry bubble. Additionally, the various Trichoderma species responsible for green mould infections adversely affect all three major types of edible mushrooms. (Nongthombam *et al*., 2021). Traditional sterilization methods such as chemical treatments, steam sterilization, and immersion in hot water are commonly used to reduce contamination (Mejía & Albertó, 2013). However; concerns about environmental toxicity, chemical residues, and sustainability have highlighted the need for safer and sustainable alternatives. Plant-based substitutes have shown potential as eco-friendly substitutes, but comparative studies evaluating their effectiveness in improving oyster mushroom growth and yield remain limited (Biswas *et al*., 2018). Therefore, this study aimed to identify the most effective botanical extract for substrate sterilization that could enhance growth, yield, and biological efficiency of oyster mushroom while providing a safer, eco-friendly, potentially lowering environmental impact and health concerns as an alternative to conventional practices.

MATERIALS AND METHODS

The experiment was carried out at the mushroom house of Prithu Technical College, which is situated at Lamahi-4, Deukhuri, Dang, Nepal, between November 2024 and February 2025. The experiment was set up using the Completely Randomized Design (CRD). At a 5% concentration, three replications of each of the six treatments were tested. For phyto extract preparation, we followed Biswas *et al.* (2018). The mother solution was prepared by combining 1000g of dehydrated and ground plant extract and 1000ml of distilled water. Followed by combining 50 ml of that mother solution per 1000 ml of water to create the standard solution, i.e., (5% concentration). Treatment details used in the study are presented in Table 1.

Table 1. Treatment details used in the study.

Symbol	Treatments
T_1	Control
T_2	Neem (*Azadirachta indica*)
T_3	Lantana (*Lantana camara*)
T_4	Papaya (*Carica papaya*)
T_5	Fenugreek (*Trigonella foenum-graecum*)
T_6	Turmeric (*Curcuma longa*)

Sanitization and preparation of substrates

First and foremost, 5% formalin solution was applied to sanitize the mushroom house. Followed by the use of potassium permanganate for fumigation. To prepare the substrate, paddy straw was then cut into pieces, i.e., 3-5 centimeters long. In plastic drums, the chopped straws were soaked in the assessed treatments for 18 hours. Before spawning, the water solution was drained off, and around 65% moisture content was maintained in the substrate.

Spawning and incubation

For the preparation of growing bags, the treated substrate was inoculated with 37.5 g of spawn and packed into 12 × 18-inch polypropylene bags, each containing 500 g of dry substrate. Spawn was placed near the periphery, forming a circular ring. Three distinct layers of spawning were done to make balls of 1.5 kg. After packing, each bag was perforated with eight to ten holes to facilitate aeration, and the holes were plugged in with cotton to minimize contamination and prevent potential insect entry. The bags were then suspended on ropes in a dark, unventilated room maintained at a temperature of 24 ± 2°C. Continuous observation was taken into consideration up until the full spawn run. The bags were then cut on either side using a sterilized scalpel to aid air exchange and therefore induce fruiting. Moisture was maintained throughout mushroom development by watering as required, in accordance with standard cultivation practices. Watering was discontinued 24 hours prior to each harvest to optimize fruiting and maintain the quality of mushroom.

Data collection and statistical analysis

The mushrooms were harvested in three consecutive flushes once the majority of the caps had attained their full size. Data were recorded on different parameters, including the number of fruiting bodies, number of leaves per fruiting body, pileus diameter (cm), stipe length (cm), total yield per flush (g), and days to full spawn run, days to pin head formation, days to fruiting body formation, along with Biological Efficiency (B.E). Fresh weight of mushroom yield on each harvest was recorded, and then total

yield and biological efficiency were calculated. Equation 1 depicts Modeste (2022) approach to determining biological efficiency. Data were analyzed using R-Studio 4.5.1. To distinguish between treatment means at 5% level of significance, the Least Significant Difference (LSD) was utilized.

$$B.E = \frac{\text{Total weight offresh mushroom}}{\text{Dry weight of substrate}} \times 100\%$$

RESULT AND DISCUSSION

Effect on phenological parameters

Table 2 shows the timing of several stages in the mushroom growth cycle under various treatments. The days to full spawn run or the duration that it takes for the mycelium to completely colonize the substrate were very consistent across all treatments, ranging from 25 to 27 days. Turmeric took the longest (27 days) to colonize, while the others completed in about 25-26 days. And this approves with Biswas *et al.* (2018), who reported a mycelial running time of 22 to 28 days for botanical treatments and Shrestha *et al.* (2021), who found an average of 29.7 days. However, it contrasts with Girmay *et al.* (2016), who reported only 16 days, indicating growing conditions such as humidity, temperature, and CO_2 levels can influence colonization time (Muswati *et al.*, 2021). In contrast, the number of days to pin head development varied significantly, with Turmeric taking the longest (48 days) and Neem the shortest (29 days). Similar studies, such as Biswas (2015), noted pinhead development between 16 and 26 days. The delay observed in our study may be due to the presence of bioactive compounds in botanical extracts such as phenolics, alkaloids, and terpenes, which have antimicrobial properties, possibly can slow down colonization, delaying mycelial growth of *P. ostreatus* and lengthening the time required for pinhead initiation (Ramachela & Sihlangu, 2016; Yin *et al.*, 2025). Pinhead initiation may also be delayed by environmental factors such as temperature, humidity, and CO deposition (Tekeste *et al.*, 2020). Similarly, Turmeric took the longest (55 days) to develop fruiting bodies, while Neem did it distinctly sooner (37 days) (Figure 1). These results align with Fufa *et al.* (2021), who reported fruiting body formation duration of 37.55 to 59.97. The total period from the beginning of cultivation to the final harvest, also known as the Cropping Length, followed a similar profile; turmeric had the longest duration (84 days), whereas Neem had the shortest (70 days). Delays in cropping duration are likely due to antimicrobial effects of the supplements and suboptimal environmental conditions, slowing mycelial growth, ultimately affecting developmental stages (Ramachela & Sihlangu, 2016; Shrestha *et al.*, 2021). These findings show that initial mycelial growth was rather consistent, but developmental phases differed significantly depending on the treatment used.

Table 2. Effect of the botanical sterilization on phenological neem-treated substrate parameters of *P. ostreatus.*

Treatment	Days to			Cropping duration
	Full spawn run	Pin head formation	Fruiting body formation	
T_1	25^{b}	32^{b}	41^{c}	75^{bc}
T_2	25^{b}	29^{c}	37^{c}	70^{d}
T_3	26^{b}	33^{b}	41^{c}	71^{cd}
T_4	25^{b}	31^{b}	42^{bc}	73^{cd}
T_5	26^{b}	33^{b}	46^{b}	78^{b}
T_6	27^{a}	48^{a}	55^{a}	84^{a}
Mean	25.6	34.3	43.5	75.2
P value	0.0214	<0.001	<0.001	0.000369
F-test	*	***	***	***
LSD(0.05)	1.3	2.3	5	4.9
CV (%)	2.9	3.8	6.5	3.7

Values in a column with the same letter(s) are not significantly different at P=0.05, according to the LSD (Least Significant Difference) test. CV: Coefficient of variation; ***, ** and *significant different at P < 0.001, P< 0.01and P < 0.1 respectively, NS: Not significantly different.

Table 3. Effect of the botanical sterilization on the diameter of pileus and the stipe length of *P. ostreatus.*

Treatment	Diameter of pileus (cm)			Stipe length (cm)		
	First flush	Second flush	Third flush	First flush	Second flush	Third flush
T_1	8.1	6.3^{bc}	5^{bc}	4.9^{a}	3.6	3
T_2	7.4	9.3^{a}	9.5^{a}	4.4^{ab}	3.6	3.5
T_3	8.9	7.7^{ab}	6.3^{b}	4.3^{ab}	2.6	2.5
T_4	9.4	5.6^{bc}	5.7^{b}	4.8^{a}	3	2.2
T_5	6.8	3.7^{c}	3.5^{c}	3.3^{bc}	2.6	2.6
T_6	6.7	6.9^{ab}	5.5^{b}	2.4^{c}	2.4	2.6
Mean	7.9	6.6	5.9	4	3	2.7
P value	0.175	0.0117	0.000667	0.00508	0.106	0.0734
F-test	NS	*	***	**	NS	NS
LSD(0.05)	2.4	2.6	1.9	1.1	1.1	0.8
CV (%)	17.5	22.4	18.7	16.4	20.5	2.7

Values in a column with the same letter(s) are not significantly different at P=0.05, according to LSD (Least significant difference) test.CV: Coefficient of variation; ***, ** and *significant different at P <0.001, P < 0.01and P < 0.1 respectively, NS: Not significantly different.

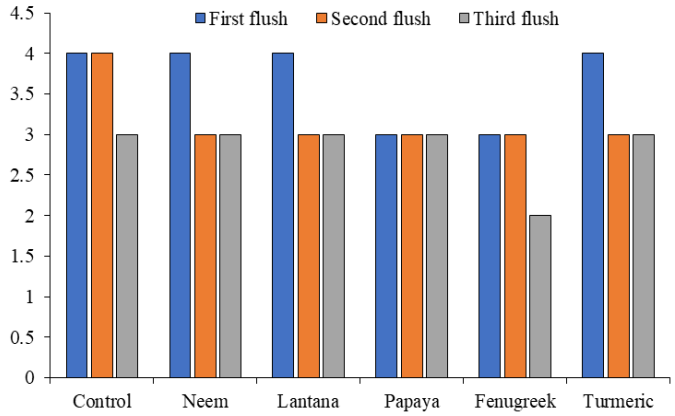

Figure 1. *Number of fruiting bodies of P. ostreatus.*

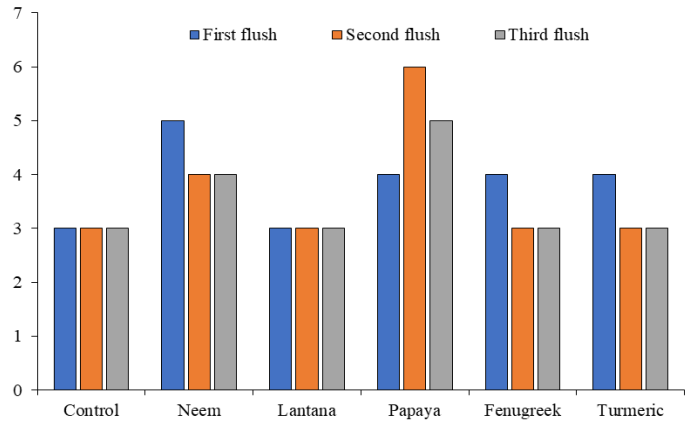

Figure 2. *Number of leaves per fruiting bodies of P. ostreatus.*

Table 4. Effect of the botanical sterilization on fresh weight and biological efficiency of *P. ostreatus.*

Treatment	Fresh weight of mushroom (gm)			Total yield (gm)	Biological efficiency (%)
	First flush	Second flush	Third flush		
T_1	225^{ab}	95.6^{b}	60^{c}	380.6^{bc}	76.1^{bc}
T_2	280^{a}	187.3^{a}	125^{a}	593^{a}	118.6^{a}
T_3	237.6^{ab}	104.3^{b}	91.9^{b}	433.9^{b}	86.8^{b}
T_4	203.6^{bc}	83.3^{b}	64.3^{c}	351.3^{bc}	66.9^{cd}
T_5	133.3^{d}	85.8^{b}	41.4^{c}	245.3^{d}	52.2^{d}
T_6	155.3^{cd}	95^{b}	63^{c}	313.3^{cd}	62.6^{cd}
Mean	205.9	108.5	74.2	386.2	77.2
P value	0.00217	<0.001	0.000159	<0.001	<0.001
F-test	**	***	***	***	***
LSD(0.05)	61.4	29.8	25.1	87.5	16.2
CV (%)	16.7	15.4	19	12.71	11.7

Values in a column with the same letter(s) are not significantly different at P=0.05, according to LSD (Least significant difference) test.CV: coefficient of variation; *** and ** significant different at P< 0.001 and P< 0.01 respectively.

Effect on the diameter of the pileus and stipe length

Table 3 displays the physical traits of the mushrooms that were noted during three consecutive flushes or harvests. Although there was no noticeable difference between treatments, in the first flush the Papaya treatment had the largest pileus diameter (9.4 cm) and the Control treatment had the longest stipe length (4.9 cm). However, during the second flush, the Neem treatment developed the biggest cap diameter (9.3 cm but there were no significant differences in stipe length among treatments. Likewise, in the third flush, the Neem demonstrated superior performance with the biggest pileus diameter (9.5 cm), and as in the previous flush and there were no statistically significant variations in stipe length across treatments. Overall, Neem consistently showed greater cap development over repeated flushes, whereas stipe length was mostly unaffected by the various treatments.

Effect on the number of fruiting bodies and the number of leaves per fruiting bodies

There was no significant difference in the number of fruiting bodies or the number of leaves per fruiting body between the treatments, proving that the selection of substrate supplement had no significant effect on the overall number of mushrooms developed. This suggests that botanical supplements can influence growth rate but yet they do not alter the fundamental reproductive output of *P. ostreatus* under the conditions tested (Figures 1 and 2).

Effect of the botanical sterilization on yield parameters

Table 4 displays the total fresh weight and biological efficiency of mushrooms obtained from three consecutive flushes. The Neem-treated substrate produced 280 g of fresh mushrooms in the first flush, 187.3 g in the second flush, and 125 g in the third flush. The highest yield of 593 g was obtained from Neem-treated substrate, followed by Lantana (433.9g), Control (360.6g), Papaya (351.3 g), and Turmeric (313.3g). Fenugreek, on the other hand, produced the lowest fresh weight (245.3 g), making it the least effective treatment. Neem's superior productivity throughout the cropping cycle is evident in the table 4 below. Biological efficiency is a crucial measure for the conversion of substrate into fruiting body mass. Neem outperformed all other treatments with the highest biological efficiency (BE) value of 118.6%. Similar results were found by Biswas *et al*. (2018), who observed that *Azadirachta indica* treated substrates had higher biological efficiency (109.25%) when compared to the untreated substrate. On the contrary, Fenugreek had the lowest biological efficiency (52.2%), indicating that it was not very effective at enhancing yield performance. These results demonstrate that, in comparison to other treatments, Neem supplementation significantly improved fresh yield and overall biological efficiency of *P. ostreatus.*

Conclusion

In conclusion, botanical sterilizing agents had a significant effect

on the growth and yield of *P. ostreatus*. Among the assigned treatments, Neem extract was the most effective, producing the highest total yield (593g) and biological efficiency (118.6%), while also promoting larger fruiting bodies and shorter cropping duration (70 days). Lantana showed a moderate positive effect with total yield of 433.9 g and BE of 86.8%, whereas papaya supported good early stage growth but had lower overall productivity (351.3g yield, 66.9% BE). In contrast Turmeric and Fenugreek had reduced growth performance with yield 313.3g (62.6% BE) and 245.3g (52.2% BE), respectively. Therefore, the findings of this study indicates that neem leaf extract could be a reliable and environmentally friendly alternative to chemical sterilizers, capable of improving yield of oyster mushrooms.

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DECLARATIONS

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