

OPTIMIZATION AND UTILIZATION OF VARIOUS SUBSTRATES FOR *PLEUROTUS OSTREATUS* PRODUCTION

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ABSTRACT

With growing recognition of the nutritional and medicinal benefits of mushrooms, there's been a surge in interest in their production and consumption. This research investigation aims to investigate the impact of different substrates, composed individually or through various mix ratios of wheat straws, sugarcane bagasse, sawdust, and coco-peat, on the growth, yield, and related parameters of oyster (*Pleurotus ostreatus*) mushrooms. The experiment, carried out between August to November 2023, included six different treatments (T1-T6), each replicated three times, using a randomized complete design. Quantitative data were gathered and analysed, revealing that the shortest spawn running duration for complete colonization was observed in T1 (12 days), while the longest was in T4 (17.66 days). Treatment T3 and T4 exhibited the shortest duration from colonization to primordial formation (2.5 days), whereas T5 took the longest (4 days). The largest cap diameter (8.46 cm) was found in mushrooms grown on T3, while the smallest (4.26 cm) was on T5. The shortest stipe length was noted in mushrooms from T5 (2.16 cm), and the longest in T3 (3.26 cm). T1 had the highest number of fruits (20.33), while T5 had the lowest (12.33). The highest biological efficiencies were observed in T1, which used substrates composed of wheat straw only followed by T3 which used substrates composed of wheat straw and sawdust in combination, suggesting their suitability for pilot or large-scale production of oyster mushrooms.

KEYWORDS: *Pleurotus ostreatus*, Morphological characteristics, Yield, Biological Efficiency.

INTRODUCTION

Oyster mushrooms, scientifically known as *Pleurotus species*, are widely cultivated edible fungi found across the globe, particularly in regions like South East Asia, India, Europe, and Africa (Mandeel Q *et al.*, 2015). Over the past decade, the cultivation of oyster mushrooms, specifically *Pleurotus ostreatus*, has seen a significant rise, making it one of the most cultivated mushrooms worldwide (Lee *et al.* 2002; Sa'nchez 2010; Royse 2013). While initially ranking as the third largest commercially produced mushroom (Obodai *et al.*, 2003), recent reports of Sánchez during 2010 suggest it may now hold the second position after *Agaricus bisporus* in the global market.

This surge in production can be attributed to various factors. *Pleurotus ostreatus* exhibits remarkable adaptability to diverse agricultural and forest waste materials, enabling cultivation through different methods. Moreover, its nutritional and medicinal benefits have garnered attention (Bonatti *et al.*, 2004; Chang and Miles, 2004; Sa'nchez, 2010). Additionally, its relative ease of cultivation compared to other mushroom species makes it suitable for both small and large-scale production. The rapid growth rate of oyster mushrooms allows for multiple harvests within a short span, providing a quicker income source for farmers.

A notable characteristic of *Pleurotus ostreatus* is its ability to thrive on various agricultural waste materials such as straw, sawdust, coffee grounds, and other lingo-cellulosic substrates. This presents an opportunity to repurpose organic waste and by-products, contributing to waste management and sustainability efforts (Sanchez, 2010). By converting waste into a valuable food source, the cultivation of oyster mushrooms aligns with the principles of the circular economy.

The research aims to assess the optimal cultivation of *Pleurotus ostreatus* by utilizing substrates and resources that are readily accessible locally.

2. MATERIAL AND METHOD

2.1. Isolation and maintenance of Fungal Culture

The pure culture of *Pleurotus ostreatus* was sourced from the Directorate of Mushroom Research, Punjab Agricultural University, Ludhiana (Punjab). The cultures were maintained on potato dextrose agar (PDA) slants at 20 -25°C and stored in the refrigerator at 4 °C (Jong, 1978). To maintain the pure line of culture sub-culturing was performed every 35 days intervals.

Potato Dextrose Agar

Material	Quantity
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Potato	400.00 gm
Dextrose anhydrous	20.00 gm
Agar- Agar Type I	20.00 gm
Distilled Water	1ltr B.

2.2. Preparation of spawn:

Following the methodology of Singh and his coworkers (2009), mycelial inoculum was prepared in Petri dishes and conserved in test tube slants. Spawn preparation was conducted based on the procedures outlined by Tandon and his associates (2004), as depicted in plate 1: a & b.

For spawn production, wheat grains were boiled for 15-20 minutes, and then drained and cooled. Addition of calcium carbonate and gypsum at the rate of 5g/kg and 25g/kg, respectively was done. Then, transferred the grains to double polypropylene bags, with thickness of 0.6 mm, and it's tops were plugged with non-absorbent cotton. The grains were also filled into 500 ml glass bottles up to two-thirds full, with the bottles similarly plugged with non-absorbent cotton. These were sterilized at 121°C (15 lbs pressure) for one and half hour. After sterilization, 5 mm discs of 10-day-old mycelial inoculum were aseptically introduced into the spawn bottles. The bottles were then incubated in a dark chamber at 25±2°C. The grains were incubated for 15-20 days, during which the mycelium was allowed to fully colonize the grains. The bottles were shaken every three days to evenly distribute the mycelium throughout the grains until the end of the incubation period.

2.3 Substrate Preparation

Wheat, sugarcane, sawdust and coco-peat were collected from nearby Chandigarh University, Gharuan. Hot water treatment was given to all the substrates for 30-40 minutes. After that the substrates were spread on the black polythene sheet having holes in it for the allowance of excess water drainage. Bags were prepared with the substrates having 60-70% moisture in them, alone and in various combinations as listed in table 1. After that 2 gm's of spawn was added in each bag by following layering method.

2.4 Initiation of Pinheads:

To stimulate pinhead formation, light and temperature adjustments were employed after the mycelium had completely colonized and whitened the substrate. The previously dark-grown mycelium was moved to a fully lit area. Simultaneously, the containers and bags, which were incubated at 25°C, were transferred to an ambient temperature environment. During the experiment, the ambient temperatures ranged from 3°C to 19°C.

2.5 Fruiting Process:

When the mycelium had fully enveloped the substrate, the cotton plugs were removed. The top portions of the plastic bags were partially cut away, specifically the parts covering the primordia, ensuring the mycelium remained unharmed. To boost relative humidity, water was sprayed on the plastic bags containing the primordia three times a day (Dawit A, 1998).

2.6 Biological efficiency (yield):

Biological efficiency serves as a quantifiable measure to assess the growth capacity of oyster mushrooms. It is determined using the following formula:

$$\text{Biological efficiency} = \frac{\text{Weight of fresh mushroom}}{\text{Dry weight of substrate}} \times 100\%$$

Dry weight of substrate

2.7. Data Analysis:

The economic evaluation of different substrate treatments included calculating the benefit-to-cost ratio for each treatment. Statistical analysis of these parameters was carried out using one-way ANOVA. Mean comparisons were conducted using Fisher's protected LSD at a significance level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Days Required for Full Spawn Development

The findings from our study reveal that spawn run initiation occurred across all substrate combinations, albeit with significant variations in the duration, ranging from 12.00 to 17.66 days, with a mean of 15.85 days. There was a notable difference ($p < 0.05$) in the duration required for spawning to complete the spawn run. T1 exhibited the shortest duration (12 days) to complete the spawn run, followed by T3 and T6 with 14.66 and 15.33 days, respectively. In contrast, T4 required the longest duration (17.66 days) to complete the spawn run compared to the other substrates.

Our findings are consistent with prior research by Gupta and his coworkers (2009) and Dehriya and Vyas (2013), which reported accelerated spawn run on various *Pleurotus species* cultivated on substrates such as paddy straw, wheat straw, and sugarcane bagasse. Gupta and colleagues (2018) also noted significantly faster spawn run in substrates supplemented with wheat bran. These observations align with the recommendations of Oei (2003), advocating for the inclusion of wheat bran supplementation at 5-10%, serving as a nutrient source and optimizing substrate conditions for mushroom cultivation. Earlier studies by Chen (1999) highlighted wheat bran as a nitrogen and thiamine (vitamin B1) source essential for the growth and reproduction of mushroom mycelium.

3.2 Morphological characters

Six different substrates were used for the cultivation of *Pleurotus ostreatus*, and their morphological attributes were recorded. The following table offers a comprehensive summary of the observed morphological characteristics of oyster mushrooms grown on these various substrates.

According to our study, the morphological traits of *P. ostreatus* were largely consistent with the observations of Chhetry and Pfoze (2007). They observations indicated that the cap (pileus) of *Pleurotus species* typically exhibited a soft, smooth

texture, with a light yellow colour and a diameter ranging from 4.5 to 11 cm. The mushrooms possessed broad decurrent gills that were white, often with a lateral stipe, typically elongated between 5 to 8 cm. The stipe was white, solid, and sometimes multiple fruit bodies were clustered at the base, forming a common structure. Fresh samples were tender, white, and emitted a mild aroma.

Ram (2007) also described *Pleurotus spp.* with white spores and a lateral or off-center stem with a fleshy texture. The pileus diameter ranged from 2 to 15 cm, with a length of 3 to 11 cm. The upper surface of the cap was smooth, with shapes ranging from spatulae to kidney-shaped, and the margin typically curved inward. The stipe was generally short or appeared as a stalk, forming clusters of 5 to 20 cm.

3.3 Impact of different substrates on the yield and biological efficiency

Mushroom cultivators prioritize yield, making it a key focus. The study examined how different substrate treatments affected yield, revealing statistically significant differences ($p < 0.05$). Table 3 presents results detailing the number of fruit bodies, as well as the length and diameter of both pileus and stipe across six substrates.

Treatments T1 (20.33) and T2 (19.00) displayed the highest average number of fruiting bodies, with T1 slightly surpassing T2. Conversely, T5 (12.33) and T6 (15.66) exhibited the lowest average number of fruiting bodies. These variations in fruiting body production indicate the substantial impact of substrate composition on mushroom yield.

Regarding pileus thickness, T3 stood out with the largest average thickness of 1.09 cm, closely followed by T1 at 1.05 cm. In contrast, T5 showed the smallest average pileus thickness of 1.00 cm. The observed differences in pileus thickness highlight how substrate composition influences the size of mushroom caps. Moreover, T3 recorded the highest average pileus diameter of 8.46 cm, while T5 (4.26 cm) and T2 (6.56 cm) displayed the lowest average pileus diameter. This underscores how substrate composition also affects the diameter of mushroom caps, similar to pileus thickness.

There were significant variations observed in yield-related factors such as pileus diameter and stipe length across different substrate combinations. The largest average stipe diameter (5.29 cm) and length (3.26 cm) were noted in T3. Conversely, T5 generally exhibited smaller stipe diameter (2.20 cm) and length (2.16 cm) compared to other treatments. These differences in stipe morphology suggest that various substrate materials may impact the development of mushroom stalks differently. In summary, these results emphasize that the selection of substrate material plays a significant role in influencing various growth parameters of mushrooms.

Among the 2 kg substrate materials used, treatment T1 exhibited the highest yield (1657.33g), followed by treatment T2 (1444.33g). Notably, only treatments T1, T2, and T3 surpassed the mean yield (1034.55 g) of all substrate treatments investigated. Conversely, the lowest yield was observed in T5 (409.33 g). In our experiments, the yield was notably higher during the first flush, followed by a slight decrease in the second flush, and reaching its lowest point in the third flush. This pattern may be attributed to the maximal substrate degradation by the mushroom's extracellular enzymes during the first flush, leading to the highest availability of nutrients for fruiting body growth. These findings align with those reported by Masevhe and his associates (2015).

The matured fruit bodies are harvested and weighed for calculating yield. The results are tabulated as follows:

The utilization of different substrates was observed to affect the yield components of *P. ostreatus*. Notably, wheat straw, both alone and in combination with sawdust, demonstrated superior growth in terms of pileus diameter and thickness, as well as stipe diameter and length. This study affirmed that the choice of substrate significantly influences the yield of *P. ostreatus*. The highest yield was obtained from wheat straw, followed by a combination of wheat and sawdust, while the lowest was observed with coco-peat and sawdust. Additionally, biological efficiency varied across different substrates, aligning with the findings of previous studies which noted varying ranges of BE when employing different lignocellulosic materials for oyster mushroom cultivation (Liang *et al.*, 2009).

In 2015, Hoa and colleagues noted that substrates yielding higher mushroom yields also yielded higher biological efficiency (BE). Conversely, 100% sawdust substrate exhibited the lowest mushroom yield and BE. The highest BE was achieved with 100% corncob and 100% sugarcane bagasse substrates, recording values of 66.08% and 65.65%, respectively.

CONCLUSION

The process of converting agricultural and agro-processing by-products into mushroom biomass is regarded as a potent microbial biotechnology, leading to the production of nutritionally rich and medicinally beneficial mushrooms. Mushrooms, classified as macro fungi due to their visible large fructification without the aid of a microscope, play a significant role in this process.

Samples of mushroom fungus were collected and subsequently identified based on various morphological characteristics. By examining features such as whitish mycelium growth, as well as the length and diameter of both stipe and pileus, the fungus was identified as *P. ostreatus*.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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TABLES

Table 1: The detail of various substrates is as follows:

Substrates	Ratio
Wheat	100%
Sugarcane Bagasse	100%
Sawdust	100%
Sawdust + Coco-peat	70:30
Wheat + Sugarcane Bagasse	70:30
Wheat + Sawdust	70:30

Table 2: The morphological characteristics of *Pleurotus ostreatus* grown on various substrates.

Treatments	Morphological Characteristics
Wheat Straw (T1)	Fast , uniform, dense, white
Wheat and Sugarcane bagasse (T2)	Patchy growth, cottony, dull white
Wheat and sawdust (T3)	Fast , uniform, less dense, white
Sugarcane bagasse (T4)	Slow, patchy, cottony, fermented odour
Coco-peat and sawdust (T5)	thread like patchy growth , slow growth , white in color, not uniform
Sawdust (T6)	medium, white (light to medium white), not uniform and less dense

Table 3: Result of yield components of *P. ostreatus* on six different substrates.

Treatments	No. of Fruiting Bodies	Diameter of Pileus (cm)	Pileus Thickness (cm)	Diameter of Stipe (cm)	Length of Stipe (cm)
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Wheat Straw (T1)	20.33	7.86	1.05	4.06	2.93
Wheat and Sugarcane bagasse (T2)	19.00	6.56	1.04	3.55	2.86
Wheat and sawdust (T3)	17.33	8.46	1.09	5.29	3.26
Sugarcane bagasse (T4)	13.33	7.46	1.02	2.99	3.20
Coco-peat and sawdust (T5)	12.33	4.26	1.00	2.20	2.16
Sawdust (T6)	15.66	7.6	1.03	4.50	3.70

Table 4: Result of yield and Biological Efficiency of *Pleurotus ostreatus* on six different substrates.

Treatments	Yield (gm's)*	Biological Efficiency (%)
Wheat Straw (T1)	1657.33	82.86
Wheat and Sugarcane bagasse (T2)	1311.33	65.55
Wheat and sawdust (T3)	1444.33	72.21
Sugarcane bagasse (T4)	566.00	28.30
Coco-peat and sawdust (T5)	409.33	20.46
Sawdust (T6)	819.00	40.95

*Yield of three flush PLATES



Pic 1: (a) Mycelial inoculum in test tube slants; (b) Spawn preparation

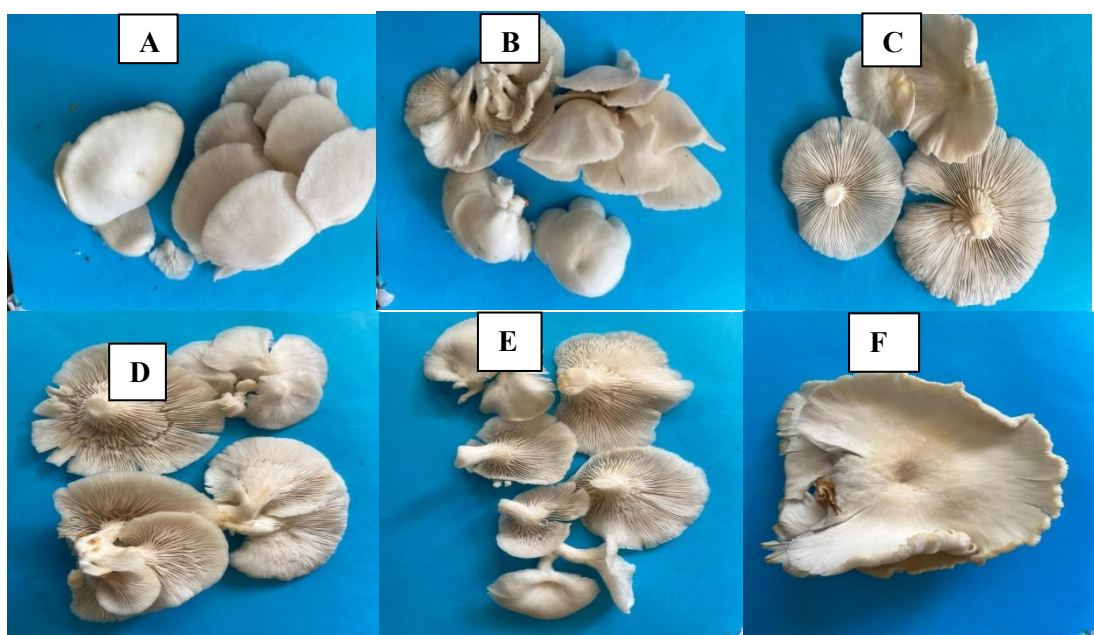


Plate 2: Fruiting bodies growth on substrates viz., (a) Sawdust; (b) Wheat and Sawdust; (c) Sugarcane bagasse; (d) wheat; (e) Coco peat; (f) wheat and sawdust