



## **Effect of Different Kinds of Substrates on the Growth and Yield Performance of *Pleurotus sapidus* (Oyster Mushroom)**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors NAK, WA, and MAK designed the study. Authors WA and OY performed the experiment and collect data. Authors WA, SA, and SM performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. All authors contributed to the final draft of the manuscript.*

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### **ABSTRACT**

Cultivation of edible fungi (mushrooms) is a useful method for bio-conservation in the agriculture industry. For the better production of mushrooms, we used the agricultural waste material as a substrate for the cultivation of mushrooms. After mushroom harvesting, these substrates are available as an excellent source of soil conditioner. In this study, we used the sawdust of a simbal tree (*Bombax ceiba* L.), office scrap paper, and poultry manure as a substrate to cultivate the *Pleurotus sapidus* (oyster mushroom). Data recorded from the inoculation of spawn to the harvesting of fruiting bodies by using different parameters. The results revealed that the Treatment-T4 (sawdust of simbal tree 50% + poultry manure 50%) required the least number of days (16 days) for 100% spawn-running, development of pinhead (6.5 days), and fruiting bodies (5.5 days), produced the maximum number of fruiting bodies (24.25), maximum yield (388.40 g), and highest biological efficacy (77.68%). The Treatment-T5 (office scrap paper 50% + poultry

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manure 50%) took the maximum number of days (32 days) for 100% spawn-running, development of pinhead (18 days) and fruiting bodies (17 days), produced least number of fruiting bodies (9.5), minimum yield (224.50 g), and lowest biological efficacy (44.90%). Therefore, the cultivation of oyster mushrooms is an excellent or environment-friendly technique because the substrate used for mushroom cultivation is economical and readily available.

**Keywords:** *Oyster mushroom; simbal tree sawdust spawn; yield; and biological efficiency.*

## 1. INTRODUCTION

Mushrooms are achlorophyllous, spore-producing, macro-fungi having a heterotrophic mode of nutrition usually grow above ground that contains cap, stem, and hymenium [1]. Oyster-mushroom fit into the kingdom; fungi, and order; Agaricales. Mushroom usually called a white vegetable, queen of vegetables, and boneless vegetarian meat. Protein contents are 20-35% higher in mushrooms as compared to vegetables and fruits [2]. Mushrooms are rich in amino acids, vitamins, potassium, protein, fibers and have a low level of cholesterol and fats [3]. Cultivation of mushrooms is the only economical and feasible method for the conservation of agriculture waste material and plant residues from the forest [4].

Oyster mushroom is famous for its excellent flavor and taste. It has a wide range of growing ability, as it has grown on many substrates at 20-30°C temperature and 70-80% relative humidity [5]. *Pleurotus spp.* have grown on a wide variety of crop residues by using these crop residues as substrate [6]. The fungus had a saprophytic mode of nutrition and utilized carbon-based organic matter, which contains lignin, cellulose, and lignocellulose for food. The substrate has a pH range 6-8 is appropriate for the cultivation of oyster mushroom [7,8]. Globally, about 10000-14000 species are discovered as a medicinal and edible mushroom; among them, only 20-60 species commercially cultivated for human consumption [9-11].

In Pakistan, different kinds of agriculture waste material are used as substrates to cultivate the edible mushrooms. These substrates comprise of wheat straw, paddy straw, cotton waste, kicar sawdust, and rice husk. Shah et al. [12] conduct a study to check the yield and biological efficacy of *Pleurotus spp.* on the substrates as mentioned above and their results showed that the sawdust was an ideal substrate for the commercial production of *Pleurotus* species. Seven different types of agriculture waste material like; date palm leaves, cotton waste, sawdust, farmyard manure, sugarcane leaves, and wheat straw utilized as a growth medium to cultivate the

oyster mushroom. The outcomes revealed that wheat straw and cotton waste were the most excellent substrates for commercial level farming of mushrooms [13].

Oyster mushroom cultivated on various farming waste materials like; kicar sawdust, paddy straw, cotton waste, and wheat straw alone and in different concentrations. Among all the substrates, cotton waste (100%) found to be a suitable substrate for the commercial and profitable production of oyster mushroom [14]. Pakistan is a state having the ideal climatic conditions and contains four seasons, which is suitable for the commercial production of mushroom on naturally and artificially terms. In the regions of Punjab, Khyber Pakhtunkhwa, and Kashmir, the commercial production of different kinds of mushroom (oyster, button, and milky mushroom) has been made by using the different types of agriculture raw materials (wheat straw, sawdust, cotton waste, and rice husk). The reason behind using these materials as a substrate is this; because these things are readily available and accessible at minor costs. To fulfill the nutritional and medicinal necessity, a mushroom is a cheap and easily accessible source and consumed all over the world with an average annual production of about 40 million tons [15].

In Pakistan, National Logistic Cell (NLC) Islamabad started production of mushrooms at a commercial level for the very first time, with an average annual output about 200 tons [16]; and among 80% of the total production was exported to Europe and the USA to make foreign reserve [17-19]. Keeping in mind the medicinal and nutritional importance of oyster mushroom, the current study was designed to find out the suitable substrate for the yield enhancement of *P. sapidus*, which is readily available at a very low cost.

## 2. MATERIALS AND METHODS

The methodology used in this research was standardized and following the methods of Siddiqui [20].

## 2.1 Collection and Preparation of the Substrate

Simbal tree (*Bombax ceiba* L.) sawdust, poultry manure, and office scarp papers were used as a substrate in this study. Simbal tree sawdust was collected from the local wood market of Faisalabad, Pakistan. Poultry manure was collected from the poultry-farm of the University of Agriculture, Faisalabad. Office scrap papers were collected from the local photocopy shops of Faisalabad, Pakistan. Preparation of the substrates was done by the fermentation process of these agricultural waste materials, for fermentation, the substrates were first soaked in distilled water drum for 24 hours. After this, the soaked substrates were thoroughly spread on polyethylene sheet to remove the extra water, and the moisture level was adjusted about 65-70%; pH 7.0 of the substrates was maintained by adding 5% gypsum on substrate dry weight. The substrates were covered with polyethylene sheets to keep the anaerobic fermentation process. The substrates were fermented for five days before filling the bags and further mixed as per treatment ratios [21]. The following combinations of cellulosic and lignocellulogic materials used as substrate for the production of *P. sapidus* (Table 1).

## 2.2 Filling and Sterilization of Bags

For each treatment, 500 g of the substrate was filled in the polypropylene bags of (8"×12") size. After filling, these bags were autoclaved for sterilization in country style (drum) autoclave for 2 hours and keep the bags overnight at room temperature to cool down the bag's temperature for the inoculation of spawn.

## 2.3 Spawn Inoculation and Incubation of Bags

The prepared spawn of *P. sapidus* was used for the inoculation of pasteurized bags, and 20 g of *P. sapidus* spawn was used to inoculate the bags. For the inoculation of spawn, the mouths of bags were opened and disturb the upper 2-3 cm

layer of the substrate and thoroughly mixed the spawn. After the inoculation of spawn, the inoculated bags were incubated at 20–25°C and 80-90% relative humidity in the mushroom growing room under complete darkness till the substrate colonized with mycelium.

## 2.4 Recording of Data

Data were collected in the total number of days required for spawn-running, development of pinhead, and development of fruiting bodies.

### 2.4.1 Time for the accomplishment of spawn-running

The data was collected in the number of days for 25%, 50%, 75%, and 100% development of mycelium on substrates after spawn inoculation.

### 2.4.2 Phases of pinheads formation

The data related to the pinhead formation was collected in the number of days after the full mycelium growth to the formation of pinheads.

### 2.4.3 Phases of fruiting bodies formation

Data was recorded in the number of days after the pinhead formation to the development of fully mature fruiting bodies on the selected substrates.

## 2.5 Yield and Biological Efficacy

Harvesting of mature fruiting bodies was done three times at the maturity of each flush, and the data for each picking was recorded in grams. The total yield of mushroom was recorded in mass (grams) by adding the weight of all three pickings and taken there means (grams). The biological efficiency of *P. sapidus* was calculated with the help of this formula [22].

$$\text{Biological Efficiency \%} = \frac{\text{Fresh weight of the harvested mushroom (g)}}{\text{The dry weight of used substrate (g)}} \times 100$$

**Table 1. Treatment combinations used as a substrate for the production of oyster mushroom**

Treatments	Combinations of treatments
T <sub>1</sub>	Sawdust of simbal tree 100%
T <sub>2</sub>	Sawdust of simbal tree 50% + office scrap paper 50%
T <sub>3</sub>	Sawdust of simbal tree 50% + office scrap paper 25% + poultry manure 25%
T <sub>4</sub>	Sawdust of simbal tree 50% + poultry manure 50%
T <sub>5</sub>	Office scrap paper 50% + poultry manure 50%

## 2.6 Statistical Analysis

The experimentation was arranged in a completely randomized design (CRD), with five treatments and each treatment has five bags as replications, while each treatment was repeated three times. Data was examined statistically using (ANOVA) analysis of variance with a probability level of  $p \leq 0.05\%$  to achieve the significance of the research.

## 3. RESULTS AND DISCUSSION

Results revealed that the treatment T4 was taken a minimum number of days (16) for 100% mycelial growth followed by treatments; T1 (18.50 days), T2 (24 days) and T3 (26.50 days), whereas, T5 was taken a maximum number of days (32) to achieve the 100% mycelial growth. The substrate in which cellulosic and lignocellulosic substance was more showed most suitable substrates for mushroom growth as also mentioned by [23]. Dhoke et al. [24] also found that different agricultural wastes have significant

effects on mushroom development. Regarding pinhead formation, decreasing trend viz. T5 (18 days) > T3 (14.50 days) > T2 (12 days) > T1 (8.50 days) was observed, whereas, the treatment T4 was taken a minimum number of days (6.50) for pinhead formation. In the case of fruiting bodies development, the treatment T5 was taken maximum days (17) over treatments; T3 (13.75 days), T2 (11.25 days), and T1 (7.75 days). In comparison, the treatment T4 was taken a minimum number of days (5.50) for the development of fruiting bodies (Table 2). Khan et al. [25] and Bhattacharjya et al. [26] reported that the pinhead formation took seven to eight days. After pinhead formation, it took only three to five days for fruiting body development, which supports the results of treatment T4 where pinhead formation was completed in 6.50 days, and the fruiting body was developed in 5.50 days which is just half day more than reported fruiting body formation days (Table 2). This difference may be due to the changes in the growth environment and conditions and verities, which are also reported by Bhattacharjya et al. [26].

**Table 2. Assessment means for the growth development of oyster mushroom (days)**

Treatment	100% Mycelial Growth	Pinhead Formation	Dev. of Fruiting Bodies
T <sub>1</sub>	18.50 ± 0.22 <sup>d</sup>	8.50 ± 0.22 <sup>d</sup>	7.75 ± 0.37 <sup>d</sup>
T <sub>2</sub>	24.00 ± 0.22 <sup>c</sup>	12.00 ± 0.44 <sup>c</sup>	11.25 ± 0.33 <sup>c</sup>
T <sub>3</sub>	26.50 ± 0.22 <sup>b</sup>	14.50 ± 0.44 <sup>b</sup>	13.75 ± 0.11 <sup>b</sup>
T <sub>4</sub>	16.00 ± 0.35 <sup>e</sup>	6.50 ± 0.22 <sup>e</sup>	5.50 ± 0.22 <sup>e</sup>
T <sub>5</sub>	32.00 ± 0.41 <sup>a</sup>	18.00 ± 0.44 <sup>a</sup>	17.00 ± 0.44 <sup>a</sup>
LSD ( $p \leq 0.05$ )	0.88**	1.10**	0.94**

In a column; means having the same superscript letters are not significantly different at  $p \leq 0.05$ . \*\*= Highly significant. T<sub>1</sub>= Sawdust of simbal tree 100%; T<sub>2</sub>= Sawdust of simbal tree 50% + office scrap paper 50%; T<sub>3</sub>= Sawdust of simbal tree 50% + office scrap paper 25% + poultry manure 25%; T<sub>4</sub>= Sawdust of simbal tree 50% + poultry manure 50%; T<sub>5</sub>= Office scrap paper 50% + poultry manure 50%

**Table 3. Assessment means of treatments for the yield (g) and biological efficiency of oyster mushroom**

Treatment	1 <sup>st</sup> Flush	2 <sup>nd</sup> Flush	3 <sup>rd</sup> Flush	Total Yield	B.E (%)
T <sub>1</sub>	130.70 ± 1.30 <sup>b</sup>	112.75 ± 1.87 <sup>b</sup>	95.50 ± 1.89 <sup>b</sup>	338.95 ± 1.84 <sup>b</sup>	67.79 ± 0.36 <sup>b</sup>
T <sub>2</sub>	114.45 ± 1.72 <sup>c</sup>	99.60 ± 1.57 <sup>c</sup>	89.73 ± 2.37 <sup>b</sup>	303.78 ± 3.99 <sup>c</sup>	60.75 ± 0.79 <sup>c</sup>
T <sub>3</sub>	98.50 ± 2.09 <sup>d</sup>	86.84 ± 2.21 <sup>d</sup>	74.25 ± 1.31 <sup>c</sup>	259.59 ± 3.76 <sup>d</sup>	51.91 ± 0.75 <sup>d</sup>
T <sub>4</sub>	153.25 ± 3.71 <sup>a</sup>	127.50 ± 1.75 <sup>a</sup>	107.65 ± 2.90 <sup>a</sup>	388.40 ± 4.21 <sup>a</sup>	77.68 ± 0.84 <sup>a</sup>
T <sub>5</sub>	89.65 ± 2.79 <sup>e</sup>	73.38 ± 2.08 <sup>e</sup>	61.47 ± 3.68 <sup>d</sup>	224.50 ± 4.73 <sup>e</sup>	44.90 ± 0.94 <sup>e</sup>
LSD ( $p \leq 0.05$ )	7.28**	5.63**	7.55**	11.29**	2.25**

In a column; means having the same superscript letters are not significantly different at  $p \leq 0.05$ . \*\*= Highly significant. T<sub>1</sub>= Sawdust of simbal tree 100%; T<sub>2</sub>= Sawdust of simbal tree 50% + office scrap paper 50%; T<sub>3</sub>= Sawdust of simbal tree 50% + office scrap paper 25% + poultry manure 25%; T<sub>4</sub>= Sawdust of simbal tree 50% + poultry manure 50%; T<sub>5</sub>= Office scrap paper 50% + poultry manure 50%

First harvesting was done after ten days of fruiting body formation, as suggested by Khan and Khan, [27]. Picking of fruiting bodies was done at the ripeness of every flush, three numbers of flushes were picked from each treatment, and mass of each flush was recorded in grams. Treatment T<sub>4</sub> exhibited maximum yield in 1<sup>st</sup> (153.25 g), 2<sup>nd</sup> (127.50 g), and 3<sup>rd</sup> (107.65 g) flush, whereas, treatment T<sub>5</sub> exhibited the minimum yield in 1<sup>st</sup> (89.65 g), 2<sup>nd</sup> (73.38 g) and 3<sup>rd</sup> (61.47 g) flush. Total yield was maximum in the case of T<sub>4</sub> (388.40 g) over T<sub>1</sub> (338.95 g), T<sub>2</sub> (303.78 g), T<sub>3</sub> (259.59 g), and T<sub>5</sub> (224.50 g). A similar trend was assessed concerning biological efficiency (%) as T<sub>4</sub> (77.68%) > T<sub>1</sub> (67.79%) > T<sub>2</sub> (60.75%) > T<sub>3</sub> (51.91%) > T<sub>5</sub> (44.90%) (Table 3). As discussed earlier that as the cellulosic and lignocellulosic material increased the yield of mushroom. Dey [28] also reported that the average yield is significantly affected by the application of different substrates. Bhoyan [29] also found similar findings with a change in sawdust composition with animal dung and claimed that the supplement provided with sawdust increased the fruiting body weight. Yoshida *et al.*, [30] and Siwulski [31] also supported the results that pure sawdust is not too effective for mushroom growth, but if different supplements were mixed, nutrients provided by them have significant effects on mushroom yield. The same results were also reported by [32] and Ahmed [33].

#### 4. CONCLUSION

It is concluded that the cultivation of oyster mushrooms is an excellent or environment-friendly technique because the substrate used for mushroom cultivation is economical and readily available.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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