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Cultivation of Oyster Mushroom (*Pleurotus ostreatus*) Using Ricebran, Sawdust and their Mixed Bed as Substrates

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ABSTRACT

Oyster mushrooms (*Pleurotus ostreatus*) are nutritious and medicinal valuable food source, with growing demand globally due to their high protein content, dietary fiber, and potential health benefits. However, their cultivation is hindered by poor substrate selection, leading to reduced yields, lower quality, and decreased profitability. This study was aimed to determine the effect of different substrates on the performance of oyster mushrooms and identifying the optimal substrate for cultivation. Various agricultural residues were evaluated as potential substrates, including ricebran, sawdust, and other locally available materials. The physical and chemical properties of each substrate and their impact on optimum mycelial growth, fruiting body yield, and nutritional content of the mushrooms was assessed. The effects of different substrate types mixed bed, rice bran, and sawdust with composting intervals of 4, 8, and 12 weeks on the growth and yield of *Pleurotus ostreatus* was also investigated. The results showed that the mixed bed substrate supported the best growth and yield of *P. ostreatus,* with a significant increase in fruit weight (84.94g), average fruit weight (5.66g), and biological efficiency (42.47%) compared to rice bran and sawdust. Composting interval also had a significant effect on the growth and yield of *P. ostreatus*, with 12-week composting interval resulting in the highest fruit weight (84.94 g), biological efficiency (42.47%), and production efficiency (45.30%). The mixed bed substrate with a 12-week composting interval resulted in the highest number of fruits (15.0), longest length of stipe (4.33 cm), and largest width of pileus (6.07cm) indicating that a mixed bed substrate with a 12-week composting interval is the optimal combination for the cultivation of *P. ostreatus.* Identifying the best substrate will improve oyster mushroom production; enhance the economic and ecological values of this valuable crop, supporting rural development and income generation for small-scale farmers. This will also address malnutrition and health concerns associated with red meat consumption, and providing a sustainable solution for organic waste management. By addressing the challenges of substrate selection, this study has contributed to the sustainable development of oyster mushroom production, improving food security, and enhancing the livelihoods of rural communities.

Keywords: Oyster Mushroom, Substrate, Cultivation, Ricebran, Sawdust, Food Security.

Introduction

Mushrooms are protein based foods that are important component of human's diet globally (Ajonina and Tatah, 2012). This is because of their high nutritional value and medicinal properties. They are known worldwide as the vegetarian's meat. It contains a high percentage of proteins, which is higher than any other vegetative protein, but it is low in calories. Mushroom has low starch content; this makes it a good diet for people suffering from diabetes (Xu *et al*., 2011). There are over 16,000 known mushroom species, about 200 species are used as functional foods around the world (Alananbeh *et al*., 2014) and of these about 35 species are commercially cultivated.

The oyster mushrooms (*Pleurotus ostreatus*) ranks third after the white button and second among the world mushroom production. Globally China is the major producer (~90% share) and consumer of oyster mushroom (Kalac, 2013). The demand for mushroom has been mounting day by day due to population growth, market expansions, changing of consumer behaviour, and developments. Cultivation of oyster mushroom (*Pleurotus ostreatus*) has increased tremendously throughout the world because of their abilities to grow at a wide range of temperature and utilizing various agro-based residues. *Pleurotus* species are efficient lignin degraders, which can grow on different agricultural wastes with broad adaptability to varied agro-climatic conditions.

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Growing oyster mushrooms convert a high percentage of the lignocellulosic substrate to fruiting bodies increasing profitability. *P. ostreatus* demands few environmental controls, and their fruiting bodies are not often attacked by diseases and pests, and they can be cultivated in a simple and economic way (Bellettini *et al*., 2019). It requires a short growth time in comparison to other edible mushrooms.

Substrate composition has significant impact on growth and nutritional composition of mushrooms (El Sebaaly *et al*., 2019). Mushrooms require carbon, nitrogen and inorganic compounds as their nutritional sources and the main nutrients are carbon sources such as cellulose, hemicellulose and lignin. Oyster mushrooms require less nitrogen and more carbon source. Thus, most organic matters containing cellulose, hemicellulose and lignin can be used as mushroom substrate e.g. rice and wheat straw, cottonseed hulls, corncob, sugarcane baggase, sawdust, waste paper, leaves, and so on. However, demanded amount of each nutritional source differs according to mushroom species and substrate used (Sánchez, 2010). Mushroom cultivation involves substrate and compost preparation, spawn production, and seeding for mycelial growth and fruiting body production (Pandey, 2004). Sawdust and Rice bran are the most popular basal ingredient in synthetic substrate formulation for mushroom production. Supplementing with protein-rich materials like rice and wheat bran enhances substrate efficiency (Carrasco *et al*., 2018).

The aim of this study was to evaluate the performance of rice bran, saw dust and a mixture of these two substrates on the growth performance of *Pleurotus ostreatus* as to identify the best substrate for the optimal growth performance and yield of oyster mushroom as well as the nutrient content of *Pleurotus ostreatus*.

Materials and Methods

Sample collection

The sawdust and rice-brand used for this research were obtained within Jos-Katako main market, in Jos-North, Plateau State, Nigeria. The Oyster Mushroom (*Pleurotus ostreatus*) was obtained from Taminus main market in Jos.

Substrate composting

The substrate (rice bran, saw dust and 1:1 mixture of rice bran and saw dust) 200g each were soaked with 1000mls of water, at the w/v ratio of 1:5 overnight for 24 hours and pressed at 20 psi the following morning to remove excess water until the moisture content was about 65% and about 1% calcium carbonate ($CaCO_3$) was added to adjust the pH to 7.5. Polyethylene bag of size 25 x 15 cm was filled with the substrate with two bags weighing 200g each and packed tightly. The neck of the bag is made of heat resistant PVC (Poly Vinyl Chloride) tube. The opening was covered with a cotton plug. The level of composting interval used in this research was 4 wks, 8 wks and 12 wks (Stamets, 1993).

Inoculation of mushroom spawn

The bags were sterilized in an autoclave at 121ºC for 15 min, and allowed to cool to room temperature. After cooling to room temperature of 30 ± 2ºC, each of the bags were inoculated with 30 g of the inoculum (10% by weight of the substrate in each bag) of the selected cultivated mushrooms (*P. ostreatus*) spawn through the neck opening**.** They were incubated in the dark room for 30 days from the day of spawning to allow the mycelia to ramify in the substrates. The fully colonized bags were brought out for weighing with an electronic weighing balance and then transferred into the cropping house where fruiting bodies emerge and then harvested manually. These procedures were carried out on each of the three substrates at 4 weeks of composting intervals (Chang and Miles, 2004).

Evaluation of growth and yield characters in *Pleurotus ostreatus*

The following data were collected on the mushroom: number of fruiting bodies (NF), fruit weight (FW), average fruit weight, width of pileus, length of stipe, primordial initiation and days to full colonization, biological and production efficiencies.

The average fruit weight, production efficiency and biological efficiency were determined by equations 1, 2 and 3 respectively (Oei, 2003).

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1. Average Fruit Weight

$$= \frac{\text{TFW}}{\text{Total number of fruit harvested}}$$

2. Productio Efficiency(%)

$$= \frac{(TBY)(TFW + AFW)}{\text{Weight of substrate used}} \times 100$$

3. Biological Efficiency(%) $= \frac{\text{MLW}}{\text{WS}} \times 100$

where; MLW is Mushroom Life Weight i.e. the total weight of mushrooms harvested till the nutrients is exhausted, WS is Weight of Substrate is transferred to the cropping house, TBY is total Biological Yield, TFW is Total Fruit Weight until nutrient of substrate is exhausted, AFW is Average Fruit Weight.

Data Analysis

The data obtained during this study were analyzed using descriptive statistics and ANOVA. Post-hoc tests were performed using Tukey's HSD test to further compare mean values (Kumar *et al*., 2023).

Results

The effect of different substrate type on the growth of the mycelia and fruiting bodies of *P. ostreatus* is presented in Table 1. It represents the Mean effects of different substrates on the number of fruits, fruit weight, and average fruit weight, width of pileus and length of stipe of *Pleurotus ostreatus*. For the mixed bed, the number and weight of fruits bodies (NF) produced was 15 and 84.9 g respectively. This gives an average fruit weight of 5.7g. Additionally, the largest width of pileus recorded was 5.34 cm while the longest length of stipe recorded was 4.46 cm. No growth was observed for the rice bran and saw dust.

Table 2: shows the biological efficiency (BE) and production efficiency (PE) of *Pleurotus ostreatus* of the mixed bed substrate. The BE was calculated as 42.47% while the PE was calculated as 45.30%. No growth was observed for the rice bran and saw dust.

The various stages of spawn preparation and growth are shown in the photo gallery of spawn preparation and cultivation of oyster Mushroom (*Pleurotus ostreatus*) as seen in Plates 1-3. Pasteurization Process of Mushroom is seen in Plate 4. The growth stages on the mixed bed are seen in Plates 5-11.

Table 1: Mean effect of substrate variety interaction on the yield parameters of *Pleurotus ostreatus*

Substrate	Yield parameters of *Pleurotus ostreatus*				
	Number of fruits	Fruit weight (g)	Average fruit weight (g)	Width of pileus (cm)	Length of stipe (cm)
Mixed Bed	15.0	84.94	5.66	5.34	4.46
Rice bran	0.00	0.01	0.04	0.03	0.04
Sawdust	0.00	0.02	0.02	0.03	0.03

Table 2: Effect of substrate variety interaction on biological and production efficiencies of *Pleurotus ostreatus*

Substrates	Biological Efficiency (%)	Production Efficiency (%)
Mixed bed	42.47	45.30
Rice bran	0.03	0.04
Sawdust	0.02	0.03

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Photo Gallery of Spawn Preparation and Cultivation of Oyster Mushroom (*Pleurotus ostreatus*)

Plate 1: Spawn preparation (Sorhum seeds in bottles ready for sterilization)

Plate 2: Growth of spawn in bottle one week after inoculation

Plate 3: Mushroom spawn fully grown in bottles and ready for use

Plate 4: Pasteurization Process of Mushroom

Plate 5:Substrates after One Month of Inoculation

Plate 6: Mushroom Ready for Fruitening

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Plate 7: Mushroom Ready for Harvest

Plate 8:Mushroom Harvested at week four (4)

Plate 9: Mushroom harvested at week eight (8)

Plate 10: Mushroom harvested at week twelve (12)

Plate 11: Packaged Mushroom for distribution

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Table 3 shows the mean effect of substrates mycelia extension as number of days from inoculation to mycelia colonization, mushroom primordial initiation and extension of *Pleurotus ostreatus*.

For the mixed bed, the longest mycelia extension observed was 8.25 cm, the number of days to full mycelia colonization was 36 days, the number of days for primordial initiation was 24 days and the extension per day is calculated as 0.68cm/day. No growth was observed for the rice bran and saw dust.

Table 4 shows the mean effects of weeks composting interval on the number of fruits, fruit weight, average fruit weight and width of pileus of *P. ostreatus.* For the mixed bed, the number of fruit (NF) at 4, 8 and 12 weeks composting interval was 6, 11 and 15 respectively. The fruit weight (FW) at 4, 8 and 12 weeks composting interval was 28.59g, 65.02g and 84.94g respectively. The Average fruit weight (FW) at 4, 8 and 12 weeks composting interval was 4.77 g, 5.91 g and 5.66 g respectively. The width of pileus at 4, 8 and 12 weeks composting interval was 5.37cm, 4.60cm and 6.07cm respectively.

Table 3: Mean effect of substrates variety interaction on the growth parameters of *Pleurotus ostreatus*

Substrates	Growth parameters of *Pleurotus ostreatus*			
	Mycelia Extension (cm)	**Full Mycelia Colonization (days)**	**Primordial Initiation (days)**	**Extension per Day (cm)**
Mixed bed	8.25	36	24	0.68
Rice bran	0.02	0	0	0.03
Sawdust	0.03	0	0	0.04

Table 4: Mean effects of substrates variety and weeks of composting intervals (WCI) interaction on the yield parameters of *Pleurotus ostreatus*

Substrate	Yield parameters of *P. ostreatus* and Weeks of composting intervals (WCI)											
	Number of fruits			**Fruit weight (g)**			**Average fruit weight (g)**			**Width of pileus (cm)**		
	4	**8**	**12**	**4**	**8**	**12**	**4**	**8**	**12**	**4**	**8**	**12**
Mixed bed	6	11	15	28.59	65.02	84.94	4.77	5.91	5.66	5.3.7	4.60	6.07
Rice bran	0	0	0	0.01	0.01	0.03	0.0 4	0.02	0.02	0.02	0.03	0.03
Sawdust	0	0	0	0.01	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.03

Table 5 shows the mean effect of weeks of composting intervals (WCI) on the length of stipe, biological efficiency, production efficiency and mycelia extension of *Pleurotus ostreatus*. For mixed bed, the longest length of stipe at 4, 8 and 12 weeks of composting interval was 4.33 cm, 4.70cm and 4.33cm respectively. The Biological Efficiency (BE) obtained at 4, 8 and 12 weeks of composting interval was 14.30%, 30.96% and 42.47% respectively. The production efficiency obtained at 4,8 and 12 weeks composting interval was 16.68%, 35.47% and 45.30% respectively. The longest mycelial extension recorded at 4, 8 and 12 weeks composting interval was 9.6cm, 6.1cm and 9.0 cm respectively. No growth was recorded for rice bran and saw dust.

Table 6 show the mean effects composting intervals (WCI) interaction on the number of days for full mycelia colonization, days to mushroom primordial initiation and extension per day of *Pleurotus ostreatus*. For the mixed bed substrate, the longest number of days recorded for full mycelial colonization at 4, 8 and 12 weeks composting interval was 51.00 days, 36.00 days and 27.00 days. The longest number of days for primordial initiation after 4,8 and 12 weeks composting interval 28.00 days, 26.00 days and 20.00 days respectively. The longest mycelia extension per day at recorded at 4, 8 and 12 weeks composting interval was 0.55 cm/day 0.72 cm/day and 0.76 cm/day respectively. No growth was observed for the rice bran and saw dust.

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Table 5: Mean effects of substrates variety weeks of composting intervals (WCI) interaction on the growth parameters of *Pleurotus ostreatus*

Substrate	Growth parameter of *Pleurotus ostreatus* and WCI (Weeks)											
	Length of stipe (cm)			Biological Efficiency (%)			Production Efficiency (%)			Mycelia Extension (cm)		
	4	8	12	4	8	12	4	8	12	4	8	12
Mixed bed	4.33	4.70	4.33	14.30	31.96	42.47	16.68	35.47	45.30	9.60	6.10	9.00
Rice bran	0.03	0.01	0.02	0.04	0.03	0.03	0.03	0.02	0.04	0.03	0.02	0.02
Sawdust	0.04	0.02	0.02	0.03	0.02	0.02	0.03	0.03	0.02	0.04	0.01	0.02

Table 6: Mean effects of substrates variety weeks of composting interval (WCI) interaction on the growth parametersof *Pleurotus ostreatus*

Substrate	Growth parameters of *Pleurotus ostreatus* and WCI (Weeks)								
	Full Mycelia Colonization (days)			Primordial Initiation (days)			Extension per Day (cm)		
	4	8	12	4	8	12	4	8	12
Mixed bed	51.00	36.00	27.00	28.00	26.00	20.00	0.55	0.72	0.76
Rice bran	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.03
Sawdust	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.04

Discussion

Pleurotus species are widely cultivated due to their ability to utilize various lignocellulosic substances and rapid growth (Sher *et al.*, 2011). They have been grown on different agricultural and industrial wastes, including cotton wastes, sawdust, cereal stover, corncob, wheat, paddy straw, and sugarcane bagasse (Ragunathan and Swaminathan, 2003) Singh *et al.*, 2020). These works on *pleurotus* spp agrees with the finds of this research. Some strains can be adapted for cultivation on conifer sawdust-based substrates (Zhang *et a*l., 2020). *Pleurotus* spp. can also be cultivated on wood waste or unused wood residues, enhancing economic returns and supporting ecosystem management. Different substrates significantly affect mushroom yield, with varied yield amounts obtained from different substrate media (Kumar *et al.*, 2023). Using varied substrate media causes different yield amounts due to biological and chemical differences between substrates and the genotype of the cultured mushroom (Sharma *et al*., 2019).

Mixed bed substrates have been consistently reported to be the best substrate supporting mycelia growth and fruitification (Singh *et al*., 2020).

The yield of mushrooms is directly related to the spread of mycelium into the substrate (Sharma *et al*., 2019). Recent studies have highlighted the potential of *Pleurotus* species for sustainable mushroom production, including the use of lignocellulosic wastes as substrates (Sher *et al*., 2011; Kumar *et al*., 2020) and the importance of substrate composition and decomposition time on mushroom yield (Zhang *et a*l., 2020).

The biological and production efficiencies of mixed bed substrates have been reported to be highest, resulting from heavy mycelia ramification (Singh *et a*l., 2020). The use of mixed bed substrates has been shown to result in higher yields and better mycelia growth (Sharma *et a*l., 2019). The ability of *Pleurotus* species to colonize and produce mushrooms on various substrates makes them a promising option for sustainable mushroom production.

In conclusion, the mixed bed of sawdust with rice-brand is the most suitable substrate for the production of *Pleurotus ostreatus* fruiting bodies, outperforming other substrates in terms of fruit number and weight, pileus width, stipe length, and mycelia extension, resulting in higher yields and efficiencies.

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Mushroom cultivation offers numerous benefits, including a pollution-free environment, land-saving, and a short duration crop that can be embraced by small-scale farmers. Mushrooms complement protein availability and reduce malnutrition. The ability to cultivate mushrooms on various substrates makes them a promising option for sustainable production and food security.

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