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Oyster Mushroom [*Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm.] Cultivation using Agricultural Wastes

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Abstract

A study was conducted to investigate the nutritional profile and growth performance of mushrooms cultivated on different substrates. Substrates consisted of agricultural wastes (fresh and composted sawdust, paper and fluted pumpkin stems) and their combinations. In this study, number of fruiting bodies produced, stipe length and girth, pileus diameter, and fresh and dry weights of mushrooms were recorded. The results showed that there was a significant difference (P<0.05) in the number of days taken for each substrate to obtain a complete mycelia run with fresh sawdust substrate (FS) having the highest (43), and mixture of composted sawdust, paper and fluted pumpkin stem (CSPPuS) having the least (30). Accordingly, there was a significant difference (P<0.05) in the number of fruiting bodies harvested from each substrate (mushroom yield) with CSPPuS having the highest fresh (32.99±0.15 g) and dry weight (5.82±0.68 g) of mushroom and also the highest number of fruiting bodies (36). There was also a significant difference (P<0.05) in the nutritional content of fruiting bodies among substrates. The highest protein content was obtained from CSPPuS (28.88±0.41 %). In conclusion, fluted pumpkin stem and paper can serve as good substrates for Pleurotus ostreatus cultivation.

Keywords: Agro-waste; Oyster mushroom; *Pleurotus ostreatus*; Substrates; Yield

Introduction

Pleurotus ostreatus (Jacq. ex Fr.) P. Kumm. is a cheap, nutritious and edible mushroom that can be added to diets in both rural and urban areas in order to reduce deficiencies of micronutrient in developing countries (Effiong *et al*., 2024). Oyster mushrooms are widely cultivated, contributing roughly 27 percent of the total global production (Aditya *et al*., 2024). Due to their remarkable nutritional composition, speedy growth and development, and versatility on different substrates, oyster mushrooms are profitable for both commercial and small-scale farmers (Nandeha, 2024). They can grow on a variety of substrates such as sawdust, cotton seed hulls, oil palm fibre wastes, leafy vegetable stems, maize cobs and husks, cassava peel, cocoa pods, cereal straw and husk, rice straw, sugarcane wastes etc. Oyster mushrooms are also important because of their great yield potential and quick growth, they provide a high return on investment. Additionally, the possibility for year-round production and the comparatively inexpensive initial capital commitment make oyster mushroom farming a desirable business venture for many (Royse *et al*., 2017). Oyster mushrooms are cherished not only because of their flavor and meat-like texture but also because they have a lot of health benefits. They are a beneficial component of an atherosclerotic diet because of their high fiber and low fat content. They also give nutritional value through proteins, carbs, fatty acids, vitamins, and minerals (Sałata *et al*., 2018). They possess therapeutic properties due to the presence of important bio-active compounds in them (Paul *et al*., 2017). They are considered a good source of several anti-microbial drugs as they have been recorded to exhibit significant anti-bacterial effect against Gram-positive and Gram-negative bacteria (Pauliuc and Botău, 2013; Bawadekji *et al*., 2017).

Pleurotus ostreatus (Jacq. ex Fr.) P. Kumm. is the second most widely grown edible mushroom globally after *Agaricus bisporus* due to its medicinal and economic values (Sánchez, 2010). Its straightforward and low-tech cultivation method is one of the main reasons growers choose to cultivate it. It is a mushroom species that is perfect for novice growers or those in resource-poor areas because sterile environment or conditions and expensive equipment are not required for its cultivation, unlike many other mushroom species (Chavan *et al*., 2022). In the cultivation of *Pleurotus ostreatus*, the ideal initial water content of a substrate is between 65 to 75 %. Substrates with water content of 75 % exhibit high enzymatic activity (Wiesnerová *et al*., 2023). The ideal temperature for mycelia development of *P*. *ostreatus* is 22 °C. This gives a growth rate of 0.56 cm/d and a biological efficiency of 99.53 % (Hu *et al*., 2023).

Oyster mushrooms can be employed in the bio-remediation of contaminated soil and water as well as the bio-fermentation of agro-industrial wastes (El-Ramady *et al*., 2022). *Pleurotus ostreatus* aids in environmentally friendly resource development and cleanup by decomposing pollutants and oil (Sharma, 2022). Agricultural wastes can be reduced and valorized by using them to grow mushrooms thereby turning low-value by-products into new material for products with additional value (Kumla *et al*., 2020). By using wastes for mushroom production, food waste can be sustainably managed, high-quality food can be produced from low-quality waste, and environmental problems can be resolved (Doroški *et al*., 2022). Mushroom cultivation using agricultural wastes can help farmers achieve sustainable agricultural production and increased yield (Grimm and Wosten, 2018). Organic waste such as palm chaff, oil palm empty bunch, yam peel and saw dust have been used in the cultivation of *Pleurotus ostreatus* in Nigeria, and the results revealed significant variations in various nutritional components among the substrates (Gbenga *et al*., 2024). The average yearly revenue of small-scale mushroom producers in North Central Nigeria is N426,092.55 (Okwuokenenye *et al*., 2024) with an average income increase of about N65,552.97 (Ayodele *et al*., 2024). Socio-demographic characteristics have a positive impact on oyster mushroom industry. Oyster mushroom cultivation is growing worldwide, and small-scale farmers in Kenya are being taught basic methods of cultivation and how to preserve them after harvest by drying them to add value (Sigot and Muliro, 2021). China produced 86% of the world's oyster mushrooms by weight in 2016, and by 2021, the production rose to about 90%, mainly due to increased output in Shandong province (Botta *et al*., 2020; Li *et al*., 2024). The aim of this study is to determine the growth and yield of *Pleurotus ostreatus* on some agricultural wastes, some of which have been used in recent studies but in different combinations and quantities.

Materials and Methods

Study Area

The study was conducted at the mushroom unit in University of Port Harcourt Demonstration farm, Choba, Rivers State, Nigeria, during August to November 2023.

Collection of Materials

Pleurotus ostreatus fruiting body was obtained from University of Port Harcourt Demonstration farm. Sawdust was obtained from a sawmill in Rumosi, Rivers State. Old newspapers were obtained from a nearby vendor around Choba junction, Rivers State, Nigeria, while fluted pumpkin stems were obtained from traders in Choba market, Rivers State, Nigeria.

Other materials used for the cultivation of mushroom include: wheat bran, lime (calcium carbonate), water, spawn (mushroom seed), weighing scale, transparent polyethene bag, ethanol (disinfectant), cotton wool, plastic corks, rubber bands, and masking tape.

Methods

The experiment was laid in complete randomized design with four treatments. Each treatment had five replicates. The study was carried out in five different phases required for the cultivation of *Pleurotus ostreatus*. They include: composting and bagging, sterilization, inoculation, incubation, cropping and harvesting (Kumera *et al*., 2021).

Preparation of Substrates

The pumpkin stems were cut into tiny pieces and sun-dried for 7 days while the newspapers were shredded into tiny pieces and soaked in water for 30 minutes. These materials were weighed and mixed to form the substrates. One thousand grams of fresh sawdust was mixed with 20 g of wheat bran, 5 g of calcium

carbonate (lime) and 1.5 litres of water to obtain the fresh sawdust (FS) substrate. Shredded newspaper (500 g), fresh sawdust (500 g), wheat bran (20 g), lime (5 g) and water were thoroughly mixed to obtain the fresh sawdust and paper (FSP) substrate. Composted sawdust was prepared by covering fresh sawdust with a jute bag in order to allow the sawdust to decompose. The sawdust was regularly turned every two days to produce homogenous compost. The sawdust was allowed to decompose for two weeks. The composted sawdust and pumpkin stem substrate (CSPuS) consisted of composted sawdust (500 g), pumpkin stem (500 g), wheat bran (20 g), lime (5 g) and water. Composted sawdust (400 g), pumpkin stems (300 g), shredded newspapers (300 g) wheat bran (20 g), lime (5 g) and water formed the composted sawdust, paper and pumpkin stem substrate (CSPPuS).
Sterilized spoke was used to disassemble the spawn which were introduced into each of the substrate bags before they were corked. The inoculated bags were labeled and transferred to the incubation room. The inoculated bags were kept in the incubation room for a period of one month for the mycelium to colonize the substrates. The bags were checked regularly for mycelia growth and contamination.

Harvesting of Mushroom

Fully mycelia colonized bags were transferred to the cropping house and opened to aid watering and to provide enough surface area for the growth of the mushrooms. The bags were watered at least twice a day. The matured mushrooms were harvested by holding and twisting the stalk in an anti-clockwise direction. Harvesting was done in the morning and substrates attached to the mushroom stalk were cut off with a knife. The fresh mushrooms from each substrate were weighed in the laboratory using an analytical balance. The mushrooms were then placed in the oven to dry for 3 hrs at 60°C.

Data Collection and Analysis

The number of days taken for mycelia to fully colonize each substrate bag was recorded. Mushroom pileus diameter was obtained by measuring the distance from one end of the mushroom cap to the other going through the center of the cap. This was measured in centimeters with a meter rule. The average cap was calculated for a given harvest per treatment. The mushroom stipe length was measured with a meter rule in centimeters from the point where the gills of the cap started on the stalk. Mushroom stipe girth was measured using a rope. The rope was then measured using a meter rule to determine the girth. Fresh weight of mushroom was determined by weighing harvested mushroom on a weight scale measured in grams. The mushrooms were then dried and weighed again to obtain the dry weight.

After harvesting, the fruiting bodies were analyzed for total protein, fibre and moisture content, according to the procedures described by Association of Official Analysis Chemists (AOAC, 1984).

Statistical Analysis

Data obtained were analyzed using analysis of variance (ANOVA) procedure of SAS statistical software package (2002). Least significant difference (LSD) test was used to determine the significant difference between treatments means.

Results and Discussion

Mycelia Colonization and Mushroom Harvest

The results from this study shows that mushroom grew on all the substrates (CSPPuS, FSP and FS), apart from CSPuS which showed no growth. This may be as a result of contamination by microorganisms, probably due to partial breakdown of cellulose and hemicelluloses, inadequate temperature during sterilization and determination of the appropriate proportions of the constituents. The time taken for mycelia initiation was between 2 to 3 days as presented in Table 1. Mycelia initiation to full mycelia colonization varied from 25 to 43 days. This is in line with the findings of Joie and Ambos (2022) and Nwachukwu and Adedokun (2014). The time taken from mycelia initiation to optimal fruiting of the different substrate ranged from 40 to 56 days.

Table 1: Growth performance of *Pleurotus ostreatus* on different substrates

Substrate	Mycelia initiation (day)	Full mycelia growth (day)	Optimal fruiting (day)	No of fruiting bodies
CSPPuS	2	30	40	36
FSP	3	25	35	5
FS	2	43	56	7
CSPuS	-	-	-	-

Mean with the same value are not significantly different, while different values are significantly different at P≤0.05. Mean ± standard deviation.

Key:

CSPPuS - Composted sawdust, paper and pumpkin stem
FSP - Fresh Sawdust and paper
FS - Fresh sawdust
CSPuS - Composted sawdust and pumpkin stem

Stipe and Pileus Performance of *Pleurotus ostreatus* on Different Substrates

The stipe length, stipe girth and pileus diameter of harvested mushrooms are presented in Table 2. There was a significant difference (P≤0.05) in the mean stipe length among all the samples. There was no significant difference between the stipe girth of CSPPuS and that of FSP but there was a significant difference between that of CSPPuS and FS. There was no significant difference between the pileus diameter of CSPPuS and FSP whereas there was a significant difference between CSPPuS and FS.

Table 2: Stipe and pileus performance of *Pleurotus ostreatus* on different substrates

Substrate	Stipe length (cm)	Stipe girth (cm)	Pileus diameter (cm)
CSPPuS	3.71±0.90	2.44±0.41	3.22±1.09
FSP	2.62±0.92	2.46±1.72	2.88±0.89
FS	1.37±0.32	1.73±0.65	4.19±1.70
CSPuS	-	-	-

Mean with the same value are not significantly different, while different values are significantly different at P≤0.05. Mean ± standard deviation.

Weight of Fruiting Bodies on Different Substrates

The fresh and dry weights of fruiting bodies are presented in Table 3. There was a significant difference (P≤0.05) in the fresh weight and dry weight of mushrooms harvested from the different substrates. The use of the mixture of composted sawdust, paper and fluted pumpkin stem as substrate for mushroom cultivation in this study revealed that fluted pumpkin stem can serve as a good source of nutrient for mushroom cultivation. Fruiting was more and fastest on this substrate. This is probably due to the high fiber content in the pumpkin stem. The fruiting bodies also recorded a higher weight compared to the other substrates. This may be due to the nutritional content of fluted pumpkin stem. Fluted pumpkin has higher physicochemical qualities in the pod husk and high levels of cellulose (49 %) and hemicellulose (26 %) (Nwajiobi *et al*., 2019). The stem and pod of fluted pumpkin can be used as an additive in oyster mushroom cultivation, to add nutrients such as fiber, minerals and carbohydrates (Ofomana and Adedokun, 2020). Fluted pumpkin leaves are rich in carbohydrate (70 %) and protein (29 %), and also contains 1.7 % fibre and 1.8 % fat (Akpasi *et al*, 2023). The use of pumpkin stem for mushroom cultivation should be encouraged in view of the observations made in this study, especially considering its abundance and availability.

Table 3: Fresh and dry weights of fruiting bodies on different substrates.

Substrate	Fresh weight (g)	Dry weight (g)
CSPPuS	32.99±0.15	5.82±0.68
FSP	20.18±0.18	2.79±0.80
FS	15.67±0.16	1.11±0.20
CSPuS	-	-

Mean with the same value are not significantly different, while different values are significantly different at P≤0.05. Mean ± standard deviation.

Proximate Analysis of Fruiting Bodies Obtained from Different Substrates

The proximate composition of mature fruiting bodies of *Pleurotus ostreatus* grown on different agricultural substrates varied significantly. The percentage protein, crude fiber and moisture contents of fruiting bodies harvested from the various substrates are presented in Table 4. The result showed that the different substrates produced *P. ostreatus* with high protein content. There was a significant difference (P≤0.05) between the protein content of CSPPuS and FSP. There was a significant difference between the crude fiber content of CSPPuS and FSP. Moisture is required for the normal biochemical activities in living things. There was a significant difference between the moisture content of CSPPuS and FS. In a study by Gbenga *et al.* (2024) using agricultural wastes (oil palm empty bunch, palm chaff, saw dust and yam peel) for the enhancement of *P. ostreatus* cultivation, oil palm empty bunch had the highest crude protein (2.56 %) and crude fiber (48.27 %) contents while yam peel had the highest moisture content (8.46 %). Composted sawdust substrate has been reported to produce king tuber mushroom with the highest protein (10.06 %) and moisture (57.78 %) contents while composted sawdust and paper yielded the highest crude fibre content (38.02 %) (Nwachukwu and Adedokun, 2014). Sawdust supplemented with fluted pumpkin stem produced the highest carbohydrates (28.06 %) in *Pleurotus floridanus* (Ofomana and Adedokun, 2020). The growth of *Pleurotus ostreatus* fruiting bodies on different substrates is presented in Figure 1.

Table 4: Proximate analysis of mushroom fruiting bodies

Substrate	Protein (%)	Crude fibre (%)	Moisture (%)
CSPPuS	28.88±0.41	14.75±0.77	72.21±0.40
FSP	19.25±0.85	47.16±0.79	80.83±0.12
FS	21.00±0.25	44.86±0.64	89.87±0.10
CSPuS	-	-	-

Mean with the same value are not significantly different, while different values are significantly different at P≤0.05. Mean ± standard deviation.

Figure 1: *Pleurotus ostreatus* growing on different substrates. a: fresh sawdust (FS); b: fresh sawdust and paper (FSP); c: composted sawdust, paper and pumpkin stem (CSPPuS); d: composted sawdust and pumpkin stem (CSPuS)

This study has demonstrated that locally available organic substrates are suitable for the cultivation of mushrooms. The ability of mushroom mycelia to degrade organic substrates can also be utilized in the management of organic wastes in the environment, which are left to decompose therefore, causing health hazards. Mushroom cultivation using organic wastes is one of the methods that can help in recycling organic wastes into profitable products. *Pleurotus ostreatus* is a powerful organism that can biodegrade and detoxify a variety of wastes and contaminants. It effectively breaks down agricultural wastes such as sawdust, sugarcane bagasse, and maize stalk (Amadioha, 2021). Adedokun (2014) reported the bio-conversion of residues of lignin and cellulose origin through mushroom cultivation. *P. ostreatus* has also been reported to be easily cultivated on agricultural wastes (Kumera *et al*., 2021; Doroški *et al*., 2022; Jimoh *et al*., 2023).The use of sawdust for mushroom cultivation is a common practice in Nigeria. Few researchers have also reported the use of waste paper as substrate for mushroom cultivation both as bulk substrate and supplementation for soil (Tesfay *et al*., 2020; Mihai *et al*., 2022; Saha *et al*., 2023).

Cultivation of oyster mushrooms is an environmentally friendly and financially sustainable venture that produces delicious, nutritious, and high-quality fruiting bodies (Pavlik et al., 2023).

Conclusion and Recommendation

This study has shown that agricultural wastes serve as good substrates for mushroom cultivation. Oyster mushroom cultivation on agricultural wastes has shown to be a highly efficient method of managing agricultural wastes and waste papers as well as producing protein-rich food. The substrate composed of composted sawdust, paper and pumpkin stem gave the best result. This substrate is good, affordable and easily available. In Nigeria, the agricultural wastes that are most commonly used as substrates by mushroom farmers are wheat bran, rice bran, sawdust, corn hubs and corn husks. These substrates are seasonal and may not be available to farmers all year round, unlike the ones used in the present study. Waste papers are found in almost every vicinity and fluted pumpkin is cultivated all year round. More investigation should be carried out on the growth of oyster mushroom using the mixture of composted sawdust and pumpkin stem as a substrate in order to determine why there was no growth with this substrate in this work even after the experiment was severally repeated. This future study can involve varying the time of composting and determining appropriate proportions of the constituents. Mushroom farmers and the general public should be educated on the use of organic wastes such as fluted pumpkin stem and paper for mushroom cultivation as this will help to create a more robust and flexible mushroom farming system by reducing reliance on more expensive or difficult-to-access substrates. It is evident that many organic substrates have high potential for utilization as substrates in mushroom cultivation. Mushroom farmers should be advised to use these substrates on commercial scale in order to improve productivity.

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