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EFFECT OF ORGANIC SUBSTRATES ON THE PROPAGATION OF *Pleurotus ostreatus* (Oyster Mushroom) IN OBUBRA, CROSS RIVER STATE, NIGERIA

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ABSTRACT

The production of indigenous mushroom in a commercial scale is constrained by lack of information on its growth requirement, threatened natural habitat resulting from deforestation and rising temperature. Rice offal, decaying mango wood, sawdust and decaying palm bunch were used as substrates. Dry substrates were heated, soaked and pasteurized by boiling in water, and inoculated into bags containing pasteurized substrates. Highest (0.721 days) mycelium running rate was found in mango wood ($P<0.05$) but the lowest (0.507 days) in control ($P<0.05$). Completion of mycelium running time was lowest in rice offal (T1). Number of total primordia and effective primordia, was highest (51.34cm) in sawdust (T2) but the maximum (7.70 cm) pileus diameter was measured from sawdust (T2). Highest (205.22 %) biological efficiency was obtained from rice straw (T1) which was much higher than the control (T4). Utilizing local waste for mushroom cultivation reduces cost and encourages indigenous participation. Sawdust and mango wood should be used by rural farmers for Oyster mushroom propagation.

Keywords: Rice straw, rice offal, oyster mushroom, substrate, mycelium.

INTRODUCTION

Mushrooms are edible fungi belonging to the genus *Pleurotus* under the class Basidiomycetes. Oyster mushroom (*Pleurotus spp*) is an edible mushroom having excellent flavor and taste. Mushroom substrate is a lignocellulose material which supports the growth, development and fruiting of mushroom (Chang and Miles, 2004). The quality of waste products like rice offal, saw dust, decaying mango wood and palm bunch is improved due to the degradation of cellulose and hemicellulose by mushroom enzymes and hence has been seen to be more digestible. Thus rice straw is transformed to valuable roughage and mushrooms provide an additional income without an extension of the limited hectarage. (Murthy and Manonmani, 2008). Rice straw yielded about 10% more mushroom than wheat straw propagated under the same cultivation conditions (Ukoima *et al.*, 2002). Oyster mushroom may be grown on a wide range of plant waste as substrate such as sawdust, paddy straw, palm bunch, corn stalk, corn cobs, waste cotton, leaves and pseudo stem of banana, water hyacinth, duck weed and rice straw. This does not require expensive processing methods and enrichment materials.

Mushrooms are the fruiting bodies of macro-fungi. They include both edible, medicinal and poisonous species. However, the word "mushroom" was used for the edible members of macro-fungi. Mushrooms may be saprophytic, parasitic and mycorrhizal in their mode of living. Most of the cultivated mushrooms are saprophytic; they feed on organic matter which has already been manufactured by plants or animals. In nature they grow on fallen leaves, animal droppings and stumps of dead wood (Werma and Beelman, 2002).

Mushroom belongs to the kingdom *Myceteae* and order *Basidomycota* due to unique fungal characteristics (Liu *et al*., 1995). The protein value of dried mushroom has been found to be 30-40% containing all the essential amino acids. Mushrooms supply more protein per unit area than most agricultural crops like legumes (Gupta, 1986), reduce serum cholesterol and high blood pressure (Mori *et al.,* 1986). Mushrooms are renowned for their medicinal properties (Noble, 2005) and often is associated with longevity, wisdom, and happiness (Stamets, 1990).

In Nigeria, huge amounts of agricultural wastes are produced annually, and are not put to use. These wastes could be used as a source of food, that is substrate for mushroom cultivation.

Oyster mushroom is one of the leading mushrooms in terms of both customer preference and production worldwide because its propagation method is simple and at a low cost (Mswaka and Tagwira, 1997; Baysal *et al.,* 2003). Many organic substrates have high potential for utilization as substrate in mushroom cultivation (Kimenju *et al.,* 2009; Onyango *et al.,* 2011).

Oyster mushroom is known for its ability to degrade lignocellulosic residues from agricultural fields and forests, converting them into protein-rich biomass (Rowell *et al.,* 2000). Oyster mushroom grows on a wider array of forest, industrial and agricultural wastes than species from any other group (Zadrazil and Brunnert, 1981; Jadhav and Bagal, 1998; Baysal *et al.,* 2003). In Nigeria, the main substrates used for *P. ostreatus* cultivation are decaying palm bunch (Kashangura *et al.,* 2005). However, Zireva *et al.,* (2007) highlighted the need to test performance of oyster mushroom under different substrates. Furthermore, the hectarage under decaying palm bunch and rice straw is low as compared to sawdust (FAO/WFP, 2010).

This study therefore, set out to assess the productivity potentials of some agricultural and industrial waste materials for mushroom production in the study area.

MATERIALS AND METHODS

The research was carried out in the Museum and Herbarium Unit of the Department of Forestry and Wildlife Management, Faculty of Agriculture and Forestry, Cross River University of Technology, Obubra Campus. The area lies between latitude 6^0 05^I and 8^0 20^I N and longitude 6^0 08^I and 8^0 33^I E (Liu *et al.,* 1996). The wet season starts from April to October while the dry season spans from November to March. Temperature is fairly uniform with mean monthly average of 27^0C. The annual rainfall is between 2000mm – 2250mm, while the relative humidity varies from 60% - 70% in January and 70% - 80% in July.

Experimental Layout and Procedure

The fungus sclerotia of *Pleurotus ostreatus* was purchased from a local market in Idomi, Yakurr Local Government Area, Cross River State, Nigeria. The treatments were rice offal (T_1), sawdust (T_2), Mango wood (T_3) and Palm bunch (T_4) as control, since palm bunch is mostly used for the cultivation of *P. ostreatus* in the study area.

The design for the experiment was Completely Randomized Design (CRD), with four treatments. Polythene bags with width of 25cm and depth 18cm were assigned to the four treatments of rice offal, sawdust, mango wood and palm bunch. Thereafter they were arranged on the ground. A total of 48 polythene bags were used for the experiment. Four bags to each treatment and replicated thrice.

Dry substrates each measuring 500g was put differently in a metal container filled with water so as to immerse the substrate. The substrates were soaked overnight so that they will achieve a moisture content of about 70 – 80% (Ha *et al*., 2015). They were then pasteurized by boiling in water for 1 hour using firewood. The pasteurized substrates was collected and allowed to cool to a temperature of about 25 – 37^0C.

Inoculation

The fungus sclerotia was soaked in water over night to allow for maximum absorption of water, thereafter it was inoculated into the bags filled with the pasteurized substrates (WenJie *et a*l., 2013). This was followed by periodic watering of the bags with clean water to ensure that the environment was humid.

Data Collection
The yield of *P. ostreatus* on the different substrate supplementation was determined by recording the number, weight and sizes of the fruiting bodies after sprouting. The measurements from the various replicates were added and the average values determined. The following parameters of growth / yield were measured

Diameter of the Pileus
The diameter of the pileus was measured in centimeters with measuring tape from one edge of the pileus across the stipe to the other.

Diameter of Stalk Height
The diameter of stalk height was measured in centimeter with a measuring tape from the base to the edge.

Fresh Weight
Fresh weight was determined using a sensitive weighing scale. Biological Efficiency (BE) was calculated thus;

$$\boldsymbol{BE} = Mushroom\ Fresh\ Weight\ / \ Substrate\ Dry\ Weight\ X\ 100\%$$

Data Analysis
The collected data was subjected to analysis of variance (ANOVA) using SPSS 20.0 (2004 edition) and their means were separated by Fisher's Least Significant Differences (P<0.05).

RESULTS
Mycelium running rate: Highest mycelium running rate was observed in mango wood and similar to rice offal, while the lowest was recorded on control as presented in table 1.

Table 1: Effect of Different Substrates on Mycelial Growth of *Pleurotus ostreatus* (Oyster Mushroom)

TREATMENTS	Colonization Period (days)	First Harvest (days)
RICE OFFALS (T1)	0.69^{a}	21.33^{a}
SAWDUST (T2)	0.51^{b}	22.00^{a}
MANGO WOOD (T3)	0.72^{a}	21.33^{a}
PALM BUNCH (T4)	0.50^{b}	23.00^{a}

Values with different superscript letters along the same column are significantly different (p < 0.05).

Time required for primordia initiation and harvest
Twenty-one to twenty-three days were required to complete mycelium running in spawn packet on different substrates. Twenty-three days was required to complete mycelium running on the control, but was however, lowest in mango and rice straw as presented in table 2.

Number of primordia and effective fruiting bodies
The time for primordia initiation recorded on control was (6.67). While (8.33 days) for primordia initiation was found in mango wood (table 2). The minimum number of total primordial was recorded on sawdust, different from that for mango wood and palm bunch, while the minimum number of effective fruiting body was recorded on palm bunch (Table 3).

Table 2: Effect of Different Substrates on Time required For Primordia Initiation and Harvest in *Pleurotus ostreatus*

TREATMENTS	Time Required for Primordia Initiation (days)	Time Required for Harvesting (days)
RICE OFFALS (T1)	6.67^{a}	7.00^{a}
SAWDUST (T2)	7.67^{ab}	9.67^{b}
MANGO WOOD (T3)	8.33^{a}	11.00^{a}
PALM BUNCH (T4)	6.67^{b}	10.67^{a}

Values with different superscript letters along the same column are significantly different (p < 0.05).

Table 3: Effect of type of substrate on the number of Primordia and Effective Fruiting body in *Pleurotus ostreatus*

TREATMENT	No. of Total Primordia			No. of Effective Fruiting Body		
	1st flush	2nd flush	3rd flush	1st flush	2nd flush	3rd flush
RICE STRAW(T1)	40.13 ^{b}	40.40 ^{b}	38.71 ^{a}	22.35 ^{b}	32.05 ^{a}	20.51 ^{a}
SAWDUST (T2)	29.39 ^{d}	51.34 ^{a}	32.50 ^{b}	35.03 ^{a}	20.33 ^{b}	17.00 ^{b}
MANGO WOOD (T3)	44.78 ^{a}	35.64 ^{c}	18.98 ^{d}	24.53 ^{b}	19.75 ^{b}	10.12 ^{c}
PALM BUNCH (T4)	35.77 ^{c}	35.98 ^{c}	26.21 ^{c}	10.58 ^{c}	19.00 ^{b}	9.50 ^{c}

Values with different superscript letters along the same column are significantly different (p < 0.05).

Oyster mushroom yield attributes from different substrates

Diameter of pileus was different for all substrates in all the flushes, recording (7.78 cm) on sawdust, (4.13 cm) on palm bunch, and (4.20) in the 1st flush. The highest stalk length (3.58 cm) was found in sawdust of first flush and the lowest (1.66 cm) was found in rice offal of third flush as presented in table 4.

Table 4 Effect of different substrates on some yield attributes of *Pleurotus ostreatus* (oyster mushroom)

TREATMENT	Pileus Diameter (cm)			Stalk Length (cm)		
	1st flush	2nd flush	3rd flush	1st flush	2nd flush	3rd flush
RICE OFFALS(T1)	4.62 ^{b}	4.07 ^{b}	4.20 ^{a}	3.24 ^{a}	2.83 ^{a}	1.66 ^{b}
SAWDUST (T2)	7.78 ^{a}	4.52 ^{a}	3.11 ^{b}	3.58 ^{a}	3.30 ^{a}	3.03 ^{a}
MANGO WOOD (T3)	4.53 ^{b}	4.20 ^{ab}	4.00 ^{a}	3.54 ^{a}	3.28 ^{a}	3.11 ^{a}
PALM BUNCH (T4)	4.13 ^{b}	3.97 ^{b}	3.28 ^{b}	2.69 ^{a}	3.00 ^{a}	2.63 ^{a}

Values with different superscript letters along the same column are significantly different (p < 0.05).

Fresh and dry weight of Oyster mushroom after harvest

For all flushes, maximum and minimum weights was recorded on mango wood, sawdust and palm bunch were between (6.40g – 2.14g) as presented in table 5.

Table 5: Effect of different substrate on weight of individual fruiting body and biological efficiency of *Pleurotus ostreatus*

TREATMENT	Weight (g)		
	1st flush	2nd flush	3rd flush
RICE OFFALS (T1)	6.21 ^{b}	4.31 ^{b}	3.32 ^{b}
SAWDUST (T2)	3.33 ^{a}	2.14 ^{d}	2.75 ^{c}
MANGO WOOD (T3)	6.40 ^{a}	3.88 ^{c}	4.16 ^{a}
PALM BUNCH (T4)	5.34 ^{c}	4.49 ^{a}	3.36 ^{b}

Values with different superscript letters along the same column are significantly different (p < 0.05).

Biological Efficiency

Maximum biological efficiency (205.22 %) was obtained from rice offal, while minimum biological efficiency (181.65 %) was obtained from palm bunch. However, rice offal yielded better than the control as presented in table 6.

Table 6: Total fresh weight, dry weight of substrats (g) and biological efficiency (%) of *Pleurotus ostreatus* mushrooms at different harvest periods.

Treatments	Total fresh weight in 3 harvests	Dry weight of substrate after all harvest	Biological efficiency (%)
Rice offals	123.79^{ab}	60.32^{b}	205.22^{a}
Sawdust	73.43^{c}	60.00^{b}	122.38^{c}
Mango wood	130.08^{a}	65.02^{a}	200.06^{a}
Palm bunch	118.22^{b}	65.08^{a}	181.65^{b}

Values with different superscript letters along the same column are significantly different ($p < 0.05$).

DISCUSSION

Mycelium running rate

Mycelium running rate (MRR) in spawn packs ranged from 0.50 to 0.72 cm/day. The highest mycelium running rate was observed in mango wood which was similar to rice offal, the lowest mycelium running rate was recorded on control which was similar with sawdust. The presence of the right proportion of alpha-cellulose, hemicelluloses, lignin and the degree of decay in the wood was the probable cause of higher rate of mycelium running in mango wood and rice offal. The substrate, palm bunch having the lowest mycelium running rate might be due to the presence of different kinds of polyphenolic substances in them (Wang, 1997) and low content of cellulose (Gohl, 1993). Suitable Carbon to Nitrogen ratio might be responsible for the higher mycelial growth in mango wood and rice offal (Jolanta *et al*., 2015), who supported the results and found that the optimum carbon /nitrogen ratio for mycelial growth of *P. ostratus* ranged from 40:1 to 90:1.

Time required for completion of mycelium running

The number of days required to complete mycelium running in spawn packet ranged from 21.33 days to 23.00 days on different substrates. The number of days needed to complete mycelium running was significantly lowest for mango and rice straw compared to other treatments. The highest number of days (23) was required to complete mycelium running on the control, which was not significantly different from the number of days observed for other substrates. The appreciable days to complete mycelium running of oyster mushroom on different substrates might be due to variation in their chemical composition and Carbon and Nitrogen ratio (Liu *et al*., 1996). The results recorded on all substrates were almost similar to the findings of Ukoima *et al*., (2009) that the spawn running took 16-25 days after inoculation, and confirmed by Tan (1981).

Time required for primordia initiation

The lowest time (6.67 days) for primordia initiation was recorded on control which was similar to rice offal. The highest time (8.33 days) for primordia initiation was found in mango wood. According to Wang *et al.*, (1996) the spawn heads appeared 6 days after the spawn running. Al Amin (2004) stated that *P. ostreatus* completed spawn running in 17-20 days on different substrates and time for pinhead formation was noted at 23-27 days.

Time required for harvesting

The lowest time (9.67 days) for harvesting was recorded from rice offal. The highest time (11.00 days) was required for mango wood. The results of this experiment is in agreement with the findings of Bugarski *et al.*, (1994) who found that the first fruiting occurred on different days depending on the substrates.

Number of total primordia

In the case of the 1st flush number of total primordia ranged from 29.39 to 40.13 and the maximum number of primordia was recorded on rice offal (Kholoud *et al*., 2014). The minimum number of total primordial was recorded on sawdust which was significantly different from results for mango wood and palm bunch. On the other hand in 2nd flush no. of total primordia was maximum (51.34) on sawdust. The lowest number of primordia was recorded on mango wood. From the result it can be said that the highest number (51.34) of total primordia was recorded in 3rd flush on sawdust and the lowest number (18.98) was recorded in first flush on mango wood.

Number of effective fruiting bodies

Effective fruiting body is the edible part of mushroom. There was significant variability on different substrates under this study. For the 1st flush number of effective fruiting bodies ranged from 10.58 to 35.03 and the maximum number of effective fruiting body was recorded on sawdust. The minimum number of effective fruiting body was recorded on palm bunch. For the 2nd flush number of effective fruiting body was recorded maximum (32.05) on rice offal. The minimum number (19.00) was recorded on mango wood. In 3rd flush, the number of effective fruiting body ranged from 9.50 to 20.51 and the maximum number was recorded on rice offal, and minimum number recorded on palm bunch. Higher yield in number of effective fruiting bodies might be due to the presence of glucose, fructose and trehalose in the substrate (Vetayasupron, 2007). Indole Acetic Acid (IAA) increased the number of fruiting body of mushroom (Noble, 2005).

Pileus Diameter

Diameter of pileus differed on different substrates. In the case of 1st flush pileus diameter was found highest (7.78 cm) on sawdust and the lowest diameter (4.13 cm) was recorded on palm bunch. In 2nd flush pileus diameter ranged from 3.97 cm to 4.52 cm and the maximum (4.52cm) was recorded on sawdust, while the minimum (3.97) diameter was recorded on palm bunch. In 3rd flush, the highest pileus diameter (4.20 cm) from rice straw which was similar with mango wood and the lowest pileus diameter (3.11 cm) was recorded on sawdust (Zenebe *et al*., 2016).

Stalk Length

Highest stalk lengths recorded in sawdust for 1^{st} and 2^{nd} flush were 3.58cm and 3.30cm respectively. This was similar to rice straw, mango wood and palm bunch. For the 3^{rd} flush the maximum (3.11cm) was recorded on mango wood. Comparing the flushes, the highest stalk length (3.58 cm) was found in sawdust of first flush and the lowest (1.66 cm) was found in rice offal of third flush. The quality of oyster mushroom *Pleurotus spp.* depends on the length of stalk, the higher the length of stalk, the poorer the quality of mushroom.

Weight of individual fruiting body

In case of 1st flush the maximum (6.40 g) weight was recorded on mango wood and the minimum (3.33 g) was recorded on sawdust. On the other hand in 2nd flush the highest (4.49 g) was recorded on palm bunch.

Biological Efficiency

Biological efficiency varied significantly due to effect of different substrates. Maximum biological efficiency (205.22 %) was obtained from rice offal, though it was not significantly different from mango wood and the minimum biological efficiency (181.65 %) was obtained from palm bunch. From the overall result, rice offal yielded better than the control. Obodai *et al.* (2003) evaluated eight lignocellulosic by-products as substrates for cultivation of the Oyster mushroom, *Pleurotus ostreatus*.

CONCLUSION

Sawdust and mango wood showed the best performance compared to the other substrates, in terms of growth parameters such as mycelium running rate, time required for primordia initiation, harvesting, number of effective fruiting bodies, stalk length and pileus diameter. High mushroom yield with relatively large pileus diameter, were obtained using sawdust and mango wood. Palm bunch was the least performing substrate in terms of mushroom yield and size.

Recommendations

1. Sawdust and mango wood are relatively abundant in rural communities where resource poor farmers reside and they are therefore recommended for use in oyster mushroom cultivation.
2. The government should engage and train rural communities on the use of local waste in the cultivation of mushroom through micro credit schemes.

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