

GROWTH PERFORMANCE OF THE MUSHROOM *PLEUROTUS OSTREATUS* VAR. *FLORIDA* ON OIL PALM FRUIT BUNCH WASTE, SAWDUST AND ELEPHANT GRASS STRAW

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ABSTRACT

The effects of three substrates, namely oil palm fruit bunch (*Elaeis guineensis*) waste, sawdust of obeche (*Triplochiton scleroxylon*) and elephant grass (*Pennisetum purpureum*) straw on the spawn running period, number and weight of pin heads and carpophores, number of flushes and biological efficiency (BE) of *Pleurotus ostreatus* var. *florida* were studied. Growth experiments were carried out for the period of three months. Mycelial culture of the mushroom was prepared on potato dextrose agar. Seven day-old cultures of the mushrooms were inoculated on sorghum grain for spawn preparation. Spawned Oil palm bunch waste (OPFBW), sawdust (SD) and grass straw (EGS) were incubated till complete ramification in five replicates. *P. ostreatus* var. *florida* produced three flushes on the substrates. Primordia took a longer time to emerge from the SD (14 days) than on OPFBW (5days) and EGS (3 days) at $p=0.05$. The mushroom had significantly shortest spawn run period of 8 days on OPFBW but the lowest biological efficiency (BE) of 4.38%. EGS took 12 days with BE 13.32% and SD in day 30 with BE of 26.72% at $p=0.05$. Sawdust best supported the growth of the mushroom followed by EGS, making them good substrates for *P. ostreatus* var. *florida* cultivation.

Key words: *Pleurotus ostreatus*; substrates; spawn; fruiting bodies; biological efficiency

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INTRODUCTION

Nigeria's mushroom biodiversity contains different species of edible mushrooms which include *Pleurotus tuberregium*, *Termitomyces mammiformis*, *Morchella* species, *Pleurotus pulmonarius*, *Pleurotus squarosurus* and *Pleurotus sajo-caju* (Ofofodile and Yusuf, 2010; Arowosoge *et al.*, 2017). Edible mushrooms are good sources of B vitamins (such as thiamine, riboflavin, niacin and pantothenic acid), vitamin C, zinc, calcium, phosphorus, potassium, sodium, carbohydrates, proteins and fat (FAO, 2007). Mushrooms and their extracts are also used in medication, biological remediation, bio-degradable packaging and dyeing wool (Adams *et al.*, 2008). Some mushrooms are medicinal are, therefore, able to synthesise a great amount of secondary metabolites with anti-tumour, anti-viral, anti-inflammatory (Owaid *et al.*, 2017), anti-bacterial, anti-fungal (Owaid *et al.*, 2015a) and anti-yeast attributes (Owaid *et al.*, 2015b). The world production of edible mushrooms has almost doubled in the past decade from 5.9 million tons in 2007 to 10.2 million tons in 2017 (Triyono *et al.*, 2019).

Increasing demand and usage of plastics and their disposal has resulted in environmental pollution burden on both land and water habitats, threatening the safety and health of wild life, aquatic life and humans (Verma *et al.*, 2016). Plastics are non-biodegradable and difficult to eliminate from the environment; plastics are disposed into landfills and burnt causing air, water and soil pollution in Nigeria. Hence the need for recycling methods for plastics in the country.

Pleurotus spp. are the most preferred ones among the edible mushrooms due to their ability to grow quickly and productively in various lignocellulosic media (Frimpong-Manso *et al.*, 2011; Gothwal *et al.*, 2012). The common name for *Pleurotus* species is Oyster mushroom. *Pleurotus* is a genus in the class Basidiomycetes and a group of fungi known as white-rot fungi as they produce white mycelia (Tsujiyama and Ueno, 2013).

Pleurotus spp. are the second most cultivated worldwide and are sold in the world mushroom market (Imran *et al.*, 2011, Royse *et al.*, 2017; Bellettini *et al.*, 2019).

Pleurotus ostreatus accounted for 14.2% of the total world production of edible mushroom in 1997 (Ofodile and Yusuf, 2010). The cultivation of mushroom includes selection of adequate mushroom species, preparation of quality fruiting culture, development of active spawn, preparation of substrate, mycelia spawn running, mushroom development and harvest (Ofodile and Yusuf, 2010). The species has a unique flavour, aromatic properties, rich in protein, fibre, carbohydrates and minerals (Ofodile *et al.*, 2011; Lavelli *et al.*, 2018). *Pleurotus ostreatus* showed protein contents similar to or higher than those found in many vegetables, but lower than meat and cheese contents (Mena and Manzi, 2019). Also, *Pleurotus ostreatus* is reported to contain attributes such as anti-viral, anti-bacterial, anti-tumour, anti-oxidant, anti-mutagenic, anti-parasitic and immunomodulatory (Ofodile *et al.* 2011). *Pleurotus* spp. are selective degraders, degrading lignin and hemicellulose rather than cellulose. In this way, it remains exposed and can be utilised by ruminants (Cohen *et al.*, 2002).

In Nigeria, indigenous mushrooms are harvested from forests and farmlands for sale. The need for commercial production of all edible mushrooms in Nigeria cannot be over-emphasised because of its potential contribution to agricultural production and as a source of cheap protein. Generally, agro-industrial wastes are inappropriately disposed (Sadh *et al.*, 2018) with the consequence of threatening the environment and public health. Agro-industrial wastes are generally characterised by high biological oxygen demand (BOD) and chemical oxygen demand (COD) (Ravindran and Jaiswal, 2016) and they are also easily susceptible to bacterial contamination due to the high water content (Ravindran and Jaiswal, 2016). Therefore, their disposal is difficult.

In Nigeria oil palm mills process a large amount of oil palm generating large quantities of wastes from the fruit bunches. According to Dalino (1995) as cited by Triyono *et al.* (2019), for each bunch of fresh oil palm bunches, about 21% palm oil, 6-7 % palm kernel, 14-15% fiber, 5-7% shell and 23% empty bunches are obtained. The waste of oil palm fruit bunch disposed in the environment constitutes pollution. The only way to preserve edible mushroom and make them available for consumption is to cultivate them because many mushrooms eaten of old are nonexistent because of the impact of climate change (Ofodile, 2006). Therefore, to alleviate hunger and malnutrition in a world of rising food prices, cultivation of mushroom is a very reliable and profitable option. Report on the cultivation of the variety *florida* of *P. ostreatus* in Nigeria is scarce.

This work, therefore, reports the comparative study of the utilisation and the effects of some agro-industrial wastes, namely sawdust (*Triplochiton scleroxylon*), elephant grass (*Pennisetum purpureum*) and oil palm fruit (*Elaeis guineensis*) bunch waste on *Pleurotus ostreatus* var. *florida*

MATERIALS AND METHODS

Study Location

The experiments were carried out at the Mushroom Research and Training Laboratory, Yaba College of Technology, Yaba Lagos, Nigeria.

Mycelial growth studies

Internal tissue of the carpophores was transferred onto sterile potato dextrose agar (PDA 1.5%) and incubated at 28°C for 72 hr to prepare pure cultures that were maintained on PDA at 4°C for further studies. Agar blocks of seven-day old culture of the mushroom were inoculated unto PDA (2%) in five replicates and incubated at 28°C. Linear mycelia growth was measured and recorded daily for 10 days on 100 x 15 mm agar plate.

Spawn Production

Uninfected *Sorghum bicolor* grains (1kg) mixed with 5 g calcium carbonate and made up to 65% moisture content was prepared, divided into 500 g units, and loaded into whisky bottles. The bottles were sterilised for 1 hr at 121°C at 15 Psi and allowed to cool. Each grain bottle was inoculated with five pieces of agar blocks (1 cm diameter) from 10-day old plate culture of *P. ostreatus* var. *florida*. After 14 days of incubation, when all bottles were fully colonised, they were used to inoculate test substrates used in growth studies.

Growth Studies

Oil palm fruit (*Elaeis guineensis* Jacq.) bunch waste was collected from Badagry area of Lagos State. Sawdust of obeche (*Triplochiton scleroxylon* K. Schum.) was collected from a Sawmill in Mushin area, Lagos while the Elephant grass (*Pennisetum purpureum*) waste was collected from a field at Mowe area of Ogun State. Elephant grass was cleaned to obtain the straw only and the oil palm bunch waste was beaten on a hard surface to the internal fiber. The wastes were sun-dried for seven days before shredding sawdust to sizes 40-100 mesh. The grass straw was chopped to 4-5 cm lengths. Rice bran was purchased from a rice mill at Abeokuta. Calcium carbonate (CaCO_3) was obtained from the chemical market at Ojota, Lagos. Moisture content in all materials was determined before use. *P. ostreatus* var. *florida* was plated on 2% PDA plates (90 mm diameter) and incubated at 25°C for one week before use in spawn preparation.

Preparation of Substrates

Three substrates, namely oil palm fruit bunch waste (OPFBW), Sawdust (SD) and Elephant grass straw (EGS) were prepared for cultivation of *P. ostreatus* var. *florida*. Dried OPFBW (5000 g) and EGS (5000 g) wastes were pasteurised separately for 1½ h and 1h, respectively in water (100 L) mixed with calcium oxide (12.9 g). OPFBW was further boiled with fresh water (100 L) only for 1 hour. After cooling, EGS was mixed with calcium carbonate (10 g); OPFBW and EGS were separated into portions each and used for further cultivation studies. Rice bran (800 g) and CaCO_3 (200 g) were appropriately mixed with SD (5000 g) and the moisture content adjusted to 64 %. The substrates OPFBW (1000 g weight) and SD (1200 g dry weight) were loaded into polyethylene bags (30 × 15 cm). EGS (1000 g weight) was packed into perforated used plastic pet bottles. Substrates were in five replicates. The bags of sawdust substrate were pasteurised for 6 hours in a locally-made steamer and allowed to cool overnight. The three substrate bags and plastic bottles were inoculated with the grain spawn (50 g) per substrate unit.

The inoculated substrates were incubated in the dark for eight weeks (SD), two weeks (OPFBW and EGS) at room temperature (20-25°C at night and 25-30°C during the day time). At the end of the incubation period, the fully colonised substrates were transferred to a fruiting room maintained at 28±2°C and 90-95% RH. Lighting was provided using sunlight coming into the fruiting house through glass windows during the day time. Aeration was achieved by opening the door to the fruiting room 30 min each, three times per day. Data collected during the fruiting experiment included the spawn run period, number of days taken until pinhead formation and number of primordial, number of days from commencement of fruit body induction to first flush, weight and number of basidiocarps per flush. The biological efficiency (BE) was calculated as the weight of fresh mushroom harvested/divided by dry weight of substrate and multiplying by 100 (Ofodile *et al.*, 2022).

Experimental Design and Data Analysis

The randomised complete block design was used in the mushroom cultivation experiment. Data collected from all experiments were expressed as mean ± Standard Error of mean (SEM). Analysis of Variance (ANOVA) with Turkey's post-hoc was used for analysis at 5% level of significance.

RESULTS

Growth of *P. ostreatus* var. *florida*

The results of the hyphal extension of *P. ostreatus* var. *florida* on potato dextrose agar is shown in Figure I. The mushroom grew well on Potato Dextrose Agar. The mycelial growth of the mushroom growth was first observed on day 2 after incubation. The mycelial growth was exponential and took 10 days to full ramification. There was significant difference in growth between day 3 and day 7.

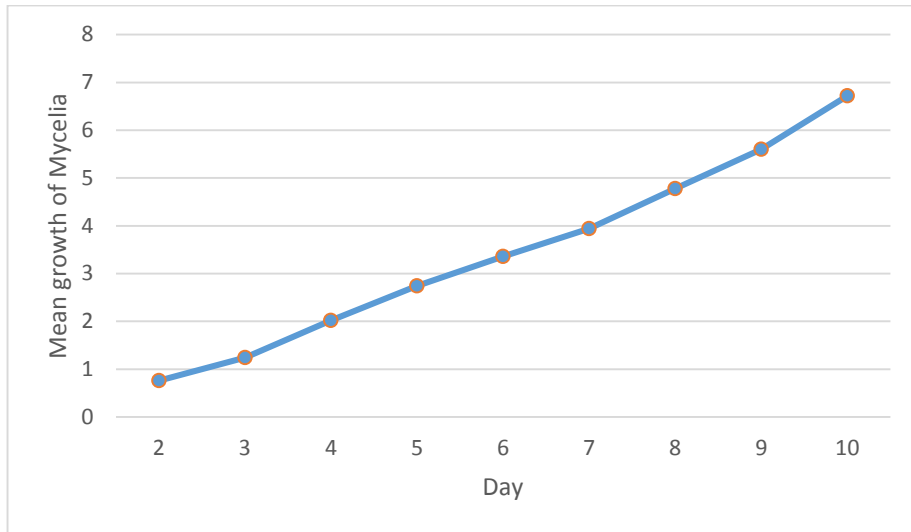


Fig. 1: Mean radial mycelial growth of *Pleurotus ostreatus* var. *florida* on potato dextrose agar

Cultivation of *P. ostreatus* var. *florida*

P. ostreatus var. *florida* produced the shortest spawn run period on EGS (8 days), compared to OPFBW (12 days) and SD (30 days). The mushroom had a comparatively short incubation period on OPFBW. Figure 2 shows the different flushes during the cultivation of *P. ostreatus* var. *florida*. The production of mushroom differed significantly amongst the three flushes on the same substrates for OPFBW and SD but was significantly lower in the third flush for EGS. Results of the time taken for pin head emergence, number of pin heads, number and weight of carpophores, day to carpophore emergence and biological efficiency of the mushroom are shown in Table 1. The mushroom produced the highest number of pinheads on EGS. The pinheads of *P. ostreatus* var. *florida* emerged significantly faster on Oil Palm Fruit Bunch Waste (OPFBW) and Elephant Grass Straw (EGS) than on Sawdust substrate (Fig. 2).

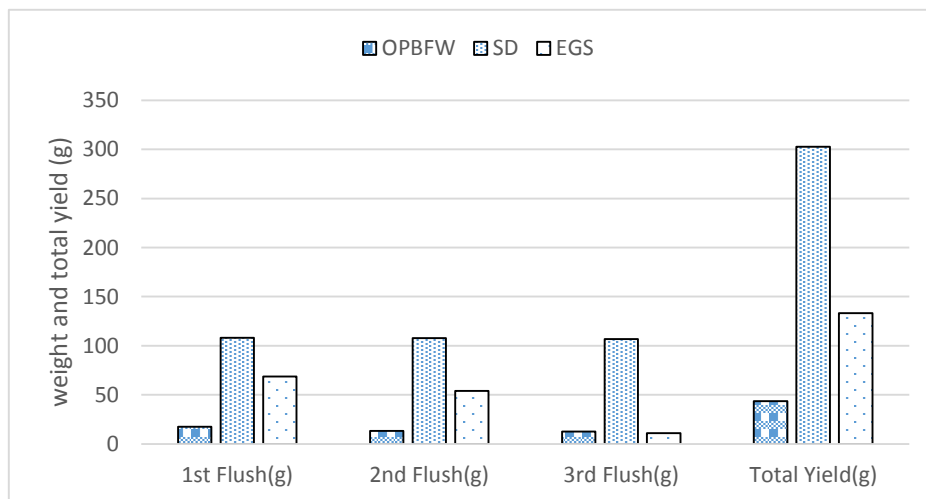


Fig 2: Effects of substrates on weight and total yield of harvested *P. ostreatus* var. *florida*
Key: OPFBW = Oil palm bunch waste, SD= Sawdust, EGS= Elephant grass straw

The weight of carpophore (g) by the mushroom on EGS was significantly higher than on OPFBW; the SD had the highest weight of carpophores in all the flushes. The total yield of mushroom carpophore from sawdust substrate was also significantly higher than on all the other substrates. Intervals between flushes on the three substrates (OPFBW, SD and EGS) were 14 days, 30 days and 10 days, respectively. The B.E on SD (26.72 %) was higher than on EGS while the B.E of OPFBW (4.32 %) was lower than on EGS (13.32 %) at 5% level of probability.

Table 3: Growth performance of *P. ostreatus var. florida* on different substrates

Substrate	Pin Head Emergence	Number of Pin Heads	Carpophore Fresh Weight (g)	No of Carpophores	Days to Carpophore Formation	Biological Efficiency (%)
OPFBW	5.80±0.37 ^a	40.80±4.12 ^c	43.40±9.81 ^e	49.40±9.66 ^{hi}	14.00±0.63 ^J	4.38±0.97 ^L
SD	16.20±1.20 ^b	36.20±0.86 ^c	322.60±5.35 ^f	27.20±2.31 ^h	46.40±1.08 ^K	26.72±0.5 ^m
EGS	5.20±0.73 ^a	207.00±20.25 ^d	133.20±6.1 ^g	70.20±7.29 ⁱ	16.20±0.73 ^J	13.32±0.62 ⁿ

Key: OPFBW = Oil palm bunch waste; SD= Sawdust, EGS= Elephant grass straw.

Substrates with different superscripts are significantly different from each other at 5% level of probability

The mushroom produced the highest number of carpophores on Elephant Grass Straw (EGS) when compared with the number of carpophores on the other two substrates (Fig. 2). The number of carpophores on OPFBW was higher than on SD. The mushroom produced carpophores on OPFBW at 14 days, 46 days on SD and 16 days on EGS after inoculation.



Plate 1: Top Left -Right A1, B1, C1 *P. ostreatus* var. *florida* pin head formation on the different substrates (EGS, SD, OPFBW)

Lower Left -Right A2, B2, C2 *P. ostreatus* var. *florida* carpophore formation on the different substrates (EGS, SD, OPFBW)

Key: OPFBW = Oil palm fruit bunch waste, SD= Sawdust, EGS= Elephant grass straw

DISCUSSION

Pleurotus ostreatus has been reported to be cultured on Potato Dextrose Agar (PDA) effectively (Sudirman *et al.*, 2011) as was also observed in this study. *P. ostreatus* var. *florida* grew well on Potato Dextrose Agar and this may be as a result of the presence of glucose which has been proven to be the best carbon for the mushroom (Kuforiji and Fasidi, 1998). The effective production of spawn on *Pleurotus ostreatus* has been reported by Narh *et al.* (2011). It was observed that sorghum was an efficient medium for the preparation of mushroom seed from this study. Naraian *et al.* (2009) associated earlier appearance of pin head in corn straw to the high content of lignin which the fungus breaks down to release sufficient nutrients for its growth. *P. ostreatus* var. *florida* was also reported to have the fastest pin head emergence on EGS.

Pleurotus spp. have a high saprophytic ability and can successfully grow on a variety of lignocellulosic substrates (Machado *et al.*, 2016; Iwuagwu *et al.*, 2017), which agrees with results of this work. The formation of fruiting bodies is initiated by mycelial growth, followed by the production of primordia (El-Fallal *et al.*, 2015). The period of mycelial growth on the different substrates in this study ranged from 8 days in OPFBW to 12 and 30 days in EGS and SD, respectively. The difference seen in the different treatments in spawn run period may be due to the composition of the substrates. Iwuagwu *et al.* (2017) reported that the variation in the number of days taken for the spawn to complete colonisation of a given substrate is a function of the fungus strain, growth condition and substrate type. An interval between flushes of 10 days has been reported for *Pleurotus* spp. cultivated on sawdust (Mandeel *et al.*, 2005). Ten days between flushes were also reported for OPFBW and EGS.

Mintesnot *et al.* (2014) observed that the total fresh weight ranged from 377 to 665 g/kg dry substrate in the case of *Pleurotus florida* on different substrates, which differ from the present work. According to Jonathan *et al.* (2013), *P. pulmonarius* performed least on oil palm waste compared to coir fiber, sawdust and rice straw in flushes, weight and total yield. *P. ostreatus var. florida* also had the least performance on oil palm fruit bunch waste in the present study. The low yield recorded by oil palm waste might be due to the complex lipid present in it. This is in agreement with the findings of Lim (1981), who reported that oil palm waste may contain complex lipids which may hinder easy access of the fungus to simpler carbon sources, thus reducing the mycelial colonisation and yield.

CONCLUSION

The study demonstrated the utilisation efficacy of *Pleurotus ostreatus var. florida* in oil palm fruit bunch waste, sawdust and elephant grass straw. The macrofungus performed best on sawdust although with a long incubation period. Mushroom cultivation can be leveraged on by a country like Nigeria for the recycling of used plastic bottles and other agro-industrial wastes in mitigating environmental pollution.

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AUTHORS' CONTRIBUTIONS

OFODILE, L.N. conceptualised the project, supervised and wrote the manuscript; ANI, E. contributed to writing the manuscript; NWANYA, C.R. carried out the research; FAGEYINBO, F.V. participated in the project; ADAMU G.O.L. contributed to writing the manuscript; ADEKUNLE, A.A. carried out the statistical analysis.

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