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Comparative Study on the Productivity, Fructification and Macro-Morphological Properties of *Pleurotus ostreatus* and *Pleurotus pulmonarius* Cultivated on two Substrates.

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Abstract

Mushroom is nutritious, medicinal and provides food, employment and foreign exchange. Potentials inherent in macrofungi cultivation has not been fully harnessed in Nigeria. Cultivation makes use of cheap and availabe agro wastes.Substrates such assaw dust (SD) and Sugarcane bagasse (SB) were sorted, sterilized and inoculated separately with spawns of *Pleurotus ostreatus* and *Pleurotus pulmonarius.* Incubation was done in a dark room and subsequently transfered to a ventilated growth room for fruiting.Fructification and the impact of each substrate on the different species were observed and recorded bymeasurement of stipe length (SL), pileus length (PL) and weight (W) of each harvested fruiting body. The *P.pulmonarius* on SB had the shortest fruiting period of 12 days and a first flush on the 18th day after inoculation and an impressive average yield of 485.5g.*P.pulmonarius* on SD fruition within16 days and an average yield of 46.4g. The biological efficiency (B.E) was adequate at 53.9% for *P. pulmonarius* on SB and 5.2% for *P. ostreatus* on SB. Comparisons on the morphology of the Pleurotus species showed that *P.ostreatus* is oyster shaped, cream coloured and gills seen as undulating straight margins all over the back of the cap. *P. pulmonarius* was observed with its fleshy texture, convex and lung shaped pileus and stipesec centrically attached to the pileus (cap). The *P. pulmonarius* and *P.ostreatus* are highly recommended as viable mushroom strains to commercialize based on their yield and biological efficiency on sugarcane bagasse with little or no contamination observed. The choice of sugarcane bagasse for faster fructification is also recommended.

Keywords: Mushroom, Substrates, Morphology, Fructification

Introduction

Mushrooms, over the years, had grown in the wild and had been either termed poisonous or edible leaving few locals or experts with the knowledge of identifying and enjoying the edible ones (Oei & Nieuwenhuijzen, 2005). They are defined as fruit bodies of macrofungi (Nwoko *et al*., 2018) and are recognized for their ability to convert waste products into a protein rich food (Mane *et al*., 2007) thus, the need to engage in its cultivation is on the increase. Mushrooms are low in energy and fat content with appreciable amounts of vitamins, minerals, dietary fibre and protein (Cheung, 2010).Mushrooms aid in medicinal purposes and the promotion of good health as they are good sources of phytochemicals, proximate components and minerals (Okwulehie & Ogoke, 2013).

In the cultivation of edible mushroom, *Pleorutus* species have ranked highest for their less complex method of production, faster fructification and larger yield from the collection of the needed substrates, availability of spawn, growth observation and harvesting *(*Husein *et al*., 2015). Among the 25 species of mushroom considered to be intensively cultivated commercially, the oyster mushroom is said to rank the third world wide (Obodoi *et al*., 2003).

From the survey reported by Josephine (2014), different kinds of wastes are useful for the cultivation of oyster mushroom. The use of this lignocellulosic materials such as saw dust, sugarcane bagasse, cereal straws, cotton waste, food waste, corn cobs, rice straw, wood chips, paper, cocoa pod husk, cassava peels, coffee pulp, barley, wheat straw etc, have curbed waste challenges in diverse regions (Ritika & Ishika 2017;Subu, 2013).Interestingly, the spent substrates obtained from post cultivation of mushroom serve as a fertilizer in animal feed and biogas production(Zenebe *et al.,* 2016)

Engaging in mushroom farming has proven to be of great benefit with numerous advantages such as in the provision of food (Mshigeni, 2001), relatively quick returns which can curb poverty (Masarirambi *et al*., 2011), utilization of little space for its cultivation and its economic advantage (Dzomeku, 2009). Remarkably, the medicinal properties of mushroom especially the oyster mushroom has been found suitable for the treatment and prevention of diverse ailments (Pramanikel *et al*., 2005; Alarcon & Aguila, 2006; Jedinal& Silva, 2008; Hernadex *et al., 2016*).

P. pulmonarius is known for its anti-nociceptive, anti- inflammatory and anti-proliferative actions (Smiderle *et al*., 2008; Lav i*et al*., 2010), high nutritive value, increased yield and simple cultivation as well as its outstanding role in the breaking down of lignin due to the enzymes released by them, thus aiding their growth onlignocellulosic materials such as paper, saw dust, and other agro wastes (Croan, 2000).

Okwulehie *et al.* (2008) reported that substrates used for mushroom cultivation contain high levels of protein, fats and oil, carbohydrate and vitamins. The influence of different substrates and environmental factors on the cultivation (Oei, 2012) and nutritional composition of *P. pulmonarius* was studied and said to have impact on the bioactive nutrients and vitamin constituents of oyster mushrooms (Okwulehie *et al.*, 2007).

This study reports and evaluates the productivity, fructification and some macro-morphological comparisons of *Pleurotus ostreatus* and *Pleurotus pulmonarius* fruit bodies cultivated on two substrates

Materials and Methods

Location of the Study

The study was conducted in a mushroom house attached to the Microbiology Department of Federal University of Technology, Owerri. South Eastern region of Nigeria has a humid tropical climate and is characterized by rainfall with peaks between the months of July and September of each year with an annual rainfall between April and November each year.

Collection of Substrates

The substrates used are classified as agro wastes sourced within the locality. Sawdust (SD) was collected from a popular local timber market in Naze, while the sugarcane bagasse (SB) was collected from a sugarcane processing outlet in Avu all in Imo State, South Eastern Nigeria.

The source of Spawn

Pure mycelia culture of *P. ostreatus* and *P. pulmonarius* were obtained from the Department of Biology, Federal University of Technology, Owerri, Imo State.

The Experimental Design.

The experiment was done in a completely randomized design (CRD) format. Each substrate was prepared in 6 replicates such that each mushroom strain was inoculated into three sets of sawdust and three sets of sugarcane bagasse making a total of 12 runs.

Spawn Development

Spawn of *P. ostreatus* was prepared using sorghum grains which were washed in tap water and soaked overnight. The grains were boiled in water in the ratio of 1:1 (Sorghum grain: water) for 15 – 20mins in an autoclave. The grains were removed from water and spread on a clean mat to drain off excess water. The sorghum grains were later mixed with 4% (W/W) $CaCO_3$ and 2% (W/W) $CaSO_4$ to optimize pH as well as prevent clumping of grains respectively. Completely drained sorghum grains were stuffed in empty wide-mouth bottles and tightly sealed with aluminum foil, sterilized at 121^0C for 30min. and allowed to cool afterwards before inoculated with the actively growing mycelia of *P. ostreatus*. The strains of *P.pulmonarius* were also aseptically inoculated into separate bottles with the prepared sorghum grains. The inoculated grains were incubated in the dark room (at 27 ± 2^0C) till the spawn run is achieved, that is, when the grains were fully colonized by the mycelia (Shyam *et al.,* 2010).

Preparation of Substrates

The sugarcane bagasse was macerated to 2 – 4cm lengths. One kilogram (1kg) of each substrate was steeped separately in tap water overnight in a plastic containers and excess water drained off. They were later transferred into a metallic drum and heated at 80^0C for 1.30min. and allowed to cool overnight (Muhammad *et al.,* 2007).Each substrate was prepared in six replications as 200g of each were separately stuffed into thoroughly washed 2.5 litres plastic buckets perforated randomly from base to the top.

Inoculation of Substrates

Inoculation was done as 30 g spawns, each of *P. pulmonarius* (*P. p)* and *P. ostreatus* (*P. o)* were inoculated in triplicates into plastic buckets containing sawdust (SD) and sugarcane bagasse (SB).Each inoculation was carried out by placing the spawn between 4 layers of the substrate. The buckets were then covered and placed on wooden shelves in the dark room.

Transfer of the set up to cropping house

When the substrates were fully colonized by mycelium (spawn run), shown by the formation of pin heads in the buckets, they were transferred from the dark room to the growth house to allow for more light intensity, ventilation and humidity (Oei and Nieuwenhuijzen, 2005). Water was frequently sprinkled till fruition and maturity was achieved.

The Harvest/Packaging

Fruit bodies were harvested at maturity by carefully holding the basal region of the stalk with fingers and slightly twisting them as they break off easily from their substrates (Oei & Nieuwenhuijzen, 2005). The harvested mushrooms were then oven dried for preservation and packaged in a transparent nylon.

Evaluation of Morphological Properties:

Measuring the Stipe size of fruit bodies

The stipe size was measured according to the method of Okwulehie and Okwujiako(2008). This was done by placing a meter rule along the length of fruit body stipe and the readings recorded.

Measuring the Cap diameter

This measurement was done with the aid of a transparent meter rule placed at the margin and across the center of the pileus. The diameter was read off from the ruler and recorded.

Effect of substrates on fruit body number of mushrooms

The impact of the substrate on the number of fruiting body of the mushroom was determined by harvesting the mushrooms, weighing the fruiting bodies and recording the values for each substrate to determine the yield (Table 1).

Results

The Fructification

The fruiting duration of each test organism indicating the time taken for the pre-mordial formation (fruiting bodies) is shown in Table 1 below. The result on the fructification shows that the P.p grown on SB had a fruiting duration (FD) of 12 days and was matured for harvesting by the 18th day while P.o grown on the same SB fruited within 24 days and was ready for harvest by the 30th day. The former gave rise to stipes with length ranging from 2.0-5.3 cm with a Pileus Diameter (PD) of 3.1 -6.9cmwhile the latter (SB on P.O) had stipe length ranging from 4.0-7.1 cm in length and a P.D of 3.9 – 6.8cm. The results on the growth on the SD substrate is as shown below.

Table 1:Fructification of the various samples after inoculation

Substrate	Mush (Test organism)	FD	First Flush	SL range (cm)	PD range (cm)
SB	P.p	12 days	18 days	2.0- 5.3	3.1- 6.9
SD	P.p	16 days	22 days	1.2 – 3.5	2.2- 4.8
SB	P.o	24 days	30 days	4.0- 7.1	3.9-6.8
SD	P.o	28 days	32 days	4.2-6.0	4.4- 6.2

Key: SB- Sugarcane Bagasse, SD- Saw Dust, SL- Stipe Length, PD- Pileus Diameter.
FD- Fruiting Duration, P.p- P. pulmonarius P.o - P.ostreatus

Morphological Characteristics

***Pleurotus ostreatus*.** The morphological features of the harvested *P. ostreatusare* as follows:

Shape of the pileus/cap: Oyster shaped

Stipe: This is bare with no stripes.

Hymenium: It has gills and undulating straight margins all over the hymen (back of the cap)
Coloration of the pileus at harvest: Cream

Basidiocarp: The surface is smooth(Figure 2 and 5)

***Pleurotus pulmonarius* (grey oyster):**The morphological features are as follows:

Sporophore: Grey coloured

Texture: Fleshy

Pileus: Convex and lung shaped

Stipe: Eccentrically attached to pileus/cap(Figure 3 and 4)

Figure 1. Fructification on-going from the P. p on SB substrate in the cropping house

Figure 2. Fructification observed from P. o on SB substrate in the cropping house

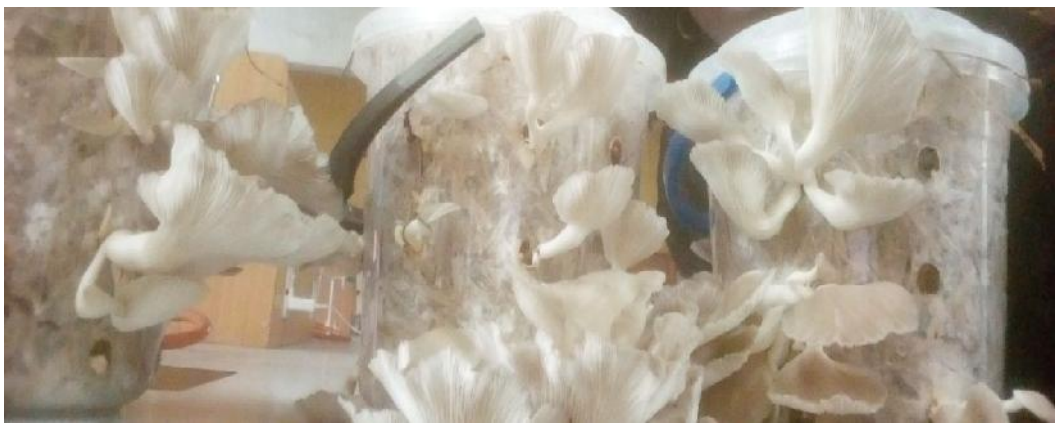

Figure 3. The P. p on SB ready for harvest

Figure 4. Harvested P.p from SD substrate

(a)

(b)

Figure 5 (a) & (b). Matured P.o on SD substrate

Biological Efficiency (B.E)

The result on the B.E (which is the rate at which the dry substrate was converted or utilized to produce mushroom)was calculated with the formula below.

$$B.E = \frac{\text{Weight of fresh mushroom harvested} \times 100}{\text{Weight of dry substrate}} \quad 1$$

The weight of fresh mushroom (yield) harvested for P.pis 485.5g and the dry weight of substrate (DWS) for SB on which the organism (P.p) grewis 900 thus, the biological efficiency when calculated with the formula is 53.9% as shown in the Table 2.The B.E of each harvested strain in relation to the substrate it degraded is shown in the table below.

Table 2: Biological Efficiency (B.E) of the Substrates made in triplicates

Substrate	Mush	DWS	Yield (g)	B.E (%)
SB	P.p	900	485.5	53.9%
SD	P.p	1350	46.4	3.4%
SB	P.o	900	46.9	5.2 %
SD	P.o	1350	34.6	2.6%

DWS- Dry Weight of Substrates; B.E- Biological Efficiency

Discussion

The morphological features of the harvested *P. ostreatus* with its shape as that of an oyster and having a creamycolour matched that described by OECD (2014) while that of *Pleurotus pulmonarius* (grey oyster) is as described by Lechner and Wright (2004) having the characteristic fleshy texture, convex and lung shaped pileus.

Fructification in P.p on its SB substrate was faster with a 12 days fruition and maturity by the 18th day than in its SD substrate. As stated in table 2, the yield and B.E(carried out with the formulae given by Stamets(2000) varied with the type of substrate used with the largest yield recorded for P.p on SB and the least observed in P. p on SD. According to Chang and Miles 2004, the variation in the spawn run and eventual fruition as well as yield can be related to the fungal strain, growth conditions and type of substrate used. Similar to this, it is a fact from other studies that the growth of Pleurotus species on SB, SD and other agro-wastes would definitely vary in primordial initiation, and fruition (Vetayasuporn, 2006; Islam *et al*., 2009;Birham, 2010).

The fructification for P.o which had a range of 24-28 days in the different substrates and maturity between 30-32 days is similar to the work done by Zenebe *et al*.(2016) were in the P.o fruited and matured within 30-38 days on a SD substrate when compared with paper waste and wheat dust substrates. The lignocellulosic materials in the substrate used are believed to have an effect on the yield as reported by Liang *et al*.(2009). With a higher yield and B.E of 53.9% in P.p on SB and the least B.E of 2.6% in P.o on SD, it is obvious that the lignocellulose materials present in saw dust may be low in protein content as equally confirmed by studies made by Obodai *et al*.(2002). This, as recommended by Zenebe *et al*. (2016) would possibly require some measures like composting so as to aid the SD substrate further breakdown its inherent materials or the addition of Nitrogen, Phosphorus and Potassium to enrich the SD.

Conclusion

The study has shown that Pleurotus species grow fast and this must have proven its choice as one of the most commercially cultivated mushroom as stated by Husein *et al*. (2015), with a range of 18 – 30 days observed for their fructification. Likewise, they can grow well on SB and SD substrates which are common wastes available in the environment. The B.E and yield from the Pleurotus species especially from the P.p on SB is of great economic advantage. There was no major contamination observed in the process among the Pleurotus species possibly due to their faster rate of fruition, giving no room for competitive microorganisms to thrive. For substrate, the sugarcane bagasse had the fastest fruiting period and is thus recommended when in season.

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