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# Use of Different Agro-wastes in the Cultivation of *Pleurotus ostreatus* (Jacq.) Kummer

## Cover Page Footnote

The authors are grateful to Prof. I.C. Okwulehie for his invaluable assistance

## USE OF DIFFERENT AGRO-WASTES FOR CULTIVATION OF *PLEUROTUS OSTREATUS* (Jacq.) Kummer

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### ABSTRACT

Agro-wastes are abundant in both rural and urban areas and when carelessly disposed off to the environment by dumping or burning, they lead to environmental pollution, and consequently to health hazards. Growing of edible mushroom on these wastes can contribute to decrease in environment pollution. This work investigated the growth and yield of *Pleurotus ostreatus* on different agro-wastes: plantain midrib (PM), corn straw (CS), sugarcane bagasse (SB) and their combinations (PM+CS, PM+SB, CS+SB, CS+PM+SB) with a view to finding the best substrate for the growth of *P. ostreatus*. The experiment was carried out using completely randomized design with seven treatments and three replications. Results revealed that mycelia growth period and days to pinhead formation were faster in corn straw (7 and 11 days, respectively from the day of inoculation), this was followed by CS+SB (8 and 12 days, respectively) while CS+PM+SB recorded the longest days (13 and 17, respectively). Stipe length ranged from 2.6 cm in PM to 3.3 cm in CS+PM while the pileus diameter was between 3.3 cm in CS and PM and 4.3 cm in PM+SB. Number of fruiting bodies, fresh and dry weights of the mushroom were significantly higher in CS than in the other substrates. The least number of fruiting bodies, fresh and dry weights were recorded in CS+PM+SB.

**Keywords:** Agro-wastes, mycelium, growth period, *Pleurotus ostreatus*, pollution.

### INTRODUCTION

The genus, *Pleurotus* is commonly known as Oyster mushroom. It belongs to the class, Basidiomycetes and a group of fungi known as white-rot fungi as they produce a white mycelium (Tsujiyama and Ueno, 2013). These are edible mushrooms, highly nutritious with high contents of protein, fiber, vitamins and minerals (Mattila et al., 2001; Shin et al., 2007). They can be effectively used as appetizers and also as an ingredient in soups, sauces, salads, and meat dishes (Mattila et al., 2001). *Pleurotus* sp. is reported to be suitable for inclusion into calorie-controlled diet because of its characteristic high water content and low calorific value (1510 kJ-

edible parts) (Jaworska and Bernas, 2009). They have several beneficial properties (anti-fungal, anti-oxidant, etc.) as they contain important bioactive molecules such as phenolic compound, terpenes, steroids and polysaccharides (Yang et al., 2013; Zhang et al., 2016).

There are over 2000 species of mushrooms that exist in nature (Manzi et al., 2001) but only about 35 species have been commercially cultivated (Aida et al., 2009). *Pleurotus* spp. are cultivated worldwide and rank second in the world mushroom market after *Lentinus edodes* (Royse et al., 2017; Bellettini et al., 2019). *Pleurotus* spp. are one of the most extensively studied white-rot fungi because of their ability to degrade cellulose, hemi-cellulose and lignin (Machado et al., 2016). Hence, they can be

cultivated on various lignocellulosic substrates (Savoie et al., 2007; Li and Shah, 2016). These substrates are majorly agro-industrial wastes, which are produced in enormous quantities causing disposal problems and consequently environmental pollution/health risks (Garg and Gupta, 2009). Utilization of these wastes in mushroom cultivation can help reduce these problems.

Many different lignocellulosic materials have been tested for their suitability in the cultivation of *Pleurotus* spp. including grass straws (Okwulehie and Okwujiaku, 2008), sawdust, cassava peels, corn cobs, melon chaff, paper waste and water hyacinth (Ambi et al., 2011), soybean straw, wheat straw, paddy straw, sugarcane bagasse, sunflower stalks, maize stalks, domestic waste, used tea leaves, fruit waste, newspaper, bamboo leaves, saw dust (Dehariya and Vgas, 2013), corn cobs (Itelima, 2012), cotton waste (Shah et al., 2004), palm leaves (Alananbeh et al., 2014), maize stover and thatch grass (Fanadzo et al., 2010) among others.

There is an ever-increasing demand for mushrooms with the total demand for all the species estimated at 60.0 million tons per annum and the world output at about 34 million tons (Panjikkaran and Mathew, 2012). China contributes about half of the world's mushroom production. Whereas, South Africa (the only African country estimated) contributes less than 0.3 % (FAO, 2009). There is therefore no gainsaying that the production level in Nigeria and the whole of Africa is insignificant. Research efforts directed in the area of exploring more substrates and determining the best for the cultivation of mushroom more especially *Pleurotus* spp., that is widely adapted, will no doubt enhance its production.

The aim of this study was to compare the growth and yield responses of *P. ostreatus* cultivated on different agro-

wastes, namely corn straw, plantain midrib, sugarcane bagasse and their combinations with a view to determine the best substrate for the cultivation of *P. ostreatus*.

## **MATERIALS AND METHODS**

### ***Study Location***

The study was conducted at the mushroom unit of Michael Okpara University of Agriculture, Umudike, in Southeastern Nigeria.

### ***Sources of Materials***

One bottle of *Pleurotus ostreatus* mother spawn was obtained from the mushroom house of Plant Science and Biotechnology Department, Michael Okpara University of Agriculture Umudike, Abia State. Red sorghum grain (*Sorghum bicolor*) was purchased from Umuahia main market, Abia State, Nigeria and was utilized as substrate for spawn multiplication. Plantain midrib, sugarcane bagasse and corn stalk were obtained from the agricultural farm of Michael Okpara University of Agriculture, Umudike, Abia State.

### ***Spawn Preparation***

The method described by Stamets (2000) was adopted in the multiplication of spawn. Substrate preparation was carried out according to the method described by Sharma (2003).

### ***Inoculation of the Substrates***

After cooling, equal amount (30 g) of *Pleurotus ostreatus* grain spawn was inoculated into each of the buckets containing the substrate, by gently placing it on layers of the substrate. The inoculated buckets were covered and transferred onto wooden racks in the cropping room. The buckets were covered with a black polythene

bag to serve as dark incubation for ramification to take place. The cropping room was well-lit, ventilated and constantly flooded with clean water to maintain the environmental conditions (good aeration, temperature between 18-32 °C, high humidity) needed for the vegetative growth of the mushroom.

### ***Induction of Fruiting Bodies***

In order to induce fruiting body formation in the substrates, the polythene bag was removed on the 10<sup>th</sup> day, when it was assumed that the mycelia have fully colonized the substrate (Plate 1). The bucket covers were removed to increase aeration and flooding of the floors was reduced so as to reduce the humidity of the room. The buckets were routinely observed until immature mushrooms started to emerge as primordia.

### ***Experimental Design and Treatments***

The experiment was laid in a completely randomized design. Each treatment was replicated thrice. The treatments were: plantain midrib (PM), corn straw (CS), sugarcane bagasse (SB), 500 g plantain midrib + 500 g corn straw (PM + CS), 500 g plantain midrib + 500 g sugarcane bagasse (PM + SB), 500 g corn straw + 500 g sugarcane bagasse (CS + SB) and 400 g corn straw + 300 g plantain midrib + 300 g sugarcane bagasse (CS + PM + SB).



**Plate 1: Substrates fully colonized by *P. ostreatus* mycelium**

### ***Assessment Parameters***

#### ***i. Vegetative Growth***

The mycelium growth period was recorded as the time (in days) taken from inoculation of the spawn to the time the spawn fully ramified and colonized the substrate. While days to pinhead formation were recorded as the number of days taken from the time (days) the spawn fully colonized the substrate to the time fruiting bodies appeared on the substrates.

#### ***ii. Yield and Yield Attributes***

At each flush (three flushes), the fruiting bodies produced (Plate 2) from each substrate were harvested by hand-picking. The number of fruiting bodies in different substrates was counted and total of the three flushes was recorded. The diameter of the cap was measured in centimeter (cm) with a transparent plastic ruler from one edge to the other edge when it had fully expanded. The length of the stripe was also measured (cm) using a transparent plastic ruler. The fresh weight of the fruiting-bodies was determined by weighing them immediately after harvesting, using an electronic

weighing balance. The dry weight was taken after drying them in an oven at 60 °C.

### **Statistical Analysis**

Data collected was analyzed statistically using ANOVA. Means that were found significant were separated using Fisher's Least Significant Difference (LSD) at a significance level of  $P < 0.05$ .

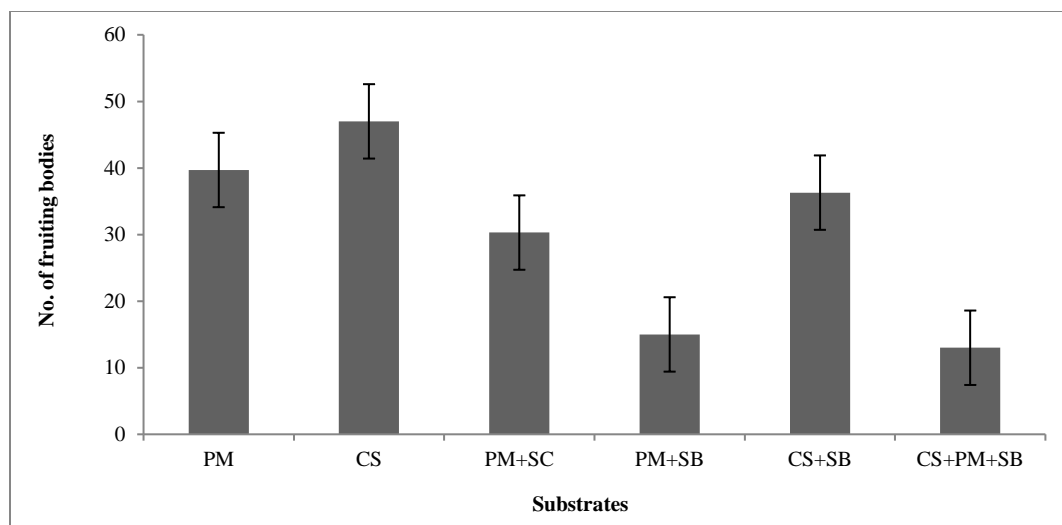
## **RESULTS**

The results for period of mycelium growth, days to pinhead formation, stipe length and pileus diameter are presented in Table 1. There was a significant ( $P < 0.05$ ) difference in the period of mycelium growth, which is the number of days taken by the fungus to fully colonize the substrate.

**Table 1: Effect of substrates on period of mycelium growth (PMG), days to pinhead formation (DPH), stipe length (SL) and pileus diameter (PD)**

<b>Treatment</b>	<b>PMG (days)</b>	<b>DPH (days)</b>	<b>SL (cm)</b>	<b>PD (cm)</b>
PM	16	10	2.6	3.3
CS	11	7	3.1	3.3
SB	-	-	-	-
PM+ CS	16	12	3.3	3.5
PM+SB	16	12	2.8	4.5
CS+SB	12	8	2.8	3.4
CS+PM+SB	17	13	2.7	3.4
LSD	4.0	2.2	0.5	1.0

*PM = plantain midrib; CS = corn straw; SB = sugarcane bagasse; PM+CS = plantain midrib + corn straw; PM+SB = plantain midrib + sugarcane bagasse; CS+SB = corn straw + sugarcane bagasse; CS+PM+SB = corn straw + plantain midrib + sugarcane bagasse*



**Figure 1: Effect of substrates on the number of fruiting bodies on *P. ostreatus*.**

*PM* = plantain midrib; *CS* = corn straw; *SB* = sugarcane bagasse; *PM+CS* = plantain midrib + corn straw; *PM+SB* = plantain midrib + sugarcane bagasse; *CS+SB* = corn straw + sugarcane bagasse; *CS+PM+SB* = corn straw + plantain midrib + sugarcane bagasse

Statistical analysis revealed significant effect of substrate formulation on time (days) taken for pinhead formation. This ranged from 7 days in corn straw to 13 days in the three combinations (*CS + PM + SB*). It took significantly shorter time for the pinheads to appear on corn straw alone in contrast to the other substrates except in the combination of corn straw and sugarcane bagasse. The number of days taken for the pinheads to appear on the other substrates did not differ significantly ( $P < 0.05$ ).

The stipe length of the fruiting bodies of *P. ostreatus* was significantly affected by the substrate formulation. Fruiting bodies harvested from plantain midrib plus corn straw had the highest stipe length that was significantly ( $P < 0.05$ ) different from the stipe lengths of those harvested from the three combinations *CS+PM+SB* and sole plantain midrib, which recorded the least. Highest pileus diameter was recorded in fruiting bodies harvested from combination of plantain midrib plus sugarcane bagasse.



**Plate 2: *P. ostreatus* fruiting bodies growing in different substrates contained in a bucket.**

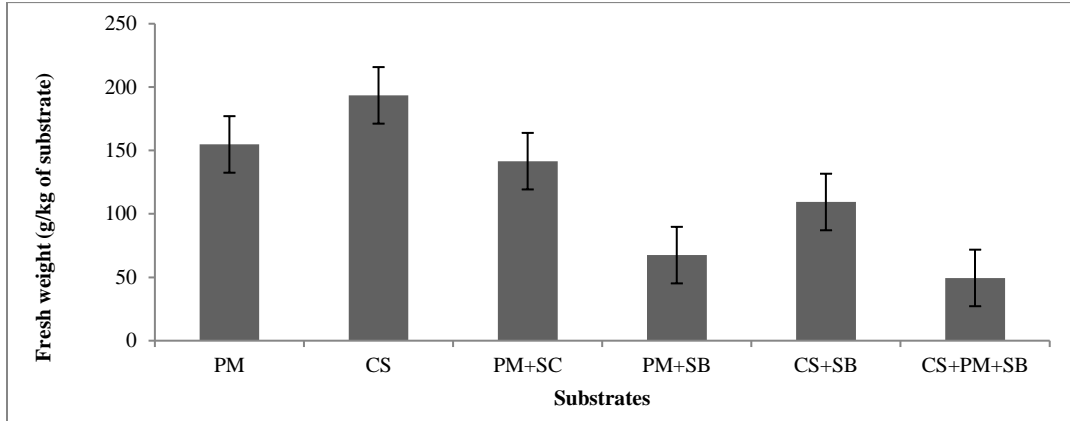
The number of fruiting bodies produced was significantly highest in corn straw substrate (Figure 1). The least number of fruiting bodies was produced by the combination of all three substrates.

The highest fresh weight was obtained in sole corn straw substrate with a mean value of 193.5 g/kg of substrate and this was significantly ( $P < 0.05$ ) different from the others. The least fresh weight (49.5 g/kg) was recorded in the all-three substrates combination (*CS+PM+SB*), though it was not significantly different from that of

plantain midrib mixed with sugarcane bagasse (67.5 g/kg) (Figure 2).

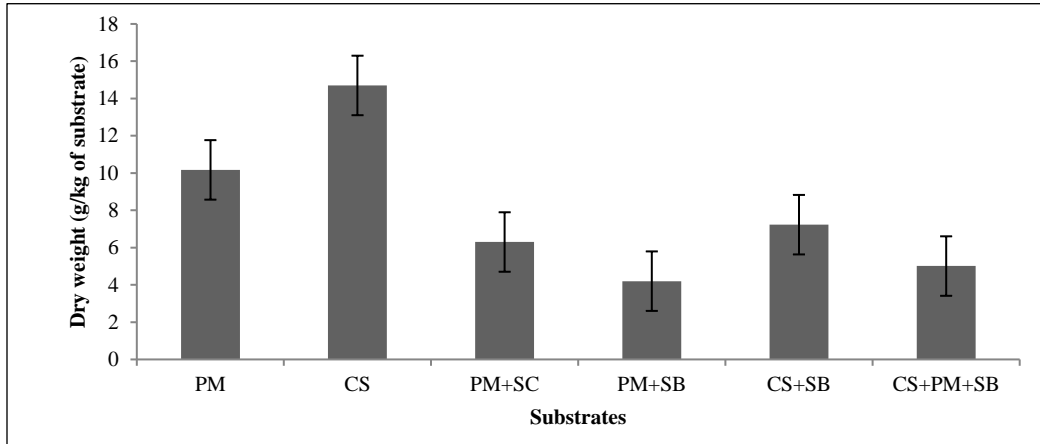
Similarly, the highest mushroom dry weight of 14.7 g/kg, was obtained in sole corn straw substrate. This value was

significantly different from those obtained from the other treatments (Figure 3). The least dry weight of 4.2 g/kg was obtained in plantain midrib mixed with sugarcane bagasse.



**Figure 2: Effect of substrates on fresh weight of *P. ostreatus*.**

*PM* = plantain midrib; *CS* = corn straw; *SB* = sugarcane bagasse; *PM+CS* = plantain midrib + corn straw; *PM+SB* = plantain midrib + sugarcane bagasse; *CS+SB* = corn straw + sugarcane bagasse; *CS+PM+SB* = corn straw + plantain midrib + sugarcane bagasse



**Figure 3: Effect of substrates on dry weight of *P. ostreatus*.**

*PM* = plantain midrib; *CS* = corn straw; *SB* = sugarcane bagasse; *PM+CS* = plantain midrib + corn straw; *PM+SB* = plantain midrib + sugarcane bagasse; *CS+SB* = corn straw + sugarcane bagasse; *CS+PM+SB* = corn straw + plantain midrib + sugarcane bagasse



## DISCUSSION

The most important phases in the cultivation of mushrooms are spawn running and pinhead formation. Spawn running or period of mycelium growth refers to the period of mycelia growing on the substrate from inoculation to full ramification. The period of mycelia growth in this study ranged from 11 days in CS to 17 days in CS+PM+SB. This range was shorter than the ranges of 14-21 days and 17-23 days reported by Tan (1981) and Shah et al. (2004), respectively. The observable difference among the treatments in period of mycelia growth may be due to the composition of the substrate. Chang and Miles (2004) reported that the variation in the number of days taken for the spawn to complete colonization of a given substrate is a function of the fungus strain, growth condition and substrate type. According to Stamets (2000), longer spawn run can be attributed to the compactness of the substrate owing to small particle size of the substrate, which interferes with mycelial growth of the mushroom.

Pin head formation (primordium initiation) was observed following the invasion of the substrates by *P. ostreatus* mycelia. The result of the present study indicated that corn straw (CS) substrate positively influenced the period of appearance of the pin head as the fruiting bodies appeared earlier on corn straw (7 days after full colonization and 18 days from spawning) than the other substrates. This is in agreement with the report of Stanley et al. (2010). Naraian et al. (2009) attributed earlier appearance of pin head in corn straw to its high content of lignin, which upon enzymatic breakdown by the fungus, releases enough nutrients for the fungal growth.

The time required for the formation of pinhead, which was between 18-30 days from spawning was comparable with other

similar studies where pinhead appeared around 20-23 days and 28-34 days on different substrates (Fan et al., 2000; Islam et al., 2009). Shah et al. (2004) found that pinheads appeared in about 24 – 30 days from spawning. Variation in mycelia growth rate, colonization and primordial initiation has also been observed when other *Pleurotus* mushroom species were grown on a wide range of substrates (Islam et al., 2009; Dehariya and Vyas, 2013; Hwang et al., 2015).

The results of the study showed that fructification of *P. ostreatus* occurred in all the substrates (Plates 3 and 4) except on undecomposed sugarcane bagasse. This indicates that these substrates contained nutrients that supported the growth of the mushroom (Okwulehie and Okwujiako, 2008; Wabali and Wocha, 2013). In addition, *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).

The stipe height ranged from 2.6 – 3.3 cm. This is in conformity with the range of 2.6 – 3.6 cm earlier reported by Iwuagwu et al. (2017) in saw dust, corn husk and their combination. Dehariya and Vyas (2013) reported that stipe height of *P. sajor-caju* ranged from 2.5 – 2.9 cm in conventional substrates (straws of soybean, wheat, paddy, sunflower and maize) and 2.3 – 3.5 cm in non-convectional substrates (domestic waste, fruit waste, used tea leaves, bamboo leave and sawdust). Islam et al. (2009) observed a stipe height of *P. flabellatus*, an oyster mushroom cultivated on various substrates as between 2.20 and 3.59 cm.

Pileus diameter differed significantly among the substrates and ranged from 3.3 to 4.5 cm. This range was within the range (1.92-4.78 cm) reported by Okwulehie and Okwujiako (2008). Bonatti et al. (2004) had earlier observed that *P. ostreatus* cap ranged

from 2–15 cm in diameter while the stipe was very short. However, the significant difference in the diameter of the pileus could be due to texture and substrate formulation as well as nutrients in the substrate, which possibly affected the composition of the final mushroom growth substrate qualities such as water holding capacity and degree of aeration (Reyes et al., 2009).

## CONCLUSION

This work investigated the use of different agro-wastes namely corn straw, plantain midrib, sugarcane bagasse, and their combinations on the cultivation of *P. ostreatus*. Results of this study have shown that *P. ostreatus* can be cultivated successfully on sole corn husk and plantain mid rib. Hence, corn straw may be considered as a good substrate for the cultivation of *P. ostreatus*.

## CONFLICT OF INTEREST

Authors declare no conflict of interest.

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