Original Research Article

OYSTER MUSHROOM (*Pleurotus ostreatus*) CULTIVATION USING SAWDUST AND DIFFERENT ORGANIC MANURES

ABSTRACT

Aims: The aim was to evaluate the performance ~~efficacy~~ of cultivating oyster mushroom (*Pleurotus ostreatus*) using sawdust and different organic manures.

Study Design: The experimental design used was the complete randomized design (CRD) and the Data obtained was subjected to Analysis of Variance (ANOVA) followed by Turkey's Least Significant Difference(LSD) test to compare treatment means; differences was considered significant at 95% (P≤0.05) (SPSS Version 21 software).

Place and Duration of Study: National Biotechnology Development Agency, South East Center, University of Nigeria, Nsukka between September 2019 and November 2019.

Methodology: Saw dust was prepared as substrate with different organic manures and coded SD, Saw dust; SDP, Sawdust+ Poultry; SDC, Saw dust + cow dung; SDD, Saw dust+ pig dung) in the ratio of 100:0 and 50:50 respectively to cultivate oyster mushroom (*Pleurotus ostreatus*). Subsequently, the rate of growth, time of harvest, yield and average weight were recorded and proximate composition determined using standard methods.

Results: Oyster mushroom harvested showed Moisture content ranged (4.63-7.14%), ash content (4.84-6.77%), crude fat (0.98-3.28), fiber (16.02-18.23%), protein (19.27-33.41%) and carbohydrate (38.18%-48.89%). Average weight yield was highest in saw dust substrate (10.2g) with total yield (980g) and least average weight (~~7.9g~~check from result and correct it) and total yield ~~(111g~~) in sawdust and cow dung substrate. Oyster mushroom from all the substrate differed significantly (p<0.05).

Conclusion: Sawdust was the most suitable substrate for oyster mushroom

Key words: Saw dust, Cow dung, ~~Organic manures,~~ Oyster mushroom, Pig dung, Poultry litters

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1. INTRODUCTION

Mushroom belong to the class Basidiomycetes and are fruit bodies of macroscopic, filamentous and epigeal fungi. Mushroom species are cosmopolitan, heterotrophic organisms and are specific in their nutritional and ecological requirements. Nigeria with her unique climatic conditions of tropical rain forest in the south and sub-Saharan condition in the north is a home to diverse species of mushrooms. The common mushrooms in Nigeria include:*Termitomyces, Pleurotus, Lentinus, Lenzites, Trametes, Ganoderma, Pycnoporus, Coriolopsis* and others (Ola and Oboh, 2001). Mushrooms have high nutritional and functional values and are accepted as nutraceutical (Ergonul *et al.,* 2013). In most parts of Africa, mushrooms are consumed based on their organoleptic properties such as aroma, taste, flavour and texture and not on the nutritional and medicinal properties (Osemwegie *et al*., 2006).

Naturally oyster mushroom grow on dead and decaying wooden logs or sometimes on dying trunk of deciduous or coniferous woods, in both temperate and tropical forests. They also grow on decaying organic matter but due to changes in climatic patterns harvesting wild mushroom has become difficult. The alternative is to grow mushrooms domestically. The production of mushrooms with better flavor, appearance, texture, nutritional qualities, and medicinal properties at a sustainable cost constitutes a challenge for both industry and independent farmers, for operational reasons (Sanchez, 2004). The main substrate for production is sawdust. Saw dust is a mixture of shavings from trees and depending on the type of tree and amount of lignin present, the growth of the spawn can be inhibited. Other alternative substrates include: dehulled corn cob, straw off paddy and wheat, stalk and leaves of maize, millet and cotton, sugarcane bagasses, jute and cotton waste, straw, coffee grounds, peanut shells, banana leaves, corn husk, palm fruit shaft, cotton wastes, cassava peel, cocoa pods, coconut husk and dried grasses (Adedokun, 2014; Onyeka *et al.,* 2017*).* The spawn usually in grain form is inoculated into the substrate and allowed to grow. Mushroom cultivation requires carbon, nitrogen and inorganic compounds as their nutritional sources, and main nutrients are carbon sources such as cellulose, hemi- cellulose and lignin. Oyster mushrooms require less nitrogen and more carbon source. Mushrooms are reported to be easily grown on different lignocelluloses wastes such as banana leaves, cereal straw, paper wastes, sawdust and poultry droppings (Shah *et al.,* 2004). This study was carried out to evaluate the performance of saw dust with different organic manures such as poultry litters, pig dung and cow dung.

2. MATERIALS AND METHODS

2.1 Sample Collection and Cultivation of Oyster mushroom (*Pleurotus ostreatus*)

Oyster mushroom (*Pleurotus ostreatus*) spawn was grown/cultivated the National Biotechnology Development Agency, South East Center, University of Nigeria, Nsukka. The spawn was cultivated using sawdust and in combination with organic manures (poultry litters, pig dung and cow dung) in different ratio as substrate as shown in Table 1. Saw dust was collected from Nsukka saw mill market while the organic manures where obtained from the University of Nigeria Research Farm. The study lasted for 90 days from September 2019 to November, 2019.

2.2 Substrate preparation

The substrate (SD, Saw dust; SDP, Sawdust+ Poultry litters; SDC, Saw dust + cow dung; SDD, Saw dust+ pig dung) were gotten dried and shredded and were mixed in the ratio presented in Table 1. Substrates were soaked with 40-60 ml of water depending on the level of dryness until 60% moisture content was achieved. The wetted substrates were bagged in 10×12 cm polyethylene bags in triplicates and tied up.

Table 1: Substrate formulation for oyster mushroom cultivation

Substrate	Ratio
SD	100:0
SDP	50:50
SDC	50:50
SDD	50:50

Key: SD, Saw dust; SDP, Sawdust+ Poultry liters; SDC, Saw dust + cow dung; SDD, Saw dust+ pig dung.

2.2.1 Sterilization

The bagged substrates in 10×12 polyethylene were sterilized in autoclave for 15 minutes prior to inoculation at the temperature of 121°C to eliminate any microbial contamination and allowed to cool overnight (Falana *et al.,* 2011).

2.2.2 Inoculation of substrate

Inoculation was done after sterilization aseptically into the sterilized bags of the substrate. Ten grams (10g) of spawn was weighed and inoculated to each substrate and tied up and taken into a dark room for mycelium colonization/ growth at 25°C - 30°C and 90% relative humidity. Within seven days, mycelium colonization or spawn run was observed. The spawn run time depend on the size of the bag, amount of spawn used, the strain used and the temperature.

2.2.3 Fructification

After 25 days, substrate bags that were ramified completely were moved to mushroom growth chamber for photosynthesis to enable sprouting and fructification. Tiny holes were created on the polyethylene bags using a wire. The polyethylene bags were opened and watered with 3 ml of

water each using different syringes daily to avoid cross-contamination. Environmental conditions such as temperature, ventilation, light and humidity are necessary for fructification.

2.2.4 Harvesting/Packaging

Fresh mushroom was harvested on maturation was carefully by hand from the substrate bags after 31 days of inoculation. The harvested mushroom fruits were weighed, recorded and oven dried at 45^{o}C, to ensure phyto-chemicals are not lost.

2.2.5 Processing of mushroom powder

The oyster mushroom (*Pleurotus ostreatus*) powder was processed in the laboratory using the method described by Okeke *et al*. (2003). The fresh mushroom was cleaned properly to remove dirt and damaged portion. The fresh mushroom was blanched in hot water at 32 °C for three minutes, which contained 3% salt and 0.01% citric acid. Then, water was drained, and the mushroom dried in an oven at temperature of 105 °C for 3 hours. The dried mushroom was milled in the laboratory using hammer mill and passed through a 60-inch mesh sieve (British Standard Screen) and packaged in a low-density polyethylene bag, stored in the refrigerator (4 °C) until required for use.

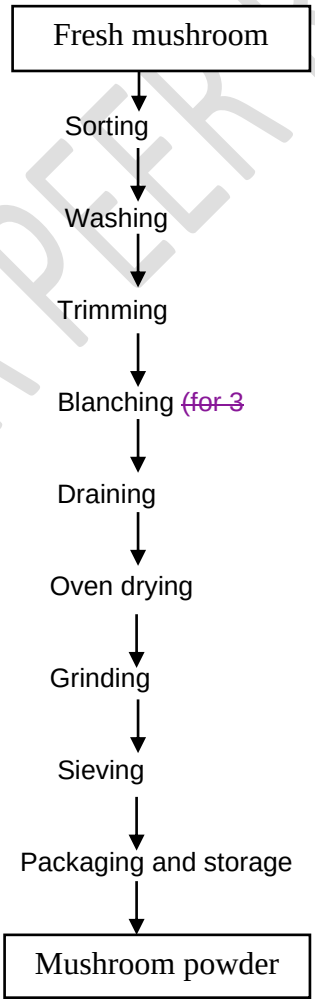

Figure 1: Flow chart for the preparation of mushroom powder

Source: Okeke *et al.* (2003).

2.3 Analysis

2.3.1 ~~Proximate Composition~~

2.3.~~2~~1 Determination of moisture content

This was determined in triplicate by hot air oven method in an indirect distillation method. According to AOAC (2010) method, three grams (3 g) each of the product sample was weighed (W_2) using a digital balance into a cleaned, dried, cooked and weighed crucible (W_1). The sample, which was in the crucibles, was transferred into a hot air oven at 150ºC and dried for 2 hours. The crucible was weighed periodically until constant weights (W_3) was obtained. The percentage of moisture was calculated as follows:

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where;

W_1 = Initial weight of empty crucible;

W_2 = Weight of crucible + food before drying

W_3 = Final weight of crucible + food after drying;

% Total solid (dry matter) = 100 - % moisture

2.3.~~3~~2 Determination of crude fibre

The crude fibre was determined using the method described by AOAC (2010). Two grams (2 g) of each of the sample (W_1) was weighed on a digital balance, boiled underreflux for 30 minutes with 200 ml of a solution containing1.25 g of H_2SO_4 per 100 ml of solution which was put into a 250ml beaker. Then, filtered through a muslin cloth on a fluted tunnel and heated with hot water for 2 times. The residue was transferred into a 250 ml beaker and boiled for 30 minutes with 200 ml of a solution containing 1.25 g of carbonate free of NaOH per 100ml. The final residue was then filtered through a muslin cloth and dried using a hot-air oven (GallenKamp oven, United Kingdom). The final residue was ashed in a furnace and put into the desiccator to cool and weighed (W_3) using a digital balance;

$$\% \text{ Crude fiber} = \frac{W_2 - W_3}{W_1} \times 100$$

W_1 = Weight of the sample

W_2= Weight of the sample before ashing

W_3 = Weight after ashing

2.3.~~3~~4 Determination of crude fat

Crude fat was determined using Soxhlet extraction method as described by AOAC (2010). A 250 ml clean boiling flask (B) was dried in an oven at 100ºC using hot air oven. Three gram (3 g) of the sample (A) was weighed (using a digital balance, Model number: No.T 320N) into thimble. The thimble and its content were placed in the extraction apparatus and extraction was with ethyl ether in Soxhlet extractor for 6–8 h at a condensation rate of at 3–6 drops per second. During this process, the fat was extracted and transferred into a pre-weighed evaporated dish. The petroleum

ether was recovered by evaporation using a bath and the remaining fat in the flask was dried in the oven at 80ºC for 30 minutes and cooled in a desiccator and finally weighed using digital balance. The difference in the weight of the empty flask and the flask with oil (C) gave the oil content was calculated as percentage fat content:

$$\% \text{ Crude fat} = \frac{C - B}{A} \times \frac{100}{1}$$

A = Weight of sample

B = Weight of empty flask

C = Weight of flask + oil

2.3.~~5~~4 Determination of crude protein

The crude protein content of the sample was determined by the semi-micro Kjeldahl technique as described by AOAC (2010). One gram (1.0 g) of the sample was put into a Kjeldahl flask and 3.0 g of hydrated cupric sulphate (catalyst) was added in the flask. Then, 20 ml of anhydrous sodium sulphate and 1.0 g of concentrated tetraoxosulphate VI acid (H_2SO_4) was added to digest the samples, which was topped and swirled. The flask and liquid was clear and free of coloration. The clear solution was cooled and made up to 100 ml with distilled water and a digest of 5 ml was collected for distillation. Then, 5 ml of 60% sodium hydroxide solution (NaOH) was put into the distillation flask and distilled for some minutes. Boric acid indicator absorbed the ammonia which was distilled off and this was titrated with 0.1 ml hydrochloric acid (HCl). The titer value or the end point at which the colour changes from green to pink was taken. The crude protein was calculated as:

Percentage crude protein = 0.001410 x 6.25 x 25 x T x 100

Where: T = Titre value

2.3.~~6~~.5Determination of ash content

Ash was determined in a muffle furnace as described by AOAC (2010). Crucibles were heated in an oven for five minutes to eliminate moisture, cooled in desiccators and weighed. Two grams of the sample was amassed into a crucible, heated over a Bunsen burner to char after which complete ashing is conducted in a muffle furnace at about 600ºC for 6 hours. The heating continued until whitish grey ash was left in the crucible. The crucible was removed from the furnace, cooled in a desiccator and re-weighed to obtain the weight of ash.

Calculation

$$\text{Percentage ash} = \frac{\text{weight of crucible + ash} - \text{weight of crucible}}{\text{Weight of sample}} \times 100$$

2.3.~~7~~6 Determination of carbohydrate content

The determination of the carbohydrate content of the product was obtained by difference as described by Ihekoronye and Ngoddy (1985).This was done by subtraction of the sum of moisture, ash, protein, fibre and fat from the total weight of 100.

% Carbohydrate = 100 – (% moisture + ash + protein + fibre + fat)

2.4 Experimental Design and Statistical Analysis

The Experimental design was based on complete randomized design(CRD) and data analyzed using one-way analysis of variance (ANOVA) and mean separation by Duncan's New Multiple Range Test. Statistical Package for Service Solution (SPSS) version 21 software was used and significance accepted at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Ramification and mycelia colonization of substrate

Ramification and substrate colonization were observed within seven days of inoculation in all the four substrates. Substrate were completely ramified after 21 days of spawn inoculation and had whitish compact mass with cottony growth formed due to complete impregnation of mycelium into the substrates. The results agreed with the findings by Peter *et al*., (2019).

Mycelia colonization was much more observed in substrate SD (Saw dust) followed by substrate SDD (saw dust + pig dung), substrate SDC saw dust + cow dung) and substrate SDP (saw dust + poultry litter) had the least condensed colonization performance. It had the longest time until 25 days of inoculation after which it was moved to the open mushroom chamber.

3.2 Oyster mushroom fructification, maturation and harvest time

Pre-mordial formation (fruiting bodies) was first observed after 3 days in the open mushroom chamber in substrate SD and SDD. This was 28 days after inoculation with substrate SDC and substrate SDP just sprouting with no fruiting bodies. This could be attributed to the nutrient composition of the organic manures used with saw dust.

The first flush which is matured fruiting bodies was harvested firstly in SD and SDD. This was after 31 days of inoculation and six days of removal from the dark room to the open mushroom chamber. Fruiting was just recorded in substrate SDC and substrate SDP. It took 38 days after inoculation and 13 days in the open mushroom chamber to harvest the first flush in substrate SDP. Meanwhile substrate SDC took 45days after inoculation and 20 days in the open mushroom chamber to harvest the first flush. The time from inoculation to harvest indicate the performance of saw dust along the different organic manures used as substrate for growth of oyster mushroom. The growth performance in substrate SD and SDD can be attributed to the chemical composition. This study agreed with Tsegaye and Tefera (2017) who observed that the number of fruit bodies recorded is related to their mycelia colonization. The researchers pointed that variation in the number of fruiting bodies in mushroom can be associated with the physical nature of the substrates as well as the nature of the mushroom species. The processes are shown in Plate 1. The picture A shows harvested oyster mushroom dried in an oven, B is harvested oyster mushroom, C is a picture of mature oyster mushroom ready for harvest and D shows sprouted oyster mushroom.

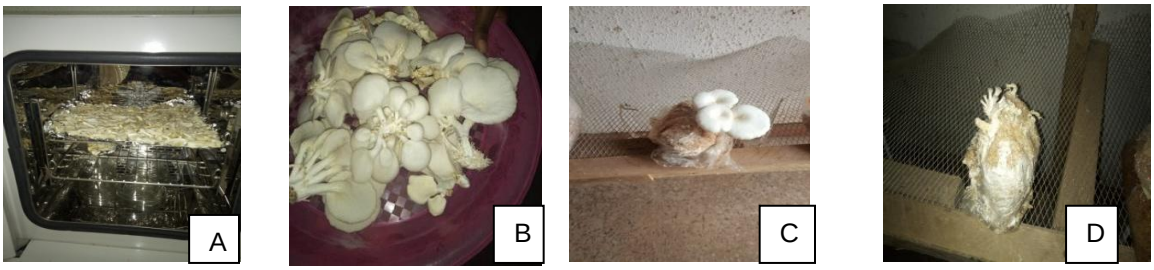

Plate 1: Oyster mushroom (*Plearotus ostreatus*) at different stages of production

3.3 Weight and yield of oyster mushroom *(pleurotus ostreatus*) by the substrate

The average weight of oyster mushroom harvested in each substrate was 10.2g in substrate SD with total yield of 980 g and 9.8 g in substrate SDD with total yield of 548 g. Substrate SDC had average weight of 7.9 g with total yield of 111 g and SDP (saw dust + poultry litter) with average weight of 7.5 g/ and total yield of 105 g Substrate SD (Saw dust) and SDD (saw dust + pig dung) had up to three (3) flushes while SDC (saw dust + cow dung) and SDP (saw dust + poultry litter) had only one(1) flush each. This is as shown in Table 2.

Table 2: Weight and yield of oyster mushroom grown on different substrate

Substrate	Average weight (g)	Total Yield (g)
SD	10.2	980
SDP	7.5	105
SDC	7.9	111
SDD	9.8	548

Key: SD, Saw dust; SDP, Sawdust + Poultry liters; SDC, Saw dust + cow dung; SDD, Saw dust + pig dung.

3.4 Proximate evaluation of oyster mushroom (*Pleurotus ostreatus*)

Proximate composition of oyster mushroom varied significantly ($p<0.05$; Table 3) and can be attributed to the various interactions of composting periods, substrates and environmental conditions. The results agreed with Fasidi *et al*. (2008) who stated *Pleurotus* species could grow on wide varieties of agro-industrial wastes.

The moisture composition in the oyster mushroom cultivated is highest (7.14%) in substrate SDP and the least (4.63%) in substrate SD. Substrate SDC had 6.30% while substrate SDD had 5.2% moisture content. All oyster mushroom grown in the different substrate are significantly different ($p<0.05$). Moisture content in the study is similar to the limit of 7.12% reported by Adejumo *et al.* (2015). Since the mushroom is shelf-stable, it would limit microbial growth and enzymatic activities. Ash is the inorganic residue that remains after the organic matter has been burnt off and used to measure minerals in foods. Substrate SD produced oyster mushroom with 6.77% ash and the least of 4.84% in substrate SDP. SDC had ash content of 4.91% with 6.45% in substrate SDD. The oyster mushroom samples cultivated on the substrate differed significantly ($p<0.05$). The result of ash content was lower than the range of 8.5 to 13% (Bhattachariya *et al*., 2015) and 8.24% (Duru *et al.,* 2019) but within the range of 5.65 % (Adejumo *et al.,* 2015). The result implies that the oyster mushroom cultivated is not so rich in mineral content.

Substrate SD produced oyster mushroom with the lowest fat content of 0.98% with the highest of 3.28% in substrate SDP. Sample SDC had 2.39% and 1.86% of fat in substrate SDD. The oyster

mushroom produced had significant (p<0.05) differences in the fat content. Fat content was within the range of up to 3.27% reported by Peter *et al.* (2019) in a similar study for oyster mushroom using sawdust as the major substrate but lower than the range of 3.43 to 4.25% reported by Bhattachariya *et al.* (2015). Differences in fat content can be attributed to type of substrate, formulation and method of collection. Kurtzman (2005) reported that mushrooms are generally low in fat and suggested it as an ideal vegetable for the obesity.

Fibre content is high in all the oyster mushroom cultivated on the substrates with the highest of 18.23 in substrate SDC and the lowest of 16.02% in substrate SD. Substrate SDP had 18.14% and 17.10% in substrate SDD. Significant (p<0.05) differences were observed in all the oyster mushroom produced. Fiber content in the oyster mushroom is similar to that reported by Jonathan *et al* (2012) and the range of 17.13 to 20.53% reported by Bhattachariya *et al.* (2015)

Protein has a high content in oyster mushroom and important in building of body tissues and cells. Substrate SD had the most of 33.41% with the lowest of 19.27% in SDC. SDP had protein content of 20.45 and 26.92% in substrate SDD. Oyster mushroom from all the substrate differed significantly (p<0.05) which could be attributed to substrate composition and interaction Protein content for substrate SDD is similar to 25.08% reported by Peter *et al*. (2019) for oyster mushroom cultivated with saw dust. The one produced by SDC and SDP is within the range of 16.30 to 21.92 reported by Jonathan *et al* (2012) and SD similar to 32.31 reported by Adejumo *et al.* (2015). Bhattachariya *et al*., (2015) reported a range of 25.35 to 27.30% protein from saw dust substrate which is similar to this study. Saiful (2015) reported that not only the protein content of the substrate but also nature of protein in the substrate influences the protein content of the fruiting bodies.

Carbohydrate is vital for energy and calories in the body. The oyster mushroom produced on the different substrate was most available in substrate SDC with 48.89 and the least of 38.18%. SDP had 46.15% 2 with 42.43% produced in substrate SDD. The oyster mushroom produced were significantly different (p<0.05). Carbohydrate content in the samples produced in substrate SDC and SDP was similar to 47.86% by Duru *et al.* (2019) but higher than 33.57% by Adejumo *et al.* (2015). Carbohydrate occurs in the mushroom in the form of arabitol, mannitol, tetrahalose, and xylose (Tasvina, 2013).

Table 3: APproximate composition of oyster mushroom grown on different substrate

Substrate	Moisture %	Ash %	Crude fat %	Crude Fibre %	Protein %	Carbohydrate %
SD	4.63±0.04^{a}	6.77±0.02^{d}	0.98±0.28^{a}	16.02±0.01^{a}	33.41±0.01^{d}	38.18±0.02^{a}
SDP	7.14±0.01^{d}	4.84±0.01^{a}	3.28±0.01^{d}	18.14±0.01^{c}	20.45±0.07^{b}	46.15±0.07^{c}
SDC	6.30±0.01^{c}	4.91±0.00^{b}	2.39±0.01^{c}	18.23±0.01^{d}	19.27±0.01^{a}	48.89±0.00^{d}
SDD	5.22±0.00^{b}	6.45±0.00^{c}	1.86±0.00^{b}	17.10±0.01^{c}	26.92±0.07^{c}	42.43±0.02^{b}

Key: SD, Saw dust; SDP, Sawdust + Poultry liters; SDC, Saw dust + cow dung; SDD, Saw dust + pig dung.

4. CONCLUSION

~~The study has shown oyster mushroom could be cultivated using saw dust with organic manures from pig dung, cow dung and poultry litters. Pig dung could be used along with sawdust to produce high yield and nutritious oyster mushroom comparable to that produced by sawdust only substrate, thereby reducing waste of the manures since agricultural waste could be transformed. Organic manures can be used with sawdust at 10-20% to cultivate nutritious and high yield mushroom since results from the study indicate high levels of supplementation affects performance of *Pleurotus ostreatus* mushroom.~~

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