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Cultivation of *Pleurotus pulmonarius* utilizing substrates derived from local agricultural waste

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Abstract

This study was carried out to determine the most suitable substrate for the cultivation of *Pleurotus pulmonarius.* Three substrates were used for this study; palm fruit waste, maize cobs and yam peels. The three substrates were packaged in heat resistant polythene bags and pasteurized for four hours at 100 ˚C. The substrates were inoculated with mycelia under aseptic conditions. The spawn of *Pleurotus pulmonarius* was used to inoculate each bag of substrate. The substrates were incubated in darkness and covered with newspapers to keep a high concentration of carbon dioxide (15-20 %) in the room to promote mycelia growth. Small incisions were made on the bags to give a cold shock to initiate fruiting. The result of the study showed that palm fruit waste produced the highest fresh weight of mushrooms (147.67 ± 24.583 g) while maize cobs produced the lowest fresh weight (119.01 ± 23.065 g). Maize cobs produced the highest dry weight of mushrooms (106.67 ± 4.041 g) while palm fruit gave the lowest dry weight (93.67 ± 2.082 g). Furthermore, palm fruit waste gave the highest biological efficiency of 91.60 ± 13.022 % while yam peels gave the lowest biological efficiency of 82.13 ± 35.072 %. The effects of the various substrates were discussed in relation to the dry weight and the biological efficiency of the cultivated mushroom. Based on the impacts of the palm kernel waste in the growth and yield of the cultivated mushroom, we recommend their use in the cultivation of mushrooms.

Keywords – Anambra – Awka – biological efficiency – fungi – Nigeria

Introduction

Pleurotus pulmonarius is a widely cultivated mushroom with high commercial value. The genus *Pleurotus* accounts for over 25 % of the total cultivated mushrooms across the globe (Raman et al. 2020). It is the second most cultivated mushroom globally following *Agaricus bisporus* (Sánchez 2010). *Pleurotus* species require rich nutritional sources such as carbon, nitrogen and inorganic compounds for growth. They thrive on substrates containing cellulose, hemicellulose and lignin because these materials contain less nitrogen and more carbon (Chang 1989). The yield and the quality of edible and medicinal mushrooms depend on the chemical and nutritional content of substrates (Pérez-Rodríguez et al. 2014).

Large amounts of organic wastes are generated annually through agricultural and industrial activities. Sadly, much of these wastes are burnt, shredded or used in landfills even though these

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wastes constitute a potentially valuable resource and can be recycled for the production of edible food and medicine (Oyetayo 2011). These can serve as substrates for the cultivation of mushrooms. For example, palm oil fruit fibre and empty bunches are the major components of all solid waste produced from the palm oil industry. These palm oil wastes are heterogeneous water insoluble materials consisting of cellulose hemicelluloses and lignin and to a lesser extent pectin, starch and other polysaccharides (Thomsen 2005). The fruit fibre has been shown to possess a high potential to be used as a mushroom growing substrate without any further treatment (Abd-Razak et al. 2012).

Mushroom cultivation involves a wide range of technologies. The choice of these technologies is usually dependent on the species of mushroom, the substrates and the financial resources available. Some of the commonly cultivated mushrooms are *Agaricus bisporus, Pleurotus pulmonarius var*. stechangii, *Lentinula eddoes*, and *Volvariela volvacea*. In the last three decades in addition to *Agaricus* species many other species have been cultivated at a larger scale (Wach 2016, Okigbo et al. 2012). This study is aimed at comparing the efficacy of local agricultural wastes in the cultivation of mushroom.

Materials & Method

Experimental Site

This study was carried out at the Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University Awka, Anambra State, Nigeria and lies between latitude 60 06'N and 60 16'N, longitude 70 01'E and 70 10'E. The climatic condition of the area is tropically dominated by rainfall patterns ranging from 1828–2002 mm.

Mushroom spawn determination

Mushroom spawn was used for Sorghum grain (*Sorghum bicolor*), and prepared at the Federal Institute of Industrial Research Oshodi (FIIRO). The mushroom spawn preparation methods were in accordance with those described by Akinrinola-Akinyemi et al. (2017) and Karuppruaj et al. (2014). Tissue culture was performed on malt agar and subsequently incubated at 25 °C in an incubator oven for 10 days. The mother spawn was generated using sorghum grains. The grains were carefully washed in water and boiled for 15 minutes to achieve gelatinization. After draining, 150 grams of grain were filled into each media bottle and subjected to sterilization in an autoclave at 121°C for 40 minutes. After cooling down, the active mycelium was transferred to a grain medium, and incubated at 28 °C for 14 days. The mushroom bags were prepared in triplicates.

Substrate preparation

Three agricultural substrates, namely palm fruit waste (*Elaeis guineensis*), yam peels (*Dioscorea* sp.), and maize cobs (*Zea mays*) were used for the cultivation of the *Pleurotus pulmonarius*. All agricultural substrates were obtained from the Eke Awka market, Awka, Anambra State, Nigeria. The procedures for the cultivation of mushrooms were as described by Peter (1996). The experiment was designed following the completely randomized block design (Akinrinola-Akinyemi 2017). All substrates were air-dried for 7 days and subsequently crushed into grits using a manual blender before use. The palm fruit fibres were defatted by boiling them in hot water at 100°C for 20 minutes. Afterwards, they were placed in a sieve and thoroughly rinsed to remove excess water and oil. Appropriate 500 grams of each palm fruit waste, yam peels and maize cobs wastes was prepared per bag. All substrates were pasteurized at 100 °C for 4 hours and allowed to cool down at room temperature. The mushroom bags were prepared in triplicates.

Mushroom spawn was provided by used sorghum grain (*Sorghum bicolor*), and prepared at the Federal Institute of Industrial Research Oshodi (FIIRO). The inoculation method was carried out under aseptic conditions. Then, 150 grams of mushroom spawn was transferred to inoculate each mushroom substrate bag, and incubated in the dark.

Mushroom cultivation and determination of fruiting body production

All substrate bags were incubated at 60% relative humidity and maintained in the dark to induce mushroom mycelial formation. After the mycelia completely colonized the substrate bags, the growth of mycelia on the substrates was measured in centimetres (cm). The data obtained were analysed using Analysis of Variance (ANOVA) and the test of significance was carried out using the least significant difference (LSD) at 0.05 probability level.

Harvesting and yield data

Mushroom fruiting bodies were harvested three days after pinhead's formation. Data were recorded for fruiting bodies at 1st, 2nd and 3rd flush. Total weight and total dry weight were also determined. The Biological Efficiency (BE) and total yields (g/Kg-1) were determined by following the protocols described by Karuppruaj et al. (2014). The Biological Efficiency (BE) was calculated using the formula below:

BE = (Weight of fresh mushroom fruiting bodies) / (Weight of dry substrate) × 100

Data analyses

The data were analysed using ANOVA according to the method described by Besufekad et al. (2020). The least significant difference (LSD) test was used to compare between the treatment.

Results

The mean of the measured variables from the cultivated mushroom

Table 1 shows the mean values of the measured variables obtained gotten from the cultivated mushroom. From the table, there was a decrease in the mean values from 1st flush (93.22) to the 3rd flush (37.44).

Table 1 Mean of measured variables

Variables measured	Mean±SD
1st flush	93.22 ± 39.59
2nd flush	52.0 ± 25.66
3rd flush	37.44 ± 18.70
Total weight (g)	166.85 ± 80.29 g
Total dry weight (g)	120.94 ± 52.78 g
Biological efficiency (%)	104.23 ± 31.38 %

Comparative analysis of the different treatment means for the three substrates.

Table 2 provides a one–way ANOVA that compares the different treatment of the substrates used in this study.

Table 2 One-way ANOVA comparing the different treatment for the three substrates.

Variables	Substrate			f-value	p-value
	Maize hub (n=6)	Palm fruit (n=6)	Yam peels (n=6)		
1st flush	79 ± 23.68	129.16 ± 44.64	71.5 ± 20.46	5.95	0.010*
2nd flush	50.83 ± 21.74	64.83 ± 35.65	40.33 ± 11.57	1.45	0.266
3rd flush	34.67 ± 16.13	45.167 ± 25.47	32.5 ± 13.05	0.77	0.482
Total weight (g)	164.5 ± 58.84	239.17 ± 102.43	144.33 ± 42.18	2.86	0.088
Total dry weigh(g)	160 ± 58.52	103.5 ± 11.07	136.83 ± 47.17	2.52	0.114
B.E %	116.62 ± 42.15	107.82 ± 19.72	88.27 ± 26.01	1.33	0.293

*=significant p-values<0.05

Effects of palm fruit waste, maize cobs and yam peels on mushroom cultivation

The result of fresh and dry weight of mushrooms cultivated on 150 g substrates of palm fruit waste, maize cobs and yam peels showed that palm fruit waste produced the highest fresh weight of mushrooms (147.67 ± 24.583 g) while maize cobs produced the lowest fresh weight (119.01 ± 23.065 g). Maize cobs produced the highest dry weight of mushrooms (106.67 ± 4.041 g) while palm fruit waste produced the lowest dry weight (93.67 ± 2.082 g). Furthermore, palm fruit waste gave the highest biological efficiency of 91.60 ± 13.022 % while yam peels gave the lowest biological efficiency of 82.13 ± 35.072 %. The LSD value of 3.68 reveals that the differences between the substrates of the dry weight of mushrooms were significant (Table 3).

Table 3 Fresh and dry weight of mushrooms cultivated on 150 g substrates of palm fruit waste, maize cobs and yam peels

Substrates	**Fresh weight (g)**	**Dry weight (g)**	**B.E (%)**
Palm fruit waste	147.67 ± 24.583	93.67 ± 2.082	91.60 ± 13.022
Maize cobs	119.01 ± 23.065	106.67 ± 4.041	82.27 ± 18.756
Yam peels	132.01 ± 57.888	94.01 ± 3.000	82.13 ± 35.072
p-value	0.679	0.004	0.863
LSD	N/A	3.68	N/A

Note: LSD is only relevant in variables with p-value < 0.05

The result of the fresh and dry weight of mushrooms cultivated on 300 g substrates of palm fruit waste, maize cobs and yam peels showed that palm fruit waste produced the highest fresh weight of mushrooms (330.67 ± 22.502 g) while yam peels produced the lowest fresh weight (156.67 ± 25.325 g). Maize cobs produced the highest dry weight of mushrooms (213.33 ± 3.512 g) while palm fruit waste produced the lowest dry weight (113.33 ± 3.512 g). Furthermore, maize cobs gave the highest biological efficiency of 148.30 ± 32.569 % while yam peels gave the lowest biological efficiency of 94.40 ± 18.665 %. The LSD values of 37.39 and 5.85 for fresh and dry weight of mushrooms revealed that the differences between substrates were significant (Table 2).

Biological efficiency of the mushrooms cultivated on 150 g and 300 g substrates of palm fruit waste, maize cobs and yam peels

The figure above shows the biological efficiency of the mushrooms cultivated on 150 g and 300 g substrates of palm fruit waste, maize cobs and yam peels. It revealed that maize cobs gave the highest mean biological efficiency of 115.28 %. This implies that over 1 lb. of fresh mushrooms was harvested from 1 lb. of dry substrate of maize cob over multiple flushes (Fig. 1).

Table 4 Fresh and dry weight of mushrooms cultivated on 300 g substrates of palm fruit waste, maize cobs and yam peels

Substrates	**Fresh weight (g)**	**Dry weight (g)**	**B.E (%)**
Palm fruit waste	330.67 ± 22.502	113.33 ± 3.512	124.03 ± 3.683
Maize cobs	210.01 ± 43.715	213.33 ± 3.512	148.30 ± 32.569
Yam peels	156.67 ± 25.325	179.67 ± 7.095	94.40 ± 18.665
p-value	0.001	0.000	0.061
LSD	37.39	5.85	N/A

Note: LSD is only relevant in variables with p-value < 0.05

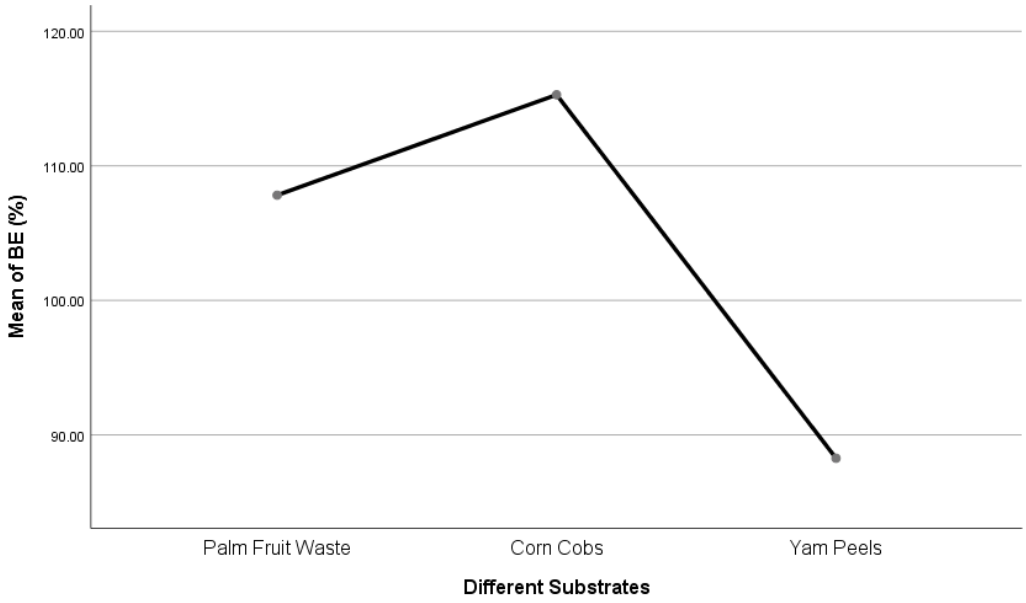

Fig. 1 – Mean plot of the biological efficiency of the mushrooms on 150 g and 300 g substrates

Mean Flush levels among the three substrates

Fig. 2 shows the mean flush levels among the substrates. The graphical presentation of the result revealed that palm fruit showed the highest mean flush levels.

Comparison of Total weight, Total dry weight & B.E % among the three substrates

Fig. 3 shows the results of total weight, total dry weight and B.E % that exist among the samples used in this study. Palm fruit showed a significant increase among the substrates.

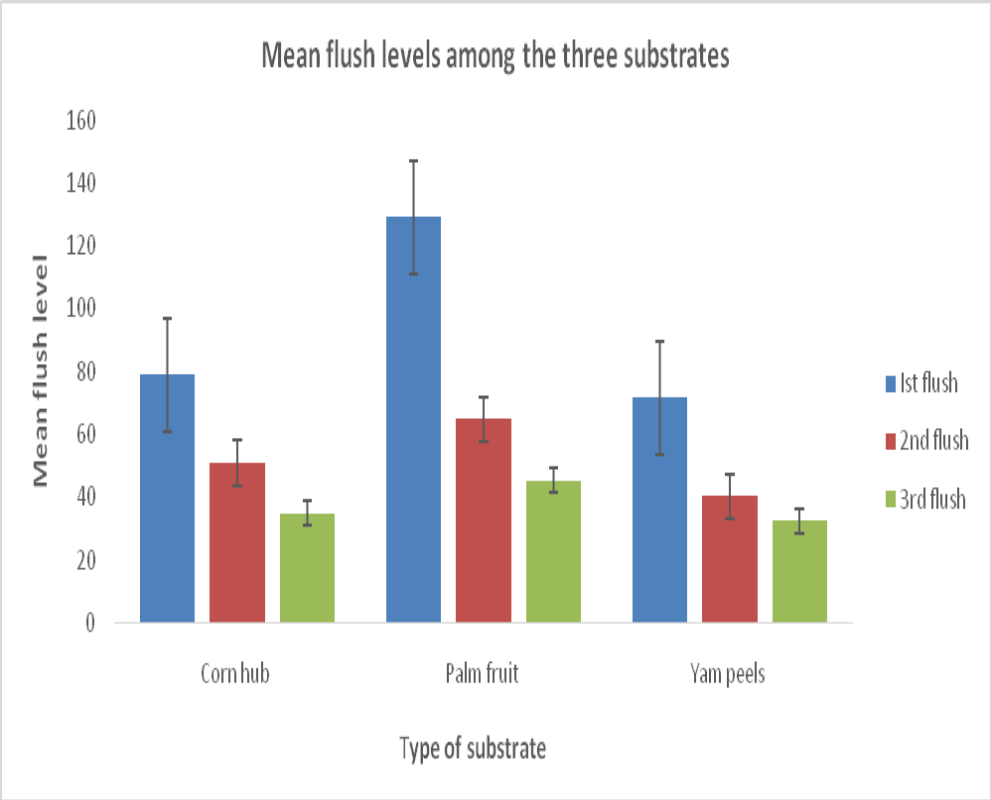

Fig. 2 – Mean flush levels among the three substrates

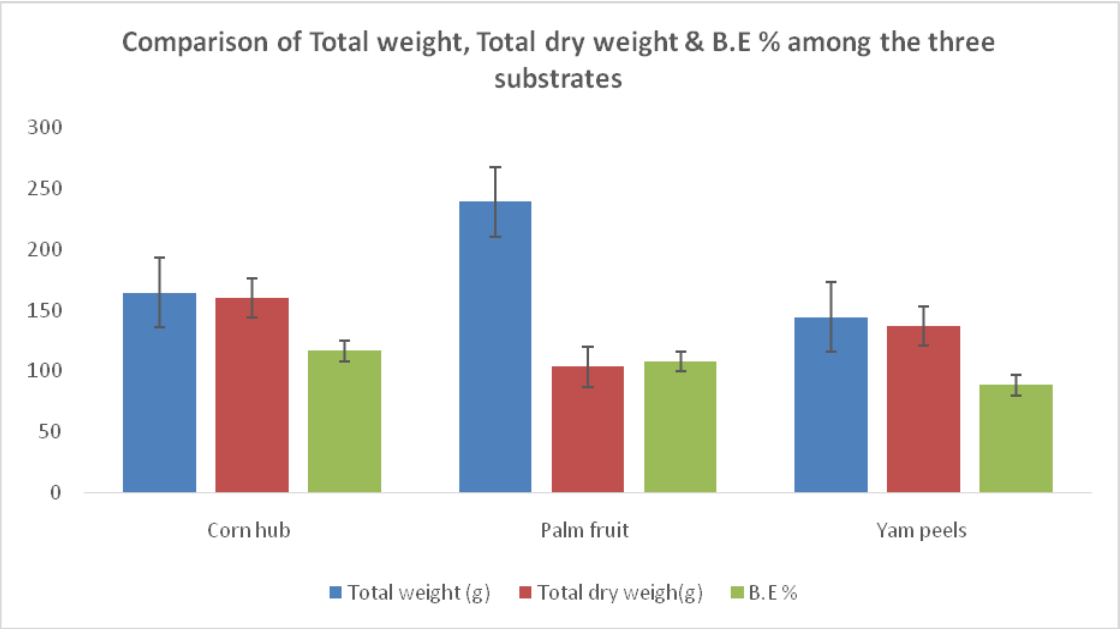

Fig. 3 – Comparison of total weight, total dry weight & B.E % among the three substrates

Discussion

The inference of this study showed that both 150 g and 300 g of palm fruit waste produced the highest fresh weight of mushrooms. This could be attributed to the high contents of complex carbohydrates and oil. This is supported by Chang et al (2004), who pointed out that mushrooms use glucose for growth and energy. They further stated that mushrooms metabolize complex carbohydrates in their substrates into glucose which is then transported through the mycelium as the main source of energy for growth.

From the tables above, 150 g and 300 g of maize cobs produced the highest dry weight of mushrooms. This could mean that maize cobs are a rich source of cellulose for the production of celluloid mycelium of the mushrooms. This was noted by Shinners and Binversie (2007) stating that maize cobs are a rich source of cellulose and hemicellulose and contain a significant amount of lignin and dietary fibre. These are converted into iron, calcium and phosphorus for absorption by mushrooms, hence resulting in high dry weight. Meanwhile Perez-Rodriguez et al (2014) who stated that maize cob is an inexpensive natural source for fungal growth and production of enzymes and other value-added compounds. It is reasonable to say that maize cob is the most appropriate substrate for the production of mushrooms. Thus, maize cobs are good for fungal growth and culture and it can be considered as a raw material for a broad number of applications in biotechnology processes, especially in the production of mushrooms rich in fibres (Kumla et al. 2020, Anuagasi et al. 2017).

Conclusion

The mushroom species *Pleurotus pulmonarius* can grow in an environment which meets its required germination condition. The various substrates used in the study showed some level of responses for the cultivation of the mushroom as seen in Fig. 1. The substrate palm fruit showed the highest response when compared to the other substrates. This might give better information to plant scientists on how to utilize the processes of cultivating mushrooms using a suitable substrate like the palm fruit.

Recommendation

Mushroom cultivation in Nigeria is of immense importance because it converts industrial and agricultural wastes which are environmental pollutants into edible protein. It can also be exported as a foreign exchange revenue source for Nigeria.

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Conflict of interest

No conflict of interest

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