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Comparison of the Growth Response and Nutritional Properties of *Pleurotus ostreatus* on Substrates Formulated from Three Different Plant Dusts

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ABSTRACT

This study was designed to assess the growth response and nutritional profile of *Pleurotus ostreatus* grown on substrates formulated from three different plant dusts namely *Piper guineense, Irvinia gabonensis* and *Pterocarpus erinaceus.* The substrates were formulated from the dusts of the plant following standard protocols. After inoculation of spores, the set up was allowed to stand in a dark room until full mycelial ramification. The radius of the ramifying mycelia was measured at 7 days interval while the dry weight of the mushroom fruiting body was taken at the end of the experiment. The result show that the mushroom grown on *P. erinaceus* had the widest radius of 4.23 cm followed by 3.17 cm in *I. gabonensis* while the least radius of 1.82 cm was recorded in those grown on *I. gabonensis*. However, at day 21 the ramification radius of the mushroom mycelia was higher in those grown on *I. gabonensis* (9.15 cm) compared with those grown on *P. guineense* and *P. erinaceus* which were comparable. Overall, the *Pleurotus ostreatus* showed a better yield performance (92.15 %) in *I. gabonensis* dust with respect to the dry weight of the harvested mushrooms as compared to others which had 84.06% and 78.8 % dry weight in *P. guineense* and *P. erinaceus* respectively. The least moisture content was found in sample grown on P. erinaceus (7.58 %) while the highest was found in sample grown on P. guineense. The lowest fat (0.38 %) and fibre (10.12 %) content were found in sample grown on P. guineense whereas protein (27.15 %), ash (4.09 %) and carbohydrate (51.68 %) were higher in sample grown on I. gabonensis compared with the other samples. The Nitrogen content of the sample grown on Piper dust (6.28 mg/g) was significantly lower than those grown on Irvinia (7.16 mg/g) and Pterocarpus (6.73 mg/g). Phosphorus was significantly higher in mushrooms cultivated on Irvinia dust (0.71 mg/g) compared the others. Therefore, domestication of the oyster mushroom may be done using the wood dust from *I. gabonensis*.

INTRODUCTION

Mushrooms have been considered as a source of human healthy food since ancient times (FAO, 2004). In addition, they have been used as in traditional medicine to cure various types of diseases. Men gain benefit from the natural antibiotics produced by mushroom to overcome bacterial infections. Mushrooms are rich in carbohydrate and protein. Proteins elaborated by these fungi have shown several biological activities like antiproliferative, immunodulatory, antiviral, antifungi, and antibacterial effects (Jonathan and Fasidi, 2003; Ngai and Ng, 2004).

Organically produced mushrooms are now popular among different sets of people ll over the world. More than two hundred (200) different genus of mushrooms contain useful species to man. About ten of the numerous species of useful mushrooms are grown for culinary and pharmaceutical purposes. Those commonly cultivated include species like *Agaricus* spp, *Lentinus* spp, *Pleurotus* spp, *Volvariella* spp, *Hericium* spp, *Auricularis* spp, *Reishi* spp, *Grifola frondosa*, *Flammulina* spp, *Tremella* spp, *Pholiota* spp and *Coprinus* spp. Out of these, only three species are produced in commercial quantity all over the world, they are *Pleurotus* spp, *Agaricus bisporus*, and *Lentinula edodes*; and they represent about 70% of global production of mushrooms (Kayode, 2010).

According to the FAO (2004), production of mushroom may help to alleviate poverty among rural farmers by generating a fast and continuous source of income. It can also aid food security and sustainability by making highly nutritious food available to people in a very short time. The advantage of mushroom farming is that it does not require soil or large spaces. Moreover, the cultivation of mushroom is not a capital intensive venture since the growing substrate can be formulated from any conceivable wastes particularly from agricultural processes.

The income generated from cultivation of mushrooms as well as improved nutrition may go a long way in ensuring the wellbeing of the local population. When mushroom farming is successful, the capcity of individuals engaged along the market chain is enhanced in such a way that other economic opportunities can be accessed.

Pleurotus spp. (Oyster mushroom) grows well on woods and woody materials, it is a good degrader of lignin and it can grow very well on various cellulosic materials such as wstes generated from crop production and agroforestry enterprises (Murthy and Manonmani, 2008). Mushroom is the choicest food of nutritionists because of its hypolipidemic, hypocholesterolemic, hypoglycaemic and antitumor properties. Mushroom contains 20-30% protein which is higher than vegetables and fruits and is of high quality (Jose and Kayode, 2009).

Pleurotus spp has been gaining popularity of recent since it has a very high nutritional value, the yield potential is great and it is easy to cultivate

(Banik and Nandi, 2004 and Gregori *et al.*, 2007). It also helps in environmental protection by attenuating toxic substances in agricultural wastes (Fan *et al.*, 2000). Oyster mushroom has the ability to breakdown directly the cellulose and lignin bearing materials without chemical or biological preparation (Chang and Miles, 1989). Thus, numerous agricultural wastes can be incorporated into the substrate for the production of mushrooms without adversely affecting its yield.

These wastes can be used by simple moistening or untreated, pasteurized, fermented or sterilized for cultivation oyster mushroom (Sharma *et al.*, 2013). Supplements or additives are normally used in the substrate of oyster and other mushrooms to enhance the yield of the mushroom or increase the bio-efficiency (Neelam *et al.*, 2014). It has been reported that increase in the nitrogen content of substrates leads to larger mycelial growth and reduces fruiting bodies formation in Basidiomycetes (Chang and Miles, 1989). Considering the tremendous nutritive values of edible mushrooms and their bioconversion ability we are attempting the production of these nutritionally versatile food sources particularly oyster mushroom from locally available agro-waste materials and also to make it available throughout the year across different seasons.

Population of the world is growing fast and this implies that there are more mouths to be fed. The major sources of protein for human consumption are of plant and animal origin which require considerable time for their production. Other sources of protein are being explored to combat protein deficiency. One of these alternatives is mushrooms which are easy to cultivate and requires short time with very low inputs. However, these mushrooms are not readily available throughout the year as they are seasonal and they grow, they easily get spoiled (Uddin *et al.*, 2011. Moreover, the edible mushrooms collected from the wild are usually contaminated with dangerous fumigants which may have been sprayed to protect the economic trees on which these mushrooms are generally found. This study is aimed at intensive production of this important food source using waste materials generated from agricultural practices which could promote their wider use and provides solution to protein deficiency at affordable price in Nigeria.

2.0 Materials and methods

2.1 Source of mushroom spawn and preparation

The spawn of the oyster mushroom used for this study was obtained from the stock of the Cocoa Research Institute, Ibadan, Oyo state, Nigeria.

2.2 Preparation and Inoculation of substrate

Substrate used for the domestication experiment was formulated from the wood dust of *Gmelina arborea* a lignocellulosic waste of wood processing centre in Owo. The following materials were added to the dust; 9.0 % wheat bran, 1.0 % $CaCO_3$, 0.05 % NH_4Cl, 0.03 % each of Potassium phosphate and Magnesium sulphate. These were thoroughly mixed and water was added until it was approximately 67 %. This preparation was filled inside transparent polythene bags and sterilized by autoclaving for 5 hours as described by Hoa *et al.* (2015). The prepared substrate was allowed to cool to room temperature. Thereafter, each bag containing sterilized substrates was inoculated with two spoonful of the spawns.

2.3 Incubation and harvest

The inoculated substrate bags were placed in a dark mycological room where the temperature and relative humidity were maintained at 28±2 °C and approximately 70 % respectively. The mycelial ramification was monitored on weekly basis until the entire substrate was covered with the mushroom mycelia. After complete ramification, the substrates were moved to fruiting room where room temperature was maintained and the relative humidity increased to about 90 % by generating water vapour in the room.

2.4 Samples Preparation

Freshly harvested mushroom samples were gently cleaned and then dried in the hot air oven at 65 °C until constant weight was achieved.

2.5 Proximate and Mineral analysis

Proximate composition including moisture content; crude protein, lipid content, crude fibre, ash content and nitrogen free extract was determined according to the method of AOAC (2005). The mineral content including N, P, K, Ca and Mg were assessed by atomic absorption.

2.6 Statistical analysis

The growth experiment was done in five replicates and data obtained subjected to a one way analysis of variance (ANOVA) and Duncan's multiple range test was used to compare the mean at $p < 0.05$ using the SPSS software (version 25).

3.0 RESULTS AND DISCUSSION

The results of the growth response of the mushroom grown on different substrates formulated from three different trees is presented in figure 1. The figure shows that 7 days after inoculation, the ramification of the mycelia of the growing mushroom varies with the species of the plant used in the substrate formulation. The mushroom grown on *P. erinaceus* had the widest radius of 4.23 cm followed by 3.17 cm in *I. gabonensis* while the least radius of 1.82 cm was recorded in those grown on *I. gabonensis*. However, at day 21 the ramification radius of the mushroom mycelia was higher in those grown on *I. gabonensis* (9.15 cm) compared with those grown on *P. guineense* and *P. erinaceus* which were comparable. The differences in the mycelial growth pattern may be attributed to the differences inherent in these woods such as phytoconstituents and other bioactive substances as well as the nutrient density in the wood resources. Oyster mushroom can easily be grown on agricultural waste and does not require compositing as a step in its cultivation. The slower growth of the mycelia on the *I. gabonensis* substrate may be due to the presence of inhibitory substances in the wood which may have hindered the growth of the ramifying mycelia. Although, both *I. gabonensis* and *P. erinaceus* are classified as hard woods, they may contain different phytochemicals and nutrients which may explain why the mycelial growths were different. However, the lower growth response of the mushroom to substrate formulated from *P. guineense* is expected as reports abound that mushrooms grow less on soft woods compared with hard woods (Chang and Miles, 2004).

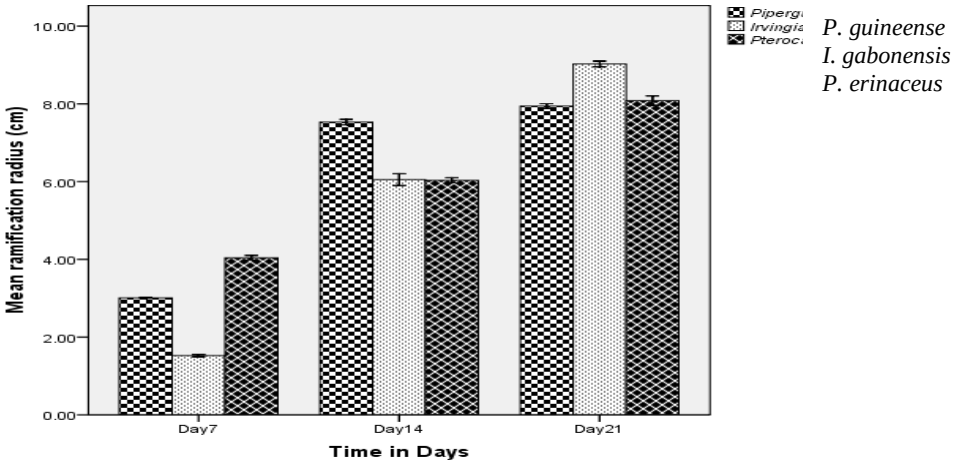

Figure 1: **The radius of the ramifying mycelia of *P. ostreatus* grown on dusts of three different plants**

Oyster mushrooms has been reported to be cultivated and grown on different types of substrates such as wheat straw, grass, wood shavings, sawdust, compost waste and other organic nutrients. It is reported that *Pleurotus* species can efficiently degrade agricultural wastes and they grow at a wide range of temperatures. In comparison to other edible mushrooms, *Pleurotus* species need a short growth time, this was evident in the complete ramification of the mycelia on the substrates after 21 days. However the time taken to fruitification was slower in this study compared with earlier reports (Stamets, 2005). *Pleurotus* species are reported to require carbon, nitrogen and inorganic compounds as their nutritional sources. The main nutrients are less nitrogen and more carbon so materials containing cellulose, hemicellulose and lignin can be used as mushroom substrates. Oyster mushroom can grow on a wide variety of substrate as shown in the growth of the mushroom on the three different substrates used in this study. However, the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates (Sharma *et al.*, 2013).

The time taken for complete maturity of the mushroom is similar to the study carried out by Siwulski *et al.* (2019), who also reported spawn running of *P. ostreatus* completed within 17–20 days on different substrates. The variation in the number of days taken for a spawn to complete colonization of a given substrate is a function of the fungal strain, growth conditions and substrate type (Chang and Miles, 2004). Similarly, the period for the pin head formation (fruit initiation stage) was found to be in the range of 25 days in this study which was also found similar to the study carried out by Fan *et al.* (2000), they reported pin-head formation of oyster mushroom cultivated in different substrates ranged from 20–23 days.

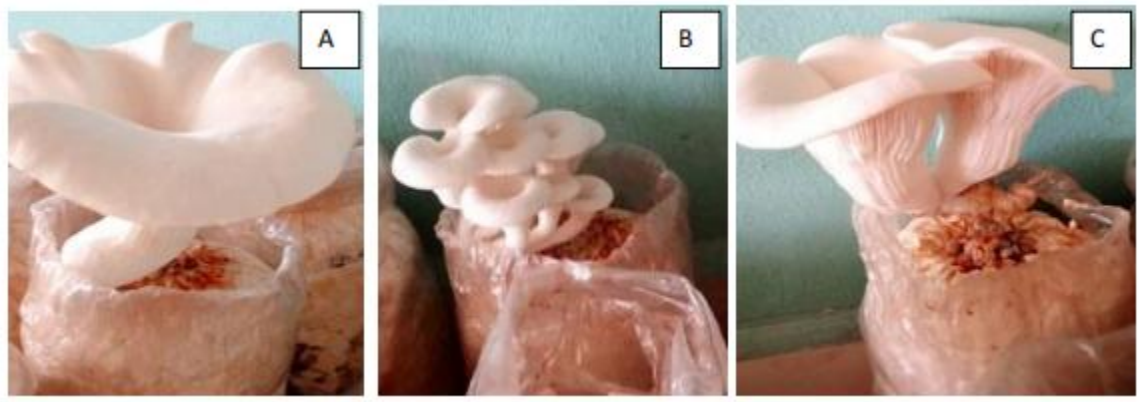

Plate 2: Matured *Pleurotus ostreatus* mushroom cultivated on *P. guineense (A)*, *I. gabonensis (B)* and *P. erinaceus* dusts

Overall, the *Pleurotus ostreatus* showed a better yield performance (92.15 %) in *I. gabonensis* dust with respect to the dry weight of the harvested mushrooms as compared to others which had 84.06% and 78.8 % dry weight in *P. guineense* and *P. erinaceus* respectively. Siwulski *et al.* (2015), has explained that hard wood dust is suitable substrates for *Pleurotus ostreatus*, which was also proved by this study. The increase in the yield of mushroom in the *I. gabonensis* dust may be due to easier way of getting glucose from the cellulosic substances. Whereas, *P. guineense* and *P. erinaceus* sawdust also contain high amount of lignin. Low degradation of lignocellulosic substances in their dusts by *P. ostreatus* might be another factor affecting the overall low yield values from these plants (Sharma *et al.*, 2013).

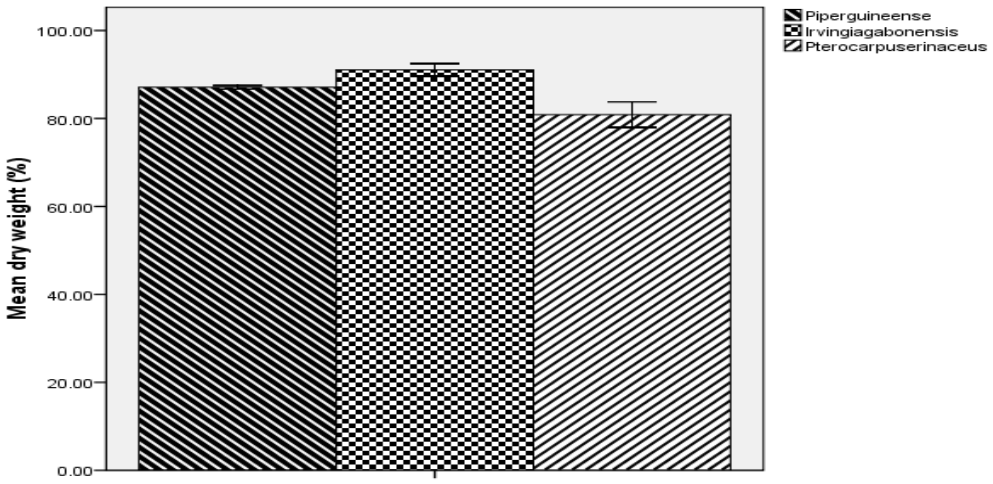

Figure 2: Comparison of yield potential of the *P. ostreatus* Mushrooms cultivated on three different substrates (%)

The effect of the various substrate on the proximate composition of the mushrooms is presented on table 1. The least moisture content was found in sample grown on P. erinaceus (7.58 %) while the highest was found in sample grown on P. guineense. The lowest fat (0.38 %) and fibre (10.12 %) content were found in sample grown on P. guineense whereas protein (27.15 %), ash (4.09 %) and carbohydrate (51.68 %) were higher in sample grown on I. gabonensis compared with the other samples. The low moisture content of the mushrooms may be due to the poor nature of this substrate in water holding capacity. The result was quite close to the value stated by Anchang (2014) who reported that 8.56~9.34% moisture content of *Pleurotus sajor-caju.* Similar moisture content (8.0~9.2%) was reported for *Pleurotus* species grown on different agrowastes (Ahmed *et al.*, 2013). Moisture content have also been reported to be influenced by mushroom age, growing environments, mushroom strains, and postharvest environments. The results also indicated that the fruiting bodies of oyster mushrooms grown on all the substrates are quite rich in protein, carbohydrate, fiber and low in fat content making them excellent foods that can be used in low caloric diets. The values obtained in this study was slightly similar to the range of Angadam *et al.* (2021) in which the protein on dry matter basis in oyster mushroom could range between 20~40%. The differences in protein content of the mushrooms could be due to the varying nitrogen content of substrates. The result was also in line with Bhattacharjya *et al.* (2008), the protein contents of mushroom depended on biological, chemical differences and the C/N ratio of substrates. The fiber content obtained in this study is higher than that reported by Tesfaw *et al*. (2015) as 5.97~6.42%. The ash content in this experiment was in accordance with the value reported by Mashudu *et al*. (2015) when *P. sajor-caju* was grown on different lignocellulosic wastes. Substrate not only affected on protein, carbohydrate, and fat but also influenced on total energy of oyster mushrooms.

Table 1: Proximate composition of oyster mushroom grown on substrate formulated from three different plant

Parameter	*P. guineense*	*I. gabonensis*	*P. erinaceus*
Moisture	9.19 ± 0.01^{c}	8.05 ± 0.00^{b}	7.58 ± 0.00^{a}
Protein	27.15 ± 0.00^{b}	28.20 ± 0.02^{c}	25.72 ± 0.05^{a}
Fat	0.38 ± 0.00^{a}	0.41 ± 0.01^{b}	0.44 ± 0.02^{c}
Fibre	10.12 ± 0.03^{a}	12.19 ± 0.01^{b}	13.17 ± 0.01^{c}
Ash	3.68 ± 0.02^{a}	4.09 ± 0.05^{b}	3.59 ± 0.00^{a}
Carbohydrate	49.87 ± 0.01^{a}	51.68 ± 0.12^{b}	50.45 ± 0.15^{a}

The mineral content of the *P. ostreatus* mushrooms grown on the dusts are presented in table 2. The Nitrogen content of the sample grown on Piper dust (6.28 mg/g) was significantly lower than those grown on Irvinia (7.16 mg/g) and Pterocarpus (6.73 mg/g). Phosphorus was significantly higher in mushrooms cultivated on Irvinia dust (0.71 mg/g) compared the others. Moreover, the calcium content of those mushrooms cultivated on Irvinia dust (0.93 mg/g) was significantly higher than others. Both magnesium and potassium contents were not significantly different in all the samples grown on different saw dusts. The nitrogen contents were predominantly high in all samples cultivated on different saw dusts compared with Calcium (Ca), Magnesium (Mg), Phosphorus (P) and Potassium (K). The contents of these minerals are in agreement with earlier works. It has been reported that the level of minerals in mushrooms is related to the species of mushroom, collecting area of the sample, age of fruiting bodies and mycelium, and distance from any source of pollution. Minerals such as Ca, K, Mg, N and P are essential minerals, since they play an important role in biological systems (Funda *et al.*, 2017).

Table 2: mineral content of oyster mushroom grown on substrate formulated from three different plants

Mineral	*P. guineense*	*I. gabonensis*	*P. erinaceus*
Nitrogen	6.28±0.01[a]	7.16±0.01[b]	6.73±0.00[ab]
Phosphorus	0.56±0.01[a]	0.71±0.01[c]	0.69±0.01[b]
Potassium	1.04±0.01[a]	1.00±0.01[a]	1.10±0.01[a]
Calcium	0.70±0.01[a]	0.93±0.01[c]	0.89±0.01[b]
Magnesium	0.81±0.01[b]	0.75±0.01[a]	0.84±0.01[b]

When mineral concentration levels are low in human diet, it can cause morphological abnormalities, reduce growth and increase mortality and mutagenic effects in humans. The level of the minerals in the mushrooms cultivated on these agrowastes falls within the average daily required allowance recommended by the WHO. This daily nutrient intake is likely to pose no risk of adverse effects. The uptake of mineral ions in mushrooms is different from plants in many ways. For this reason, the concentration variations of minerals depend on mushroom species and their ecosystems. The trace metal contents in the mushrooms are mainly affected by acidic and organic matter content of their ecosystem and soil (Ashraf *et al.*, 2013).

CONCLUSION

From the results obtained in this study, it can be concluded that the substrates formulated from the three species of plants (*P. guineense*, *I. gabonensis* and *P. erinaceus*) dust supported the growth of P. ostreatus. However, substrate from *I. gabonensis* gave the best performance as revealed in the mycelial ramification as well as the yield of the mushroom. However, optimization studies should be carried out on the conditions necessary for the growth and development of the oyster mushrooms on *I. gabonensis* dust having given the best results.

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