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Oil palm fruit fibre promotes the yield and quality of *Lentinus squarrosulus* (Mont.) Singer, an edible Nigerian mushroom

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Agricultural production and the agro-food industry furnish large volumes of solid wastes, which when unutilized could lead to environmental pollution. An attempt was made to utilize wastes from the oil palm and timber industries for the cultivation of *Lentinus squarrosulus*, a Nigerian edible mushroom. Mahogany sawdust (MSD), *Gmelina* sawdust (GSD), oil palm fruit fibre (OPFF) and oil palm empty fruit bunch (OPEFB) significantly influenced crop cycle time, yield, nutritional properties and market quality of the mushroom. The shortest crop cycle time achieved (47 days) was with *Gmelina* sawdust. Oil palm fruit fibre proved a better substrate for the production of mushrooms with higher yields and protein content (30.10 g/kg substrate and 27.42%). Yield and protein content of harvested mushrooms were strongly correlated with the nitrogen content of the substrates. Fruit bodies with the lowest fat content were harvested from *Gmelina* sawdust. Fat contents of the mushrooms showed a positive and significant correlation with the cellulose content of the waste. Oil palm fruit fibre yielded the highest quality mushrooms, with 26% in the >7 cm group while GSD and OPEFB had 0% in the same quality group. Considering the desirable characteristics of yield, protein content and market quality, OPFF proved to be a good substrate which could be exploited for large scale production of *L. squarrosulus*.

Key words: *Lentinus squarrosulus*, yield, market quality, crop cycle time.

INTRODUCTION

Huge quantities of a wide variety of organic wastes are generated annually through the activities of the agricultural, forest and processing industries. Sadly, much of these wastes are either burnt, shredded or used as landfill even though these wastes constitute a potentially valuable resource and can be recycled for the production of edible food in the form of mushrooms for man (Chang, 1996). Oil palm 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

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Abbreviations: MSD, Mahogany sawdust; **GSD,** Gmelina sawdust; **OPFF,** oil palm fruit fibre; **OPEFB,** oil palm empty fruit bunch; **PDA,** potato dextrose agar; **CRD,** completely randomized design; **DMRT,** Duncan multiple range test.

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 high potential to be used as mushroom growing substrate without any further treatment (Abd Razak et al., 2012). On the other hand, sawdust is the most popular basal ingredient used in the formulation of substrate for producing various types of mushroom and is abundantly available during timber processing. Christopher and Custodio (2004) revealed that hardwoods like mahogany contain much higher amounts of structural carbohydrate than softwood trees and hence have more nutrients that could be utilized by mushrooms for their growth and fructification.

Lentinus squarrosulus is a highly prized Nigerian mushroom, which is appreciated for its meaty taste and texture (Kadiri, 2005). The fungus is widely distributed across sub- Saharan Africa and many parts of Asia and is currently attracting interests due to its rapid mycelia growth and potential for use in food and biodegradation (Isikhuemhen et al., 2012). Several reports have shown that various lignocellulosic residues from agro-industrial sector, such as oil palm and timber wastes among others, can provide this mushroom with nutrients required for its spawn run and fructification which under controlled conditions and procedures result in an optimum mushroom yield (Okhuoya et al., 2005; Ayodele and Akpaja, 2007). Nutritional compositions of mushrooms are known to vary with strain, substrates, cultivation and fruiting conditions and the developmental stage of the mushroom (Jo et al., 2013). Due to the development of varying cultivation techniques which in turn affect the chemical compositions of mushrooms, new nutritional data are needed, based on the substrates and the cultivation protocols adopted. The global upsurge in the market for mushrooms could be attributed to their culinary, nutritional and medicinal properties. However, in order to exploit the market potentials of this group of fungi, there is need for better understanding of the influence of locally available organic residues on their marketable qualities such as pilei and stipe sizes. Innovations in cultivation using the abundant low value agricultural residues in Africa could open up new market opportunities through the production of high quality mushrooms with higher demands.

In the present study, four locally available organic wastes; oil palm fruit fibre, oil palm empty fruit bunch, sawdust of mahogany and *Gmelina arborea* were utilized for the cultivation of *L. squarrosulus*. The influence of these wastes on the crop cycle time, yield, nutrient compositions and market quality of the fruit bodies were evaluated.

MATERIALS AND METHODS

Sources of materials

Stock culture of *L. squarrosulus* used for this study was obtained from the Pathology Unit of Forestry Research Institute Ibadan, Nigeria. The culture was maintained on potato dextrose agar (PDA, Difco) until used for spawn preparation. Fresh hardwood sawdust of mahogany (*Khaya ivorensis*) and *G. aborea* were collected from a local sawmill in Nsukka, Nigeria while the oil palm empty fruit bunch (OPEFB) and oil palm fruit fibre (OPFF) were sourced from a local oil palm industry in Nru, Nsukka, Nigeria.

Spawn preparation

The spawn was prepared using Sorghum (*Sorghum bicolor*) grains as substrate. Jars measuring 11 × 9 cm each were filled with parboiled sorghum grains (75% water) to three quarter full and covered with cotton wool. The jars were autoclaved at a temperature of 121°C and 103 kPa pressure for 1 h and allowed to cool down overnight. Each jar was inoculated with the mycelium growing in a 9 cm diameter Petri dish under aseptic conditions on a clean bench. The inoculated grains were incubated at 25 ± 2°C for one to two weeks in the dark. After the grains had been fully colonized, they were stored in a refrigerator at 4°C until required.

Substrate preparation and spawning

Fresh OPEFB chopped into small pieces of about 1 to 5 cm and OPFF were soaked in distilled water overnight in order to wash out the remaining oil in the fibre and to gain 75% moisture content (Chiejina and Olufukunbi, 2010). The moisture content of the sawdust was adjusted to 75% with distilled water by sprinkling using the squeeze test method (Oei, 1991). Three hundred grams oven-dry-weight equivalent of the moistened substrates were filled into 10 high porosity polypropylene plastic bags measuring 17.5 × 15 cm each. Thereafter, the mouth of each bag was plugged with cotton wool and covered with aluminium foil paper. The bags were autoclaved at a temperature of 121°C and 103 kPa pressure for 1.5 h. Sterilized substrates were allowed to cool down to ambient temperature. The bags were randomly picked and spawned with 25 g spawn per 500 g substrate (5%, w/w) under aseptic conditions and incubated at a temperature of 25 ± 2°C in the dark.

Fruit body induction and harvesting

After complete mycelia colonization of the substrates, bags were exposed to temperature of 25 ± 2°C with a 12 h photoperiod in a mushroom growth room. Fruiting bags were sprayed daily with sterile water using a hand sprinkler and free water was placed in a reservoir on the floor to maintain high humidity. Fresh air was circulated in the growing room using an electric fan. Fruit bodies were harvested three days after primordia emergence when the lamellae were fully exposed. Average number of days to spawn run, primordia initiation and fruit body development were recorded. Stipe height and pileus diameter of harvested fruit bodies were measured and expressed in (cm) while yield was the average fresh weight of the mushrooms harvested per kilogram dry weight of substrate and expressed in (g/kg substrate). The market quality of the harvested basidiocarps was evaluated as described by Rossi et al. (2003). Marketable mushrooms were more than 3 cm in diameter and those of highest commercial value were those with diameter of more than 5 cm.

Analytical methods

Cellulose, hemicellulose, lignin (Datta, 1981) and nitrogen (N) (AOAC, 2002) contents of the organic waste substrates were determined before mushroom cultivation on an air dry weight basis. Moisture content of mushrooms was determined by direct

Table 1. Chemical analysis of the main constituents of oil palm fruit fibre (OPFF), oil palm empty fruit bunch (OPEFB), *Gmelina* sawdust (GSD) and mahogany sawdust (MSD) before mushroom cultivation.

Substrate	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Nitrogen (%)
OPFF	44.29	12.00	8.50	1.36
OPEFB	42.86	11.50	10.00	0.69
GSD	27.14	13.50	17.50	0.30
MSD	51.71	13.00	19.30	0.20

Table 2. Mean crop cycle time of *Lentinus squarrosulus* mushrooms grown on oil palm fruit fibre (OPFF), oil palm empty fruit bunch (OPEFB), *Gmelina* sawdust (GSD) and mahogany sawdust (MSD).

Substrate	Spawn run (days)	Primordia induction period (days)	Fruit body formation (days)
OPFF	6.40^{d}	39.40^{a}	48.80^{a}
OPEFB	9.90^{c}	34.40^{b}	47.30^{ab}
GSD	15.40^{a}	28.20^{c}	46.60^{b}
MSD	12.30^{b}	33.30^{b}	48.60^{a}

* Means followed by the same letter(s) in the same column are not significantly different at $p > 0.05$ according to DMRT.

oven-drying to constant weight at 105°C in a hot air oven. Mushroom samples harvested from the same substrates were mixed and homogenised before analyses. The fruiting bodies were oven dried at 60°C in a hot air oven and analysed for nutrient contents; total nitrogen, crude fibre, ash and crude fat using standard procedures (AOAC, 2002). The crude protein content was calculated by using the adjusted conversion factor (N x 4.38) for mushroom protein, due to the significant content of non-protein nitrogen in the form of glucosamine in mushrooms chitinous cell walls (Shashirekha et al., 2005).

Experimental design and data analysis

The experimental design was a completely randomized design (CRD) with 10 replicates. Data were subjected to one way analysis of variance (ANOVA) using SPSS program. Mean separation was done using Duncan multiple range test (DMRT). All analysis was done at 5% level of significance. Correlation analyses were carried out to ascertain the level of relationship between the constituents of the organic waste substrates and mushroom growth, yield and nutrient compositions.

RESULTS AND DISCUSSION

Chemical analyses revealed that initial substrate composition varied among the organic wastes substrates evaluated in this study (Table 1). Total nitrogen content (N) determined in OPFF was found to be higher than values recorded for the other wastes. Cellulose contents of the wastes substrates varied between 27.14 and 51.71% in GSD and MSD, respectively. Tshinyangu and Hennebert (1995) demonstrated that the nutrient composition of substrates is one of the most important factors limiting saprobiotic colonization and fruiting of cultivated mushrooms. The influence of the different organic waste substrates on the duration of colonization phase and primordia induction period of the mushroom is presented on Table 2. OPFF supported the fastest substrate colonization for the mushroom followed by OPEFB, while spawn run was completed after 13 and 16 days on MSD and GSD, respectively. The shortest spawn run on OPFF could be attributed to the waste providing adequate aeration for the growth of the mushroom mycelia. Tinoco et al. (2001) observed that the larger the surface area and pore size of substrates, the more the mycelial growth rate. In the present study, spawn run showed significant positive correlation with lignin content of the waste (Table 5). It is however reasonable to assume that the low lignin content of OPFF could have resulted to the vigorous colonization of the waste by the fungus. Previous studies had shown that high lignin contents of substrates impede mycelial growth as cellulose may not be readily available as carbon source (Freer and Detroy, 1982).

From results presented in Table 2, GSD supported the shortest primordia induction period (29 days) despite presenting a long colonization phase which lasted for 16 days, while on OPFF the pin initiation process commenced considerably later (after 40 days). Results of the correlation studies show that primordia induction period was positively correlated with N content of the substrates (Table 5). It is therefore rational to assume that the N content of GSD may have played a significant role in the earliness of primordia induction of the mushroom on the waste. This is also the case of OPFF where the high N content of the waste had a negative effect on the primordia induction period despite emerging as the best substrate for spawn run. This result therefore confirms

Table 3. Mean stipe height (cm), pilei diameter (cm), yield (g/kg substrate) and dry weights of *L. squarrosulus* harvested from mahogany sawdust (MSD), *Gmelina* sawdust (GSD), oil palm fruit fibre (OPFF) and oil palm empty fruit bunch (OPEFB).

Substrate	Stipe height (cm)	Pileus diameter (cm)	Yield (g/kg substrate)	Dry weight (g)
MSD	4.50[a]	4.86[b]	16.05[b]	2.78[b]
GSD	2.46[c]	4.06[c]	9.02[c]	1.56[c]
OPFF	3.17[b]	5.76[a]	30.10[a]	4.31[a]
OPEFB	2.10[c]	3.01[d]	4.12[d]	1.06[c]

*Each value is a mean of 10 replicates. Values in the same column followed by the same letter (s) are not significantly different at p > 0.05 according to DMRT.

Table 4. Nutrient compositions of harvested mushrooms from Oil palm fruit fibre (OPFF), Oil palm empty fruit bunch (OPEFB), *Gmelina* sawdust (GSD) and Mahogany sawdust (GSD).

Organic wastes	Protein content (% d.w.)	Crude fibre (% d.w.)	Ash (% d.w.)	Moisture (% d.w.)	Fat (% d.w.)
OPFF	27.42[a]	5.09[a,b]	7.34[a,b]	12.73[b]	0.68[b]
OPEFB	17.57[b]	4.58[b]	6.86[b]	13.33[a]	0.39[c]
GSD	19.79[b]	5.40[a]	7.97[a]	12.28[b]	0.38[c]
MSD	13.27[c]	5.17[a]	6.71[b]	13.33[a]	0.91[a]

*Values (means of at least three replicates) in the same column followed by the same letter (s) are not significantly different at p > 0.05 according to DMRT.

Table 5. Simple correlations between constituents of the organic waste substrates and mushroom growth, yield and nutrient compositions.

Properties	Nitrogen (%)	Cellulose (%)	Lignin (%)	Hemicellulose (%)
Spawn run	-0.9053**	-0.5449	0.8485**	0.8110**
Pin initiation	0.8532**	0.6444*	-0.7491*	-0.7352*
Yield	0.6672*	0.3539	-0.3023	-0.0687
Protein	0.8821**	-0.2630	-0.7108*	-0.2992
Fat	-0.0363	0.7934**	0.3086	0.1505
Fibre	-0.3048	-0.4038	0.6332*	0.9078**
Moisture	-0.0982	0.8473**	-0.0807	-0.5364
Ash	0.0422	-0.9171**	0.0768	0.5114
Pilei diameter	0.4458	0.3167	-0.0348	0.1873

*Significant at P < 0.05, **Significant at P < 0.01

the conclusion previously drawn by Zadrazil and Brunnert (1979) that although N increases yields, above a certain level, it inhibits fruiting. The highest yield (30.10 g/kg substrate) in the current study was obtained from OPFF (Table 3). MSD and GSD also furnished significantly higher crop yields than OPEFB, which was also the least productive as regards mushroom height and pilei diameter. Fruit bodies with the widest pilei diameter and highest stipe height were recorded on OPFF and MSD respectively (Table 3). Correlation studies revealed that yield was positively correlated with N contents of the waste substrates (Table 5). Although, the higher N content of OPFF may have contributed to the significant yield of the mushroom, probably, the residual oil from the waste could also have played an important role in promoting higher yield of the mushroom on OPFF. Earlier reports had shown that plant oils are stimulatory to mushroom mycelial growth and sporophore production, since they are needed for expanding cell membranes and hence result in higher yields (Schisler, 1967; Song et al., 1989). The lowest yield recorded for fruit bodies harvested from OPEFB could be attributed to the complex nature of the waste and/or the presence or little or no vital nutrients needed for mushroom growth in the substrate.

Table 4 shows the influence of the organic waste substrates on the nutritional content of *L. squarrosulus*. Chemical composition of the substrates used in cultivation

Table 6. Substrate effect of *Gmelina* sawdust (GSD), mahogany sawdust (MSD), oil palm empty fruit bunch (OPEFB) and oil palm fruit fibre (OPFF) on *Lentinus squarrosulus* market quality evaluated by cap size group.

Substrate	Market quality groups (cm)				
	<3 cm	3-5 cm	5-7 cm	>7 cm	Deformed
GSD	8.00^{b2}	82.00^{a1}	10.00^{b2}	0.00^{b2}	0.00^{a2}
OPFF	14.00^{b2}	36.00^{c1}	34.00^{a1}	26.00^{a1}	0.00^{a3}
OPEFB	44.00^{a2}	56.00^{b1}	0.00^{b3}	0.00^{b3}	0.00^{a3}
MSD	8.00^{b3}	52.00^{b1}	30.00^{a2}	10.00^{b3}	0.00^{a3}

*Values are expressed as a percentage of the pileus diameter and represent the mean of 10 replicates. Mean values in the same row followed by the same figure (s) are not significantly different at $p > 0.05$. Mean values in the same column followed by the same alphabet (s) are not significantly different at $p > 0.05$ according to DMRT.

has been shown to have a direct effect on the chemical composition of the fruit bodies (Shashirekha et al., 2005). In the present study, protein, crude fibre, ash, moisture and fat contents of the fruit bodies were affected by the substrates. OPFF produced mushrooms with the highest protein content (27.42%) followed by GSD (19.79%) while MSD yielded mushrooms with the lowest protein (13.27%). The fruit bodies of *L*. *squarrosulus* grown on GSD were richer in crude fibre and ash than those harvested from the other waste substrates evaluated in this study. The variations in the protein content of fruit bodies harvested from the different substrates may be attributed to the differential availability of usable nitrogen after spawn run, which in turn influenced the amount of nitrogen available for utilization during sporophore development. From the results presented in Table 5, it is evident that protein content of fruit bodies was positively correlated with N contents of the substrates. This positive relationship justifies the importance of N contents of mushroom cultivation substrates in the production of protein rich fruit bodies. Moreover, N has been reported to be an important nutrient required for fungal growth due to its involvement in protein, chitin and nucleic acid synthesis (Adebayo et al., 2009). In the present study, mushrooms with the lowest fat content (0.38%) were harvested from GSD while MSD stimulated the production of fruit bodies with the highest fat content (0.91%). One of the main reasons why mushroom is a favoured item for human nutrition is its abundance of unsaturated fatty acids and its hypocholestrolemic property (Shashirekha et al., 2005).

Correlation studies between the constituents of the different organic waste substrates and the growth parameters of the mushroom (Table 5) revealed a positive correlation of spawn run with hemicellulose content. Primordia induction period was also found to be positively correlated to cellulose content. These findings were further supported by Moyson and Verachtert (1991) who demonstrated that substrate decomposition by *Lentinula edodes* is initially associated with its hemicellulose content. Gaitan-Hernandez et al. (2011) also reported a positive correlation of days to primordia formation with cellulose content indicating that cellulose in each substrate was directly proportional to primordia formation. In the current study, fibre content of the fruit bodies were significantly positively related with hemicellulose content of the wastes. Fat and moisture contents of the mushroom were also found to be positively correlated with cellulose. However, spawn run showed a significant negative correlation with N content of the substrates. Primordia induction period was also negatively correlated with lignin and hemicellulose contents of the waste. Mushroom market quality in terms of basidiocarp pileus diameter was affected by substrate type (Table 6). OPFF yielded the highest quality mushrooms, with 26% in the >7 cm group while GSD and OPEFB had none in the same quality group (Table 6). GSD stimulated highest production (82%) of basidiocarp in the 3 to 5 cm size group (Table 6). OPEFB had many of the basidiocarps in the 3 to 5 and <3 cm size groups with 56 and 44%, respectively (Table 6). It is noteworthy that no fruit bodies were deformed throughout the experiment and hence none was recorded for the deformed group. Mushroom size is essential for its market evaluation since mushrooms with wide pilei could be of interest in the promotion of mushroom marketing. Although, the large sized fruit bodies harvested from OPFF and MSD are considered to be of good market quality and are rated highly, Shen and Royse (2001) observed that this could be an inferior quality since such fruit bodies tend to break during packaging thereby reducing their quality. In the present study, GSD and OPEFB also produced much of the mushrooms in the 3 to 5 cm quality group which are also marketable. The differences in mushroom quality in the different waste substrates could be due to the nutrient status and the nature of lignocellulose in the respective wastes. This observation corroborates the findings of Fung et al. (2005) who demonstrated that nutrient

concentration in the substrates determined the productivity and quality of the mushroom crop.

Conclusion

The results of the present study indicate that unsupplemented and uncomposted locally available organic wastes (MSD, GSD, OPFF and OPEFB) could support the growth and fructification of *L. squarrosulus*. It also showed that *L. squarrosulus* with good yields, high protein and market quality could be cultivated using OPFF. This is of particular interest in the developing countries where the availability of cheap sources of protein is paramount in order to counter protein malnutrition. The current study also shows that the overall nutritional properties of the fruit bodies harvested from GSD is promising. However, due to the low yield recorded on GSD, there is need for detailed investigations on the supplementation of this waste with nitrogen sources to increase yield.

Conflict of interests

The authors did not declare any conflict of interest.

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