CROP CYCLE TIME, YIELD AND PROTEIN CONTENT OF *LENTINUS SQUARROSULUS* PRODUCED ON SOME ORGANIC WASTES

NNEKA VIRGINIA CHIEJINA AND DANDY AHAMEFULA OSIBE*
Department of Plant Science & Biotechnology, University of Nigeria, Nsukka, 410001
dandy.osibe@unn.edu.ng

ABSTRACT

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 production of *Lentinuss quarrosulus*, 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 and protein content of the mushroom. The shortest crop cycle time achieved (47 days) was with *Gmelina* sawdust, while oil palm fruit fibre produced mushrooms with the highest protein content (27.42%). Although, there were no significant differences ($p > 0.05$) in yield of mushrooms produced with oil palm fruit fibre and mahogany sawdust, oil palm fruit fibre gave the highest and most consistent yields. Considering the desirable characteristics of yield and protein content, oil palm fruit fibre proved the best waste for commercial production of *L. squarrosulus*.

Keywords: *Lentinus squarrosulus*, yield, protein content, crop cycle time.

INTRODUCTION

Nigeria is one of the largest timber and oil palm producing countries in the world. Unfortunately, much of the wastes from the oil palm and timber industries are either burned, shredded or used as landfill even though these wastes constitute a potentially valuable resource and can be recycled for the production of edible food for man [1]. Several reports have shown that various lignocellulosic residues from agro-industrial sector, such as oil palm and timber wastes among others, can produce the mushroom with nutrients required for spawn run and fructification, which under controlled conditions and procedures result in an optimum product yield [2].

Sawdust is the most popular basal ingredient used in the formulation of substrate for producing various types of mushroom. Christopher and Custodio [3] 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. 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 contain heterogenous water insoluble materials consisting of cellulose, hemicelluloses and lignin and to a lesser extent pectin, starch and other polysaccharides [4]. The fruit fibre has been shown to possess high potential to be used as mushroom growing substrate without any further treatment [5].

The greatest difficulty in feeding humans is to supply a sufficient quantity of the body building protein [6]. In Africa, the gap between the increasing population and supply of protein is somewhat wide since the traditional sources of protein have not kept pace with population growth. In view of the general shortage of animal protein in some developing countries, the need to explore vegetable protein as an alternative source has been duly recognized. Mushrooms are a nutritious food source being rich in protein, vitamins and minerals. The Food and Agricultural Organization (FAO) recognizes mushrooms as food contributing to the protein nutrition of the countries which depend largely on cereals, because of their high protein quantity and quality [7].

The objective of this study was to evaluate the suitability of locally available organic wastes for the production of *L. squarrosulus*. The study therefore, outlines the production protocols for *L. squarrosulus* that can be adopted by rural farmers for commercial, nutritional and conservation purposes.

MATERIALS AND METHODS

Sources of materials

Stock culture of *Lentinus squarrosulus* used for the experiment was obtained in January 2013 from the Pathology Unit of Forestry Research Institute Ibadan, Nigeria. Fresh hardwood sawdust of mahogany (*Khaya ivorensis*) and *Gmelina aborea* were collectedfrom a local sawmill in Nsukka, Nigeria while the oil palm empty fruit bunch (OPEFB) and oil palm fruit fibre (OPFF) were obtained from a local oil palm industry in Nru, Nsukka and Nigeria in February 2013.

Spawn preparation

The spawn was prepared using Sorghum (*Sorghum bicolor*) grains as substrate. Empty salad cream jars 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 ^{o}C and 103 KNM^{-2} pressure for 30 min and allowed to cool down overnight after which they were inoculated with the pure culture of *Lentinus squarrosulus* under aseptic conditions in the inoculation chamber. The inoculated grains were incubated at room temperature (25 ± 2 ^{o}C). After the grains had been fully colonized, they were stored in a refrigerator at 4 ^{o}C until required.

Substrate analysis

The cellulose, hemicellulose and lignin contents of the organic wastes were determined before mushroom growth by the method of Datta [8] while the nitrogen content of the wastes was determined by the method of AOAC [9].

Substrate preparation

The fresh OPEFB (chopped into small pieces of about 1-5 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. The moisture content of the sawdust was adjusted to 75% with distilled water by sprinkling. Three hundred grams oven-dry-weight equivalent of the moistened substrates were each filled into ten (10) high porosity polypropylene plastic bags measuring 17.5x15 cm each. A polyvinyl chloride pipe measuring 5 cm wide and 3 cm long was passed through the top of each bag. Thereafter, the mouth of each bag was plugged with cotton wool and covered with foil paper. The bags were autoclaved at a temperature of 121 ^{o}C and 103 KNM^{-2} pressure for 30 min.

Substrate spawning and incubation

Autoclaved bags 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) [10] under aseptic conditions and incubated at a temperature of 28-30 ^{o}C .

Fruit body induction and harvesting

Bags were transferred to a growing room once primordia had formed. Fruit body induction was achieved by reducing the temperature of the environment by spraying water into the room and opening of the mouth of the bags. Fruiting bags were sprayed daily with sterile water using a hand sprinkler and water was placed in a reservoir on the floor to maintain humidity at about 85%-90%. Fresh air was circulated in the growing room using an electric fan. The room was lit on a 12 h on/off cycle using an electronic fluorescent lamp of 85 watts. Fruiting bodies were harvested three days after primordia emergence when the lamellae were fully exposed.

Determination of crop cycle time and yield

Data were collected on the average number of days to spawn run, primordia initiation and fruiting body development on the different wastes. Stipe length and pileus diameter of harvested fruiting bodies were measured and expressed in centimetres (cm) while yield was expressed as the average fresh and dry weights of the mushrooms harvested per bag and expressed in (g). Harvested mushrooms were placed in a paper bag and oven dried at 60 ^{o}C for 48 h. Dried mushrooms were placed

on a laboratory bench for 2 h to cool down before weighing. Mushroom weights were determined using an Ohaus weighing balance (Model AR3130, 0.001 g accuracy, made in England).

Protein Analysis

The protein analysis was conducted at the National Centre for Energy Research and Development, University of Nigeria, Nsukka. The mushrooms harvested from the different wastes were analyzed for their protein content using Micro-Kjeldahl's method as described in AOAC [9].

Experimental design and data analysis

The experiment comprising four substrate types (mahogany sawdust, *Gmelina* sawdust, oil palm fruit fibre and oil palm empty fruit bunch) was laid out using a Completely Randomized Design (CRD) with ten replicates per treatment.

Data on mushroom yield and crop cycle time were analyzed by one way ANOVA using SPSS version 17.0. Mean separation was done using Duncan Multiple Range Test (DMRT). All analysis was done at 5% level of significance.

RESULTS AND DISCUSSION

Mushroom crop cycle time

Variations in the number of days to spawn run, primordia development and fruiting body formation as influenced by the organic wastes can be seen in Table 1. The shortest spawn run of the mushroom (6.4 days) was observed on oil palm fruit fibre. Analysis of variance showed that mushroom spawn run varied significantly ($p < 0.05$) in all the organic wastes

Table 1. 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 development (days)	Fruit body formation (days)
OPFF	6.4^{d}	39.4^{a}	48.8^{a}
OPEFB	9.9^{c}	34.4^{b}	47.3^{ab}
GSD	15.4^{a}	28.2^{c}	46.6^{b}
MSD	12.3^{b}	33.3^{b}	48.6^{a}

ð Means followed by the same letter (s) in the same are not significantly different at $p > 0.05$ according to Duncan multiple range test (DMRT).

evaluated. Pinheads emerged as small rounded lumps that were grouped at various parts of the substrate surfaces. The rate at which the mushroom developed mycelium on oil palm fruit fibre could probably be due to the residual oil and the appreciable nitrogen content of the waste (Table 2). Nitrogen content of substrates has been shown to affect earliness of fructification and productivity of mushrooms [11]. Schisler and Patton [12] had also reported that lipids are stimulatory to mushroom growth. Substrate structure is an important factor for the growth of fungal mycelium as it should allow for the ramification of the fungus. The shortest spawn run on oil palm fruit fibre may be attributed to the waste providing adequate

Table 2. 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	8.50	17.50	0.30
MSD	51.71	10.00	19.30	0.49

aeration for the ramification of the mushroom mycelia. Tinoco *et al*. [13] observed that the larger the surface area and pore size of substrates the more the mycelium growth rate. It is worth noting that *Gmelina* sawdust showed, on the average, the slowest rate in spawn run (Table 1). However, in terms of fruiting, it had the shortest time for primordia development and fruiting body formation. This probably suggests that *Gmelina* sawdust may contain some kind of compound that induces the development of mushroom reproductive phase. The shortest crop cycle time of the mushroom on *Gmelina* sawdust could also be due to the initial composition of the waste, which may present a C:N ratio that delays spawn run but accelerates the formation of fruiting bodies as demonstrated by Stamets [14] for some mushroom species.

Mushroom yield parameters

The various organic wastes significantly influenced the fresh and dry weights, stipe height and pilei diameter of the mushroom (Table 3). The highest mushroom mean fresh weight (16.05 g) and dry weight (2.78 g) were obtained from oil palm fruit fibre; however, fresh weight was not significantly different ($p > 0.05$) from the mahogany sawdust (Table 3). Mushrooms with the highest mean stipe height (4.5 cm) were harvested from oil palm fruit fibre while mahogany sawdust produced fruiting bodies with the widest mean pilei diameter (5.76 cm) (Table 3). The considerable yield of the mushroom in this experiment on oil palm fruit fibre may be attributed to the residual oil of the waste. Reports have shown that developing sporophores require a supply of lipids and proteins (ratio 50/50), that are needed for expanding cell membranes and hence, promote higher yields [15]. Mahogany sawdust also influenced yield and pilei diameter of the mushroom. This could probably be due its appreciable cellulose content of 51.71% (Table 2) and compactness on wetting when compared to the other organic wastes evaluated. Thomas *et al*. [16] had earlier demonstrated that cellulose content of substrates is an important factor for fruit body development. The lowest fresh and dry weights recorded for fruit bodies harvested from oil palm empty fruit bunch could be due to the complex nature of the waste and/or the presence of little or no vital nutrients needed for the mushroom growth in the substrate. Schisler [15] associated the reduction in fresh weight of mushrooms to the absence of certain specific nutrients in the substrate required by the mushroom for its growth.

Table 3. Mean stipe height (cm), pilei diameter (cm), fresh and dry weights (g) 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)	Fresh weight (g) per bag	Dry weight (g)
MSD	3.17^{b}	5.76^{a}	15.10^{a}	1.31^{bc}
GSD	2.46^{c}	4.06^{c}	9.02^{b}	1.56^{b}
OPFF	4.50^{a}	4.86^{b}	16.05^{a}	2.78^{a}
OPEFB	2.10^{d}	3.01^{d}	4.12^{c}	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 Duncan multiple range test (DMRT).

Mushroom protein content

The percentage protein content of harvested mushrooms varied significantly as a result of the influence of the various organic wastes. Table 4 shows that the percentage protein content ranged from 13.27% for mushrooms produced from mahogany sawdust to 27.42% for those grown on oil palm fruit fibre. In the present study, harvested mushrooms were evaluated for their nutritional status on the basis of their chemical composition. The observed variations in the protein content of the mushrooms harvested from the different organic wastes may be due to the differential availability of usable nitrogen in the wastes after spawn run which in turn influenced the amount of nitrogen available for the formation of fruit bodies. Nitrogen has been reported to be an important nutrient required for fungal growth due to its involvement in protein, chitin and nucleic acid syntheses [17]. However, other factors

Table 4. Percentage protein content 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 of harvested mushrooms (%)
OPFF	27.42
OPEFB	17.57
GSD	19.79
MSD	13.27

such as the size of pileus, harvest time, stage of development and species of mushrooms have been reported to influence the protein content of the fruit bodies [18]. The crude protein content of this mushroom compared favourably with and in some instances surpassed those reported for most legumes except groundnut and soybeans grown in West Africa [19]. Using this protein content as approximate indices of nutritional quality, it would appear that this mushroom falls between most legumes and meat. However, while the protein content of the mushroom is still lower than that found in eggs, meat and fish, it is adequate to be used as a substitute in the diets of the populace in the developing countries.

CONCLUSION

In conclusion, the dwindling forests and the absence of commercial cultivation of *L. squarrosulus* has resulted to its scarcity with the few available very expensive. This study suggests that many locally available organic wastes have high potentials for utilization as substrates for growth and production of *L. squarrosulus*. Our experiment reveals the possibility of the production of adequate protein containing *L. squarrosulus* on oil palm fruit fibre (OPFF) and the waste is also recommended as a very good substrate for spawn production.

REFERENCES

[1] Chang ST and Buswell JA. (1996). Mushroom nutriceuticals. *World J. Microbiol.Biotechnol.* 12: 473-476.

[2] Fung YW *et al*. (2005). Evaluation of different Colombian agro industrial wastes as substrates for the growth and production of *Lentinula edodes* Berk. Pegler (shiitake). *Acta Edulis Fungi.* 12: 285-290.

[3] Christopher J and Custodio D. (2004). Coco lumber sawdust. In: Gush R (Ed.). Mushroom Grower's Handbook I. Mush world Inc.

[4] Thomsen MH. (2005). Complex media from processing of agricultural crops for microbial fermentation. *Appl. Microbiol. Biotechnol.* 68: 598-606.

[5] AbdRazak DL *et al*. (2012). Comparative study of mycelia growth and sporophore yield of *Auricularia polytricha* (Mont.) Sacc.on selected palm oil wastes as fruiting substrate. *Appl. Microbiol.Biotechnol.* 97: 3207-3213.

[6] Chang ST. (2008). Overview of mushroom cultivation and utilization as functional foods. In: Peter, C. and Cheung, K. (Eds.). *Mushrooms as Functional Foods.* John Wiley and Sons Inc. pp. 1-31.

[7] Kuforiji OO and Fasidi IO. (2009). Biodegradation of agro-industrial wastes by an edible mushroom *Pleurotus tuberregium* (Fr.). *Journal of Environmental Biology.* 30 (3): 355-358.

[8] Datta R. (1981). Acidogenic fermentation of lignocellulose-acid yield and conversion of components. *Biotechnol. Bioeng.* 23 (9): 2167-2170.

[9] AOAC. (2002). *Official Methods of Analysis* (17th Edition). Association of Official Analytical Chemist, Maryland, 1106pp.

[10] Quimio TH *et al*. (1990). Technical Guide for Mushroom Growing in the Tropics. Plant Production and Protection Study. FAO., Rome.

[11] Philippoussis A *et al*. (2007). Productivity of agricultural residues used for the cultivation of the medicinal fungus *Lentinula edodes*. *Int Biodeter. Biodegr.* 59 (3): 216-219.

[12] Schisler LC and Patton TG. (1971). Yield response of selected mushroom strains to vegetable oil supplementation. *Mushroom Sci.* 8: 707-712.

[13] Tinoco R *et al*. (2001). Kinetic differences of purified laccases from six *Pleurotus ostreatus* strains. *Lett. Appl. Microbiol*. 32 (5): 331-335.

[14] Stamets P. (2000). Growing Gourmet and Medicinal Mushrooms (3rd Edition). Ten Speed Press, Berkeley, California, USA.

[15] Schisler LC. (1982). Innovations for efficient mushroom growing. In: Wuest, P. J. and Bengston, G. D. (Eds.). *Pennsylvania State* Handbook for Commercial Mushroom Growers. Pennsylvania Agriculture Experiment Station, University park, Pennsylvania USA. pp. 117-118.

[16] Thomas GV *et al*. (1998). Evaluation of lignocellulosic biomass from coconut palm as substrate for cultivation of *Pleurotus sajor-caju* (Fr.) Singer. *World J. Microbiol. Biotechnol.* 14 (6): 879-882.

[17] Adebayo GJ *et al*. (2009). Evaluation of yield of oyster mushroom (*Pleurotus pulmonarius*) grown on cotton waste and cassava peel. *Afr. J. Biotech.* 8 (2): 215-218.

[18] Wani BA *et al*. (2010). Nutritional and medicinal importance of mushrooms. *Journal of Medicinal Plants Research.* 4(24): 2598-2604.

[19] Okoro IO and Achuba FI. (2012). Proximate and mineral analysis of some wild edible mushrooms. *Afr. J. Biotechnology.* 11(30): 7720-7724.