

Full Length Research Paper

Comparative study of yield performance and nutrient composition of the edible mushroom *Pleurotus pulmonarius*, cultivated on different substrates

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Sawdust from four types of woods (*Milicia excelsa*, *Gmelina arborea*, *Azelia africana* and *Khaya senegalensis*) was used for cultivation of *Pleurotus pulmonarius*. Growth and yield of fruiting bodies were monitored and biological efficiency (BE) determined. Proximate composition of fruiting bodies was determined; total phenol content (TPC) and total flavonoid content (TFC) of the extract from the mushrooms were investigated. Results obtained show significant yield ($P<0.05$) of 361.30 ± 4.55 g/kg substrate (BE = 36.13%) on *G. arborea* followed by *K. senegalensis*. The highest crude protein content ($19.69\pm0.09\%$) was observed in mushrooms harvested on *A. africana*. Crude fibre was significantly ($P<0.05$) higher in mushrooms harvested from *A. africana*. Magnesium, sodium, potassium and calcium content of all the mushrooms were significantly different but all had significantly ($P<0.05$) higher potassium content (3.52 ± 0.11 to 7.09 ± 0.004 mg/kg). The highest TPC and TFC were recorded in mushrooms harvested from *M. excelsa* and the least TPC was observed for mushrooms harvested from *K. senegalensis* while the least TFC was recorded in *G. arborea* and *A. africana*. These findings indicate effective economic bioconversion of sawdust with varying effects on yield, nutritional composition, TPC and TFC of *P. pulmonarius*. *G. arborea* gave a better yield and can be further exploited in various capacities.

Key words: Sawdust, biological efficiency, *Pleurotus pulmonarius*, proximate composition.

INTRODUCTION

Sawdust is one of the major residues or waste produced during milling and re-sawing of wood and is one of the solid wastes available in large quantities in most developing countries. Its proper disposal has constituted a great challenge in most African countries. In Nigeria

and most developing countries in Africa, residue generated from wood processing are regarded as waste and this has led to open burning practices, dumping in water bodies or dumping in an open area which constitutes environmental pollution (Aina, 2006; Aiyelaja

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et al., 2013). It has been reported that less than 5% of the total wood waste generated in Nigeria is being utilized (Ogunwusi, 2014). This leaves huge quantity of wood waste unutilized which also constitute health hazards if not properly disposed and they may also become breeding places for worms and insects (Dosunmu and Ajayi, 2002).

Mushroom cultivation is one of the economically viable biotechnological processes for the bioconversion of plant wastes and residues (Wood and Smith, 1987). Many agricultural and industrial by-products have been used in mushroom cultivation (Chinda and Chinda, 2007). Extracellular enzymes secreted during growth (Cohen et al., 2002) of Basidiomycetes have the capability of degrading lignin in plant cell walls (Yamakawa et al., 1992). This makes them able to decompose and mineralize plant cell components by converting the easily digestible carbohydrates into simpler sugars. Among these basidiomycetes are white rot fungi (WRF) including oyster mushrooms, *Pleurotus* species. Many species of the genus *Pleurotus* (Jacq.:Fr.) P. Kumm. are edible species and have been cultivated on a wide range of lignocellulosic materials (Guzman, 2000). Oyster mushrooms have been shown to have nutritive and medicinal properties such as antitumor, antiviral, antineoplastic and antioxidant potentials and properties (Akinyele et al., 2011; Weigand-Heller et al., 2012; Yashvant et al., 2012). Investigations on the use of agricultural wastes as substrate for cultivation of *Pleurotus* spp. have been reported by a number of researchers (Anyakorah and Olatunji, 2001; Yildiz et al., 2002; Oei, 2003; Peksen and Küçüközü, 2004; Jonathan et al., 2012a).

Pleurotus pulmonarius is an edible fungus belonging to the Class: Agaricomycetes, Order: Agaricales, Family: Pleurotaceae, Genus: *Pleurotus* (Alexopolous et al., 1996; Jonathan et al., 2012b). The cultivation of *Pleurotus* spp. is common but not as much as the white button and shiitake in terms of global mushroom production, with versatility in terms of growth substrates and cultivation conditions (Gyorfi and Hajdu, 2007). The cultivation of mushroom serves as an alternative source of income for small holder farmers (Sánchez and Royse, 2001; Valencia and López, 2005).

In Nigeria, the commercial cultivation of mushrooms is still at the tender stage because most people still collect from the wild during rainy seasons for sale in the local market place. Among the cultivated edible mushrooms in the country, *Pleurotus* spp. ranks the first due to availability of suitable substrate, conducive growth condition and acceptability (Kues and Liu, 2000; Amuneke et al., 2011). Sawdust from different hard wood represents some of the solid waste in the country with the challenge of its proper disposal. The present study was undertaken to demonstrate economical bioconversion of sawdust and determine the most suitable wood sawdust substrate for the cultivation of *P. pulmonarius* and its

effect on the nutrient composition of mushrooms harvested from the substrates.

MATERIALS AND METHODS

Collection of materials

Matured fruiting bodies of the mushroom (*P. pulmonarius*) used for this work were obtained from a commercial mushroom farm along Odo-ona Kekere road within Ibadan, Oyo State, Nigeria. Sawdust was obtained from freshly processed woods at a major sawmill located within Ogbomosho, Oyo State, Nigeria. The sawdusts used as substrates for mushroom cultivation were obtained from four different woods: *Milicia excelsa*, *Gmelina arborea*, *Azela africana* and *Khaya senegalensis*.

Tissue culture

Mycelia were obtained by tissue culture on freshly prepared PDA (Lab M, Neogen, Lancashire, UK) plate according to the method of Stanley (2010). Plates were incubated at 27°C±2 for 5 days when visible mycelia were observed. Subsequently, subcultures were made onto PDA slants and after incubation for visible mycelia growth; slants were stored for further analysis.

Preparation of spawn

Whole rice grains were used as substrates for the planting spawn. The grains were washed to remove dead seeds, shaft and dirt and boiled for 2 min to soften. Boiled grains were drained and spread out to air-dry on absorbent sheets. Two percent (w/w) of CaCO₃ and 4% (w/w) of rice bran were added to the grains mixed thoroughly and filled into clean empty jars till ¾ full, plugged with cotton wool and loosely covered with aluminium foil before autoclaving for 1 h. Autoclaved grains were inoculated with five 9.0 mm mycelia plugs from actively growing margins of 7-days old plate cultures of *P. pulmonarius* and incubated at 27°C±2 until full ramification.

Preparation and spawning of substrate

Sawdust (*M. excelsa*, *G. arborea*, *A. africana* and *K. senegalensis*) were spread separately on a flat surface in the shade to air-dry, sorted separately to remove wood chip and foreign particles. Substrates were then prepared by adding 2% (w/w) CaCO₃ and 4% rice bran to each of the 4 types of sawdusts and mixed thoroughly. Water was added to the mixture to moisten to about 60% moisture content. The indoor method of mushroom cultivation was used (Oei, 1996; Quimio, 2002) and carried out in the mushroom house. The substrate was packed separately in triplicates into heat resistant polythene bags (40×60 cm) with each bag containing 1 kg of the substrate. Polyvinyl chloride (PVC) tubes cut into sizes of 2 inches each were used as the bottle necks plugged with a piece of cotton wool and covered loosely with aluminium foil. Bags were steamed in 200 L capacity drum with tightened lid for 2½ h. The steamed substrates were allowed to cool overnight in the drum and inoculated under aseptic conditions with fully ramified grain spawn. Sterile pins were used to puncture bags randomly. After full ramification of substrates, the PVCs (used as bottle necks for the polythene bags) were removed and the polythene bags cut open for watering and aeration of ramified substrate in order to initiate ramification of substrates, the PVCs (used as bottle necks for the polythene bags) were removed and the polythene bags cut open for watering and aeration of ramified substrate in order to initiate

Table 1. Details of fructification of *P. pulmonarius* (in days) on different sawdust substrate.

Sawdust substrate	Full ramification	Primordia formation	Fruit body formation
ME	19.67±0.67 ^{ab}	25.33±0.33 ^b	29.67±0.33 ^b
GA	18.00±0.58 ^a	21.67±1.20 ^a	27.33±0.67 ^a
AA	21.33±0.88 ^b	27.67±0.58 ^b	34.00±0.58 ^c
KS	20.00±1.00 ^{ab}	28.69±0.33 ^b	35.00±0.00 ^c

Each value is a mean of 3 replicates ± standard error, values accompanied by identical superscript letters are not significantly different ($p \leq 0.05$) in columns. ME is sawdust from *M. excelsa*, GA is sawdust from *G. arborea*, AA is sawdust from *A. africana*, and KS is sawdust from *K. senegalensis*.

fruiting. Fully matured mushroom were harvested after about 5 to 7 weeks of spawning and weighed.

Yield of mushrooms and biological efficiency

Mushroom yield was determined by the division of total weight of fruiting bodies from all flushes (each bag) by the dry weight of the substrate. Biological efficiency (BE) which is defined as the ratio of fresh weight of mushrooms to dry weight of compost at spawning was expressed as a percentage using the following formula:

$$\text{Biological Efficiency (BE)} = \frac{\text{Fresh weigh to fmushroom}}{\text{Dry weight of substrate}} \times 100$$

Determination of proximate composition of fruiting bodies of mushrooms

Harvested fruiting bodies of mushroom were oven-dried at 60°C until constant weight, cooled in desiccators and powdered in an electric blender. Moisture content was determined by the direct oven drying method. The weight loss after oven drying of each sample (1 g) at 105°C to constant weight was expressed as % moisture content (Sivrikaya et al., 2002). The nitrogen content was determined using the micro-Kjeldahl method and crude protein was calculated by multiplying the nitrogen content with a factor of 6.25 (Thimmaiah, 2004). Crude fibre was determined according to the standard method of AOAC (2005) where the sample was digested successively with acid and base. The difference in the weight of the residue obtained after digestion expressed in percentage gave the crude fibre content. Crude fat was determined by using the Soxhlet extraction method using petroleum ether as the solvent (AOAC, 2005). Ash content of 1 g powdered sample was determined as the residue of incineration at 550°C in a muffle furnace (AOAC, 2005). Mineral constituents (magnesium, sodium, potassium, calcium) were determined by atomic absorption spectrophotometry (AOAC, 2005). All proximate analyses of the mushroom powder were carried out in triplicate and reported in percentage.

Sample extraction with ethanol

Extraction was performed according to the method of Tibuhwa (2014). The residual solvent of ethanol extract was removed using a rotary evaporator to dryness. The obtained concentrated extracts were stored in dark at 4°C until further analysis.

Estimation of total phenol content (TPC) of fruiting body

Total phenol content in the mushroom extracts was determined using a Folin-Ciocalteu colorimetric assay following the methods of

Ainsworth and Gillespie (2007) and Chai and Wong (2012) with modifications. A mixture of extract (0.1 mL) and 10% v/v Folin-Ciocalteu reagent from Sigma-Aldrich, St. Louis, USA (0.2 mL) was first incubated at room temperature for 3 min. A volume of 0.8 mL of 700 mM Na₂CO₃ was added and the mixture was incubated at room temperature for 2 h. Absorbance of the mixture was read at 765 nm. A standard curve was prepared from 0 to 42 mg/L gallic acid. Total phenolic content was expressed in mg gallic acid equivalents/g dry matter.

Estimation of total flavonoid content (TFC) of fruiting body

Total flavonoid content in the mushroom extracts was determined following the method of Chai and Wong (2012). Mushroom extract (0.2 mL) was added to 0.15 mL of NaNO₂ and the mixture was incubated at room temperature for 6 min. A volume of 0.15 mL of AlCl₃.6H₂O (10 % w/v) was added to the mixture, which was then left at room temperature for 6 min. Exactly 0.8 mL of NaOH (10% w/v) was added and the absorbance of the mixture was read at 510 nm after standing at room temperature for 15 min. For the blank, the extracts were replaced with water. A standard curve was prepared from 0 to 500 µg/mL quercetin dissolved in 80% ethanol. Total flavonoid content was expressed in mg quercetin equivalents/g dry matter.

Statistical analysis

Each data value was presented as the mean standard error. Differences between the means of individual groups were assessed by one-way analysis of variance (ANOVA) with Duncan's multiple-range test using Statistical Package for the Social Science (SPSS 16.0).

RESULTS

The details of growth and fructification of *P. pulmonarius* from spawning of substrates to development of mature fruiting bodies is shown in Table 1. Time (in days) of full ramification of substrates by mycelia of *P. pulmonarius* and development of mature fruiting bodies was significantly ($P \leq 0.05$) different in the four types of sawdust used. Mycelia growth (spawn run) resulting in the full ramification of the substrates was fastest in *G. arborea* sawdust taking 18.00±0.58 days followed by *M. excelsa*, while *A. africana* took the highest number of days (21.33±0.88) to completely ramify. Formation of primordial was also fastest in *G. arborea* sawdust taking 21.67±1.20 days from spawning but there was no

Table 2. Yield and biological efficiency of *P. pulmonarius* cultivated on different sawdust substrate.

Sawdust substrate	First flush (g)	Second flush (g)	Yield g/kg of dry substrate	Biological efficiency (BE, %)
ME	165.57±2.98 ^b	135.80±2.49 ^b	301.37±3.67 ^b	30.14±0.60 ^b
GA	193.60±5.91 ^c	167.70±4.36 ^c	361.30±4.55 ^d	36.13±0.24 ^d
AA	140.33±4.88 ^a	115.28±5.87 ^a	255.61±2.05 ^a	25.56±0.60 ^a
KS	159.97±2.91 ^b	124.67±5.33 ^{ab}	284.64±2.42 ^c	28.46±2.42 ^c

Each value is a mean of 3 replicates ± standard error, values accompanied by identical superscript letters are not significantly different ($p \leq 0.05$) in columns.

Table 3. Proximate composition (%) of *P. pulmonarius* cultivated on different sawdust substrate.

Sawdust substrate	Moisture	Ash	Crude fibre	Crude protein	Fat
ME	2.17±0.09 ^a	7.75±0.05 ^c	5.54±0.27 ^a	18.70±0.40 ^a	1.85±0.05 ^c
GA	2.98±0.06 ^b	5.92±0.01 ^a	5.89±0.11 ^a	18.15±0.13 ^a	1.56±0.17 ^b
AA	3.72±0.04 ^c	6.91±0.05 ^b	6.73±0.12 ^b	19.69±0.09 ^b	1.19±0.06 ^a
KS	3.03±0.04 ^b	5.64±0.07 ^a	5.56±0.30 ^a	18.67±0.18 ^a	1.55±0.06 ^b

Each value is a mean of 3 replicates ± standard error, values accompanied by identical superscript letters are not significantly different ($p \leq 0.05$) in columns.

significant difference in the number of days for primordial formation in the other substrates. Number of days for fruiting body formation also followed a similar trend with *G. arborea* emerging as the fastest in fruiting body formation but there was no significant difference in the number of days for fruiting body formation on *A. africana* and *K. senegalensis*.

The weight of harvested mushroom in the first and second flushes for all the sawdust substrates used were significantly different at $P \leq 0.05$ and followed a similar trend in both flushes. The highest yield of 361.30±4.55 g/kg of substrate occurred in *G. arborea* sawdust, followed by *M. excelsa* while the least yield of 255.61±2.05 g/kg of substrate was recorded on *A. africana* sawdust (Table 2). The BE of mushrooms harvested from each of the substrates ranged from 25.56±0.60 to 36.13±0.24%. The BE of *G. arborea* was significantly ($P \leq 0.05$) higher than the BE of the other substrates with *A. africana* recording the least BE.

Proximate composition of *P. pulmonarius* harvested from the different substrates is shown in Table 3. The result shows significant differences ($P \leq 0.05$) in the proximate composition of mushrooms harvested from the different sawdust substrates. Moisture content of the dried and pulverized mushroom samples ranged from 2.17±0.09 to 3.71±0.04% with dried mushroom from *A. africana* showing the highest moisture content. Ash content was significantly higher in mushroom harvested from *M. excelsa* (7.75±0.05%) and least in mushroom harvested from *K. senegalensis* (5.64±0.07%). The crude fibre and crude protein content were significantly higher whereas there was no significant difference in the crude fibre and crude protein content of mushrooms harvested from the other substrates. Fat content was low for all mushroom harvested from all the substrate and ranged

from 1.19±0.06 to 1.85±0.08% with *M. excelsa* having the highest fat content and *A. africana* having the least value. Fat content in *G. arborea* and *K. senegalensis* were not significantly different. Mineral content (Mg, Na, K and Ca in mg/kg) determined for fruiting bodies harvested from the different saw dust used showed that all the mushrooms had significantly higher potassium content followed by calcium (Table 4). All the mushrooms had low magnesium content compared to the other minerals investigated in this study. However, magnesium, sodium and calcium content for mushrooms harvested from *M. excelsa* were significantly higher than in mushrooms harvested from the other sawdust substrate. There was no significant difference in the magnesium and sodium content of mushrooms harvested from *G. arborea*, *A. africana* and *K. senegalensis*.

The TPC and TFC represented in Figure 1 for mushrooms harvested from *M. excelsa* were significantly higher ($P < 0.05$), however, there was no significant difference between the TPC of mushrooms harvested from *K. senegalensis* and *G. arborea*. There were no significant differences ($P < 0.05$) in the TFC of mushrooms harvested from *G. arborea*, *A. africana* and *K. senegalensis*.

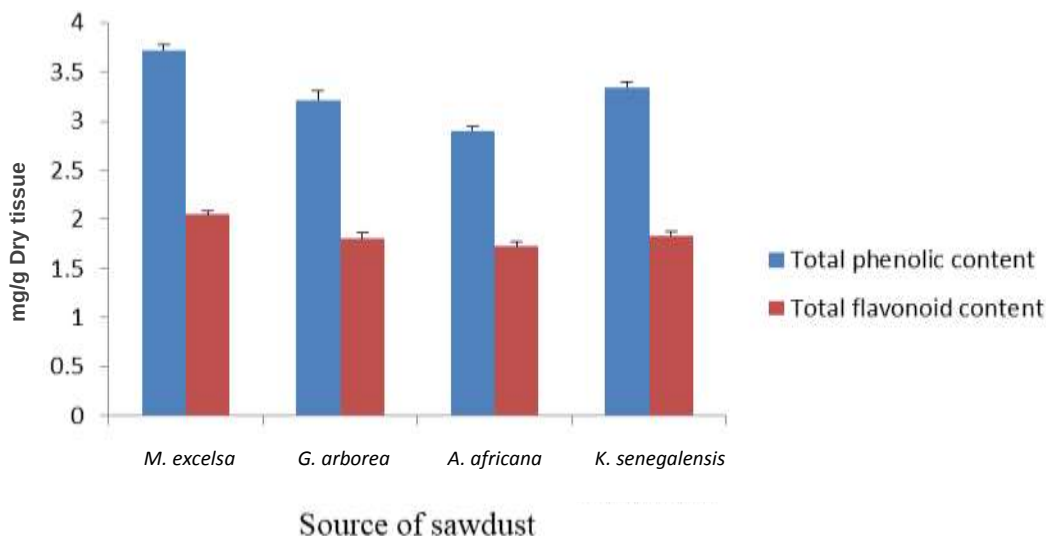
DISCUSSION

The period for complete spawn run obtained in this study falls within the range reported by Liang et al. (2011) for *P. pulmonarius* cultivated on grass plants in Taiwan, but higher than what was reported by Shah et al. (2004). Sopit (2006) on the other hand reported as high as 34 days for completion of spawn run of *Pleurotus ostreatus* on saw dust while Akinmusire et al. (2011) reported 35

Table 4. Mineral composition (mg/kg) of *P. pulmonarius* cultivated on different sawdust substrate.

Sawdust substrate	Mg	Na	K	Ca
ME	0.41±0.007 ^b	0.14±0.023 ^b	6.51±0.012 ^d	4.06±0.020 ^c
GA	0.34±0.006 ^a	0.12±0.011 ^a	4.49±0.056 ^b	2.77±0.031 ^a
AA	0.36±0.007 ^a	0.12±0.008 ^a	7.09±0.004 ^c	3.90±0.067 ^b
KS	0.35±0.020 ^a	0.10±0.007 ^a	3.52±0.011 ^a	2.65±0.033 ^a

Each value is a mean of 3 replicates ± standard error, values accompanied by identical superscript letters are not significantly different ($P \leq 0.05$) in columns.

**Figure 1.** Effect of substrate on TPC and TFC of mushrooms

days for completion of spawn run of *P. pulmonarius* on saw dust which is higher than what is reported in this study.

The yield of *P. pulmonarius* recorded on all the saw dust substrates used in this work (expressed in BE) is lower than the yield obtained by some workers (Liang et al., 2011; de Siqueira et al., 2012), however, the yield from all the saw dust substrates was higher than what was reported earlier (Anyakorah and Dike, 2012; Adenipekun and Omolaso, 2015) for *P. pulmonarius*. The result obtained from the cultivation of oyster mushrooms on cassava waste showed that substrates containing up to 75% cassava peels and supplemented with rice bran have productions well comparable to yields obtained from the traditional saw dust based substrates (Anton et al., 2014). Jonathan et al. (2013) however reported greater yield of *P. pulmonarius* on rice straw and coir fibre than saw dust.

The variation in the rate of growth, spawn run and yield of *P. pulmonarius* on the different substrate in this present study corroborates the findings of Nasir et al. (2012) in the use of sawdust of different woods for the cultivation of oyster mushroom (*P. ostreatus*). These

differences in growth and yield could be, in part, as a result of the variation in substrate type (Muhammad et al., 2007), substrate pre-treatment, additives, difference in strains of *P. pulmonarius* cultivated and the climatic condition at the time of cultivation.

Protein content reported in this present study falls within the range reported by earlier researchers (Ali et al., 2010; Khatun et al., 2012; Joshi and Shekhawat, 2014), but lower than that which was reported by Ashraf et al. (2013) for *P. ostreatus*. Protein and ash content was higher than that which was reported by Anyakorah and Dike (2012) for *P. pulmonarius* cultivated on saw dust, the fibre content was however lower. The lipid and protein content reported in the study fall within the range reported by Moni et al. (2004). The moisture content on the other hand for all the mushrooms harvested from all the saw dust substrate was lower than what was reported by Adenipekun and Omolaso (2015). The crude fibre content reported compares favourably with earlier finding (Anyakorah and Dike, 2012; Adenipekun and Omolaso, 2015). The high concentration of potassium compared to other nutrients in mushrooms may be due to a high content of potassium in agro-waste used for the

mushroom cultivation. The findings in this work are in agreement with earlier findings that substrates used in mushrooms cultivation have effect on chemical characteristics of mushrooms (Oyetayo and Ariyo, 2013) indicating a variation in the capability of such substrates to support mushroom growth (Kuhad and Singh, 1997).

The TPC reported in this study is higher than what was reported for both *Pleurotus eryngii* and *P. ostreatus* (Kim et al., 2008), falls within the range reported by Kumari and Acha (2008), but lower than what was reported by Abaaayah and Umi (2013). The TFC on the other hand, falls within the range reported by Abaaayah and Umi (2013). All mushroom harvested from the different sawdust substrates used showed varying TPC and TFC indicating that *P. pulmonarius* cultivated on different saw dust is able to synthesize phenolic compound thereby serving as a potential source of natural antioxidant for consumers. Further investigation is however necessary to determine ways of improving the TPC and TFC.

Conclusion

Based on the results obtained in this study, it can be concluded that most of the commonly available saw dust that constitute nuisance because of improper disposal are able to support growth and fructification of *P. pulmonarius* in varying degrees. And this indicates that the substrate recording the highest yield can be exploited for its cultivation thereby representing a bioconversion process of saw dust. Additionally, the fruiting bodies harvested from the substrates are nutritionally acceptable and posses antioxidant potential due to the TPC and TFC. Further investigations are necessary to determine influence of growth promoting additives so as to enhance higher yield of mushrooms on the different saw dust.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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