

Cultivation of *Pleurotus pulmonarius* Fr. Singer on fermented and non fermented sawdust*JonathanSG¹, Adegboyega AA¹ and Oyelakin AO²¹Mycology and Biotechnology Unit, Department of Botany, University of Ibadan, Ibadan, Nigeria³College of Agriculture, Igbo-Ora, Nigeria.sg.jonathan@mail.ui.edu.ng

Abstract: Composting were carried out (under natural environmental condition) on wood wastes of five economically important Nigerian trees (*Mansonia altissima*, *Nauclea diderrichii*, *Gmelina arborea*, *Funtumia africana* and *Anogeissus leiocarpus*). Both composted and non composted wood wastes (control) were used to propagate mycelia biomass and sporophores of *Pleurotus pulmonarius* that have been tissue cultured. The pH of these plank wastes (*Nauclea diderrichii*) significantly dropped from 6.3 to 4.0 after 90 days of fermentation ($P \leq 0.05$). Likewise, that of *Anogeissus leiocarpus* also dropped from 6.2 to 4.2. It was rather observed that the amino nitrogen content (ANC) generally increased in all the sawdust. For, *Gmelina arborea*, the value of ANC increased from 2.24 to 4.20 mg/100g from 0 and 90 days. Besides, lignin content of the composted wastes decreased considerably at end of the solid state fermentation. The greatest lignin reduction for the fermented and non-fermented wood wastes were observed in *Gmelina arborea* followed in order by *Funtumia africana*, *Anogeissus leiocarpus* and *Mansonia altissima*. The best substrate for fruitbody production (29.5 g kg⁻¹) was the composted sawdust of *Funtumia africana* while non composted wastes of this wood gave fruit body yield of 8.3 g kg⁻¹. The second best woodwaste was *Nauclea diderrichii* with values of 3.5 and 22.65g kg⁻¹ for the non fermented and fermented saw dusts. The significance of these observations was discussed.

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1. All *Pleurotus* species belong the class basidiomycetes and are referred to as 'oyster mushrooms'. They have been found to be excellent delicacies in all regions of the world (Iwase *et al.*, 2000; Jonathan and Esho, 2010; Jonathan and Adeoyo, 2011a). The common edible oyster mushrooms are *P. ostreatus*, *P. florida*, *P. tuber-regium* and *P. pulmonarius* (Kuforiji, 2005; Fasidi *et al.*, 2008). In Nigeria, the most popular edible species are *Pleurotus*, *Termitomyces*, *Tricholoma*, *Psathyrella*, *Lentinus* and *Volvariella* (Gbolagade, 2006; Jonathan *et al.*, 2011a). Mushroom cultivation could be regarded as an economically viable biotechnology for the conversion of agro-industrial wastes into high quality protein in term of mushroom fruit bodies (Fasidi and Ekuere, 1993; Agahar-Marugkar and Subbulakshmi, 2005; Jonathan *et al.*, 2011b; Aina, 2012a). Edible fungi like *Agaricus*, *Volvariella* and *Pleurotus* species are produced on a large scale and sold commercially in America, Europe and Asia countries (Asghar and Rehman, 2007; Aina, 2012b; Jonathan *et al.*, 2012a). In Nigeria, wild mushrooms are still being hunted for in the villages that are exposed to natural vegetation. The need for commercial production of edible mushrooms in Nigeria cannot be over emphasized in view of their potential contribution to agricultural, environmental and health values (Jonathan *et al.*, 2008a; Oluranti *et al.*, 2012). Mushrooms could be regarded as source of

cheap protein especially for adults that required low cholesterol in their diet (Aina, 2012b; Jonathan *et al.*, 2008b). They could be cultivated on various waste products of human, agricultural, forestry and industries, sources. (Chang, 1989; Jonathan and Esho, 2010). Thus, the growth of fungi on these substrates has helped to prevent environmental and health hazards posed by indiscriminate dumping of these materials (Jonathan *et al.*, 2012b). Edible fungi may also be utilized medically (Jonathan *et al.*, 2008a; Oluranti *et al.*, 2012; Jonathan and Adeoyo, 2011b). Mushrooms is highly nutritious and are important features of human diet worldwide (Gbolagade *et al.*, 2006). High protein content of as much as 50 to 84% dry matter has been detected in the fruit bodies and mycelia of *P. ostreatus*, *Lentinus edodes*, *Volvariella esculenta* and *Termitomyces clypeatus* (Jonathan *et al.*, 2012). Edible mushrooms have also been reported of containing amino acids like glycine, valine, threonine, serine, leucine, proline, methionine, asparagine, glutamine, lysine, arginine, histidine, cysteine and alanine (Agahar-Marugkar and Subbulakshmi, 2005;). *Pleurotus pulmonarius* has been used by human cultures all over the world for their nutritional value, medicinal properties and other beneficial effects. It is a good source of dietary fibre and other valuable nutrients (Fasidi and Ekuere, 1993; Alam *et al.*, 2010; Oluranti *et al.*, 2012).

The objective of the present investigations is to use fermented and non fermented wood wastes to cultivate *P. pulmonarius* in order to determine which of these substrates will support vegetative growth and fruit bodies' production in this fungus.

2. Materials and Methods

2.1 Substrate collection and preparation Sawdust of five economically important trees: *Gmelina arborea*, *Funtumia africana*, *Nauclea diderrichii*, *Anogeissus leiocarpus* and *Mansonia altissima* were collected from Bodija Plank industry, Bodija, Ibadan, Nigeria and composted separately in a natural environment for 90 days for fermentation to take place. The procedures of Gbolagade, (2006) was used for solid state fermentation

2.2 Biomass yield and fruitbody production of *P. pulmonarius* on different wastes

One thousand two hundred grammes (1200.0 g) of each substrate (fermented and non fermented) were separately mixed with 300.0ml of distilled water. Ten grammes of each wastes (in triplicates) were separately added into petri dishes and autoclaved. After cooling, they were inoculated using 7.00mm mycelia disc from actively growing culture. Mycelial extension and densities were measured after 10 day using the procedure of Fasidi *et al.*, (2008) For fruit bodies production, 500.0 g of each of the substrate was put inside transparent nylon bags. These substrates were tied with rubber bands and sterilized at 1.02 kg cm⁻² pressure at 121°C for 30 minutes. After cooling, a hole was made at the centre of each bag with the aid of a sterilized peg, under aseptic condition and they were inoculated with spawns of *P. pulmonarius* and tied immediately. They were kept in a clean dark cupboard in the laboratory at 30°C and 100%RH. They were incubated for 48 days for fruit body production. Each experiment was replicated thrice (Jonathan *et al.*, 2012b).

2.3 pH :

Eight grammes (8.0 g) of each substrate were soaked in 100 ml of distilled water for 18 hrs at 30±2°C. The pH was determined using microprocessor based Bench pH Mv meter (Hanna Instruments Inc Rhode Island, USA).

2.4 Amino nitrogen: Amino nitrogen determination was carried out using the method of Association of Analytical Chemists (AOAC, 1990).

2.5 Lignin analysis:

Three grams (3.0 g) of each substrate was mixed with 20 ml of freshly prepared 72% H₂SO₄ at 15.20°C for 2 hrs. It was later refluxed with 244.0 ml of distilled water for 4 hrs. Insoluble lignin was allowed to settle overnight and filtered. The residue

was then transferred into a crucible of known weight and dried in the oven at 60°C to a constant weight in a desiccator and weighed. (Asghar and Rehman, 2007).

Percentage lignin was obtained using this formula:

$$\% \text{ lignin} = \frac{\text{weight of insoluble lignin} \times 100}{\text{Oven dried weight of the sample}}$$

2.6 Moisture content: The loss in weight after oven drying fresh samples at 80°C for 72 hrs was taken as the moisture content.

2.7 Statistical analysis: The data obtained were subjected to analysis of variance (ANOVA) and tests of significance were carried out using Pearson chi-square on SPSS computer package.

Table 1a: Mycelia biomass production in *P. pulmonarius* on non composted sawdust

Wood wastes	Mycelia growth (mm)	biomass density
<i>Gmelina arborea</i>	41.7±0.30a	4 ⁺
<i>Nauclea diderrichii</i>	31.3±0.22b	3 ⁺
<i>Funtumia Africana</i>	35.2±0.03b	4 ⁺
<i>Anogeissus leiocarpus</i>	27.7±0.07c	2 ⁺
<i>Mansonia altissima</i>	19.3±0.37d	1 ⁺

Values followed by the same letter(s) are not significantly different by Duncan's multiple range test (P≤ 0.05).

Table 1b: Mycelia biomass production of *P. pulmonarius* on composted sawdust

Wood wastes	Mycelia growth(mm)	biomass density
<i>Gmelina arborea</i>	80.3±0.6a	6 ⁺
<i>Nauclea diderrichii</i>	60.2±0.01b	5 ⁺
<i>Funtumia africana</i>	60.7±0.3b	
<i>Anogeissus leiocarpus</i>	50.3±0.2c	4 ⁺
<i>Mansonia altissima</i>	40.4±0.1d	2 ⁺

Values followed by the same letter(s) are not significantly different by Duncan's multiple range test (p≤ 0.05).

Table 2: Water loss and pH changes during fermentation of sawdust of *P. pulmonarius*

Wood wastes	Incubation period	Water loss(%)	pH values
<i>Funtumia africana</i>	0	48.12±2.9	5.2±0.1b
	30	70.80±0.3	4.0±0.1d
	60	73.67±0.2	4.0±0.1d
	90	76.17±0.2	4.0±0.1d
<i>Nauclea diderrichii</i>	0	67.83±0.9	6.3±0.1a
	30	71.47±2.5	4.0±0.5d
	60	73.31±0.7	4.0±0.1d
	90	75.29±1.4	4.0±0.1d
<i>Anogeissus leiocarpus</i>	0	66.27±0.1	6.2±0.1a
	30	69.17±0.1	4.6±0.1c
	60	73.31±0.7	4.3±0.1c
	90	74.30±0.1	4.2±0.1c
<i>Mansonia altissima</i>	0	68.30±0.2	6.1±0.1b
	30	71.42±0.6	4.4±0.1c
	60	82.15±0.3	4.3±0.1c
	90	85.23±0.1	4.1±0.1c

Values followed by the same letter(s) in each column are not significantly different by Duncan's multiple range test ($p \leq 0.05$)

Table 3: Amino nitrogen content during fermentation of wood wastes by *Pleurotus pulmonarius*

Substrates	Days of Incubation	Amino nitrogen Content (mg/100g)
<i>Gmelina arborea</i>	0	2.24±0.6c
	30	3.15±2.0b
	60	3.25±1.5b
	90	4.20±1.3a
<i>Funtumia africana</i>	0	2.40±2.3c
	30	3.39±1.6b
	60	4.27±2.1a
	90	4.83±2.6a
<i>Nauclea diderrichii</i>	0	2.38±0.9c
	30	3.96±1.0b
	60	4.12±1.5a
	90	4.24±0.7a
<i>Anogeissus leiocarpus</i>	0	1.47±2.2d
	30	1.56±0.8d
	60	2.96±2.3b
	90	4.27±2.6a
<i>Mansonia altissima</i>	0	1.58±0.9d
	30	1.74±1.5d
	60	2.87±2.4c
	90	3.96±1.8b

Values followed by the same letter(s) are not significantly different by Duncan's multiple range test ($p \leq 0.05$)

Table 4: Characteristics of *P.pulmonarius* growth on fermented compost

Examination	Observations	
	C _x	C _z
Appearance or colour of compost	Compact, deep brown	Loose
Smell	Fresh	Fresh wood
Mycelium growth	Deep and dense	Weak and shallow
Rate of mycelium (spawn running)	Rapid	Very rapid
Emergency of mushroom pinheads	Early	Late
Nature of fruiting bodies	Strong, big and healthy	Weak and tiny
Yield of first flush	High (250g)	Low (130g)

C_x – Well fermented compost (90 days)

C_z - non fermented compost (0 day)

Table 5: Influence of the test fungus on lignin contents of the wood wastes

	(days)	(g)	
<i>Gmelina arborea</i>	0	98.33±7.1a	41.96
	30	56.37±1.3d	43.00
	60	55.33±8.3d	45.33
	90	53.00±3.1d	
<i>Funtumia Africana</i>	0	96.67±5.4a	39.00
	30	57.67±1.5d	41.00
	60	55.67±2.3d	44.17
	90	52.50±2.3d	
<i>Nauclea diderrichii</i>	0	92.67±6.0a	34.67
	30	58.00±3.0d	38.00
	60	54.67±2.3d	39.17
	90	53.50±2.3d	
<i>Anogeissus leiocarpus</i>	0	95.00±1.5a	37.00
	30	58.00±6.5d	38.67
	60	56.33±7.1d	41.33
	90	53.67±7.8d	
<i>Mansonia altissima</i>	0	92.20±1.8a	33.99
	30	58.21±2.4d	36.86
	60	55.34±1.6d	41.90
	90	50.30±3.5d	

Values followed by the same letter(s) are not significantly different by Duncan's multiple range test ($p \leq 0.05$)

Table 6: Yield of *P. Pulmonarius* fruit bodies on fermented and non-fermented wood wastes

Wood wastes	Yield of fruit bodies on non fermented wood wastes (g/kg)	Yield of fruit bodies on fermented wood wastes (g/kg)
<i>Gmelina arborea</i>	2.6c	19.3c
<i>Funtumia Africana</i>	8.3a	29.5a
<i>Nauclea diderrichii</i>	3.5b	22.6b
<i>Anogeissus leiocarpus</i>	-	10.5d

Values followed by the same letter(s) are not significantly different by Duncan's multiple range test ($p \leq 0.05$)

Results and discussion

From these investigations, it was observed that all the wood wastes studied supported the vegetative growth of *P. pulmonarius* (Tables 1a & b). This observation is in agreement with findings of Fasidi and Ekuere, (1993) on *Volvariella esculenta*. Likewise, Jonathan *et al* (2008c) reported that *P. tuberregium* was able to biodegrade sawdust of selected Nigerian economic trees and utilized these agro-industrial wastes for its vegetative growth. Chang (1989), Fasidi and Ekuere (1993), Kuforiji (2005), and Fasidi *et al* (2008) reported that *Pleurotus species* as a group of basidiomycetes and other wild mushrooms have high saprophytic ability to grow on variety of

agro industrial wastes. The ability of this fungus to flourish on different wastes may be linked to its ability to secrete hydrolyzing and oxidizing enzymes, which could aid the decomposition of recalcitrant compounds in the wastes into utilizable compounds (Jonathan *et al.*,2011b). It was also revealed from Tables 1a and 1b that fermented substrates significantly supported mycelia growth than non fermented saw dusts. This results could be linked with the contribution of Gbolagade(2006) that composting is a solid-state fermentation process, which exploits the principles of elimination of competing microorganisms. After the succession of different microorganisms in the composting substrates. The fermented wastes will now be utilized for the growth of mushroom mycelia with little or no competitors. The main focus of solid state fermentation or composting to a mushroom grower is to prepare a substrate in which the growth of mushroom is promoted to the practical exclusion of other microorganisms. According to Gbolagade (2006) ,composted substrates will also improve oysters mushroom fruit body yield and also reduced infestation by insects, fungi and bacteria pests(Jonathan *et al.*,2012c)

The rapid colonization of *P. pulmonarius* mycelia on selective substrates such as wood wastes of *Gmelina arborea*, *Nauclea diderrichii*, *Funtumia africana*, *Anogeissus leiocarpus* and *Mansonia altissima* as observed in this study will considerably reduce the growth of other competitive microorganisms thereby reducing spawn contamination. Likewise, the sawdust of *Gmelina arborea*, *Nauclea diderrichii*, *Funtumia africana*, *Anogeissus leiocarpus* and *Mansonia altissima*, which are nuisance to our environment, could be successfully utilized as substrates for cultivation of *P. pulmonarius* and other Nigerian edible mushrooms. The results of this study also revealed that composting of agricultural substrates for the growth of *P. pulmonarius* is necessary since luxuriant growth of mycelia and higher fruit bodies yield were obtained on fermented wood wastes (Tables 1a,1b and 6). The change in pH value of the different substrates as the incubation period increased may be linked with the increase in amino nitrogen content and the presence of metabolic waste products within the substrates. Similar pH changes were observed by Jonathan and Adeoyo(2011b) for the enzyme production in wildy collected mushrooms in submerged liquid medium. The increase in amino nitrogen content may be due to hydrolysis of protein within the substrates.

From the results, all the substrates used were found to enhance vegetative growth of *P. pulmonarius* (Table 2). The best mycelial growth was observed on the wood wastes of *Gmelina arborea* followed in order

by *Funtumia africana* and *Nauclea diderrichii* ($P \leq 0.05$). The growth of this white rot fungus caused a decrease in the pH of the wastes (Table 3). The pH of *Mansonia altissima*, which was initially 6.1 dropped to 4.1 after 90 days of incubation. This change was observed in the other substrates with pH values reduced to 4.2 and 4.0. In *Gmelina arborea*, *Anogeissus leiocarpus* and *Mansonia altissima*, the pH values decreased as the incubation period increased but for *Funtumia africana* and *Nauclea diderrichii* there was no change in pH after 30 days of incubation(Table3). Generally, it was observed that the amount of amino nitrogen in the substrates increased with incubation time (Table 4). The greatest amount of nitrogen was found in sawdust of *Funtumia africana* (4.90 mg) followed by *Anogeissus leiocarpus* (4.33 mg). Although there was amino nitrogen accumulation in all the substrates with time, there was however no statistical difference in the nitrogen contents of the different substrates used after 90 days ($P \leq 0.05$).

During the fermentation of the wood wastes by *Pleurotus pulmonarius*, water loss occurred. The amount of water lost from the substrates was also observed to increase as the incubation period increased (Table 3). As the fungus degraded the wood wastes, lignin content decreased as the incubation period increased (Table 5). For fermented wood wastes, at zero days, *Gmelina arborea* had the highest lignin content (98.33 g), which reduced significantly to 53.00 g after 90 days. The greatest lignin reduction was noticed in *Gmelina arborea* followed by *Funtumia africana* and *Anogeissus leiocarpus* while the lowest lignin reduction was observed in *Nauclea diderrichii* and *Mansonia altissima* respectively.

From Table 6, it was shown that composted wood wastes enhanced greater fruit body yield than non fermented sawdust. There were no fruit body production in *Anogeissus leiocarpus* and *Mansonia altissima* in non fermented wood wastes whereas, there were yield of 10.5 and 5.3 g/kg in composted *Anogeissus leiocarpus* and *Mansonia altissima* respectively .Likewise, low yield of 2.6,3.5and 8.3g/kg were observed on non fermented sawdust of *Gmelina arborea*, *Nauclea diderrichii* and *Funtumia africana* respectively, whereas, very good yield 19.3,22.6 and 29.5g/kg of fruit body yield were obtained from the composted wood wastes of *Gmelina arborea*, *Nauclea diderrichii* and *Funtumia Africana* respectively(Table6).These observation is similar to those of Fasidi *et al* (2008) on *Pleurotus tuber-regium*.

4.0 Conclusion:

It was observed that the amino nitrogen content (ANC) generally increased in all woodw. Foaster, *Gmelina arborea*, the value of ANC increased from

2.24 to 4.20 mg/100g from 0 and 90 days. Besides, lignin content of the composted wastes decreased considerably at end of the solid state fermentation. The greatest lignin reduction for the fermented and non-fermented wood wastes were observed in *Gmelina arborea* followed in order by *Funtumia africana*, *Anogeissus leiocarpus* and *Mansonia altissima*. The best substrate for fruitbody production (29.5 g kg⁻¹) was the composted sawdust of *Funtumia africana* while non composted wastes of this wood gave fruit body yield of 8.3 g kg⁻¹. The second best woodwaste was *Nauclea diderrichii* with values of 3.5 and 22.65g kg⁻¹ for the non fermented and fermented saw dusts

Correspondence to: Dr Jonathan Segun Gbolagade
Mycology & Biotechnology unit,
Department of Botany,
University of Ibadan, Ibadan, Nigeria.
Email: sg.jonathan@mail.ui.edu.ng
Tel: +2348164746758

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