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Utilization of fermented and non fermented wood wastes by *Pleurotus pulmonarius* (Fr.) Singer, for vegetative growth and fruit bodies production

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ABSTRACT

Solid state fermentation was carried out on wood wastes of five economically important Nigerian trees(*Mansonia altissima, Nauclea diderrichii, Gmelina arborea, Funtumia africana and Anogeissus leiocarpus*)for 90 days. The fermented wood wastes of each tree and non fermented sawdust(control) were used to generate mycelia biomass and fruit bodies of *Pleurotus pulmonarius*, an edible mushroom for 48days. The pH of the composted wastes dropped to 4.0/4.1 after 90 days of composting while amino nitrogen content increased significantly. Conversely, lignin content of the fermented wastes decreased considerably at end of 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.* Utilization of different wood wastes such as *Gmelina arborea, Funtumia africana, Nauclea diderrichii, Anogeissus leiocarpus* and *Mansonia altissima* by *Pleurotus pulmonarius*, gave *Gmelina arborea* as the best substrate for the mycelia growth and fruit bodies production of this edible mushroom.

KEYWORDS

Mycelia;
Fermentation;
Substrates;
Incubation.

INTRODUCTION

'Oyster mushroom' refers to several species of edible mushrooms belonging to the genus *Pleurotus*[1, 2, 4]. Among listed *Pleurotus* species are *P.osteatus, P.florida, P.tuber-regium and P. pulmonarius*[17,20]. In Nigeria, the most prized edible species are *Pleurotus, Termitomyces, Tticholoma and Volvariella*[6, 9, 10, 16]. Mushroom cultivation could be regarded as an conomically viable biotechnology for the conversion of largo-industrial wastes into high quality protein food in term of mushroom fruit bodies[11, 15, 17]. Edible fungi like *Agaricus, Volvariella* and *Pleurotus species* are produced on a large scale and sold commercially in America, Europe and Asia countries[1, 2, 3]. 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 its potential contribution to agricultural and environmental

FULL PAPER

values. Edible fungi could be regarded as source of cheap protein especially for an adults that required low cholesterol in their diet[7, 5, 8]. Mushrooms are cultivated on various waste products of human, agricultural, forestry and industries, sources[16, 18, 20]. Thus, the growth of fungi on these substrates has helped to prevent environmental and health hazards posed by indiscriminate dumping of these materials[23]. Edible fungi may also be utilized medically[18, 19]. Mushrooms are highly nutritious and are important features of human diet worldwide[12, 13, 14]. 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 clypeatu*[4, 11, 21]. 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[10, 19]. *Pleurotus pulmonarius* (oyster mushroom) 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[13]. The objective of the present investigations is to use fermented and non fermented wood wastes to cultivate *P.pulmonarius* to determine which of these substrates will support vegetative growth and fruit bodies production in this fungus.

MATERIALS AND METHODS

Wood wastes

Wood wastes of five economically important trees. These were: *Gmelina arborea, Funtumia africana, Nauclea diderrichii, Anogeissus leiocarpus* and *Mansonia altissima*. These saw dusts were collected from Bodija Plank Market, Bodija, Ibadan, Nigeria and composted separately in a natural environment for 90 days for fermentation to take place. The procedures of Gbolagade[24] was used for solid state fermentation

Vegetative growth and fruit body production of *P. pulmonarius* on different wood wastes

One thousand two hunded grammes (1200.0 g) of each substrate (fermented and non fermented)were separately mixed with 300. ml 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 mycelial disc from actively growing culture. Mycelial extension and densities were measured after 10 day using the procedure of Fasidi[10]. For fruit bodies production, 250.0 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/cm2 pressure at 121^{0}C for 60 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^{0}C and 100%RH. They were incubated for 48 days for fruit body production. Each experiment was replicated thrice[21, 22].

pH Determination

Eight grammes (8.0 g) of each substrate were soaked in 100 ml of distilled water for 18 hrs at 30±2^{0}C. The pH was determined using microprocessor based Bench pH Mv meter (Hanna Instruments Inc Rhode Island, USA).

Amino nitrogen: Amino nitrogen determination was carried out using the method of Kadiri(1990).

Lignin determination

Three grams (3.0 g) of each substrate was mixed with 20 ml of freshly prepared 72% H_2S0_4 at 15.20^{0}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^{0}C to a constant weight in a desiccator and weighed[17].

Percentage lignin was obtained using this formula

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

Moisture content

The loss in weight after oven drying fresh samples at 80^{0}C for 72 hrs was taken as the moisture content.

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.

RESULTS AND DISCUSSION

In this study, all the wood wastes investigated were found to support the vegetative growth of *P. pulmonarius*(TABLE 1). This result is in agreement with findings of Jonathan *et al*[17] on *Pleurotus tuber-regium*. Chang[4] and Fasidi and Ekuere[6] repored that *Pleurotus species* as a group of basidiomycetes 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[22, 23, 24]. 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 myceliaand higher fruit bodies yield were obtained on fermented wood wastes(TABLES 1 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 *et al*[18] for the growth of *V. esculenta* in submerged 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 = 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 altisima*, 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(TABLE 3). 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= 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.

Generally, fermented wood wastes enhanced greater fruit body yield than non fermented.

TABLE 1 : Mycelial Growth of *P. pulmonarius* on wood wastes of some selected Nigerian Economic Trees

Substrates	Mycelia extension	Mycelia density
	cm	
Gmelina arborea	8.3±0.6a	6^+
Nauclea diderrichii	6.2±0.01b	5^+
Funtumia Africana	6.7±0.3b	3^+
Anogeissus leiocarpus	5.3±0.2c	4^+
Mansonia altissima	4.4±0.1d	2^+

Each value is the mean of 3 readings ± SE taken over a period of 10 days. Values followed by the same letter(s) are not significantly different by Duncan's multiple range test (p= 0.05)

FULL PAPER

TABLE 2 : Water Loss and PH Changes during Fermentation of Wood Wastes of *Pleurotus pulmonarius*

Substrates	Incubation period	Water loss	pH values
	(days)		
Gmelina arborea	0	66.56±0.1	5.1±0.1b
	30	70.00±0.3	4.6±1.7cd
	60	81.67±4.8	4.4±0.1d
	90	94.40±0.4	4.3±0.1d
Funtumia Africana	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
Nauclea diderrichii	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
Anogeissus leiocarpus	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
Mansonia ltissima	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

Each value is the mean of 3 readings ± SE taken over a period of growth. Values followed by the same letter(s) are not significantly different by Duncan's multiple range test (p= 0.05)

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

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

Each value is the mean of 3 readings ± SE taken over a period of growth. Values followed Values by the same letter(s) are not significantly different by Duncan's multiple range test (p= 0.05)

TABLE 4: Mushroom growth observations in compost fermented at different levels

	Observations	
Examination	**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 (90days); C_z - non fermented compost (0 day)

TABLE 5 : Effect of fungal growth on the lignin content of some wood wastes

Fermented substrates	Incubation period	Lignin value	Lignin reduction
	(days)	(g)	
Gmelina arborea	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	
Funtumia Africana	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	
Nauclea diderrichii	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	
Anogeissus leiocarpus	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	
Mansonia altissima	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	

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

Woodwastes	Yield of fruit bodies on non fermented wood wastes (g/kg)	Yield of fruit bodies on fermented wood wastes(g/kg)
Gmelina arborea	2.6c	19.3c
Funtumia Africana	8.3a	29.5a
Nauclea diderrichii	3.5b	22.6b
Anogeissus leiocarpus	-	10.5d
Mansonia altissima	-	5.3e

Each value is the mean of 3 readings ± SE taken over a period of growth. Values followed Values by the same letter(s) are not significantly different by Duncan's multiple range test (p= 0.05)

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