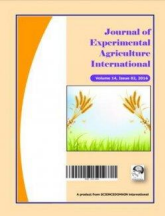

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Abilities of *Tectona grandis* and *Celtis zenkeri* (Hardwood) Sawdust as Substrates of *Pleurotus* Species and Their Indigenous Fungi

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Authors' contributions

This work was carried out by all the authors. The first author ISO did all the bench work and wrote the first draft. The second author AAS designed the study (together with the third author), identified the isolated fungi, interpreted the analyzed data and supervised the write up. The third author COA designed the study (together with the second author), supplied the mushroom technology aspect of the work and also supervised the write up. All authors read and approved the final manuscript.

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ABSTRACT

Mushroom cultivation has continued to receive growing attention because of its nutritional and medicinal values. However, this study examined the effect of hardwood sawdust on the growth of *Pleurotus ostreatus* and *Pleurotus pulmonarius* were investigated. Relationship between fungal incidence of the substrates (sawdust) and that of the mushroom were examined. Both *Pleurotus ostreatus* and *Pleurotus pulmonarius* were inoculated on fermented and unfermented sawdust of *Tectonal grandis* and *Celtis zenkeri*. The fruiting bodies of the mushrooms were harvested and the growth parameters and biological efficiency was recorded. The isolated resident fungi were identified after obtaining pure cultures. The collected data were subjected to analysis (ANOVA) using Generalized Linear Model Procedure (GLM) of Statistical Analysis software (SAS). Means were separated using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

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Some of the growth parameters of *P. ostreatus* were significantly ($p \leq 0.05$) better than that of *P. pulmonarius*. *Tectona grandis* and *Celtis zenkeri* sawdust had significant ($p \leq 0.05$) impact on different growth parameters of the two mushrooms. Fermentation or non-fermentation of the substrates (sawdust) had no significant ($p \leq 0.05$) impact on growth parameters of the mushrooms. Growth parameters of the two mushrooms were significantly better in 0% additive ($p \leq 0.05$) than in the other additive concentrations. Five fungi were identified as indigenous fungi of the unfermented sawdust which did not significantly differ from those of the fermented sawdust and mushrooms. Nutritional composition of the mushrooms that grew on fermented and unfermented sawdust were good and comparable. The mushrooms were rich in protein, fibre, ash, moisture, fat and carbohydrate. Cultivation of mushrooms on hardwood sawdust is thus an effective means of managing such waste.

Keywords: Fermented; resident fungi; Tectonal grandis; Celtis zenkeri; Pleurotus ostreatus and Pleurotus pulmonarius.

1. INTRODUCTION

Mushrooms, locally referred to as 'Olu' in Yoruba, 'Ero atakata' in Igbo and 'naman kaza' in Hausa, is currently gaining global attention due to its nutritional value and medicinal properties [1]. There are many species of edible mushrooms which have been reported to grow on agro-industrial wastes [2]. Specifically, the ability of *Pleurotus* species to grow on any agro-industrial wastes have been documented [3]. The estimated amount of agro industrial waste generated in Nigeria has been reported to be more than 3.2 million tonnes per annum causing environmental pollution as a result of improper disposal and burning. Such wastes have been reported to be used as substrates for mushroom cultivation [4]. However, the yield of various mushrooms is known to be affected by agricultural substrates such as sawdust [5]. It was also reported that fermented sawdust as substrate [6] improves the yield of mushroom and prevent infestation by insects. Addition into substrates, of certain additives such as rice bran and wheat bran are as well known to improve the growth of mushroom [7].

Mushrooms on the other hand are reported to be suitable as substrates for different micro-organisms [8]. Different fungi have also been reported to be isolated from decaying sawdust [9].

The experiment was thus set up to examine effect of hardwood sawdust on the cultivation of *Pleurotus ostreatus* and *Pleurotus pulmonarius* and to also examine probable relationship between fungal incidence of the substrates (sawdust) and that of the mushroom.

2. MATERIALS AND METHODS

2.1 Collection of Substrates and Additive

The substrates, which are the sawdust of *Celtis zenkeri* and *Tectona grandis* were obtained from Sango and Bodija sawmills in Ibadan, Oyo State, while the additive was bought from the feed mill in Bodija Market, Ibadan.

2.2 Collection and Multiplication of Spawn and Substrate Preparation

The spawn was collected and multiplied at the Plant Physiology laboratory, Department of Botany, University of Ibadan using the method of [10]. Fermentation of the substrates was done using the method of 6. Eighty grams each of fermented and unfermented sawdust was weighed into 350 ml bottles and sterilized using standard procedures.

2.3 Inoculation and Fructification of Mushrooms and Proximate Analysis

The bottles were inoculated with 10g spawn of *P. ostreatus and P. pulmonarius* and were incubated at 28±2°C for 21 days. They were later taken out and watered regularly for fructification. Harvesting of the fruiting bodies was done afterwards and growth parameters, total yield and biological efficiency (BE) of the mushroom were recorded. Proximate composition of *Pleurotus ostreatus* and *Pleurotus pulmonarius* was determined using the method of [11].

2.4 Isolation and Identification of Fungal Species

Isolation of resident fungi of the mushrooms and sawdust was done at the Plant Pathology

laboratory, Department of Botany. Two methods were used to isolate fungi from the sawdust. The first method was pour plate, where 0.1 g of sawdust was sprinkled in sterile Petri plates and molten Acidified Potato Dextrose Agar (APDA) was later poured into the plates after sterilization of the agar (at 121°C for 15 minutes) and cooling. The plates were swirled gently to allow even dispersion of the sawdust in the molten agar and later left to gel. In the second method, 0.1 g of the sawdust was sprinkled on sterile plates of APDA. Isolation from mushroom was done by cutting small pieces of the mushroom unto sterile plates of APDA. All experiments were done in three replicates. All plates were incubated at room temperature and were observed daily for fungal growth. The isolated fungi were later sub-cultured to obtain pure cultures and later identified using morphological characteristics both on Petri plates and under the microscope.

2.5 Data Analysis

The data obtained were subjected to analysis (ANOVA) using Generalized Linear Model Procedure (GLM) of SAS (version 9.3). Means were separated using Duncan's Multiple Range Test (DMRT) at p≤0.05.

3. RESULTS

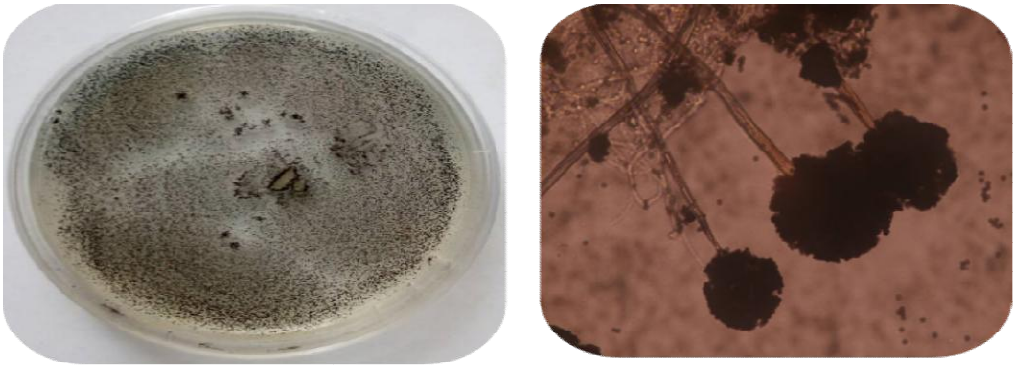
The effect of sawdust on the growth parameters of *Pleurotus ostreatus* and *Pleurotus pulmonarius* is given in Table 1. Some of the growth parameters of *P. ostreatus* and *P. pulmonarius* were significantly ($p \leq 0.05$) higher than themselves.

Generally, growth parameters (i.e. cap length, cap width, stipe width and fruiting bodies) of the mushrooms were significantly ($p \leq 0.05$) better on *Tectona grandis* than on *Celtis zenkeri*. However, the fermented and unfermented substrates had no significant ($p \leq 0.05$) impact on the growth parameters (Table 1). Most of the growth parameters of the two mushrooms were significantly ($p \leq 0.05$) better on 0% additive than on the other additive concentrations (Table 2).

Table 3 shows resident fungi isolated from the substrates and the mushrooms. Five fungi were isolated from the unfermented sawdust. These are *Aspergillus niger, A. tamarii, A. flavus, Trichoderma harzianum* and *Trichoderma* species (Plate 1 - 3). Similar fungi were isolated from the fermented sawdust and mushrooms which are *Aspergillus niger, A. tamarii, A. flavus.* The number of resident fungi in fermented substrate was higher than those from unfermented substate. *Aspergillus niger* was the most predominant of all the resident fungi isolated.

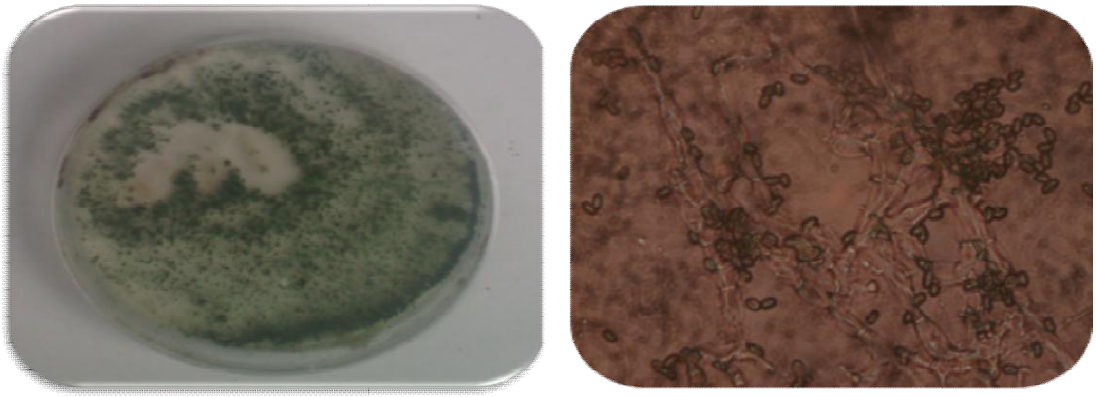
3.1 Effect of Fermentation pH and Temperature on the Two Sawdust during Fermentation

Figs. 1 and 2 show the pH and temperature values of fermented *Celtis zenkeri and Tectonal grandis* for 12 days of fermentation. At days 1, 2, 4, 5, 7 and 9 of fermentation, there were significant differences in their pH values (Fig. 1). There was no significant difference in their temperature at days 1 and 2 of fermentation but there was significant difference in temperature among days 3 to 12 of fermentation (Fig. 2).



a b

Plate 1. *A. niger* obtained from *T. grandis* and *C. zenkeri* sawdust and from the mushrooms (a); Photomicrograph (b)



a b

Plate 2. *Trichoderma harzianum* from unfermented *T. grandis* and *C. zenkeri sawdust* and from the mushrooms (a); Photomicrograph (b)

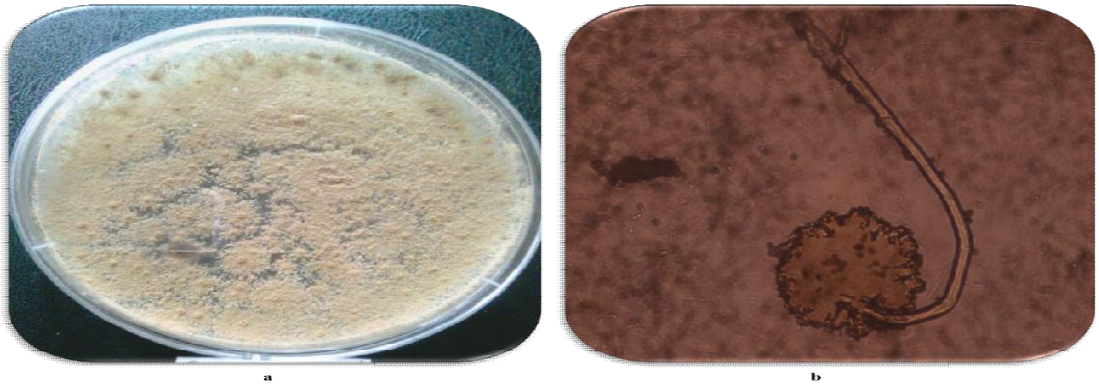


Plate 3. *Aspergillus tamarii* isolated from both the fermented and unfermented sawdust and also from the mushrooms (a); Photomicrograph (b)

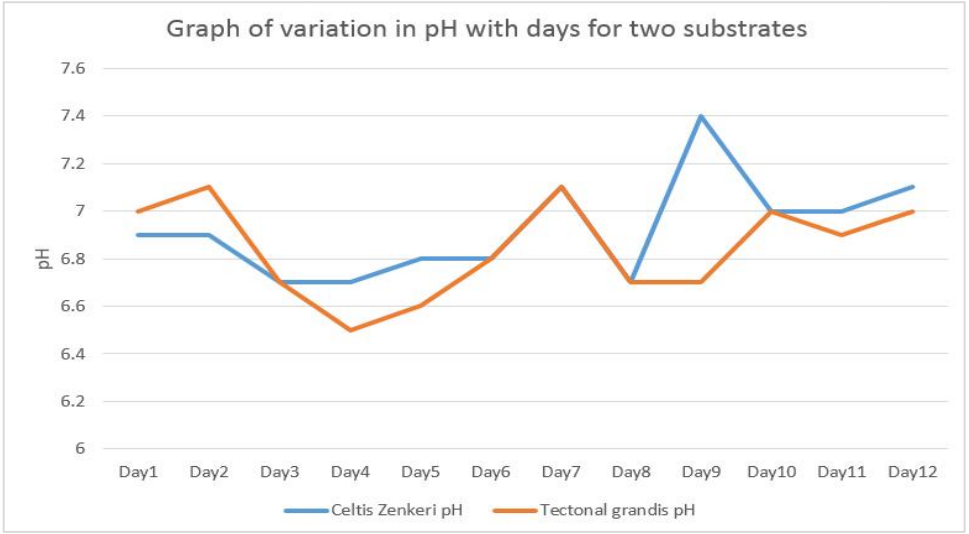


Fig. 1. Effect of pH on the two sawdust during fermentation

Table 1. Effect of sawdust on the growth parameters of *Pleurotus ostreatus and Pleurotus pulmonarius*

Parameters		Cap length (cm)	Cap diameter (cm)	Cap width (cm)	Stipe length (cm)	Stipe width (cm)	No of Fruiting Bodies	Biological efficiency (%)
Mushroom species	***Pleurotus ostreatus***	4.63a	10.59a	5.48a	6.62a	3.56a	7.71a	67.40a
	Pleurotus sajor caju	4.43a	11.05a	4.05b	5.37b	3.05b	5.98b	57.38b
Sawdust types	***Celtic zenkeri***	4.29b	9.66a	4.00b	5.81a	3.08b	5.92b	59.84a
	Tectona grandis	4.77a	11.99a	5.53a	6.18a	3.53a	7.77a	64.94a
Sawdust conditions	**Fermented**	4.57a	11.96a	4.90a	6.17a	3.44a	7.17a	62.28a
	Unfermented	4.49a	9.69a	4.63a	5.83a	3.18a	6.53a	62.50a
	$LSD_{0.05}$	0.45	3.66	0.53	0.62	0.30	1.40	6.64
	R^2	0.17	0.09	0.48	0.31	0.25	0.17	0.28

Means with different letters in a column are significantly different at p ≤ 0.05 (Statistical Analysis: ANOVA)

Table 2. Effect of supplement on the growth parameters of *Pleurotus ostreatus and Pleurotus pulmonarius*

Wheat bran concentrations (%)	Cap length (cm)	Cap diameter (cm)	Cap width (cm)	Stipe length (cm)	Stipe width (cm)	No of Fruiting Bodies	Biological efficiency (%)
0	5.13a	11.13a	5.38a	6.81a	3.34a	7.54a	73.63a
10	4.16b	8.68a	4.16c	5.35b	3.10a	7.50a	60.07b
20	4.60ab	13.05a	4.43bc	5.32b	3.33a	6.17a	53.26b
30	4.22b	10.43a	5.08ab	6.51a	3.34a	6.17a	62.60b
$LSD_{0.05}$	0.63	5.18	0.75	0.88	0.43	1.98	9.39
R^2	0.17	0.09	0.48	0.31	0.25	0.17	0.28

Means with different letters in a column are significantly different at p ≤ 0.05. (Statistical Analysis: ANOVA)

Table 3. Resident fungi isolated from the substrates and the mushrooms

S/N	Source of isolation	Isolated fungi
1	**Unfermented sawdust (Substrate)**	*Aspergillus niger, A. tamarii, A. flavus, Trichoderma harzianum* and *Trichoderma* species
2	**Fermented sawdust (Substrate)**	*Aspergillus niger, A. tamarii, A. flavus*
3	**Mushrooms**	*Aspergillus niger, A. tamarii, A. flavus*

Table 4. Proximate analysis of *P. ostreatus* and *P. pulmonarius* cultivated on sawdust of *Tectona grandis* and *Celtis zenkeri*

Parameters		Crude Protein (%)	Crude Fat (%)	Ash (%)	Crude fibre (%)	Moisture content (%)	CHO (%)
Substrate types	*Celtis zenkeri*	38.99a	1.57a	2.54a	4.76a	4.79a	52.25a
	Tectona grandis	38.92a	2.27a	3.10a	4.36a	4.16b	52.83a
Mushroom species	*Pleurotus ostreatus*	39.00a	1.60a	2.29a	4.69a	5.05a	53.31a
	Pleurotus sajor caju	38.91a	2.24a	2.72a	4.44a	3.90b	52.77a
Substrate condition	unfermented	38.84a	1.99a	2.70a	4.55a	4.54a	52.60a
	Fermented	39.07a	1.86a	2.94a	4.59a	4.41a	53.48a
	$LSD_{0.05}$	1.44	0.85	0.59	0.67	0.49	1.11
Wheat bran concentrations	0	39.66ab	0.33b	2.15b	3.55b	3.71b	53.48a
	10%	37.61b	2.72a	2.83ab	4.97a	4.82a	51.47b
	20%	38.42ab	1.99a	3.19a	4.77a	4.61a	53.45a
	30%	40.14a	2.65a	3.11a	4.96a	4.76a	53.77a
	$LSD_{0.05}$	2.03	1.2	0.84	0.95	0.69	1.57
	R^2	0.12	0.33	0.18	0.21	0.43	0.24

Means with different letters in a column are significantly different at $p \leq 0.05$. (Statistical Analysis: ANOVA)

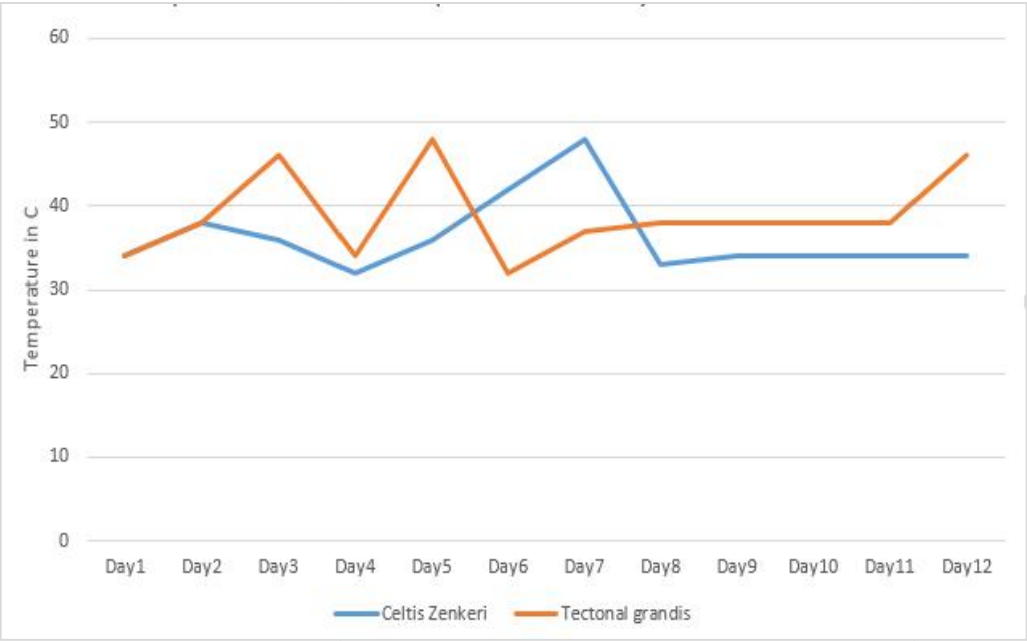


Fig. 2. Effect of temperature on the two sawdust during fermentation

3.2 Proximate Composition of *P. ostreatus* and *P. pulmonarius* Cultivated on Sawdust of *Tectona grandis* and *Celtis zenkeri*

Pleurotus ostreatus had higher crude protein (39.00%), crude fiber (4.69%), moisture content (5.05%), and carbohydrate content (53.31%) than *Pleurotus pulmonarius*. Generally, there was no significant difference in the nutrient composition of *P. ostreatus* and *P. pulmonarius* except in their moisture content where *P. ostreatus* (5.05%) was significantly higher than *P. pulmonarius* (3.90%). On the two substrates, the moisture content of *P. ostreatus* was significantly higher than that of *P. pulmonarius*. However, other nutrient parameters of the two mushrooms were not significantly different (Table 4).

There was no significant difference in the nutrient composition of the mushrooms grown on both fermented and unfermented substrates. The mushrooms cultivated on 30% wheat bran had the highest protein content (40.14%) and carbohydrate content (53.77%) when compared with other additive concentrations used. The mushrooms grown on 10% wheat bran recorded the highest fat content (2.72%), crude fiber content (4.97%) and moisture content (4.82%) when compared with other concentrations. The 20% wheat bran concentration had the highest ash content compared with others (Table 4).

The protein content of the mushrooms cultivated on 30% wheat bran was significantly higher than that of mushrooms cultivated on other concentrations. More so, the protein content of mushrooms cultivated on 0% additive compared favorably with that of mushrooms cultivated on 30% additive. The carbohydrate content of mushrooms grown on 0% wheat bran also compared favorably with that of mushrooms cultivated on other concentrations except 10% (Table 4).

4. DISCUSSION

The growth of *Pleurotus ostreatus* and *P. pulmonarius,* which was supported by *Tectona grandis* and *Celtis zenkeri* agrees with the findings of [12] who reported that *Pleurotus* species grew well on agricultural substrates. Better growth of *P. ostreatus* and *P. pulmonarius* on fermented sawdust of *T. grandis* and *C. zenkeri*, compared to that on the unfermented sawdust which was observed in this work agrees with the findings of [13,6] who reported that fermented sawdust improved the yield of oyster mushrooms and prevented infestation by insects. Also, [14] reported that *P. pulmonarius* grew well on fermented *Funtumia africana.* [15] submitted that the substrate of fermented sawdust showed

potential to prevent the growth of *Trichoderma* sp. which caused a symptom on mushroom mycelium, whereas there was nothing to inhibit the growth of *Trichoderma* sp. in the non-fermented sawdust. It may thus not be impossible that fermentation impacted negatively on growth of some resident fungi thereby freeing up more nutrients for growth of the mushroom which might have aided the better growth of *P. pulmonarius* on fermented sawdust of *T. grandis* and *C. zenkeri* than on unfermented sawdust. The variation in sizes of the harvested mushroom obtained in the experiment could be as a result of factors such as: temperature, light, humidity, substrate nutrient, moisture content, culture medium used in its cultivation and duration of cropping period as submitted by [16]. However, the optimum yield obtained at 0% wheat bran compared to other concentrations was in accordance with [17] who reported that the yield for rice straw without additives was higher than others.

The resident fungi (*Aspergillus niger, A. tamari, A. flavus, Trichoderma harzianium*) which were isolated from unfermented and fermented sawdust, as well as from the mushroom fruiting body was in line with the work of [18], who reported that substrate fermentation prevents the growth of *Trichoderma* species and also reported the isolation of fungi from decay sawdust.

Some of the fungi such as *A. niger* are famous for their mycotoxin (aflatoxin) contamination; however, fungi are generally known to produce lignocellulose enzymes that improves the quality, aeration, pH and temperature of the substrate during fermentation [19]. The variation in the pH and temperature values at different days is in accordance with [20] who reported that the pH value for grass silage fermentation at different temperatures was different. The result obtained with the pH of substrates (which ranged from 6.7 – 7.1) during the fermentation agrees with the work of [21] who posited that the required pH range for the rapid mycelial growth of mushroom is 6.4 -7.8.

In recent times, the amounts of mushroom consumption have been raised greatly because of the presence of numerous nutritional compositions. The high protein content of *Pleurotus ostreatus* and *Pleurotus pulmonarius* grown on the different substrates also agrees with the findings of [22]. They reported that an increase in available nitrogen of the substrate increases the protein content of plants, fungi and animals. The results obtained were also in line with the findings of [23] who reported high protein content in both *Pleurotus ostreatus* and *Pleurotus pulmonarius*. The crude protein and ash content of *Pleurotus ostreatus* and *Pleurotus pulmonarius* are comparable to most legumes soybeans grown in West Africa. The low moisture content observed from the dried mushrooms is in line with the findings of [24] and this indicates that the mushrooms can easily be sundried, smoked and stored soon after harvest.

The low fat content of *Pleurotus ostreatus* and *Pleurotus pulmonarius* compares favorably with the work of [25] who reported that mushrooms usually contain less fat ranging from 1-8% of dry weight and that the low fat content makes it suitable component of weight-restricted diet. The high carbohydrate content of *Pleurotus ostreatus* and *Pleurotus pulmonarius* recorded in this study is in line with the works of [26] who asserted that the carbohydrate content in mushroom ranged between 30 - 80%. The low fiber content also confirms the work of [24] as well as [23] who also reported low fiber in their own experiments. Considerable fiber content in any food aids digestion and helps in speeding up the passage of faeces from the body thereby indirectly preventing certain diseases like colon cancer and coronary heart disease [27].

Although additives are meant to improve growth of mushrooms, some can be said to grow optimally without the need for additives. The mushrooms, which were rich in protein, carbohydrate, fats, fiber and ash may be useful as supplements due to their nutritional benefits. The adequate nutritional contents of the mushrooms which grew on the sawdust underscores the capability of the sawdust from these two hardwoods to support mushroom growth. It also suggests a positive way of putting sawdust wastes to use.

5. CONCLUSION

The cultivation of *P. ostreatus* and *P. pulmonarius* on the sawdust of *Tectona grandis* and *Celtis zenkeri* can thus be said to be a good means of waste management of sawdust. The use of additive to aid optimal mushroom growth, which is a popular practice may sometimes not be necessary. A close association can also be said to exist between the resident fungi of the substrate (sawdust) and the mushroom growing on them. However, the capacity of some of the fungi like *A.niger* to produce mycotoxins such as aflatoxin must not be overlooked.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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