Abilities of *Tectona grandis* and *Celtis zenkeri* (hardwood) sawdust as substrates of *Pleurotus* species and their indigenous fungi

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 $p \leq 0.05$.

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 was 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.*

INTRODUCTION

Mushrooms are locally referred to as 'Olu' in Yoruba 'Ero atakata' in Igbo and 'naman kaza' in Hausa. There are many species of edible mushrooms which grow mostly on agro-industrial wastes (Jonathan and Babatunde, 2013). *Pleurotus* species are mushrooms that can grow on any agro-industrial wastes (hardwood inclusive) (Manpreet *et al.,* 2004). The estimated amount of agro industrial waste generated in Nigeria is more than 3.2

million tonnes per annum. This causes environmental pollution due to improper disposal and burning. Therefore, these wastes can be used as substrates during mushroom cultivation. (Musatto and Teixera, 2010). Mushrooms on the other hand have been reported to be a suitable substrate for micro-organisms (Kim *et al*., 2013).

Mushroom is currently gaining global attention due to its nutritional value and medicinal properties (Chang and Miles, 1988). Agricultural substrates such as sawdust has been reported to affect the yield of various mushrooms (Harun-or-Rashid *et al*., 2016). Mushroom growth has been reported to improve due to the addition of certain additives such as rice bran and wheat bran (Jonathan *et al.*, 2012a). Fermented sawdust have also been reported to improve the yield of mushroom and prevent infestation by insects (Gbolagade, 2006). However, different fungi has been reported to be isolated from decaying sawdust (Obire and Amadi, 2013).

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.

MATERIALS AND METHOD

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.

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 Adenipekun and Fasidi (2005). Fermentation of the substrates was done using the method of Gbolagade (2006). Eighty grams each of fermented and unfermented sawdust was weighed into 350ml bottles and sterilized using standard procedures.

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^{o}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 according to AOAC, 2002.

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 0.1g 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^{o}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, 0.1g 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.

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 $p \leq 0.05$.

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.. *Aspergilus niger* was the most predominant of all the resident fungi isolated.

Effect of fermentation pH and temperature on the two sawdust during fermentation.

Figures 1 and 2 show the pH and temperature values of fermented *Celtis zenkeri and Tectonal grandis* for 12 days of fermentation processes. At days 1, 2, 4, 5, 7 and 9 of fermentation, there was significant differences in their pH values (Figure 1). For temperature, there was no significant difference in their temperature first and second day of fermentation but there was significant difference from third day of fermentation to twelfth day of fermentation (Figure 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%), 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%). The nutrient composition of the mushrooms performance on two substrate also indicate that the moisture content of *P. ostreatus* was significantly higher than that of *P. pulmonarius* but other nutrient parameters showed no significant difference (Table 4).

There was no significant difference in the nutrient composition of the mushrooms grown on both fermented and unfermented substrate. The mushrooms cultivated on 30% wheat bran concentration 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 concentration recorded the highest fat content (2.72%), crude fiber content (4.97%) and the highest moisture content (4.82%) when compared with other concentrations. Also, 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 concentration was significantly different from others. Mushrooms cultivated on 0% additive concentration was significantly different in fat content, moisture content and crude fiber content when compared with other additive concentrations. Also, the carbohydrate content of mushrooms grown on 10% wheat bran concentration was significantly different from others (Table 4).

DISCUSSION

The growth of *Pleurotus ostreatus* and *P. pulmonarius* was supported by *Tectona grandis* and *Celtis zenkeri* which agrees with the findings of Fuwape *et al.* (2014) who reported that *Pleurotus* species grew well on

agricultural substrates. *P. ostreatus* and *P. pulmonarius* grew well on fermented sawdust of *T. grandis* and *C. zenkeri* than unfermented sawdust of *T. grandis* and *C. zenkeri* (hardwoods) which agrees with the findings of Hernandez *et al.* (2003) and Gbolagade (2006) who reported that fermented sawdust improved the yield of oyster mushrooms and prevented infestation by insects. Also, Jonathan *et al.* (2012) reported that *P. pulmonarius* grew well on fermented *Funtumia africana.* Oh *et al*. (2003) 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. during 30 days after inoculation in 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 harvested mushroom sizes is as a result of factors such as: temperature, light, humidity, substrate nutrient, its porosity, moisture content appropriate strain, culture medium used in its cultivation, duration of cropping period and particle size (Frimpong-Manso *et al*., 2010). However, the optimum yield obtained at 0% wheat bran concentration compared to other concentrations was in accordance with Soniya e*t al.* (2013) who reported that the yield for rice straw without additives was higher than others.

The resident fungi isolated from unfermented sawdust were *Aspergillus niger, A. tamari, A. flavus, Trichoderma harzianium* and *T.* species while *Aspergillus niger, A. tamari, A. flavus* were isolated from both the fermented sawdust and mushroom fruiting body which is in line with the work of Obire and Amadi (2013), 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 (Oyetayo and Ariyo, 2013). The variation in the pH and temperature values at different days is in accordance with Lancaster (1975) reports that the pH value for grass silage fermentation at different temperature was different. The pH of substrates ranged from 6.7 – 7.1 during the fermentation process which agrees with the work of Khan *et al.,* 2013 that reported 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 varied substrates ranged from 38.00 to 40.00 which agrees with the findings of Wang *et al.* (2001). They reported that an increase in available nitrogen of the substrate increases the protein content of plants, fungi and animals. Also, Adejumo and Awosanya 2005 reported high protein content in both *Pleurotus ostreatus* and *Pleurotus pulmonarius* which corresponds with my findings. 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 Kayode *et al.* (2013) and it indicate that the mushrooms can easily be sundried, smoked and stored soon after harvest.

The low fat content of *Pleurotus ostreatus* and *Pleurotus pulmonarius* correspond to the work of Breene, (1990) who reported that mushrooms usually contain less fat ranging from 1-8% of dry weight and this low fat content makes it suitable component of weight restricted diet. The high carbohydrate content of *Pleurotus ostreatus* and *Pleurotus pulmonarius* gotten from this study is in line with the works of Okwulehia and Ogoke, 2013 where they assert that the carbohydrate content in mushroom is between 30 - 80%. The low fiber content gotten confirms the work of Kayode *et al.,* 2013 and Adejumo and Awosanya, 2005 who also reported low fiber. Considerable fiber content in any food helps in speeding up the passage of faeces from the body thereby preventing them from sitting for too long which may result in several diseases like colon cancer and coronary heart disease (Deakin University, 1999).

Although additives are meant to improve growth of mushrooms, some can be said to grow optimally without the need for additives. The mushrooms were rich in protein, carbohydrate, fats, fiber and ash and may be used 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 good way of putting sawdust wastes to use.

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.

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245 **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

246 **Means with different letters in a column are significantly different at $p \leq 0.05$**

247 **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

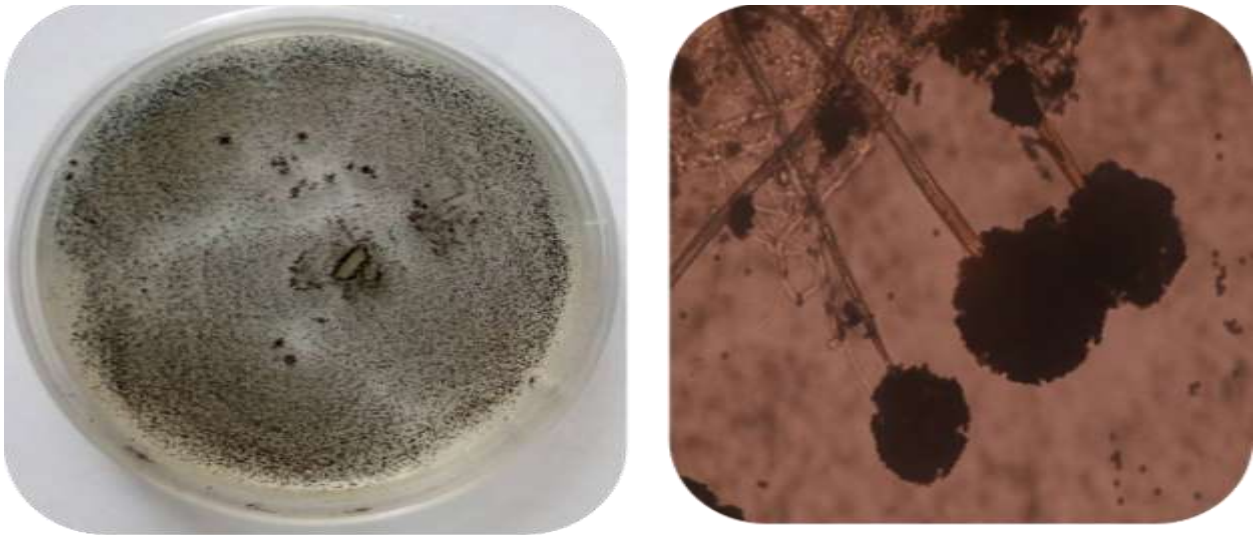
Means with different letters in a column are significantly different at $p \leq 0.05$.

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*

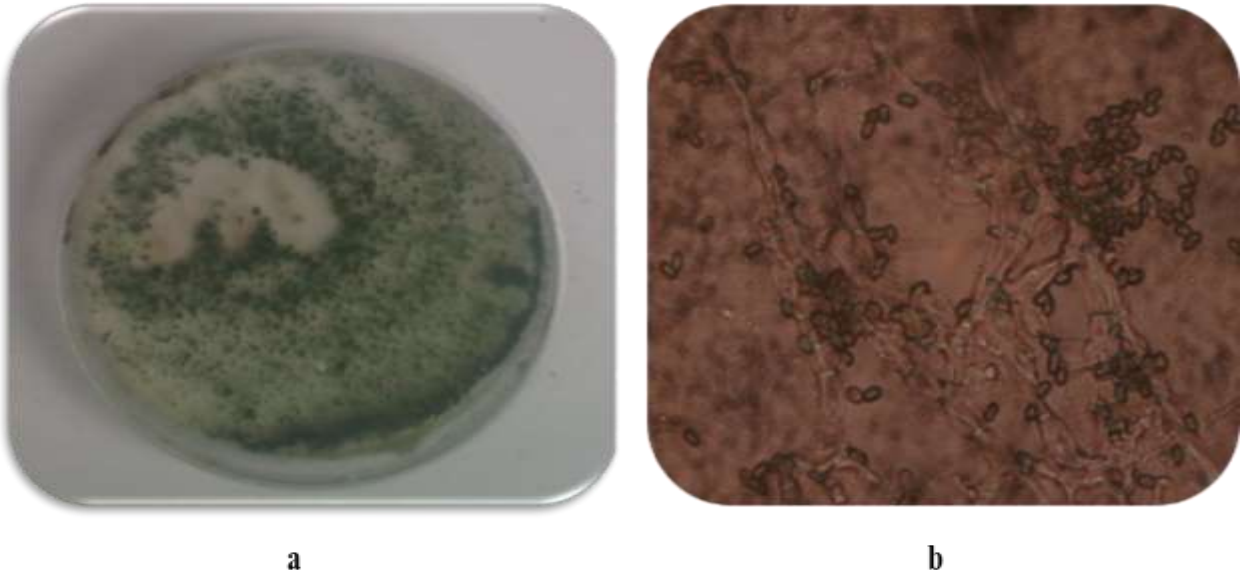
Table 4: Proximate analysis of *P. ostreatus* and *P. pulmonarius* cultivated on sawdust of *Tectona grandis* and *Celtis zenkeri*

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



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).

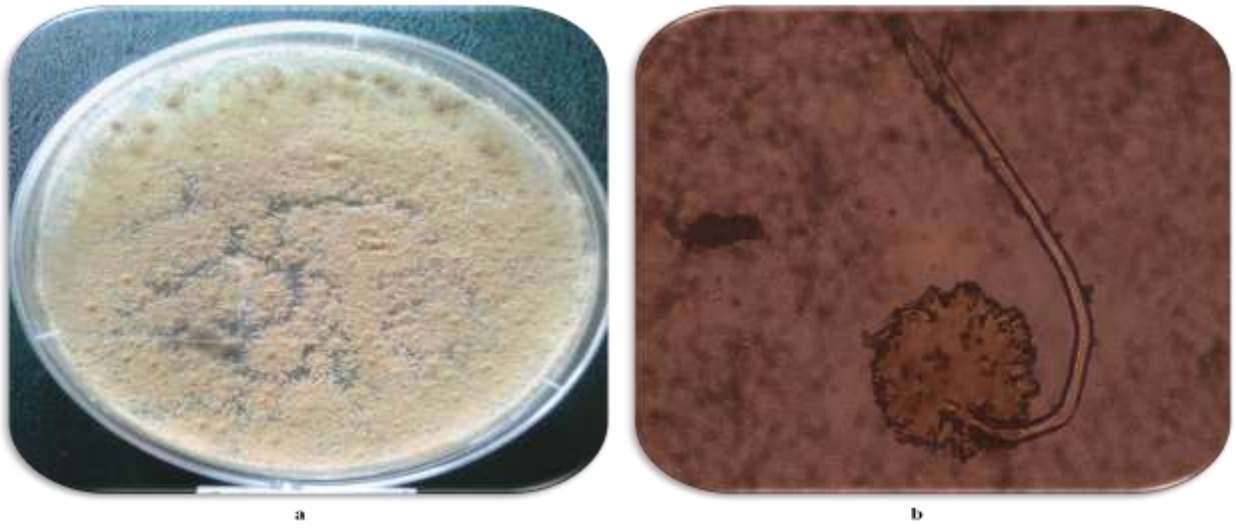


Plate 3: *Aspergillus tamarii* isolated from both the fermented and unfermented sawdust and also from the mushrooms (a); Photomicrograph (b).

Figure 1: Effect of pH on the two sawdust during fermentation.

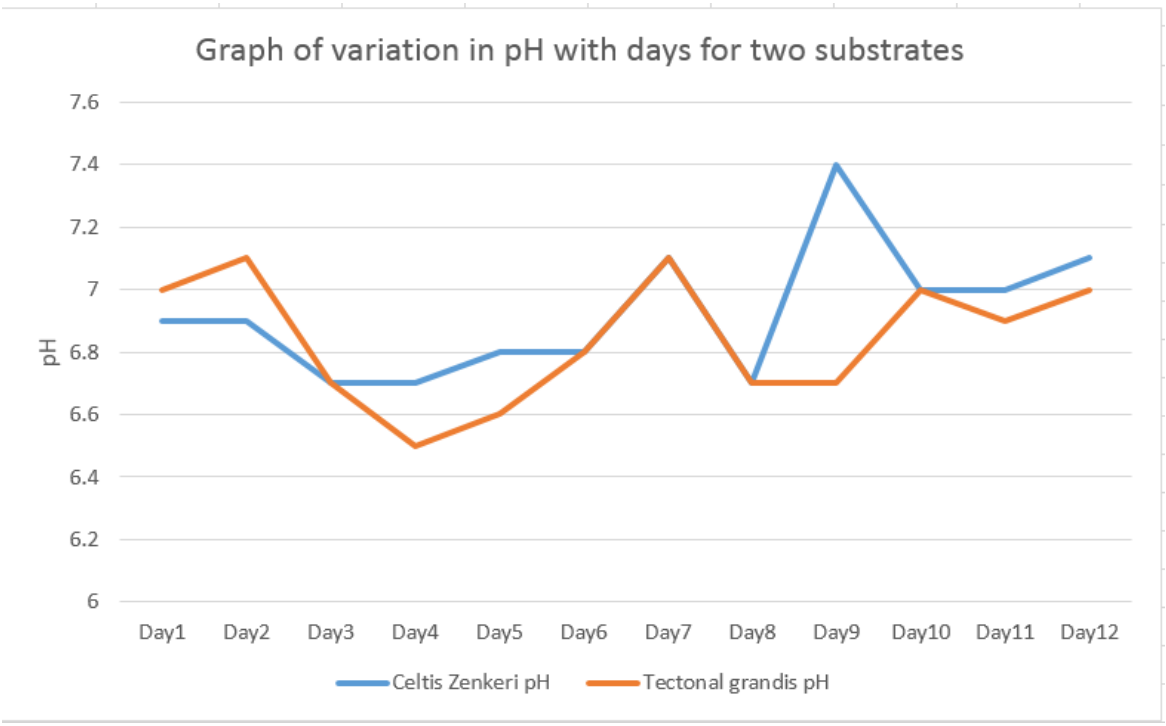
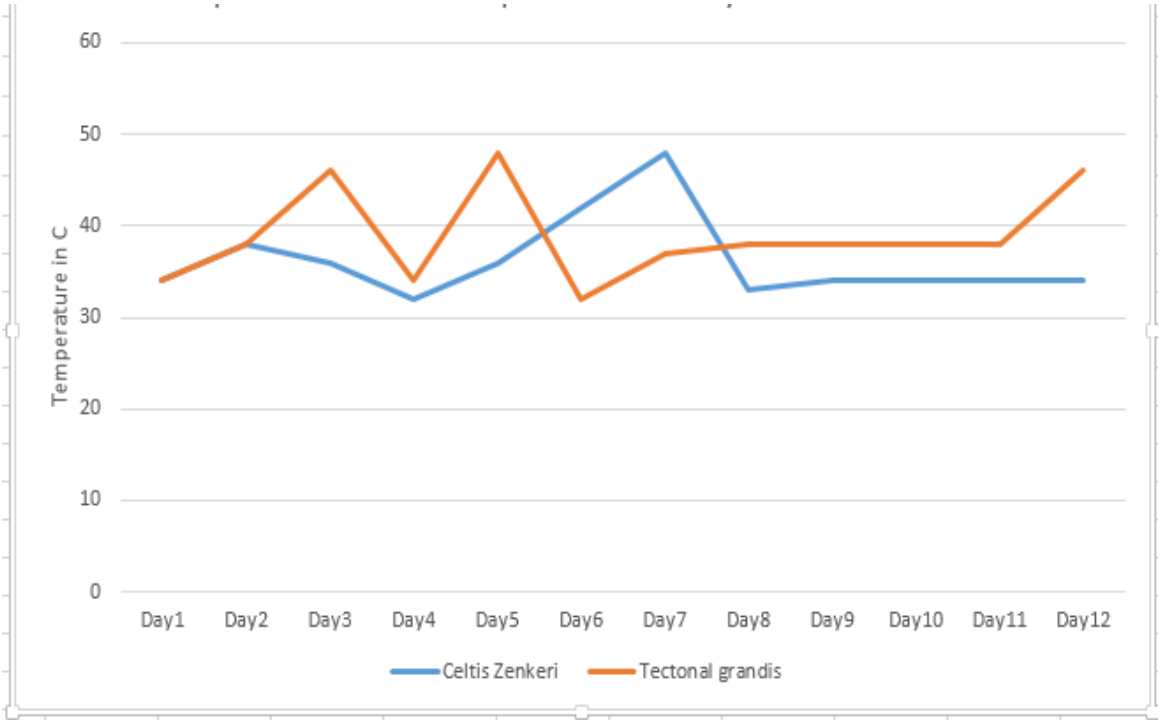


Figure 2: Effect of temperature on the two sawdust during fermentation.


60
50
40
30
20
10
0
Temperature in C
Day1
Day2
Day3
Day4
Day5
Day6
Day7
Day8
Day9
Day10
Day11
Day12
Celtis Zenkeri
Tectonal grandis

266