

# 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 sajor-caju* were investigated. Relationship between fungal incidence of the substrates (sawdust) and that of the mushroom were examined. Both *Pleurotus ostreatus* and *Pleurotus sajor-caju* were inoculated on fermented and unfermented sawdust of *Tectona 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. sajor-caju*. *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. Cultivation of mushrooms on hardwood sawdust is thus an effective means of managing such waste.

**Keywords:** fermented, resident fungi, *Tectona grandis*, *Celtis zenkeri*, *Pleurotus ostreatus* and *Pleurotus sajor-caju*.

## 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 Teixeira, 2010). Mushrooms on the other hand have been reported to be good substrate for micro-organisms. 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 (Chun-Li *et al.*, 2015). 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 sajor-caju* 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. sajor-caju* and were incubated at  $28\pm 2^{\circ}\text{C}$  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 sajor-caju* was determined according to AOAC, 2002.

### **Isolation and identification of fungal species**

Isolation of resident fungi from the mushrooms and sawdust was done at the Plant Pathology laboratory. The isolated fungi were later identified after obtaining pure cultures.

62 **Data analysis**

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

65

66 **RESULTS**

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

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

75

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

82

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

84 Figures 1 and 2 show the pH and temperature values of fermented *Celtis zenkeri* and *Tectona grandis* for 12  
85 days of fermentation processes. At day 6, 7, 8 and 10 of fermentation, there was significant differences in their  
86 pH values (Figure 1). For temperature, there was no significant difference in their temperature first and second  
87 day of fermentation but there was significant difference from third day of fermentation to twelfth day of  
88 fermentation (Figure 2).

89 **Proximate composition of *P. ostreatus* and *P. sajor-caju* cultivated on sawdust of *Tectona grandis* and**  
90 ***Celtis zenkeri***

*Pleurotus ostreatus* had higher crude protein (39.00), crude fiber (4.69), moisture content (5.05), carbohydrate content (53.31) than *Pleurotus sajor-caju*. Generally, there was no significant difference in the nutrient composition of *P. ostreatus* and *P. sajor-caju* except in their moisture content where *P. ostreatus* (5.05) was significantly different from *P. sajor-caju* (3.90). The nutrient composition of the mushrooms performance on two substrate also indicate that the moisture content of both *P. ostreatus* and *P. sajor-caju* was significantly different 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. sajor-caju* was supported by *Tectona grandis* and *Celtis zenkeri* which agrees with the findings of Fuwape *et al.* (2014) and Chun-Li *et al.* (2015) who reported that *Pleurotus* species grew well on agricultural substrates. *P. ostreatus* and *P. sajor-caju* 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*.

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 *et 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 harzianum* 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.

However, fungi produces 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. Chungi *et al.* (2012) also reported the pH value decreased (i.e more acidic) as the day of fermentation of Soy Sauce increased. The pH of substrates ranged from 6.7 – 7.1 during the fermentation process which agrees with the work of Iqbal and Shah, 1989 and 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 sajor-caju* 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 sajor-caju* which corresponds with my findings. The crude protein and ash content of *Pleurotus ostreatus* and *Pleurotus sajor-caju* high compared 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 sajor-caju* 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 sajor-caju* 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. sajor-caju* 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 good association can also be said to exist between the resident fungi of the substrate (sawdust) and the mushroom growing on them.

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220 **Table 1: Effect of sawdust on the growth parameters of *Pleurotus ostreatus* and *Pleurotus sajor-caju*.**

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

221 Means with different letters within the same column are significantly different at  $p \leq 0.05$



222 **Table 2: Effect of supplement on the growth parameters of *Pleurotus ostreatus* and *Pleurotus sajor-caju*.**

Wheat concentrations	bran	Cap length	Cap diameter	Cap width	Stipe length	Stipe width	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 <sub>0.05</sub>		0.63	5.18	0.75	0.88	0.43	1.98	9.39
R <sup>2</sup>		0.17	0.09	0.48	0.31	0.25	0.17	0.28

223 Means with different letters within the same column are significantly different at  $p \leq 0.05$ .

224 **Table 3: Resident fungi isolated from the substrates and the mushrooms**

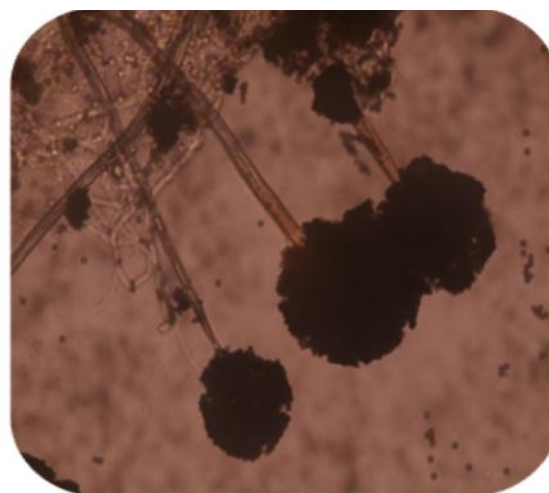
S/N	SUBSTRATE	ISOLATED FUNGI
1	Unfermented sawdust	<i>Aspergillus niger</i> , <i>A. tamarii</i> , <i>A. flavus</i> , <i>Trichoderma harzianum</i> and <i>Trichoderma</i> species
2	Fermented sawdust	<i>Aspergillus niger</i> , <i>A. tamarii</i> , <i>A. flavus</i>
3	Mushrooms	<i>Aspergillus niger</i> , <i>A. tamarii</i> , <i>A. flavus</i>

225 Table 4: Proximate analysis of *P. ostreatus* and *P. sajor-caju* cultivated on sawdust of *Tectona grandis* and  
226 *Celtis zenkeri*

Parameters		Crude Protein	Crude Fat	Ash	Crude fibre	Moisture content	CHO
Substrate types	<i>Celtis zenkeri</i>	38.99a	1.57a	2.54a	4.76a	4.79a	52.25a
	<i>Tectona grandis</i>	38.92a	2.27a	3.10a	4.36a	4.16b	52.83a
Mushroom species	<i>Pleurotus ostreatus</i>	39.00a	1.60a	2.29a	4.69a	5.05a	53.31a
	<i>Pleurotus sajor-caju</i>	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 <sub>0.05</sub>	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 <sub>0.05</sub>	2.03	1.2	0.84	0.95	0.69	1.57
	R <sup>2</sup>	0.12	0.33	0.18	0.21	0.43	0.24



a

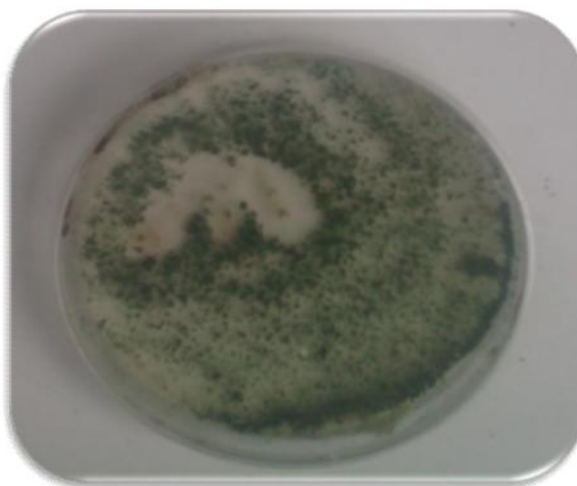


b

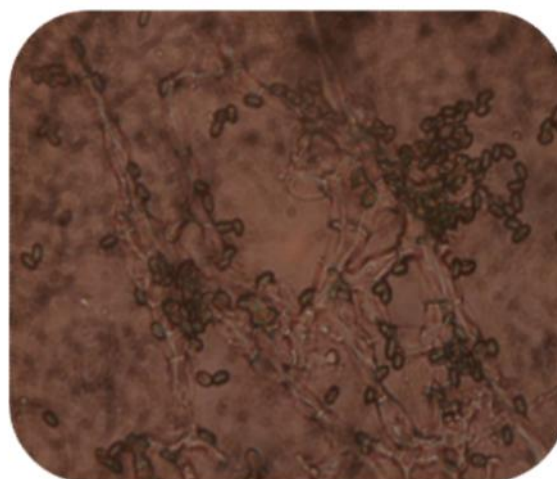
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228 Plate 1: *A. niger* obtained from *T. grandis* and *C. zenkeri* sawdust and from the mushrooms (a);

229 Photomicrograph (b).



a



b

230

231 Plate 2: *Trichoderma harzianum* from unfermented *T. grandis* and *C. zenkeri* sawdust and from the

232 mushrooms (a); Photomicrograph (b).

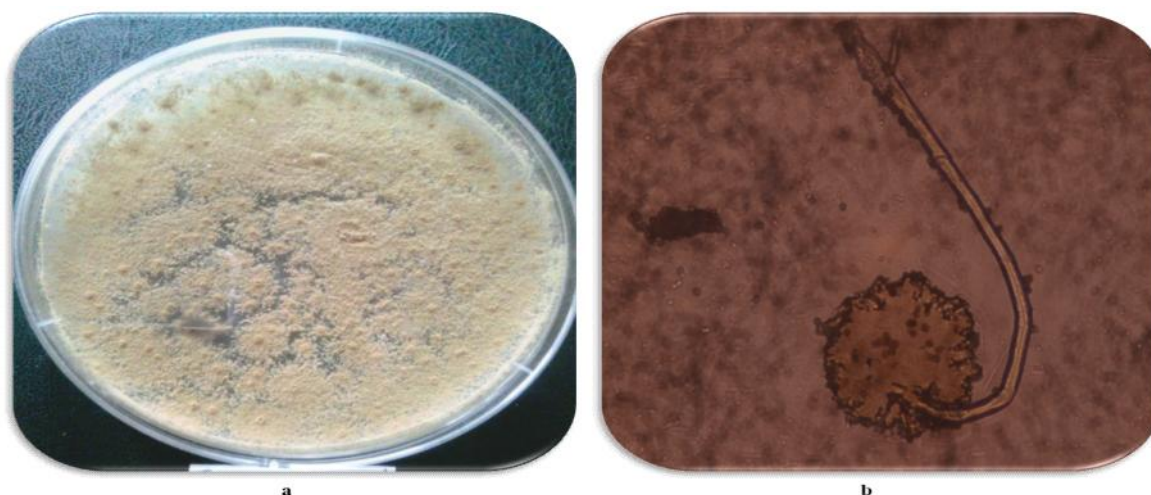


Plate 3: *Aspergillus tamarii* isolated from both the fermented and unfermented sawdust and also from the mushrooms (a); Photomicrograph (b).

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

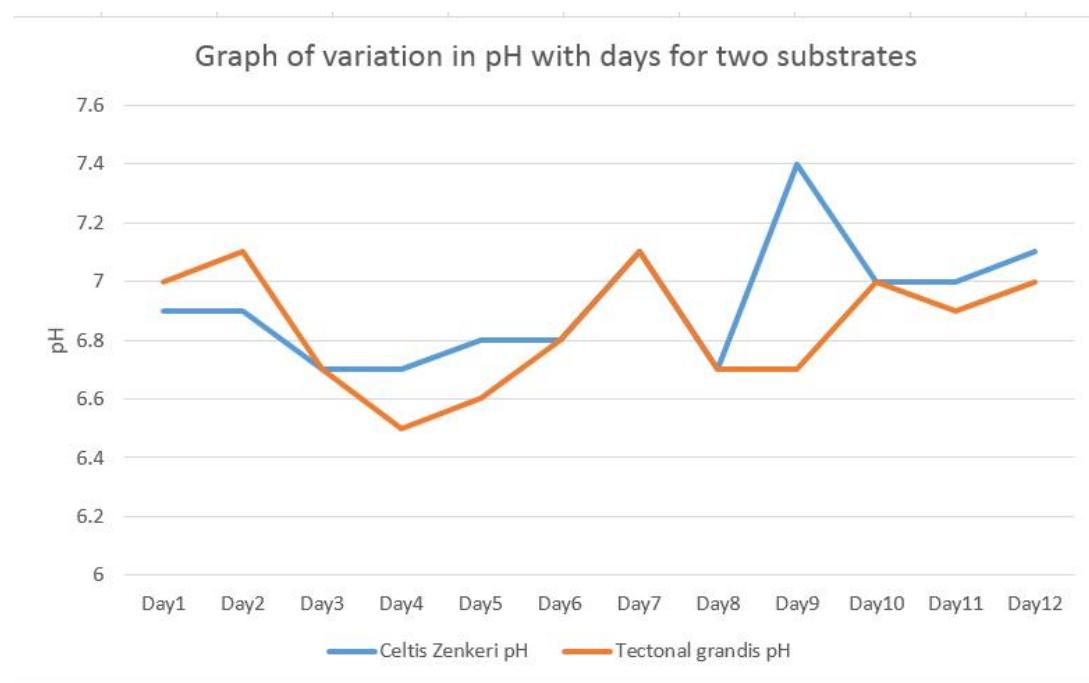
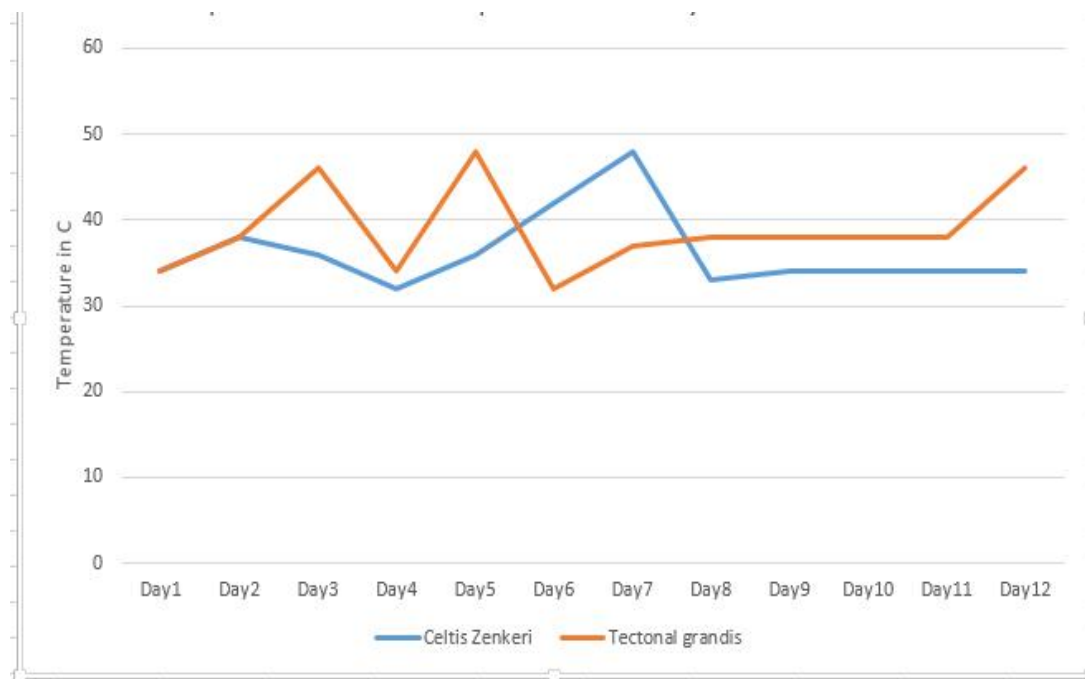


Figure 2: Effect of temperature on the two sawdust during fermentation.



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