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Fungal Incidence and Growth of Two *Pleurotus* Species on Sawdust of *Ceiba pentandra* (Linn.) Gaertn and *Ficus Mucuso* welw (Softwoods)

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Abstract

Objective: Nigeria is faced with one of the most pressing environmental challenge which is the large production of sawdust waste of which *Ceiba pentandra* and *Ficus mucuso* are among the major contributors. This work seeks to harness waste through mushroom cultivation thereby reducing environmental hazards usually caused by improper disposal and burning. Softwood sawdust of *Ceiba pentandra* and *Ficus mucuso* was evaluated for their effects on the production of *Pleurotus ostreatus* and *Pleurotus pulmonarius*. Similarly, probable relationship among the fungal incidence of the substrates (sawdust) and that of mushrooms were examined.

Methods: The treatments used were replicated three times in a completely randomized design. The fruiting bodies were harvested and growth parameters, total yield and biological efficiency (BE) of the mushroom were recorded while their nutritional analysis was carried out. Resident fungi in the sawdust and mushrooms were isolated and identified after obtaining pure cultures. The data obtained were subjected to analysis.

Results: *Ceiba pentandra* and *Ficus mucuso* sawdust (substrates) supported the growth of the two mushrooms. *P. ostreatus* grew significantly ($p \leq 0.05$) higher than that of *P. pulmonarius.* However, the substrates had significant ($p \leq 0.05$) impact on the different growth parameters of the mushrooms. Generally, fermented sawdust significantly ($p \leq 0.05$) improved some growth parameters of the mushrooms compared to unfermented one. Zero percent (0%) additive had significant ($p \leq 0.05$) impact on growth parameters of the mushrooms than other concentrations. The mushrooms was nutritionally good. Similar fungi were isolated from the substrates (sawdust) and the mushrooms.

Conclusion: Softwood sawdust is thus a conducive substrate for mushroom cultivation and could be part of the solution to environmental challenge due to sawdust in Nigeria.

Keywords: Ferment; Resident fungi; *Ceiba pentandra*; *Ficus mucuso; Pleurotus ostreatus*; *Pleurotus pulmonarius*

Introduction

Mushrooms are one of man's earliest form of fungi known [1]. They are macro-fungi, many of which are known to be edible [2]. *Pleurotus* species is an edible mushroom known to grow on any kind of lignocellulose waste (Chang and Milles 2004). Member of the species have received considerable attention due to their nutritional value, medicinal properties and biodegradation abilities [3]. Nigeria is known to produce high amount of sawdust waste among all other lignocellulose waste (softwood inclusive). Sawdust of softwood such as *Ceiba pentandra* and *Ficus mucuso* are known to be among the major contributors of this waste. Micro-fungi (Resident fungi) on the other hand are known to be among the common contaminants growing both on sawdust and mushrooms [4]. Mushrooms are widely distributed in nature with some being edible and some poisonous [1] Sawdust of certain trees such as mango and cashew have been reported to improve yield of different mushrooms [5,6]. Fermented and Unfermented sawdust have also been reported to affect the yield of different mushrooms [7]. Certain additive such as rice bran and wheat bran have also been reported to improve growth of mushroom [8]. However, different species of fungi have been isolated from sawdust [9]. Therefore, the experiment was set up to determine the effect of softwood sawdust in the production of *Pleurotus ostreatus* and *Pleurotus pulmonarius* and to also examine any probable relationship among the fungal incidence of the substrate (sawdust) and that of mushrooms.

Materials and Methods

Collection of sawdust and additive

The sawdust and additive were collected from sawmill in Isopako and the feed mill in Bodija market, Ibadan, Oyo State respectively**.** The sawdust was identified by Prof. A.A Jayeola in the Anatomy unit of Botany Department, University of Ibadan.

Collection and multiplication of spawn

Spawn was collected from the Plant Physiology Laboratory, Department of Botany, University of Ibadan. Multiplication of spawn was done using the method of Adenipekun and Fasidi [10]. The spawn bottles were stored in the refrigerator below 10°C.

Fermentation and preparation of the substrates

The fermentation of the substrates (sawdust) was carried out using the method of Gbolagade [11]. Preparation of fermented and unfermented substrates were done using the method of Adenipekun and Fasidi [10].

Inoculation and fructification of mushrooms

The bottles were inoculated with 10 g spawn of *Pleurotus pulmonarius* and *Pleurotus ostreatus* and were incubated at 28 ± 2°C for 3 weeks. They were later taken out and watered regularly for fructification.

Data collection

The fruiting bodies were harvested and the growth parameters, total yield and Biological Efficiency (BE) of the mushroom were recorded.

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Isolation and identification of fungal species

The isolation of resident fungi in the sawdust and mushroom were done in the Plant pathology and mycology department. They were identified after obtaining pure cultures.

Determination of proximate composition (Analysis)

Proximate composition of *Pleurotus ostreatus* and *Pleurotus pulmonarius* was determined according to AOAC 2005.

Data analysis

The data obtained were subjected to analysis (ANOVA) using SAS (version 9.3). Means were separated ($p \leq 0.05$) using Duncan's Multiple Range Test (DMRT).

Results

Growth parameters of *Pleurotus ostreatus* and *Pleurotus pulmonarius*

Ceiba pentandra and *Ficus mucuso* sawdust supported the growth of the mushrooms and also had significant ($p \leq 0.05$) impact on different growth parameters of the mushrooms. Generally, fermented sawdust significantly ($p \leq 0.05$) improved some growth parameters of both *Pleurotus ostreatus* and *Pleurotus pulmonarius* compared to the unfermented one. Such parameters included; pileus thickness, number of fruiting bodies and biological efficiency. However, *P. ostreatus* was significantly ($p \leq 0.05$) higher than that of *P. pulmonarius* in all the growth parameters. The mushroom's first harvest was significantly higher than that of the second harvest (Table 1). Of all the wheat bran concentrations (additives), growth parameters of mushrooms that received 0% additive was significantly ($p \leq 0.05$) higher than the growth in other concentrations (Table 2).

Fungal incidence in the mushroom species and sawdust

All the resident fungi isolated from the substrate (sawdust) were also isolated from the mushrooms (Table 3). The isolated fungi include *Aspergillus niger, A. flavus, A. tamarii, A. fumigatus, Trichoderma harzinum, Trichoderma sp.* 1, *T. viride* and *Trichoderma sp.* 2 (Figures 1-3).

Overall incidences of the fungi in the two mushrooms and sawdust

The overall incidence of the fungi ($p \leq 0.05$) in mushroom species and sawdust were not significantly different from each other (Table 4). However, the comparison of the overall incidences of the fungi in the two mushrooms and sawdust were significantly different from each other (Table 5).

Proximate composition of the mushroom species grown on the two sawdust

The two selected mushrooms grown on *Ceiba pentandra* and *Ficus mucus* sawdust were rich in protein, fibre, ash, and carbohydrate. However, the additives had different impact on the nutritional composition of the mushrooms (Table 6).

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

After fermenting the two sawdust for twelve days, the temperature and pH between the two substrates were significantly different (Figures 3-5).

Discussion

The significant effect of substrates and additives on the growth parameter of *P. ostreatus* and *P. pulmonarius* confirmed the appropriateness of the model used. The enhanced growth of *P. ostreatus* and *P. pulmonarius* recorded in *C. pentandra* and *F. mucuso* sawdust agreed with the findings of Kadiri and Fasidi [12]; Fuwape et al. [13] which reported that lignocellulose wastes are good growth media for *Pleurotus* mushrooms. The variations observed in the growth parameters of the mushrooms species might be attributed to the impact

Parameters		Pileus length (cm)	Pileus diameter (cm)	Pileus thickness (cm)	Stipe length (cm)	Stipe width (cm)	No of fruiting bodies	Biological efficiency (%)
Mushroom species	*P. ostreatus*	4.85^a	5.68^a	6.36^a	7.16^a	3.81^a	5.02^a	41.36^a
	P.pulmonarius	3.96^b	3.96^b	5.17^b	4.48^b	2.85^b	3.09^b	26.68^b
Substrate types	*Ficusmucuso*	4.26^a	4.47^b	5.53^a	6.17^a	3.38^a	3.84^a	32.82^a
	Ceibapentanda	4.55^a	5.17^a	6.01^a	5.46^b	3.29^a	4.27^a	35.22^a
Substrate conditions	Fermented	4.54^a	4.63^a	6.17^a	5.64^a	3.21^a	4.60^a	37.65^a
	Unfermented	4.27^a	5.01^a	5.36^b	6.00^a	3.45^a	3.52^b	30.39^b
Harvest regimes	First harvest	6.01^a	6.87^a	8.80^a	8.30^a	4.54^a	6.24^a	51.02^a
	Second harvest	2.79^b	2.77^b	2.74^b	3.346^b	2.12^b	1.88^b	17.02^b
	$LSD_{0.05}$	0.48	0.57	0.57	0.64	0.39	0.45	2.91
	R^2	0.53	0.59	0.73	0.64	0.5	0.73	0.83
Means with different letters within the same columns are significantly different at $p \leq 0.05$								

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

Parameters		Pileus length (cm)	Pileus diameter (cm)	Pileus thickness (cm)	Stipe length (cm)	Stipe width (cm)	No of fruiting bodies	Biological efficiency (%)
Wheat bran concentrations	0	4.13^a	5.36^a	6.14^a	5.69^{ab}	3.44^{ab}	5.04^a	51.78^a
	10	4.50^a	4.57^{ab}	5.78^{ab}	5.96^{ab}	3.27^{ab}	3.85^b	33.24^b
	20	4.79^a	5.12^a	6.09^a	6.44^a	3.68^a	3.77^b	27.79^c
	30	4.19^a	4.24^b	5.05^a	5.18^b	2.93^b	3.56^a	23.26^d
	$LSD_{0.05}$	0.67	0.81	0.81	0.9	0.56	0.64	4.12
	R^2	0.53	0.59	0.73	0.64	0.5	0.73	0.83
Means with different letters within the same column are significantly different at $p \leq 0.05$								

Table 2: Effect of additive on growth parameters of *Pleurotus ostreatus* and *Pleurotus pulmonarius*.

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Parameters	Variable	*Aspergillusniger*	*A.flavus*	*A. tamari*	*A.fumigatus*	*T.harzinum*	*Trichoderma sp. 1*	*Trichoderma sp. 2*
Mushroom Species	*P. ostreatus*	0.33[a]	0.31[a]	0.21[a]	0.17[a]	0.25[a]	0.17 [a]	0.13[a]
	P.sajorcaju	0.35[a]	0.31[a]	0.13[a]	0.15[a]	0.29[a]	0.04[b]	0.13[a]
Substrate types	*F. mucuso*	0.35[a]	0.35[a]	0.17[a]	0.15[a]	0.27[a]	0.15[a]	0.15[a]
	C.pentandra	0.33[a]	0.27[a]	0.17[a]	0.17[a]	0.27[a]	0.06[a]	0.10[a]
Substrate conditions	Fermented	0.33[a]	0.33[a]	0.10[a]	0.13[a]	0.33[a]	0.04[b]	0.15[a]
	Unfermented	0.35[a]	0.29[a]	0.23[a]	0.19[a]	0.21[a]	0.17[a]	0.10[a]
	$LSD_{0.05}$	0.13	0.18	0.15	0.15	0.18	0.12	0.13
	R_2	0.57	0.14	0.15	0.02	0.12	0.13	0.10
Means with different letters within the same column are significantly different at $p \leq 0.05$								

Table 3: Fungal incidence in the mushroom species and the two sawdust.

Parameters	Variables	Means
Mushroom species	*P. ostreatus*	0.22[a]
	P. pulmonarius	0.20[a]
Substrate types	*F. mucuso*	0.23[a]
	C. pentandra	0.20[a]
Substrate conditions	Fermented	0.20[a]
	Unfermented	0.22[a]
	$LSD_{0.05}$	0.06
Wheat bran concentrations	0	0.24[a]
	10	0.22[a]
	20	0.17[a]
	30	0.21[a]
	$LSD_{0.05}$	0.09
	R^2	0.06
Means with different letters are significantly different at $p \leq 0.05$		

Table 4: Overall incidences of the fungi in the two mushrooms and sawdust.

Parameter	Variables	Means
Pooled fungi	*A. niger*	0.34[a]
	A. flavus	0.31[a]
	A. tamari	0.17[bc]
	A. fumigatus	0.16[c]
	T. harzianum	0.27[ba]
	Trichoderma. sp. 1	0.10[c]
	Trichoderma. sp. 2	0.13[c]
	$LSD_{0.05}$	0.11
	R^2	0.06
Means with different letter are significantly different at $p \leq 0.05$		

Table 5: Comparison of the overall incidences of the fungi in the two mushrooms and sawdust.

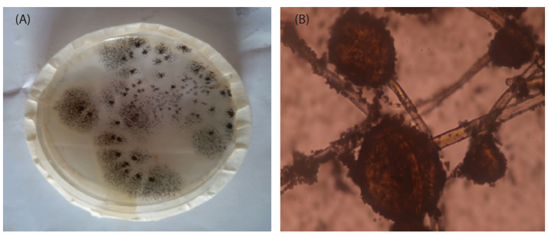

Figure 1: *Aspergillus niger* obtained from the substrates and fruiting bodies of *Pleurotus ostreatus* and *Pleurotus pulmonarius* (**a**) with its photomicrograph (**b**).

of ecological factors, texture, water holding capacity, degree of aeration formulations and nutrients in substrates possibly affected the final mushroom growth [14-16]. However, the different effect of the two sawdust on some the mushrooms' growth parameters corroborated the findings in the study of Ahmed [17] who reported significant influence of various substrates on the growth parameters. The increased

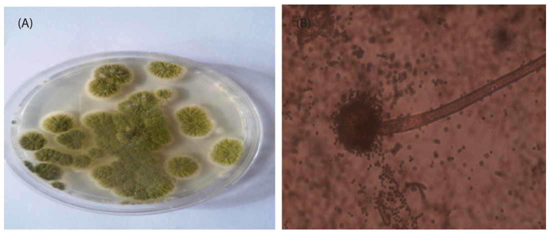

Figure 2: *Aspergillus flavus* obtained from the substrates and fruiting bodies of *Pleurotus ostreatus* and *Pleurotus pulmonarius* (**a**) with its photomicrograph (**b**).

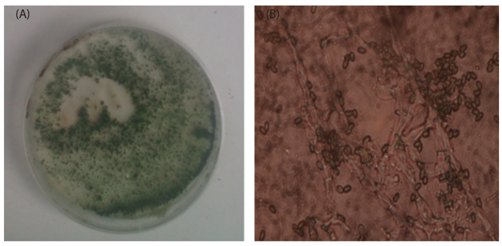

Figure 3: *Trichoderma harzianum* obtained from the substrates and fruiting bodies of *Pleurotus ostreatus* and *Pleurotus pulmonarius* (**a**) with its photomicrograph (**b**).

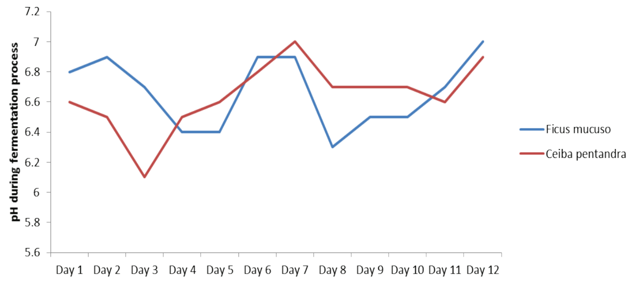

Figure 4: Effect of pH on the two-saw dust during fermentation.

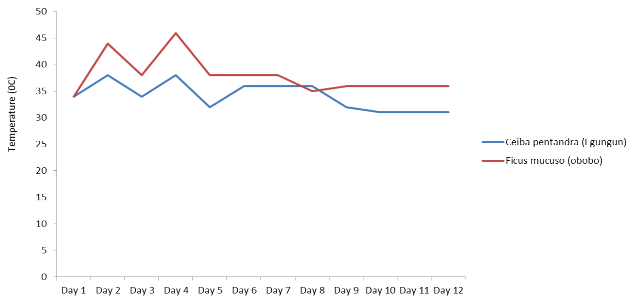

Figure 5: Effect of temperature on the two saw dust during fermentation.

Parameters		Moisture content	Crude protein	Crude fat	Crude fibre	Ash	CHO
Mushroom species	*Pleurotus ostreatus*	15.32^{a}	32.15^{b}	1.10^{a}	23.30^{a}	9.90^{b}	38.20^{a}
	Pleurotus pulmonarius	16.27^{a}	45.93^{a}	0.92^{b}	19.61^{b}	11.40^{a}	29.26^{b}
Substrate condition	Unfermented	15.61^{a}	40.19^{a}	1.05^{a}	22.55^{a}	9.83^{b}	35.63^{a}
	Fermented	15.79^{a}	37.90^{b}	0.96^{b}	20.36^{b}	11.47^{a}	31.83^{b}
Substrate types	*Ceiba pentandra*	16.33^{a}	39.16^{a}	1.08^{a}	18.96^{b}	10.89^{a}	32.83^{a}
	Ficus mucuso	15.07^{a}	38.92^{a}	0.93^{b}	23.95^{a}	10.40^{b}	34.63^{a}
	$LSD_{0.05}$	2.52	1.41	0.08	1.88	0.35	3.31
Wheat bran concentrations	0	13.00^{b}	32.97^{c}	0.79^{c}	23.46^{a}	9.48^{c}	44.61^{a}
	10	23.54^{a}	43.14^{a}	1.16^{a}	17.12^{c}	10.75^{b}	26.52^{c}
	20	11.51^{b}	40.23^{b}	1.08^{ab}	20.53^{b}	10.86^{b}	37.01^{b}
	30	14.75^{b}	39.82^{a}	1.00^{b}	24.70^{a}	11.50^{a}	26.78^{c}
	$LSD_{0.05}$	3.57	2	0.12	2.66	0.5	4.68
	R^2	0.4	0.96	0.49	0.37	0.69	0.56
Means with different letters in the same column are significantly different at $p \leq 0.05$							

Table 6: Proximate composition of mushroom species grown on the two sawdust.

biological efficiency of *P. ostreatus* and *P. pulmonarius* at the different substrate conditions indicated might be as a result of the fermented substrates as found in line with the reports of Hernandez et al. [18] who also recorded the highest biological efficiency in the fermented substrate. However, the better result obtained at 0% additive compared to other concentrations levels was in agreement with Soniya et al. [19] who revealed that the yield of rice straw without supplementation was the most significant highest among the treatments. The significant harvest rate recorded in the first harvest over the second harvest corroborated the report of Soniya et al. [19] who reported that the yield of oyster mushroom recorded the highest significance in the first flush of all treatments. This could possibly be a result of *P. ostreatus* and *P. pulmonarius* utilization of the growth nutrient mostly during the first harvest, thus resulting in declining in mushroom yield in the second harvest [20,21]. The eight resident fungi isolated from the substrates and mushrooms are in agreement with the findings of Lennox et al., Obire and Amadi [22] that also isolated *Penicillium* sp., *Mucor* sp., *Trichoderma* sp. and *Aspergillus* sp. from fermented sawdust. One of the fungi major characteristic is the ability to help in sawdust degradation according to the report of Obire and Amadi [22]. The similarity in the fungal incidence of the sawdust and mushroom species can be as a result of the interaction between them. The non-significant effect of the overall incidence of the fungi in the mushroom species and sawdust shows that the incidence of each fungus does not have any effect on the mushroom species and sawdust. However, the significance in the comparison of the overall incidences of the fungi in the two mushrooms and sawdust shows that incidence of each fungus occurred independently. This implies that when exposed to suitable conditions it can be virulent [23]. However, in spite of this, fungi produces lignocellulosic enzymes that help in the fermentation of the substrates because the medium composition, quality aeration rate, pH and temperature of the substrates used in mushrooms cultivation have an effect on the growth, chemical, functional and sensorial characteristics of mushrooms [24,25]. The pH of the substrates ranged from 6.90 - 7.00 during the fermentation process which agrees with the work of Urben [26]; Kalmis et al. [27] which reported the optimal pH range for the development of mushroom as 4.0-7.0. Fermentation is therefore necessary to adjust the pH level of the substrate pH [25]. The variation in the fermentation temperature could be due to the changes in the environmental conditions while the gradual increase in the temperature contrasted the findings of Sher et al. [28]. who reported that lower temperatures and dry condition can cause a reduction in the stalk height and cap size of mushroom. The richness of the two mushrooms supported the earlier findings that mushrooms have nutritional attributes and can be used as supplements [29,30]. Furthermore, the moisture content of mushroom depend on their harvesting time, maturation period and environmental conditions such as humidity and temperature in growing period and storage conditions [31]. The relatively low moisture content obtained in this study is in line with the report of low moisture content in some mushrooms collected in Nigeria and this indicated that mushrooms can be easily dried and have an extended shelf life [32,33]. The high crude protein and total carbohydrate content obtained in this study corroborates the works of Kadiri and Fasidi [12]; Kayode et al. [32]; Adejumo and Awosanya [34]. This indicates that mushrooms can replace animal protein and can be used as supplement in cereals or carbohydrate meals because of its affordability, medicinal and nutritive attributes. The crude fat content of the mushrooms was low and this agrees with the findings of Adebiyi et al. [35] and contracts the works of Akindahunsi and Oyetayo [36]; Khurdishul [37]. The relatively low fat content of the mushrooms showed that they have no cholesterol, suitable as a component of weight restricted diet and are less harmful to health than the saturated fatty acids [38]. The high crude fiber content obtained contrasts the work of Kayode et al. [32]. However, high fiber content in mushrooms indicated that it is a good source of roughages, thus having ability to aid easily passage of feaces from the body, reducing the risk of been exposed to several diseases [35]. The high ash content obtained in this study could be based on the role of ash content in the determination and characterization of the content of mineral substances which implies that such mushroom is a good source of minerals [39]. The substrates, substrate conditions, wheat bran concentration and mushroom species significantly ($p \leq 0.05$) affected moisture content, crude protein content, crude fiber content, total ash content and carbohydrate contents of the selected mushrooms. The notable variation in the proximate composition of the mushrooms depends on the type of mushroom, the stage of development, the part samples, level of nitrogen available, the environments, storage conditions, additives and the substrate [40,41].

Conclusion

Mushroom cultivation on sawdust is an efficient way of recycling and managing agricultural and industrial waste. Mushroom farmers are encouraged to use softwood sawdust because they are easily accessible with no cost implication and also support mushroom growth. The mushrooms, which are relatively cheap and easy to come by, were rich in nutrients and therefore should be incorporated into our daily diets to improve its quality, nutritional and medicinal benefits. Further studies, should be done on the substrates composition before addition of additives. It is also important for the environmental conditions of the mushroom house to be kept sterile to limit the fungal incidence on mushroom.

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Compliance with Ethical Standards

Conflicts of interest

All authors declare that there is no conflict of interest.

Ethical approval

This work does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Informed consent was obtained from all individual involved participants included in the study.

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