Regular Article
pISSN: 2288-9744, eISSN: 2288-9752
Journal of Forest and Environmental Science
Vol. 40, No. 1, pp. 64-71, March, 2024
https://doi.org/10.7747/JFES.2024.40.1.64

JFES
Journal of Forest and Environmental Science

Assessment of the Effect of Substrates from Different Wood Species on the Cultivation of Edible Mushroom

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Abstract

Mushroom is very important to rural dwellers due to the various economic, health and nutritional benefits that can be derived from its consumption, sales and utilization. Hence, this experiment was carried out with a view to evaluating the influence of locally available substrate i.e., sawdust of different wood species on the cultivation of *Pleurotus ostreatus* (oyster mushroom). *Pleurotus ostreatus* was grown on sawdust (indigenous and exotic species) using two pretreatment methods, which included hot water treatment to remove (a water-soluble extract) and non-extracted sawdusts (substrate). The result of this study showed that there was a significant difference in the effect of different wood species selected. *Parkia biglobosa* produced a better yield (71.06 g), followed by *Anogeissus leiocarpus* (53.17 g) and *Gmelina arborea* (51.39 g) in a non-treated unextracted states. In the treated samples, *Parkia biglobosa* has the highest yield (50.61 g) followed by *Anogeissus leiocarpus* (49.28 g) and *Gmelina arborea* (45.00 g). Thus, *Parkia biglobosa* and *Anogeissus leiocarpus* produced a higher yield of mushroom either in a treated or non-treated state and they could be recommended for growing oyster mushroom (*Pleurotus ostreatus*).

Key Words: Edible mushroom, sawdust, spawn, *Pleurotus ostreatus*, growth, wheat bran, yield

Introduction

In most countries, there is a well-established consumer acceptance for cultivated mushrooms such as *Agaricus bisporus*, *Pleurotus* spp., (that constitutes about 27% of the world's cultivated mushrooms, with 5 to 6 cultivated species), *Lentinus edodes*, *Volvariella volvacea*, and *Auricularia* spp. (Diez and Alvarez 2001). Over the decades ago, mushrooms have been recognized as an important food item because of their nutritional values and therapeutic properties. They are considered as a good source of protein and polysaccharides. The Romans ate mushrooms on special occasions. The Yoruba race, predominantly in Southwest Nigeria, has folklores, maxims and proverbs woven around mushrooms (Martinez-Carrera 1989). *Pleurotus ostreatus*, the oyster mushroom (Davidson and Jaine 2014) or oyster fungus, is a common edible mushroom. It was first cultivated in Germany as a subsistence measure during World War I (Eger et al. 1976) and is now grown commercially around the world for food. It is related to the similarly culti-

Received: August 22, 2023. Revised: February 5, 2024. Accepted: February 9, 2024.

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vated king oyster mushroom. It originates from the kingdom fungi and belongs to the division Basidiomycota and class Agaricomycetes. It has a broad, fan or oyster-shaped cap spanning 5-25 cm, natural specimens range from white to gray or tan to dark-brown the flesh is white, firm, and varies in thickness due to stipe arrangement. The mushroom's stipe is often absent, but it is short and thick when present. *Pleurotus ostreatus* may contain chemicals that stimulate the immune system. Oyster mushroom is one of the more commonly sought wild mushrooms, though it can also be cultivated on straw and other media. *Pleurotus* spp. contains various types of vitamins and amino acids, high content of fiber and protein, and low-fat content. Besides the nutritional attributes of *Pleurotus* spp, the health benefiting effects like anticancer, antihyperlipidemic, antioxidant, hepatoprotective, antiflammatory, antimicrobial activities make this species a healthy food. The growth of a diverse type of mushrooms require different type of substrates and availability of varied type of materials may dictate the type used (Shin et al. 2010). A large quantity of freely available sawdust of different trees offers a potential substrate source for mushroom cultivation in the tropics (Baysal et al. 2003).

Malnutrition is a problem in developing third world countries. Mushrooms with their flavor, texture, nutritional value, and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries (Eswaran and Ramabadran 2000). The high biofuel prices have caused an increase in food prices and food scarcity in many countries. To alleviate hunger and malnutrition in a world of rising food prices, cultivation of mushrooms is a very reliable and profitable option. *Pleurotus ostreatus* mushroom cultivation can play an important role in managing wood wastes (sawdust) whose disposal has become a problem. These wastes can be recycled into food and environment may be less endangered by pollution. Arends and Donkershoot-shooq (1985) stated that enormous quantities of sawdust are produced annually all over the world. Owonubi and Badejo (2000) also stated that the volume of wood waste generated in Nigeria increases yearly, and that the volume of sawdust creates disposal problems and environmental hazards, which are of concern to all wood industries, the society and government. Olufemi et al. (2012) revealed that enormous wood wastes are generated in the wood industries, and sawdust accounts for about 10-20% of the generation of wood waste. This implies that sawdust generation in the mill continues to increase daily. Hence, this study investigates the possibility of using sawdust from different wood species for the cultivation of *Pleurotus ostreatus* mushroom.

The present experiment was undertaken to evaluate the influence of locally available substrates containing sawdust of different wood species with wheat bran and 1% lime on growth and yield of *Pleurotus ostreatus* mushroom. The effect of the pretreatment method of substrate on the yield of mushroom (*Pleurotus ostreatus*) was also assessed.

Materials and Methods

The research was conducted at the Department of Pathology, Forest Conservation and Protection, Forestry Research Institute of Nigeria, Jericho hills, Ibadan, Nigeria. It lies within Latitude 7°26'N and longitude 3° 34'E of Ibadan, Oyo State.

Procurement of experimental material

Sawdust of two indigenous and exotic tree species were acquired from sawmills in Ibadan metropolis as well as the mushroom spawn was procured from Department of pathology, Forest Conservation and Protection, Forestry Research Institute of Nigeria, Ibadan, Oyo State. Other materials used were; Rubber glove, hydrated lime, ethanol, plastic bags, wheat bran, rubber bands, spatula, transparent ruler, big drum, gas, spirit lamp and weighing balance. These materials were consumables utilized in subsequent processes such as substrate preparation, inoculation and measurement of the substrates and mushrooms. All these materials were purchased within Ibadan metropolis while some were provided in the laboratory.

Procedure for the experiment

Preparation of substrate

Sawdust of both indigenous and exotic species was divided into two equal halves. The first half was measured using a weighing balance after which it was pretreated using hot water, it was then dried using a clean cloth or sieve. The second half was also measured but it was not treated at all. Hot pasteurization method was then used for both treated and untreated sawdust where sawdust was collected and weighed after which wheat bran (5% of the total weight of sawdust) and industrial lime (1% of the total weight of sawdust) was added and mixed thoroughly with adequate water already measured with a measuring cylinder. The substrate was bagged at 1,000 g per packet with each species of sawdust at (10) replicates per treatment. It was pasteurized by loading the already bagged mixture into a big metallic drum, cover it and ignite the gas for 4 hours. It was immediately cooled down to room temperature to allow the substrate ready for inoculation.

Inoculation & incubation of spawn

After pasteurization of the substrate, the workspace was cleaned with ethanol to disinfect the workspace in order to avoid contamination, and the spirit lamp was lighted. The bags were opened at the top to create a little space and the substrate was inoculated with 2-3 spoons of grain spawn subsequently. The inoculated substrate was covered tightly with rubber band and it was moved to the incubating room. The substrates were incubated for about 4 weeks.

Exposure of ramified bags

The incubated substrates showed full colonization by the fungi and the substrates were transferred from the incubating room to an open space, not in direct contact with sunlight and well ventilated to aid the fruiting of the mushroom. Each bag was opened at one end while watering was carried out daily.

Harvesting

Matured mushroom was harvested by twisting it gently at the base for continuous fruiting from the first flush to the final harvest. Growth and yield variables including pileus diameter, stipe height, stipe diameter and fresh weight were measured before each harvest. A transparent well-calibrated ruler was used to measure pileus diameter, stipe diameter and stipe height while weighing balance was used to measure fresh weight. Harvesting was carried out in four flushes and this was done weekly. The fresh yield of oyster mushroom was measured in weighing balance and biological efficiency of the mushroom was determined by using:

Biological efficiency, BE (%)=

$$\frac{Fresh\ weight\ of\ harvested\ mshroom}{Weight\ of\ substrates} \times 100$$

Data analysis

All data generated were subjected to analysis of variance while means were separated using Duncan's Multiple Range Test (DMRT).

Results

The analysis of variance for the mycelial growth of *Pleurotus ostreatus* mushroom grown on different sawdust substrate and pretreatment method is presented in Table 1. It was observed that there was no significant difference in the pretreatment method used and, there was no significant difference in the interaction between the pretreatment methods and the sawdust type used in the cultivation of *Pleurotus ostreatus* ($p > 0.05$). However, it was observed that there was a significant difference in the sawdust type

Table 1. Analysis of variance (ANOVA) for the mycelium growth of *Pleurotus ostreatus* mushroom indicating the differences in the sawdust type and pretreatment method

Source	Type III sum of squares	Df	Mean square	F	p-value	Conclusion
Sawdust type	85.594	2	42.797	17.414	0.000	*
Pretreatment method	4.173	1	4.173	1.698	0.199	NS
Sawdust type×pretreatment method	12.709	2	6.354	2.586	0.087	NS
Error	105.677	43	2.458			
Total	212.445	4				

*Denote that pretreatment is significant at $p<0.05$. NS denotes that pretreatment and its interaction with sawdust type are not significantly different ($p>0.05$).

Table 2. Duncan multiple range test for mycelial growth

Sawdust type	Ranking (sawdust type)	Treated (pretreatment method)	Non-treated
Parkia biglobosa	7.46[a]	7.97[a]	7.47[a]
Anogeissus leiocarpus	5.77[b]	5.96[a]	5.76[a]
Gmelina arborea	4.22[c]	5.28[a]	5.27[a]

Means with the same alphabet vertically are not significantly different ($p>0.05$).

Table 3. Analysis of variance (ANOVA) for the biological yield of *Pleurotus ostreatus* on different sawdust under non treated and treated pretreatment method

Source	Type III sum of squares	Df	Mean square	F	p-value	Conclusion
Pretreatment	1,034.296	1	1,034.296	3.278	0.079	NS
Sawdust type	1,850.567	2	925.283	2.932	0.037	*
Pretreatment×sawdust type	503.752	2	251.876	0.798	0.459	NS
Error	10,412.836	33	315.540			
Total	14,291.302	38				

*Denote that sawdust type is significant at $p<0.05$. NS denotes that pretreatment and its interaction with sawdust type are not significantly different ($p>0.05$).

Table 4. Duncan multiple range test for biological yield

Sawdust type	Ranking (sawdust type)	Treated (pretreatment method)	Non-treated
Parkia biglobosa	72.85[a]	71.06[a]	70.01[a]
Anogeissus leiocarpus	65.92[b]	53.12[a]	49.28[a]
Gmelina arborea	54.74[c]	51.39[a]	49.67[a]

Means with the same alphabet vertically are not significantly different ($p>0.05$).

Table 5. Analysis of variance (ANOVA) for the economic yield of *P. ostreatus* on different sawdust using non treated and treated pretreatment method

Source	Type III sum of squares	Df	Mean square	F	p-value	Conclusion
Sawdust type	1,879.453	1	939.726	3.159	0.056	*
Pretreatment	1,034.296	1	1,034.296	3.278	0.079	NS
Sawdust type×pretreatment method	114.728	2	57.364	0.193	0.826	NS
Error	9,518.118	32	297.441			
Total	13,509.390	37				

*Denote that sawdust type is significant at $p<0.05$. NS denotes that sawdust type and its interaction with pretreatment method are not significantly different ($p>0.05$).

used as substrate in mycelial growth. The highest mycelium growth was observed in *Parkia biglobosa* (7.46), followed by *Anogeissus leiocarpus* (5.77) and *Gmelina arborea* (4.22) had the lowest mycelium growth (Table 2).

The result of the Analysis of Variance (ANOVA) for the growth of *P. ostreatus* cultivated on *Anogeissus leiocarpus*, *Parkia biglobosa*, *Gmelina arborea* using non-treated and treated method of pretreatment was shown in Table 3. The result showed that there was a significant difference in the yield of the mushroom species between the sawdust type used as substrate ($p<0.05$). Subsequently, there was no significant difference in the pretreatment methods and the

Table 6. Duncan multiple range test for economic yield

Sawdust type	Ranking (sawdust type)	Treated	Non-treated
Parkia biglobosa	66.64[a]	59.50[a]	61.05[a]
Anogeissus leiocarpus	56.96[b]	51.45[a]	51.25[a]
Gmelina arborea	48.33[c]	47.80[a]	46.90[a]

Means with the same alphabet vertically are not significantly different ($p>0.05$).

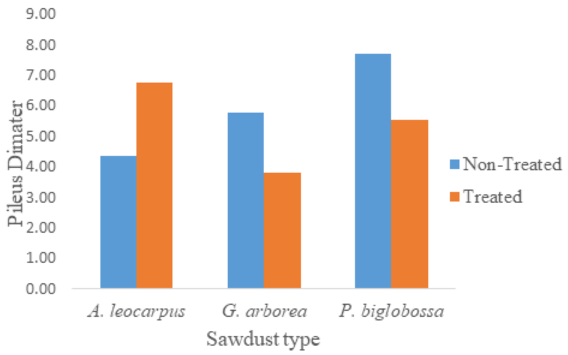

Fig. 1. Effect of sawdust type using the treated and non-treated pretreatment method in *Pleurotus ostreatus* in response to pileus diameter.

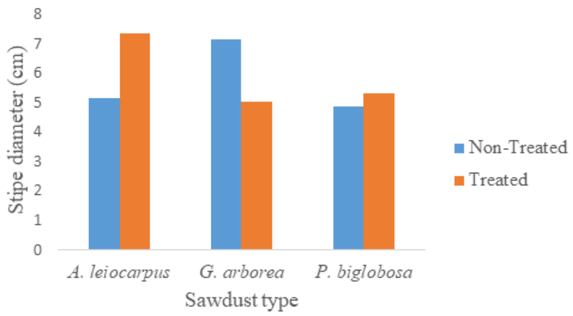

Fig. 2. Effect of stipe diameter on *Pleurotus ostreatus* in response to sawdust type using the treated and non-treated pretreatment method.

interaction between the sawdust type and the pretreatment method. The Duncan follow-up test revealed that in terms of biological yield, *Parkia biglobosa* had the best yield with 72.85 followed by *Anogeissus leiocarpus* with 65.92 and *Gmelina arborea*, 54.74 (Table 4). In terms of economic yield, there was a significant difference in the sawdust type since ($p<0.05$) but there were no significant differences in the pretreatment method used (Table 5). Hence, in terms of economic yield, *Parkia biglobosa* was adjudged the best substrate compared to *Anogeissus leiocarpus* and *Gmelina arborea* (Table 6).

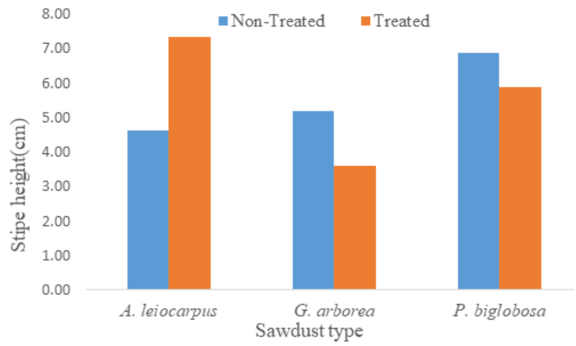

Fig. 3. Effect of stipe height on *Pleurotus ostreatus* in response to sawdust type using the treated and non-treated pretreatment method.

Growth of Pleurotus ostreatus in response to sawdust type and pasteurization method

Fig. 1 showed the pileus diameter, for non-treated, it was revealed that *Parkia biglobosa* grew wider in cap diameter followed by *Gmelina arborea* while *Anogeissus leiocarpus* had the lowest value. For treated, *Anogeissus leiocarpus* had the highest pileus diameter, followed by *Parkia biglobosa* and *Gmelina arborea* had the lowest value. The result indicated that *Anogeissus leiocarpus* performed best when treated with hot water as compared to the non-treated while *Parkia biglobosa* performed well in the non-treated for the pileus diameter.

In the stipe diameter as shown in Fig. 2, it was revealed that *Anogeissus leiocarpus* grew wider in girth for treated samples, followed by *Parkia biglobosa* and *Gmelina arborea*. Subsequently, for the non-treated, the highest value was recorded in *Gmelina arborea* followed by *Parkia biglobosa* and *Anogeissus leiocarpus*. Hence, the result indicated that *Anogeissus leiocarpus* performed best when treated with hot water compared to the non-treated while *Gmelina arborea* performed well in the non-treated for the stipe diameter.

In the stipe height of *Pleurotus ostreatus* grown on dif-

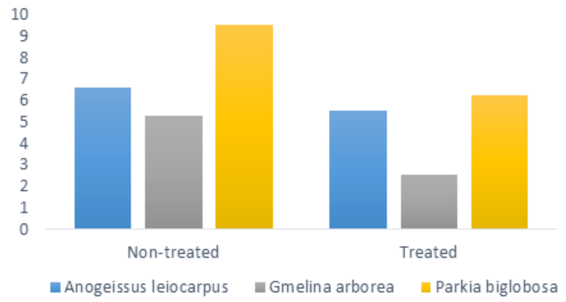

Fig. 4. Average number of fruiting body of *P. ostreatus* on sawdust type and pretreatment method.

ferent sawdust type using both non-treated and treated method as shown in Fig. 3. The result clearly indicates an increase in mushroom growth in the sawdust type where *Anogeissus leiocarpus* had the tallest stipe when treated, the lowest stipe height was recorded for *Gmelina arborea* while *Parkia biglobosa* had the tallest stipe height when not treated.

The highest average number of fruiting body was in *Parkia biglobosa* (9.50) and the lowest average number of fruiting body was in *Gmelina arborea* (5.28) for non-treated (Fig. 4). For treated, the highest average number of fruiting body/packet was observed in *Parkia biglobosa* (6.24) while the lowest average number of fruiting body/packet was revealed in *Gmelina arborea* (2.50). Therefore, the result clearly indicated that *Parkia biglobosa* proves to be the best sawdust with the highest yield for both treated and non-treated.

In Fig. 5, numerically the highest average number of effective fruiting body was observed in *Parkia biglobosa* (8.26) followed by *Anogeissus leiocarpus* (5.40) and the lowest average number of effective fruiting body/packet was in *Gmelina arborea* (4.33) for the untreated. For treated, the highest average number of effective fruiting body was observed in *Parkia biglobosa* (5.43) followed by *Anogeissus leiocarpus* (4.81) while the lowest average number of effective fruiting body was also revealed in *Gmelina arborea* (2.63). Therefore, the result clearly indicated that *Parkia biglobosa* proves to be the best sawdust with the highest yield for both treated and non-treated.

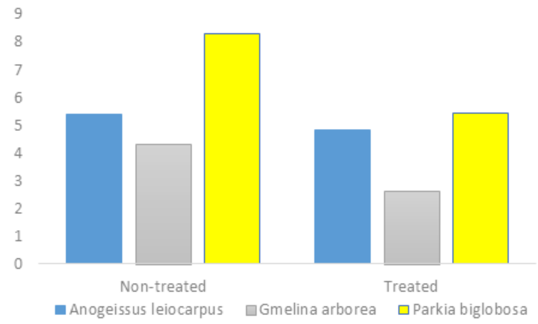

Fig. 5. Average number of effective fruiting body of *P. ostreatus* on sawdust type and pretreatment method.

Discussion

The results from this study show that colonization of *Pleurotus ostreatus* ranges between 16 to 21 days for both sawdust type and pretreatment methods. This was observed as the color of the sawdust changed to white showing the effect of colonization by the fungi. The findings in this study corroborate the earlier report by Ali et al. (2007), who reported that complete mycelia growth was recorded within 12 days for hot water treatment for different substrate type. Girmay et al. (2016) also reported that mycelium growth on each substrate takes about 16 days on average for different substrates. Outstanding growth of mycelium is a vital factor in mushroom cultivation. The spawn run time is different for each species and depends on the size of the bag, amount of spawn, the strain used and the temperature (Oei 2005).

It was observed that there was no significant difference in the pretreatment method for the mushroom but on the contrary, Ejigu and Kebede (2015), reported that hot water treated substrates had significantly earlier invasion by mycelium than cold water using wheat straw, maize stalk, Saw & Fab, fababean stalk and sawdust as substrate. It was that stated the reason for the difference could be due to the release of easily available nutrients from substrates and the reduction of unwanted green mould by effective hot water treatment. Generally, the result showed that there was significant difference in the biological yield of the mushroom species between the sawdust type used as substrate ($p < 0.05$) with the highest yield of 71.06 g. This is comparable with 72.4 g of fresh weight of mushroom reported by Gume et al. (2013). Chowdhury et al. (1998) examined the effects of adding different supplements to substrates for growing oyster mushrooms (*Pleurotus sajor-caju*) and found adding 5% supplements gave the highest yield of oyster mushroom. Our result corroborates with their study with the supplementation of substrate with 5% wheat bran which gave a higher yield of mushroom when treated or not. Numerically the highest economic yield was recorded under *Parkia biglobosa* (65.38 g). Payapanon et al. (1994) mentioned that suitable amount of supplements added to rice straw medium maximized the economic yield of oyster mushroom at optimum production cost of 349.5 g. However, our results disagree with the findings, and this could be due to the differences in the wood species study.

It was also observed that mushroom grows wider in cap diameter. The highest mean pileus diameter was observed in *Anogeissus leiocarpus* (6.8 cm) sawdust substrate when treated and *Parkia biglobosa* (7.7 cm) without treatment and the lowest in *Gmelina arborea* (3.8 cm). Kimenju et al. (2009) also reported comparable results from sugarcane bagasse (4.8 cm) and sawdust (3.4 cm) which agree with the present study. In terms of stipe height, it was observed that *Anogeissus leiocarpus* treated before hot pasteurization produced the taller stipe height. The lowest stipe height was recorded for *Gmelina arborea* while *Parkia biglobosa* had the tallest stipe height even without treatment. Girmay et al. (2016) reported that paper waste resulted in relatively better growth in terms of diameter and thickness of pileus, and diameter and length of stipe than other substrate (paper waste, wheat straw and sawdust). This study agrees with the work of Gume et al. 2013 who reported a stipe height range of 1.4 to 1.9 cm and pileus diameter of 3.8 to 5.2 cm using substrate of *C. africana* sawdust, *P. adolfi-friederici* (sawdust), Combinations of *C. africana* sawdust and coffee bean husks, *P. adolfi-friederici* sawdust and coffee bean husks, corncobs and coffee bean husks, however this study records a pileus diameter range of 3.8 to 6.8 cm. In the stipe diameter it was revealed that *Anogeissus leiocarpus* grew wider in girth for treated followed by this was *Parkia biglobosa* and *Gmelina arborea*. Subsequently, for the non-treated, the highest value was recorded in *Gmelina arborea* followed by *Parkia biglobosa* and *Anogeissus leiocarpus*. This may be because of less localized competition that existed in fewer fruit body containing bunches. Hence, the result indicated that *Anogeissus leiocarpus* performed best when treated with hot water as compared to the non-treated while *Gmelina arborea* performed well in the non-treated for the stipe diameter. The result of the present study corroborates with Ahmed (1998) who observed significant effects of various substrates on diameter and length of stalk, diameter and thickness of stipe. Two flushes of mushroom harvested in this study were observed to show a decrease in weight from the first flush to the second flush. The yield of the two flushes of mushroom harvested was significantly highest in the first flush of all treatments (Sharma et al. 2013).

Conclusion

Based on the outcome of this study, *Parkia biglobosa* produced the highest yield in term of biological yield, economic yield, stipe diameter pileus diameter, stipe height, average number of fruiting and effective body. Further, treatment of this wood species sawdust has no influence on the quality of the above parameters mentioned. The effect of natural extractives in the wood species on the growth and yield of the mushrooms is not significant except in *Anogeissus leiocarpus* which flourished well when treated especially in the yield (stipe height, pileus diameter, stipe diameter). Therefore, production of mushroom using mill waste such as sawdust can be a profitable enterprise and can save environment from the indiscriminate disposal of the wastes by sawmills. It is recommended that further studies should be carried out on other potential species for sustainable production of edible mushroom in Nigeria. One of the major limitations of this study, which also forms a reference for future studies is the investigation of bioactive compounds present in *Parkia biglobosa* substrate, which supported the growth of mushrooms when compared to substrates from other wood species. Higher yield of mushroom from *Parkia biglobosa* substrates may lead to its expanded use in large scale production of mushroom in Nigeria, thus providing increased nutritional benefits.

Acknowledgements

The authors appreciate the staff of Forestry Research Institute of Nigeria for their help in substrate preparation.

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