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Cultivation of *Pleurotus ostreatus* (Jacq Fr.) Kumm on *Gossypium hirsutum* Roxb. (Cotton waste) and *Gmelina arborea* L. sawdust

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Abstract

Agricultural wastes from *Gossypium hirsutum* and *Gmelina arborea* sawdust were used to cultivate a mushroom, *Pleurotus ostreatus*. Three hundred gram (300 g) of dried substrates were weighed and mixed individually with 10%, 20% and 30% concentration of additives (rice bran and corn cobs) and loaded into polypropylene bags. Each treatment was replicated thrice. The bags were tied and pasteurized in a drum for 4 hours. After cooling, each bag was inoculated with 10 g of vigorously growing spawn of *P.ostreatus*. The bags were incubated at 28 ± 2^0C and observed for growth parameters and fructification. The results showed that *G. arborea* sawdust with 20% rice bran additive gave the highest stipe length (9.0 cm), pileus diameter (7.7 cm) and stipe width (8.9 cm.) The proximate composition of the harvested fruit bodies showed highest protein content of (24.97%) in *G. arborea* sawdust with 20% rice bran , highest fat and ash contents (7.72% and10.95%) in *G. arborea* sawdust with 10% corncobs and highest crude fibre content (8.49%) in *G. arborea* sawdust supplemented with 20% rice bran. The highest moisture content (86.53%) was found in *G. arborea* sawdust supplemented with 20% corncobs. The highest carbohydrate value of (17.11%) was recorded in *G. arborea* sawdust (control). The highest total yield (95.44g) and highest biological efficiency (31.81%) were recorded for *G. arborea* sawdust plus 20% rice bran. This study shows that *Pleurotus ostreatus* cultivated on *G. arborea* sawdust with 20% rice bran incorporated as an additive produced better and bigger mushrooms.

Introduction

Pleurotus species are members of the Kingdom Fungi, Phylum Basidiomycota and Order Agaricales (Alexopoulos *et al.*, 2003). Mushrooms develop on various agricultural wastes thereby converting the lignocellulosic materials into digestible proteinous materials appropriate for feeding livestock. Cultivation of mushrooms may be one of the solutions to biologically convert inedible biomass residues into proteinous food in the form of edible mushrooms (Isikhuemhen and Okhuoya, 1996). The importance of mushrooms in biodegradation cannot be overemphasized. Fasidi *et al.* (2008) reported that *P. ostreatus* could be cultivated on numerous lignocellulosic wastes having hemicellulose, cellulose and lignin. Adenipekun and Omolaso (2015) found that banana leaves supplemented with wheat bran stimulate mushroom size, total yield, biological efficiency and proximate composition when compared with rice straw on *P. pulmonarius.*

Oyster mushrooms are superb delicacies in various parts of the world (Iwase *et al.*, 2000). *Pleurotus ostreatus* is treasured more than other *Pleurotus* species because of its delicious taste, high quantities of proteins, carbohydrates, minerals (calcium, phosphorus, iron) and vitamins (thiamine, riboflavin and niacin) and low fat content (Kurtzman, 2005). According to Chang (2007), many mushrooms are medicinal, possessing pharmacologically active compounds with potentials like antitumor, immunomodulatory, antigen toxic, antioxidant, anti-inflammatory,hypocholesterolaemic,antihypertensive, antihyperglycaemic, antimicrobial, antiviral activities and food (e.g. flavour compound) industries.

Oso (1977) reported that *P. tuber-regium* sclerotium when mixed with some herbs and used by fortune-tellers to wash their face enables them to see clearly into the future. The same sclerotium when ground with condiments like pepper, snail-fluid, onion and palm oil is used for curing ailments such as headache, stomach pains, fever, cold, chest pains, smallpox, boils, asthma, high blood pressure, nervous disorder, constipation and as an aid in foetus development.

Reports on the use of additive such as rice bran, wheat bran, corn cobs has been well documented in the cultivation of oyster mushroom. The addition of additive during mushroom cultivation aids in the improvement of nutritional contents, yield,

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biological efficiency, mycelium growth, quantity and quality of fruiting bodies of mushrooms grown on substrates as against when substrate along is being used (Adenipekun and Omolaso, 2015).Therefore, the objectives of this study are to compare the yield performance and nutritional quality of *P. ostreatus* cultivated on these two substrates and to determine the mineral contents of the two substrates.

Materials and Methods

Sample location and collection

Pure cultures of *Pleurotus pulmonarius* were collected from Plant Physiology Laboratory, Department of Botany, University of Ibadan, Nigeria.

Substrates

Rice straw, *Gossypium hirsutum* waste and *Gmelina arborea* sawdust. Freshly harvested rice straw was collected from International Institute of Tropical Agriculture (IITA) in Ibadan, Oyo State, Nigeria, *G. arborea* was collected from Bodija market, Ibadan while *G. hirsutum* was obtained from Bode and Sabo markets, Ibadan, Oyo state, Nigeria. The straw was cut into 0.1 – 3 mm using a guillotine.

Additives

Rice bran was collected from African Rice unit, International Institute of Tropical Agriculture (IITA) Ibadan, and Corn cobs obtained from Bodija Market, Ibadan.

Preparation of pure isolates spawn

The pure spawn was prepared according to the modified method of Adenipekun and Fasidi (2005). The substrate (rice straw) was soaked in water for one hour to moisten the straw and then squeezed using muslin cloth until no water oozed out. Wheat bran (additive) was added to the moist straw which was then put into 350 mL sterile bottles, covered with aluminium foil and autoclaved at 15 lbs. pressure and 121°C for 15 mins. The bottles were incubated at 28 2°C for 3 weeks until the substrate was completely ramified to form a spawn. These spawn bottles were preserved inside a refrigerator below 10^{o}C.

Preparation of Substrates

The culture conditions was carried out according to the method of Lawal *et al.* (2011) and modified as follows: 300 g of the dried sawdust and cotton waste were individually weighed, moistened with 75% distilled water (w/v) and mixed individually with each additives (rice bran and corn cob) at varying percentage compositions 0% (control), 10%, 20% and 30% w/w and then loaded into polyethylene bags. Each substrate bag was replicated thrice. The bags were immediately tied then sterilized in the pasteurized in drum for 4 hours. After cooling, each bag was inoculated with 10g of vigorously growing spawn of *P. pulmonarius*. In the first set of control treatment, additive (rice bran and corn cob) was not added to each substrate but inoculated with fungus while in the second set, different percentage compositions of additives were added to the substrates with the fungus. The bags were incubated at 28 ± 2°C.

Spawn run

Spawn run (mycelia extension) was observed regularly and. three times watering per day was done on the substrate until complete ramification takes place. The bags were then opened for fructification at different flushes.

Morphological parameters, determination of yield and biological efficiency

Growth parameters such as pileus diameter, stipe length and stipe width were measured in centimetres with a meter rule. Harvesting was done at the end of each flush; the yield and biological efficiency (BE) were determined.

Fresh and dry weight determination

Freshly harvested mushrooms from all flushes were weighed with an electronic meter balance and later oven dried at 45°C to a constant weight.

Mineral element analysis

The mushrooms were analysed for Zinc, Nitrogen, Phosphorus, Potassium, Carbon, Calcium, Magnesium, Sodium, Manganese, Iron and copper contents using the standard methods of AOAC (2005).

Proximate analysis/composition

Crude fibre and Crude protein contents were determined as described by Zadrazil and Brunnert, (1982); moisture content by method of Campbell *et al.* (1968); ash content (Parent and Thoen, 1977),sugar content (Dubois *et al.*, 2003) while the fat and crude fibre content according to AOAC (2005).

Biological Efficiency (BE)

$$\text{BE (\%)} = \frac{\text{Total fresh weight of the mushrooms harvested per unit production (bottle) for the two flushes (g)}}{\text{Substrates dry weight (300 g)}} \times 100$$

Data analysis

The data were subjected to Analysis of Variance while the means were separated according to Duncan multiple range test (p ≤ 0.05).

Results and Discussion

Mineral element composition

The mineral element composition of the substrates (cotton waste and *G. arborea* sawdust) is shown in Table 1. Higher values of zinc (0.003 mg/100g), calcium (0.18 mg/100g), magnesium (0.04 mg/100g), manganese (0.002 mg/100g), iron (0.02 mg/100g) were recorded in cotton waste compared to zinc (0.002 mg/100g), calcium (0.15 mg/100g), magnesium (0.03 mg/100g), manganese (0.001 mg/100g), iron (0.01 mg/100g) in *G. arborea* sawdust. Also, higher values of Nitrogen (1.95 mg/100g), phosphorus (0.15 mg/100g), potassium (0.21 mg/100g), carbon (96.50 mg/100g), sodium (0.15 mg/100g), copper (0.002 mg/100g) were recorded in *G. arborea* sawdust compared to Nitrogen (1.69 mg/100g), phosphorus (0.10 mg/100g), potassium (0.18 mg/100g), carbon (93.70 mg/100g), sodium (0.14 mg/100g), copper (0.001 mg/100g) in cotton waste.

The mineral elements in these substrates suggest that they can support growth of the mushroom since essential element as potassium plays an important role in the synthesis of amino acids and proteins while copper in combination with manganese, also play important roles in enzymatic catalyses which are crucial in all biological and physiological processes in living organisms (Saiqa *et al*., 2008; Idowu and Kadiri, 2013).

Growth parameters of P. ostreatus

The effect of substrates and different additives concentrations on the growth parameters of *P. ostreatus* is shown in Table 2. In cotton waste and *G. arborea* sawdust with 20% additives(rice bran and corn cobs), the highest stipe length ,stipe width and pileus diameter were recorded while 10% additive level for both rice bran and corn cobs recorded the least value for stipe length, stipe width and pileus diameter. These sizable fruit bodies could attract high market values as confirmed by the findings of Kadiri and Fasidi (1992).

Differences in the growth pattern of *P. ostreatus* on the substrates with the additives may be related to different chemical compositions of these agricultural wastes (Kadiri and Fasidi, 1992). Adenipekun *et al.* (2015) reported that *Auricularia auricular* exhibited an increase in performance with increase in additives used in different substrates used and concluded that wheat bran was the best out of the five additives used.

Proximate composition

The proximate composition of the harvested mushrooms on different substrates is shown in Table 3. *P. ostreatus* grown on *G. arborea* sawdust supplemented with 20% rice bran gave the highest composition of crude protein (24.97%) while the least value (22.64%) was recorded for *P. ostreatus* on cotton waste supplemented with 30% rice bran. The high protein content observed in the mushroom cultivated on *G.arborea* could be linked to richness of the substrate coupled with the additive added in carbonates and nitrogen (Isikhuemhen *et al.*, 1999). Also, the result obtained in this study is in conformity with the findings of Patil (2012) that protein content of *P. sajor-caju* fruiting bodies grown on different substrates ranged from 20.33 to 25.33%.

The least ash content was obtained from *P. ostreatus* harvested from cotton waste (control) with 9.86%. The low fat content of *P. ostreatus* indicates it is as a food with lower caloric value. Moisture contents of *P. ostreatus* fruit bodies, was relatively high in all the substrates. Similarly, Manzi *et al.* (1999) that moisture content of fresh *Pleurotus* spp range between 70 -95%. Lawal *et al.* (2011) reported that the high moisture content of *A. auricula* cannot permit it to be kept for long because high water activities encourage microbial growth.

The carbohydrate composition of *P. ostreatus* was relatively high in all substrates highest been 17.11% recorded on *G. arborea* sawdust (control) and the least in *G. arborea* sawdust supplemented with 10% rice bran (14.24%). The high carbohydrates content obtained in this study agrees with the reports of Beluhan and Ranogajec (2011), where oyster mushroom was reported to contains higher carbohydrates than their wild corresponding species from Croatia . The reduction in in the carbohydrates with the inculsion of low additive percentage could be linked to the bioconversion of carbohydrates in the colonized wastes into mycelia protein (Iyayi , 2004).

Total yield and biological efficiency

The total yield of the harvested *P. ostreatus* from different substrates at different concentration of the additives for three flushes is shown in Table 4. For the first flush, *P. ostreatus* harvested on sawdust supplemented with 20% corn cob had the highest weight (32.05 g) followed by *P. ostreatus* harvested on cotton waste supplemented with 20% corn cob (30.86 g). The second flush recorded the highest weight of (37.05 g) for *P. ostreatus* harvested on

Table 1. Mineral element composition of the substrates (Cotton) waste and *Gmelina arborea* sawdust

Substrate	Zn (mg/100g)	N (%)	P (mg/100g)	K (mg/100g)	C (%)	Ca (mg/100g)	Mg (mg/100g)	Na (mg/100g)	Mn (mg/100g)	Fe (mg/100g)	Cu (mg/100g)
Cotton waste	0.003^{a}	1.69^{b}	0.10^{b}	0.18^{b}	93.70^{b}	0.18^{a}	0.04^{a}	0.14^{b}	0.002^{a}	0.02^{a}	0.001^{b}
G. arborea sawdust	0.002^{b}	1.95^{a}	0.15^{a}	0.21^{a}	96.50^{a}	0.15^{b}	0.03^{b}	0.15^{a}	0.001^{b}	0.01^{b}	0.002^{a}

Each value is a mean of 3 replicates. Values in the same column with different letters as superscripts are significantly different by Duncan's multiple range test (p≤0.05)

Table 2. Effect of substrates on the growth parameters of *Pleurotus ostreatus*

Substrate	Additives	%	Stipe length(cm)	Stipe width(cm)	Pileus diameter(cm)
Cotton waste		0	5.2 ± 0.06^{f}	5.1 ± 0.12^{f}	4.7 ± 0.36^{f}
	Rice bran	10	4.9 ± 0.15^{g}	5.0 ± 0.06^{f}	4.4 ± 0.21^{g}
		20	6.2 ± 0.06^{de}	6.5 ± 0.45^{d}	6.4 ± 0.12^{c}
		30	5.8 ± 0.21^{e}	5.9 ± 0.33^{e}	5.3 ± 0.12^{e}
	Corncobs	10	5.6 ± 0.89^{e}	5.9 ± 0.75^{e}	4.5 ± 0.45^{c}
		20	6.7 ± 0.29^{d}	6.6 ± 0.46^{d}	5.9 ± 0.33^{d}
		30	6.6 ± 0.46^{d}	6.2 ± 0.21^{de}	5.1 ± 0.32^{e}
G.arborea sawdust		0	6.7 ± 0.15^{d}	7.1 ± 0.18^{c}	6.8 ± 0.25^{bc}
	Rice bran	10	7.5 ± 0.66^{c}	7.0 ± 0.21^{c}	6.7 ± 0.17^{bc}
		20	9.0 ± 0.35^{a}	8.9 ± 0.32^{a}	7.7 ± 0.13^{a}
		30	8.3 ± 0.21^{ab}	8.6 ± 0.15^{a}	7.2 ± 0.88^{b}
	Corncobs	10	7.9 ± 0.29^{b}	4.5 ± 0.17^{g}	5.7 ± 0.58^{b}
		20	8.8 ± 0.33^{a}	7.8 ± 0.41^{b}	6.5 ± 0.45^{c}
		30	7.5 ± 0.32^{c}	6.7 ± 0.12^{d}	6.4 ± 0.21^{c}

Each value is a mean of three replicates ± (SE=standard mean error of mean).Values in the same column with different letters as superscript are significantly different by Duncan's multiple range test (p≤0.05)

Table 3. Effect of substrates on the proximate composition of the harvested *P. ostreatus*

Substrates	Additives	%	CP%	Fat%	CF%	Ash%	Moisture%	CHO%
Cotton waste		0	22.78^{a}	6.93^{ab}	8.01^{cd}	9.86^{b}	86.30^{a}	14.96^{ab}
	Rice bran	10	22.88^{a}	6.95^{ab}	8.33^{ab}	10.43^{ab}	85.38^{bc}	15.01^{ab}
		20	23.60^{a}	6.76^{b}	7.87^{cd}	10.36^{ab}	85.26^{bc}	16.12^{ab}
		30	22.64^{a}	7.23^{ab}	8.07^{bcd}	10.12^{ab}	85.84^{abc}	15.68^{ab}
	Corn cobs	10	24.75^{a}	7.10^{ab}	8.19^{abcd}	10.62^{ab}	85.87^{abc}	14.81^{ab}
		20	23.31^{a}	6.92^{ab}	8.17^{abcd}	10.30^{ab}	85.76^{abc}	15.80^{ab}
		30	24.44^{a}	6.66^{b}	8.19^{abcd}	10.34^{ab}	85.26^{bc}	15.83^{ab}
G.arborea sawdust		0	22.95^{a}	6.67^{b}	8.26^{abc}	10.35^{ab}	85.90^{abc}	17.11^{a}
	Rice bran	10	24.59^{a}	7.09^{ab}	7.81^{d}	11.07^{a}	86.27^{a}	14.24^{b}
		20	24.97^{a}	7.38^{ab}	8.49^{a}	10.52^{ab}	86.02^{ab}	15.37^{ab}
		30	22.80^{a}	6.68^{b}	8.15^{abcd}	10.69^{ab}	85.12^{c}	15.37^{ab}
	Corn cobs	10	23.52^{a}	7.72^{a}	7.81^{d}	10.95^{ab}	85.36^{bc}	15.05^{ab}
		20	23.58^{a}	6.75^{b}	8.11^{abcd}	11.07^{a}	86.53^{a}	16.41^{ab}
		30	23.42^{a}	6.38^{ab}	8.14^{abcd}	10.09^{ab}	85.25^{ab}	16.32^{ab}

Each value is a mean of 3 replicates. Values in the same column with different letters as superscript are significantly different by Duncan's multiple range test (P≤0.05).
CP=Crude Protein, CF=Crude Fibre, CHO=Carbohydrate.

cotton waste supplemented with 30% rice bran followed by *P. ostreatus* harvested on cotton waste supplemented with 10% corncobs (35.84 g). For the third flush, *P. ostreatus* harvested on sawdust supplemented with 20% rice bran had the highest weight of 38.65 g followed by *P. ostreatus* harvested on sawdust supplemented with 10% corncobs (31.29 g).

Table 4. Effect of substrates on the weight and total yield of the harvested *P.ostreatus*

Substrate	Additives	%	1st flush (g)	2nd flush (g)	3rd flush (g)	Total yield (g)
		0	23.40c	18.12e	12.98g	54.50g
	Rice bran	10	15.08f	20.64de	27.07c	62.79f
		20	17.27e	15.19f	23.35d	55.81g
Cotton waste		30	20.26d	37.05a	30.05b	87.36b
	Corncobs	10	21.69d	35.84a	15.03f	72.56de
		20	30.86a	30.92b	12.37g	74.15de
		30	18.51de	33.78ab	19.42e	71.71de
		0	18.01de	30.02b	21.62de	69.65e
	Rice bran	10	19.17de	23.05d	26.47c	68.69e
G.arborea sawdust		20	24.50c	32.29ab	38.65a	95.44a
		30	20.53d	22.71d	19.57e	62.81f
	Corncobs	10	24.21c	20.30de	31.29b	75.80d
		20	32.05a	26.24c	28.45c	86.74bc
		30	27.08b	31.09ab	23.75d	81.92c

Each value is a mean of 3 replicates. Values in the same column with different letters as superscripts are significantly different by Duncan's multiple range test (P≤0.05)

Table 5. Biological Efficiency (BE) in the substrates

Substrate	Additives	%	Total yield (g)	Biological Efficiency (%)
		0	54.5g	18.17g
Cotton waste	Rice bran	10	62.79f	20.93f
		20	55.81g	18.60g
		30	87.36b	29.12b
	Corn cobs	10	72.56de	24.19de
		20	74.15de	24.72de
		30	71.71de	23.90de
		0	69.65e	23.22e
G. arborea sawdust	Rice bran	10	68.69e	22.90e
		20	95.44a	31.81a
		30	62.81f	20.94f
	Corn cobs	10	75.8d	25.30d
		20	86.74bc	28.91bc
		30	81.92c	27.31c

Each value is a mean of 3 replicates. Values in the same column with different letters as superscripts are significantly different by Duncan's multiple range test (P≤0.05)

The biological efficiency (BE) of the harvested *P. ostreatus* from the two substrates with the additives is shown in Table 5. *P. ostreatus* harvested from sawdust supplemented with 20% rice bran gave the highest biological efficiency (31.81%) while least biological efficiency was recorded in the control of cotton waste with value (18.17%). The values obtained for biological efficiency showed that biological efficiency is directly proportional to the total yield. This is similar to the findings of Moonmoon *et al.* (2010) who studied the growth of *Pleurotus eryngii* on sawdust and rice straw reported that sawdust supplemented with 25% wheat bran produced the highest number of fruit bodies (34.8/500 g packet), highest biological yield (153.3/500 g packet) and biological efficiency (73.5%).

Conclusion

This study has shown that cotton waste and *Gmelina arborea* sawdust with rice bran and corn cobs are excellent substrates for the cultivation of *P. ostreatus*. Additives such as rice bran and corn cobs enhanced better performance of the substrates. However, *Gmelina arborea* sawdust gave the best yield and biological efficiency coupled with the highest content of crude protein when supplemented with 20% rice bran. Rice bran should therefore be incorporated as good additive with agricultural wastes to enhance growth of *P. ostreatus*.

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