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INFLUENCE OF WHEAT BRAN ON DEGRADATION OF AGRICULTURAL WASTES BY A WHITE ROT FUNGUS, *Lentinus squarrosulus* (MONT) SINGER

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ABSTRACT
This study was aimed at evaluating the influence of wheat bran at varying percentage concentrations (0, 10, 20 and 30% w/w) on the degradation of Mansonia altissima sawdust and rice straw by Lentinus squarrosulus. The proximate composition, nutrient contents, percentage lignin loss and enzyme production on each wastes were assessed in triplicates before and after 42 days of degradation. This experiment was carried out in a completely randomized design and the means were separated with Duncan multiple range test ($p\leq0.05$). Significant increment from 10.66 to 14.65%, 11.12 to 18.07%, 11.24 to 16.18% and 12.10 to 17.84 were observed for 0, 10, 20 and 30% respectively for crude protein in M. altissima sawdust while 9.81 to 14.57%, 13.52 to 13.73%, 12.29 to 15.29% and 11.82 to 13.94% in rice straw. Similar trends were observed in crude fat, ash and moisture contents while a slight reduction was observed for the crude fibre and dry matter. Significant reductions in organic matter, phosphorus and potassium contents were observed ($p\leq0.05$). Reductions in lignin content of both wastes were recorded except in 10% and 30% levels of additive in rice straw. An increase in lignase and cellulase contents of M. altissima sawdust was recorded except for the control where a reduction in cellulase was observed. Conversely, in rice straw, significant reductions were recorded for lignase and cellulase contents except in 20% lignase and 10 to 20% cellulase. Enzyme production occurs at pH 5.34 – 6.99. This study has shown that 10 % concentration of wheat bran was most effective in enhancing degradation of Mansonia altissima sawdust by Lentinus squarrosulus while 20 % concentration was most effective on rice straw.

Keywords: Additive, *Mansonia alltisima*, Mushroom, rice straw, wheat bran.

INTRODUCTION

Accumulation of lignocellulosic biomass in our environment is on the increase and has become a major concern due to its impact as a source of environmental pollution (Adejoye and Fasidi, 2009). Cereal residues account 75% of major source of lignocellulosic biomass which is called "agro-waste" (Lal, 2008). The excessive nutrients discharged from these wastes produce an offensive odour during anaerobic decomposition of organic residues (Hamza, 1989; Adenipekun *et al*., 2012).

Different technologies have been implemented to treat wastes with varying degree of efficiency. Biodegradation is of great interest by offering an eco-friendly and low cost technique due to its natural process which does not produce toxic by-products and complete mineralization of the biomass in the environment (Perelo, 2010).

White rot fungi (edible mushrooms) have shown a great potential in the degradation of a wide range of lignocellulosic materials due to their secretion of extracellular enzymes (Buswell *et al*., 1996). Cellulase, lipase, amylase carboxymethylcellulase, proteinase and peroxidase secreted in large amounts by mushroom hyphae are responsible for the degradation of macromolecules such as cellulose, hemicelluloses, lignin and protein in the substrates (Kuforiji and Fasidi, 2008).

The nature and insufficient nutrient composition of agro-wastes affect fungi mycelium growth, mushroom quality and crop yield during degradation process (Baldrian and Val´aˇskov´a 2008). Thus, this problem led to the use of additive in increasing the production of fruiting bodies and high quality mushrooms as well as shortening mushroom production periods which indirectly brings about a rapid biodegradation of agro-wastes (Raymond *et al*., 2012). Kadiri and Kehinde (1999) reported that a mixture of rice bran with rice straw and $CuSO_4$ (4%) produced the best planting spawn for *Lentinus squarrosulus.*

Adesina *et al.* (2011) recorded that among the supplements used for cultivation and fruit body production of *L. squarrosulus*, rice bran gave the highest mycelial growth and highest yield of fruit bodies with biological efficiency on logs of *Spondias mombin* compared to other additives.

This study was undertaken to assess the effect of different levels of additive on the; proximate composition of degraded agro-wastes, degradability of the fungus over the selected agricultural wastes and the enzyme activities of the fungus on the agricultural wastes.

MATERIALS AND METHODS

Pure cultures of *Lentinus squarrosulus* were obtained from Plant Physiology Laboratory, Department of Botany, University of Ibadan. Rice straw and *Mansonia altissima* sawdust were used as substrates. Freshly harvested rice straw was collected from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria while the *Mansonia altissima* sawdust was collected from the Sango sawmill market in Ibadan, Nigeria. These substrates were air-dried in a clean open space in the Department of Botany for seven days to reduce moisture content and to prevent decomposition. The dried golden/light brown rice straw was cut into 0.1 – 3 mm pieces using a guillotine. The additive (Wheat bran) was collected from the feed mill of the Bodija Market, Ibadan.

Prepration of pure spawn.

The pure spawn was prepared according to method of Jonathan and Fasidi (2001). 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. One hundred grams of the moisten rice straw was mixed thoroughly with 10 g of wheat bran (additive) and loaded into 350 ml sterile bottles, covered with aluminium foil and autoclaved at 151bs pressure and 121°C temperature for 15 mins. The bottles were later allowed to cool before inoculating with 5 g of the pure spawn and incubated at 28 ± 2 °C for 3 weeks until the substrate was completely ramified to form a spawn. 80 bottles of the pure spawn were prepared for this experiment.

Experimental procedure.

The culture conditions followed the method of Adenipekun and Fasidi (2005) but modified as follows: 100 g of the dried sawdust and rice straw were weighed each into 350 mL sterile bottles and moistened with 75 % distilled water (w/v). Additive (wheat bran) of varying compositions 0% (control), 10%, 20% and 30% w/w were thoroughly mixed with each moistened sawdust and rice straw. The bottles were immediately covered with aluminium foil and sterilized in the autoclave at 121^0 C for 15 minutes. The substrates were prepared in three replicates. After cooling, each bottle was inoculated with 5g of vigorously grown spawn of *L. squarrosulus*. In the first set of control treatment, wheat bran was not added to each substrate but inoculated with fungus while in the second set of different level of wheat bran were added to the substrates with the fungus. The bottles were incubated at 28 ± 2 °C for 42 days.

Analytical methods.

pH determination: This was determined according to the method of Zadrazil and Brunnert (1982).

Lignin determination: The method of Association of Official Agricultural chemists (A.OA.C. 2003) was used.

Proximate composition: Crude fibre and Crude protein contents were determined as described by Zadarazil and Brunnert (1982). Ash content and moisture content were according to the method of Campbell *et al*. (1968) while, the dry matter was determined according to Pearson (1975).

Nutrient content analysis: The method of Association of Official Agricultural Chemists (A.O.A.C. 2003) was used to determine percentage organic carbon, organic matter, percentage nitrogen, phosphorus and potassium contents.

Enzyme assay: Cellulase and lignase were assayed as described by Berridge (1955).

Experimental design and data analysis: The experimental design was a completely randomized design (CRD) with 3 replicates. Data were subjected to analysis of variance using CoStat version 6.400 while treatment means were separated according to Duncan multiple range test ($p \leq 0.05$).

RESULTS

The proximate composition of the degraded agro-wastes treated and untreated with wheat bran by *L. squarrosulus* is shown in Table1. The crude protein contents of the agro-wastes for both treated and untreated with the additive significantly increased with the incubation

Table 1: Proximate composition of the *L. squarrosulus* degraded agro-wastes treated and untreated with wheat bran.

Agro-wastes	Treat-ment (%)	Incuba-tion Period (Days)	Crude Protein (%)	Crude fat (%)	Crude fibre (g/kg)	Ash Content (mg/kg)	Moisture Content (cmol/g)	Dry matter (%)
M. altissima sawdust	0	0	10.66^{c}	2.49^{d}	12.60^{ab}	4.15^{d}	37.12^{b}	62.94^{bc}
		42	14.65^{b}	2.98^{c}	12.36^{b}	6.69^{c}	37.57^{b}	62.42^{c}
	10	0	11.12^{c}	2.29^{d}	14.41^{a}	3.90^{d}	35.92^{c}	64.1^{ab}
		42	18.07^{a}	3.71^{a}	9.07^{d}	8.14^{b}	40.74^{a}	60.46^{d}
	20	0	11.24^{c}	2.59^{d}	13.32^{ab}	3.97^{d}	36.08^{c}	64.0^{ab}
		42	16.18^{ab}	3.40^{ab}	10.12^{cd}	8.20^{b}	37.69^{b}	62.30^{c}
	30	0	12.10^{c}	2.10^{bc}	12.92^{ab}	3.78^{d}	35.49^{c}	64.57^{a}
		42	17.84^{a}	3.47^{ab}	8.84^{d}	9.69^{a}	40.06^{a}	59.94^{d}
Rice straw	0	0	9.81^{e}	2.22^{c}	19.40^{a}	8.40^{a}	21.31^{c}	78.75^{a}
		42	14.57^{ab}	3.05^{ab}	7.07^{c}	7.89^{ab}	38.89^{a}	61.10^{c}
	10	0	13.52^{bc}	2.18^{c}	11.81^{b}	4.99^{c}	38.09^{ab}	63.22^{bc}
		42	13.73^{bc}	2.82^{b}	11.06^{bc}	8.35^{a}	36.78^{ab}	62.31^{bc}
	20	0	12.29^{cd}	2.34^{c}	11.92^{b}	4.90^{c}	36.14^{ab}	63.92^{bc}
		42	15.29^{a}	3.12^{a}	11.55^{bc}	7.89^{ab}	37.17^{ab}	63.87^{bc}
	30	0	11.82^{d}	2.37^{c}	12.68^{b}	4.82^{c}	34.32^{b}	65.74^{b}
		42	13.94^{ab}	2.84^{b}	10.08^{bc}	6.66^{b}	37.23^{ab}	62.77^{bc}

Each value is a mean of three replicates. Values in the same column with different letters as superscripts are significantly different by Duncan multiple range test($p \leq 0.05$)

period. On *M. altissima*, wheat bran additive at 10% concentration had the highest crude protein content (18.07%) while control (0%), has the least crude protein with 14.65%. However, 20% wheat bran concentration level resulted in the highest crude protein content with 15.29% while the lowest crude protein content was observed in the control (14.57%).

Similar trend was observed in crude fat, ash content and moisture content for the agro-wastes. After 42 days, there was no significant difference ($P \leq 0.05$) between 10% and 30% wheat bran percentage composition in crude protein. Furthermore, slight reductions were observed for the crude fibre and dry matter of the agro-wastes.

Table 2 shows the effect of additive composition on the nutrient contents of the degraded agro-waste substrates by *Lentinus squarrosulus*. The organic carbon content of the agro-wastes for both treated and untreated with the additive significantly decreased with the incubation period except for 30% in *M. altissima* and 20% rice straw which was increasing. Decrease from 22.0% to 20.93%, 24.15% to 21.58%, 23.31% to 21.71% were recorded for 10%, and 20% *M. altissima* while 23.79% to 21.03%, 22.21% to 20.72% and 24.10% to 20.33% for 10% and 30% % additive levels for rice straw. Conversely, an increase from 20.91% to 22.14 was recorded for 30% additive level in *M. altissima* and 22.28% to 22.46% for 20% additive level in rice straw. Similarly, significant reductions in the organic matter, phosphorus and potassium contents were observed except for potassium content of 30% additive level in *M. altissima* which increased from 0.61% to 0.69% and 20% additive in rice straw which increased from 0.52% to 0.66%.

Table 2. Effect of additive composition on the nutrient contents (g/100 gdm) of the *L. squarrosulus* degraded substrates.

Agro-wastes	Treatment (%)	Incubation Period (Days)	Organic Carbon (%)	Organic Matter (%)	Total Nitrogen (g/kg)	Phosphorus (mg/kg)	Potassium (cmol/g)
M. altissima sawdust	0	0	22.00^{bc}	37.91^{cd}	1.73^{d}	0.34^{a}	0.60^{ab}
		42	20.93^{d}	36.08^{e}	2.34^{bc}	0.28^{a}	0.42^{b}
	10	0	24.15^{a}	41.61^{b}	1.96^{cd}	0.33^{a}	0.53^{ab}
		42	21.58^{bcd}	37.21^{cde}	2.59^{a}	0.27^{a}	0.39^{ab}
	20	0	23.31^{a}	48.78^{a}	1.83^{d}	0.32^{a}	0.61^{ab}
		42	21.71^{cd}	36.50^{de}	2.89^{ab}	0.31^{a}	0.49^{b}
	30	0	20.91^{d}	53.17^{c}	1.81^{d}	0.30^{a}	0.61^{ab}
		42	22.14^{a}	36.03^{e}	2.85^{a}	0.29^{a}	0.69^{a}
Rice straw	0	0	23.79^{a}	40.99^{a}	1.59^{d}	0.34^{a}	0.60^{a}
		42	21.03^{c}	36.25^{c}	2.33^{ab}	0.29^{a}	0.47^{a}
	10	0	22.21^{b}	43.27^{b}	2.19^{abc}	0.31^{a}	0.61^{a}
		42	20.72^{c}	35.73^{c}	2.10^{bc}	0.28^{a}	0.47^{a}
	20	0	22.28^{b}	48.39^{b}	1.99^{c}	0.32^{a}	0.52^{a}
		42	22.46^{b}	38.72^{b}	2.45^{a}	0.29^{a}	0.66^{a}
	30	0	24.10^{a}	53.53^{a}	1.92^{c}	0.33^{a}	0.59^{a}
		42	20.33^{c}	35.05^{c}	2.23^{abc}	0.27^{a}	0.52^{a}

Each value is a mean of three replicates. Values in the same column with different letters as superscripts are significantly different by Duncan multiple range test($p \leq 0.05$)

Table 3 Effect of additives concentration on *L. squarrosulus* degraded agro-waste for enzyme production and pH

Agro-wastes	Treatment (%)	Incubation Period (Days)	pH	Lignin (%)	Lignase (%)	Cellulase (%)
M. altissima	0	0	6.21^{b}	4.92^{ab}	5.23^{ab}	7.23^{abc}
sawdust		42	5.75^{c}	3.08^{a}	4.67^{ab}	5.77^{cd}
	10	0	6.60^{ab}	4.26^{ab}	3.83^{b}	5.93^{d}
		42	5.44^{c}	3.42^{a}	5.03^{a}	9.07^{a}
	20	0	6.68^{a}	4.61^{a}	4.13^{b}	6.73^{bcd}
		42	5.69^{d}	3.92^{a}	5.43^{ab}	8.13^{ab}
	30	0	6.82^{a}	4.72^{a}	4.93^{ab}	6.93^{bcd}
		42	5.34^{c}	3.04^{ab}	5.20^{ab}	9.20^{a}
Rice straw	0	0	7.14^{a}	5.14^{a}	5.63^{a}	7.80^{b}
		42	6.31^{cde}	3.80^{a}	5.30^{ab}	6.70^{b}
	10	0	6.37^{cde}	3.79^{a}	4.63^{abc}	5.9^{cd}
		42	6.64^{bc}	5.46^{a}	4.07^{bc}	6.4^{bc}
	20	0	6.76^{c}	4.61^{a}	3.53^{c}	4.53^{d}
		42	6.99^{b}	4.55^{a}	4.37^{abc}	6.37^{bc}
	30	0	6.89^{ab}	4.72^{a}	5.33^{ab}	7.53^{bc}
		42	6.91^{b}	4.81^{a}	3.93^{c}	7.47^{bc}

Each value is a mean of three replicates. Values in the same column with different letters as superscripts are significantly different by Duncan multiple range test ($p \leq 0.05$)

As shown in Table 3, the pH of *M. altissima* sawdust significantly decreased with an increase in incubation period while that of rice straw increased except its control which decreased. The pH of the wastes after degradation ranges from 5.34 – 6.99. Total reductions were observed in the lignin content in both wastes except for 10% and 30% levels of additive in rice straw which observed a slight increase from 3.79 -5.46% and 4.72 – 4.81% respectively. Lignase and cellulase contents of *M. altissima* sawdust increased except the control of cellulase where a reduction was observed while, in rice straw, a significant reduction was recorded for lignase and cellulose contents except in 20% lignase and 10 to 20% cellulase where an increment were observed. There were no significant differences in the lignin content of all the additive levels in rice straw ($p \leq 0.05$).

DISCUSSION

The rapid degradation of these agro-wastes by *L. squarrosulus* through mycelia colonization when wheat bran was added at different percentage concentration is in agreement with Moonmoon *et al.* (2011), where additive was reported to have played a significant role in the degradation of substrate for effective growth, yield and quality of *L. edodes*.

The increase in the crude protein of the agro-wastes both supplemented with and without wheat bran may be due to the addition of fungal protein or the bioconversion of carbohydrates in the colonized wastes into mycelia protein or single cell protein by the growing fungus fermentation process (Iyayi, 2004). Reduction in the crude fibre content could be linked with the liberation of cellulose from its bonds with lignin as a result of delignification which could increase solubility of crude fibre in the presence of excessive nutrients such as additive (Abd El-Ghani *et al*., 1999).

The increase in ash content corroborates the report of El-Ashry *et al*. (2001) who observe increased in ash contents when roughages were treated with fungi. Reductions in moisture content could probably be due to partial assimilation of water into the mushroom fruit bodies coupled with partial loss to the atmosphere as carbon dioxide due to the mushroom respiration (Royse, 2003). The general increase in dry matter contents could be linked to break down of cell wall bonds of the wastes during degradation by the fungus (Fazaeli *et al*., 2004).

Agreeing with the opinion of Van der Gast *et al*. (2002), the increase in the organic carbon content of *M. altissima* sawdust supplemented with wheat bran indicates the effective degradation by the fungus. Conversely, the reductions in organic carbon content of rice straw suggest that the fungus used the carbon as its nutrient source for growth. This agrees with the report of Jonathan and Fasidi (2001) that a ratio of 2:3 of carbon to nitrogen is most suitable for mushroom (*Psathyrella atroumbonata*) growth. Decrease in organic matter content of agro-wastes could be attributed to the efficient colonization of wastes (Sharma *et al*., 2002). Similarly, deficiency in calcium and phosphorous contents of the supplemented agro-wastes may be attributed to the decrease in organic matter content (McDowell, 1985). A significant increase in nitrogen contents agrees with the report of Royse (2002) that application additive to lignocellulosic materials during mushroom growth will increased their nitrogen content. The reductions in potassium might be due to carbohydrate metabolism by the fungus (Kuforiji, 2005). The change in pH value could be associated with an increase in amino-nitrogen content coupled with the presence of metabolic waste products within the agro-wastes, since fungi are generally known to carry out their metabolic activities at acidic pH (Fasidi, 1996). Also, Hossain and Anatharaman (2006) further explained that acidic pH best enhances the lignin degradation enzymes (laccase and other peroxidases) secreted by white rot fungi.

According to Datta and Chakravarty (2001), decrease in lignin contents of agro-wastes results from an increase in cellulose content of the wastes. The significant increase in lignase and cellulase contents of the agro wastes may be attributed to complete mycelia colonization of the wastes due to its richness in nutrient as occasioned by the wheat bran additive which further enhanced the production of the enzymes such as cellulases, hemicellulases, and ligninases by the fungus. Buswell *et al*. (1996) similarly reported that the production of the enzymes such as cellulases, hemicellulases, and ligninases by the fungal mycelium is a crucial part of the colonization process and thus an important determinant of mushroom growth and yields.

CONCLUSION

This study has shown that the use of wheat bran at 10 % concentration is most effective in enhancing degradation of *Mansonia altisima* sawdust by *Lentinus squarrosulus* while that of rice bran is at 20% concentration. However, inoculation of *L. squarrosulus* on these agro-wastes could decrease the environmental problems arising from their accumulation in an environment and it could also provides a sustainable means of adding value to the farmers in terms of protein rich mushrooms.

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