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EFFECTS OF DIFFERENT CARBON/NITROGEN RATIOS ON YIELD AND BIOCONVERSION EFFICIENCY OF PLEUROTUS OSTREATUS CULTIVATED ON CASSAVA PEELS AND SAWDUST- BASED SUBSTRATE

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ABSTRACT

This study was aimed to evaluate the effects of different levels of wheat bran on the yield and bioconversion efficiency of *Pleurotus ostreatus* cultivated on an equal mixture of cassava peels and sawdust-based substrates. Two per cent lime was added to stabilise the pH of the substrate while different levels of wheat bran, namely C/N 17:0, C/N 17:1, C/N 17:3, C/N 17:6 and C/N 17:9 were added to enrich the substrate with nitrogen. Completely randomised design was used with three replications. Proximate analysis was conducted on the fruit bodies to determine the nutritional composition. Mushroom weight, mushroom number, stipe length, pileus diameter, dry weight of fruit bodies, dry matter loss, biological efficiency, bioconversion efficiency and number of mushroom flushes differed significantly with treatment. The nutritional composition of the fruit-bodies also differed significantly. C/N 17:0 was outstanding in supporting the colonisation, yield and bioconversion efficiency of *P. ostreatus*. *Pleurotus ostreatus* can be cultivated on an equal volume of sawdust (50%) and cassava peels (50%) without supplementing it with wheat bran or any nitrogen source. Higher C/N ratios hindered mycelial colonisation and fruit body formation. The lower the C/N ratio, the higher the nutrient composition of the mushroom fruit bodies.

Key words: C/N ratio; yield; *Pleurotus ostreatus*; cassava peels; sawdust; substrate
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INTRODUCTION

Pleurotus species are saprophytic macro-fungi that degrade the hemicelluloses as well as cellulose from numerous lignocelluloses to produce valuable nutrients for man and his livestock. The recent increase in the economic value of edible mushrooms could be attributed to their use as food (Kortei, 2008; Kalac, 2009) and medicinal properties (Kortei, 2011; Singh *et al.*, 2012).

The cultivation of *Pleurotus* requires carbon, nitrogen and other inorganic compounds as major sources of nutrient (Chang and Miles, 1989). Badu *et al.* (2011) and Patil *et al.* (2010) reported that the quality and yield of oyster mushroom depended on the nutritional and chemical contents of the substrates. Significant quantities of agricultural wastes are generated annually in Nigeria and other parts of Africa which are usually left to decay or are disposed of through burning (Tesfaw *et al*., 2015). Utilisation of locally available agro-wastes to cultivate edible mushroom is a value-added process and is regarded as the most eco-friendly method to bio-transform these huge inedible wastes into acceptable edible biomass with high market and nutritional qualities (Tesfaw *et al.*, 2015). The main substrate used in the cultivation of edible mushroom globally is sawdust. The possibility of shortage of sawdust and the high potential of agro and industrial wastes are the main reasons driving the need to identify other alternative mushroom substrates for the sustainable edible mushroom cultivation.

In the cultivation of edible mushroom, various agricultural and industrial wastes are used as substrates and sources of nutrient for their growth (Upadhyay *et al.,* 2002). These wastes include cereal grains, wheat straw, sawdust, soybean meal etc, which require varying levels of supplementation to improve yield (Adebayo *et al*., 2009; Baig *et al*., 2009). Lebot (2009) reported cassava (*Manihot esculenta*) as a major staple food for about 800 million people especially in the developing countries. FAOSTAT (2010) ranked cassava as the sixth globally important crop in terms of annual cultivation. This important root crop is cultivated in Africa, Asia, Pacific islands, Central and South America (McKey *et al*., 2010; Burns *et al.,* 2010; FAOSTAT, 2012). According to Egesi *et al.* (2007a), Africa accounts for the majority of cassava harvested globally with over half of the world's total production followed by Latin America and Asia. Despite its utilisation as an important source of feed for animal, cassava has emerged as a major source of biofuel resource which has been transformed as a source of cash for most resource-poor farmers (Baah, *et al.*, 2011).

Cassava peels comprise mostly lignocellulose materials which consist of three major components namely hemicelluloses, cellulose and lignin (Youri, 2003; Tewe, 2004; Youri 2004). Tewe (2004) reported that cassava peels may contain higher quantities of cyanogenic glycosides and higher protein than the rest of cassava tuber. The peels from cassava are a byproduct of processing roots into flour, starch and garri, which constitutes about 11% of the roots with about 400,000 MT (dry matter basis) of it produced annually (Naraian *et al.*, 2009). The cassava peels are used to feed livestock in Nigeria and other parts of Africa. Therefore, there is the need to investigate the ability of edible fungi to enrich it for the formulation of livestock feeds.

The ratio of carbon and nitrogen in the substrate for edible mushroom cultivation has a significant effect on the growth of mycelium, formation and development of fruit bodies. Albores *et al.* (2006) observed a positive relationship between C/N ratio and mycelial growth rate. Substrates with low C/N ratios appeared to support fruit body formation better than those with high C/N ratios. Naraian *et al*. (2009) reported that mycelial growth and primordial development of *Pleurotus florida* were dependent on the lignocellulosic materials especially the C/N ratio. Yang (2000) observed that higher C/N ratio favoured the mycelial growth while lower C/N ratio favoured the fruit body growth.

This study investigated the effects of different levels of C/N ratio on the yield and bioconversion efficiency of *Pleurotus ostreatus* and to determine the best C/N ratio for effective cultivation on cassava peels and sawdust substrates.

MATERIALS AND METHODS

Study site and source of sample

The study was carried out at the School of Agriculture Teaching and Research Farm, Federal University of Technology, Owerri, Nigeria. Cassava peels and sawdust were collected from Eziobodo community, while mushroom spawns established on *Sorghum bicolor* seeds were obtained from Dilomat Mushroom Farm and Services, Port Harcourt, Rivers State.

Substrate preparation

Substrates were prepared according to the modified method of Stamets (1993). Shredded and moistened cassava peels (50%) and sawdust (50%) were mixed with different levels of wheat bran in the following C/N ratios: 17:0 (T1 or control), 17:1 (T2), 17:3 (T3), 17:6 (T4) and 17:9 (T5). They were replicated three times and composted for one week. Two per cent lime ($CaCO_3$) was added to stabilise the pH of the substrate. One (1) kg of the composted substrate was measured and placed into high-density polypropylene bags. The bags were packed inside a drum steamer and pasteurised for three hours and later allowed to cool before they were inoculated with spawns of *Pleurotus ostreatus* grown on *Sorghum bicolor* seeds. The inoculated substrates were incubated at ambient temperature in a specially constructed chamber for mushroom cultivation where temperature and relative humidity were monitored. The bags were opened after 43 days of spawning for fruit body production.

Productivity evaluation

Mean mushroom weight was taken by weighing each harvested mushroom flush using a sensitive weighing scale and by taking the average of the weight of the flushes. Mean number of mushroom (MNM) was taken by counting the number of fruit bodies. The mean stipe length and pileus diameter were measured using a ruler. Dry matter loss was taken as the weight of the substrate at spawning and weight at the end of fruit body production. Dry weight of the fruit bodies and biological efficiency (B.E) measured the yield potentials from different substrates and calculated thus: weight of fresh mushroom produced per 100 g of dry substrate used. Bioconversion or bio-transformation efficiency (BCE) measured the rate of conversion of waste materials into usable products by biological processes or agents. It was calculated as the weight of dry mushroom produced per 100 g of dry substrate used. The number of mushroom flushes produced was also evaluated. The mushroom quality was evaluated based on different size groups according to the methods described by Elenwo and Okere (2007) and classified as follows: Pileus diameter above 13 cm (very big), 10-13 cm (big), 5-10 cm (medium), 2.5 cm (small) and 0- 2 cm (very small). The substrates were lightly watered to induce fruiting. The production cycle for this study was 65 days.

Laboratory analysis

The proximate analysis was conducted to determine the nutritional compositions of the fruit bodies using standard procedures at the Biochemistry Laboratory, School of Biological Sciences, Federal University of Technology, Owerri.

Data analysis

Data generated were subjected to analysis of variance (ANOVA). Means were separated using Fishers Least Significant Difference at p=0.05 (Steel and Torrie, 1980). The statistical package for social sciences, version 2.0 was used for the analysis.

RESULTS

Evaluation of Yield and Yield attributes

The highest mean mushroom weight of 16.7 g was produced using the C/N ratio of 17:0 (control) while the least (5.7 g) was produced at T3 (C/N 17:3). The mean number of mushrooms produced at T1 (C/N 17:0) and T2 (C/N 17:1) was statistically similar, but differed significantly from the number of mushrooms (1.0) produced at T3 (C/N 17:3) (Table 1).

The pileus diameter ranged from 4.7 cm at T1 (C/N17:0) to 6.0 cm at T3 (C/N 17:3). The pileus diameter at T2 and T3 differed significantly ($p< 0.05$) from that of T1 (Table 1). The longest stipes (9.3 cm) were observed at T1 (C/N 17:0) and these were significantly different from those observed at C/N17:1 (2.6 cm) and C/N 17:3 (3.0 cm). Dry matter loss was highest at the C/N ratio of 17:3 (40%), followed by the C/N ratio of 17:0 (31.7%) and C/N 17:1 (23.3%). Dry weight of fruit body decreased as the C/N ratio increased, being highest (3.8 g) at C/N 17:0 and lowest (1.0 g) at C/N 17:3 (Table 1). The biological efficiency was highest (1.60%) when the C/N ratio was 17:0 (control) and lowest (0.58%) when the C/N ratio was 17:3 (Table 1). The highest bioconversion efficiency of 0.40% was observed at the C/N ratio of 17:3 (T3), followed by the C/N ratio of 17:0 (0.37%) and 17:1 (0.17%). The highest number of flushes of 3.0 was observed at the C/N ratio of 17:0 (control). The number of flushes at C/N 17:1 (T2) and C/N 17:3 (T3) was similar (Table 1).

Nutritional composition of mushroom fruit bodies

Table 2 shows the nutritional composition of the mushroom fruit bodies. The dry matter content varied from 80.4% at T3 (C/N 17:3) to 94.6% at T1 (C/N 17:0). The highest Nitrogen content of 0.21% was observed at

the C/N ratio of 17:0 (T1), which differed significantly ($p< 0.05$) from those of C/N 17:1 (T2) and C/N 17:3 (T3) with nitrogen contents of 0.17% and 0.18%, respectively. Similarly, crude protein was highest at the C/N ratio of 17:0 (T1), followed by 17:1 (T2) and 17:3 (T3) with crude protein values of 1.31%, 1.13% and 1.12%, respectively.

Crude fiber, sodium and calcium contents were highest at the C/N ratio of 17:0 (control) and lowest at the C/N ratio of 17:3 (T3), and the differences ($p< 0.05$) were significant. The magnesium content did not differ significantly with the C/N ratio. The highest potassium content of 0.30 mg/kg was observed at the C/N ratio of 17:0 (control), followed by 17:1 (T2) and 17:3 (T3), with potassium content each of 0.20 mg/kg. The ash content increased as the C/N ratio increased, ranging from 1.87% at C/N 17:0 (control) to 2.26% at C/N 17:3 (T3).

Table 1: Effects of different C/N ratios on yield and yield attributes of *Pleurotus ostreatus*

Treatments	MMW(g)	MNM	PD (cm)	Stipe length (cm)	Dry matter loss (%)	Dry weight FB (g)	BE (%)	BCE (%)	Number of flushes
T1	16.7[a]	12.0[a]	4.7[b]	9.3[a]	31.7[b]	3.8[a]	1.6[a]	0.37[a]	3.0[a]
T2	7.9[b]	1.7[b]	5.3[a]	2.6[b]	23.3[c]	1.8[b]	0.77[b]	0.17[b]	1.0[b]
T3	5.7[c]	1.0[c]	6.0[a]	3.0[b]	40.0[a]	1.0[c]	0.58[c]	0.40[a]	1.0[b]
LSD 0.05)	1.35	0.54	0.90	0.74	8.07	0.29	0.16	0.208	0.474

Means followed by the same letter within the same column are not significantly different

T1- C/N 17:0 (control)
T2-C/N 17:1
T3- C/N 17:3
MMW-Mean mushroom weight
MNM-Mean number of mushroom
BE-Biological efficiency
BCE- Bioconversion efficiency
PD-Pileus diameter
FB-Fruit body

Table 2: Nutritional composition of the mushroom fruit bodies

Treatments	Dry matter (%)	N (%)	Crude protein (%)	Crude fiber (%)	Na (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	K (mg/kg)	P (mg/kg)	Ash (%)
T1	94.6[a]	0.21[a]	1.31[a]	3.72[a]	0.21[a]	0.04[a]	1.09[a]	0.30[a]	0.16[c]	1.87[c]
T2	82.4[b]	0.17[b]	1.13[b]	3.14[b]	0.15[b]	0.40[a]	0.75[b]	0.20[b]	0.29[a]	2.16[b]
T3	80.4[c]	0.18[b]	1.12[b]	3.04[c]	0.13[c]	0.30[a]	0.70[c]	0.20[b]	0.28[b]	2.26[a]
LSD (p=0.05)	1.40	0.012	0.011	0.066	0.008	0.115	0.047	0.008	0.008	0.086

Means followed by the same letter within the same column are not significantly different

T1- C/N 17:0 (control)
T2-C/N 17:1
T3- C/N 17:3

Effect of Pileus size group on mushroom quality

Table 3 shows the effect of pileus size group on the quality of mushroom harvested. A total of 18 fruit bodies were harvested from the three treatments (T1, T2 and T3). No fruit body was observed in the very small category. All the 12 fruit bodies (representing 66% of the total) were harvested from T1 (C/N 17:1), one (1) fruit body (5.6%) was classified under the small category, while 4 fruit bodies (representing 5.5% of the total fruit bodies harvested) were observed under the medium category. The only one (1) fruit body (5.6% of the total fruit bodies harvested) observed in T3 (C/N 17:3) belongs to the medium size category. None of the fruit bodies harvested belonged to the very big size category (Table 3).

Table 3 Effect of pileus size on the mushroom quality

Treatment	Very small (0 - 1.9 cm)	Small (2 - 4.9 cm)	Medium (5 - 9.9 cm)	Big (10 - 12.9 cm)	Very big (Above 13cm)
T1	-	12 (66%)	-	-	-
T2	-	1 (5.6%)	4 (22%)	-	-
T3	-	-	1 (5.5%)	-	-
Total	-	13	5	-	-

T1- C/N 17:0 (control)
T2-C/N 17:1
T3- C/N 17:3

DISCUSSION

Productivity parameters

The control (C/N 17:0) resulted in the highest mean mushroom weight, mean number of mushroom, longer stipe, higher dry matter loss, biological efficiency and high number of mushroom flushes but it produced fruit bodies with less pileus diameter. Treatments with C/N 17:2 (T3) resulted in higher yield attributes of mean mushroom weight, mean number of mushroom, dry matter loss and biological efficiency. The result suggests that as the C/N ratio increased, the mushroom productivity decreased. This finding is in agreement with the findings of Albores *et al.* (2006), who observed that low C/N ratio enhanced fruit body production better than substrates with high C/N ratio. Naraian *et al.* (2009) reported that mycelial growth and primordial development of *Pleurotus* species were dependent on the lignocellulosic materials especially the C/N ratio. Yang (2000) reported that high C/N ratio favoured mycelial growth while lower C/N ratio favoured fruit body formation. Tewe (2004) observed that cassava peels contained higher protein than other parts of the cassava tuber. Application of this resulted in higher dry matter loss and higher bioconversion efficiency, but this did not translate to higher yield. Zhang *et al.* (2002) reported that the dry matter loss was partly assimilated into the mushroom fruit bodies and partly lost into the atmosphere as carbon dioxide through respiration. Treatments with higher C/N ratios of T4 (C/N 17:6) and T5 (C/N 17:9) resulted in poor growth or low production of fruit body parts. This could be due to the higher nitrogen content in the substrate. However, Albores *et al.* (2006) reported a positive correlation between C/N ratio of the substrate and mycelial growth rate. The higher C/N ratio in these treatments did not favour mycelial growth contrary to the findings of Yang (2000)**.**

The mushroom fruit bodies from T1 (control) contained higher dry matter, nitrogen, crude protein, crude fiber, sodium, calcium, potassium, but lower magnesium, phosphorus and ash when it was compared with T2 (C/N 17:1) and T3 (C/N 17:3). The higher nutritional quality of the fruit bodies in the control (T1) could be attributed to the vigorous mycelial growth/colonisation of the substrate due to relatively lower C/N ratio (Yang, 2000; Albores *et al.*, 2006). This vigorous growth was reflected in the higher biological efficiency which degraded and biologically unlocked the abundant nutrients locked up in the lignocellulose materials used as substrate in this study. There appeared to be a negative relationship between the C/N ratio and the nutrient composition; as the C/N ratio increased, the nutrients appeared to decrease. The mushroom fruit bodies harvested were graded into the small and medium categories. This result could be due to the environmental condition especially the light in the growth chamber. This finding is in agreement with Markson *et al.* (2012).

CONCLUSION

Results of this study showed that *P. ostreatus* could be cultivated on equal volume of sawdust (50%) and cassava peels (50%) without supplementing it with wheat bran or any nitrogen source. Higher C/N ratios hindered mycelial growth or colonisation and fruit body formation. The lower the C/N ratio, the higher the nutrient composition of the mushroom fruit bodies. It is recommended that further investigation should be carried out on the effect of Carbon/Nitrogen ratio on the nutritional composition of the fruit bodies.

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