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BIO-CONVERSION OF *Isoberlinia doka.* Craib & Stapf AND *Anogeissus leiocarpa.* (DC) Guill. & Perr. WOOD RESIDUE BY *Lentinus sajor-caju* (Fr.) Fries. INTO EDIBLE PROTEIN

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ABSTRACT

Sawmills by nature generate a lot of wastes with burning being the major means of disposal. This has constituted a threat to the local environment and causing emission of green house gases into the atmosphere. Instead burning of these wastes, mushroom cultivation can harness these waste resources. In this study, comparative analysis of yield, growth and nutritional composition of oyster mushroom *(Lentinus sajor-caju)* cultivated on *Isoberlinia doka Craib & Stapf* and *Anogeissus leiocarpa* (DC) *Guill. & Perr* were conducted. Fungi colonisation of the substrates showed significant variations in L. *sajor-caju* grown on *I. doka* and the mixed substrate up to the 15th day after ramification started while from the 35th day, the rate of ramification were statistically the same for the three substrates. The length of stipe and diameter of pileus of the mushrooms were statistically the same for all the substrates for flush 1 and 2 while there were significant variation in the length of stipe among the 3 substrates for the 3rd flush. Diameter of the pileus were the same for *A. leiocarpa* and the mixed substrate. However, *I. doka* had the largest pileus diameter of 4.67 cm. There were significant variation in the weight of the mushrooms grown on *I. doka* and the mixed substrate. However, mushroom weight from *A. leiocarpa* and mixed substrates were statistically the same at p= 0.05. The weight of mushroom from *A. leiocarpa* was highest (8.21g) while the mixed substrate had the lowest (4.88g). No significant variation existed in the number of fruiting bodies. However, highest numbers of fruiting bodies were recorded in the mixed substrate even though it recorded the lowest mushroom weight. The proximate composition of the mushrooms differ significantly among the substrates with *A. leiocarpa* having the highest protein (43.65 %) and crude fibre content (33.06 %) while the mixed substrate had the lowest protein (40.20 %) and lowest (29.45 %) crude fibre content. *I. doka* had the highest crude lipid and moisture content. The highest carbohydrate content (15.29%) was recorded in the mixed substrate and the lowest (5.51%) in *A. leiocarpa*. It is therefore concluded that *A. leiocarpus* was found to be the best for the cultivation of *Lentinus sajor-caju* among the substrates used

Keywords: Wood substrate; ramification; mushroom; pileus, stipe and proximate composition

INTRODUCTION

Wood-based industrial operations in Nigeria include timber logging, sawmilling, wood-based panel products manufacturing, furniture/joinery making, paper making, match making and many more. Sawmills by nature generate a lot of wastes – sawdusts, wood off cuts, wood backs, plain shavings, wood rejects, and so on. Most times these wastes are usually disposed off by dumping into water bodied or subjected to burning thereby causing environmental pollution and consequently leading to health hazards. Mushroom cultivation can harness these waste resources (World Bank, 2004). Mushroom cultivation is the only economically viable bio-technology process for conversion of waste plant residues from forests and agriculture into human food and nutraceutical products, which have many health benefits (Wood and Smith, 1987). It might also be the only current process that combines the production of protein-rich food with the reduction of environmental pollution (Sánchez, 2010) as it represents one of the most efficient biotechnological processes for lignocellulosic organic waste recycling (Mandeel *et al*., 2005). The use of fungi for the conversion of lignocellulose into food and feed rich in protein offers an alternative for developing unconventional source of proteins as food/feed.

Wood wastes are valuable resources for the production of nutritious, gourmet, and medicinal mushrooms (She *et al*., 1998; Wasser and Weis, 1999). This is the bioconversion solution of inedible wood biomass residue into nutrition protein rich food in form of edible mushroom. The aim of the work was to investigate the biological conversion of *Isoberlinia doka* and *Anogeissus leiocarpus* wood wastes into edible protein by *Lentinus sajor-caju*.

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MATERIALS AND METHODS

Collection of materials

The two most generated wood wastes on visits to the wood mill were *Isoberlinia doka* (babo) and *Anogeissus leiocarpus* (Ayin) were used for the cultivation of mushroom. *I. doka* (Babo) was designated treatment 1 (T1), *A. leiocarpus* (Ayin) treatment 2 (T2) and a mixture of both as treatment 3 (T3) in ratio 1:1. Composted method of substrate preparation was used which entails fermentation of sawdust prior preparation for bagging.

Composting of substrates

The different species of sawdust were spread and dried under the sun after collection from sawmill.

2000 g was measured each for *I. doka* (T1) and *A. leicarpus* (T2) and 1000 g of each species of sawdust was measured and mixed together to make T3. About 5 % wheat offal, 1 % sugar and lime were added to each treatment. 2, 1.8 and 2 litres of water were used in mixing T1, T2 and T3. These were estimated through squeeze test method to determine the volume of water to be added to each substrate so that the substrate would only be moist and not water. Lime and sugar were dissolved in the measured water before mixing. Wheat offal was used as additional nutrient, lime was used to moderate the acidity and sugar was used to aid fermentation. Each treatment was thoroughly mixed together to be fully homogenize, moist and not water logged by using a squeeze test method. Each treatment was made into a heap, covered with thick black polythene and placed in an open space under direct sunlight. Each substrate was tilled every 5days till a cycle of 30 days was complete.

Each fermented sawdust was spread and dried under the sun after fermentation. About 1600g was measured each for T1, T2 and T3. About 5% wheat offal and 1% lime was added to each treatment.

After mixing each substrate, 400g of each substrate was bagged using transparent polythene bag of 27cm length and were tied with rubber band. Bagged substrates were arranged in a large pot and pasteurized for 7 hours. After, they were allowed to cool. After cooling down, a stick was used to prick the sawdust to allow easy inoculation. Twenty gram of spawn was inoculated in each bag and they were tied with rubber band. Incubation was done in a dark room at 27^{o}C. The bags were incubated for 5 weeks. Rate of ramification was checked every 5days.

After ramification, all ramified bags were cut opened and wetting followed immediately. Wetting was done every morning and as mushroom started sprouting, wetting was done every morning and evening. Harvesting was done carefully when the mushrooms were fully matured. The stalk height, pileus diameter as well as weight of the mushrooms were assessed. The nutritional composition such as crude protein, crude lipid, total ash, crude fibre and total carbohydrate were determined following the procedures recommended by AOAC (1995).

The experimental design adopted was Completely Randomized Design consisting of three treatments replicated five times. Data were analysed by analysis of variance to determine significant difference in means while Duncan's multiple range test (DMRT) was used for mean separation.

RESULTS

Growth of *Lentinus Sajor-Caju* grown on Different Wood Substrate

The total ramification period of mycelia on the mixed substrate was the fastest and was completed between 20 – 30 days and ranged between 14.30 – 15.00 cm after incubation. While total ramification on *Anogeissus leiocarpa* also varied from 20 – 30 days and ranged between 13.70 – 15.00 cm. Rate of ramification in *A. leiocarpus* was slow for ten days but later became faster. However, ramification in *Isoberlinia doka* started with a faster rate after incubation (Fig 1) but later slowed down. It was noticed that the ramification rate was stopped by day 25 and never progress. Generally, there were significant difference between the rate of ramification in *Anogeissus leiocarpus* and other substrates from day 5 to day 15 at p=0.05.

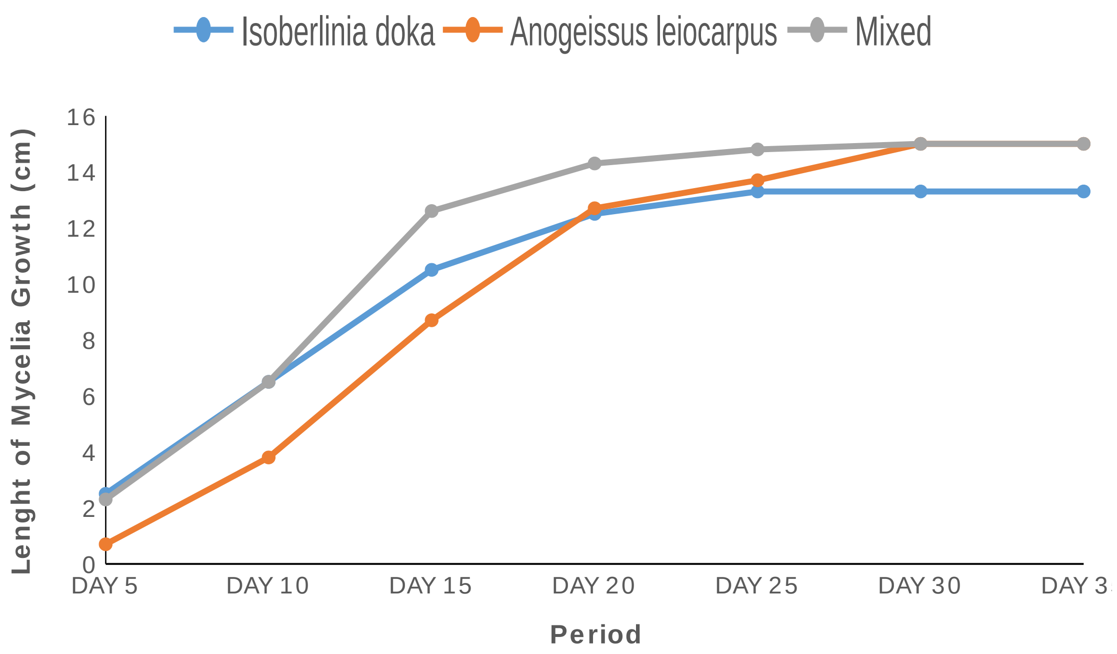

Figure 1: Rate of fungi colonisation of the different wood substrates.

Morphological Characteristics of *Lentinus Sajor-Caju* grown on Different Wood Substrate

The fruiting bodies gotten from the substrates that supported the growth of the mushrooms were measured using parameters such as diameter of pileus (cm) and length of stipe (cm) of *L. sajor-caju*. The length of stipe per flush ranges from 2.37 cm – 4.38 cm (Table 5). The highest length of stipe was found in mushroom harvested from *I. doka* from flush 1, while mushroom harvested from mixed substrate had the lowest stipe length from flush 1. There were significant variation at p=0.05

in the stipe length of the 3 substrates in flush 3. Mixed substrate had the lowest stipe length of 2.57 cm while *I. doka* had the highest (3.97cm). Also, statistical differences occurred in the diameter of pileus of mushrooms from flush 3. *I. doka* had the highest mean diameter of pileus 4.67±0.34cm which is significantly different from the ones produced from *A. leiocarpa* and mixed substrate respectively. From flushes 1 and 2, there was no significant difference between the mean diameters of pileus of mushroom produced from the three substrates.

Yield of *Lentinus Sajor-Caju* grown on Different Wood Substrate

The mushroom weight depends on the pileus diameter, the length and thickness of stipe and the number of fruiting bodies. From the result in Table 1, it can be seen that the mushrooms were harvested in 3 flushes. There was no significant variation in the weight of mushroom from each substrate from all flushes at p=0.05 except in flush 3 where there were significant differences. *I. doka had the highest weight of* 2.95±0.82g followed by *A. leiocarpa* (1.54±0.18g) while the mixed substrate had the lowest (0.77±0.12g).

Table 1: Productivity of *Lentinus sajor-caj*u Grown on Different Substrates

Growth/Yield	Substrates	FLUSH 1	FLUSH 2	FLUSH 3
Stipe length (cm)	*I. doka*	4.38±1.05 ^{a}	2.48±0.26 ^{a}	3.97±0.77^{a}
	A. leiocarpus	3.22±0.42^{a}	2.37±0.36 ^{a}	3.19±0.27^{ab}
	Mixed	2.93±0.40 ^{a}	2.61±0.17 ^{a}	2.57±0.28^{b}
Diameter of pileus (cm)	*I. doka*	3.74±0.68 ^{a}	2.97±0.38 ^{a}	4.67±0.34 ^{a}
	A. leiocarpus	4.30±0.58 ^{a}	3.50±0.87 ^{a}	3.25±0.45 ^{b}
	Mixed	3.96±0.83 ^{a}	2.65±0.14 ^{a}	2.81±0.21 ^{b}
Weight	*I. doka*	3.61±1.05^{a}	1.73±0.52 ^{a}	2.95±0.82^{a}
	A. leiocarpus	4.08±0.95 ^{a}	2.59±1.36 ^{a}	1.54±0.18^{b}
	Mixed	3.19±1.35 ^{a}	0.92±0.20 ^{a}	0.77±0.12^{b}
	I. doka	2.60±0.87 ^{a}	3.20±0.58 ^{a}	3.00±1.00^{a}

Number of fruiting bodies	*A. leiocarpus*	4.20±1.07 [a]	4.00±1.55 [a]	4.75±1.11[a]
	Mixed	4.00±1.10 [a]	5.80±0.74 [a]	5.00±1.58 [a]

Means followed by the same letter along the same column for each substrate are not significantly different at p = 0.05.

Nutritional Composition of *Lentinus Sajor-Caju* grown on Different Wood Substrate

The proximate composition of *L. sajor-caju* harvested from the three different substrate differ significantly at p=0.05. *A. leiocarpa* had the highest crude protein (43.65 %) and fibre (33.06 %) while the mixed substrate had the lowest protein (40.20 %) and fibre (29.45 %). *I. doka* had the highest (8.25%) crude lipid while mixed substrate had the lowest value of 5.47%. The moisture content was highest in *I. doka* and lowest in *A. leiocarpa* with values of 6.52% and 5.58% respectively. The mushrooms examined were high in crude fibre which is also an important part of healthy diet. The mean % ash content was lowest (6.36 %) in *I. doka* and highest (6.70 %) in *A. leiocarpa*. However, carbohydrate content was highest (15.29%) in the mixed substrate and lowest (5.51%) in *A. leiocarpus.*

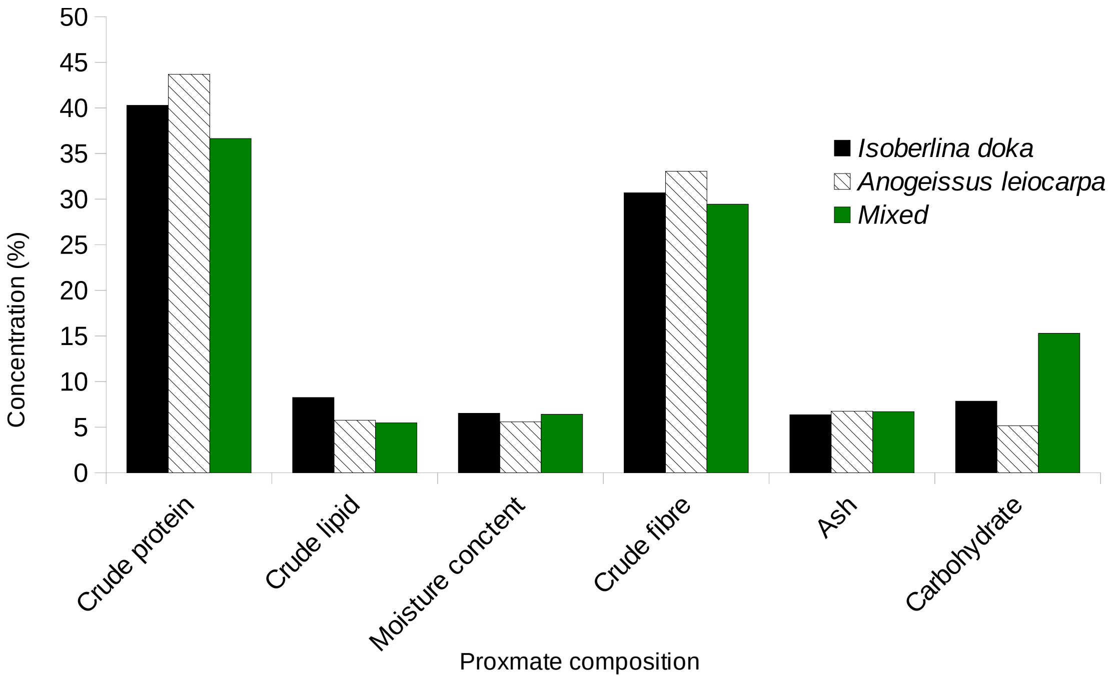

Fig 2: Nutritional composition of *Lentinus sajor-caju* as influenced by substrates type.

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DISCUSSION

The rate of ramification of *L. sajor-caju* exhibited by different substrates were observed for five weeks (Figure 1) *A. leiocarpus* and the mixed substrate reached their maximum ramification stage at week 4. This is an indication that *L. sajor-caju* can perform in the substrates selected. However, this result is in contrast to Nurudeen *et al*, (2013) who recorded that total ramification of *Pleurotus sajor-caju* took a total of 3weeks to reach full ramification on sawdust.

From Table 1, it is shown that mushroom harvested from *A. leiocarpus* and the mixed substrate reduced in weight from one flush to another, this is because the amount of nutrient available in the substrates diminished after each flush. This is in agreement with the study of Hussein *et al*, (2016) with a record that fruiting bodies of mushroom were harvested in two flushes with the last flush having the lower yield.

The first pin head appearance was noticed on *I. doka* on the second day of bag opening and the longest time for pin head appearance was noticed on the mixed substrate, 6 days after bag opening. First harvest was carried out on *I. doka*, followed by *A. leiocarpus* and then the mixed substrate but later vary according to how they flush.

The result also showed that *I. doka* from flush 3 showed the highest recorded pileus diameter (4.67±0.34cm), followed by *A. leiocarpus* from flush 1 (4.30±0.58cm) and the mixed substrate with 3.96±0.83cm in flush 1. The mean stipe recorded ranges from 2.37-4.38cm from all flushes in all substrates. This result is lower to mean stipe length values ranging from 5.7 – 7.1cm from flush 1-flush3 recorded by Nurudeen *et al*, (2013). Stipe length and pileus diameter showed variation from flush to flush, this was in line with the study of Nurudeen *et al*, (2013) with a record showing variation in the mean measurement of pileus diameter and stipe length of *P. sajor-caju* from flush to flush.

The mixed substrate had the lowest weight from all flushes, having 0.77±0.12g. *A. leiocarpus* had the highest total weight recorded. Likewise, no significant difference occurred in the number of fruiting bodies of the mushroom (Table 1) *I. doka* had the lowest mean number of fruiting bodies ranging from 2.60±0.87 in flush 1 to 3.00±1.00 in flush 3 while the mixed substrate has the highest mean number of fruiting bodies ranging from 4.00±1.10 in flush 1 to 5.00±1.58 in flush 3.

The result of the nutritional composition of *L. sajorcaju* grown on the different substrtaes is presented in Figure 2. The protein content exhibited by *L. sajorcaju* grown on these substrates was found to be relatively higher to those found in literatures (Fig. 2). The higher protein content in *L.*

sajorcaju produced by these substrates might be as a result of high nutritional content present in them. These values were greater than the range of values recorded by Celestin *et al*, (2015) for *L.sajor-caju* (< 20%) and 24.62% for *L. connatus* picked from the wild, 30.12% and 40.10% for *P. sajor-caju* harvested from sawdust of *Triplochiton scleroxylon* and coconut husk (Nurudeen *et al*, 2013) and 29.60 – 38.86% for *L. sajor-caju* cultivated on different substrates (Gupta *et al*, 2016).

The lipid content of *L. sajor-caju* in this study was low (Fig. 2), corroborating the findings of Celestine *et al*, (2015), they recorded above 2.0 % lipid for *L. sajor-caju.* It is an evident that mushrooms are low in fat and cholesterol (Syred *et al.,* 2003). Consequently, moisture content ranges from 5.58-6.41% which are comparably lower to 10.8% for *L.sajor-caju* harvested from the wild (Celestine *et al.*, 2015) and 12.58% recorded for *Pleurotus ostreatus* (Garuba *et al.*, 2017). Crude fibre value ranges from 29.45-33.06% with *A. leiocarpus* having the highest value. This result is greater than 21.0 % mean crude fibre for *L. sajor-caju* harvested in the wild recorded by Celestine *et al*., (2015) and the study of Garuba *et al.*, (2017) with values ranging from 5.31% - 5.83% for *Pleurotus pulmonarius* grown on different substrates. The ash content produced by *L. sajor-caju* was relatively low. However, the mushroom produced by *A. leiocarpa* exhibited higher value compared with the mushroom produced from other substrate. Nurudeen *et al.*, (2013) had similar values (5.71% - 7.42%) for *P. sajor-caju* cultivated on different substrates. The carbohydrate content (7.85-12.29%) was far lower than 65.68 – 69.22 % recorded for *P. sajor-caju* cultivated on different substrates (Nurudeen *et al.*, 2013) and 40 % for wild-grown *L. sajor-caju* (Celestine *et al.*, 2015).

CONCLUSION

The findings of this work showed significant variations in the growth and yield of *Lentinus sajor-caju* grown on *I. doka, A. leiocarpa* and the mixture of the two wood wastes. Generally, the growth and yield of mushroom was highest in *A. leiocarpa.* The highest nutritional content in terms of the protein and crude fibre content was also recorded in *A. leiocarpa* wood wastes. *A. leiocarpus* was found to be the best for the cultivation of *Lentinus sajor-caju* among the substrates used.

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