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Cultivation of *Pleurotus pulmonarius* on bark of common fruit trees in Nigeria namely; *Mangifera indica, Pentaclethra macrophylla, Elaise guineensis, Dacryodes edulis and Treculia africana*

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ABSTRACT

Pure culture of Pleurotus pulmonarius which was collected from the laboratory of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, was used in the investigation. The culture was used to prepare sorghum grain spawn. Fully ramified spawn was used to inoculate finely crushed, fermented and sterilized barks of selected fruit-trees: (Mangifera indica, (MI), Pentaclethra macrophylla, (PM) Elaise guineensis, (EG) Dacryodes edulis (DE) and Treculia africana. (TA), dispensed in perforated 50ml transparent plastic pails. Five grams of spawn was aseptically inoculated into each of the 1kg substrates. Fruit-bodies of the mushroom were harvested in flushes starting from 14 days after inoculation. The highest mean fruit-bodies was obtained from TA (18.00^{a}±2.15) followed by EG, (17.33a±2.27) while the least was in DE (13.00^{c}±0.95). Similarly the fruit-bodies produced in TA weighed more than those from the other substrates. Pileus diameter and stipe length of the fruit-bodies from the substrates followed the same trend as in the number of fruit-bodies. The proximate composition in percentage of the fruit-bodies 4.58^{a}±0.00 from the substrates are Ash from TA (4.61^{a}±0.01) from DE, (3.16^{b}±0.02), from DE (4.58^{a}±0.00), from MP (2.91^{bc}±0.01) and from EG (2.35^{c}±0.01) Crude fibre ranged from 3.41^{a}±0.01) in DE to (1.47^{d}±0.015) in MI. Crude protein ranged from (25.81^{a}±0.01) in TA to (18.41^{d}±0.01) in MI, while carbohydrate composition of the fruit-bodies were not significantly different but were as follows, 91.29^{a}±0.01, 91.22^{a}±0.02, 90.17^{b}±0.01, 91.34^{a}±0.035, and 90.67^{b}±0.09, for TA, MI, DE, PM, and EG respectively. The E. E. composition equally ranged from 4.76^{a}±0.04 in EG to 3.78^{e}±0.025 in TA. Moisture Content of fruit-body produced in DE (9.83^{a}±0.07), was highest compared to those produced in EG (9.33^{b}±0.085) MI (8.83^{c}±0.07), TA, (8.71^{c}±0.01) and PM (8.67^{c+}±0.035). The fruit-bodies from all the substrates differed significantly for their mineral contents. The contents ranged from Potassium (P) (1.258^{a}±0.02 in DE to 1.138^{d}±0.05 in TA), Calcium (Ca) (35.68^{c}±0.075 in PM to 24.77^{e}±0.01 in EG), Sodium (70.44^{a}±0.16 in EG to 58.66^{d}±0.04 in MI), Iron (Fe) (5.83^{a}±0.07 in PM to 4.37d±0.01 in DE), Zinc (Zn) (2.85^{d}±0.00in DE to 1.80d±0.00 in TA) and Magnesium (Mg) (92.52^{a}±0.075 in EG to 80.66^{d}±0.04 in MI). The trend is not consistent but the contents are appreciable. The investigation concluded that growing, Pleurotus pulmonarius on Barks of the trees may be worthwhile though TA could be preferred.

Keywords: Bark of fruit trees, *Pleurotus pulmonarius*, Nutritional composition

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INTRODUCTION

Mushrooms are known to grow on a wide variety of substrates and habitat. However the fact still remains that mushrooms show preference for a particular substratum within a habitat, [2]. Most of the edible fungi have strong enzyme system and are capable of utilizing complex organic compounds, which occur as agricultural wastes and industrial by-product Hence various agricultural by-products are being used as substrates for the cultivation of oyster mushrooms [14]. The agricultural wastes are converted into edible biomass in the form of fruit-bodies. In the Eastern parts of Nigeria barks of the common fruit trees involved in the investigation are not of significant use except as fuel wood in rural settings. Cultivation of mushroom on these solid residues can be viewed as an effective means to utilize bio-resource left behind and simultaneously as a sound environmental protection strategy.

Furthermore, the use of these residues in bioprocesses may be one of the solutions to bioconversion of inedible biomass residues into nutritious protein-rich food in the form of edible mushrooms). [7]. According to [14], *Pleurotus* species are found to be one of the most efficient lignocellulose solid state decomposing type of white rot fungi. Thus many agricultural and industrial wastes can be implicated in the cultivation of *Pleurotus* species. This suggestion stems from the fact that *Pleurotus* species have the ability to secrete hydrolyzing enzymes which enable them to flourish over a wide range of lignocellulosic waste materials [13], Many workers have thus exploited the aforementioned facts to investigate the use of various agricultural and industrial wastes to produce mushrooms. However in the literature no studies related to the use of *Mangifera indica, Pentaclethra macrophylla, Elaise guineensis, Dacryodes edulis and Treculia africana* in the cultivation of *P. pulmonarius* have been reported.

Therefore the present study was undertaken to investigate the cultivation of *Pleurotus pulmonarius* on bark of these common fruit trees in Nigeria.

MATERIALS AND METHODS

Collection of samples

The pure culture of *Pleurotus pulmonarius* was collected from the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike. A pure and fresh culture of the fungus was maintained by regular sub culturing on malt extract.

Substrates and their collection

The substrates used for this study were waste barks of some selected fruit trees: *Mangifera indica*, (MI), *Pentaclethra macrophylla, (*PM) *Elaise guineensis* (EG), *Dacryodes edulis* (DE) *and Treculia africana*. (TA) Barks from logs of each of the fruit trees were collected from felled trees in newly developed site within the environs of Michael Okpara University of Agriculture Umudike. The fruit trees substrates were crushed into pieces after which they were prepared according to a modified method of [1], The back of trees for substrates was crushed into pieces of approximately 1.5 cm in diameter. One kilogram (1000g) of each substrate was measured in replicates into 40 ml transparent poly bags. The substrates were soaked in water for five days to enhance fermentation. Excess water was drained off. The substrates were sterilized using a big metal drum containing stacks of sticks and water up to the level of the sticks and covered with fresh plantain leaves in order to generate enough heat. The substrates were subjected to heating up to 100^{oC} then steam-sterilized for 2 hours after monitoring the temperature with a thermometer and allowed overnight to cool while still in the drum. 1000g (1kg) each of the prepared substrates were aseptically placed into. A pure and fresh culture of the fungus was maintained by regular sub culturing on malt extract.

The substrates were inoculated with the spawn of the fungus *Pleurotus pulmonarius.* Five grams of spawn was aseptically inoculated into each of the treatment. The spawn was sprinkled on the substrates in layers and covered' Watered was lightly sprayed on the inoculated substrate every two days to maintain a high relative humidity of between 75-80%. This was done in triplicates and kept in sterile wooden racks in the mushroom house, at 30 ± 2°C.

Data collection: The yield of *Pleurotus pulmonarius* on the different substrates was determined by recording the number and size of the fruit bodies after sprouting. The measurements from the various replicates were added and their mean values calculated.

The following parameters of growth/yield were taken, Number of fruit bodies, Height of fruit body, and diameter of the pileus and fresh weight of fruit body:

Diameter of the pileus was measured in centimeter with a transparent plastic ruler from one edge of the pileus across the stripe to the other edge. Fresh weight of fruit bodies was obtained weighing using an electrical weighing balance

Preparation of samples for analysis

The fruit-bodies were prepared for analysis by drying them in the Selecta model oven at 104 °C for four hours, following the method of[6], The dried specimens were broken into smaller pieces before grinding into fine powder using the Thomas Willey milling machine [9],[10], The dried and powdered samples were dispensed into air-tight bottles and they were all taken to the laboratory for the analysis.

PROXIMATE COMPOSITION

Determination of Total Ash

This was done using the incineration gravimetric method [3] Sing a measured weight (5g) of sample.

It was given by the formula.

$$\% \text{ Ash} = \frac{W2-W2}{W0} \times \frac{100}{1}$$

Where, W1=weight of crucible W2=weight of empty crucible + ash.
W0= weight of sample used

Determination of CrudeFibre
This is determined by the Wende method [12], with 5grames of each sample.

The crude fibre content was calculated gravimetrically as:

$$\% \text{ crude fibre} = W2 - W3 \times 100$$

Where, W2= weight of crucible + sample after washing and in the oven.
W3 = weight of crucible + sample as ash.

Determination of Crude Protein
The crude protein contents were determined using the Kjedahl method described by [3], 0.2g of each sample was digested and made up of 50 ml of flask. 10ml of the each digested samples were distilled with 0.2m sulphuric acid for the nitrogen determination.

% crude protein = total nitrogen x 6.25

Determination of Moisture Content
A moisture content was determined by the gravimetric method [12], was used. A measured weight of each sample (5g) was used while the moisture content was calculated as follows

$$\text{Moisture} = \frac{W2-W3}{W2-W1} \times \frac{100}{1}$$ [8],

Where, W1=weight of empty moisture can, W2=weight of can before drying and W3=weight of can sample alter drying to a constant weight.

$$\text{Percentage moisture content} = \frac{\text{Weight of Moisture}}{\text{Weight of sample}} \times \frac{100}{1}$$

The percentages dry matter was determined by subtracting the percentage moisture content from that of the samples.

Carbohydrate was calculated as follows,

CHO (% dry wet) = 100 – CP + FO + ASH +MC + DF (in g/100g DW) [5], where CHO = Carbohydrate, CP = Crude protein, FO = Fats and oil, MC = Moisture contents, and DF = Dietary fibre.

Food Energy Value Calculation:
The Food Energy Value of the dry samples were estimated using the following factors.

Protein, 4.0 Kcal/g; fats and oil, 8.37Kcal/g and carbohydrates, 3.48Kcal/g [4], and the formula used was:

FEV - (%CP x 4.0) + (%FO x 8.37) + (%CHO x 3.48), all in Kcal/g.

Where FEV – Food Energy Value, CP 0- Crude Protein, FO – Fats and Oil and CHO - Carbohydrate

Mineral Determination
The levels of the mineral contents (P, Ca, Mg and Na) was done following the wet digestion extraction methods as described by [8]. A measured weight (5.0g) o

RESULTS AND DISCUSSION

Table 1. The yield of *P. pulmonarius* on different substrate

Parameters	TA	MI	DE	PM	EG
NO	18.00^{a}±2.15	13.75^{b}±1.52	13.00^{c}±0.95	13.25^{c}±0.93	17.33^{a}±2.27
WT (g)	35.09^{a}±1.38	21.16^{b}±5.09	19.41^{c}±4.28	15.33^{d}±2.88	12.16^{e}±3.64
PD (cm)	5.98^{a}±1.61	3.15^{d}±0.66	3.23^{d}±0.74	3.56^{c}±1.02	4.20^{b}±1.30
SL(cm)	2.55^{a}±0.66	1.83^{b}±0.41	2.55^{a}±0.64	1.95^{b}±0.43	1.40^{c}±0.37

Values are means and SD±SE standard error $^{a-b}$ *Means in the same row with different super scripts are significantly different ($p \leq 0.05$) while means in the same row with similar superscripts are not significantly different ($p \geq 0.05$).*

TA = *Treculia africana*, MI= *Mangifera indica*, DE= *Dacryodes edulis*, PM= *Pentaclathra macrophylla*, EG= *Elaeise guineensis*

Table 2. Proximate composition of *P. pulmonarius* produced on the different substrates

Parameters	TA	MI	DE	PM	EG
ASH	4.61^{a}±0.01	3.16^{b}±0.02	4.58^{a}±0.00	2.91^{bc}±0.01	2.35^{c}±0.01
Crude Fibre	1.63^{b}±0.005	1.47^{d}±0.015	3.41^{a}±0.01	1.62^{b}±0.025	1.59^{c}±0.01
Crude protein	25.81^{a}±0.01	18.41^{d}±0.01	18.47^{d}±0.00	23.35^{b}±0.11	21.66^{c}±0.06
CHO	91.29^{a}±0.01	91.22^{a}±0.02	90.17^{b}±0.01	91.34^{a}±0.035	90.67^{b}±0.09
EE	3.78^{e}±0.025	4.08^{d}±0.00	4.53^{b}±0.18	4.21^{c}±0.025	4.76^{a}±0.04
Moisture Content	8.71^{c}±0.01	8.83^{c}±0.07	9.83^{a}±0.07	8.67^{c+}±0.035	9.33^{b}±0.085

Values are means and SD±SE standard error

$^{a-b}$ *Means in the same row with different super scripts are significantly different ($p \leq 0.05$) while means in the same row with similar superscripts are not significantly different ($p \geq 0.05$).*

Table 3. Mineral composition of *P. pulmonarius* produced on barks of fruit trees and their supplementation

MINERALS	TA	MI	DE	PM	EG
P	1.138^{d}±0.05	1.205^{b}±0.00	1.258^{a}±0.02	1.156^{c}±0.19	1.148^{cd}±0.01
Ca	31.34^{b}±0.06	29.52^{c}±0.075	27.47^{d}±0.13	35.68^{c}±0.075	24.77^{e}±0.01
Na	65.83^{b}±0.03	58.66^{d}±0.04	69.525^{ab}±0.75	62.77^{c}±0.07	70.44^{a}±0.16
Fe	4.61^{c}±0.09	4.71^{c}±0.01	4.37^{d}±0.01	5.83^{a}±0.07	5.76^{b}±0.04
Zn	1.80^{d}±0.00	2.34^{c}±0.0o	2.85^{d}±0.00	2.64^{a}±0.015	2.47^{b}±0.035
Mg	85.42^{c}±0.18	80.66^{d}±0.04	91.54^{a}±0.06	89.51^{b}±0.11	92.52^{a}±0.075

Values are means and SD±SE standard error

$^{a-b}$ *Means in the same row with different super scripts are significantly different ($p \leq 0.05$) while means in the same row with similar superscripts are not significantly different ($p \geq 0.05$).*

The highest mean fruit-bodies was obtained from TA (18.00^{a}±2.15) followed by EG, (17.33a±2.27) while the least was in DE (13.00^{c}±0.95). Similarly the fruit-bodies produced in TA weighed more than those from the other substrates. Pileus diameter and stipe length of the fruit-bodies from the substrates followed the same trend as in the number of fruit-bodies. Generally the results obtained showed significant variability in the fruit-bodies obtained from the different substrates. The maximum number of fruit-bodies (18.00^{a}±2.15) obtained from *Treculia africana* followed by those from *Elaise guineense* (17.33a±2.27) suggests that in term of number of fruit-bodies TA and EG barks are preferred. Similarly the fruit-bodies produced in TA weighed more than those from the other substrates. Hence TA is most preferred. Pileus diameter and stipe length of the fruit-bodies from the substrates followed the same trend as in the number of fruit-bodies. This result implies that in terms of fruit-body yield, *Treculia Africana* bark is a preferable substrate.

The results of the proximate composition of the substrates are shown in the Table 2. All the assays were carried in triplicate (dry basis for humans.

The proximate composition in percentage of the fruit-bodies from the substrates are Ash from TA (4.61^{a}±0.01) from DE, (3.16^{b}±0.02), from DE (4.58^{a}±0.00), from MP (2.91^{bc}±0.01) and from EG (2.35^{c}±0.01) Crude fibre ranged from 3.41^{a}±0.01) in DE to (1.47^{d}±0.015) in MI. Crude protein ranged from (25.81^{a}±0.01) in TA to (18.41^{d}±0.01) in MI, while carbohydrate composition of the fruit-bodies were not significantly different but were as follows, 91.29^{a}±0.01, 91.22^{a}±0.02, 90.17^{b}±0.01, 91.34^{a}±0.035, and 90.67^{b}±0.09, for TA, MI, DE, PM, and EG respectively. The E. E. composition equally ranged from 4.76^{a}±0.04 in EG to 3.78^{e}±0.025 in TA. Moisture Content of fruit-body produced in DE (9.83^{a}±0.07), was highest compared to those produced in EG (9.33^{b}±0.085) MI (8.83^{c}±0.07), TA, (8.71^{c}±0.01) and PM (8.67^{c+}±0.035).).The analysis of the fruiting-bodies (Table 2), showed that the mushroom possessed a varied amount of nutrient composition. However, the highest percentage of protein was found in the mushroom cultivated in *Treculia africana*. The high protein percentage in the mushroom, shows that it can be a great protein source in the diet of humans. The high contents of protein in the fruit-bodies from *Treculia Africana* bark, the suggested preference of this substrate is further consolidated. Nevertheless all the

substrates produced fruit-bodies with appreciable quantities of proteins, ash, crude fibre and carbohydrates. This fact confirms the nutritional richness of *Pleurotus* mushrooms, as reported by [15], [10], [11],

The Mineral composition of *P. pulmonarius* on bark of fruit trees is summarized in Table 3. The mineral contents of the fruit-bodies ranged from P (1.258^{a}±0.02 in DE to 1.138^{d}±0.05 in TA), Ca (35.68^{c}±0.075 in PM to 24.77^{e}±0.01 in EG), Na (70.44^{a}±0.16 in EG to 58.66^{d}±0.04 in MI), Fe (5.83^{a}±0.07 in PM to 4.37d±0.01 in DE), Zn (2.85^{d}±0.00 in DE to 1.80d±0.00 in TA) and Mg (92.52^{a}±0.075 in EG to 80.66^{d}±0.04 in MI). Generally all the substrates produced fruit-bodies with appreciable quantities of all the minerals investigated. This also confirms the nutritional richness of *Pleurotus* mushrooms, as reported by [15].

CONCLUSION

The result of the investigation revealed that the organic ingredients in the dry barks of *Mangifera indica, Pentaclethra macrophylla, Elaise guineensis, Dacryodes edulis and Treculia africana* are suitable for the cultivation of *Pleurotus pulmonarius* mushroom. For higher yield and better quality however, *Treculia africana* bark is recommended. Generally, the barks pose potential alternative substrates for mass production of the mushroom. This will thus contribute a quota to the economic development of Nigeria as well as reduce waste disposal problems in the country.

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