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Evaluation of Yield, Heavy Metals and Vitamins Compositions of *Pleurotus pulmonarius* (Fries) Quell. Fruit Bodies Cultivated on three Deciduous Tree Logs

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Abstract

Studies were conducted to determine the yield potential, vitamins and heavy metals concentrations of *Pleurotus pulmonarius* (Fries) Quell. fruit bodies cultivated on different wood logs. Pure mycelium culture of *P. pulmonarius* was aseptically multiplied using sorghum grains. Fully colonized spawn was used to inoculate *Mangifera indica*, *Dacryodes edulis* and *Treculia africana* logs and incubated in the dark room of a mushroom house at 27°C ± 2. Data were subjected to analysis of variance (ANOVA), while comparison between different means were done using Duncan multiple range test (DMRT) at $p<0.05$ level of significance. Fruit body primordia of the oyster mushroom were first observed in *D. edulis* logs, followed by *T. africana* and then *M. indica* logs after 9, 10 and 12 days of inoculation respectively. *T. africana* gave the highest yield (156.0967 gm/kg) and Biological efficiency (0.836%) of *P. pulmonarius* fruit bodies while *D. edulis* was lowest at 62.3667 gm/kg and 0.200% biological efficiency. Fruit bodies of *M. indica* had the highest (3.19 mg/kg) Zn content; slightly higher than those of *T. africana* (3.14 mg/kg), while fruit bodies from *D. edulis* gave the lowest Zn (2.96 mg/kg) concentration. Fe was significantly ($p<0.05$) higher than other heavy metals studied in all the fruit body samples from the log substrates. Mushrooms of *D. edulis* gave the highest (184.87 mg/kg) Fe concentration while those of *M. indica* gave the lowest (182.91 mg/kg). Results

showed that *D. edulis* fruit bodies gave the highest vitamin A content (4.33 mg/100g) while *M. indica* (4.17 mg/100g) gave the lowest. However, thiamine content of the mushroom was 0.13mg/100g in *D. edulis* and *T. africana* but 0.12 mg/100g in *M. indica*. Mushroom growers should harness the wisdom of this wonderful innovation by utilizing wood logs first, before they can be used for other domestic purposes, such as firewood.

Keywords: *Pleurotus pulmonarius;* Yield; Heavy Metals; Vitamins; Umudike

1. Introduction

Mushrooms are fruit bodies of macro fungi. They are heterotrophic because of the absence of chlorophyll, but take up nutrients from organic sources synthesized by green plants [1]. Mushrooms have diversified into various groups. Out of 1.5 million known species of fungi, about 10,000 produce the fruit body called mushroom. More than 3000 mushroom species are said to be the most edible types, but only 100 are cultivated commercially while about 10 species are on industrial scale [2]. Approximately 70 species of *Pleurotus* have been identified, while new species are discovered more or less frequently, although, most of these are considered identical to previously recognized species [3].

1.1 Ecology and substrate for mushroom growth

Mushrooms grow wild in the tropical and sub-tropical rainforest [4]. They are capable of degrading lignin and hence are found naturally growing on different woody and non-woody agricultural residue [5]. Many workers have successfully carried out a number of investigations on mushroom cultivation using different lingo-cellulosic materials and reported favorable results. Muller, cultivated oyster mushroom on *Cassia* substrate, Okwulehie et al. [6] cultivated *P. pulmonarius* on HCl induced-oil palm bunch, Hamlyn, [7] worked using cotton wastes while Okhuoya et al. [8] grew *Pleurotus tuber-regium* on cassava peelings, corn straw, oil palm fruit fibre, rice straw, yam peelings and wild grass (*Pennisetum* sp.). In their attempt to grow shiitake on wood logs, Albert et al. [9] reported that when wood logs are inoculated with spawn and left to grow as they would in wild conditions, fruiting is triggered by seasonal changes or by briefly soaking the logs in water. Shiitake and oyster mushroom have traditionally been cultivated by outdoor log technique, although, controlled technique such as indoor tray-growing or artificial logs made by compressing sawdust substrate have been successfully substituted [10]. Hyunjong et al. [11] reported that since mushrooms primarily derive their nutrient from wood sap, any tree trunk chosen for mushroom cultivation should have a larger sapwood area. The lighter or outermost wood is the sap wood while the darker or inner wood is the heart wood. He further stated that logs with small amount of sapwood would probably produce mushroom for fewer years than those with greater amount of sap wood.

This investigation aimed to ascertain the productivity, heavy metals and vitamins constituents of *Pleurotus pulmonarius* fruit bodies grown on various trees logs.

2. Materials and Methods

2.1 Source of culture

Pure culture of *Pleurotus pulmonarius* was obtained from the laboratory of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

2.2 Spawn preparation

Spawn of *P. pulmonarius* was prepared using sorghum grains. Sorghum grains were washed in tap water and soaked overnight. Grains were then boiled in water in the ratio of 1:1 (sorghum grain: water) using kerosene stove for 15-20 mins and mixed with 4% (w/w) $CaCo_3$ and 2% (w/w) $CaSO_4$ for pH optimization and to prevent grains from clumping respectively according to the description by Muhammad et al. [12]. Completely drained Sorghum grains were then stuffed into 35cl Lucozade bottles, tightly plugged with absorbent cotton wool and autoclaved at 121°C for 30 mins. After autoclaving, the bottles were allowed to cool, prior to inoculation with actively growing mycelium of *P. pulmonarius* by grain-to-grain transfer and incubated in a dark environment (at 27°C ± 2) for 10 to15days, until the grains were completely colonized by mycelium [6, 13].

2.3 Preparation of wood logs (substrates)

Average trees size of *T. africana, M. indica* and *D. edulis* were cut down during the Hammattern (winter) season following the recommendations by Oei [14]. Trees were made into logs of 18cm long using Electric wood saw (EWS); Model: Elect. 1710, Japan. Care was taken to ensure that the barks of the logs were not peeled off as instructed by Hyunjong et al. [11].

2.4 Inoculation holes

Holes of 3 cm depth by 15 mm diameter were made hexagonally on each log with high speed drills (HSD) of 5 drill bit with respect to log size. Average number of holes per log was determined by the formula derived by Stamets, [5].

$$NH = \frac{\text{DL (cm)} \times \text{LL (cm)}}{6} \qquad (1)$$

Where NH-Number of holes; DL-Diameter of log (cm); LL-Length of Log (cm); 6-Derived constant

2.5 Mushroom cultivation

Logs were laid in open field between 8 and 9 months in alternating rains and sun for further decomposition. Dry weight of logs (g/kg) were determined before they were soaked in water for 24 hr. Logs were pasteurized at 80°C in an improvised metallic column (IMC) for 1hour, using cooking gas for heat supply and allowed to cool overnight, as recommended by Canford. Logs were inoculated by inserting about 15 g grain spawn of *P. pulmonarius* into 2/3 of the holes and subsequently sealing the logs with transparent polybags to avoid contamination. Mycelia recovery and substrate colonization were clearly visible after 24 hrs, while fully colonized polythene bags were cut open to induce primordial formation [11].

Before primordial initiation, white mycelium was visibly noticed on the cut ends of logs. Light intensity and humidity of air were increased to about 400 lux and 75% respectively. To achieve these, logs were watered at least morning and evening while the cropping room of the mushroom house was regularly flooded with water. Temperature was kept at 27°C ± 2 [14, 15] while fruit-bodies were harvested at maturity [16].

2.6 Yield and biological efficiency

Total weight (g) of all fresh fruit bodies harvested from each observation of 5 replications was measured as total mushroom yield. The Biological Efficiency B.E (%) of mushroom per (kg) weight of dry wood log substrate was calculated following the formula recommended by Chang et al. [2].

$$\text{B.E} = \frac{\text{fresh weight of mushroom}}{\text{dry weight of substrate}} \times \frac{100}{1} \qquad (2)$$

Figure 1: (a) Fully colonized logs (b) Fruit bodies growing from log holes (c) Fruit bodies growing at the cut end of logs.

2.7 Sample preparation

Harvested mushroom samples were arranged according to their source of collection, sun-dried and ground to fine powdery samples, using manual grinding machine and stored in dry air-tight containers for further nutritional analyses (Victor and Olatomiwa).

2.8 Determination of heavy metals

The concentrations of Fe, Cu and Zn in the sample were determined by Energy Dispersive X-ray Fluorescence (EDXRF) technique according to the method of Stihi et al. [17]. Using the Elvax spectrometer having an x-ray tube with Rh anode, operated at 50 kv and 100 µA. Samples were excited for 300sec and the characteristic x-rays were detected by a multi-channel spectrometer based on a solid state si-pin-diode x-ray detector with a 140 µm Be-window and an energy solution of 200 ev at 5.9 Kev. Elvax software was used to interpret the EDXRF spectra. The

accuracy of the results as evaluated by measuring a certified reference sample of good results was achieved between certified values and data obtained.

The concentrations of Cd and Pb in the samples were determined by Atomic Absorption spectrometry (AAS) [18, 19], using the AVANTA GBC spectrometer with flame and hollow cathode lamps (HCl). Cd and Pb were finally determined by calibration curve, according to the absorber concentration. Several standard solutions of various known concentrations were prepared and the elemental concentration in unknown sample was determined by extrapolating same values from the calibration curve. All sample concentrations were recorded as mg/kg dry weight of mushroom samples.

2.9 Determination of vitamins

2.9.1 Determination of vitamin A (Retinol): The vitamin A content in each sample was determined by the method of Shyam et al. [13] and AOAC [20]. About 5 g of each sample was first homogenized using acetone solution and filtered off using Whatman filter paper No. 1. The filtrate was further extracted with petroleum spirit using separating funnel, two layers, aqueous and solvent layers were obtained. The upper layer contains vitamin A and was washed with diluted water to remove residual water. This was later poured out into the volumetric flask through the tap of the separating funnel and made up to mark. The absorbance of the solution was read by spectrophotometer at wave length of 450 nm and was calculated as: Mg/g = A × Vol × 104 = A × 12 cm × sample weight.

2.9.2 Determination of vitamin B_1 (Thiamin): Thiamin was determined by the method of Nwoko et al. [21]. 5 g of each mushroom sample was homogenized with Ethanol sodium hydroxide (50 ml) and filtered into a 100 ml flask. 10 ml of the filtrate was pipette and the colour development read at the same time. Thiamin acid was used to get 100 ppm and serial dilution of 0.0, 0.2, 0.6 and 0.8 ppm was made. This was used to plot the calibration curve.

2.9.3 Determination of vitamin B_2 (Riboflavin): Riboflavin content of each mushroom sample was determined by spectrometric method. Five grams (5 g) of the dry powdery sample was inserted into an extraction plastic tube and 100 ml of 5% aqueous ethanol was added. The tube was placed in a mechanical shaker and shaken for 30mins, before it was filtered into 100 ml volumetric flask using whatman filter paper. $KmnO_4$ (0.5 g) was added to the filtrate to make up to 50 ml using hydrogen peroxide (H_2O_2) solution. The mixture was read off in a spectrophotometer to measure absorbance at 510 nm [22].

2.9.4 Determination of vitamin B_3 (Niacin): Niacin content of fruit bodies was determined by konig spectrophotometric method. 0.5 g of dry powdered sample of each mushroom was extracted with 50 ml of INHCl in a shaking water bath maintained at 30°C for 35 mins. The mixture was filtered using whatman filter paper No.1. $KmnO_4$ (0.5 g) was added to the filtrate and made up to mark. 10 ml of the extract was pipetted into 50 ml flax while

10ml of phosphate solution was added as buffer. The pH was adjusted with 5ml of INHCl and the solution was made up to mark with distilled water. After 15 mins, the extract was read by spectrophotometry at 470 nm wavelength.

2.9.5 Determination of vitamin C (Ascorbic Acid): Vitamin C content of each sample was determined by the method of Kamman et al. [23]. 5 g of each sample was homogenized in a 100 ml of EDTA/TCA extraction solution. The homogenate was filtered and the filtrate was used for analysis. Each mushroom sample filtrate was passed through a packaged cotton wool containing activated charcoal, to remove colour. The volume of the filtrate was adjusted to 100 ml of water by washing with more of the extracted solution. 20 ml of each filtrate was measured into a conical flask. 10 ml of 2% potassium iodide (KI) solution was added to each of the flasks followed by 5mls of starch solution (indicator). The mixture was titrated against 0.01M Copper sulphate ($CuS0_4$) solution using starch as indicator. The vitamin C content of the samples was given by the formula of Shyam et al. and Nwoko et al. [13, 21].

Therefore, vitamin C mg/100 g sample = $\frac{100}{Va} \times vf \times 0.88T$ (3)

Where Vf-volume of filtrate analyzed; Va-volume of acid analyzed; 0.88T-constant

2.10 Statistical analysis

All the data collected from various samples were subjected to Analysis of Variance (ANOVA) while comparison between different means were done using Duncan multiple range test (DMRT) at $p<0.05$ level of significance.

3. Results and Discussion

It was recorded that primordial formation of *P. pulmonarius* were first noticed in *D. edulis* logs followed by *T. africana* and then *M. indica* logs after 9, 10 and 12 days of inoculation respectively. This strictly conforms to the work of Okwulehie et al. [6], which recorded *P. pulmonarius* primordia on HCl acid-induced oil palm bunch substrate between 9 and 12 days. The mushroom fruited earlier than those cultivated by Shah et al. [24], which reported that *P. ostreatus* cultivated on different agro-waste fruited within 27-34 days after inoculation; Quimio [25] which maintained that fruit bodies of *P. ostreatus* cultivated on various substrates emerged within 3-4 weeks after substrate inoculation. According to their report, various factors such as: pH, temperature, nature of substrate and pasteurization technique can determine the fruiting time of oyster mushrooms. Ahmed et al. [26] worked on cultivation of oyster mushroom using different lignocellulosic substrates, and concluded that *P. ostreatus* took time (17-20days) to complete spawn running, while pin head formation started after 23-27 days of spawning. Khan et al. [27] grew oyster mushroom on different lignocellulosic substrates, but reported that pin head formation started 7-8days and matured between 10-12 days of substrates inoculation. The variation in fruiting duration could be due to density of the substrate as those with lesser amount of lignin tend to produce mushrooms faster [3].

Substrate	Yield(g)/kg Dry log	Biological Efficiency (B.E%)
D. edulis	62.3667	0.200

M. indica	91.6667	0.397
T. africana	156.0967	0.836

B.E = Biological Efficiency (%)

Table 1: Effect of different log substrates on yield of *P. pulmonarius* fruit bodies.

Results represent the yield and Biological Efficiency (B.E%) of *P. pulmonarius* cultivated on different log substrates. It was observed that *T. africana* gave the highest yield (156.0967 gm/kg) and biological efficiency (0.836%) of *P. pulmonarius* fruit bodies, followed by *M. indica* (91.6667 gm/kg and 0.397%) while *D. edulis* was lowest at 62.3667 gm/kg and 0.200% biological efficiency. The high yield of *P. pulmonarius* fruit bodies recorded in *T. africana* logs justifies heavy mycelium colonization of the log substrate observed within 5 days of spawn run. The total average yield of *P. pulmonarius* fruit bodies on various log substrates was low compared to the work of Okwulehie et al. [28], who reported higher yield and biological efficiency of *P. pulmonarius* fruit bodies after using stem back of the same trees. The lower yield of *P. pulmonarius* fruit bodies observed using logs could be due to the fact that logs provide smaller surface area to volume ratio for mycelia ramification; unlike when trees bark, sawdust or chopped pieces of straw grasses are used as substrate [2, 28]. This observation further justifies the claims by Funda [29], which reported that substrate preparation method is one of the major factors affecting yield of oyster mushrooms.

Log substrate	**Zn**	**Fe**	**Cd**	**Cu**	**Pb**
D. edulis	2.96^{c}	184.87^{a}	0.08^{c}	0.85^{b}	0.07^{c}
M. indica	3.19^{a}	182.91^{a}	0.09^{a}	0.10^{c}	0.09^{a}
T. africana	3.14^{b}	183.76^{a}	0.09^{b}	0.93^{a}	0.08^{b}

Values are means of 3 replicates and means bearing the same letter are not significantly different at ($p > 0.05$).

Table 2: Effect of wood log substrates on heavy metals (mg/kg) accumulation in *P. pulmonarius* fruit bodies.

Results reveal the heavy metals contents of *P. pulmunarius* fruit bodies grown on different wood logs. *P. pulmonarius* cultivated on *M. indica* had the highest (3.19 mg/kg) Zn content, though slightly higher than that of *T. africana* (3.14 mg/kg), while *D. edulis* gave the lowest Zn (2.96 mg/kg) concentration. Fe was significantly higher ($p<0.05$) than other heavy metals studied in all the fruit body samples from the log substrates. Mushroom of *D. edulis* gave the highest (184.87 mg/kg) Fe concentration while that of *M. indica* gave the lowest (182.91 mg/kg). Mehmet and Sevda reported that the maximum iron level in Oyster mushroom could be as high as (838.0 mgk^{-1}), making mushroom an ideal food for haemoglobin formation. Cadmium (Cd) content of *P. pulmonarius* cultivated across the various log substrates was lower than the results previously reported by Chang et al. [30], Rugunathan et al. [31] and Rugunathan and Swaminathan [32]. *M. indica* and *T. africana* had equal concentration of (0.09 mg/kg) while *P. pulmunarius* grown on *D. edulis* gave Cd concentration of (0.08 mg/kg). The quantity of Pb recorded in this

investigation was below the admitted maximum level of certain contaminants in foodstuff as established by the commission of the European communities (Commission Regulation [EC] No 466/2001). The admitted maximum level for Pb and Cd is set about 2 and 3 mg/1kg respectively; in cultivated mushrooms [33]. This suggests that that the oyster mushroom is safe for human consumption. Copper content ranged from 0.10 mg/kg in *M. indica* to 0.93 mg/kg in *T. africana.* Cu concentration of the mushroom grown on the various log substrates was lower than that obtained by Mehmet. Pb was present, though in minute concentration across all the log substrates. *M. indica* gave 0.0 gmg/kgof Pb. *T. africana* (0.08 mg/kg) while mushroom of *D. edulis* had the lowest Pb content (0.07 mg/kg). Kalac et al. [34] reported Pb concentration values between 0.1 and 40 mg/kg from mushroom collected near lead smelter. This indicates that the environment where mushroom is grown influences its chemical composition [35].

Log substrate	**Retinol (A)**	**Thiamine(B_1)**	**Riboflavin (B_2)**	**Niacin (B3)**	**Ascorbic acid (C)**
D. edulis	4.33^{a}	0.13^{a}	0.85^{a}	6.28^{a}	18.16^{a}
M. indica	4.17^{b}	0.12^{b}	0.83^{a}	6.07^{b}	17.17^{b}
T. africana	4.20^{b}	0.13^{b}	0.84^{a}	6.12^{c}	17.67^{c}

Values are means of 3 replicates and means bearing the same letter are not significantly different at ($p>0.05$).

Table 3: Effect of log substrates on vitamin contents (mg/100g DW) in *P. pulmonarius* fruit bodies.

Results showed that *D. edulis* gave the highest vitamin A content (4.33 mg/100g) while *M. indica* (4.17 mg/100g) gave the lowest. However, thiamine content of the mushroom was 0.13 mg/100g in *D. edulis* and *T. africana* but 0.12 mg/100g in *M. indica*. Results indicated that Vitamin A content of *P. pulmonarius* across the various log substrates was lower compared to the values obtained by Nwoko et al. [21] in an experiment where *P. ostreatus* was grown on the same log substrates. It was also observed that values recorded in this experiment were slightly lower than the result obtained by Okwulehie et al. [36]. Thiamine (B1) is a beriberi-preventing vitamin and plays important role in energy metabolism. Riboflavin contents were 0.85 mg/100g, 0.84 mg/100g and 0.83 mg/100g in *P. pulmonarius* grown on *D. edulis, T. africana* and *M. indica* respectively. These were slightly higher than the results of Okwulehie et al. [37]. Niacin content was highest (6.28 mg/100g) in mushroom cultivated on *D. edulis* but lowest (6.07 mg/100g) in that of *M. indica. P. pulmonarius* grown on *D. edulis* had the highest (18.16 mg/100g) concentration of ascorbic acid followed by *T. africana* (17.07 mg/100g) while *M. indica* gave the lowest composition of Ascorbic acid (17.17 mg/100g).

4. Conclusion and Recommendations

Fruit body primordia were first observed on D. edulis logs, after 9 days of spawn inoculation. Treculia africana log supported the highest fruit body production of P. pulmonarius Fruit bodies of P. pulmonarius grown on D. edulis logs had the highest composition of all the vitamins analyzed while those of M. indica gave the lowest. Mushroom growers should harness the wisdom of this wonderful innovation by utilizing wood logs first, before they can be used for other domestic purposes, such as firewood. Finally, care must be taken to ensure that forest/economic trees

are not over exploited for the purpose of mushroom cultivation as this may in turn have adverse effect on the climate.

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