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Productivity, Vitamins and Heavy Metals Analysis of *Pleurotus ostreatus* (Jacq: Fr) Kumm. Fruit Bodies Cultivated on Wood Logs

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Abstract

This research was conducted to determine the productivity, vitamins and heavy metals concentration of *Pleurotus ostreatus* (Jacq: Fr) Kumm. friut bodies cultivated on different wood logs. Pure mycelium culture of *P. ostreatus* was aseptically multiplied by grain-to-grain transfer using sorghum grains. Fully colonized spawn was used to inoculate *Mangifera indica, Dacryodes edulis* and *Treculia africana* logs and incubated in the dark at 27°C ± 2 M. Fruit body primordia were first observed in *D. edulis* followed by *T. africana* and lastly on *M. indica* logs after 11, 13 and 15 days respectively. *M. indica* log substrate gave the highest (245.8100 g/kg) yield of *P. ostreatus* fruit bodies among other log substrates. Vitamin contents were significantly highest in *P. ostreatus* cultivated on *D. edulis* logs while *P. ostreatus* cultivated on all the log substrates accumulated copper more than every other heavy metals analyzed. Both vitamins and heavy metals contents of *P. ostreatus* across various log substrates were significantly different $p<0.05$. Cultivation of *P. ostreatus* on *M. indica* logs especially before they are used for firewood will help boost food provision.

Keywords: *Pleurotus ostreatus*; Heavy metals; Logs and vitamins

Introduction

Oyster mushrooms grow wild on Logs and stumps of trees in tropical rainforest. The fruitbodies are collected by mushroom enthusiast for food and sold in local markets [1]. Oyster mushrooms are one of the most popular edible mushrooms in the world [2,3]. Approximately 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently, although, some of these are considered identical with previously recognized species. Oyster mushrooms provide a nutritionally significant content of vitamins (B1, B2, B12, C, D and E) [4]. Mushrooms have been used for anti-cancer and many other therapeutic purposes [5]. Being rich in folic acid, mushrooms counteract panicious anaemia [6]. The polysaccharide protein complex (PSPC) found in mushrooms has proven to be anti-tumour, immune modulatory, anti-malaria, anti-viral, anti-cancer etc. [7]. Cholesterol (a dreaded sterol for heart patients) is absent in mushroom although, can be converted to vitamin D by the human body [5].

Growing on a substrate with a high concentration of various heavy metals, mushrooms can become toxic by accumulating a larger amount of heavy metals [8]. Before now, studies have shown that accumulation of heavy metals in mushrooms is dependent on: species and age of mushroom, substrate and environment where the mushroom is growing [9,10].

The determination of heavy metal concentration in the fruit bodies of mushrooms is essential in dietary intake studies. Different heavy metals are toxic, such as: Arsenic (AS), Cadmium (Cd), Nikel (Ni) and Mercury (Hg). On the other hand, many elements such as Fe, Zn, Mn, Cu, Cr and Se are essential for human metabolism [8].

Materials and Methods

Source of culture

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

Spawn preparation

Spawns of *P. ostreatus* were 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) $CaSO_4$ to optimize P^H and prevent clumping of grains respectively as described by Muhammad et al. [1]. Completely drained Sorghum grains were then packed in 35 cl Lucozade bottles tightly plugged with cotton wool and sterilized in an autoclave at 121°C for 30 mins. After sterilization, the bottles were allowed to cool, before they were inoculated with actively growing mycelia of *P. ostreatus* by grain-to- grain transfer and incubated in the dark (at 27 ± 2°C) for 10-15 days until the grains were fully colonized by mycelia [11].

Preparation of wood logs (Substrates)

Average trees size of *T. africana, M. indica* and *D. edulis* were fell during the Hamattern season (winter) according to the recommendations of Oei [6]. Trees were cut into logs of 18 cm 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 and Seung [12].

Inoculation holes

Holes of depth 3 cm by 15 mm diameter were made hexagonally on each log with high speed drills (HSD) of 5 drill bit in respect to log

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size. Average number of holes per log was determined by the formula, according to Stamets [13].

$$NH = \frac{DL(cm)XLL(cm)}{6}$$

Where: NH= Number of holes

DL= Diameter of log (cm)

LL= Length of Log (cm)

6= Derived constant.

Mushroom cultivation

Logs were laid in open field for 8-9 months in alternating rains and sun to allow for decomposition. Dry weights of logs (g/kg) were determined before they were soaked in water for 24 hrs. Logs were pasteurized at 80°C in an improvised metallic drum (IMD) for 1 hr using cooking gas as a local heat source and allowed to cool overnight, as recommended by Canford.

Log inoculation was done by inserting about 15 g grain spawn of *P. ostreatus* and into 2/3 of the holes and subsequently sealing the logs with transparent polybags to avoid contaminants. Mycelium recovery and colonization were clearly visible after 24 hrs; when fully colonized polythene bags were cut open to allow for fruiting [12]. Before primordial initiation, white mycelium blotches were visibly noticed on the cut ends of the logs. Light intensity and humidity of the air were increased to about 400 lux and 75% respectively. To achieve these, logs were watered at least morning and evening and the cultivation room of the mushroom house was flooded with water. Temperature was maintained at 27 ± 2°C [6,9]. Pinheads of *P. ostreatus* were first noticed in *D. edulis* logs followed by *T. africana* and lastly on *M. indica* logs after 11, 13 and 15 days respectively. Mushrooms were harvested as soon as the fruit-bodies were fully matured [14].

Yield and biological efficiency

Total fresh weight (g) of all the fruit bodies of *P. ostreatus* harvested from each set of 5 replications were measured as total yield of mushrooms. The Biological Efficiency B.E (%) yield of mushroom per weight (kg) of woodlog substrate (on dry weight basis) was calculated following the formula recommended by Chang et al. [5].

$$B.E = \frac{\text{fresh weight of mushroom}}{\text{dry weight of substrate}} X \frac{100}{1}$$

Sample preparation

Mushroom samples were arranged according to their source of collection and dried at room temperature after which they were ground to fine powdery samples using manual grinding machine and stored in dry air-tight bottles for further laboratory analysis, following the method of Okwulehie and Okwujiako [14]; Victor and Olatomiwa [15].

Determination of Vitamins

Determination of vitamin A (Retinol)

The vitamin A content in each sample was determined by the method of Shyam et al. [11]. About 5 g of the sample was first homogenized using acetone solution and filtered off using Whatman filter No. 1. The filtrate was then extracted with petroleum spirit using separating funnel, two layers of both aqueous and solvent layer were obtained. The upper layer which contains vitamin A was washed with diluted water to remove residual water. It was later poured out to the volumetric flask through the tap of the separating funnel and made up to mark. The absorbance of the solution was read using a spectrophotometer at wave length of 450 nanometer (nm) and was calculated as:

Mg/g = A × vol × 104 = A × 12 cm × sample weight.

Determination of vitamin B_1 (Thiamin)

5 g of each mushroom sample was homogenize with ethanolic sodium hydrozide (50 ml). It was filtered into a 100 ml flask. 10 ml of the filtrate was pipetted 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.

Determination of vitamin B_2 (Riboflavin)

Riboflavin content of each 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% (aq) ethanol was added. The tube was placed in a mechanical shaker and was shaken for 30 mins and filtered into 100 ml volumetric flask using whatman filter paper. $KmnO_4$ (0.5 g) was added to the filtrate and made up to 50 ml with hydrogen peroxide (H_2O_2) solution. The mixture was read off in a spectrophotometer to measure absorbance at 510 nm [16].

Determination of vitamin B_3 (Niacin)

Niacin content was determined following 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 kept at 30°C for 35 mins. The mixture was filtered using whatman filter paper. $KmnO_4$(0.5 g) was added to the filtrate and made up to mark. 10 ml of the extractswas pipetted into a 50 ml flax and 10 ml of phosphate solution was added as buffer. The pH was adjusted with 5 ml 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.

Determination of vitamin C (Ascorbic Acid)

Vitamin C content of each sample was determined by the method of Kamman et al. [17]. Five grams (5 g) of each sample was homogenized in a 100 ml of EDAT/TCA extraction solution. The homogenate was filtered and the titrate used for the analysis. Each sample filtrate was passed through a packaged cottonwool containing activated charcoal to remove the colour. The volume of the filtrate was adjusted to 100 ml of water by washing with more of the extraction solution. 20 ml of each filtrate was measured into a conical flask. 10 ml of 2% potassium iodide solution was added to each of the flasks followed by 5 mls of starch solution (indicator). The mixture was titrated against 0.01 mol $CuSO_4$ solution, titration of the brink of the mixture; the vitamin C content was given by the relationship that 1 ml of 0.01 mol $CuSO_4$, 0.88 n vitamin C [11].

$$\text{Therefore, vitamin mg/100 g sample} = \frac{100}{va} \times vf \times 0.88T$$

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. [8]. Using the Elvax spectrometer having an x-ray tube with Rh anode, operated at 50 kv and 100 µA. Samples were excited for 300 sec and the characteristic x-rays were detected by a multichannel 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 good results were achieved between certified values and data obtained.

The concentration of Cd and Pb in the sample were determined by Atomic Absorption spectrometry (AAS), using the AVANTA GBC spectrometer with flame and hollow cathode lamps (HCL). Cd and Pb were determined by the method of calibration curve according to the absorber concentration. Several standard solutions of different known concentrations were prepared and the elemental concentration in unknown sample was determined by extrapolation from the calibration curve. All sample concentrations were reported as mg/kg dry weight of material.

Statistical analysis

The data obtained were statistically analyzed using Analysis of Variance (ANOVA) mean separation and tests of significance were carried out by Duncan Multiple Range Test (DMRT) at $p<0.05$ (Steel and Torie). This investigation was conducted to determine the productivity, vitamins and heavy metals composition of Pleurotus *ostreatus* fruit bodies cultivated on various log substrates in Abia State, Nigeria.

Results and Discussion

The results revealed the yield and Biological Efficiency (B.E) of *P. ostreatus* cultivated on three different wood log substrates. *M. indica* log substrate showed a significantly highest yield (245.8100 g) with Biological Efficiency (B.E) of 1.060%; followed by *T. africana,* with a total yield and B.E of 144.70 gm/kg log substrate and 0.763% respectively, while *D. edulis* gave the lowest yield (120.8067 gm/kg) log with (0.396%) B.E. This result conforms with the report by Oei [6] who maintained that *M. indica* log substrate supports high *P. ostreatus* fruit body yield. He also stated that *Liquidambar formosana* logs gave lower yield of the same Oyster mushroom compared *M. indica*. The high yield of *P. ostreatus* in respect to *M. indica* log substrate could suggest that *M. indica* has a larger sap wood area than D. *edulis* and *T. africana* logs as reported by Hyunjong and Seung [12] (Tables 1 and 2).

Substrate	Yield (g)/kg Dry log	Biological Efficiency (B.E)
D. edulis	120.8067	0.396
M. indica	245.8100	1.060
T. africana	144.7000	0.763

BE=Biological Efficiency

Table 1: Effect of different log substrates on yield of *P. ostreatus.*

Log substrate	Retinol (A)	Thiamine (B_1)	Riboflavin (B_2)	Niacin (B3)	Ascorbic acid (C)
D. edulis	6.81^a	0.24^a	0.97^a	5.28^a	19.86^a
M. indica	6.67^b	0.24^b	0.97^a	5.07^b	19.63^b
T. africana	6.72^b	0.24^b	0.96^a	5.16^c	19.72^c

Table 2: Vitamin Composition (mg/100 g DW) of *P. ostreatus* as affected by different woodlog substrates.

Values are means of 3 replicates and means bearing the same letter are not significantly different (P>0.05)

Vitamin contents of *P. ostreatus* grown on different wood log substrates are shown in the result above. The result indicates that *P. ostreatus* fruit bodies cultivated on various log substrates were rich in vitamins, especially Ascorbic acid. Mushroom grown on *D. edulis* logs gave the highest retinol content (6.81 mg/100 g Dw) followed by that grown on *T. africana* (6.72 mg/100 g Dw) and then *M. indica* (6.67 mg/100 g Dw). The Recommended Dietary Intake (RDI) of Retinol is 200 µg. Retinol is essential for good eyesight and prevents blindness [11], and helps in fetus development during pregnancy.

Thiamine (Vit. B1) is essential for neural functioning and carbohydrate metabolism and its deficiency results in beriberi [11]. All the wood log substrates used in the cultivation of *P. ostreatus* gave the same thiamine content (0.24 mg/100 g D.W). These were slightly lower compared to the result of Okwulehie et al. [14]. Riboflavin contents fall within the range of (0.97 mg/100 g D.W) for all the substrates. Niacin content was 4.28 mg/100 g D.W, 5.16 mg/100 g D.W and 5.07 mg/100 g D.W for *D. edulis, T. africana* and *M. indica* respectively.

Ascorbic acid content was highest (19.86 mg/100 g D.W) in *D. edulis* < (19.72 mg/100 g D.W) obtained in *T. africana* < (19.63 mg/100 g D.W) in *M. indica.* All the vitamins present were significant ($p < 0.05$) in respect to the various log substrates (Table 3).

The heavy metal contents of *P. ostreatus* fruit bodies across various log substrates are presented. Heavy metals concentration of fruit bodies on dry weight basis, show *P. ostreatus* grown on *D. edulis* logs had the highest Fe (116.49 mg/kg) concentration, which is significantly ($p<0.05$) higher than Zn (2.15 mg/kg) followed by Cu (0.73 mg/kg), Cd (0.06 mg/kg) and Pb (0.04 mg/kg). Fe concentration gained significant increase in *M. indica* (165.13 mg/kg) and followed the same trend in Zn (2.77 mg/kg), Cu (0.83 mg/kg), Cd (0.08 mg/kg) and Pb (0.06 mg/kg). Logs of *T. africana* had the overall highest Fe concentration (165.85 mg/kg) but showed a slight decrease in Zn, Cu, Cd and Pb as 2.45 mg/kg, 0.76 mg/kg, 0.07 mg and 0.05 mg/kg respectively when compared to *P. ostreatus* grown on *M. indica.* The respective high and low concentrations of Fe and Pb in *P. ostreatus* cultivated on the various wood log substrates were also reported by Stihi et al. [8].

A great attention was done to the Cd and Pb contents in the analyzed mushrooms, because these are toxic elements included in the hygiene norms concerning the foods security Stihi et al. [8]. The implication of this result is that the cultivation of *Pleurotus ostreatus* fruit bodies on *M. indica* logs should be encouraged; especially before logs are used as firewood. *Pleurotus ostreatus* fruit bodies cultivated on the various log substrates were rich in all the Vitamins studied. Cadmium and Lead concentrations were found in very small amount and place the mushroom safe for consumption. It also shows that the fruit body samples were rich in Zn and Fe, which are highly needed in the body for healthy especially for wound healing.

Log substrate	Zinc (Zn)	Iron (Fe)	Cadmium (Cd)	Copper (Cu)	Lead (Pb)
D. edulis	2.15^c	116.49^a	0.06^c	0.73^b	0.04^c
M. indica	2.77^a	165.13^a	0.08^a	0.83^c	0.06^a
T. Africana	2.45^b	165.85^a	0.07^b	0.76^b	0.05^b

Values are means of 3 replicates and means bearing the same letter are not significantly different (P>0.05)

Table 3: Effect of substrates on heavy metals (mg/kg) accumulation in *P. ostreatus*.

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