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Research Article

PRODUCTIVITY, VITAMINS AND HEAVY MET ALS ANALYSIS OF *PLEUROTUSOSTREATUS* (JACQ: FR) KUMM. FRUITBODIES CULTIVATED ON WOOD LOGS

[1]Nwoko, M. C.,[2]Onyeizu, U. R., [3]Chukunda, F.A. and[3]Ukoima, H.N.

[1]Department of Environmental Management and Toxicology, Michael Okpara University of Agriculture, Umudike, Nigeria
[2]Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria
[3]Department of Forestry and Environment, Rivers State University of Science and TechnologyPort Harcourt, Nigeria

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ABSTRACT

This research was conducted to determine the productivity, vitamins and heavy met als presence in *Pleurotusostreatus* (Jacq: Fr) Kumm, fruitbodies cultivated on different wood logs. Pure mycelium culture of *P. ostreatus* was aseptically multipliedby grain-to-grain transfer using sorghum grains. Fully colonized spawn was used to inoculate *Mangiferaindica, Dacryodesedulis* and *Treculiaafricana* logs and incubated in the dark at 27±2°C.Fruit body primordia were first observed in *D. edulis* followed by *T. africana* and *M. indica*was the least.*M. indica* woodlogs gave the highest yield (245.8100g/kg) of *P. ostreatus* fruit bodies among other wood log substrates. Vitamin contents were significantly high in *P. ostreatus* cultivated on *D. edulis*wood logs. *P. ostreatus* cultivated on all the log substrates accumulated copper more than every other heavy met als analyzed. The vitamins and heavy met als contents in *P. ostreatus*on various log substrates were significantly different (P≤0.05). Cultivation of *P. ostreatus* on *M. indica*wood logs produced reasonable quantity of mushroom. Therefore, the use of wood logs should not be used as fuel wood only since it was found to boast mushroom production especially *P. ostreatus.*

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 (Muhammad *et al*., 2007). Oyster mushrooms areone of the most popular edible mushrooms in the world (Sturion and Oetterer, 1995; Justo *et al.,*1998). Approximately 70 species of *Pleurotus* have been recorded and new species are discovered.Although, some of these are considered identical with previously recognized species (Chang, 2013). Oyster mushrooms provided significant vitamins content B_1, B_2, B_{12}, C, Dand E. (Mattila*et al.,* 2001). Mushrooms have been used for anti-cancer and many other therapeutic purposes (Liu *et al.,* 2001; Chang and Miles, 2004). Being rich in folic acid, mushrooms can solve the anaemicpatients (Oei, 2003). The polysaccharide protein complex (PSPC) found in mushrooms has proven to be anti-tumour, immune modulatory, anti- malaria, anti-viral and anti-cancer (Wang *et al.,*2001; Liu *et al.,* 2001).

Corresponding author: Onyeizu, U. R.,
Department of Environmental Management and Toxicology, Michael Okpara University of Agriculture, Umudike, Nigeria.

Cholesterol is absent in mushroom although, can be converted to vitamin D by the human body (Chang *et al.,* 2004). Growing on a substrate with a high concentration of various heavy met als, mushrooms can become toxic by accumulating a larger amount of heavy met als (Stihi *et al.,* 2011). Before now, studies have shown that accumulation of heavy met als in mushrooms is dependent on: species and age of mushroom, substrate and environment where the mushroom is growing (Turkekul *et al.,* 2004; Ita *et al.,* 2006; Ukoima *et al.,* 2009a, 2009b, 2009c). The determination of heavy met al concentration in the fruit bodies of mushrooms is essential in dietary intake studies (Stihi *et al.,* 2011). Different heavy met als 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 (Stihi *et al.,* 2009).

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 kerosine stove for 15-20 minutes and mixed with 4% (w/w) $CaCo_3$ and 2 % (w/w) $CaSO_4$ to optimize pH and prevent clumping of grains respectively as described by Muhammad *et al.* (2007). Completely drained Sorghum grains were then packed in 35cl Lucozade bottles tightly plugged with cotton wool and sterilized in an autoclave at 121^{o}C for 30 minutes. 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^{o}C for 10-15days until the grains were fully colonized by mycelia (Shyam *et al.,* 2010).

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 (2003). Trees were cut into logs of 18cm 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.

Inoculation Holes

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

$NH = \frac{\mathrm{DL(cm)XLL(cm)}}{6}$ (Stamets, 2003),

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 8-9months in alternating rains and sun to allow for the wood decomposition. Dry weights of logs (g/kg) were determined before they were soaked in water for 24hrs. Logs were pasteurized at 80^{o}C in an improvised met allic drum (IMD) for 1hr using cooking gas as a local heat source and allowed to cool overnight (Hyunjong and Seung, 2004). Log inoculation was done by inserting about 15g grain spawn of *P. ostreatus*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 24hrs when fully colonized polythene bags were cut open to allow for fruiting (Hyunjong and Seung, 2004). 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^{0}C (Oei, 2003, Chen, 2004, Ukioma*et al.,* 2009a, b,c). Mushrooms were harvested as soon as the fruit-bodies were fully matured (Okwulehie and Okwujiako, 2008; Nwoko*et al.,* 2016, Chukunda *et al.,* 2017).

Yield and Biological Efficiency

Total fresh weight (g) of all the fruit bodies of *P. ostreatus* harvested from each set of 5 replicates were measured as total yield of mushrooms. The Biological Efficiency (B.E) the yield of mushroom per weight (kg) of woodlog substrate (dry weight basis) was calculated following the formula.

B.E $= \frac{\text{fresh weight of mushroom}}{\text{dry weight of substrate}} \text{ X } \frac{100}{1}$(Chang *et al.,* 2004, Ukioma*et al.,* 2017).

Determination of Vitamins

Determination of Vitamin A (Retinol)

The vitamin A content in each sample was determined by the method of Shyam *et al.* (2010). About 5g 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 x vol x 104

= A x 12cm x sample weight.

Determination of Vitamin B_1 (Thiamin)

5g of each mushroom sample was homogenize with ethanolic sodium hydroxide (50ml). It was filtered into a 100ml flask. 10ml of the filtrate was pipetted and the colour development read at the same time. Thiamin acid was used to get 100ppm and serial dilution of 0.0, 0.2, 0.6 and 0.8ppm was made. This was used to plot the calibration curve (AOAC, 1980).

Determination of Vitamin B_2 (Riboflavin)

Riboflavin content of each sample was determined by spectrometric method. Five grams (5g) of the dry powdery sample was inserted into an extraction plastic tube and 100ml of 5% (aq) ethanol was added. The tube was placed in a mechanical shaker and was shaken for 30mins and filtered into 100ml volumetric flask using whatman filter paper. $KmnO_4$ (0.5g) was added to the filtrate and made up to 50ml with hydrogen peroxide (H_2O_2) solution. The mixture was read off in a spectrophotometer to measure absorbance at 510nm (Okoi, and Iboh, 2015).

Determination of Vitamin B_3 (Niacin)

Niacin content was determined following konig spectrophotometric method. 0.5g of dry powdered sample of

each mushroom was extracted with 50ml of INHCL in a shaking water bath kept at 30°c for 35mins. The mixture was filtered using Whatmanfilter paper. $KmnO_4$ (0.5g) was added to the filtrate and made up to mark. 10ml of the extractswas pipetted into a 50ml flax and 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 15mins, the extract was read by spectrophotometry at 470nm wavelength (AOAC, 1980).

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 (AOAC, 1980).The concentration of Cd and Pb in the sample were determined by Atomic Absorption spectrometry (AAS) (Wagner, 1999; Dima*et al.,* 2006), 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.

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

Substrate	Yield(g)/kgDry log	Biological Efficiency (B.E)
D. edulis	120.8067 ± 0.02	0.396 ±0.01
M. indica	245.8100 ±0.04	1.060 ±0.02
T .africana	144.7000 ±0.01	0.763 ±0.03

B.E = Biological Efficiency

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

Woodlog substrate	Retinol (A)	Thiamine (B_1)	Riboflavin (B_2)	Niacin (B_3)	Ascorbic acid
D. edulis	6.81^{a}±0.02	0.24^{a}±0.01	0.97^{a}±0.01	5.28^{a}±0.01	19.86^{a}±0.02
M. indica	6.67^{b}±0.03	0.24^{b}±0.01	0.97^{a}±0.02	5.07^{b}±0.04	19.63^{b}±0.01
T .africana	6.72^{b}±0.02	0.24^{b}±0.02	0.96^{a}±0.03	5.16^{c}±0.02	19.72^{c}±0.02

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*

woodlog substrate	Zinc(Zn)	Iron(Fe)	Cadmium(Cd)	Copper(Cu)	Lead(Pb)
D. edulis	2.15^{c}±0.01	116.49^{a}±0.01	0.06^{c}±0.02	0.73^{b}±0.03	0.04^{c}±0.02
M. indica	2.77^{a}±0.02	165.13^{a}±0.02	0.08^{a}±0.01	0.83^{c}±0.03	0.06^{a}±0.01
T. Africana	2.45^{b}±0.01	165.85^{a}±0.03	0.07^{b}±0.02	0.76^{b}±0.02	0.05^{b}±0.02

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

Determination of Vitamin C (Ascorbic Acid)

Vitamin C content of each sample was determined by the method of Okwulehie (2009). Five grams (5g) of each sample was homogenized in a 100ml 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 100ml of water by washing with more of the extraction solution. 20ml of each filtrate was measured into a conical flask. 10mls of 2% potassium iodide solution was added to each of the flasks followed by 5mls 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 1ml of 0.01,molCuSO4, 0.88n vitamin C. (Shyam *et al.,* 2010).

Vitamin mg/100g sample = $\frac{100 \times vf \times 0.88T}{va}$

Determination of Heavy Met als

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.* (2011). Using the Elvax spectrometer having an x-ray tube with Rh anode, operated at 50kv and 100µA. Samples were excited for 300sec and the characteristic x-rays were detected by a multichannel spectrometer based on a solid state si-pin-diode x-ray detector with a 140µm Be- window and an energy solution of 200ev at 5.9 Kev. Elvax software was used to 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, 1980).This investigation was conducted to determine the productivity, vitamins and heavy met als 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 (245.8100g) 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 (2003) who maintained that *M. indica*log substrate supports high*P.ostreatus* fruit body yield. He also stated that *Liquidambarformosana*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 byHyunjong and Seung, (2004).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 (6.81mg/100g Dw) followed by that grown on *T. Africana* (6.72mg/100g Dw) and then *M. indica* (6.67mg/100g Dw). The Recommended Dietary Intake (RDI) of Retinol is 200µg (Bobek *et al.,* 2010). Retinol is essential for good eyesight and prevents blindness. (Shyam *et al.,* 2010) and helps in fetus development during pregnancy (Caglarimak, 2007). Thiamine (Vit.B1) is essential for neural functioning and carbohydrate metabolism and its deficiency results in beriberi (Shyam *et al.,* 2010). All the wood log substrates used in the cultivation of *P. ostreatus* gave the same thiamine content (0.24mg/100g D.W). These were slightly lower compared to the result of Okwulehie *et al.,* (2009). Riboflavin contents fall within the range of (0.97mg/100g D.W) for all the substrates. Niacin content was 4.28mg/100g D.W, 5.16mg/100g D.W and 5.07mg/100g D.W for *D. edulis, T. africana* and *M. indica*respectively.Ascorbic acid content was highest (19.86mg/100g D.W) in *D. edulis* (19.72mg/100g D.W) obtained in *T. africana*(19.63mg/100g D.W) in *M. indica.* All the vitamins present were significant (P≤0.05) in respect to the various log substrates. The heavy met al contents of *P. ostreatus* fruit bodies across various log substrates are presented. Heavy met als 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 (2.15mg/kg) followed by Cu (0.73mg/kg), Cd (0.06mg/kg) and Pb (0.04mg/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 (165.85mg/kg) but showed a slight decrease in Zn, Cu, Cd and Pb as 2.45mg/kg, 0.76mg/kg, 0.07mg and 0.05mg/kg respectively when compared to *P. ostreatus* grown on *M. indica.* The varying degree of Iron and lead quantity in the fruit bodies of *P. ostreatus* was earlier reported by Shiti*et al*, 2010. The present result findings agreed with the earliest report of Shiti *et al*., 2011. It also shows that the fruit body samples were rich in Zn and Fe, whichare highly needed in the body for healthy especially for wound healing. Though the presence of cadmium and lead concentrations were small in quantity which placed the mushroom safe for consumption as supported by Stihi *et al.* (2010) in their report of norm concerning food security.*Pleurotusostreatus* fruit bodies cultivated on the various log substrates were rich in all the Vitamins studied.

Conclusion

Pleurotus ostreatus fruit bodies were successfully cuitivated on wood logs of *D. edulis*, *M. indica* and *T. africana.* The cultivated fruit bodies showed variation in yield with *M. indica* logs being the highest. Vitamins and heavy met als contents of the fruit bodies; with respect to their substrates indicated they were safe for consumption and hence, beneficial in human nutrition. Therefore cultivation of *Pleurotusostreatus* fruit bodies on *M. indica* logs should be encouraged; especially before logs are used as firewood.

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