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REVIEW ARTICLE

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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

M. C. Nwoko, *J.N. Achufusi and C. R. Ehumadu

Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State Nigeria

***Department of Biology, Nwafor-orizu College of Education, Nsugbe, Anambra State, Nigeria**

ABSTRACT

Studies were conducted to determine the yield potential, vitamins and heavy metals concentrations of Pleurotus pulmonarius (Fries) Quell. friut 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 (3.19mg/kg) Zn content; slightly higher than those of T. africana (3.14mg/kg), while fruit bodies from D. edulis gave the lowest Zn (2.96mg/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 (184.87mg/kg) Fe concentration while those of M. indica gave the lowest (182.91mg/kg). Results showed that D. edulis fruit bodies gave the highest vitamin A content (4.33mg/100g) while M. indica (4.17mg/100g) gave the lowest. However, thiamine content of the mushroom was 0.13mg/100g in D. edulis and T. africana but 0.12mg/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 fire wood.
Key words: Pleurotus pulmonarius, Yield, Heavy Metals, Vitamins and Umudike.

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 (Song, 2004).

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 (Chang and Miles, 2004)

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 (Chang, 2013).

Ecology and Substrate for Mushroom Growth

Mushrooms grow wild in the tropical and sub-tropical rainforest (Chirinang and Intarapitchet, 2009). They are capable of degrading ligning and hence are found naturally growing on different woody and non-woody agricultural residue (Stamets, 2003). Many workers have successfully carried out a number of investigations on mushroom cultivation using different lingo-cellulosic materials and reported favourable resultrs. Muller (2007) cultivated oyster mushroom on cassia substrate, Okwulehie *et al.,* (2018) cultivated *P. pulmonarius* on HCl induced-oil palm bunch, Hamlyn (1987) worked using cotton wastes while Okhuoya and Okogbo (1991) grew *Pleurotus tuber regium* on cassava peelings, corn straw, oil palm fruit fiber, rice straw, yam peelings and wild grass (*Pennisetum* sp.). In their attempt to grow shiitake on wood logs, Albert and Frank, (2001) reported that when tree log are inoculated with spawn, then allowed 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 produced using the outdoor log technique, although, controlled technique such as indoor tray-growing or artificial logs made of compressed sawdust substrate have been substituted (Davis, 2001). Hyunjong and Seung, (2004) reported that since mushrooms feed primarily on sap wood, any tree trunk selected for inoculation 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 a log with a small amount of sapwood will probably produce mushroom for fewer years than another log with a 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

MATERIALS AND METHODS

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.

SPAWN PREPARATION

Spawn of *P. ostreatus* and *P. pulmonarius* 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 15-20mins 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 30mins. After sterilization, the bottles were allowed to cool, before they were inoculated with actively growing mycelia of *P. pulmonarius* 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; Okwulehie *et al.,* 2018).

PREPARATION OF WOOD LOGS (SUBSTRATES)

Average trees size of *T. africana, M. indica* and *D. edulis* were cut down during the Hammattern (winter) season according to the recommendations by Oei (2003). Trees were cut 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 and Seung; 2004).

INOCULATION HOLES

Holes of depth 3cm by 15mm 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, according to Stamets (2013).

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

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 8-9months in alternating rains and sun to allow for decomposition. Dry weight of logs (g/kg) were determined before they were soaked in water for 24hr. Logs were pasteurized at 80^{o}c in an improvised metallic drum (IMD) for 1hr using cooking gas as a local heat source and allowed to cool overnight, as recommended by Canford, (2004). Log inoculation was done by inserting about 15g grain spawn of *P. pulmonarius* into 2/3 of the holes and subsequently sealing the logs with transparent polybags to avoid contaminants. Mycelia recovery and colonization were clearly visible after 24hrs while fully colonized polythene bags were cut open to allow for fruiting. (Hyunjong and Seung, 2004). Prior to pinhead initiation, white mycelium was 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). Mushrooms were harvested as soon as the fruit-bodies were fully matured (Okwulehie *et al.*, 2008).

YIELD AND BIOLOGICAL EFFICIENCY

Total fresh weight (g) of all the fruit bodies 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 wood log substrate on dry weight basis) was calculated following the formula recommended by Chang and Miles (2004).

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

(a)

(b)

(c)

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

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 nutritional analysis, according to the method of Victor and Olatomiwa (2013).

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.,* (2008) 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 multi-channel 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 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 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. 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.

DETERMINATION OF VITAMINS

Determination of Vitamin A (Retinol)

The vitamin A content in each sample was determined by the method of AOAC (1980), Shyam *et al.,* (2010). 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 nm and was calculated as: Mg/g = A × Vol × 104 = A × 12 cm × sample weight.

Determination of Vitamin B_1 (Thiamin)

Thiamin was determined by the method of Nwoko *et al*., (2017). 5 g of each mushroom sample was homogenized 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 30mins 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 (Okoi and Iboh, 2015).

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 35mins. 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 extract was pipetted into 50ml 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 15mins, 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.,* (1980). 5g of each sample was homogenized in a 100ml of EDTA/TCA extraction solution. The

homogenate was filtered and the filtrate was used for analysis. Each sample filtrate was passed through a packaged cottonwool containing activated charcoal, to remove colour. The volume of the filtrate was adjusted to 100ml of water by washing with more of the extracted solution. 20ml of each filtrate was measured into a conical flask. 10mls 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.,* (2010) (Shyam *et al.,* 2010; Nwoko *et al*., 2017).

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

Where: Vf = volume of filtrate analyzed
Va = volume of acid analyzed
0.88T = constant.

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.

RESULTS AND DISCUSSION

It was recorded that priordia 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.,* (2018) 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*, (2004) which reported that *P. ostreatus* cultivated on different agro-waste fruited within 27-34days layer after inoculation; Quimio, (1976) which reported that fruit bodies of *P. ostreatus* grown on various substrates emerged within 3-4 weeks after substrate inoculation. According to them, various factors such as pH, temperature, nature of substrate and method of pasteurization can determine the fruiting time of oyster mushrooms. Ahmed (1986) studied the cultivation of oyster mushroom on different lignocellulosic substrates and concluded that *P. ostreatus* took time (17-20days) to complete spawn running, while pin head formation started after 23-27days of spawning. Khan *et al*, (1981) 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 (Chang, 2013).

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

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 (%)

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 (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 and Nosike, (2015) 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 (Okwulehia and Nosike, 2015, Chang and Miles, 2004). This observation further justifies the claims by Funda (2016) which reported that substrate preparation method is one of the major factors affecting yield of oyster mushrooms.

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

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).

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 (3.19mg/kg) Zn content, though slightly higher than that of *T. africana* (3.14mg/kg), while *D. edulis* gave the lowest Zn (2.96mg/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 (184.87mg/kg) Fe concentration while that of *M. indica* gave the lowest (182.91mg/kg). Mehmet and Sevda, (2008) reported that the maximum iron level in Oyster mushroom could be as high as (838.0mgk-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.,* (1981), Rugunathan *et al.,* (1999) and Rugunathan and Swaminathan (2000). *M. indica* and *T. africana* had equal concentration of (0.09mg/kg) while *P. pulmunarius* grown on *D. edulis* gave Cd concentration of (0.08mg/kg). The quantity of Pb recorded in this investigation was below the admitted maximum level of certain contaminants in foodstuffs 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 d.w respectively; in cultivated mushrooms (Das, 2005). This suggests that that the oyster mushroom is safe for human consumption. Copper content ranged from 0.10mg/kg in *M. indica* to 0.93mg/kg in *T. africana.* Cu concentration of the mushroom grown on the various log substrates was lower than that obtained by Mehmet and Sevda (2008). Pb was present, though in minute concentration across all the log substrates. *M. indica* gave 0.0gmg/kgof Pb. *T. africana* (0.08mg/kg) while mushroom of *D. edulis* had the lowest Pb content (0.07mg/kg).

Kalac and Svoboda, (2000) reported Pb concentration values between 0.1and 40mg/kg from mushroom collected near lead smelter. This indicates that the environment where mushroom is grown influences its chemical composition. (Ita *et. al.,* 2006).

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

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).

Results showed that *D. edulis* gave the highest vitamin A content (4.33mg/100g) while *M. indica* (4.17mg/100g) gave the lowest. However, thiamine content of the mushroom was 0.13mg/100g in *D. edulis* and *T. africana* but 0.12mg/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.,* (2017) 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.,* (2007).

Thiamine (B1) is a beriberi-preventing vitamin and plays important role in energy metabolism (Demirci, 2006). Riboflavin contents were 0.85mg/100g, 0.84mg/100g and 0.83mg/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.,* (2009). Niacin content was highest (6.28mg/100g) in mushroom cultivated on *D. edulis* but lowest (6.07mg/100g) in that of *M. indica. P. pulmonarius* grown on *D. edulis* had the highest (18.16mg/100g) concentration of ascorbic acid followed by *T. africana* (17.07mg/100g) while *M. indica* gave the lowest composition of Ascorbic acid (17.17mg/100g).

CONCLUSION AND RECOMMENDATIONS

Fruit body primordia were first observed on *D. edulis* logs, after 9days 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 fire wood.

Finally, care must be taken to ensure that forest/economic trees are not over exploited for the purpose of mushroom cultivation.

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Corresponding author: Dr. M. C. Nwoko, Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State Nigeria
Email: magnuswhitemush@gmail.com