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Pleurotus pulmonarius cultivation on amended palm press fibre waste

Mojeed Olaide Liasu[1], Adeyemi Ojutalayo Adeeyo[1]*, Emmanuel Oluwafunminiyi Olaosun[1] and Rebbeca Oyedokun Oyedoyin[2]

[1]Department of Pure and Applied Biology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria.
[2]Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria.

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In the last few decades, rapid global demand for edible oils has resulted in a significant increase in the land area of oil crop cultivation. In the process of extraction of palm oil from oil palm fruit, biomass materials such as palm pressed fibre (PPF) are generated as waste products. This research was undertaken to evaluate the use of palm pressed fibres as the substrates for the cultivation of *Pleurotus pulmonarius* which currently use sawdust. Seven different substrates (A to G) were prepared from saw dust, palm press fibre (PPF), palm press fibre ash (PPFA), distilled water and Hoagland solution either alone or in combinations. These substrates were combined to investigate a probable effect of substrate combination on yield of *P. pulmonarius*. The highest yields were observed from substrates D (comprising 50% PPF, 50% PPFA and water with a mean fresh weight of 95.0 g) and substrate F (comprising of 50% PPF, 50% PPFA and Hoagland solution with a mean fresh weight of 89.20 g). The performance of substrate combination of PPF, PPFA and water however compare favourably with that of PPF, PPFA and Hoagland solution combination under all growth and yield parameters investigated. Therefore, this study has revealed that with optimum use of the biomass generated from the palm waste, prevention of environmental pollution problems and conversion of low quality waste biomasses into a valuable high protein food can be achieved.

Key words: *Pleurotus pulmonarius*, substrates, palm pressed fibre, palm press fibre ash, Hoagland solution.

INTRODUCTION

Oil palm is a fast-growing crop, which grows in the tropical lowlands where rainfall is distributed evenly. It can grow in a wide range of soil types with relatively low pH but is susceptible to high pH (Hartley, 1988). In the last few decades, rapid global demand for edible oils has resulted in a significant increase in the land area of oil

*Corresponding author. E-mail: firstrebby@gmail.com. Tel: +2348060894335.

Abbreviations: PPF, Palm pressed fibre; **PPFA,** palm press fibre ash.

crop cultivation, including oil palm (Yacob, 2008). Due to increasing demand for palm oil, enormous quantities of wastes/bye products are generated which may include palm kernel shell, palm kernel cake, decanter cake, empty fruit bunch, palm press fibre, palm oil fuel ash, palm oil mill sludge and palm oil mill effluent. Thus, a need for the sustainable management of products, which if left un-attended to, may perhaps lead to environmental problems. While research is on-going on the use of by-products as biofuels, the products are sometimes incinerated or released directly on field (Tabi et al., 2008). These practices create environmental pollution problems as incineration emits gases with particulates such as tar and soot droplets of 20 to 100 microns and a dust load of about 3000 to 4000 mg/nm (Igwe and Onyegbado, 2007). Indiscriminate dumping of empty fruit bunch also causes methane emission into the atmosphere. In recent time composting and vermicomposting have been gaining grounds as good options for the management of these wastes because they are organic in composition (Yusri et al., 1995; Thambirajah et al., 1995; Danmanhuri, 1998). To also minimize pollution effect of such waste, newer usage ought to be investigated.

Pleurotus pulmonarius commonly known as Indian Oyster, like other spp in the genus, have been reported to directly breakdown lingo-cellulosic materials (Zadrazil, 1978) which makes them economically viable in biotechnological conversion of wastes to high quality protein food (Onuoha et al., 2009). The ability to bio convert lignocelluloses materials as substrates results from the presence of lignocellulolytic or fibrolytic enzymes such as xylanases, cellulases and lacasses (Sun et al., 2004) which convert cellulose and lignin into useful carbohydrates for energy generation by the fungi (Baysal et al., 2003). Mushrooms are seasonal organisms and are always available in short supply (Onuoha et al., 2009). While, mushrooms such as *Pleurotus* spp are commercially produced and sold in markets in Asia, America and Europe, they are still being hunted for in forests and farmland for sale in Africa (Onuoha et al., 2009) hence the need for their commercial production. *P. pulmonarius* is selected for this study because it is one of the species commonly eaten in Nigeria (Zoberi, 1972).

A wide range of plant waste such as saw dust, paddy straw, bagasse, cornstalks, waste cotton, banana stalks and leaves can be used for *Pleurotus* mushroom production without a requirement for costly processing methods and enrichment materials (Chang and Miles, 2003). In Nigeria, the traditional substrate for cultivation of *P. pulmonarius* is sawdust (Onuoha et al., 2009). The low availability of sawdust coupled with the pollution effects of oil palm waste are reasons while the usability of oil palm fibre for the production of *P. pulmonarius* ought to be looked into. *Pleurotus* spp may be a good candidate in this study because; they are efficient degraders of lignocellulosic materials, easy to grow with simple technology, short life span, available and native to Nigeria (Stanley et al., 2011).

Table 1. Substrate combination for *P. pulmonarius* cultivation.

Substrate	Composition of substrate
A	100% Sawdust + water
B	100% PPF + water
C	100% PPFA + water
D	50% PPF + 50% PPFA + water
E	100% PPF + Hoagland
F	50% PPF + 50% PPFA + Hoagland
G	100% PPFA + Hoagland

PPF; Palm press fibre; PPFA; Palm press fibre ash.

MATERIALS AND METHODS

The spawn (fungal) spores of *P. pulmonarius* used for the cultivation of the fungus were collected from Federal Institute of Industrial Research Oshodi, Lagos State in Nigeria. The sawdust was obtained from Sabo market sawmill Ogbomoso, Oyo State, palm pressed fibre was obtained from a palm oil processing industry in Masifa, Ogbomoso, Oyo State in Nigeria while all salts and reagents used were of analytical grade and supplied by Sigma Aldrich through Labtrade Chemicals Limited, Nigeria. Seven different substrate combinations were prepared as shown in Table 1 with each substrate combination prepared in triplicate.

Substrate preparation

One kilogram (1000 g) of each substrate was used for the mixed substrates, they were in equal proportion of 500 g (Oei, 2005) for each component and were done using a weighing balance (APX 200, Denver Instrument, Arvada, Colorado). Dried palm pressed fibre (PPF) were chopped into smaller pieces of 2 to 4 cm while palm pressed fibre ash (PPFA) were prepared by burning PPF to ash and allowed to cool. 1000 g individual substrate or a combination of 500 g each of PPF and PPFA for mixed substrate was added to 295 ml of sterile distilled water or Sterile Hoagland solution. Substrates were mixed until all the water was absorbed and packaged in a separate polythene bags. The bags of substrates were then compressed and closed with PVC necks which were covered with cotton and wrapped with papers to prevent entry of insects. The bags were pasteurized at 100°C for 8 h to avoid microbial contamination and were allowed to cool and inoculated with about 8 g of spawn. The substrate were subsequently placed vertically in a spawn running room maintained at 25°C and watered daily to maintain a relative humidity between 70 to 80% for spawn colonization while mycelia density was measured according to the method of Kadiri (1998). After the colonization, the upper parts of the bags were opened for fructification. The method employed was as describe by Sarkar et al. (2007).

Data collection

The yield of *P. pulmonarius* on the different substrate combination was determined by recording the number, weight, diameter of pileus and size of the fruit bodies after sprouting. The measurements from the various replicates were added and their mean value

Table 2. Reagent composition of modified Hoagland solution used in experimental design.

Reagent	Amount of reagent in water (Stock) (g/L)	Amount of stock in 1 L of water (ml/L)
KNO_3	202	2.5
$Ca(N0_3)_2.4H_2O$	236	2.5
Fe-EDTA	15	1.5
$MgSO_4.7H_2O$	493	1
NH_4NO_3	80	1
H_3BO_3	2.86	1
$MnCl.4H_20$	1.81	1
$ZnSO_4.7H_2O$	0.22	1
$CuSO_4$	0.05	1
$Na_2MoO_4.2H_2O$	0.12	1
KH_2PO_4	136	0.5

Hoagland and Arnon, 1950.

calculated. Other data collected include time of mycelia growth after inoculation and mycelia growth rate after 14 days,initial day of botton formation, fruiting body formation day and average number of fruiting body.

Number and height fruit bodies

Number of fruit bodies was obtained by directly counting the number of fruit bodies on each substrate. The height was measured in centimeters using a steel ruler of dimension 50 cm by 2.5 cm (Dongguan Hust Tony Instrument Co. Ltd, Guandong, China) from the base of the stipe to the pileus.

Diameter of the pileus

This was also measured in centimeters with ruler from one edge of the pileus across the stripe to the other edge.

Fresh and dry weight of fruit bodies

This was done using an electrical weighting balance (APX 200, Denver Instrument, Arvada, Colorado).

Biological efficiency

This was calculated as:

$$\frac{\text{fresh weight of harvested mushroom}}{\text{substrate weight}} \times 100$$

Preparation of Hoagland solution

Hoagland solution was prepared as indicated in Table 2 according to the method of Hoagland and Arnon (1950) using the following salts KNO_3, $MgSO_4.7H_2O$, KH_2PO_4, Fe-EDTA, H_3BO_3, $CuSO_4$, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, $Na_2MoO_4.2H_2O$, $Ca(NO_3)_2.4H_2O$.

Location of experiment

The experimental set up was carried out at the laboratory complex of the department of Pure and Applied Biology, Ladoke Akintola University of Technology Ogbomoso Oyo State, Nigeria. The University is located at the lower limit of southern guinea savannah zone, between Latitude 8°10'N and Longitude 4°10'E.

Proximate and mineral content analyses

These were carried out at Nigeria Stored Product Research Institute (INSPRI), Ilorin, Nigeria according to standard methods.

Statistical analysis

Analysis of variance (ANOVA) was further employed to assess similarities and differences between the growth parameters.

RESULTS AND DISCUSSION

In this research *P. pulmonarius* was cultivated on seven combinations of substrates consisting of Palm press fibre (PPF), Palm press fibre ash (PPFA), sawdust, Hoagland solution and water. The effect of substrate combination on mycelia and fruit body initiation as well as botton formation and number of mushroom fruit body is presented in Table 3 while Table 4 shows the effect of various substrates combination on other growth parameters. Figure 1 shows the growth of *P. pulmonarius* on substrate A, D and F.

Mycelia initiation and growth assessment

Mycelia initiation after inoculation on various substrates vary between 48 to 72 h with substrates E, G and F having the least period of mycelia initiation of 48 h while A, B, C and D have mycelia growth initiation period of 72 h. Mycelia were abundant on substrate F, moderate on substrate A and B, and scanty on substrates C, D, E and G after 14 days.

Botton initiation and fruit body formation

Earliest botton initiation was recorded on day 25 on substrates D and F followed by Substrate C on day 26, substrate A and B on Day 27, and substrate E and G on day 28 and 29, respectively. Earliest mushroom fruiting body were observed on day 26 on substrates D and F followed by substrate C on day 27, substrate A and B on Day 28, and substrate E and G on day 30.

Number of fruit body, height of stipe and diameter of pileus

Average number of fruiting body was observed to be higher on substrate D (27) and F (31), followed by A (16) and E (18), while the least were recorded on substrate B (15), G (13) and C (12). Highest height of stipe were

Table 3. Effect of substrate combination on mycelia and fruit body initiation as well as botton formation and number of mushroom fruit body.

Substrate	Mycelia initiation (days)	Mycelia growth after 14 days	Botton initiation (day)	Fruit body formation (day)	Average no of fruit body formed
A	3	++	27	28	16
B	3	++	27	28	15
C	3	+	26	27	12
D	3	+	25	26	27
E	2	+	28	30	18
F	2	+++	25	26	31
G	2	+	29	30	13

+, Scanty growth of mycelia; ++, moderate growth of mycelia; +++, abundant growth of mycelia.

Table 4. Effect of various substrate combinations on growth parameters measured.

Substrate	Average height of stipe (cm)	Average diameter of pileus (cm)	Average fresh weight of fruit bodies (g)	Average dry weight of fruit bodies (g)	Biological efficiency (%)
A	1.40±0.23^{a}	2.80±0.21^{a}	13.60±3.91^{a}	5.47±3.91^{a}	1.37±0.39^{a}
B	4.70±0.81^{bc}	5.37±0.93^{a}	14.13±1.02^{a}	5.47±1.02^{a}	1.43±0.09^{a}
C	1.60±0.10^{a}	1.60±0.20^{a}	4.30±1.80^{a}	1.50±1.80^{a}	0.45±0.15^{a}
D	8.00±0.24^{c}	13.47±2.98^{b}	95.37±8.69^{b}	24.20±8.69^{b}	9.53±0.87^{b}
E	1.30±0.11^{a}	2.80±0.21^{a}	13.60±3.91^{a}	5.87±3.91^{a}	1.37±0.39^{a}
F	7.00±1.48^{c}	12.50±2.95^{b}	89.20±8.63^{b}	21.87±8.63^{b}	8.93±0.84^{b}
G	1.80±0.40^{a}	2.90±0.40^{a}	4.90±1.80^{a}	1.60±1.80^{a}	0.55±0.15^{a}

Mean values followed by the same alphabets in the column are not significantly different by Duncan's Multiple range Test (DMRT) ($P \leq 0.05$).

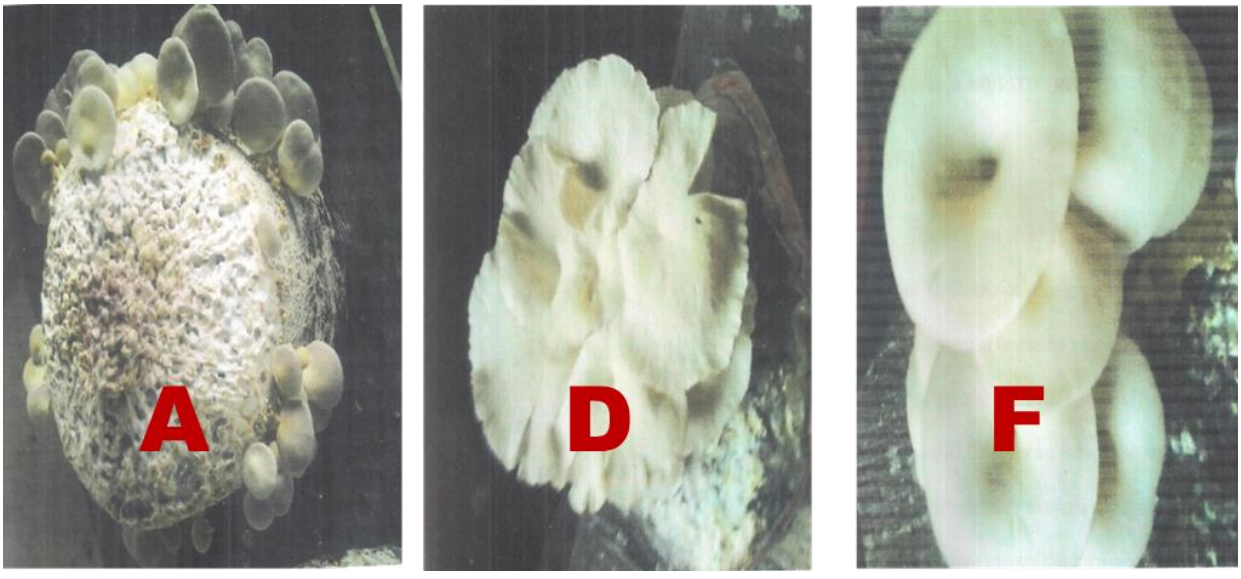

Figure 1. *P. pulmonarius* growing on substrate A (100% Sawdust + water), D (50% PPF + 50% PPFA + water) and F (50% PPF + 50% PPFA + Hoagland).

recorded on substrate D and F as 8 and 7 cm, respectively, while the least height were recorded on substrate A and E as 1.4 and 1.3 cm. Substrate B, C and G have heights of 4.7, 1.6 and 1.8 cm, respectively. Best

fruit bodies of average diameter 13.5 and 12.5 cm were equally recorded on D and F while the least were recorded on A and E as 1.2 and 2.8 cm. Substrate B, C and G have average diameter of pileus recorded as 5.3, 3.2 and 2.9 cm, respectively.

Fresh and dry weight of fruit bodies

The mean fresh weight recorded on substrate D and F were 95.0 and 89.2 g followed by that of substrates B (14.1 g), A (13.6 g) and E (13.6 g). The least amount was recorded on substrates C (4.3 g) and G (4.9 g). The mean dry weight recorded on substrate D and F were 24.6 and 21.9 g followed by that of substrates B (5.4 g), A (5.4 g) and E (5.9 g). The least amount was recorded on substrates C (1.5 g) and G (1.6 g). The highest yield of *P. pulmonarius* was recorded on substrates D and F with average fresh weight of fruit bodies recorded as 95.37 and 89.20 g, respectively. Substrates D and F also recorded the highest average number of fruit body, height of stipe and diameter of pileus (27, 8 and 13.5 cm for D) and (31, 7 and 12.5 cm for F). The yields of *P. pulmonarius* recorded for D and F in this work is greater than the highest recorded for the growth of *P. pulmonarius* cultivated on sawdust, cassava peels and oil palm fibre (15 g) in the work of Onuoha et al. (2009), that of *P. pulmonarius* cultivated on corncorb and rice bran substrates (53.2 g) in the work of Stanley et al. (2011) and that of *Pleurotus ostreatus* cultivated on cotton waste, rice straw and sawdust (4.3 g) substrates in the work of Jonathan et al. (2012). Onuoha et al. (2009) recorded highest number of fruit body, height of stipe and diameter of pileus as 6, 2.6 and 5 cm on sawdust while Stanley et al. (2011) recorded highest number of fruit body, height of stipe and diameter of pileus as 12, 3.6 and 5.5 cm and no growth was recorded on palm press fibre substrate only. This result therefore compare favourably with such previously published work. Oil palm fibre have been reported to improve the mineral content of soil such as N, K, and organic C, and to improve C:N ratio (Akinyele et al., 2013) which have been reported to favour the growth of *P. pulmonarius*. Oil palm fibre has also competed favourably in enhancement of mineral content of substrates than sewage and animal dung (Mbah and Mbagwu, 2006). Fibre has lesser phosphorus content which inhibit the uptake of micronutrients and contain a high C:N ratio which favours the growth of mushroom (Zadrazil, 1980; Onuoha et al., 2009).

The outstanding performance of *P. pulmonarius* on substrate D and F amended with oil palm ash may be due to the potential of ash to influence substrate pH and enhance nutrient bioavailability as yield and nutrient availability have been associated with pH (Altomare et al., 1999). Wood ash has previously been used to amend soil pH (Lerner and Utzinger, 1986; Naylor and Schmidt, 1986; Hakkila, 1989) and supply plant nutrient (Ohno and Erich, 1990; Mbah and Nkpaji, 2010). Ashing has also been reported to release most nutrient otherwise locked up within the body of substrate (Mehdi et al., 2013). Ash has been reported for potential use in organic agriculture and as good source of K, P, Mg, Ca and micronutrients (Kakier and Summer, 1996; Demeyer et al., 2001). Wood ash has shown significant impact on growth and yield of maize in the absence of synthetic fertilizers (Nottidge et al., 2005; Mbah and Nkpaji, 2010). Patterson (2001) reported that ash application up to 25 $Mgha^{-1}$ significantly increased barley and canola yield, and canola oilseed yield. Effective and timely utilization of ash for maintaining soil quality and reducing the harmful effects of acidify-cation of surface waters have also been reported (Fransman and Nihlgard, 1995; LeBlanc et al., 2006). The ability of ash to increase bioavailability of N, P, K^+, Ca^{2+} and Mg^{2+} have been documented (Mehdi et al., 2013).

From previously reported works, various agricultural wastes reported for cultivation of mushrooms have been documented to include; cotton waste, sugar cane bagasse, wheat straw, rice straw, paper waste, saw dust and cassava peels (Onuoha et al., 2009; Jonathan et al., 2012). Previous attempts in cultivation of *P. pulmonarius* on oil palm fibre have met various degrees of difficulties. This report therefore adds to the history of successful cultivation of *P. pulmonarius* on rarely utilized substrate such as oil palm fibre. The work of Onuoha et al. (2009) which reported the previous trial of cultivation of *P. pulmonarius* on oil palm fibre recorded no growth while the limited growth recorded on the mixture of oil palm fibre, cassava peel and sawdust substrate was attributed to the presence of cassava peel and sawdust component of the substrate. This result negates the observation of Okwujiako (1992) and Onuoha et al. (2009) that asserts that sawdust is the best artificial substrate for the growth of *P. pulmonarius* and which remains the current practice in Nigeria. The addition of fibres in substrates used for agricultural cultivation has been reported to increase the mineral contents of such substrates such as C, N, P and K. The comparative mineral contribution of fibres to substrate correlates with the mineral composition of the oil palm fibre applied to such substrates (Akinyele et al., 2013).

Analysis of variance (ANOVA), proximate, mineral content analysis and biological efficiency

The analysis of variance (ANOVA) of the growth parameters measured and biological efficiency obtained by separating and subjecting the mean results to Duncan Multiple Range Test (DMRT) indicates that substrate combination in treatment D and F resulted in optimal yield of *P. pulmonarius.* The mean of D and F were signifi-cantly different from those recorded on all other substrates combination. The mineral content and proximate analysis of *P. pulmonarius* cultivated on substrates D and F were then investigated for further comparison. The percentage (%) proximate analysis of *P. pulmonarius*

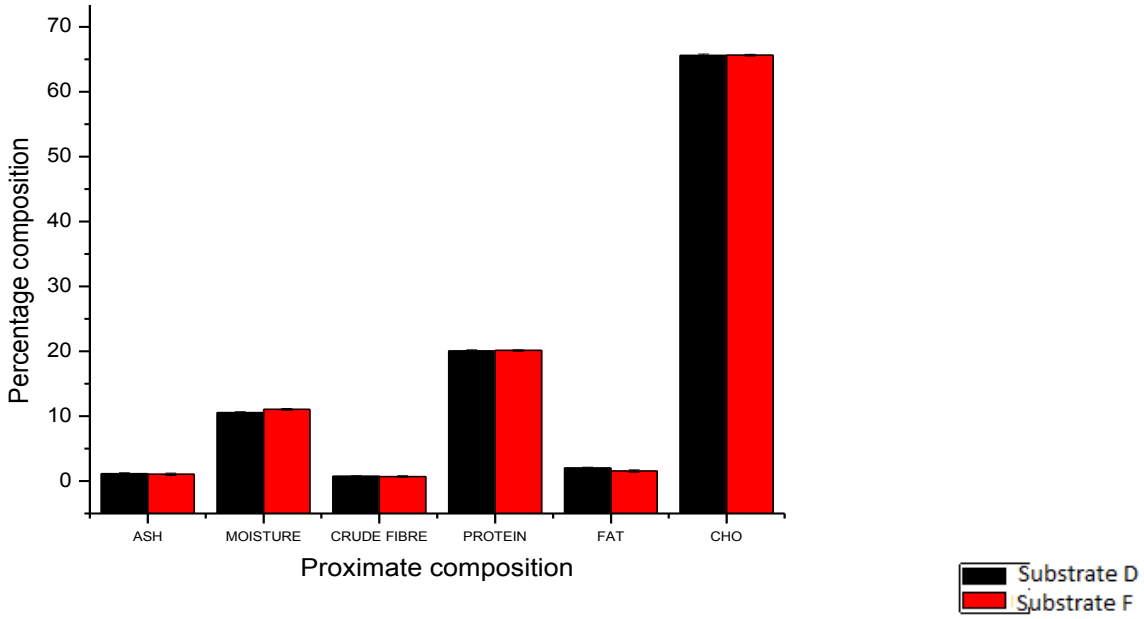

Figure 2. Proximate composition of mushroom obtained from substrate D (50% PPF + 50% PPFA + water) and F (50% PPF + 50% PPFA + Hoagland). Values are the means ± standard deviation of three replicate determinations.

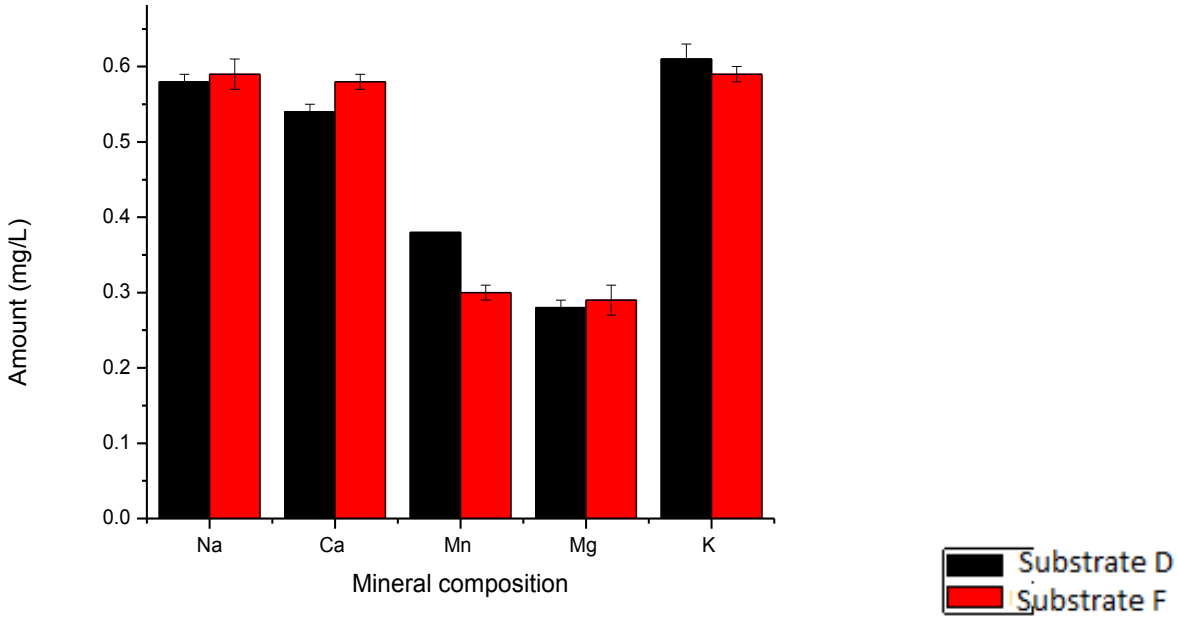

Figure 3. Mineral composition of mushroom obtained from substrate D (50% PPF + 50% PPFA + water) and F (50% PPF + 50% PPFA + Hoagland). Values are the means ± standard deviation of three replicate determinations.

cultivated on substrate D and F shows similar results (Figure 2) with mushroom on substrate D containing Ash (1.14), moisture (10.54), crude fibre (0.71), protein (20.03), fat (2.00) and carbohydrate (55.59), while that cultivated on substrate F contains Ash (1.03), moisture (11.03), crude fibre (0.70), protein (20.13), fat (1.53) and carbohydrate (65.59). The mineral content analysis of *P. pulmonarius* cultivated on substrates D and F also shows similar result (Figure 3) with D containing in mg/L of Na (0.58), Ca (0.54), Mn (0.38), Mg (0.28), and K (0.61) and F containing in mg/L of Na (0.59), Ca (0.58), Mn (0.3), Mg (0.29) and K (0.59). Results indicate a similar proximate

and mineral composition for mushroom cultivated on substrate D and F.

The similarity in the proximate and metal mineral contents of *P. pulmonarius* despite its cultivation on different substrate and Hoagland solution may be attributed to the ability of the mushroom to extract sufficient amount of nutrient from those locked within the organically rich lignocellulolytic fibre substrate for its yield and quality. Ability of various fungi to extract mineral nutrients from their solid phase compounds to satisfy nutritional requirements have been reported (Glowa et al., 2003). Fungi have been reported to degrade and solubilize minerals from rocks, metallic zinc, basalt, alumno-silicates, biotite, microcline and chlorite (Sterflinger, 2000; Glowa et al., 2003) by the production of organic and inorganic acids, alkalis, CO_2 and other complexing agents (Sterflinger, 2000; Cunningham and Kuiack, 1992; Goldstein, 1995). Organic acids, peptides, proteins, phenolics, lignilolytic enzymes, chiting (Altomare et al., 1999) and other complexing agents provides both sources of solubilization and metal chelating anion to complex the metal cation (Devevre et al., 1996). The solubilization, extraction and absorption by fungi have been explain to include biomechanical weakening and biochemical solubilization (Kumar and Kumar, 1999; Sterflinge, 2000; Hoffland et al., 2004; Gadd, 2010) while other mechanisms involved include acidolysis, complex-lysis, redoxolysis and mycelia metal accumulation.

Highest biological efficiency was recorded for substrates D (9.5%) and F (8.9%) followed by A (1.4%), B (1.4%) and E (1.4%) while the least biological efficiency was recorded against substrates C (0.4%) and G (0.5%). The result shows that substrate D is better converted and supports a higher yield of mushroom that is significantly similar to that of substrate F but differs from that of all other substrate investigated and reveals the suitability of the combination of ash, water and palm press fibre (Substrate D) over Ash, Hoagland solution and palm press fibre (Substrate F), and other substrate combination investigated. The above result suggests that substrate D may contain higher nutrient suitable for *Pleurotus* cultivation than substrate F and other substrates being investigated. Previous report have shown that improved growth is directly associated with amount of nutrient concentration until a threshold after which the effect of such addition may not be further noticed (Kang and Iersel, 2004). Kang and Iersel (2004) reported increase in shoot and total dry weight with increasing nutrient solution concentrations until a threshold after which little or no additional increase in dry weight was observed. The improved yield observed in substrate D and F therefore suggests that the two substrates provided sufficient amount of nutrient needed for improved yield of *Pleurotus* mushroom. This result may explain why mushrooms grow naturally and are picked on fields by locals on burnt palm trees in Nigeria. The performance of substrate combination of palm press fibre ash, water and palm press fibre however compare favourably with that of palm press fibre ash, Hoagland and palm press fibre combination under all growth and yield parameters investigated.

This result corroborates the findings of Ajibade et al. (2013) which noted that addition of chemical fertilizers during cultivation may not necessarily give corresponding high yield. This result also confirms the usability of palm waste in the improvement of soil fertility and cultivation of mushroom as reported by Tabi et al. (2008); Sudirman et al. (2011) and Ajibade et al. (2013). The differences in the nutritional composition of substrate used have been suggested for the variation in the results obtained as reported by Tabi et al. (2008). Factor such as amount of nitrogen, carbon content and substrate particle size have also been suggested to contribute to yield of cultivated mushrooms. From this research, all substrates applied in this study show potentials for *Pleurotus* mushroom cultivation either alone or in combination as all substrates produce *Pleurotus* fruit bodies. However, Substrate D with the composition of 50% of PPF and 50% of PPFA and water yielded the highest amount (fresh/dry weight, average diameter of pileus and height of stipe) of *Pleurotus* fruit bodies as well as improved mycelia, botton initiation, and fruit body formation day per replicate compared to the other substrates. With optimum use of the biomass generated from the palm waste, it will not only solve the environmental pollution problem but it can also offer a promising way to convert low quality bio-masses into a valuable high protein food.

Conflict of interests

The authors did not declare any conflict of interest.

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