

Full Length Research Paper

Effect of substrate on the growth, nutritional and bioactive components of *Pleurotus ostreatus* and *Pleurotus florida*

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Received 26 November, 2015; Accepted 16 June, 2016

Mushrooms are increasingly being recognized as important food products for their significant role in human health, nutrition and disease. This study was carried out with the aim of comparing the effect of substrate on growth, determining nutritional and bioactive components of two oyster mushroom, *Pleurotus ostreatus* and *Pleurotus florida*. A completely randomized block design with two treatments replicated three times was done and a laboratory analysis was carried out on the nutritional and bioactive components. The results obtained indicated that the growth and yield of *P. ostreatus* and *P. florida* varied widely depending on the kind of substrate used. It was observed that sawdust had the greatest influence on both growth and total yield because it had mean height, weight and pileus length significantly higher than those cultivated on corn cobs. It took least days for pin head formation and maturity period but had the highest number of fruiting bodies produced. *P. ostreatus* had the highest weight, height and biological yield while *P. florida* had the least pin head formation, maturity period and number of fruiting bodies. The biological efficiency and moisture content was the highest for sawdust substrate as a whole. The substrate had an effect on the nutritional value as maximum protein (29.45%) was observed on sawdust while minimum (25.12%) on corn cobs. Maximum lipid (4.62%) was observed on corn cobs while minimum (1.97%) was still on corn cobs. Regarding substrate, maximum ash (8.67%) was observed on sawdust while minimum (8.215%) on corn cobs; also maximum crude fibre (16.69%) was observed on corn cobs while minimum (5.08%) was on sawdust. The bioactive components analysis revealed the presence of major bioactive compounds such as flavonoids, polyphenols, saponins, triterpenoids and steroids. Oyster mushroom cultivated on sawdust possesses better growth and nutritional properties than those cultivated on corn cobs.

Key words: Bioactive components, cultivation, nutritional analysis, oyster mushroom.

INTRODUCTION

Mushroom has been defined as a macro-fungus with a distinctive fruiting body, which can be hypogenous or

epigeous, large enough to be seen with the naked eye and to be picked by hand (Chang and Miles, 1989).

Oyster mushroom (*Pleurotus* species) belongs to the family of Tricholomataceae and is usually found clustering naturally on dead trees at spring season (Lee, 1993). Among all species of mushroom, the oyster mushroom is the second widely cultivated mushroom worldwide following the *Agaricus bisporus* (Kües and Liu, 2000). *Pleurotus* spp. are popular and widely cultivated throughout the world mostly in Asia, America and Europe because of their simple, low cost production technology and high biological efficiency (Mane et al., 2007).

Mushrooms are increasingly being recognized as important food products for their significant role in human health, nutrition and disease. Several species of mushrooms are of great importance because of their medicinal properties, for example, they are active against hypercholesterolemic conditions, hypertension, diabetes, cancer and other infections (Alam et al., 2007). The nutritional and chemical compositions of mushroom are responsible for their medicinal values. However, nutritional composition of mushroom is affected by many factors among which the composition of growth substrate and the method of cultivation are of major importance (Benjamin, 1995). During an investigation of the cultivation of mushroom on agricultural residues, it was found that rice husk, sorghum stover, saw dust, cotton waste, cocoa bean shell, and saw dust such as *Gliricidia* mixture were suitable substrates for the cultivation of edible mushroom (Belewu, 2003). Various substrates have different effects on the growth, yield and quality of mushrooms (Ponmurugan et al., 2007).

The genus *Pleurotus* is a heterogeneous group of economic importance. Several species are of nutritional and/or medicinal importance (Cohen et al., 2002). *Pleurotus* spp. have the ability to absorb microelements from different cultivation media and thus they may present an excellent dietary source (Stajic et al., 2002). Fungi of the *Pleurotus* genus have an important place among the commercially cultivated *Basidiomycetes*, because they have gastronomic, nutritional and medicinal properties and can be easily cultivated on a large range of substrates (Kumari and Achal, 2008).

Mushrooming in Cameroon rainforest zones is often possible only during the rainy season and is usually inefficient in terms of time spent to collect sufficient mushroom. Most edible species rot quickly and collector must be at the right time at the right place. Hence, there is a need for a cultivation of mushroom for lasting availability all year round. Also, there is an inadequate food supply in most rural areas, diminishing quality of health and increasing environmental deterioration. Many children and people in Cameroon are malnourished and most families cannot afford meat in their daily meal, there is also high prevalence rate of HIV/AIDS, malaria, and

tuberculosis amongst others. Hence, mushrooms can help to improve health and nutrition. When used as food, mushrooms promote good human health, being rich sources of protein and vitamins (Kinge et al., 2014).

Also, there is a very high incidence of malnutrition, especially of protein deficiency in most developing countries. This study would help to provide the community with an additional vegetable of high quality and enrich the diet with high quality proteins, minerals and vitamins which can be of direct benefit to the human health and fitness. The extractable bioactive compounds from medicinal mushrooms would enhance human's immune systems and improve quality of life. This study will serve as means of generating employment, particularly for rural women and youths in order to raise their social status. The harvested fruiting bodies can be sold in local markets for additional family income or exported for an important source of foreign exchange that will definitely improve the economic standards of the people in and around the study area. Hence, this study is very important because it takes little time, less energy and the use of wastes to provide a good and nutritious mushroom that is also medicinal. The aim of this research was to investigate the effect of two substrates on the growth, nutritional and bioactive components of two oyster mushroom species.

MATERIALS AND METHODS

Study area

This work was carried out in the Mbeng Adio Mushroom Farm, Banjah village; in Bamenda III sub division of the Mezam division in the North West Region of Cameroon. It is located between latitude 5° 57'23.72 and longitude 10° 13'06.86. The climatic variations of this area fit into two seasons (the dry and rainy season), and experience the tropical rain forest climate in general. Thus this area has normal rainfall like all other villages in Bamenda III sub division as a whole which the rains begin in February or March ending up to October and the dry season which runs from October to February or March ending. Although places are normally wet during this period, the temperatures are relatively warmer (28 to 30°C) favouring the cultivation of mushroom. People in this area carry out agricultural activities in which most of them carry subsistence agriculture, cultivating crops like cocoyam, Irish potatoes, corn, beans, huckleberry and some engaged in the mushroom cultivation with little or no knowledge on the agro waste that would give the best quality and productivity. Since most of the people in this area are farmers, mushroom should be encouraged, since the main substrates needed in the cultivation of mushroom is agriculture wastes. The map of the study area is as shown in Figure 1.

Collection and preparation of samples

The spawn was bought from the Mbeng Adio Mushroom farms, Banjah village, Bamenda of the North West Region of Cameroon.

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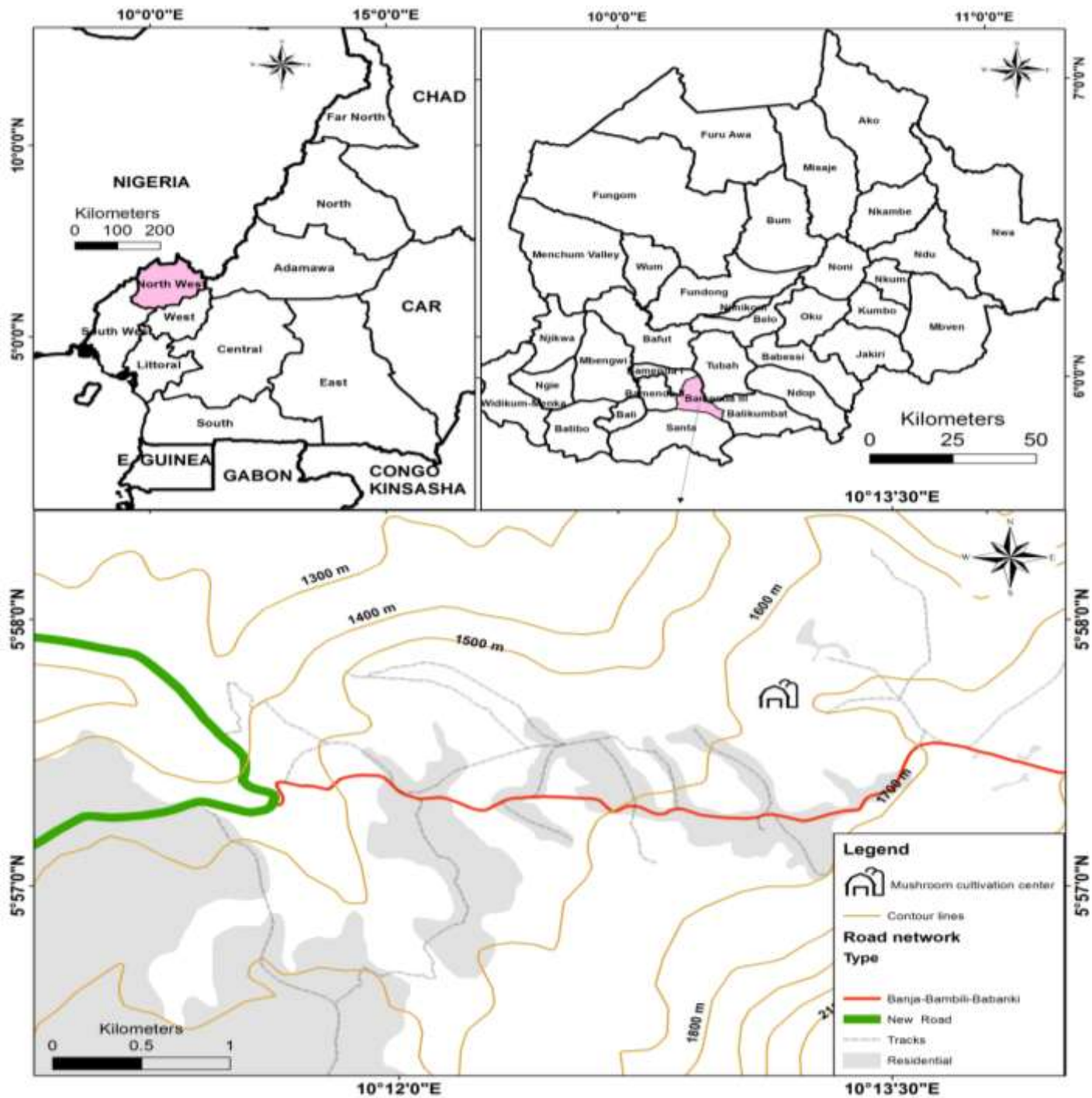


Figure 1. Locational map of Mushroom Research Center in Banjah, Cameroon.

The sawdust was collected from a saw mill in Nkwen, Bamenda and was identified to be from Eucalyptus. Corn cobs were collected from household in Banjah village. The rice husk was collected from church centre rice mill while the corn flour was collected from a milling machine in Bamenda town. Cultivation bags, slake lime were bought from a provision store in Bamenda. The sawdust and corncobs were well selected to remove physical contaminants such as sticks, stones, plastic paper and rotten corn cobs. The corn cobs

were further crushed into smaller pieces using a crusher. The materials were further weigh and taken to the mixing room.

Spawn preparation

Two buckets of sawdust, dried corn flour, rice husk and slake lime were mixed using a cleaned spade. Water was added to 65%

Table 1. Treatments, substrates and their composition used in the cultivation of *P. ostreatus* and *P. florida*.

S/N	Treatment	Substrates and composition
1	Treatment-1	Corn cob+corn flour = 3:1
2	Treatment-2	Sawdust + rice husk + corn flour = 6:3:1

moisture content. The mixture was put in bottles, wiped, covered and sterilized for 2 h in an oil drum. The bottles were removed from the drums after 6 h and allowed to cool. The bottles were then transferred to the inoculation room where they were sent to the inoculation box and they were inoculated with the mother culture bought from Mbeng Adio Mushroom farms Bamenda. The inoculated bottles were then transferred to a room for maturity. It took the spawn 25 days to mature. The spawn were then used in the inoculation of the substrates.

Mushroom cultivation

Cultivation of the two species of oyster mushrooms *Pleurotus ostreatus* and *Pleurotus florida* on two different substrates was done following the procedure of Anagho (2008). The cultivation experiment was laid out in a completely randomized design with two treatments replicated three times. The composition of the different treatments is shown in Table 1.

Shavings and pieces of wood that were too big or too sharp were removed by hand from the saw dust because these pieces absorb water poorly and easily pierce plastic bags during handling. Rice husk and corn flour wastes were supplemented as organic nitrogen sources. Slake lime was diluted with water, and then showered onto the sawdust to adjust pH. The following are the cultivation of the different treatments.

Treatment 1

In treatment 1, three 15 L buckets of corn cob was measured using a scale. The weight of each bucket of crushed corn cobs was 3 kg (total of 9 kg). One bucket of corn flour was measured using 15 L bucket and weight as 8 kg. 30 g of powdered calcium carbonate was measured and added to the mixture. The three ingredients were then mixed dry using a spade until a homogenous mixture was obtained to about 65% moisture content. The moisture content was tested by squeezing the mixture in the palms of the hands. After testing the moisture content, calcium carbonate was then applied on the hand to avoid microbial contamination and substrates was then filled in black polythene bags (25 × 18 cm) using the hand. The substrate was pressed in the bag while filling. The bag was filled such that each bag weight as 2 kg. The bag was tight with a knob so that it could be easily untied later. These bags were then transferred to the sterilization unit.

Treatment 2

In treatment 2, six buckets of volume 15 L sawdust of fine type was measured using a measuring scale balance; the weight of each of the bucket of saw dust was 4 kg (total 24 kg). Three buckets of rice husk was measured in 15 L buckets, each weigh 1.8 kg (total of 5.4 kg); one bucket of the corn flour was also measure using 15 L bucket and weight of bucket was 8 kg. The measured corn flour and rice husk was added to the measured sawdust. 30 g of calcium carbonate was measured and added to the mixture of sawdust, rice husk and corn flour. The four components were homogeneously mixed using a spade. The mixture was done on a cemented floor

that has been well cleaned. Water was then added to the homogenous mixture to about 65% moisture content. The moisture content was tested by squeezing the mixture in the palms of the hands. After testing the moisture content, calcium carbonate was then applied on the hand to avoid microbial contamination and substrates was then filled in black polythene bags (25 × 18 cm) using the hand. The substrate was pressed in the bag while filling. The bag was filled such that each bag weight as 2 kg. The bag was tight with a knob so that it could be easily untied later. These bags were then transferred to the sterilization unit.

Sterilization of substrates

A tripod was placed in the 1000 L oil drum such that it occupied 1/5 of the height of the drum. This was done to prevent direct contact of substrates and water. Water (15 L) was then poured into the drum and jute bags were placed on top of the tripod and around the walls of the drum. The polythene bags containing the substrates were then paced in the drum. Twelve substrates were placed round the drum and three each in the middle up till the drum got full. Each drum carried a total of 60 polythene bag substrates for sterilization. The drum was then covered on the top with jute bags and plastic papers to prevent heat from escaping. Wood was used to set up heavy flames on which the substrate was sterilized (at about 100 °C) for 4 h. Sterilization of substrate bags was done under high temperature using an oil drum with fuel from wood. The substrate had enough heat and started boiling after 30 min. From the boiling point, the substrate was sterilized (at about 100°C) for 4 h in order that all other microorganisms were destroyed and the substrate well cooked. The sterilization took place in the evening and bags were allowed to cool overnight and removed the next day ready for spawning.

Spawning or inoculation

The bags were then removed from the oil drum and transferred to the spawning room. The first step of spawning was washing of hands and knives to be used. The bags were then untied and allowed to cool. The spawns were introduced using a knife to avoid contamination. 20 bottles of spawns weighing 750 g each were used to plant 60 substrates. The substrates were planted at a ratio of 1 bottle to 3 substrates of 2 kg (1:3). The spawn was introduced deep into the substrates and was well mixed with the help of the knife such that the spawn covered almost all the whole substrates. The bags were then tied and transferred to a room with shelves.

The mushroom substrates were stored in a dark room for colonization to take place. The substrates were placed on shelves that had been painted with calcium carbonate and the window was covered with black polythene bag to reduce the light in the room. The substrates were left in this dark room for 21 days at 25°C. Spawn run for mushroom differ from species to species, the size of the polythene bag, the colour of the bag and the nature and compactness of substrates. After the incubation period of 21 days, the windows were open for proper ventilation. At the beginning of fruiting, the substrates were transferred to a fruiting room. The mushroom basidiocarps were seen to shoot in different direction from each of the substrates. The number of days taken for the initiating of primordial and harvesting was noted for the different substrate mixture and species. To maintain proper temperature, moisture and humidity, the room was watered daily and by watering the bags one after each harvesting by pouring water on the bags.

Harvesting

Harvesting was carried out when the fruiting bodies were matured.

Harvesting was made three times for each bag of substrate. The process of harvesting involves the removal with the hand of the matured fruiting bodies from their substrate without any destruction on the substrate bag. The mature mushroom was held on their stipe below the pileus and close to the substrate level and was gradually pulled out. All fruiting bodies of a particular substrate bag were harvested at the same time since each bag had to be watered after harvest. Watering was done by immersing the bags in a bowl of water for 5 s. This is to enable the substrate to have moisture that enables fruiting to occur again for harvest.

Morphological data collection

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

The following parameters of growth and yield were measured. From three bags of each treatment and replicates of the two species were sampled by measuring the growth and yield parameters thus.

Biological yield

The biological yield was obtained by taking the total fresh weight of the fruiting bodies per bag. The average for the three bags per treatment and per replicate were calculated and recorded.

Biological efficiency

It was calculated as the weight of fresh mushroom as a percentage of the dry weight of the substrate.

Time required for primordial initiation

Three bags for a particular treatment were sampled at random after the incubation process completion. These three bags were observed on daily basis to note the number of days it took after incubation to the formation of the first primordial.

Time required for harvest

Time taken for harvest was done for the three sample bags from initiation stage to the time of maturation of fruiting body. The average of the time taken for primordial initiation and the time taken for harvesting were calculated and recorded.

Number of total primordial

After the primordial formation, the number of primordial was counted for each of the three sample bags and the average for the three bags were calculated and recorded.

Number of total effective fruiting body

The number of effective mature fruiting body was counted just before harvesting was done for the three sample bags. The average number of fruiting bodies for the three bags were calculated and recorded.

Weight of individual fruiting body

The weight of the individual fruiting bodies per bag for the three

sample bags were measured using a scale balance. The average of the three bags were calculated and recorded. The weight taken was the fresh weight.

Height of fruit bodies

This entails measuring the distance from the substrate where the stalk starts growing. The height was measured in centimeters using transparent ruler from the base of the stipe to the pileus. The height of the stalk was carried out for at least three fruiting bodies for each of the three bags of the particular treatment. The average height was recorded and calculated. Morphological data were subjected to student t test to see if there is any significant difference in the growth substrates used or the species used.

Nutritional analysis

A laboratory analysis (AOAC) was done in the Nutritional Laboratory in the University of Dschang, Cameroon to compare composition of nutrient in *P. ostreatus* and *P. florida* cultivated on the two substrates. The data was recorded on moisture, crude protein, crude fiber, crude fat, ash, organic matter and dry matter according to Raghuramulu et al. (2003).

Moisture analysis

Twenty gram of fresh mushroom was weighed into a weighed moisture and dried in an oven at 100 to 105°C and cooled in a dessicator. The process of heating and cooling was repeated till a constant weight was achieved. The moisture content of mushroom was also expressed in percent and calculated by the formula;

Moisture content (%) = $\frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$

Determination of total protein

Five grams of ground mushroom was taken with 50 ml of 0.1 N NaOH and boiled for 30 min. The solution was cooled at room temperature and centrifuged at 1000 × g by a DSC-200T tabletop centrifuge. The supernatant was collected and total protein content was measured according to the method of Lowry et al. (1951).

Determination of total lipid

Total lipid was determined by slightly modifying the method of Folch et al. (1957). Five grams of ground mushroom was suspended in 50 ml of chloroform: methanol (2: 1 v/v) mixture then mixed thoroughly and let stand for 3 days. The solution was filtrated and centrifuged at 1000 g by a table centrifuge machine. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid.

Determination of crude fiber

Ten grams of moisture and fat-free sample were taken in a beaker and 200 ml of boiling 0.255 N H₂SO₄ was added. The mixture was boiled for 30 min keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker, and 200 ml of boiling 0.313 N NaOH was added. After boiling for 30 min

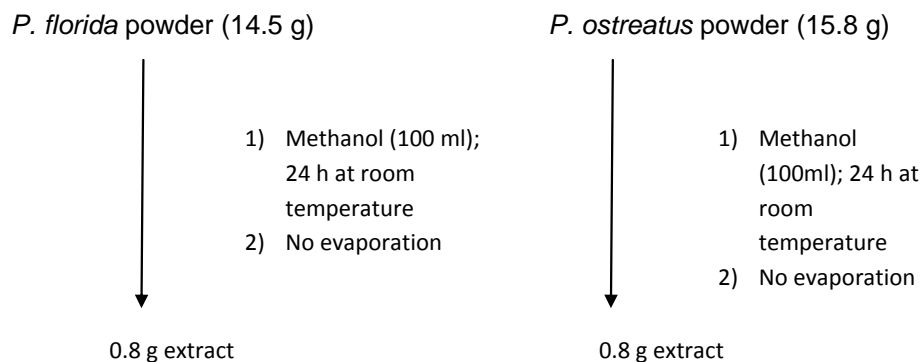


Figure 2. The extraction method.

(keeping the volume constant as before), the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80 to 100°C and weighed (W_e) in an electric balance (Key: JY-2003; China). The crucible was heated in a muffle furnace (Nabertherm: Mod-L9/11/c6; Germany) at 600°C for 5 to 6 h, cooled and weighed again (W_a). The difference in the weights ($W_e - W_a$) represents the weight of crude fiber. Crude fiber (g/100 g sample) = $[100 - (\text{moisture} + \text{fat})] \times (W_e - W_a) / W_t$ of sample (Raghuramulu et al., 2003).

Determination of total ash

One gram of the sample was weighed accurately into a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5 to 6 h at 600°C. It was then cooled in a desiccator and weighed. To ensure completion of ashing, the crucible was then heated in the muffle furnace for 1 h, cooled and weighed. This was repeated till two consecutive weights were the same and the ash was almost white or grayish white in color. Then total ash was calculated as: Ash content (g/100 g sample) = $\text{Weight of ash} \times 100 / \text{Weight of sample taken}$ (Raghuramulu et al., 2003).

Total carbohydrate estimation

The content of the available carbohydrate was determined by the following equation:

Carbohydrate (g/100 g sample) = $100 - [(\text{moisture} + \text{fat} + \text{protein} + \text{ash} + \text{crude fiber}) \text{ g/100 g}]$ (Raghuramulu et al., 2003).

Detection of bioactive components

The bioactive component analysis was done using standard procedures (AOAC, 1984).

Preparation of extracts

After collection, the mushrooms samples were wrapped in newspaper and stored in moisture free open places. The removal of all foreign matters was done. Thereafter they were cut in small pieces of around 2 to 3 cm across using a knife. They were

incubated for 2 days at a temperature of 50°C. Then, they were ground using metal mortar and pestle. The powder was collected and ground again at the end. The extraction was done as shown in Figure 2. The bioactive components of oyster mushroom were determined using standard procedures (Sofowora, 1982; Trease and Evans, 1983; Sofowora, 1993).

Test for saponin

Sample (0.5 g) was weighed in a beaker; 5 ml of distilled water was added and heated to boil. Persisted foaming on warming was taken as an evidence for the presence of saponin.

Test for alkaloid

Sample (0.5 g) was weighed in a beaker and it was extracted with 10 ml of 2% hydrochloric acid (HCl) by heating gently for about 5 min. The HCl extract was filtered with Whatman No.1 filter paper to have a clear solution and prevent false result; 2.5 ml of the filtrate was treated with few drops of Dragendoff's reagent. Appearance of precipitate indicated the presence of alkaloid in the extract.

Test for flavonoids

This was done using the Shinoda test (Magnesium Hydrochloride reduction test). To the test solution, few fragments of magnesium ribbon were added and concentrated HCl was added drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes indicated the presence of flavonoids.

Test for triterpenoids and steroids

The Libermann Burchard's test was used. The extract was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added from the sides of the test tube, showing a brown ring at the junction of two layers and the upper layer turning green shows the presence of steroids and formation of deep red colour indicated the presence of triterpenoids.

Test for phenolic compound and tannins

Ferric chloride test

To 5 ml of the extract, few drops of neutral 5% ferric chloride

Table 2. Biological yield determinants on three flushes of *Pleurotus ostreatus* and *Pleurotus florida*.

Growth parameters and substrate	Flush 1 on P. os	Flush 1 on P.fl	Flush 2 on P.os	Flush 2 on P.fl	Flush 3 on P.os	Flush 3 on P.fl	Mean on P.os	Mean on P.fl	t value and significance
Mean height on sawdust	39.1	22.5	38.2	22.0	31.3	19.7	36.3	21.3	9.226*
Mean height on corn cobs	25.2	19.8	18.0	19.2	15.3	16.0	19.5	18.3	0.5499 ^{ns}
t value and significance	-	-	-	-	-	-	9.017*	9.6442*	-
Mean weight on sawdust	410.0	238.4	316.7	211.6	176.6	200.0	301.1	216.7	1.4752 ^{ns}
Mean weight on corn cobs	106.6	133.3	133.3	106.6	76.7	76.4	98.9	98.8	0.00642 ^{ns}
t value and significance	-	-	-	-	-	-	3.3113 ^{ns}	17.989**	-
Mean pileus on sawdust	42.7	20.5	37.7	16.3	29.3	14.0	36.6	16.9	9.0104*
Mean pileus on corn cobs	32.5	14.5	11.7	13.8	9.8	11.1	18.0	13.1	0.7406 ^{ns}
t value and significance	-	-	-	-	-	-	4.049 ^{ns}	3.4356 ^{ns}	-

^{ns}P > 0.05; *P < 0.05; **P < 0.01.

solution were added. A dark green colour indicated the presence of phenolic compounds.

Test for glycosides

Sample (0.5 g) was stirred with 10 ml of boiling distilled water. This was filtered and 2 ml of the filtrate hydrolyzed with a few drops of concentrated HCL and the solution rendered alkaline with a few drops of ammonia solution. 5 drops of this solution was added to 2 ml of Benedict's qualitative reagent and boiled. Appearance of reddish brown precipitate showed the presence of glycosides.

Test for resins

To 0.5 g of each sample was added 5 ml of boiling ethanol. This was filtered through Whatman No.1 filter paper and the filtrate diluted with 4 ml of 1% aqueous HCL. The formation of a heavy resinous precipitate indicated the presence of resins.

Test for tannins

Sample (0.5 g) was stirred with 10 ml of boiling distilled

water. This was filtered and a few milliliters of 6% ferric chloride added to the filtrate. Appearance of deep green coloration indicated the presence of tannins. The second portion of the filtrate was treated with a few milliliters of iodine solution. Appearance of a faint bluish coloration confirmed the presence of tannins.

RESULTS AND DISCUSSION

Effect of two substrates on the growth and yield of *P. ostreatus* and *P. florida* is shown in Tables 2 and 3. There was a significant difference between mean height of *P. ostreatus* and *P. florida* cultivated on sawdust and corn cobs. Sawdust as a substrate had mean height significantly higher than those cultivated on corn cobs. The mean weight of the fruiting bodies of *P. florida* cultivated on sawdust was highly significantly higher than *P. florida* cultivated on corn cobs. Also, there was a significant difference in pileus length for *P. ostreatus* and *P. florida* cultivated on sawdust.

The growth and yield of *P. ostreatus* and *P. florida* varied widely, depending on the kind of

substrate used. It was noted that sawdust had the greatest influence on both growth and total yield. It demonstrated excellent biological yield, greater height, and pileus size. There was a significant difference on pin head formation for *P. florida* cultivated on sawdust and corn cobs. There was no significant difference in maturity period for the two species and the two substrates. For the number of fruiting bodies, there was a highly significant difference in the number of fruiting bodies which sprouted from sawdust for *P. ostreatus* and *P. florida*.

Various substrates have different effects on the growth, yield and quality of mushroom (Zhang et al., 2002). Cultivation of oyster mushroom is becoming popular throughout the world, because of their abilities to grow at a wide range of agricultural wastes. The development of oyster mushrooms depends on the specific environment, nutritional and genetic factors according to species. The two species of oyster mushroom cultivated on two substrates (sawdust and corn cobs) gave the following growth outcomes. The

Table 3. Mean pin head formation, maturity period and number of fruiting bodies of *Pleurotus ostreatus* and *Pleurotus florida* for three flushes grown on sawdust and corn cobs.

Growth parameters	Species	Flush 1 on sawdust	Flush 2 on sawdust	Flush 3 on sawdust	Mean flushes on sawdust	Flush 1 on corn cobs	Flush 2 on corn cobs	Flush 3 on corn cobs	Mean flushes on corn cobs	t value for media
Pin head formation (Days)	<i>P. ostreatus</i>	10.3	11.7	11	11	14	12.3	14.3	13.5	- 2.60 ^{ns}
	<i>P. florida</i>	9.4	9.6	10.4	9.8	11.4	12.4	12	11.9	- 6.05*
	t value Species	-	-	-	2.62 ^{ns}	-	-	-	1.87 ^{ns}	-
Maturity period (Days)	<i>P. ostreatus</i>	5.7	5.7	6	5.8	6	5.7	6.3	6	- 2 ^{ns}
	<i>P. florida</i>	5.4	6	6	5.8	5.3	5.7	6	5.7	1.51 ^{ns}
	t value Species	-	-	-	0.0	-	-	-	1.64 ^{ns}	-
Number of fruiting body	<i>P. ostreatus</i>	54	59	63	58.7	44	28	21	31	2.95 ^{ns}
	<i>P. florida</i>	37	43	45	41.7	30	20	15	21.7	2.94 ^{ns}
	t value Species	-	-	-	29.44**	-	-	-	3.88 ^{ns}	-

^{ns}P > 0.05; *P < 0.05; **P < 0.01.

biological efficiency varied significantly due to the effect of different substrate composition on the different flushes. *P. ostreatus* growth was better in sawdust than in corn cobs as it had the highest height, weight, and pileus length. This study is in the same line with the study of Shah et al. (2004) who reported that sawdust gave maximum yield of *P. ostreatus*. Overall, on the two species of mushroom cultivated on sawdust and corn cobs, *P. florida* on sawdust gave lesser days for pin head formation, maturity period and number of fruiting body. The least results was indicated by *P. ostreatus* on corn cobs which took the longest time for pin head formation, longest maturity days and the average number of fruiting body. This variability in pin head formation, maturity period and number of effective fruiting body has been reported by Shah et al. (2004). They reported that significant variability on the number of days of pin head formation, maturity period and number of effective body is due to presence of different composition of substrate. The number of days for

pin head formation is supported by Bughio (2001) who stated 25 to 50 days for pinhead formation and reported that maturation of fruiting bodies took 5 to 6 days after pinhead formation as in the same line with this study.

The biological efficiency and moisture content was the highest for sawdust substrates as a whole. Biological efficiency was the highest on *P. ostreatus* cultivated on sawdust with 50.2% and least on corn cobs substrates of both *P. ostreatus* and *P. florida* with 16.5%. However, moisture content was also the highest for *P. ostreatus* cultivated on sawdust with 90% and least for *P. florida* on corn cobs with 80% as shown in Table 4.

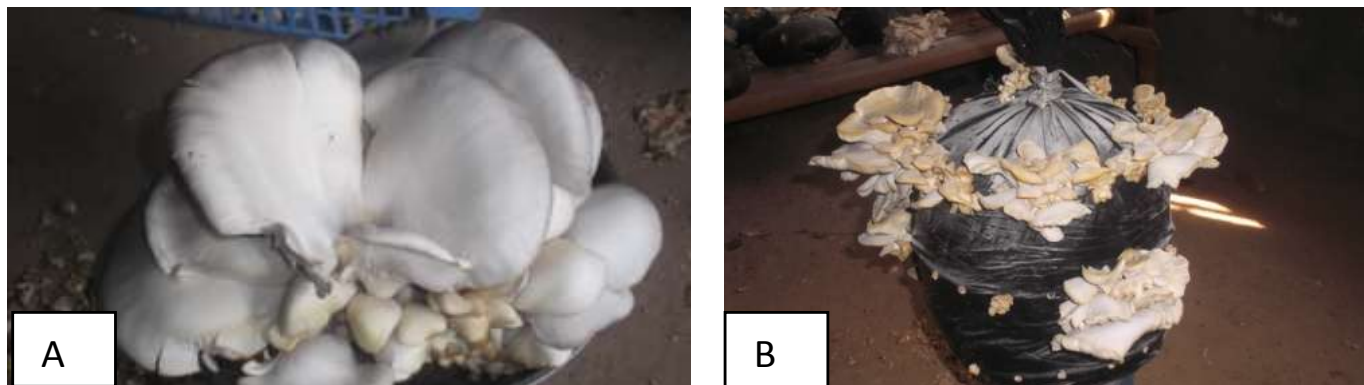
Fully and broadly opened basidiocarps were obtained from *P. ostreatus* than for *P. florida*. This is shown in Figure 3. It can be concluded that *P. ostreatus* cultivated on sawdust is the best species of oyster mushroom which can be cultivated for commercial purposes as it had the highest biological yield value than those grown on

corn cobs. The substrates used in this study can be considered practical and economically feasible due to their availability throughout the year at little or no cost in large quantities. Utilization of these agro-wastes for the production of oyster mushrooms could be more economically and ecologically practical. The effect of two substrates on nutritional content of *P. ostreatus* and *P. florida* is shown in Table 5.

Nutritional composition of mushroom is affected by many factors among which the composition of the substrate is of major importance. This can be supported by the results in the findings where *P. florida* and *P. ostreatus* on sawdust gave a significant nutritional value than that cultivated on corn cobs Shah et al. (2004). The nutritional properties of mushroom are also influenced by the species. This is supported by the fact that *P. florida* gave the best nutritional properties than *P. ostreatus* in terms of amount of proteins, lipids, fibre and ash content. The protein content ranged from 25.12 to 29.45% in 100% dry matter. Crude

Table 4. Culmulative *Pleurotus ostreatus* and *Pleurotus florida* biological efficiency (BE) and mean moisture content on two substrates.

Species and substrates	Biological efficiency (%)	Mean moisture content (%)
<i>P. ostreatus</i> on sawdust	50.2	90
<i>P. ostreatus</i> on corn cobs	16.5	82
<i>P. florida</i> on saw dust	36.1	86
<i>P. florida</i> on corn cobs	16.5	80

**Figure 3.** Fully opened Basidiocarps of *Pleurotus ostreatus* and *Pleurotus florida*.**Table 5.** Nutritional analysis of *Pleurotus ostreatus* and *Pleurotus florida* on different substrate.

Species and type of substrate	DM (%)	Ash (%DM)	Organic matter (%DM)	Crude protein (%DM)	Lipids (%DM)	Crude fibre (%DM)
<i>P. florida</i> Corn cobs	91.1	7.0	93.0	25.1	4.6	16.7
<i>P. florida</i> Sawdust	90.0	9.0	90.9	29.5	2.2	5.1
<i>P. ostreatus</i> sawdust	88.7	8.3	91.7	26.0	3.8	12.5
<i>P. ostreatus</i> corn cobs	90.9	9.5	90.5	25.8	2.0	16.0

protein percentage of oyster mushroom was affected by different substrates. Regarding substrate, maximum protein (29.45%) was observed on sawdust while minimum (25.12%) on corn cobs. Genotypic variable was observed in *Pleurotus* spp. to protein percentage, the highest percentage (29.45%) was observed in *P. florida*, whereas *P. ostreatus* showed the lowest amount (25.78%). The percentage content of protein was similar as reported in earlier studies (Patil et al., 2010). Mushroom protein is intermediate between that of animals and vegetables and it is of superior quality because of the presence of all the essential amino acids. The value of lipid ranged from 1.97 to 4.62% in 100% dry matter. Lipid percentage of oyster mushroom was affected by different substrate. Regarding substrate, maximum lipid (4.62%) was observed on corn cobs, while minimum (1.97%) was still on corn cobs. Genotypic variable was observed in *Pleurotus* spp. to lipid percentage, the highest percentage

(4.62%) was observed in *P. florida*, whereas *P. ostreatus* showed the lowest amount (1.97%).

Lipid value is contrary to that of Wang et al. (2001) as lipid range from 2.5 to 2.8%. Linoleic acid makes up to 76% of unsaturated fatty acids and 90% of polar lipids. It is the presence of linoleic acid, one of the omega 6 fatty acid that contributes to mushrooms being a healthy food. The crude fibre ranged from 5.08 to 16.69% and the ash content range from 6.94 to 9.46. Regarding substrate, maximum ash (8.67%) was observed on sawdust, while minimum (8.215%) on corn cobs. *Pleurotus* spp. contain high rate of potassium to sodium, which makes it an ideal food for patients suffering from hypertension and heart diseases (Purkayastha and Nayak, 1981). Regarding substrate, maximum crude fibre (16.69%) was observed on corn cobs, while minimum (5.08%) was still on sawdust. Genotypic variable was observed in *Pleurotus* spp. to crude fibre percentage, the highest percentage

Table 6. Bioactive components of *P. florida* and *P. ostreatus*.

Bioactive components	<i>Pleurotus florida</i>	<i>Pleurotus ostreatus</i>
Alkaloids	–	–
Flavonoids	+	+
Polyphenols	+	+
Triterpenoids	+	+
Steroids	+	+
Saponins	+	+
Glycosides	–	–
Resins	–	–
Tannins	–	–

The -sign indicates the absence of the compounds while the + sign indicates the presence of the compound.

(16.69%) was observed in *P. florida*, whereas *P. ostreatus* showed (16.01%).

The nutritional composition of edible mushrooms is affected by many factors among which the composition of substrate is of major importance also mentioned by Belewu (2003). Nutritional properties also differ according to species, but this difference also depends on the substrates. These results also indicate that the studied oyster mushroom species have good nutritive value for human. Protein is an important nutritional component and protein deficiency is the world's most serious human nutritional problem, especially in third world countries like Cameroon. So oyster mushroom is a promising food that may overcome protein energy malnutrition problem and mineral deficiency in the third world. While the protein content is lower than that found in eggs, meat and fish, it is adequate to be used as a substitute in the diet of the general public. These species of oyster mushroom contain low fats of unsaturated fatty acids which makes it a healthy food for all type of people.

As concerns nutritional properties of *P. florida* on sawdust which had the highest amount of protein of 29.5%. This protein is of great importance to health as they help the body to build, repair and maintain body tissue. On the other hand, *P. florida* cultivated on corn cobs had the highest amount of fibre which aids in digestion, weight management and has the highest amount of lipids that protect the vital organs. It has been reported that not only the protein content in fruiting body but also the nature of protein depends on used substrate (Wang et al., 2001). *P. florida* cultivated on sawdust and corncobs are the most preferred species according to nutritional properties because of the presence of essential nutrients such as proteins, ash, lipids and fibers. Effect of two substrates on the bioactive components of *P. florida* and *P. ostreatus* is shown in Table 6.

Lindequist et al. (2005) stated that the nutritional and chemical compositions of mushroom are responsible for their medicinal values. The bioactive component analysis

of edible mushrooms *P. ostreatus*, and *P. florida* revealed the presence of major bioactive components such as flavonoids, polyphenols, saponins, triterpenoids and steroids. This result is similar to that of (Iwalokun et al., 2007). The compound alkaloids, glycosides, resins and tannins were absent in the extracts. Bioactive compounds found in edible mushroom are known to play a vital role in promoting health. The absence of alkaloids and glycosides confirms the report of Hamzah et al. (2014). These phytochemicals play a vital role in medicinal properties of plants. Saponins for instance comprise a large family of structurally related compounds containing steroids or triterpenoid. They are reported to have a wide range of pharmaceutical properties, such as anti-inflammatory and anti-diabetic effects. Thus these mushrooms can be used in the management of diabetes and inflammation related diseases. Terpenoids have been reported to show a wide range of pharmacological benefits that include anti-malarial, anti-inflammatory and anti-cancer effects among others. Phenolic compound are antioxidant and exhibit a wide range of spectrum medicinal properties such as anti-cancer and anti-inflammatory. These mushrooms can therefore be harnessed in the management of oxidative stress induced disease since phenol and flavonoids have been shown to possess various antioxidant functions (Pandimeena et al., 2015).

Flavonoids have been isolated from hundreds of species of mushroom that are effective against many chronic diseases and the bioactive constituents of *P. florida* confirmed it to be one among these. The medicinal value of the mushroom may be due to the presence of secondary metabolites (Pandimeena et al., 2015). These metabolites present in the human diet possess a number of beneficial effects on human health such as anti-oxidant, anti-allergic, anti-viral, anti-diabetic and anti-carcinogenic. The triterpenoid in *P. florida* and *P. ostreatus* might be responsible for its anti-oxidant and anti-inflammatory activity (Price et al., 1987). Saponins cause cytotoxic effect and are beneficial in lowering

cholesterol and blood sugar levels (Price et al., 1987).

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

This work was financed by the Research Modernization grant from the Cameroon Ministry of Higher Education.

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