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Yield potentials, nutritional and mycochemical properties of fruit-bodies of *Pleurotus ostreatus var. florida*, grown on *Andropogon gayanus* straw; supplemented with *Anthonotha macrophylla*

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ABSTRACT

The study involved the investigation of the effect of *Andropogon gayanus* straw and *Anthonotha macrophylla* bark on the yield and mycochemical composition of *Pleurotus ostreatus var. florida*. The highest gross yield of fruit-bodies of *P. ostreatus* was obtained from substrate E, while the least was found in substrates B. Similarly, the fresh and dry weights of the fruit-bodies of substrate E were higher than those from other substrates. The pileus diameter and stipe lengths of the fruit-bodies from the substrate followed the same trend as in the number of fruit-bodies. Based on the mycochemicals composition, the highest alkaloid content was obtained from substrate C, while substrates E had the least. The highest tannin content was obtained from substrates D, while substrate E had the least. However, flavonoid content of the fruits-bodies followed the same trend as in tannin having the highest flavonoid content for substrates D and the least was found in substrate E. The highest hydrogen cyanide content was obtained from substrate D, while the least was obtained from substrate A. Substrate C had the highest anthocyanin content, while substrate E had the least. The appreciable levels of the mycochemicals obtained from the mushrooms, as affected by the substrates and its supplementations, indicate their medicinal values and property which support the safety of mushroom for consumption. The results of the investigations suggest that growing *P. ostreatus* on 50:50 *A. gayanus* straw and *A. macrophylla* bark produced the highest number and best quality fruit-bodies among the whole substrates. This fact makes the substrate suitable for the cultivation of mushrooms healthy for consumption purposes. The use of *A. gayanus* and *A. macrophylla* in the ratio of 1:1 is therefore recommended for potential commercial production of mushroom for the Nigerian market.

INTRODUCTION

Mushroom is a general term applied to fungi that belong to the division Basidiomycetes and a few members of the division Ascomycotina. They are saprophytes and are known to grow on a wide variety of substrates and habitats. However, the fact still remains that mushrooms show preference on particular substrata within a habitat (Adesina et al., 2011). The fruiting-bodies of most mushrooms are shaped like an umbrella with central stalk (stipe) supporting a cap (pileus), which bears gills (lamellae) that produce spores on the lower side. In some

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species of *Pleurotus*, the stipe may be absent, especially those growing on wood (Jose et al., 2002).

Many genera of mushrooms are edible and are rich in essential nutrients such as carbohydrates, low fats and oil content, proteins, vitamins, minerals, fibres, various amino acids and one third of the iron in it is in available form (Okwulehie and Odunze, 2004).

Cultivation of mushroom can be viewed as an effective way to extract bio-resources left behind in agricultural residues and as a source of environmental protection strategy. The use of residues in bioprocesses may be one of the solutions to bio-conservation of inedible biomass residue into nutritional protein rich food in the form of edible mushroom (Chui et al., 2003). However, mushroom cultivation is not easy, it involves many steps, from selecting a suitable technique and strain to spawn manufacturing, growing the crops and marketing the final crop (Oei, 2003).

The nutritional values of edible mushroom depend on the type of the agricultural waste used for its production (Wabali and Wocha, 2013). Some authors have investigated the nutritional and chemical contents of the substrates used to produce mushroom fruit-bodies and sclerota so as to determine their influence on the yield and quality of the mushroom. For instance, Badu et al. (2011) reported that the yield and quality of Oyster mushroom on any substrate is dependent on the chemical content of the substrate.

Some mushroom extracts are used or studied as possible treatments for diseases, such as cardiovascular disorders (Gullamon et al., 2010). Some mushroom materials, including polysaccharides, glycoproteins and proteglycan are under basic research for their potential to modulate immune system responses and inhibit tumor growth, whereas other isolates show potential antiviral, antibacterial, antiparasitic, anti-inflammatory and antidiabetic properties in preliminary studies (Borchers et al., 2008). Currently, several extracts have widespread use in Japan, Korea and China as adjuncts to radiation treatments and chemotherapy (Borchers et al., 2008).

According to United Nation Food and Agricultural Organisation (FAO Statistics) 2009, the global mushroom production was estimated at about 2.18 to 3.41 million tons over a period of last ten years (1997-2007). Mushrooms in FAO database have been classified into Button mushroom (*Agaricus bisporus*), Shiitake mushroom (*Lentinula edodes*) and Oyster mushroom (*Pleurotus* spp.) and accounted for nearly 76% of global mushroom market size in 2013. Since there was an increase of about 56% world mushroom production in last decades and guesstimates can be put on current production to be around 3.5 million tons. China, USA, Netherlands, Poland, Spain, France, Italy, Ireland, Canada and UK are the leading producer. World mush room production (FAOSTAT) is continuously increasing from 0.30 to 3.41 million tons over period of about last 50 years from 1961 to 2010.

Documented literature indicates that mushroom has phytochemicals and other compounds which are strong antioxidants (Fang et al., 2002). Phenolic compounds, alkaloids, saponins, flavonoids, tannins and cyanide have also been detected from wild edible mushrooms in Abia State, Nigeria (Okwulehie and Enwere, 2014).

According to Food and Agricultural Organization (FAO, 2011), mushrooms are recognized as food contributing protein nutrition to countries depending largely on cereals. Lebo (2004) reported that mushrooms are eaten as snacks during festivals. The rural dwellers consume mushrooms as delicacies in soup and ingredients for seasoning or part of the local melon cake (a local snacks called usu in Igbo). Zoberi (1973) reported that many still depend largely on mushroom harvested from the wild and that improper identification of edible mushroom has led to cases of mushroom poisoning, aliment and death. It has also been reported as therapeutic food, useful in preventing disease such as hypertension, hyper-cholesterollamia and cancer.

MATERIALS AND METHODS

Source of stock spawn and substrates for cultivation

The spawn used for the research was prepared in the Laboratory of Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State. The substrates, *A. gayanus* straw and *A. macrophylla* bark used for the cultivation of the *P. ostreatus* were collected from farms and forest strip in Michael Okpara University of Agriculture Umudike, respectively. The substrates were moisturized, heaped and covered to allow fermentation process to take place.

Preparation of substrates for inoculation of spawn

The *A. gayanus* straw and *A. macrophylla* bark substrates were chopped into pieces of about 5 cm average length and soaked overnight in basins of clean tap water, according to the method of (Sharma, 2003). The substrates were drained the next day and separately poured into a drum and pasteurized by heating on gas for 2 h at 100°C (Okwulehie and Okoro, 2013).

After cooling, 100 kg of *A. gayanus* straw supplemented with *A. macrophylla* bark was weighed into perforated transparent buckets at various concentration to be equivalent to 100 grams as follows; *A. gayanus* + *A. macrophylla* bark, 90% + 10%, 80% + 20%, 70% + 30%, 60% + 40%, 50% + 50%, respectively. The control was 100 g *A. gayanus* straw. Each of the supplementation and the control were replicated three times.

Figure 1. Bottle of spawns.

Figure 2. Experimental layout in MOUAU cropping room.

Spawn running

The substrates concentration were mixed thoroughly and equal amount of the spawn of the *P. ostreatus* mushroom was inoculated in layers (using hands inserted disposable globes), in each of the buckets containing the substrates. Thereafter, they were transferred into wooden racks (Figures 1 and 2) in the Mushroom House of the Department of Plant Science and Biotechnology, and were covered with black polyethylene bags for ramification that lasted for 8 days. The polyethylene bags were removed on the 10th day, during which time immature mushroom started to emerge as tuff of small bottons or premodials.

Fruiting and harvesting

Fruiting of the mushroom commenced on the different concentration of the substrates from the 10th day after inoculation. The first flush was harvested from the 12th day due to the different nutrients concentration of the substrates. The second flush was harvested from 16th day, and the third flush was harvested from the 30th-40th day (Figures 3 and 4).

Measurement parameters

During each of the flushes, the yield of the *P. ostreatus* on different substrates concentration was determine by recording the number and size of the fruiting bodies. The measurements from the various replicates were added and their mean values calculated. The following parameters of growth/yield were taken.

Yield/Number of fruit-bodies

The harvested fruit-bodies were counted directly after

Figure 3. Fruit-bodies (under side).

Figure 4. Fruit-bodies (upper side).

splitting them from their bunch from each bucket.

Pileus diameter (cm)

The diameter of the pileus was measured in centimetre (cm) with a transparent plastic ruler from one edge of the pileus across the stipe to the other edge.

Stipe length (cm)

Similarly, the length of the stipe was also measured in centimetre (cm) as well using a transparent plastic ruler.

Weighing of the fresh and dry fruit-bodies

The fresh and dry weight of the fruit-bodies were weighed using a digital scale (Model, 2000) made in USA.

Drying of the fruit-bodies

The drying of fresh fruit-bodies was done using a hot air oven, at 80°C for 48 h.

Biological efficiency

The biological efficiency (yield of mushroom per kg

substrate on dry wt. basis) was calculated using the following formula:

$$B.E\ (\%) = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times \frac{100}{1}$$

Phytochemicals analysis

Anthocyanin determination

This was done gravimetrically by the method of Harbone (1973). Five grams (5 g) of each test sample was treated with ethyl acetate and allowed to separate into two layers. The ethyl acetate layer (extract) was recorded, while the aqueous layer was discarded. The extract was separated to dryness in the crucible over a steam bath. The dried extract was then treated with concentrated amyl alcohol to extract the anthocyanins. The weight of anthocyanin was determined and expressed as percentage of the original sample.

Hydrogen cyanide (HCN) determination

Hydrogen cyanide was determined by alkaline pikrate colorimetric method by Palogopalan et al. (1988). Two grams (2 g) of the sample was dispersed in 50 ml of distilled water in a conical flask. An alkaline pikrate paper was hung over the sample mixture and the blank in their respective flasks. The set up were incubated overnight and each of the pikrate paper was eluted or dipped into a 60 ml of distilled water. A standard cyanide solution was prepared and diluted to a required concentration. The absorbance of the eluted sample solutions were measured with colorimeter at 540 nm wavelength with the reagent blank at zero.

Flavonoids determination

Flavonoids were determined using the method described by Harbone (1973). A measured weight of the processed sample (5 g) was boiled in 100 ml of 2 M HCl solution under reflux for 40 min. It was allowed to cool before being filtered. The filtrate was treated with equal volume of ethyl acetate and the mixture was transferred to separation funnel. The flavonoid extract (contained in the ethyl acetate portion) was received by filtration using weighed filter paper. The weight was expressed as a percentage of the weight analysed. It was calculated as shown below.

Percentage of flavonoid = W1 - W2 × 100

Where, W1: weight of filter paper × flavonoid precipitate; W2: weight of filter paper alone.

Alkaloids determination

Modified precipitation gravimetric methods of Harbone (1973) and Maxwell et al. (1995) was applied. A measured weight (5g) of each of the dried samples of mushroom was dispersed in 100 ml of 10 % acetic acid in ethanol solution. The mixture was well shaken and left to stand for 4 hours at room temperature; being shaken every 30 minutes. The extract was filtered to remove debris using weighed Whatman No. 42 filter paper and then concentrated to a quarter of the original volume. After washing with 1% NH_4OH solution, the precipitate in the filter paper was dried at 65°C and re-weighed after cooling in a desiccator. The alkaloid content was calculated as shown below.

$$\%\ \text{Alkaloid} = \frac{\text{Weight of residue}}{\text{Weight of residue}} \times \frac{100}{1}$$

Tannin determination

The tannin content of the sample was determined by Folin Denmscolometric method (Harbone, 1973). The treated filtrated was mixed with 2.5 ml of saturated Na_2CO_3 solution. Then each flask was diluted to 50 ml mark with distilled water and incubated for 90 min at room temperature. Their absorbance was measured at 760 nm in a colorimeter with the reagent blank at zero. The tannin content was calculated as shown below.

$$\%\ \text{Tannin} = \frac{100}{w} \times \frac{au}{as} \times \frac{c}{1000} \times \frac{vt}{va}$$

Where, w: weight of sample, au: absorbance of test sample; as: absorbance of standard tannin solution; c, concentration of standard tannin solution; vt, total volume of extract; va: volume of extract analyzed.

RESULTS AND DISCUSSION

The results of the investigation of yield and myco-chemical composition of *P. ostreatus* fruit-bodies produced from different substrates and substrate supplementations are summarized in Tables 1 and 2.

Values are means of 3 replicates of the observation. Mean on the same column with the same letters are not statically different at 5% confidence level ($p>0.05$). The result summarized in Table 1, represents the Gross yield and qualities of the effects of the substrates and

Table 1. The effect of the substrates and substrates supplementation on the gross yield and qualities of *P. ostreatus.*

Substrates and substrates supplementation	Total	Pileus diameter	Slipe length	Fresh 2wt	Dry wet	BE (%)
Control	54.00[a]	78.10[a]	51.20[b]	103[a 00]	11.00[b]	10.3
A	55.00[a]	78.70[a]	53.10[b]	105[a] .00	13.00[a]	10.5
B	36.00[a]	52.00[c]	34.50[d]	88.00[b]	9.70[c]	8.8
C	41.00[a]	65.50[b]	43.90[c]	89.00[b]	11.00[b]	8.9
D	58.00[a]	80.20[a]	59.50[b]	99.00[ab]	12.00[b]	9.9
E	66.00[a]	95.40[a]	67.90[a]	123.00[a]	14.00[a]	12.3

Table 2. Myco-chemical compositions of the fruit-bodies of the mushroom as affected by substrates and substrate supplementations.

Treatment	Alkaloid	Tannin	Flavonoid	HCN	Anthocyanin
Control	5.20[a]±0.02	1.62 ±0.00	2.50[d]±0.02	0.018[c]±0.00	2.08[c]±0.00
A	5.80[d]±0.01	1.71[b]±0.01	2.74[c]±0.02	0.017[d]±0.00	4.78[a]±0.00
B	6.10[b]±0.02	1.81[c]±0.01	2.85[b±]0.01	0.018[cd] ±0.001	4.78±0.59
C	6.30[a]±0.01	1.92[b]±0.01	2.92[a]±0.02	0.018[bc]±0.01	4.93[a]±0.02
D	6.00[c]±0.04	1.95[a]±0.01	2.97[a]±0.03	0.020[a]±0.01	3.85[b]±0.62
E	4.00[f]±0.04	1.58[f]±0.01	1.47[e]±0.06	0.018[bc]±0.00	1.07[d]±0.01

Values are mean ± SD. Values with the same letters(s) on the same column are not statistically different at 5% confidence level.

substrates supplementation on the *P. ostreatus* of the investigation. The result indicates that the highest total fruit-bodies number was recorded in substrate E (50:50 *Andropogon* straw and *Anthonotha* bark) having (66.00[a]) total fruit-bodies with highest weight of 123.00[a] cm and biological efficiency (BE) of 12.3%, followed by substrate D (60:40 *A. gayanus* straw and *A. Macrophylla*), having (58.60[a]) fruit-bodies, while the least is found in the substrate B (80:20 *Andropogon* straw and *Anthonotha*) having 36:00[a] with the least fresh weight of 88.00[b].

The myco-chemical composition of the fruit-bodies of the mushroom as affected by substrates and substrate supplementation are shown in the Table 2. The result shows that the mushroom fruit-bodies obtained from the different substrates and substrates supplementation contain alkaliods, tannin, flavonoid, HCN and anthocyanin in varying quantities. The highest alkaloids content was (6.30[a] ± 0.01) obtained from substrate C, while the lowest (4.00[f] ± 0.04) was obtained from substrate E. The highest content of tannin (1.95[a]0 ± 0.01) was obtained from substrate D, while the least content (1.58[f] ± 0.01) was found in substrate E. The highest content of flavonoid (2.97[a] ± 0.05) was obtained from substrate D, while the lowest (1.47[e] ± 0.06) were obtained from substrate E. The highest content HCN (0.020[a] ± 0.01) was obtained from substrate D, while the lowest (0.017[d]±0.00) were obtained from substrate A. The highest content of anthocyanin (4.93[a] ± 0.02) was obtained from substrate C, while the lowest were obtained from substrate D while the lowest (1.07[d] ± 0.06) were obtained from substrate E.

DISCUSSION

The result of the study shows that fructification of *P. ostreatus* occurred in all the substrates and substrate supplementations. This indicates that these substrates contained nutrients that supported the growth of the mushroom. This is in line with the report of Wabali and Wocha (2013) that nutrient concentration of substrates has effect on yield of *P. ostreatus*. The results also supported the earlier report by Okwulehie and Okwujiako (2008) that *Pleurotus* spp. have a high saprophytic ability and can grow well in a variety of cellulosic substrates (Thambidurai et al., 2006). The ability of the *P. ostreatus* to successfully grow on substrates E (50:50 *Andropogen* straw and *A. macrophylla* bark) may be associated with the nutrients contained in the substrates (Badu et al., 2011). The various observations of high yield number of fruit-bodies produced by substrates E, followed by substrates D and substrates A respectively (Table 1), may be associated with differential nutrients status of this substrates and to some extent the physical nature of the

substrates, as well as environmental factors with the nature of the mushroom. This results obtained agree with findings that some supplements when added to substrates, in a favourable condition improved yield of mushrooms (Ukoima et al., 2009).

Phytochemical composition of matured fruited-bodies of the mushroom as affected by substrates and substrates supplementation in Table 2 shows that the mushrooms produced from substrates E and B contain little quantities of tannins, HCN and anthocyanin which means that the mushroom is safety for consumption (Okwulehie et al., 2013). The flavonoids contents of the mushroom indicate their medicinal value, meaning that the consumption of the mushroom may prevent oxidative cell damage and have strong anti-cancer activity (Okwu, 2004). Edeoga and Eriata (2001) observed powerful effect of alkaloids in animal physiology and showed their considerable pharmacological activities. The high alkanoids in the mushrooms and their synthetic derivative are used as basis for recommendation of mushrooms as medicinal agents for analgesic, antispasmodic and bactericidal effects (Stray, 1998).

Similarly, tannin concentrations detected in the mushrooms suggests their astringent properties that could play a role in the healing of wounds and inflamed mucous membrane (Okwu, 2004). The wide spread occurrence of cynaide in an edible mushrooms has been reported (Akinyama et al., 2006). According to Akinyama et al. (2006), high cyanide exposure could occur from intake of *Surihiratake* mushroom in one diet and high accumulation of cyanide in blood of patients with chronic kidney diseases might be associated with onset of encephalopathy. Epidemiological studies show that small doses of cyanide given over a long of time produced histological changes in the central nervous system of a rat (Smith, 1964). However the cyanide content of the mushroom under investigation is low. The low cyanide contents in the mushrooms makes it nutritional safe for consumption.

Conclusion

In conclusion, the result of the investigation suggests that substrates supplementation could be vital in production of *P. ostreatus* var. Florida. There is appreciable level of phytochemicals in the mushrooms from all substrates and clear cut influence of the substrates and substrates supplementation in support of yield and growth of the mushroom. The result of the investigation reveals that the substrates E (50:50 *A. gayanus* straw and *A. macrophylla* bark) produced the highest fruit-bodies and fresh weight among the whole substrates used for the study. The mushroom fruit-bodies produced from the substrates, contained little quantities of tannin, anthocyanin and HCN. This supports the safety of mushroom for consumption.

Alkaloids and flavonoid levels in the mush rooms support the report its antimicrobial properties. The investigation suggests that there is great potential in the production of good fruit-bodies of *P. ostreatus* using the substrates at the ratio of 1:1.

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Abbreviations:

ANS, *A. gayanus* straw; **AM,** *A. macrophylla*; Substrate A, 90:10 ANS & AM; **Substrate B,** 80:20 ANS & A.M; **Substrate C,** 70:30 ANS & A.M; **Substrate D,** 60:40 ANS & A.M; **Substrate E,** 50: ANS & A.M; **Control,** 100% (1000 g) ANS only.