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Full Length Research Paper

The effects of different substrates on sporophore yield, mineral and nutrient composition of *Pleurotus tuber-regium* fries singer in Calabar, Nigeria

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The effects of four agricultural wastes-yam (*Dioescorea rotundata*) peels, plantain (*Musa paradisica*) peel, cocoyam (*Colocasia esculenta*) peels and cassava (*Manihot esculenta*) peels on sporophore yield, nutrient and mineral composition of *Pleurotus tuber-regium* were investigated. Yield was highest in plantain peel substrate (63.50±2.30 g), while yam peel substrate (36.60±3.80 g) recorded the lowest yield. There was variation in the nutrient status of the fruit bodies harvested from the different substrates. Fruit bodies from yam peel recorded the highest moisture (15.85±0.30%) and carbohydrate content (14.90±4.50%), while plantain peel recorded the highest protein (18.52±1.52%) and crude fibre content (13.75±4.51%). The least fat content was recorded in fruit bodies from yam peel substrate (3.20±0.23%). However, there was more calcium than magnesium, zinc or iron in fruit bodies.

Key words: Agro waste substrates, sporophore yield, nutrient content, mushroom.

INTRODUCTION

Pleurotus tuber regium (Fr) Sing, is an edible mushroom of the class Basidiomycetes. It occurs in both tropical and sub tropical regions of the world (Zoberi, 1972, 1973). In Nigeria, the mushroom is particularly harvested in the wild and consumed in the southern parts of Nigeria for its delicacy and high protein content. *P. tuber regium* exists as sclerotium, which is an underground resting spore, with a large mass of asexual cells with thick, hard walls (mycelia) that survives the fungus through adverse environmental conditions (Pandey, 2011). The sclerotia of *P. tuber regium* vary in size, from a few centimeters to several centimeters in diameter. They are spherical or oval in shape, and appear dark brown on the outside and whitish to grayish colour on the inside (Okhuoya and Okogbo, 1991).

In Nigeria, both the sclerotium and sporophore are eaten (Okhuoya and Okogbo, 1990). The sclerotium in particular is a delicacy and is used in thickening stews and various soups, and is used in traditional medicine as well (Oso, 1977; Zoberi, 1973). Apart from *P. tuber regium,* mushrooms generally are consumed by humans not only for their protein but because they possess highly desirable taste and aroma (Kurbanoglu and Algur, 2002; Jonathan and Fasidi, 2003; Kadiri, 2005b). Oso (1977) also reported that *P. tuber regium* is combined with some herbs and other ingredients for the cure of headaches, stomach disorders, colds and fever; and also asthma, small pox and high blood pressure (Fasidi and Olorunmaye, 1994; Dzomeku, 2009). In China, its use has been reported in folk recipes as a tonic and in traditional medicine for the treatment of asthma and coughs (Chenghua et al., 2000). The phytochemical analysis of *P. tuber regium* showed the presence of alkaloids, saponins, tannins and cyanogenic glycodides (Ikewuchi and Ikewuchi, 2008), thus confirming the fungus as useful in traditional medicine for the treatment of several ailments. Apetorgbor et al., (2013) also reported that only plantain leaves (Musa sapientum) substrate significantly produce maximum fruit bodies and sclerotia of *P*. tuber regium than millet stalk and obeche saw dust used as substrate. Chemical analysis of fresh plantain and banana leaf substrates we reported to be less toxic and possess large quantities of cynanogenic glycosides (Sharrock and Lusty, 2000). The nutritional status of *P. tuber* regium showed that it contains protein, carbohydrates fats as well as mineral elements like

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calcium, magnesium, zinc and iron (Ogbo and Okhuoya, 2006; Ogundana and Fagade, 1981; Agomuo, 2011) and also vitamins A, B, B2, B3 and B6 (Danso et al., 2006). Also, the chemical composition and nutrient status of mushrooms generally varies and depends on the type of substrate used and the composition of the substrate (Stamets, 2000; Ogbo and Okhuoya, 2006).

Although there is a high demand for the consumption of mushrooms in Nigeria, its cultivation by farmers is still done by collection from the wild (Okhuoya and Okogbo, 1990), thus causing scarcity of this delicacy. Also several edible species that would have been cultivated are known only in the wild state, where their sclerotia infect dry wood causing decay and other organic vegetation buried in the top sub layers of the soil. *Pleurotus* species as edible mushrooms were reported to possess a high saprophytic ability and to grow on a variety of agro-wastes (Ruganathan et al, 1996), thus bioconverting lignocellulosic raw materials present into proteins (Dianxia et al., 2002; Obodai et al., 2003; Adejoye and Fasidi, 2009). Researchers in Nigeria reported that the sclerotium of *P. tuber regium* obtained by farmers from the forest when buried in warm humid soil induced the growth of fruit bodies (Oso, 1977; Okhuoya and Etugo, 1993; Isikhumhen and Okhuoya, 1995) within a relatively shorter period of 14-21 days (Patrabansh and Madan, 1997). The ideal condition for optimum mycelia growth of *Pleurotus sajor caju* was reported to be 24 h darkness, while 12 h light and 12 h darkness was the best condition for fruit bodies production of the fungus (Idowu et al., 2013).

Several workers have tried the cultivation of *Pleurotus* species and other mushrooms on various agro-waste residues (Sangwan and Saini, 1995; Rugunathan et al., 1996; Das et al., 2000; Kadiri, 2005a; Ogbo and Okhuoya, 2006). However, most of the studies in Nigeria on *P. tuber regium* concentrated on the nutrients available in the various agro-waste substrates that enhanced the vegetative growth and fruit bodies. In Nigeria, available literature on the cultivation of *P. tuber regium* showed that no work has been done to examine cocoyam and plantain peels as agro-waste substrates and their effect in the cultivation of edible mushrooms in Nigeria. In the 1960s when cocoyam was cultivated in large quantities with little or no deforestation of Nigerian virgin soils, some species of edible mushrooms like *Pleurotus ostreatus* have been observed to grow wild on rotted cocoyam (*Colocasia* sp.) barns and in forest soils. In Ghana, Ighodaro (2012), while assessing the mineral content of unripe plantain peels, reported the presence of high levels of mineral iron than the other elements present. The search for still cheaper and better agro-waste residues vis-à-vis the few that have been researched on, for the cultivation of edible mushrooms in Nigeria is what informed this study. The aim of this study therefore is to investigate the effect of cocoyam peel, plantain peel, cassava peel, and yam peel in the cultivation of *P. tuber regium*, and to determine the yield, mineral and nutrient composition of the mushroom in Calabar, Nigeria.

MATERIALS AND METHODS

Collection of farm waste materials

The sclerotia of *P. tuber regium* used for this study were purchased from Watt Market in Calabar Metropolis, Cross River State, Nigeria. It was identified by the Herbarium unit of the Department of Biological Sciences, Cross River University of Technology, Calabar to confirm that they were *Pleurotus tuber regium.* They were taken to the laboratory and soaked in clean tap water for 12 h at 26°C before use. The farm wastes used were yam (*Dioscorea rotundata*) peelings from some yam farmers at Idomi, Yakurr Local Government Area, Cross River State, Nigeria; plantain (*Musa paradisica*) peelings from an abandoned plantain farm at Akpet I, Biase Local Government Area; cocoyam (*Colocasia esculenta*) peelings from a private farm at Agoi Ibami, Yakurr Local Government Area, and cassava (*Manihot esculenta*) peelings from a cassava mill in Calabar South Local Government Area.

Induction of sporophore from *P. tuber regium* sclerotium

The sclerotium of *P. tuber* regium was reduced into pieces of 10×10 cm in size and planted in five perforated plastic buckets (22×22 cm in size) in the screen house, with top loamy soil and watered daily where necessary. The mushroom was germinated after 10 days of planting and the vegetative growth harvested and used as inoculum source for tissue culture preparation of the fungus.

Preparation of tissue culture and spawn of *P. tuber regium*

Fresh tissues from the growing sporophores of *P. tuber regium* were carefully and aseptically plated in sterile potato dextrose agar (PDA) medium in Petri dishes and allowed to grow for 10 days and form tissue cultures of *P. tuber regium* in PDA. The grain used for the spawn was unthreshed rice, obtained from a local market at Adim, Biase Local Government Area of Cross River State. Unthreshed rice (100 g) was each poured into 10 jam jars and a mixture of calcium carbonate (4 g) and 2 g of calcium sulphate (gypsium) were also added into each bottle. The jam bottles with their contents were sterilized in the autoclave at 1.1 kg/cm^3 at 121°C for 15 min. The media bottles were allowed to cool down and 5 mm mycelia disc from a 5 day old pure culture isolate of *P. tuber regium* were inoculated into the grains in each bottle using a sterile cork-borer. The spawn was allowed

Table 1. Morphometric values of *P. tuber regium* grown in different substrates.

Substrate	Pileus diameter (cm)	Stipe height (cm)	Stipe girth (cm)
Yam peel	8.30±3.60	4.20±0.92	3.15±0.53
Plantain peel	15.54±1.65	6.90±2.40	3.30±0.70
Cocoyam peel	10. 35±2.40	5.50±1.21	3.10±0.60
Cassava peel	7.50±3.70	3.70±0.75	3.20±0.50

Table 2. Average yield of *P. tuber regium* in different substrates.

Substrate	Fresh weight (g)	Dry weight (g)
Yam peel	36.60±3.80	19.90±085
Plantain peel	63.50±2.30	29.63±1.15
Cocoyam peel	53.70±1.60	24.70±0.70
Cassava peel	48.20±1.53	23.52±0.63

to incubate for a period of 14 days.

Substrate preparation and sterilization

Fresh peelings from yam, plantain, cocoyam and cassava were each, separately cut into smaller pieces (2×2 cm), rinsed in sterile distilled water and sun dried for 7 days. The substrates were separately bulked and surface sterilized by soaking in 5% bleach (sodium hypochlorite) (v/v) for 30 min. They were further rinsed in three changes of sterile distilled water, and then pressed to expel excess water until the moisture content was maintained at 85% relative humidity. Exactly 100 g of each substrate was put into 2000 ml plastic bowls and covered tightly with aluminum foil and autoclaved at 1.1 kg/cm^3 at 120°C for 20 min.

Inoculation of spawn and vegetative growth of *P. tuber regium*

Each plastic bowl with its substrate was allowed to cool. Exactly, 10 g of spawn was aseptically inoculated into the centre of each substrate in the plastic bowl in an inoculating chamber and incubated at 28±2°C. Mycelia growth along the sides of the plastic bowls was measured weekly for 3 weeks. The plastic bowls were left to induce formation of fruit bodies and were collected during the 6 months growth period of the mushroom. The experiment was a completely randomized design with five treatments and 3 replicates.

Statistical analysis

Data obtained were analysed by analysis of variance (ANOVA) and means were separated by least significant difference (LSD) test at 5% probability level.

Determination of morphometric characteristics of the mushroom

Parameters like Pileus diameter, stipe height and girth of the fungus were determined after harvest by measuring with a meter rule.

Determination of fresh and dry weight of *P. tuber regium*

The mycelia mat cultured in each plastic bowl was carefully scrapped out onto a Whatman No. 1 filter paper with a scapel. The mycelia fresh weight was obtained by weighing the mycelia on an analytical (Top loader) digital balance and subtracting the weight of the filter paper from that of the filter paper plus the mycelia (Nwanze et al., 2005). The dry weight was obtained by placing the mycelia on dry filter papers and dried in the oven at 40°C for 48 h until a constant weight was obtained.

Chemical analysis of sporophores

Chemical analysis was carried out by digesting the dried fungal specimen harvested from the substrates with HNO_3, H_2SO_4 and H_2O_2 in a Kjedahl flask. Standard solutions were then prepared and fed into the Varian AA-1475 series Atomic Absorption Spectrophotometer. Values of mineral elements like calcium, magnesium, zinc and iron were read from the standard curve.

Nutrients like carbohydrates, protein, fat ash, moisture and crude fiber contents were determined by using the methods outlined in the AOAC (1984). Protein determination was carried out using the Kjedahl method (AOAC, 1984). Fat determination was carried out using a Soxhlet apparatus (AOAC, 1984). Also determination of fiber content was done according to the enzymatic gravimetric method (AOAC, 1984).

RESULTS

The various substrates used affected the size of the mushroom cultivated. The widest pileus was produced in plantain peel substrate (15.54±1.65 cm) while the narrowest pileus was recorded in cassava peel substrate (7.50±3.70) (Table 1). Similarly, plantain peel substrate recorded the highest stipe height (6.90±2.40 cm), while the lowest stipe height of 3.70±0.75 cm was observed in cassava peel substrate. All the substrates used did not significantly ($p<0.05$) affect the size of the stipe girth.

The highest yield was recorded in plantain peel substrate (63.50±2.30 g), closely followed by cocoyam peel (53.70±1.60 g) while yam peel substrate recorded

Table 3. Nutrient composition of *P. tuber regium* in different substrates (%).

Substrate	Moisture	Protein	Fat	Carbohydrate	Ash	Crude fiber
Yam peel	15.85±0.30	4.50±2.30	3.20±0.23	14.90±4.50	3.24±0.42	3.2±177
Plantain peel	13.70±2.40	18.52±1.52	4.65±1.65	10.45±2.77	14.45±0.32	13.75±4.51
Cocoyam peel	10.53±1.52	7.33±0.90	3.34±0.70	12.61±2.30	10.65±0.44	11.32±2.85
Cassava peel	14.74±1.60	5.32±1.50	4.25±0.48	13.54±6.32	16.57±0.25	12.41±7.35

Table 4. Mineral composition of *P. tuber regium* in different substrates (mg/100 g).

Substrate	Ca	Mg	Zn	Fe
Yam peel	7.60±7.60	2.32±0.40	2.31±0.75	0.06±0.06
Plantain peel	16.50±2.23	6.30±1.22	1.95±0.85	1.76±1.02
Cocoyam peel	10.24±0.96	3.50±0.53	2.40±0.76	0.53±0.04
Cassava peel	12.13±1.18	4.53±0.75	3.95±0.17	0.37±0.13

the least with 36.60±3.80 g (Table 2).

The different substrates used affected the nutrient concentration in the fungus. Fruit bodies from yam peel substrate recorded the highest moisture content (15.85±0.30%) and the least crude fiber content (3.21±1.77%) (Table 3). The highest protein content (18.52±1.52%) was shown by the fruit bodies of the fungus when grown in plantain peel substrates; and was significantly ($P<0.05$) higher than the rest of the three substrates. Plantain peel substrate also had the least carbohydrate content. Generally, cassava peel substrate had the highest ash content (16.57±0.25%) but with the least protein (5.32±1.50%) content (Table 3). Calcium was more abundant in the fruit bodies generally, than any other element tested in this work (Table 4). It was highest in plantain peel substrate (16.50±2.23 mg/100 g) closely followed by cassava peel substrate (12.13±1.18 mg/100 g), while yam peel substrate was least (7.60±0.78 mg/100 g). Also, plantain peel substrate generally recorded highest magnesium (6.30±1.22 mg/100 g) and iron (1.76±1.02 mg/100 g) than the rest. It was also observed that there was more magnesium than zinc and iron, and more zinc than iron in the fruit bodies cultivated from the substrates. Except for calcium, the four substrates used did not show much variation in the quantity of the mineral contents of the mushroom cultivated in them (Table 4).

DISCUSSION

This study had successfully grown *Pleurotus tuber regium* on four different agro-waste substrate for the production of fruit bodies with compact mycelia growing and ramifying in all of them (Ogbo and Okhuoya, 2006; Das et al., 2000; Isikhuemhen et al., 1995; Songwan and Saini, 1995; Okuoya and Okogbo, 1991). All the substrates used showed sporophore (mycelia) yields with highest yields recorded in plantain (63.50±2.30 cm), while yam peel substrate was least with 36.60±3.80 cm. This result also agreed with that of Ogbo and Okhuoya (2006) who reported that banana leaf blades (*Musa sp*) gave the highest sporophore yields of *P. tuber regium* than the other substrates used during cultivation of the mushroom.

Analysis of the fruit bodies (fructification) of the mushroom showed that the nutrient content varied depending on the type of substrate used during cultivation. This infers that the medium of cultivation can also influence the nutritional values of the mushroom produced (Ogbo and Okhuoya, 2006; Stamets, 2000). However, the chemical analysis of the vegetative structures of this mushroom was higher in calcium than any other mineral element analyzed for. This could also reflect the chemical composition of the substrate used as the mushroom is able to carry out extracellular digestion of the decomposed substrate and take up the elements into its tissues (Obodai, 2003; Adejoye and Fasidi, 2009). Generally, the mineral element of the highest concentration in the fruit bodies was iron, recorded in plantain peel substrate (1.76±1.02 mg/100 g), while the lowest was yam substrate (0.06±0.06 mg/100 g), indicating that, except for plantain peel, the rest of the substrates had very low presence of iron. This could be due to the presence of high levels of mineral iron in unripe plantain peel than the other agro-waste substrates used here. The high levels also corroborate earlier reports that unripe plantain peels is rich in iron (Ighodoro, 2012). The high levels of mineral iron (Fe), a micronutrient element in unripe plantain peels used as one of the agro-waste substrates promoted the cultivation of *P. tuber regium* in this study. Unripe plantain, apart from the peel, also contains high levels of minerals like potassium (K), calcium (Ca) and manganese (Mn), and

vitamins A, B, B_2, B_3, B_6, hence, it is very rich nutritionally (Danso et al., 2006). It is low in fat, sodium (Na) and cholesterol and that makes it like *P. tuber regium* sclerotium, useful in managing obese, high blood pressure and cardiovascular disease patients in traditional medicine (Fasidi and Olorunmaye, 1994). It also promotes and purifies blood (Oxyhaemoglobin) in the bone marrow, and is also safer for consumption because it contains less toxic and anti-nutrient substances like cyanogenic glucosides (Sharrock and Lusty, 2000).

Based on this research, it could be concluded that the type of substrate used for cultivation affects the nutrient status and morphological characteristics of the mushroom. The study also affirms unripe plantain peels as a very good substrate for the growth and yield of *P. tuber regium*.

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