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Studies on *Pleurotus tuber-regium* (Fries) Singer: cultivation, proximate composition and mineral contents of sclerotia

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Studies were conducted on the growth and cultivation of *Pleurotus tuber-regium* on local cellulosic wastes. *Andropogon tectorum* straw supported the greatest mycelial growth, and cotton and oil palm pericarp wastes supported the least. For cultivation of edible sclerotia, cotton waste and rice straw gave the highest yield and banana leaves gave the lowest. However, with regard to protein, lipid, ethanol-soluble sugar, crude fibre, calcium and magnesium contents, the sclerotia grown on banana leaves were the richest. In contrast, the sclerotia cultivated on corn cob were the poorest in sodium, potassium, calcium, magnesium and phosphorus contents. In all the sclerotia cultivated on banana leaves, corn cob, cotton waste and rice straw, protein and potassium were the most abundant nutrients. These results are discussed in relation to the prospect of cultivating *P. tuber-regium* in Nigeria.

INTRODUCTION

Pleurotus tuber-regium (Fries) Singer is an economically important edible mushroom which is popularly consumed in Nigeria (Oso, 1977). Zoberi (1973) observed that this fungus is common in Nigeria and often found growing around the African breadfruit (*Treculia africana*). It attacks dead wood, on which it produces globose to ovoid sclerotia which can sometimes be up to 30 cm or more in diameter (Oso, 1977). Both the sclerotia and the mushroom fruitbodies are eaten in Nigeria.

Kadiri and Fasidi (1990*a*) have shown that *P. tuber-regium* fruitbody is highly nutritive and very rich in proteins. In Nigeria, *P. tuber-regium* is consumed, not only for its flavour and nutritive value, but also for its beneficial medicinal effects (Zoberi, 1973).

The cultivation of *P. tuber-regium* by people of Nigeria has been considered to be the most primitive (Oso, 1977). Sclerotia are collected from their natural habitats, planted and watered to induce frutification. The cultivation amounts essentially to cropping of sclerotia. In view of rapid agricultural and urban development, which are destroying the natural habitats of this fungus, commercial cultivation will not only ensure that Nigerians obtain this delicacy regularly but also help in preserving the germplasm of the fungus. In our preliminary investigation we have established that *P. tuber-regium* mycelium grows in a temperature range of 20–40°C (optimum 30°C) and pH range of 4–9 (optimum 6·0–7·0). Hence an attempt was made to cultivate *P. tuber-regium* on local cellulosic wastes and to determine the chemical composition of the cultivated sclerotia.

MATERIALS AND METHODS

Inoculum preparation

Sclerotium purchased from Igbara-Oke Market in Ondo State, Nigeria, was used as the initial inoculum source. The sclerotium was washed many times with distilled water, blotted dry and sliced open to obtain mycelial pieces which were cultured and maintained on potato dextrose agar (PDA) throughout the investigation period.

Growth on cellulosic wastes

Andropogon tectorum straw, rice straw, banana leaves, corn cob and cassava peel were chopped into 1–3-cm pieces. These wastes, cotton waste, oil palm pericarp waste and rice husk were separately soaked in hot water (80°C) for 30 min and pressed to expel excess water until the moisture content was about 60%. Test-tubes (2·3 cm × 15 cm) were filled with each waste, sealed tightly with aluminium foil and sterilized at

121°C for 15 min in an autoclave. Each treatment was replicated three times. On cooling, each waste was inoculated with fresh mycelium (5-mm in diameter) and incubated at 30°C for 17 days.

Cultivation of sclerotia

Samples (500 g) of chopped corn cob, rice straw, banana leaves and cotton waste were weighed and soaked in hot water as described above. Aluminium containers were filled with each waste and sterilized in an autoclave at 121°C for 30 min. On cooling, each substrate was mixed thoroughly with 50 g of spawn.

Spawn was produced from a mixture of cotton waste and rice bran (9:1 w/w). Cotton waste, moistened as described above, was mixed thoroughly with rice bran and placed in 500-ml screw-capped bottles. The bottles were autoclaved for 30 min, cooled and inoculated with fresh *P. tuber-regium* mycelium. After incubation for 8 days at 30±2°C, the spawn was ready for use. Spawned substrate was placed in perforated transparent polyethylene bags which were tied at the open end and incubated in the dark at 30±2°C. Each treatment was replicated three times. Sclerotia were harvested 3½ months after spawning, weighed and analysed for mineral contents and proximate composition. The biological efficiency of the substrates was calculated according to the method of Khanna and Garcha (1982). Some sclerotia were stored at 12°C in the refrigerator to test their viability in storage.

Proximate composition

Moisture content of each sample was determined by drying in an oven at 100°C, and crude fibre was determined according to the standard method (Association of Official Agricultural Chemists (AOAC), 1950). Ethanol-soluble sugar was extracted for 6 h in a Soxhlet extractor in boiling 80% ethanol and quantified by the phenol–sulphuric acid method of Dubois *et al.* (1956). Total lipid was determined by the petroleum ether method of Mukiibi (1973) and Parent and Thoen (1977).

Minerals

Phosphorus, potassium, sodium, calcium, magnesium, manganese, iron, copper and zinc were determined at the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, by automated atomic absorption spectrophotometry, flame photometry, and the phosphovanadomolybdate reaction according to the methods described in the IITA manual (1979).

Statistical analysis

The data obtained were analysed by ANOVA and tests of significance were carried out using Duncan's multiple range tests.

RESULTS AND DISCUSSION

The growth of *P. tuber-regium* mycelia was supported on all the substrates, of which *A. tectorum* straw was the best and cotton and oil palm pericarp wastes were the least stimulatory (Table 1). There was, however, no significant difference in the growth of *P. tuber-regium* on *A. tectorum* straw and banana leaves ($P > 0{\cdot}05$). *Pleurotus* species as a class of edible mushrooms are reputed to have a high saprophytic ability and to grow on a variety of cellulosic wastes (Jandaik, 1974; Chang, 1980; Garcha *et al.*, 1984). Their capability to flourish on a wide variety of wastes is attributed to their ability to secrete hydrolysing and oxidizing enzymes (Toyama & Ogawa, 1974; Ulezlo *et al.*, 1975; Daugulis & Bone, 1977; Rajarathnam *et al.*, 1979; Kadiri & Fasidi, 1990*b*). The result of *P. tuber-regium* growth on *A. tectorum* straw is similar to that obtained by Kadiri (1990), who observed that *A. tectorum* straw simulated the highest mycelial growth of *Lentinus subnudus*. The implication of the present finding is that *A. tectorum*, a grass weed that grows luxuriantly on highways in Nigeria, can be used as a substrate for the cultivation of *P. tuber-regium*. The poor growth of *P. tuber-regium* recorded on cotton and oil palm pericarp wastes is surprising because they are good substrates for mushroom cultivation (Chen & Graham, 1973; Nout & Keya, 1983). However, the compactness of these wastes in the test-tubes probably stimulated fermentation which eventually inhibited mycelial growth (Ali & Khan, 1981).

Banana leaves, corn cob, cotton waste and rice straw supported the cultivation of *P. tuber-regium* sclerotia. Cotton waste produced the highest yield (30·11%) and banana leaves produced the lowest (13·58%) (Table 2). These yields are comparable with those recorded for other pleuroti (Graham *et al.*, 1980; Nout & Keya, 1983). There was, however, no significant difference in the yield of sclerotia produced on cotton waste and rice straw. The implication of this is that rice straw can be a good replacement for cotton waste, which is not readily available in Nigeria because of its various domestic and commercial uses. Cotton waste and rice straw, as reported by many workers, are superior

Table 1. Growth of *P. tuber-regium* mycelium on cellulosic wastes (data are means of three replicates ± SE)

	Growth (cm)
Andropogon tectorum straw	$11{\cdot}3^{a} \pm 0{\cdot}8$
Banana leaves	$10{\cdot}8^{ab} \pm 0{\cdot}1$
Cassava peel	$9{\cdot}2^{bc} \pm 2{\cdot}6$
Corn cob	$8{\cdot}6^{c} \pm 1{\cdot}3$
Cotton waste	$4{\cdot}7^{d} \pm 0{\cdot}1$
Oil palm pericarp waste	$4{\cdot}7^{d} \pm 0{\cdot}8$
Rice husk	$10{\cdot}2^{b} \pm 0{\cdot}8$
Rice straw	$88{\cdot}8^{c} \pm 0{\cdot}1$

Means having the same superscript letter(s) are not significantly different ($P > 0{\cdot}01$) by Duncan's multiple range test.

Table 2. Yield and proximate composition of sclerotia cultivated on cellulosic wastes (data are calculated as % dry weight except 'A' and 'B' which are % fresh weight)

	Yield/kg waste (g)	Biological efficiency (%)	Moisture A	Dry matter B	Protein (%)	Lipid (%)	Ash (%)	Crude fibre (%)	Ethanol-soluble sugar (%)
Banana leaf	135·84[c]	13·58[c]	50·94[a]	49·06[b]	16·8[a]	1·02[a]	4·00[c]	8·00[a]	0·24[a]
Corn cob	228·52[b]	22·85[b]	45·62[ab]	54·38[ab]	15·4[b]	0·00	1·00[d]	5·00[b]	0·23[a]
Cotton waste	301·06[a]	30·11[a]	43·77[b]	56·23[a]	15·1[c]	0·20[c]	7·03[a]	8·00[a]	0·16[b]
Rice straw	295·08[a]	29·51[a]	51·35[a]	48·65[b]	13·0[d]	0·72[b]	5·50[b]	7·00[a]	0·19[a]

Means followed by the same superscript letter(s) within each column are not significantly different ($P > 0{\cdot}01$) by Duncan's multiple range test.

to other cellulosic wastes (Ali & Khan, 1981; Nout & Keya, 1983).

Protein was the most abundant nutrient in the cultivated sclerotia, and its value ranged from 13·0% (on rice straw) to 16·8% (on banana leaves) (Table 2). This result agrees with the earlier work done on sporophores of *P. tuber-regium* (Kadiri & Fasidi, 1990*a*), *L. subnudus* and *Termitomyces robustus* (Fasidi & Kadiri, 1990). Hence, the sclerotia of *P. tuber-regium* can be eaten as a protein supplement or an alternative to fish and meat in rural and urban areas where these items are expensive. Mushroom proteins are generally higher in value than those of green vegetables (Chan, 1981).

The ethanol-soluble sugar and lipid contents of the cultivated sclerotia were generally low (Table 2). This means that the sclerotia of *P. tuber-regium* can be consumed by diabetics and those with heart or weight problems (Khan & Kausar, 1981). With regard to protein, lipid, crude fibre and ethanol-soluble sugar, the sclerotia produced on banana leaves were the richest (Table 2). This may be due to the abundance of nutrients in banana leaves (Muirhead & Nicholas, 1985). Banana leaf is known to be a good substrate for mushroom cultivation (Alicbusan, 1981). Cotton waste produced sclerotia which contained the highest amount of dry matter, ash and crude fibre (Table 2), whereas the sclerotia produced on corn cob had the lowest crude fibre content.

Potassium was the most abundant mineral element in the cultivated sclerotia (Table 3). This result is similar to that obtained by earlier workers (Parent & Thoen, 1977; Khanna & Garcha, 1982; Fasidi & Kadiri, 1990; Kadiri & Fasidi, 1990*a*). The preponderance of potassium in the sclerotium tissue may be due to absorption and accumulation of this element from the substrate. *Lentinus tigrinus* has been shown to absorb and translocate ^{32}P from the medium to the sporophores (Littlefield *et al.*, 1965). With regard to potassium and sodium contents, the sclerotia produced on cotton waste were the richest, whereas the sclerotia produced on banana leaves were the richest in calcium and magnesium. In contrast, the sclerotia cultivated on corn cob were the poorest in potassium, sodium, magnesium, calcium and phosphorus (Table 3).

In this study, sclerotium production commenced 5–6 weeks after spawning, as a globose structure outside the substrate. Sclerotia were formed first on the cotton waste. Further growth and development continued until harvesting at 14 weeks after spawning. At harvest, the mycelia have turned light brownish with age. Harvested sclerotia were viable and able to produce fruitbodies 9 months after storage in the refrigerator at 12°C. The implication of this result is that cultivated sclerotia can be eaten, stored or cropped to produce fruitbodies after harvest. The ability to produce sclerotia and fruitbodies makes *P. tuber-regium* a unique fungus with a high potential for commercial cultivation.

From the foregoing it is clear that *P. tuber-regium* sclerotia can be cultivated on local agricultural wastes in Nigeria. Instead of incinerating some of these wastes as is practised now, they can be converted into edible protein-rich sclerotia. Three or four crops can be produced a year, as it takes 3½ months to obtain a crop.

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Table 3. Mineral composition of sclerotium (data are calculated as % dry weight)

	Major elements					Trace elements			
	K	Na	Mg	Ca	P	Mn	Zn	Cu	Fe
Banana leaves	5·78	1·70	1·51	2·10	1·83	0·10	0·03	0·01	0·03
Corn cob	2·45	0·30	0·58	0·62	0·82	0·01	0·03	0·01	0·02
Cotton waste	9·56	2·70	0·93	1·41	1·11	0·06	0·05	0·05	0·05
Rice straw	7·88	2·55	1·03	0·76	1·37	0·08	0·03	0·01	0·01

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