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Spawn production and cultivation of *Pleurotus tuber-regium* on agricultural wastes

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ABSTRACT: Spawn of *Pleurotus tuber-regium* an edible Nigerian mushroom was prepared. It was tested for growth on four organic wastes viz. rice straw, tea leaves, cocoa pods and sawdust. The wastes were supplemented with 10% rice bran. Two types of fruiting bodies were observed, the open and the unopen fruiting bodies. Tea leaves gave the unopen type while sawdust, rice straw, rice straw plus additive gave the open type which is preferred. The yield of 31.4g, 50.0g, 80.0g, 126.0g and 136.6g of *Pleurotus tuber-regium* per kilogramme were harvested on sawdust, tea leaves plus additive, tea leaves, rice straw and rice straw plus additive respectively.

Key Words: Spawn production; Edible mushroom; *Pleurotus tuber-regium*; Agricultural wastes.

Introduction

Mushrooms are protein rich delicious vegetable which is increasingly becoming popular. The cultivation requires little space but much labour and it can be an important means for small scale farmers and land labourers to increase their income (Mante, 1973).

Pleurotus species are found in nature on dead wood as saprophytes and primary 'wood fungi'. They have white oyster – like layers. The fruit bodies of *pleurotus* which are white and pigmented are familiar sight in tropical, subtropical and temperate regions (FAO, 1990).

Pleurotus tuber regium is an economically important edible mushroom popularly consumed in Nigeria (Oso, 1997). Its local name is "Olu – ayunre". Zoberi (1973) observed that this mushroom is common in Nigeria and often found growing around the African breadfruit tree, *Treculia africana*. It attacks and lives on dead wood where it produces globose to ovoid sclerotia which are sometimes as big as 30cm or more in diameter (Oso, 1977). If the sclerotium or "tuber" is kept in a cool moist place, it continues to produce sporophores over a long period of time hence the name *Pleurotus tuber-regium*. The sclerotia and the fruiting bodies are both eaten in Nigeria (Fasidi and Ekuere, 1993).

Mushroom spawn is the mushroom mycelium growing on a given substrate. In nature mushrooms use spores for generative multiplication and these are microscopic and difficult to handle (Oei, 1991). Alternatively, tissue cultures taken from cap-tissues may be used to prepare spawn.

In time the mycelium completely grows through the grain or wood pieces. The mixture of grain or sawdust fully covered by the mycelium is the spawn. This is used to seed bed logs, or other substrates. There are different types of Spawns, Virgin Spawn, Flake Spawn, brick Spawn, Grain Spawn (Atkinson,

1961). Grain Spawn was used in this study because of its ability to ramify the substrate faster and ease of planting (Bahl, 1988).

The production of spawn is a critical process which require expertise. This study was conducted to investigate the procedures for the production of *Pleurotus tuber-regium* spawn and to assess its growth performance on different organic waste.

Materials and Method

Source of Materials

Mushroom culture of *Pleurotus tuber-regium* used for this work was supplied by Professor I.O. Fasidi of the Department of Botany and Microbiology, University of Ibadan. Organic wastes used for the experimental work were obtained as follows:
Rice straw and rice bran were obtained from International Institute of Tropical Agriculture (IITA), Ibadan. Cocoa pods were collected from Sapala village, Abeokuta. Used tea leaves were obtained from students of Awolowo Hall of the University of Ibadan while sawdust was collected from a sawmill at Bodija, Ibadan.

Preparation of culture medium

Potato Dextrose Agar (PDA) was employed as medium of growth for culturing *Pleurotus timber-regium*. PDA was prepared as described by oxoid manual. For one litre of the culture medium prepared, 200g of thinly sliced peeled Irish potatoes; 20g of glucose; 20g of agar powder and one litre distilled water were used.

Culture of fungus

A fresh culture of *Pleurotus tuber-regium* was used. With a sterilized needle, a small piece of the mushroom tissue was removed and placed on PDA medium in Petridishes. The inoculated dishes were incubated at 27°C for ten days. Several sub-culturings were made until pure cultures were obtained.

Spawn preparation

250g Sorghum seeds were soaked in water, drained and put in the spawn bottle. 5g of Calcium Carbonate ($CaCO_3$) were mixed with content in bottle and auto claved at 121°C for 15 minutes. A vigorously growing mycelia culture was used to inoculate the substrate bottles, which were incubated at 27

Test of spawn of Pleurotus tuber-regium on different wastes.

50g of each substrate was measured into spawn bottles except for rice straw where 15g was used because it has large volume though light weight. The bottles were autoclaved at 121°C for 15 minutes consecutively for 3 days. The bottles were inoculated with 5g of the spawn. growth was monitored during a 2 week period.

Radial growth of Pleurotus tuber-regium Mycelia on different wastes.

Petridishes were used as containers. 8g of each substrate were used. A cork borer (1cm diameter) was used to punch the culture to get inoculum. This was placed at the centre of the petridishes and incubated for ten days.

Effect of additive on cocoa powder

Cocoa pods were cleaned and ground to powder. 10g was measured into petridishes. Four treatments were given as follows:

A: 10g of cocoa powder

B: 9.9g cocoa powder, 0.1g rice bran

C: 9.5g cocoa powder, 0.5g rice bran

D: 9.0g cocoa powder, 1.0g rice bran

Growth was observed during a 14 days period.

Result

Fungus culture

White mycelia of the fungus spread over the plate eight days after inoculation. The first culture made is referred to as the starter culture. Subculturing was made from it (Plate 1).

Spawn of Pleurotus tuber-regium

White mycelia started spreading through the sorghum grains on 3rd day; while complete ramification of grains was observed on the 14th day. The substrated in the bottle turned white at the end of the ramification and this was actually 'the spawn' which was used for cultivation (Plate 2). Unused spawn kept in the refrigerator at 150°C was found to be viable for five months. This is because experimental trials after five months with the spawn produced neither mycelia nor fruiting body.

Performance of Pleurotus tuber-regium on different substrates.

After the inoculation of the different substrates with the spawn, growth initiation was observed in all substrates between 3 to 4 days. Total Mycelia Run (TMR) varied between 14 to 18 days for the different substrates (Plate 3). As mycelia ramified the substrates, some thick strand of hypae were noticed which are referred to as 'hyphae strands'.

This was most obvious on tea leaves.

Appearance of fruiting body on substrates

Among the substrate that produced fruiting bodies, sawdust gave the least yield with 31.4g/kg while rice straw plus additive gave 136.6g/kg. Two types of fruiting bodies were observed – open and unopen types. Tea leaves gave the unopened type while other substrates gave fully opened fruiting bodies with oyster shape. Cocoa pods did not support the growth of fruiting bodies although mycelia growth was observed on it. Growth of fruiting body differs from one substrate to another (Table 1). Plates 4 and 5 show the appearance of fruiting body on the substrates.

Radial growth of Pleurotus tuber-regium on substrates.

Growth pattern on three replicates for each substrate was recorded for ten days (Table 2). There were no significant difference at 5% level of significance between the radial growth of *Pleurotus tuber-regium* on cocoa powder, sawdust, rice straw and tea leaves. The coefficient of variation CV was 16.4%.

Effect of additive on cocoa powder

Growth pattern was recorded for ten days on three replicates of the four treatments and data analysed by ANOVA. Tests of significance were carried out using Duncan's Multiple Range Test (Table 3 and Plate 6).

Plate 1: *Pleurotus tuber-regium* culture.

Plate 2: Spawn of *Pleurotus tuber-regium*.

Plate 3: The appearance of Pleurotus tuber-regium mycelia on each substrate.

A: Cocoa pod; B: Cocoa pod + 10% Rice bran; C: Sawdust; D: Sawdust + 10% Rice bran; E: Tea leaves; F: Tea leaves + 10% Rice bran; G: Rice straw; H: Rice straw + 10% Rice bran.

Table 1: Appearance and yield of fruiting bodies.

Substrates	**Yield 9/kg***
Sawdust	31.4
Sawdust + 10% rice bran	-
Rice straw	126.0
Rice straw + 10% rice bran	136.6
Cocoa powder	-
Cocoa powder + rice bran	-
Tea leaves	80.0
Tea leaves + 10% rice bran	50.0

*Yield was based on dry matter of fruiting body in g/kg dry weight of the substrate.

Table 2: Radial growth of *Pleurotus tuber-regium* mycelia

Substrates	**Mycelia growth (cm)**	**Mycelia density**
Cocoa	4.86	4+
Sawdust	5.66	+
Rice straw	5.06	2+
Tea leaves	4.32	3+

Plate 4: Fruiting bodies on (A) Tea leaves, (B) Tea leaves + 10% Rice bran, (C) Sawdust.

Plate 5: Fruiting body on rice straw.

Plate 6: Effect of rice bran additive on cocoa powder.

(1) 9.0g Cocoa powder + 1.0g Rice bran; (2) 9.5g Cocoa powder + 0.5g Rice bran; (3) 9.9g Cocoa powder + 0.1g Rice bran; (4) 10g Cocoa powder + 0g Rice bran;

Table 3: Effect of additive on cocoa pod

Treatments	**Mycelia growth (cm)**
10g cocoa powder, 0g rice bran	4.07[a]
9.9g cocoa, 0.1g rice bran	4.87[a]
9.5g cocoa powder, 0.5g rice bran	5.97[b]
9.0g cocoa powder, 1.0g rice bran	6.60[b]

Means followed by the same letter are not significantly different at 5% probability by Duncan's Multiple Range test.

Table 4: pH and nutrient contents (g/kg) of substrate

	pH	Nutrient content (g/kg)						
		Ca	K	Na	P	N	Organic	Carbon
Cocoa pod	6.3	2.3	43.5	0.2	0.10	25.2	251.7	
Rice straw	4.8	1.2	16.5	1.7	0.04	38.3	353.5	
Sawdust	8.1	7.2	6.6	0.2	0.00	36.8	368.0	
Tea leaves	3.7	0.3	0.4	0.4	0.04	25.6	255.7	
Cocoa + 10% rice bran	5.7	1.45	31.0	0.2	0.20	39.5	395.1	
Rice straw + 10% rice bran	-	5.0	0.9	10.0	0.9	0.20	36.8	368.0
Sawdust + 10% rice bran	-	5.3	0.1	8.0	0.2	0.10	34.9	348.7
Tea leaves + 10% rice bran	-	4.4	0.8	7.0	0.2	0.20	31.0	309.9
Rice bran	5.8	-	-	-	-	31.8	317.7	

Source: Department of Agronomy, Analytical Research Laboratory, 1997.

Discussion

All the substrates used in this study supported the growth of *pleurotus tuber-regium. Pleurotus* species are reported to have a high saprophytic ability to grow on a variety of cellulosic wastes (Jandaik, 1974). Kadiri and Fasidi (1990) observed that their capability to flourish on a wide variety of wastes is attributed to their ability to secrete hydrolysing and oxidizing enzymes.

Mushroom spawn is the mushroom mycelium growing on a given substrate. It serves as the planting material in mushroom cultivation. There is need to culture mushroom and use the mycelia to produce the spawn as described earlier under materials and method because natural mushroom spores are microscopic (although large in numbers) and difficult to handle.

There are different types of spawns such as virgin, flake, brick and grain spawn (Atkinson, 1961). However, grain spawn was used in this study because of its ability to ramify the substrates faster and because of ease of planting as similarly noted by Bahl (1988).

Mushroom spawns could be stored if they are not to be used immediately. From this study, the viability of the spawns lasted five months at 4°C of refrigeration after which it became non-viable. This is because the older the spawn, the drier it becomes. This agrees with the observation of Heltay and Barber (1959) that the storage condition of spawns affect its productivity. They observed that the productivity of spawns was reduced by 5, 6 and 8 percent if the spawn was kept at –20°C for 68, 128 and 256 days respectively. Stroller (1962) similarly observed that spawn taken directly from the growing room grew faster than spawn kept in the refrigerator at 2°C. Lemke (1968) reported that cream and white strains give different reactions to storage condition of wheat grain spawns. Sengbusch (1968) stored the spawn of the cream variety for four months at 20°C and white strains for up to six months at the same temperature. Spawn stored at 2°C for 2 to 6 months reduced the productivity of mushroom (Heltay and Barber, 1959).

The fastest growth of mycelia observed on sawdust may be due to the lightness of the substrate and also its loose texture which allows for aeration and quicker penetration.

This is in agreement with the observation of Awashi and Mehrotra (1986) that aeration is an important factor in growth and fruiting of edible mushrooms. The thick hyphae strands which were observed on tea leaves may be due to favourable nutritional materials.

As quality of rice bran added to cocoa powder increases, radial growth of mycelia on cocoa powder increased. This effect shows that better result may be achieved by increasing the quantity of additives on

substrates. From the analysis made (ANOVA) there were significant difference among the treatments (Table 3). This further buttressed the fact that the higher the quantity of additive, the better the result obtained. According to Visscher (1989), there may be a positive correlation, no correlation or negative correlation between substrate and additive depending on how it is added.

The fastest mycelia growth was observed on sawdust, followed by rice straw, cocoa powder and tea leaves. This is explained by lightness of substrates. The lighter the substrate, the faster the mycelia ramification. Cocoa powder sustained the highest mycelia density while sawdust, the least. This is because of the compact nature of the cocoa powder particles.

No fruiting was observed on cocoa, cocoa plus additive, and sawdust plus additive. This most probably is due to environmental problem. Compactness of the materials might have also contributed to this result. Oei (1991) wrote that pH, aeration, available nutrient, water contents and microbial activity determine the quality of substrates which in turn determines yield.

The number of fruit bodies produced per bottle is small. This is mainly due to the size of bottle and the substrate it could accommodate for the purpose of this experiment. A bigger bottle would accommodate more substrate and most likely produce more fruiting bodies during fruiting.

There are two types of fruiting bodies produced. Fruiting bodies produced on tea leaves are not opened (cap not expanded). This may be due to acidity of the substrate. Rice straw, sawdust, rice straw plus additive gave the open type because of the favourable pH (Table 4). During grading, the two types were separated. The open types are preferred. Burden (1972) and Verdder (1971) explained that mushrooms should be graded into unopen fruit bodies, opened but cupped and fully opened fruit bodies.

From the observation in this study the use of rice straw with or without additive as substrate for growing *Pleurotus tuber-regium* is recommended.

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