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DETERMINATION OF APPROPRIATE GROWTH MEDIUM FOR PRODUCTION OF SCLEROTIA IN*PLEUROTUS TUBER-REGIUM*

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ABSTRACT

This study determined appropriate growth medium for production of sclerotia in Pleurotus tuber-regium. Due to climate change and other anthropogenic activities, such as forest depletion, sclerotia sourcing form the wild is tending towards extinction. An alternative production of sclerotia is therefore imperative. This research was conducted in the Mushroom Farm of the University of Port-Harcourt, Nigeria. Fresh and Composted sawdust were used for the cultivation of Pleurotus tuber-regium to determine a better substrate medium for sclerotia production. The composted sawdust was left to decompose for two weeks while the fresh sawdust was used without decomposition. Mycelia ramification and sclerotia production were observed in the course of the research. Full mycelia ramification for composted substrate took more days (26 days from inoculation of substrate with mushroom spawn) compared with fresh sawdust (25 days from inoculation of substrate with mushroom spawn). However, there was no significant difference for mycelia ramification between the substrates. Sclerotia production took shorter days on composted substrate (47 days from inoculation of substrate with mushroom spawn)and significantly different (P≤0.05) from production on the fresh sawdust (49 days from inoculation of substrate with mushroom spawn).Fresh weight of sclerotia for the composted sawdust weighed 21.6± 5.6 grams while the fresh weight of sclerotia for the fresh sawdust weighed 15.6±3.9 grams; dry weight of sclerotia for the composted sawdust was 11.6±4.5 grams while that of the fresh sawdust was 8.2±3.0 grams. Composted sawdust is recommended for the cultivation of Pleurotus tuber-regium for production of sclerotia.

Key words: *Pleurotus tuber-regium*, Sclerotia sustainability, Fresh sawdust, Composted sawdust.
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INTRODUCTION

Mushrooms are super food with increased consumption and other uses because of their various benefits to humans and their environment. They are often referred to as vegetables. Mushroom is a spore bearing fruiting body of a fungus typically produced above ground, on soil or its substrate. Like all other fungi, mushroom are not plants and do not undergo photosynthesis. However, Oei (2016), reported a high technology approach where a greenhouse could be built on top of mushroom house thus allowing gas exchange from both types of growing houses. The unique feature of *Pleurotus tuber-regium* among other oyster mushrooms is the production of underground tubers, often referred to as sclerotia. Fruiting bodies are produced as well and both the sclerotia and the fruiting bodies of the mushroom

are edible. The mushroom is similar to oyster mushroom (*Pleurotus ostreatus*) in look but when mature the cap curves upward to form a cup-like shape. The sclerotium is spherical to ovoid in shape and varies in diameter (Onuoha and Obi-Adumanya, 2010).

Sclerotia are resistant to extreme environmental conditions found in some distribution areas, for example the hot dry season of West Africa. They can serve as food, medicine and inoculums as well as induced to produce sporophores (Fasidi and Ekuere, 1993). Sporophores harvested at young stage are delicious and can be eaten when fresh or dried for future use (Isikhuemhen *et al*., 2000a). *Pleurotus tuber regium* is one of the popular mushrooms indigenous to and consumed in Nigeria. In the southern part of Nigeria, it is used as condiment and food thickening especially in "egusi" melon soup. As reported by Okhuoya *et al.* (2010), it is used as food, serving as meat as well as food supplement. It is a great medicinal source for native doctors in the treatment of many ailments including asthma, small pox, high blood pressure, heart diseases and diabetes (Oso, 1977).

Various laboratory studies have been carried out on *Pleurotus tuber-regium* (Isikhuemhen *et al*., 2000a, Adedokun *et al*., 2003); and on its medicinal uses, and nutritional properties ((Fasidi and Ekuere, 1993, Afieroho *et al.,* 2013). However, this mushroom is not being produced as other oyster species. The natural habitat for sclerotia is the forest. Due to climate change and other anthropogenic activities, such as forest depletion, sclerotia sourcing from the wild is tending towards extinction.An alternative production of sclerotia is therefore imperative. The general objective of this study is to cultivate sclerotia of *P. tuber-regium* on fresh and composted sawdust substrates. The specific objective of this study is to determine the better substrate to produce sclerotia of *Pleurotus tuber-regium*.

MATERIALS AND METHOD

The experiment was carried out at the University of Port-Harcourt Teaching and Demonstration Farm Choba Campus and Microbiology laboratory in the Faculty of Pharmacy, Abuja campus.

Sources of Materials: The fruiting body of *Pleurotus tuber-regium* used in this study was the strain UP1307 obtained from the Mushroom Farm, Faculty of Agriculture, University of Port- Harcourt. Sawdust and wheat bran were obtained from the sawmill and the market near the campus respectively.

Preparation of Substrate: Modified method of Gbolade (2006), was used. Sawdust, wheat bran and Calcium carbonate ($Caco_3$) were thoroughly mixed together in ratio 1:0.5:0.01. Water was added until the moisture content of 40-60% was attained. For fresh substrate, 500 grams of the mixture was weighed into transparent polyethylene bag and sterilized using industrial autoclave for 15 min. at 121°C to eliminate unwanted microorganisms.This was replicated ten times and inoculated with 10% cotton waste spawn. Incubation was at room temperature 28±2 °C for six (6 weeks). Sawdust for composted substrate after mixture as described above, was stacked into heaps, covered with jute bags and left for 2 weeks with

regular turning and re-stacking every other day, this was done to produce homogenous compost. The process of sterilization, inoculation and incubation as described above was applied after two weeks.

Data Collection

Weight of sclerotia harvested from fully ramified bags was recorded for both fresh and composted samples using electronic weighing balance. Fresh sclerotia were sun dried for one week. When there was no reduction in weightafter weighing, the final reading was recorded as dry weight (DW) of the sclerotia. This was done twice during the period of the experiment.

Data analysis

The results obtained were statistically analyzed using analysis of variance (ANOVA), and tests of significance carried out by Duncan's multiple range test (Steel and Torrie 1980) at $P \leq 0.05$.

Results and Discussion

Mycelia ramification

Mycelia ramification which is the first stage to sclerotia production was observed on both fresh and composted substrates. Dense mycelia of *P. tuber-regium* ramified the two substrate type at different rates. Initiation of mycelia was observed from the fifth day and complete ramification was after one month (Table 1). Full mycelia ramification for composted substrate took more days (26 days from inoculation of substrate with mushroom spawn) than with fresh sawdust (25 days from inoculation of substrate with mushroom spawn). However, there was no significant difference for mycelia ramification between the substrates. The observation about initial mycelia ramification was similar to the report of Isikhuemhen and Okhuoya (1996) in a related study where mycelia initiation was observed within seven days.

Production of Sclerotia

Performance of sclerotia on fresh and composted sawdust is as presented in Tables 1 and 2. Fresh and composted sawdust substrates produced sclerotia of *P. tuber-regium.* Initial signs of sclerotia formation (white roundish mass of dense hyphae) were first observed on composted sawdust 47 days from inoculation of substrate with mushroom spawn prior to the observation on fresh sawdust (49 days from inoculation of substrate with mushroom spawn) and significantly different ($P \leq 0.05$) from fresh sawdust. This shows that the composted sawdust substrate attained full mycelia ramification faster than the fresh substrate. Plate (1 A and B) (appendix) shows fresh and dried sclerotia produced in the course of this study. There was no appreciable difference in physical observation (except for dryness) between sclerotia from fresh and composted substrates.

Harvest of sclerotia was after three months. The number of sclerotia harvested from each substrate is represented in Table (2) and significantly different ($P \leq 0.05$). The yield and

weight of sclerotia are also indicated (Table 2). The yield on composted substrate was better than fresh sawdust. This probably could be due to the effect of composting on the substrate where nutrients were easily made available for the use of the fungus. Addition of wheat bran as a nitrogen source enhanced the process of composting (Richard, 1996).

The higher yield of *P. tuber-regium* observed on composted sawdust agrees with the report of (Olufokunbi and Chiejina, 2010), where a mixture of rice bran and fermented sawdust (composted sawdust) were used for sclerotia production. Other studies with similar reports are Onuoha and Obi-Adumanya(2010), and Isikhuemhen and Okhuoya(1996). Visual observation of sclerotia harvested on fresh and composted sawdust did not reveal any significant difference. Since no nutritional analysis was conducted, no conclusion could be drawn at this time as to the chemical constituents of sclerotia from the different treatments.

Conclusion

Sclerotia of *Pleurotus tuber- regium* which is often sourced from the forest as natural habitat could be cultivated in a controlled environment. This study has shown that the usage of composted sawdust for sclerotia production yielded better, faster and higher than the fresh substrate.

Recommendation: Composted sawdust substrate is recommended for timely and sustainable production of *Pleurotus tuber regium* sclerotia.

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APPENDIXES

Plate 1:
A: Fresh Sclerotia of *P. tuber-regium* produced in the course of research
B: Dry Sclerotia of *P. tuber-regium* produced in the course of research

Source: Field Survey, 2012

Table 1: Number of days for Mycelia Ramification, Sclerotia Initiation and Sclerotia Harvest

Treatment	Duration (Days)		
	Mycelia Ramification	**Sclerotia Initiation**	**Sclerotia Harvest**
Fresh Sawdust	25.00[a]	49.00[a]	80.00[a]
Composted Sawdust	26.00[a]	47.00[b]	76.00[a]
S.E.D	4.150	0.979	1.871

Note: Values in the column bearing the same letters do not differ significantly at P = 0.05. Means were separated using S.E .D (standard error for difference of means).

Source: Field Survey, 2012

Table 2:Sclerotia Yield of Fresh and Composted Sawdust

Treatment	Fresh Weight (g)	Dry Weight(g)	Number of Harvested Sclerotia
Fresh Sawdust	15.60±3.9[a]	8.20±3.0[a]	1.0± 0.0[b]
Composted Sawdust	21.60±5.6[a]	11.60±4.5[a]	1.8± 0.84[a]
S.E.D	3.053	2.408	0.140

Note: Values in the column bearing the same letters do not differ significantlyat P = 0.05.

Means were separated using S.E .D (standard error for difference of means).

Source: Field Survey, 2012