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Full Length Research Paper

Effects of different substrates on the yield and protein content of *Pleurotus tuberregium*

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The effects of seven substrates for the cultivation, yield and protein content of the mushroom, *Pleurotus tuberregium* (Fries) Singer were investigated. The experimental design used was completely randomized design (CRD) of 7 treatments and 10 replicates. The highest fresh weight yield was obtained from mushrooms grown in river sand and sawdust mixture (T6) while the least was from those grown in top soil mixed with sawdust (T4). Oil palm fruit fibre (OPFF) substrate (T3) produced no fruit bodies at all. Protein analysis of the mushrooms revealed that those grown in river sand alone (T1) had the highest protein content while fruit bodies harvested from sawdust substrate alone (T2) had the least protein content. River sand plus sawdust substrate (T6) is recommended as the best substrate for the production of *P. tuberregium* in view of the fact that it produced the highest fruit body weight and its protein content was not significantly lower than that of river sand alone (T2).

Key words: Mushroom, *Pleurotus tuberregium*, substrates, protein.

INTRODUCTION

A mushroom is the fleshy, spore-bearing fruit body of a fungus, typically produced above ground or on a substrate (Boa, 2004; Oei and Nieuwenhuijzen, 2005; Stevenson and Lentz, 2007). *Pleurotus tuberregium* (Fries) Singer also known as the king tuber oyster mush-room is an example of an edible mushroom found in tropical and subtropical regions of the world (Oso, 1977b; Isikhuemhen and LeBauer, 2004). The sclerotium is often of a dark brown colour externally and white inside. It is popularly used in Nigeria as food and/ or medicine (Oso, 1977a, 1977b; Isikhuemhen and LeBauer, 2004). Mushrooms have been considered as a source of rich food because they contain proteins, sugars, glycogen, lipids, vitamins, amino acids and crude fibres. The protein value of mushrooms is twice that of asparagus and potatoes, four times that of tomatoes and carrots and six times that of oranges (Adejumo and Awosanya, 2005; Jonathan et al., 2006). Apart from being a relatively much cheaper source of protein compared with animal proteins, mushrooms could also hold special attraction and may be recommended for people with cholesterol-related ailments (Adejumo and Awosanya, 2005; Jonathan et al., 2006).

The sclerotia are usually harvested from decaying logs; the dark brown exterior is peeled off and the white compact mycelial tissue used for food or medicine (Oso, 1975, 1977a, 1977b; Isikhuemhen and LeBauer, 2004). Proximate analysis of both sclerotia and sporophores carried out by Ogundana and Fagade (1981), Isikhuemhen and LeBauer (2004) and Jonathan et al. (2006) showed that they are rich in carbohydrates, proteins, vitamins and minerals and low in fats. Recent scientific studies have shown that sclerotia of *P. tuberregium* contain polysaccharides and other compounds with positive medicinal benefits (Ogundana and Fagade, 1981; Isikhuemhen and LeBauer, 2004; Gregori et al., 2007).

Apart from the nutritional value and the medicinal uses, many workers like Atlas and Bartha (1992), Isikhuehmen et al. (2003) and Adenipekun (2008) have reported the use of *Pleurotus* species in bioremediation exercises. *P. tuberregium* (a white-rot fungus) has been reported to ameliorate crude oil polluted soil and the resultant soil sample supported germination and growth of *Vigna unguiculata* (Isikhuehmen et al., 2003).

It is of interest to note that this mushroom has always

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been grown mainly from agricultural or domestic wastes which are readily available (Okhuoya and Okogbo, 1991; Oei, 2003; Isikhuemhen and LeBauer, 2004). The focus of this work, therefore, was to determine the best substrate for maximum yield and the nutritional quality of the mushroom with respect to its protein content, with a view to advising farmers on its production.

MATERIALS AND METHODS

Sources of materials

The sclerotia used in this study were obtained from Ugbogo-Nike, Enugu east local government area of Enugu State, Nigeria. Fresh hard wood sawdust was collected from timber-shed, Ugbene II, Abakpa, Enugu, Enugu State, while oil palm fruit fibre (OPFF) was collected from Ugwuafor, Akpugo in Nkanu west local government area of Enugu State. All the other substrates used like top soil and river sand were collected from the school farm and premises of the School of Preliminary Studies, Doyen Academy, Enugu.

Preparation of substrates

To a heap of sawdust on a cement platform, water was added in the ratio of 1:2 (v/v) and the substrate piled up into a heap of 1.3 m high by 1.2 m diameter and covered with a black plastic poly-ethylene sheet to undergo fermentation for four weeks. Fermented sawdust was mixed with fermented OPFF in a ratio of 1:1 (v/v) and water was added to the substrate in a ratio of 1:2 (v/v). The sawdust and OPFF substrate was piled into a heap of 1.5 m high by 1.5 m diameter and covered with a black polyethylene sheet to undergo fermentation for four weeks. The substrates used were: top soil (T0), river sand (T1), fermented sawdust (T2), fermented OPFF (T3), mixture of topsoil and fermented sawdust (T4), mixture of fermented OPFF and fermented sawdust (T5), mixture of river sand and fermented sawdust (T6). These mixtures were in the ratio of 1:1 (v/v).

Experimental layout

All treatments for the experiment were laid out following the complete randomized design (CRD) and each treatment was replicated ten times.

Inoculation and incubation

Two hundred grams (200 g) of each substrate was placed in a poly-propylene plastic bag (17.5 cm high x 15 cm width). The sclerotia were soaked in water for 15 h and sliced into sets of about 6 cm^3. The sliced sclerotia were seeded into the bags containing the sub-strates and watered enough to create a humid environment required for fructification. The bags of the inoculated substrates were placed on laboratory benches at room temperature (25 ± 2^0C) for observation of fungal growth for 3 weeks. The cultures were slightly watered daily to keep them damp.

Data collection

The growth of the mushrooms in the different substrates was recorded weekly. The yield of the mushrooms from the different substrates involved was determined in terms of the height and diameter of the stipe, diameter of the pileus and the fresh and dry weight of the harvested mushrooms. The duration of the stipes to produce pinheads and to mature was also noted and recorded for statistical analysis.

Protein analysis

The mushrooms harvested from the different substrates were dried before they were analysed for their protein content using Micro-Kjeldahl's method (AOAC, 1990). Oven dried samples of 0.5 g from each substrate was separately put into 30 ml Kjeldahl flask and 15 ml conc. H_2SO_4 added. The mixture was cautiously heated in a fume hood until a greenish clear solution appeared. The digest was allowed to cool for 30 min and 10 ml distilled water added to prevent caking. The digested sample was distilled and 35 ml of the distillate collected in a receiver flask. This was titrated with 0.01 M HCl until a pink colour emerged. Percentage protein was calculated as percentage nitrogen x 6.25.

Data analysis

The results obtained were statistically analysed using analysis of variance (ANOVA), and tests of significance carried out by Duncan's multiple range test.

RESULTS

The results indicated that river sand substrate (T1) mushrooms had the highest stipe height (6.62 cm) and the mixture of top soil and fermented sawdust substrate (T4) mushrooms had the least stipe height (0.96 cm) and no growth at all (0.00 cm) in the fermented OPFF substrate (T3) (Table 1). The table also shows the fruit bodies with widest stipe diameter (1.63 cm) as those grown in both top soil substrate (T0) and the mixture of river sand and fermented sawdust (T6), while the least (1.11 cm) was from the mushrooms grown in the mixture of fermented OPFF and fermented sawdust substrate (T5). The effect of the different substrates on pileus diameter is shown in Table 1. Mushrooms grown on top soil substrate (T0) had the widest pileus diameter (10.40 cm) while the mixture of top soil and fermented sawdust substrate (T4) produced mushrooms with the least pileus diameter (0.79 cm). Table 1 also shows that the highest fresh weight (13.759 gm) mushrooms were obtained from those grown in the mixture of river sand and fermented sawdust (T6) and the least (1.423 gm) from those grown in the mixture of top soil and fermented sawdust sub-strate (T4). It is note worthy that no fruit bodies were produced in fermented OPFF substrate (T3) throughout the experiments (Table 1) but rather, extensive mycelial ramifications were observed in the substrate.

The histogram, (Figure 1), shows that the mixture of river sand and fermented sawdust (T6) gave the highest dry weight yield (5.45 gm) of the mushrooms while the

Table 1. Effect of different substrates on *P. tuberregium* stipe height and diameter, pileus diameter and fresh weight.

Treatment	Stipe height (cm)	Stipe diameter (cm)	Pileus diameter (cm)	Fresh weight (g)
T0	4.82b	1.63a	10.40a	9.443a
T1	6.62a	1.45a	6.05b	10.893a
T2	5.63a	1.29a	5.94b	10.270a
T3	0.00c	0.00c	0.00c	0.00c
T4	0.96c	1.30c	0.79c	1.423b
T5	4.15b	1.11b	5.99b	7.619a
T6	5.46a	1.63a	6.05b	13.759a

Each value is a mean of 10 replicates. Values in the same column followed by the same letter (s) are not significantly different according to Duncan's multiple range test (P≤ 0.05). T0 = Top soil substrate; T1 = river sand substrate; T2 = fermented sawdust substrate; T3 = fermented OPFF substrate; T4 = mixture of topsoil and fermented sawdust substrate; T5 = mixture of fermented OPFF and fermented sawdust substrate; T6 = mixture of river sand and fermented sawdust.

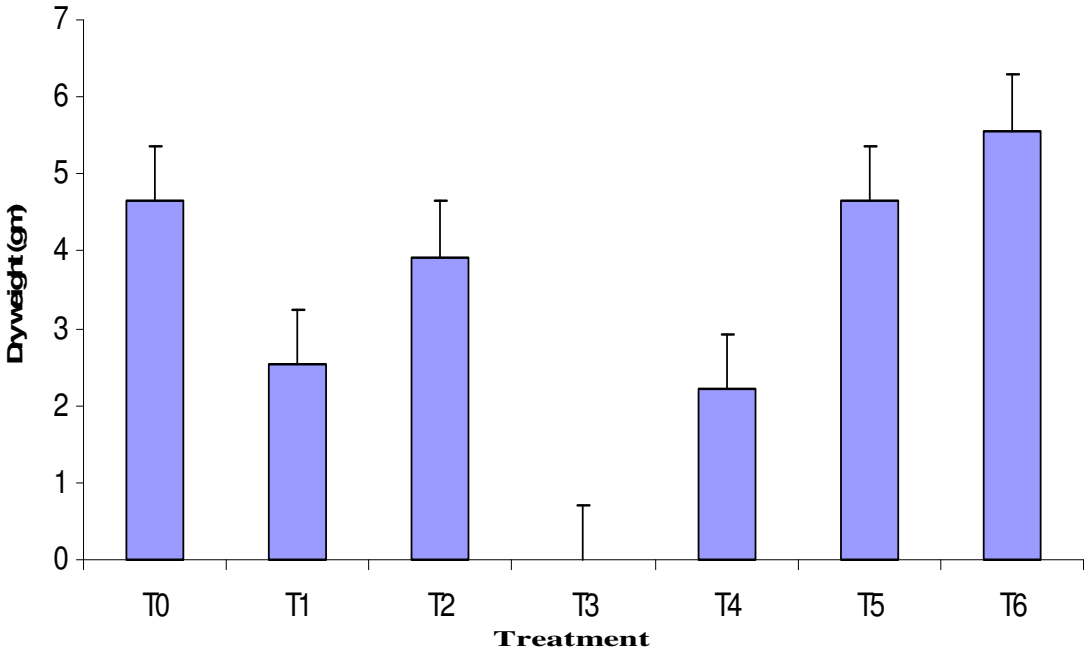

Figure 1. Dry weight of harvested mushroom. T0 = Top soil substrate; T1 = river sand substrate; T2 = fermented sawdust substrate; T3 = fermented OPFF substrate; T4 = mixture of topsoil and fermented sawdust substrate; T5 = mixture of fermented OPFF and fermented sawdust substrate; T6 = mixture of river sand and fermented sawdust. LSD (P = 0.05) = NS. Protein content (%).

mixture of top soil and fermented sawdust substrate (T4) produced the least dry weight (2.20 gm).

River sand substrate (T1) mushrooms gave the highest percentage protein content (25.19%) while fermented sawdust substrate alone (T2) produced mushrooms with the least percentage protein content (20.5%) (Figure 2).

DISCUSSION

The results obtained in this study revealed that the mixture of river sand and fermented sawdust (T6) produced mushrooms with highest fresh and dry weights (Table 1 and Figure 1). This could probably be due to the river sand providing good aeration for the germination and fructification of the mushrooms. Sawdust has been reported as the best substrate for mycelial growth and fructification (Kadiri and Fasidi, 1990). Also sandy soils of which river sand is one offer the least resistance to enlargement of sporophores unlike compact soils like top soil that would have to combat with microbial antagonism as well. The combination of the qualities of the river sand

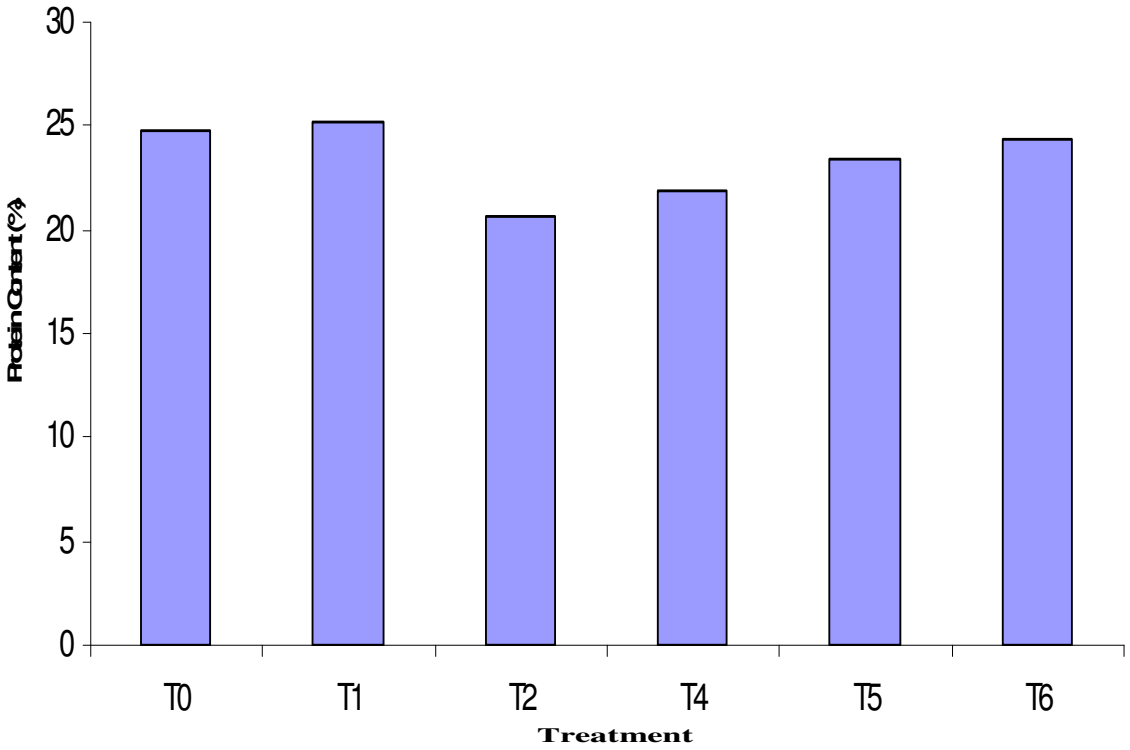

Figure 2. Percentage protein content. LSD (P = 0.05) = NS.

and the sawdust may have been responsible for the highest yield recorded in that substrate. A mixture of top soil and sawdust (T4) gave the least yield as can be seen in Table 1 apart from the no growth at all in fermented OPFF (T3). The observation in the latter could probably be due to the inhibitory effects of pathogens present in the fermented OPFF which might have had antagonistic effects on the growth of mushrooms in that substrate. However, the ability of top soil substrate (T0) to produce good stipe and pileus diameter (Table 1) and dry weight yield of the mushrooms (Figure 1) agrees with the findings of Okhuoya and Etugo (1993) who reported loam soil (very similar to top soil) as the best for planting sclerotia, and that could be due to its high water holding capacity.

The fact that sclerotia inoculated into a mixture of river sand and fermented sawdust (T6) could yield the highest fresh and dry weights of the mushrooms and were significantly different from those of some treatments as seen in Table 1 and Figure 1 (P = 0.05) is an indication that nutrients required for growth and fructification of the mushrooms are inherent in the sclerotia. This observation agrees with the result of Okhuoya and Okogbo (1991). Extensive mycelial production was observed in the fermented OPFF (T3) substrate and it is not clear whether this extensive mycelia produced in (T3) had any inhibitory effects on sporophore production and it would be interesting if this observation is investigated. The very high fertility of the fermented OPFF substrate may be responsible for the extensive mycelia (a type of vegetative growth) produced. The fermented OPFF nutrients were not depleted within the duration of the experiment and together with those already present in the sclerotia, there appeared to be too much for fruit body production to commence.

The highest percentage protein occurred in mushrooms grown in river sand (T1) substrate and the least was in those grown in sawdust (T2) substrate (Figure 2). River sand offers least resistance to sporophore enlargement unlike compact soils, so the large sporophores from the river sand substrate would have more proteins while the fermented sawdust addition would make the river sand more compact; so the sporophores would not grow as large as in the river sand alone, so less proteins. Also the microbes from the fermented sawdust may feed on the proteins in the sporophores hence less proteins than in river sand alone (T1) substrate. Okhuoya and Okogbo (1991) reported that sclerotia have already stored in them all the nutrients required for fruiting, this therefore, explains why river sand with little or no fertility could produce mushrooms with the highest percentage protein while the other substrates may have to combat first with the microbial antagonists in them.

P. tuberegium mushroom in this study was grown from

its sclerotia inoculated into the various substrates investigated. Some other workers (Okhuoya and Okogbo, 1990; 1991; Isikhuehmen and LeBauer, 2004; Onuoha, 2007) have also employed the same procedure of growing it from its sclerotia because it is much faster than growing it from the spawn raised from the spores.

Conclusion

The study suggests that *P. tuberregium* can be grown in essentially the same ways as other *Pleurotus* species using lignocellulosic agricultural wastes as substrates. Although, there have been a few studies on nutritional values of cultivated Nigerian mushrooms, there appears to be no available data on the protein content of *P. tuberregium* mushroom.

Since *P. tuberregium* mushroom was confirmed to have a higher percentage of proteins than most leguminous plants and vegetables (Adejumo and Awosanya, 2005; Jonathan et al., 2006), it is hereby suggested that it be used as a substitute for meat and fish which are very expensive for most middle class families in developing nations like Nigeria.

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