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EFFECTS OF DIFFERENT SUBSTRATES ON THE YIELD AND NUTRITIONAL VALUE OF *Pleurotus tuberregium* (FR.) SING

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ABSTRACT

Two experiments were conducted to study the effects of different substrates on the yield and nutritional content of *Pleurotus tuberregium* (Fr.) Sing. Seven different substrates were used in both experiments to grow the mushrooms and sclerotia, respectively. The experiments were carried out using a completely randomized design (CRD). Fresh and dry weights of the harvested mushrooms and sclerotia were recorded and the proximate analysis of the mushrooms conducted using Association of Official Analytical Chemists (AOAC's) methods. Analysis of variance (ANOVA) was used for data analysis and test of significance carried out by the Duncan's multiple range test. Results of the mushroom cultivation experiment showed that mean dry weights varied from 0.22 g for mixture of topsoil and fermented sawdust substrate (M5) to 3.34 g for mixture of river sand and fermented sawdust substrate (M7), while the mean fresh weights varied from 1.42 g for M5 to 13.76 g for M7. The mean fresh weights of M2, M3 and M7 were not significantly different from one another but were significantly different from those of M4 and M5. Furthermore, percentage carbohydrate content ranged from 59.03% in M1 to 65.41% in M2 while that of crude protein varied from 14.88% in M3 to 17.78% in M1. For the second experiment, the rate of substrate colonization differed significantly ($P \leq 0.05$) for the treatments. The mean colonization rate varied from 0.0 day in S2, S3 to 31.2 days in S5, S4 and S6, which were not significantly different from each other but were significantly different from S1, S5 and S7 and from S2 and S3. The mean dry weight yield varied from 46.26 g in S5 to 127.48 g in S1. The biological efficiency of sclerotia harvested from S1, S6 and S7 were not significantly different from one another but were significantly different from those of S4 and S5 substrates. Considering all the parameters investigated; a mixture of river sand and fermented sawdust substrate (M7) is recommended as the best substrate for the cultivation of *P. tuberregium* mushrooms while a mixture of corn waste and fermented sawdust substrate (S6) is recommended for sclerotial cultivation. Mushrooms grown on topsoil substrate (M1) are recommended as the best for nutritional supplement.

Key words: *Pleurotus tuberregium*, mushroom, nutrition, sclerotia

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INTRODUCTION

Pleurotus tuberregium (Fr.) Sing is a basidiomycete found in the tropical and subtropical regions of the world [1, 2]. It is also known as the "King Tuber Oyster Mushroom". Chen and Huang revealed that the mushroom is a nematode-trapping mushroom and it is best known as "tiger milk mushroom" or "sclerotia-producing *Pleurotus*" in China [3]. In Nigeria, it is known as "osu", "ohu" or "katala". *P. tuberregium* is the only species that produces true sclerotia [4]. *P. tuberregium* is of great economic importance in Nigeria. Both the sclerotium and the mushrooms grown from it are eaten. The outer brown portion of the sclerotium is peeled off and the inner white portion cut into small pieces, ground and used in making soup. In this form it may replace melon in okro or vegetable soup. It was stated that the pileus and stipe of the mushroom are cut into pieces, boiled and added to okro or vegetable soup [1]. The local people who use this fungus for food and medicine usually collect the sclerotia from the wild. However, easy growing method of this fungus was established to produce sclerotia using many lignocelullosic agricultural wastes as cultivation substrates [2]. Chiejina and Olufokunbi confirmed that basidiocarps can be easily induced by burying the sclerotia in soil [5]. This mushroom is of economic importance in food and medicine preparations [1, 3]. African herbalists have used *P. tuberregium* sclerotia to solve a variety of health problems, ranging from skin diseases to small pox and even in embalmment of bodies [1, 3, 6]. Badalyan *et al*. [7] reported that the antifungal activity of *P. tuberregium* against filamentous fungi is utilized in treating mycoses in mammals. Many studies have reported the use of *Pleurotus* species in bioremediation exercises. *P. tuberregium* (a white-rot fungus) has been reported to ameliorate crude oil polluted soils and the resulting soil sample supported the germination and seedling of *Vigna unguiculata* [8, 9]. Yongabi confirmed that the sclerotium of *P. tuberregium* is a good coagulant and disinfectant, which can be used in natural water and waste water purification [10]. Equally, aerial hyphae of *P. tuberregium* cultures on agar by Hibbett and Thorn produced droplets of toxin on stalked secretory processes [11]. Nematodes that came in contact with the toxin droplets were paralysed and then colonized by hyphae. Today, this fungus has attained international recognition and is actively studied in laboratories in the US, Europe and Asia for its potential application in modern medicine [2, 12].

In view of its popularity (use as food condiment and in medicine), it became necessary to study the simplest and cheapest substrate that would give the highest weight yield and nutritional content for its production. This study was designed to investigate the effect of using different substrates, especially farm waste products in the cultivation and yield of *Pleurotus tuberregium* (King tuber oyster mushroom) sporophores and sclerotia. Data from this work could be used to recommend the best substrate for *Pleurotus tuberregium* cultivation in developing countries.

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OBJECTIVES

Specific objectives of the research were to investigate the cultivation of *P. tuberregium* to produce sporophores and sclerotia in column bags in a tropical environment; investigate the effects of different substrates used to supplement sawdust on the yield of sporophores and sclerotia; and investigate the relationship between various substrates and the nutritional content of *P. tuberregium* mushroom cultivated on supplemented sawdust.

MATERIALS AND METHODS

Mushroom Cultivation

Treatments

The substrates used were: top soil (M1), river sand (M2), fermented sawdust (M3), oil palm fruit fiber-OPFF (M4), mixture of topsoil and fermented sawdust (M5), mixture of OPFF and fermented sawdust (M6) and mixture of river sand and fermented sawdust (M7). These mixtures were in the ratio of 1:1 (v/v).

Substrate preparation

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, covered with a black plastic polyethylene sheet to undergo fermentation for four weeks. Fermented sawdust was mixed with 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.

Experimental layout

All treatments for the experiment were laid out using a Completely Randomized Design (CRD) and each treatment was replicated ten times in a 1.5 X 2.4 m^2 portion of a laboratory.

Inoculation and incubation

Two hundred grams (200 g) of each substrate was placed in a polypropylene 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 substrates 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^oC) to observe fungal growth for 3 weeks. The cultures were slightly watered daily to keep them damp.

Data collection

Growth of the mushrooms in the different substrates was recorded weekly. Yield of the mushrooms was determined in terms of height and diameter of the stipe, diameter of the pileus and the fresh and dry weights of the harvested mushrooms.

Proximate analysis

Protein, fat, ash, carbohydrate, fiber and moisture contents of the harvested mushrooms were determined using AOAC methods [13]. No mushroom grew in OPFF (M4) substrate while the mushrooms harvested from the mixture of top soil and fermented sawdust (M5) substrates were exhausted before analysis. Therefore, mushrooms of M4 and M5 substrates were omitted in the proximate analysis conducted on the harvested mushrooms.

Sclerotia Cultivation

Substrate preparation

To a heap of sawdust on a cement platform water was added in the ratio of 1:2 (v/v). The substrate was piled up into a heap of 1.3 m high by 1.2 m diameter and covered with polyethylene sheet to undergo fermentation for at least four weeks. Sun dried corn straw was shredded with a wood chipper and soaked in water overnight. Also, dried OPFF was soaked in water overnight before putting into substrate bags. The substrates used were: fermented sawdust (S1), mixture of topsoil and fermented sawdust (S2), mixture of OPFF and fermented sawdust (S3), mixture of rice bran and fermented sawdust (S4), mixture of chopped corn straw and fermented sawdust (S5), mixture of corn waste and fermented sawdust (S6) and mixture of millet waste and fermented sawdust (S7) (w/w on dry weight basis).

Experimental layout

All treatments for the experiment were carried out using a Completely Randomized Design (CRD) and each treatment was replicated five times.

Bagging and pasteurization

Two hundred and forty grams of each substrate was placed in polyethylene plastic bags (26 cm high x 17.5 cm width). Five replicate bags were prepared for each treatment. The tops of the substrates in the bags were covered with cotton wool and secured with rubber bands. The bagged substrates were sterilized in an autoclave for 30 min at 121^{o}C and 15 lb pressure. The pasteurized substrates were later cooled to ambient temperature (30^{o}C) [14, 15].

Inoculation and incubation [16]

The fungus sclerotia were soaked overnight in tap water to allow for maximum accumulation of water. Sclerotial cubes (4 x 4 cm^{3}) sterilized in 40% sodium hypochlorite for 15 minutes and rinsed in 3 changes of sterile distilled water were sown into the substrate bags (sealed with cotton wool and rubber bands) for colonization. The substrate bags were put into a growth chamber at ambient temperature of 30^{o}C for 90 days. This was followed by periodic watering of the bags with clean water to ensure that the environment was humid.

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Data collection

Data were collected and recorded daily from the different replicates and the means of each set of data calculated.

Collected data included the following:

1. Number of days for total colonization in each bag of substrate.
2. Wet weight of each sclerotium harvested from each bag.
3. Dry weight of each sclerotium harvested from each bag.
4. Calculated biological efficiency [2].

$$\text{Biological Efficiency} = \frac{\text{dry weight of harvested sclerotium}}{\text{dry weight of substrates}} \times 100\%$$

Statistical analysis

The results obtained were statistically analysed using analysis of variance (ANOVA), and tests of significance carried out by Duncan's multiple range test at $P \leq 0.05$ [14].

RESULTS

Results of the mushroom cultivation experiment showed that mean dry weights varied from 0.22 g for mixture of topsoil and fermented sawdust substrate (M5) to 3.34 g for mixture of river sand and fermented sawdust substrate (M7), while the mean fresh weight of M2, M3 and M7 were not significantly different from one another. Table 1 shows that the OPFF substrate (M4) had no growth and the mean fresh weight yield from the substrates ranged from 1.42 g for M5 to 13.76 g for M7. The sclerotia cultivation experiment showed that mean dry weight yield varied from 46.26 g in mixture of chopped corn straw and fermented sawdust substrate (S5) to 127.48 g in fermented sawdust substrate (S1). Using Duncan's multiple range test ($P \leq 0.05$), the sclerotial mean fresh weights of S2 and S3 were not significantly different from each other but have the least significant difference followed by S5, S4, S7 and S6 which were not also significantly different from each other. Fermented sawdust substrate (S1) displayed the highest level of significance at 127.48 g (Table 3). The biological efficiency of sclerotia harvested from S1, mixture of corn waste and fermented sawdust substrate (S6) together with mixture of millet waste and fermented sawdust substrate (S7) were not significantly different from one another but were significantly different from those of mixture of rice bran and fermented sawdust (S4) and S5 substrates. Proximate analysis results show that the percentage protein content ranged from 14.88% for fermented sawdust (M3) substrate to 17.78% for top soil substrate (M1). The percentage carbohydrate content ranged from 59.03% for M1 substrate to 65.41% for river sand substrate (M2) while all the mushrooms harvested from all the substrates have the same value for percentage fat (2.00%) (Table2). The highest percentage fiber content (3.50%) is from mushrooms of M7 while the least (2.50%) is from M2 substrate mushrooms. Table 2 also revealed that mushroom harvested from M1 substrate had the highest percentage moisture content (13.00%), while the least

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(9.00%) is from M7 substrate. The percentage ash composition was highest (6.75%) in mushrooms harvested from M6 substrate (Table 2), while the least (5.00%) was from M2 substrate mushrooms.

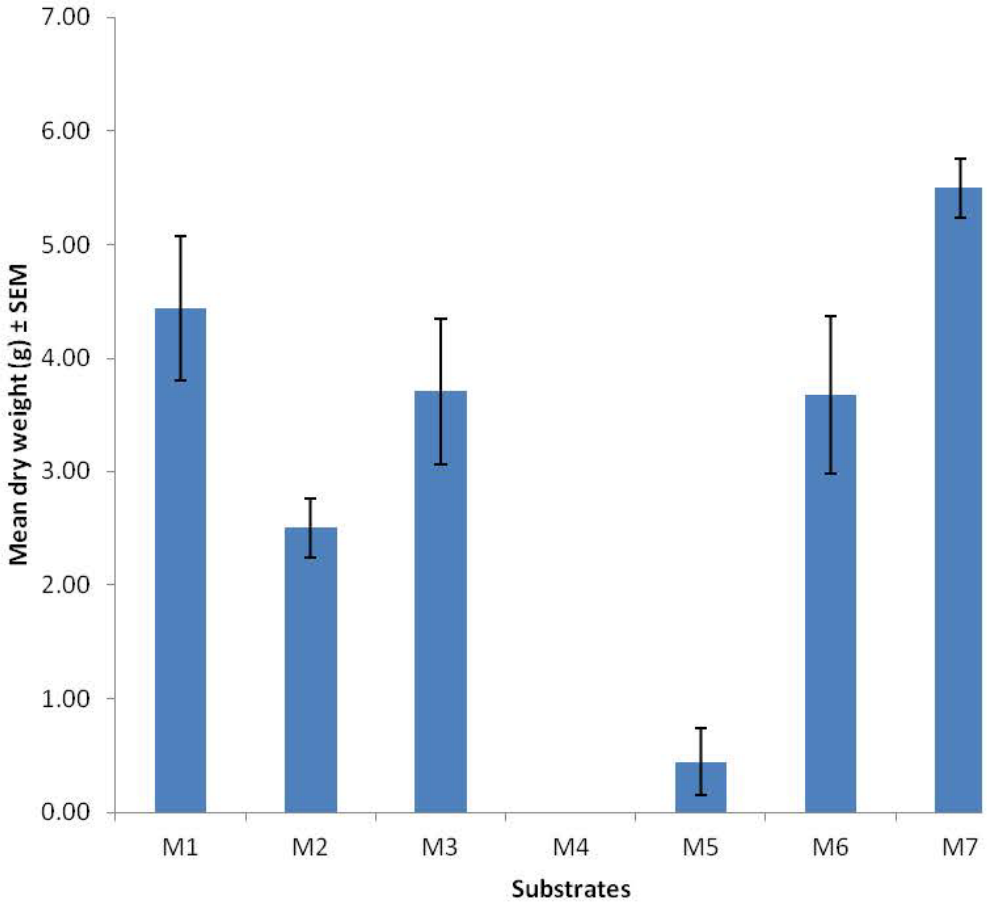

Figure 1: Dry weight(g) of harvested mushroom

SEM = Standard error measured
M1 = Top soil substrate;
M2 = River sand substrate;
M3 = Fermented sawdust substrate
M4 = Oil palm fruit fiber (OPFF) substrate;
M5 = Mixture of topsoil and fermented sawdust substrate;
M6 = Mixture of OPFF and fermented sawdust substrate;
M7 = Mixture of river sand and fermented sawdust.

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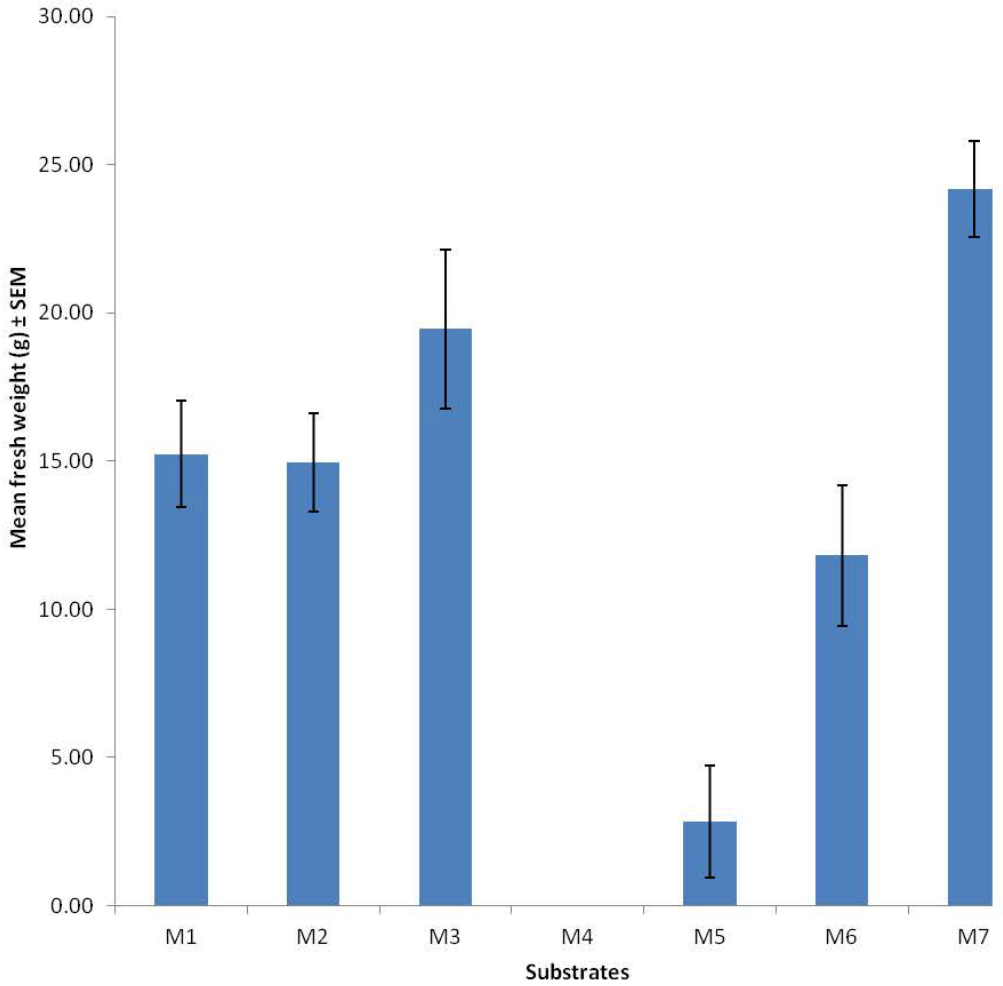

Figure 2: Fresh weight (g) of harvested mushroom

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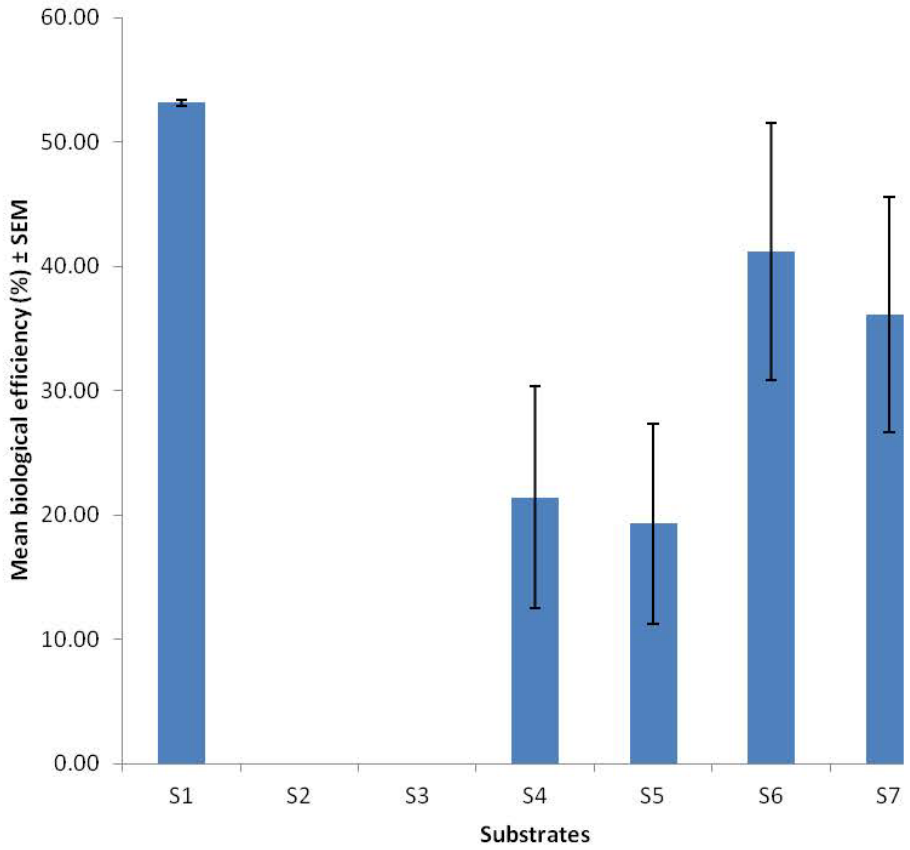

Figure 3: Biological Efficiency of harvested sclerotia

SEM = Standard error measured
S1 = Fermented sawdust;
S2 = Mixture of topsoil and fermented sawdust;
S3 = Mixture of oil palm fruit fiber and fermented sawdust;
S4 = Mixture of rice bran and fermented sawdust;
S5 = Mixture of chopped corn straw and fermented sawdust;
S6 = Mixture of corn waste and fermented sawdust;
S7 = Mixture of millet waste and fermented sawdust.

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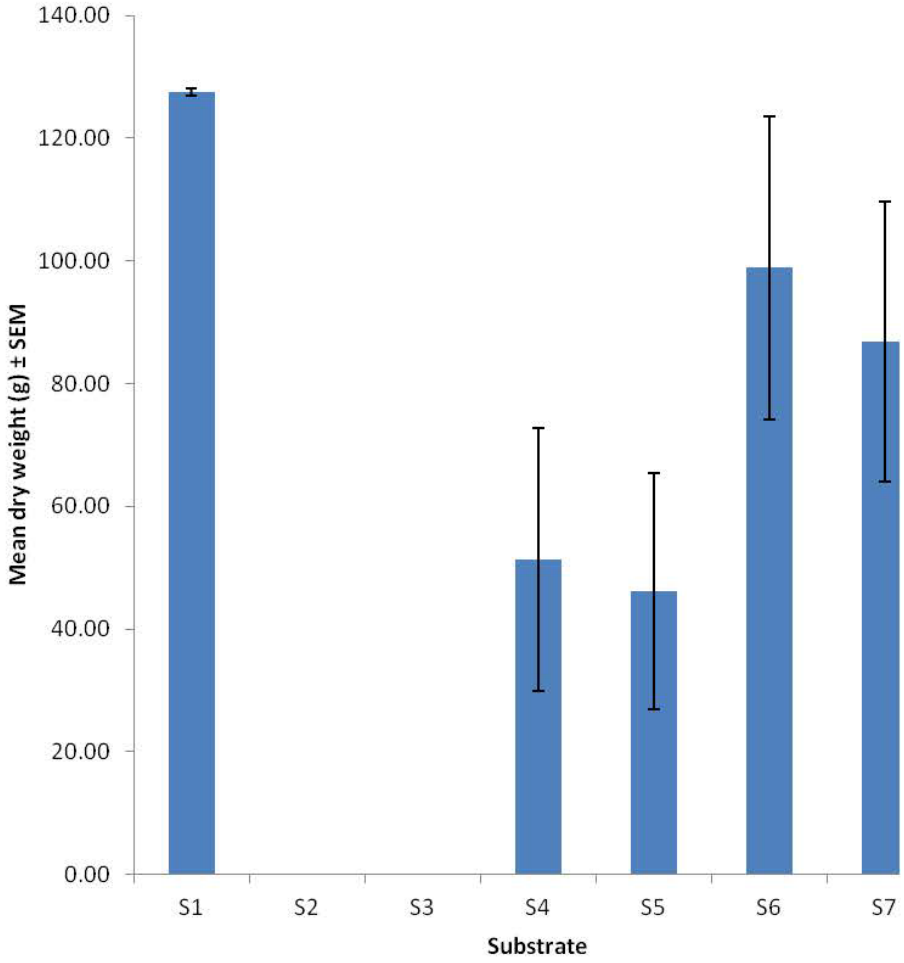

Figure 4: Mean dry weights of harvested sclerotia

DISCUSSION

Results obtained in the first experiment revealed that the mixture of river sand and fermented sawdust (M7) produced mushrooms with highest fresh and dry weights. 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 [15]. The combination of the qualities of the river sand and the sawdust may have been responsible for the highest yield recorded in that substrate. A mixture of top soil and sawdust (M5) gave the least yield while no growth at all was observed in OPFF (M4). The observation in the latter could probably be due to the inhibitory effects of pathogens present in the OPFF

which might have had antagonistic effects on the growth of mushrooms in that substrate. This observation agrees with the results of Okhuoya and Okogbo [16]. Extensive mycelial production was observed in the OPFF (M4) substrate and it is not clear whether this extensive mycelia produced in (M4) had any inhibitory effects on sporophore production; this observation requires further investigation. The very high fertility of the OPFF substrate may be responsible for the extensive mycelia (a type of vegetative growth) produced. The 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. However, the ability of top soil substrate (M1) to produce good stipe and pileus diameter and dry weight yield of the mushrooms agrees with the findings of Okhuoya and Etugo who reported loam soil (very similar to top soil) as the best for planting sclerotia, and that may be due to its high water holding capacity [17]. Using Duncan's multiple range test, mean fresh weight yields from M2 and M3 were not significantly different from one another but were significantly different from those of M5 (mixture of topsoil and fermented sawdust substrate) and M6 (mixture of OPFF and fermented sawdust). The highest percentage protein occurred in mushrooms grown in top soil (M1) substrate and the least was in those grown in sawdust (M3) substrate. This is probably due to the nutrients already present in topsoil and absent in sawdust. River sand substrate (M2) producing mushrooms with the highest carbohydrate percentage agree with the findings of Okhuoya and Okogbo, who had earlier reported that sclerotia have already stored in them all the nutrients required for fruiting [16]. 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 first combat the microbial antagonists in them [7].

For the sclerotia cultivation experiment, the mean colonization rate varied from 0.0 day (S2, S3) to 31.2 days (S5). S4 and S6 were not significantly different from each other but were significantly different from S1, S5 and S7 and from S2 and S3 which were not significantly different from one another. The mycelial density/colonization was rated by visual observation as described by Oghenekaro *et al.* [18]. This observation may be due to the high nutrient content in the rice bran and the seed coat of the corn waste, respectively, which ultimately resulted in a rich source of nutrients for fungal mycelial ramification and quick sclerotial formation [19]. Fermented sawdust substrate (S1) produced sclerotia with the highest fresh and dry weights. Sawdust has been reported as one of the best substrates for mycelial growth, sclerotia formation and fructification because sawdust substrates offer the least resistance to enlargement of sclerotia unlike other supplemented-sawdusts, where the sclerotia would have to combat with microbial antagonism from the supplements [15]. No sclerotium was formed in the mixture of topsoil and fermented sawdust (S2) as well as in the mixture of OPFF and fermented sawdust (S3). The sclerotial mean fresh weights of S2 and S3 were not significantly different from each other but have the least significant difference followed by S4 and S5 and then S6 and S7 which were not also significantly different from each other. However, the ability of sawdust substrate alone (S1) to produce highest fresh and dry weights yield of sclerotia agrees with the

findings of Okhuoya and Etugo as well as Okhuoya *et al*. [12, 17] which explained why sawdust alone could produce sclerotia with the highest percentage biological efficiency while the other supplemented substrates may have to combat first with the microbial antagonists in them. *P. tuberregium* sclerotia could thrive well in sawdust because they are wood decaying saprophytes which can digest extra cellular lignocelluloses and hemicelluloses deriving nutrients from them [9, 20]. Mixture of corn waste and fermented sawdust (S6) produced the second mean highest fresh weight, dry weight and biological efficiency.

CONCLUSION

In conclusion, the study suggests that sclerotia and sporophore of *Pleurotus tuberregium* can be grown in lignocellulosic agricultural wastes as substrate, which is much faster, economical and easier than growing it from the spawn raised from the spores [3, 12]. Considering all the parameters investigated, mixture of river sand and fermented sawdust (M7) substrate is recommended as the best substrate for the production of *P. tuberregium* mushrooms while mixture of corn waste and fermented sawdust (S6) substrate is recommended for sclerotia production. Since *P. tuberregium* was confirmed to have a higher percentage of proteins than most leguminous plants and vegetables, sclerotia and mushrooms of *P. tuberregium* can be used as substitute for expensive meat and fish in developing nations like Nigeria [1, 21, 22].

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Table 1: Effects 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 (cm)
M1	4.82^{b}	1.51^{a}	10.40^{a}	9.44^{ab}
M2	6.62^{a}	1.45^{ab}	6.05^{b}	10.89^{a}
M3	5.63^{ab}	1.29^{ab}	5.94^{b}	10.27^{a}
M4	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}
M5	0.96^{c}	0.13^{c}	0.79^{c}	1.42^{bc}
M6	4.15^{b}	1.11^{b}	5.99^{b}	7.62^{abc}
M7	5.46^{ab}	1.63^{a}	9.95^{a}	13.76^{a}

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 ($P \leq 0.05$)

M1 = Top soil substrate;
M2 = River sand substrate;
M3 = Fermented sawdust substrate
M4 = Oil palm fruit fiber (OPFF) substrate;
M5 = Mixture of topsoil and fermented sawdust substrate;
M6 = Mixture of OPFF and fermented sawdust substrate;
M7 = Mixture of river sand and fermented sawdust.

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Table 2: Proximate composition of *Pleurotus tuberregium* mushroom

Properties (%)	M1	M2	M3	M6	M7
Moisture content	13.00	9.50	10.00	9.50	9.00
Total ash	5.50	5.00	6.50	6.75	5.75
Crude protein	17.78	15.59	14.88	16.46	15.24
Crude lipid	2.00	2.00	2.00	2.00	2.00
Total carbohydrate	59.03	65.41	63.63	62.29	64.51
Crude Fiber	2.70	2.50	3.00	3.00	3.50

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Table 3: Effects of different substrates on the yield of *P. tuberregium* sclerotia

Treatment	Full mycelial colonisation (days)	Sclerotia Fresh weight (g)	Sclerotia Dry weight (g)
S1	22.20[a]	415.48[a]	127.48[a]
S2	0.00[b]	0.00[c]	0.00[d]
S3	0.00[b]	0.00[c]	0.00[d]
S4	12.00[ab]	184.44[b]	51.32[bcd]
S5	31.20[a]	210.30[b]	46.26[cd]
S6	16.80[ab]	311.20[ab]	98.86[ab]
S7	24.20[a]	300.16[ab]	86.78[abc]

Each value is a mean of 5 replicates. Values in the same column followed by the same letter (s) are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

S1 = Fermented sawdust;
S2 = Mixture of topsoil and fermented sawdust;
S3 = Mixture of OPFF and fermented sawdust;
S4 = Mixture of rice bran and fermented sawdust;
S5 = Mixture of chopped corn straw and fermented sawdust;
S6 = Mixture of corn waste and fermented sawdust;
S7 = Mixture of millet waste and fermented sawdust.

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