# Impact of substrate on protein content and yield of mushrooms and sclerotia of *Pleurotus tuberregium* in Nigeria

**Olufokunbi JO[1*] and Chiejina NV[1]**

[1]Department of Botany, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria. joecomolu@yahoo.com

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Fruitbodies of *Pleurotus tuberregium* are universally used as food while sclerotia are used in Nigeria as food condiment and in medicine. Seven different substrates supplemented with fermented sawdust were used to produce mushrooms and sclerotia of *P. tuberregium*. Mean dry weights of fruitbodies varied from 0.22 g for mixture of topsoil and fermented sawdust substrate to 3.34 g for mixture of river sand and fermented sawdust substrate, while percentage protein content ranged from 20.59% for fermented sawdust substrate to 25.19% for river sand substrate. The rate of substrate colonization had a significant effect on sclerotium production. The mean dry weight yields varied from 46.26 g for mixture of rice bran and fermented sawdust substrate to 127.48 g for fermented sawdust substrate alone. The highest sclerotial protein content (8.40%) was from mixture of rice bran and fermented sawdust substrate although it was not significantly different from those of other substrates. A mixture of river sand and fermented sawdust substrate is recommended as the best substrate for the production of *P. tuberregium* mushrooms while a mixture of corn waste and fermented sawdust substrate is recommended for sclerotial production.

**Key words** – mycelium – nutrition – sawdust – sporophore – supplement

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*Corresponding author: Olufokunbi JO – e-mail – joecomolu@yahoo.com

## Introduction

*Pleurotus tuberregium* (Fr.) Sing., a nematode-trapping mushroom is best known as “tiger milk mushroom” or “sclerotia-producing *Pleurotus*” in China (Chen & Huang 2004). It is also known as the “king tuber oyster mushroom”. It is found in tropical and subtropical regions of the world (Oso 1977, Isikhuemhen & LeBauer 2004). In Nigeria, it is known as “osu” or “ero nsu” in Ibo, “ohu” in Yoruba and “katala” or “rumbagada” in Hausa language. *P. tuberregium* is the only species of *Pleurotus* that produces true sclerotia (Isikhuemhen et al. 2000). The mushroom is indigenous to tropical Africa and the Australasian-Pacific region (Isikhuehmen & LeBauer 2004).

Chen & Huang (2004) defined sclerotium as a non-fertile or sterile mycelial structure, tightly woven. It is an organ for survival and resistant to adverse environment conditions such as drought. Sclerotia are hard in nature and may be viable for 7 years or more after harvest. The sclerotium is often dark brown on the surface and white inside. The size and weight of the sclerotium varies; they may be as large as 30 cm in length and weigh over 5 kg. It enjoys popular use in Nigeria as food and medicine. The sclerotia are usually harvested from decaying logs; the dark brown exterior is peeled off and the white compact mycelial tissue is used for food or medicine. One of the most common dietary applications of

*P. tuberregium* in Nigeria is as a soup thickener. The white tissue is blended into fine powder and when added to soup, it swells and adds bulk to the soup (Iwuagwu & Onyekweli 2002). The sclerotia can also be cut into pieces and buried in the soil, and then watered regularly to produce the sporophore (mushroom) which is consumed. Sclerotia give rise to fruiting bodies in most environments at high temperatures. Chiejina & Olufokunbi (2010) confirmed that basidiocarps can be easily induced by burying the sclerotia in soil. It is of economic importance in food and medicine preparations (Oso 1977, Stamets 2001, Chen & Huang 2004). African herbalists have used *P. tuberregium* sclerotia to solve a variety of health problems, ranging from skin diseases, inflammation, childhood malnutrition, headache, stomach problem, cold, asthma, fever, high blood pressure, diabetes to small pox and even in embalmment of bodies (Oso 1977, Okhuoya et al. 1998, Chen & Huang 2004). Badalyan et al. (2008) 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 (Isikhuemhen et al. 2003, Adenipekun 2008) and the resulting soil sample supported the germination and seedling of *Vigna unguiculata*. Yongabi (2004) confirmed that the sclerotium of *P. tuberregium* is a good coagulant and disinfectant, which can be used in natural water and waste water purification. Today, this fungus has attained international recognition and is actively studied in laboratories in the US, Europe and Asia for its application in modern medicine (Akpaja et al. 2003).

The aims of the present study were to investigate the cultivation of *P. tuberregium* to produce mushrooms and sclerotia in column bags in a tropical environment; to investigate sclerotia yield when different local agricultural wastes are used to supplement sawdust substrate; and to investigate the relationship between the various substrates and the protein content of *P. tuberregium* sclerotia cultivated on supplemented sawdust substrates.

## Material and methods

### Collection of Materials

Fresh hard wood sawdust of *Daniella oliveri* called "Agba" in Igbo language was collected from the timber shed at Nsukka, Enugu State, Nigeria, while dried oil palm fruit fibre of *Elaeis guineesis* (OPFF) was collected from a local farm at Ibagwa-Ani, Enugu State. Rice bran (*Oryza sativa*) was collected from Adani rice mill, Adani town, Enugu State. Corn waste (*Zea mays*) and millet waste (*Pennisetum glaucum*) were collected from Ugwuaji market in Enugu, while corn straw was collected from a local farm in Nkpologwu, Emene, Enugu. Sclerotia used in this study were obtained from Oba town, Enugu State, Nigeria; while top soil and river sand were collected from the Botanical Garden, Department of Botany, University of Nigeria, Nsukka, Nigeria.

### Experiment I - Mushroom cultivation

### Substrates Preparation

Water was added to sawdust on a cement platform in the ratio of 1:2 (v/v). The substrate was piled up into a heap about 1.3 m high by 1.2 m diameter and covered with a black plastic polyethylene sheet and allowed to undergo fermentation for 4 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 4 weeks. The substrates used were: top soil (M1), river sand (M2), fermented sawdust (M3), 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).

### Experimental Layout

All treatments for the experiment were laid out using a completely randomized design and each treatment was replicated ten times.

### Inoculation and Incubation

Two hundred grams (200 g) of each substrate was placed in a polypropylene plastic

bag (17.5 cm high × 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 ± 2ºC) for observation of fungal growth for 3 weeks. The cultures were 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.

## Experiment II – Sclerotia cultivation

### Substrates Preparation

A heap of sawdust on a cement platform was fermented as described above. Sun dried corn straw was shredded with a wood chipper and soaked in water overnight. Dried OPFF was also soaked in water overnight before loading into substrate bags. The substrates used were: fermented sawdust (S1), mixture of top-soil 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 laid out using a completely randomized design and each treatment was replicated five times.

### Bagging and Pasteurisation

Two hundred and forty grams of each substrate was placed in polyethylene plastic bags (26 cm high × 17.5 cm width). Five replicate bags were prepared for each treatment. The top of the substrate in the bags were covered with cotton wool and secured with rubber bands. The bagged substrates were sterilised in an autoclave for 30 minutes at 121ºC and 15 lb pressure. The sterilised substrates were later cooled to ambient temperature (30ºC).

### Inoculation and Incubation

The fungus sclerotia were soaked overnight in tap water to allow for maximum accumulation of water. After this, sclerotial cubes (4 × 4 $cm^3$) sterilised in 40% sodium hypochlorite for 15 minutes and rinsed in 3 changes of sterile distilled water were added to the substrate bags (a cube per substrate bag), sealed with cotton wool and rubber bands for colonization. The substrate bags were put into a growth chamber at ambient temperature of 30 ± 2ºC for 90 days. The bags were periodically watered with clean water to ensure that the environment was humid.

### Data Collection

The data was collected and recorded daily and the means of each set of data calculated.

### The data collected include the following

1. Number of days to achieve 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. Biological efficiency was calculated.

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

### Protein Analysis

The protein analyses for the experiments were conducted at the Analytical Laboratory of the Department of Crop Science, University of Nigeria, Nsukka. The mushrooms harvested from the different substrates were oven dried at 100ºC for 24 hours 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. Ten gram $NaSO_4$, 20 ml concentrated $H_2SO_4$ and 1 g $CuSO_4$ were later added. The mixture was cautiously heated in a fume hood until a bluish

green clear solution appeared. The digest was allowed to cool and solidify for 24 hours while the colour changed to white. Distilled water (20 ml) was added to the solidified sample and allowed to cool in a refrigerator. Sixty milliliter (60 ml) of 40% NaOH was added to the sample and two pieces of zinc metal connected to the mixture in a distillation column. In the set up, 100 ml of 4% boric acid was added to a conical flask with two drops of screen methyl red indicator. A light pink colour appeared when boric acid and screen methyl red indicator came in contact. The distillation was stopped and collected in a receiver flask when it reached 200 ml. This was titrated with 0.1M $H_2SO_4$ until a pink colour emerged.

## Calculation

$$\textbf{Formula} = \frac{100 \times \text{titre value} \times 0.0014 \times 6.25}{\text{Weight of sample used}}$$

Where,
100 = conversion to %
0.0014 = constant which means that 0.0014 is liberated by 1ml of 0.100 $H_2SO_4$
6.25 = protein constant according to Kjeldahl's method.

$$\textbf{Percentage Protein} = \frac{100 \times \text{titre value} \times 0.0014 \times 6.25}{0.5}$$

## 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 (Steel & Torrie 1980) at $P \leq 0.05$.

## Results

The results of the first experiment, involving mushrooms, showed that river sand (M2) substrate produced the highest mean stipe height (6.62 cm) while the lowest (0.96 cm) was from the mixture of topsoil and fermented sawdust (M5) substrate. There was no growth in the oil palm fruit fibre (OPFF), M4 substrate (Table 1). Fruit bodies with the widest mean stipe diameter (1.63 cm) were formed in top soil substrate (M1) and the mixture of river sand and fermented sawdust (M7), while the narrowest stipes (1.11 cm) was from the mushrooms grown in the mixture of OPFF and fermented sawdust (M6) substrate. Mushrooms grown on top soil substrate (M1) had the widest mean pileus diameter (10.40cm) while the mixture of top soil and fermented sawdust (M5) substrate produced mushrooms with the narrowest (0.79 cm) (Table 1). The highest mean fresh weight (13.76 g) mushrooms were obtained from those grown in the mixture of river sand and fermented sawdust (M7) and the lowest (1.42 g) from those grown in the mixture of top soil and fermented sawdust substrate (M5). No fruit bodies were produced in OPFF substrate (M4) throughout the experiment but rather, extensive mycelial ramifications were observed in the substrate.

Mean dry weights varied from 0.22 g for mixture of topsoil and fermented sawdust (M5) substrate to 3.34 g for mixture of river sand and fermented sawdust (M7) (Fig. 1); while percentage protein content ranged from 20.59% for fermented sawdust (M3) substrate to 25.19% for river sand substrate (M2) (Fig. 2).

In the second experiment the fastest substrate colonization rate of 12 days came from the mixture of rice bran and fermented sawdust substrate (S4) while the slowest rate of 31 days came from the chopped corn straw and fermented sawdust mixture (S5) substrate (Table 2). There was no growth in S2 (mixture of topsoil and fermented sawdust) and S3 (mixture of OPFF and fermented sawdust) substrates. Mean fresh weight yields of the sclerotia ranged from 184.44 g for mixture of rice bran and fermented sawdust (S4) substrate to 415.48 g for fermented sawdust (S1) substrate, while mean dry weight yield varied from 46.26 g for (S4) to 127.48 g for (S1). There were no growths in S2 (mixture of topsoil and fermented sawdust) and S3 (mixture of OPFF and fermented sawdust) substrates.

Fermented sawdust (S1) substrate gave sclerotia with the highest mean percentage biological efficiency (53.14%) while mixture of chopped corn straw and fermented sawdust (S5) substrate produced the least (19.30%) (Fig. 3). Mixture of rice bran and fermented sawdust (S4) substrate sclerotia gave the highest percentage protein content (8.40%) while fermented sawdust (S1) substrate produced sclerotia with the least percentage protein content (2.45%) (Fig. 4).

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

| Treatment | Stipe height (cm) | Stipe diameter (cm) | Pileus diameter (cm) | Fresh weight (cm) |
|---|---|---|---|---|
| M1 | 4.82b | 1.63a | 10.40a | 9.44a |
| M2 | 6.62a | 1.45a | 6.05b | 10.89a |
| M3 | 5.63a | 1.29a | 5.94b | 10.27a |
| M4 | 0.00c | 0.00c | 0.00c | 0.00c |
| M5 | 0.96c | 1.30c | 0.79c | 1.42b |
| M6 | 4.15b | 1.11b | 5.99b | 7.62a |
| M7 | 5.46a | 1.63a | 6.05b | 13.76a |

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).

**Key to substrates:** M1 = Top soil, M2 = River sand, M3 = Fermented sawdust, M4 = OPFF, M5 = Top soil and fermented sawdust, M6 = OPFF and fermented sawdust, M7 = River sand and fermented sawdust.

**Table 2** Effects of different substrates on the yield of *Pleurotus tuberregium* sclerotia.

| Treatment | Full mycelial colonization (days) | Fresh weight (g) | Dry weight (g) |
|---|---|---|---|
| S1 | 22.20a | 415.48a | 127.48a |
| S2 | 0.00b | 0.00c | 0.00d |
| S3 | 0.00b | 0.00c | 0.00d |
| S4 | 12.00a | 184.44b | 46.26c |
| S5 | 31.20a | 210.30b | 51.32b |
| S6 | 16.80a | 311.20a | 98.86a |
| S7 | 24.20a | 300.16a | 86.78a |

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≤ 0.05).

**Key to substrates:** S1 = Fermented sawdust, S2 = Top soil and fermented sawdust, S3 = OPFF and fermented sawdust, S4 = Rice bran and fermented sawdust, S5 = Chopped corn straw and fermented sawdust, S6 = Corn waste and fermented sawdust, S7 = Millet waste and fermented sawdust

## Discussion

The mixture of river sand and fermented sawdust (M7) produced mushrooms with highest fresh and dry weights The river sand may provide good aeration for the germination and fructification of the mushrooms while sawdust has been reported as the best substrate for mycelial growth and fructification (Kadiri & Fasidi 1990). Also, sandy soils offer the least resistance to enlargement of sporophores unlike compact soils like top soil that would also contain microbial antagonists. The combination of the qualities of river sand and sawdust may have been responsible for the highest yield recorded in that substrate. A mixture of top soil and sawdust (M5) gave the lowest yield. No growth was observed in OPFF (M4), perhaps due to the inhibitory effects of pathogens present in the OPFF. This observation agrees with the result of Okhuoya & Okogbo (1991). Extensive mycelial production was observed in the OPFF (M4) substrate and it is not clear if this had any inhibitory effects on sporophore production. The very high fertility of the OPFF substrate may be responsible for the extensive vegetative mycelia growth. The OPFF nutrients were not depleted within the duration of the experiment and together with nutrients 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 Okhuoya & Etugo (1993) who reported loam soil (very similar to top soil) as the best for planting sclerotia, perhaps due to its high water holding capacity. Mean fresh weight yields from M4 and M5 were not significantly different but were significantly different from those of M1 (topsoil substrate) and M6 (mixture of OPFF and fermented sawdust) as well as those of M2, M3 and M7 which were not significantly different from one another. The highest percentage protein occurred in mushrooms grown in river sand (M2) substrate and the lowest was in

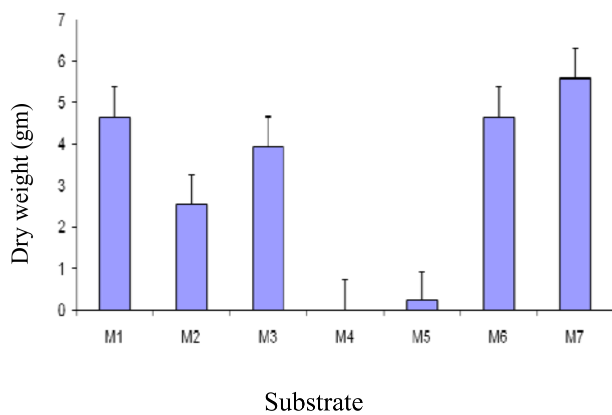


**Fig. 1** – Dry weight of harvested fruitbodies of *Pleurotus tuberregium*. Key to substrates: **M1** = Top soil, **M2** = River san, **M3** = Fermented sawdust, **M4** = OPFF, **M5** = Top soil and fermented sawdust, **M6** = OPFF and fermented sawdust, **M7** = River sand and fermented sawdust

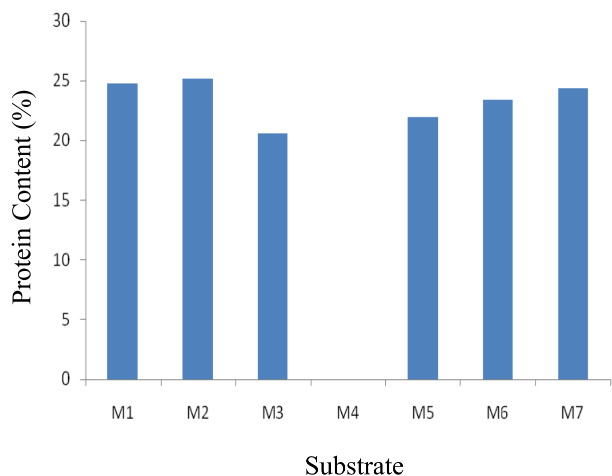


**Fig. 2** – Percentage protein content of harvested fruitbodies of *Pleurotus tuberregium*. LSD (P = 0.05) =NS. Key to substrates: **M1** = Top soil, **M2** = River san, **M3** = Fermented sawdust, **M4** = OPFF, **M5** = Top soil and fermented sawdust, **M6** = OPFF and fermented sawdust, **M7** = River sand and fermented sawdust

those grown in sawdust (M3) substrate. 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

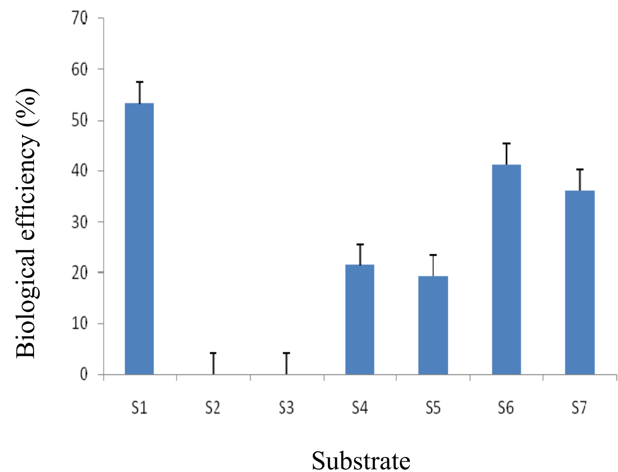


**Fig. 3** – Biological efficiency of harvested sclerotia of *Pleurotus tuberregium*. Key to substrates: **S1** = Fermented sawdust, **S2** = Top soil and fermented sawdust, **S3** = OPFF and fermented sawdust, **S4** = Rice bran and fermented sawdust, **S5** = Chopped corn straw and fermented sawdust, **S6** = Corn waste and fermented sawdust, **S7** = Millet waste and fermented sawdust

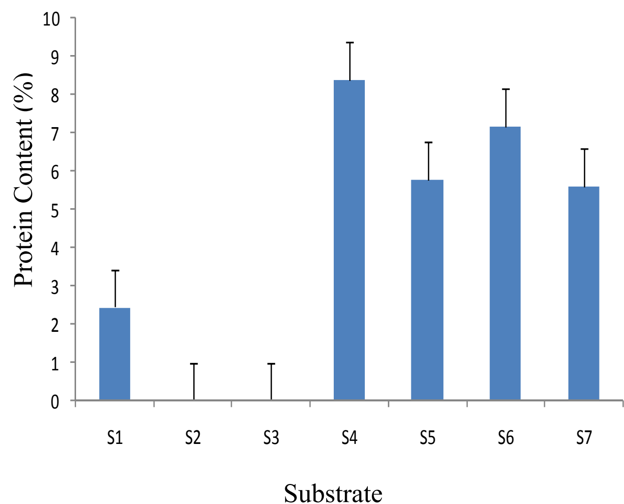


**Fig. 4** – Percentage protein content of harvested sclerotia of *Pleurotus tuberregium*. Key to substrates: **S1** = Fermented sawdust, **S2** = Top soil and fermented sawdust, **S3** = OPFF and fermented sawdust, **S4** = Rice bran and fermented sawdust, **S5** = Chopped corn straw and fermented sawdust, **S6** = Corn waste and fermented sawdust, **S7** = Millet waste and fermented sawdust

sporophores hence less proteins than in river sand alone (M2) substrate. Okhuoya & 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.

The rate of substrate colonization was significantly different between some treatments. The mycelial density/colonization was rated as described by Oghenekaro et al. (2008) by visual observation. Rice bran and the seed coat of the corn waste are a rich source of nutrients for the fungal mycelial ramification and quick sclerotial formation (Gyar & Attah 2007). 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 it offers the least resistance to enlargement of sclerotia unlike other supplemented-sawdusts, where the sclerotia would have to combat with microbial antagonism from the supplements (Kadiri & Fasidi 1990). 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). This may be due to the lack of nutrients due to leaching of the soil and drying of OPFF, respectively. The ability of sawdust substrate (S1) to produce highest fresh and dry weights yield of sclerotia agrees with the findings of Okhuoya & Etugo (1993) and Okhuoya et al (1998), 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 saprobes, which can digest extracellular lignocelluloses and hemicelluloses deriving nutrients from them (Atlas & Bartha 1992, Adenipekun 2008).

The highest sclerotial protein content (8.40%) was from S4 which was not significantly different from those from S1, S5, S6 and S7. Mixture of corn waste and fermented sawdust produced the second mean highest fresh weight, dry weight, biological efficiency and percentage protein content values. This is in line with the fact that rice bran and corn waste have been confirmed to have a higher percentage of protein content than other farm wastes (Gyar & Attah 2007), which is one of the reasons they are usually used to supplement animal feeds. On the other hand, fermented sawdust substrate (S1) had the lowest percentage protein content (2.45%) because of the lack of supplemented nutrients.

In conclusion, the study suggests that *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 (Isikhuehmen & LeBauer 2004, Chiejina & Olufokunbi 2010). 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 (Okhuoya & Ajerio 1988, Chen & Huang 2004, Ikewuchi & Ikewuchi 2008), it is 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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