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Molecular Identification and Cultivation of *Pleurotus Tuber-Regium* for Sclerotium Production Using Supplemented Lignocellulosic Wastes

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Abstract

This study was conducted not just to cultivate *Pleurotus tuber-regium* to produce sclerotium but also to know the conditions that enhance the production of sclerotium instead of fruit bodies. Supplemented Sawdusts of White Afra (*Terminalia superba*), Teak tree (*Tectona grandis*) and *Gmalina arborea* were used as substrates. Some portions were incubated at room temperature (25±2°C) and others at 36±2°C. There was faster colonisation of the substrates with Afra sawdust compared to other substrates. Afra sawdust also produced sclerotium 17 days earlier than the other substrates. The first harvesting was done 90 days from the day of inoculation of substrates and the second followed the same pattern too. The third and last harvest was done after eleven months. At first harvest, Afra substrates gave the highest average yield of 140 g/bag and the highest bio-efficiency of 20.05%. Gmelina has the least average yield of 103 g/bag. *Gmalina* had the best yield of 100 g/bag at second harvest with an average bio-efficiency of 31.5% while teak has the lowest average yield of 80 g/bag and bioefficiency of 19.61%. At third harvest, Afra gave the best yield of 155 g/bag but Gmelina gave the best bioefficiency of 42.45% while teak gave the poorest yield of 30 g/bag and a bioefficiency of 18.28%. The average overall yield of the substrates showed that Afra gave the best average yield of 120 g/bag and a bioefficiency of 27.95% while teak came a distant third with an average yield of 65 g/bag and a bioefficiency of 18.51%. Statistical analysis also showed that substrates incubated at high temperature gave better yields than those incubated at room temperature as follows Afra HT 130 g/bag, Afra RT 110 g/bag; Gmelina HT 130 g/bag, Gmelina RT 60 g/bag; Teak HT 70, Teak RT 60 g/bag.

Keywords

Pleurotus tuber-regium, Sclerotium, Yield, Bio-efficiency, Substrates

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1. Introduction

Pleurotus tuber-regium is known in different places by different names such as “tiger milk mushroom”, “sclerotia-producing Pleurotus” or “king Tuber Oyster mushroom” as it is normally referred to in China [1]. It is established that it is indigenous to tropical Africa and the Australasian-Pacific region of the world [2; 3]. The mushroom is a saprotroph and has been found to frequently grow on trees like *Daniella oliveri*, African breadfruit (*Treculina africana*), white and black Afra (*Terminalia superba), Blighia sapida* and so on [4, 5, 6]. It is differentiated from all other Pleurotus species

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because of its non-pleurotoid habit [7]. *P. tuber-regium* is the only oyster mushroom species known to produce true sclerotia [8]. The sclerotium is a dry compact mass of fungal hyphae or dense aggregations of fungal tissue which can be spherical to ovoid and can be quite large - up to 30 cm (11.8 inches) or larger in diameter [9, 10]. The sclerotium helps the mushroom to survive challenging conditions such as freezing temperatures, desiccation, microbial attack, or the long-term absence of a host [11]. In nature, sclerotia typically form in response to adverse growing conditions as a method of carrying the life of the fungus through difficult conditions. Both the sclerotium and the fruiting bodies are edible [2].

It has been difficult to determine the taxonomic position of *P tuber-regium* because of its production of leathery fruiting bodies and possession of a dimitic hyphal system with intercalary skeletal hyphae. Some researchers have earlier classified it as either *Panus* or *Lentinus.* Of recent however, *P. tuber-regium* was demonstrated to produce nematotoxic microdroplets in culture, supporting its classification in *Pleurotus* by Singer [12]. Other compelling evidence from comprehensive systematic molecular studies however show that authorities are right to place *P. tuber-regium* in the genus *Pleurotus* [7].

In Nigeria, both the fruiting body and the sclerotia are used in the preparation of soups [13]. The sclerotia is commonly used as substitute or mixed with melon to prepare soups. Furthermore, *Pleurotus tuber-regium* is used to treat bronchitis, breastmilk stimulation for lactating mothers and bed wetting in the Democratic Republic of Congo (14) Consumption of the sclerotia is widespread in Nigeria and across many tribes in sub-Saharan Africa and it continues to produce white sporophores when kept in a cool moist place [15].

Different materials have been used or combined with others to formulate substrates to grow *P. tuber-regium* over the years [2; 6; 16; 17] etc. Examples of such materials are cotton, cottonseed hull, corncob, sugarcane waste, sawdust, corn, rice and wheat straw, oil palm fruit fiber, cassava, banana leaves, cotton waste, (*Zea mays*, *Oryza sativa*, *Triticum aestivum*, *Elaeis guineesis*, *Manihot esculenta*, *Musa paradisica* and *Gossypium herbaceum* respectively) corncobs, hard wood sawdust, paper and cardboard papers [18; 3]. However, required amount of each of the materials may differ depending on whether the fruiting body or the sclerotium is the target. However, most of these materials are not available throughout the year, making it difficult for researchers or mushroom growers to use them for cultivation.

The mycelia of the mushroom can survive between 5 and 40°C. Mushroom mycelia grow well at a temperature range of 20-30°C while pinheads grow best at 10-20°C. Over 80% of the fruit body is water. Substrate moisture content should be 60-75% and log moisture content 34-45%. [19].

Nutritionally, Ikewuchi and Ikewuchi, [20] reported the proximate composition of *Pleurotus tuberregium* (Fr) sclerotia as follows; protein (64.31% WW and 71.21% DW) and carbohydrate (20.00% WW and 22.15% DW), with moderate contents of ash (2.20% WW and 2.44% DW), and crude fiber (2.89% WW and 3.20% DW). The sclerotium is also reported to contain the following; soluble sugars, lipids, glycogen, protein, and ash contents of 7.7, 1.7, 10.7, 16.3 and 9.2 g respectively [21]. One hundred grams of the sclerotia is also found to contain 25.93 g of essential amino acids, 1.50 g of sulphur-containing amino acids and 6.15 g of aromatic amino acids. It is also high in essential amino acids like histidine, leucine and phenylalanine, with the sulphur-containing amino acids (Methionine and Cystine) as its limiting amino acid(s). Chemical scores of 36, 88 and 26% with reference to human milk, adult requirements, and egg protein, respectively were also recorded [20]. Experiments conducted by [22] showed that ethanol-soluble sugar and lipid content of the mushroom were generally low. This suggests that diabetics and those with heart or weight problems can consume this mushroom. *Pleurotus. tuber-regium* has the highest amount of crude fiber compared with other wild edible mushrooms [22]. Ohiri [23] reported that the sclerotium contains fairly high concentrations of potassium and magnesium as major minerals with values of 60.66 ± 4.13 and 41.79 ± 3.14 mg/kg while manganese and zinc were micronutrients with the highest values of 1.20±0.10 and 0.95±0.07 mg/kg. Glutamic acid and aspartic acid were also observed in high concentrations with values of 11.51 ± 1.01 and 5.52 ± 0.86 mg/kg respectively.

Over the years, several researchers have investigated the medicinal benefits of this mushroom and there are so many breakthroughs. Okolo *et al.,* [24] and Kamalebo *et al*., [14] reported that *Pleurotus tuber regium* is used to treat bronchitis, breastmilk stimulation for lactating mothers and bed wetting in the Democratic Republic of Congo. Adebayo et al., [25] also reported remarkable antioxidant and antibacterial properties in *P. tuber-regium*. Bamgboye et al., [26] successfully demonstrated the effectiveness of the extracellular polysaccharides (EPS) fraction from *P. tuber-regium* in preserving hepatic cells against paracetamol-induced damage. Afieroho, [27] used dichloromethane extract of *Pleurotus tuber-regium* sclerotium to successfully inhibit the growth of clinical isolates of *Mycobacterium tuberculosis* at the test concentration of 32.5 µg/ml.

It has also been successfully employed in the amelioration of crude oil polluted soil and the resulting soil sample supported germination and seedling growth of *Vigna unguiculata* [28]. Also, aerial hyphae of *P. tuber-regium* cultures on agar by

[29] produced droplets of toxin on stalked secretory processes. Nematodes that came in contact with the toxin droplets were paralysed and then colonized by hyphae.

It is however worthy of note that despite the huge economic, medicinal, nutritional and environmental benefits of the sclerotia of this mushroom, most attempts or researches on it have focused on the fruiting body. Therefore, this attempt was focused on the development of a scientific and profitable protocol to produce the sclerotia to meet the demand for it from various sectors. This is also because sclerotium-forming fungi are excellent targets for the discovery of antibacterial, antifungal, and antiherbivore compounds. Thirdly, many non-parasitic fungi are known to form sclerotia, so it is likely that this life history trait is ecologically important for many fungal species and not just for plant pathogens [11]. Finally, many other mushrooms such as *Tuber magnatum pico* (the Italian white truffle), *Tuber melanosporum* (the perigord truffle), and fungi like *Claviceps purpurea*, *Claviceps fusiformis* etc are known to produce truffles or sclerotia which may affect proper identification. Therefore, there is a need for molecular identification in order to ascertain that a given sclerotium belongs to the reasonably suspected mushroom.

2. Research Significance

This effort will assist researchers to grow this mushroom for the purpose of sclerotium production better than in history. The importance of temperature in making the mushroom produce the sclerotia instead of the fruiting body has also been revealed here. This work has also revealed the importance of the type of substrate in determining the yield of the mushroom with respect to sclerotium production. In contrast to other oyster mushrooms that can only produce fruiting bodies for some few weeks, this work has revealed that *P. tuber-regium* can continue to produce sclerotia for a period of a year under proper management.

3. Materials and Methods

3.1. Tissue Culture

Sclerotia purchased from Ajoke Market in Oka-Akoko, Ondo State, Nigeria were employed for the tissue culture process. They were transported to the laboratory in clean polyethylene bags and kept in dry conditions before analysis. They were sliced to 30 g each and planted in containers filled with loamy soil [30]. These were kept in a well-ventilated place in the laboratory while being wetted with 50 ml of water every 48 h. The fruiting bodies emerged from them twenty-five days after planting.

3.2. Spawn Production

The fruiting bodies that emerged from the planted sclerotia were carefully harvested and sliced and cultured on potato dextrose agar (PDA) according to the methods of Zheng et al., [31] and Adebayo et al [32]. PDA was prepared as described by Oxoid manual. This lead to the PDA plates being covered in rapidly growing mycelium in just six days.

3.3. Multiplication of Spawn

For the purpose of adequacy of spawn for substrate inoculation, the spawn produced was multiplied by inoculating 1 kg of parboiled and sterilised sorghum in transparent plastic bags with the spawn from the plates [31].

3.4. Preparation of Substrates

Sawdust of three different trees White Afra (*Terminalia superba*), Teak tree (*Tectona grandis*) and *Gmalina arborea* were used. They were compounded using a modified method of [31] that is, 78% sawdust, 10% wheat bran, 10% cotton waste and 2% gypsum. The substrates were tightly packed into plastic bags measuring 40 cm by 23 cm. Twenty of such bags were made for each treatment. Each weighed averagely two (2) kilograms. They were then sterilised in a boiler at a temperature of 100°C for 4 h. Since the mushroom is usually dug from the soil, ten plastic bags of the same size as those used for the substrates were filled with the same loamy soil on which the initial sclerotia were planted but were not sterilised.

3.4.1. Inoculation of Substrates

The sterilised bags were allowed to cool to room temperature and inoculated each with 60 g of the spawn while the unsterilized bags containing soil were inoculated with 100 g of spawn.

3.4.2. Incubation of Substrates

It has been established that the production of sclerotia by this mushroom is a strategy to cope with unfavourable environmental conditions, especially temperature. Therefore, half (10 bags) of each of the groups of the inoculated substrates bags were incubated at normal room temperature (25±2°C) while the other half were incubated at 36±2°C. The steps involved in the cultivation of this mushroom are indicated in figure 12.

Biological efficiency of the substrates was calculated using the formula below;

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

3.5. Molecular Characterisation of *P. tuber-regium*

3.5.1. The DNA Extraction and Purification

This was done using a modified procedure of Vesty et al., [33], using Zymo Research Kit. 75 mg (wet weight) fungal cells were centrifuged at 10,000 x gram re-suspended in 200 ul isotonic buffered (PBS) ZR BashingTM Lysis Tube. A lysis solution was added to help lyse cells during the mechanical lysis step. The supernatant of the lysis solution was filtered using a Zymo-SpinTM IV Spin Filter, then DNA was bound to a Zymo-SpinTM IIC Column in the presence of DNA Binding Buffer, containing 0.5% (v/v) beta-mercaptoethanol. DNA was washed and then eluted with 100 µl of DNA Elution Buffer.

3.5.2. Quantification of Isolated Genomic DNA

DNA quantitative and qualitative analysis of the isolated DNA was carried out using Nanodrop Spectrophotometer (Nanodrop-2000).

3.5.3. DNA Amplification

This was done using a modified method of Gardes and Bruns [34] with the use of ITS4-Fand ITS5-B primers using amplication of the ITS region of rRNA gene. The final concentration of 25 µl PCR reaction volume consists of 200 µM each of dATP, dCTP, dGTP and dTTP, 2.5 mM $MgCl_2$, 10X Taq, DNA polymerase and 20 picomoles of each of the two primers (Banglore Genei). There is an initial denaturation level of 94°C for 85 s in PCR profile and after that 25 amplification cycles of denaturation, annealing, and extension. The temperature and times for these steps were 95°C for 35 s, 55°C, and 55 s and 72°C for two minutes, with further incubation at 72°C for ten minutes. The amplified PCR products were resolved on a 1.2% agarose gel, and stained with ethidium bromide. A 1 kb ladder DNA marker (GeneRulerTM) was used as a size standard.

3.5.4. DNA Sequencing

This was done using the Sanger method [35] and the DNA obtained was sequenced. 3 µl of the sample and 1 µl of primer diluted with 3 µl milliq water. The sequence was analyzed using Sequence Analysis software v6.2 from Thermos Fisher Incubator.

3.5.5. Phylogenetic Analysis of *Pleurotus tuber-regium*

The mushroom which had earlier being identified and assigned an ascension number MW376907 was subjected to comparative genome analysis in order to determine common ancestor and positions of significant difference between it and selected sequences with close relatedness, downloaded from NCBI on 13th of January 2021 by constructing tree using Neighbour-Joining with a bootstrap value of 1000, distance matrix and alignment view. The ascension number (MW376907) was blasted on NCBI database and 13 sequences were selected based on the percentage of close relatedness (% identity and Query covered) to the query sequence. Alignment of all sequence including the query sequence were conducted with Geneious Alignment tool with a cost matrix of identity 1.0, gap open penalty of 12 and extension of 3. Phylogenetic tree was constructed using Neighbor-Joining and the maximum likelihood based on genetic distance model with a bootstrap value of 1000 and 100 numbers of replicates to indicate the revolutionary process analysed over time, edited with Geneious tree builder version 9.0.5 to determine the evolutionary relatedness and diversities. Comparative analysis with a distance matrix of the tree was performed on Geneious (https://www.geneious.com) based on statistical analysis to determine positions of significant difference between the samples.

4. Statistical Analysis of Data

Data were analyzed by SPSS (version 17) and mean ± SEM were determined for all parameters. Significant differences among the groups were determined by one-way analysis of variance (ANOVA) and levels of significance were evaluated using Duncan's Multiple Range Test (DMRT) at $P< 0.05$.

5. Results and Discussion

The base sequence from the molecular identification of the mushroom was blasted through the NCBI database and it was identified as *Pleurotus tuber-regium*. Since the database did not show any base sequence that has 100% similarity to the sample, it was subsequently registered and given an Ascension number of MW376907.

Phylogenetic analysis of all sequences, using geneious tree builder revealed that, during the evolution process over time, data MW376907 had 100% similarity to the outgroup, however going down the group, sequence data exhibited diverse genetic profiles and that random mutations occurred indicating that all data sequences are orthologous to each other. Out of all clustered branches on the tree, each with a percentage identity determining the evolutionary relatedness of all branch, the cluster with data AF109983.1 and a branch with data AF109983.1, AF109985.1 inclusive shows to be relatively close to MW376907 with 97 percent identical at the root compared to the rest, exhibiting more diverse profiles going down the tree as shown in figure 1. The last

cluster shows a more diverge phyloevolutionary difference with a percentage root value of 52.

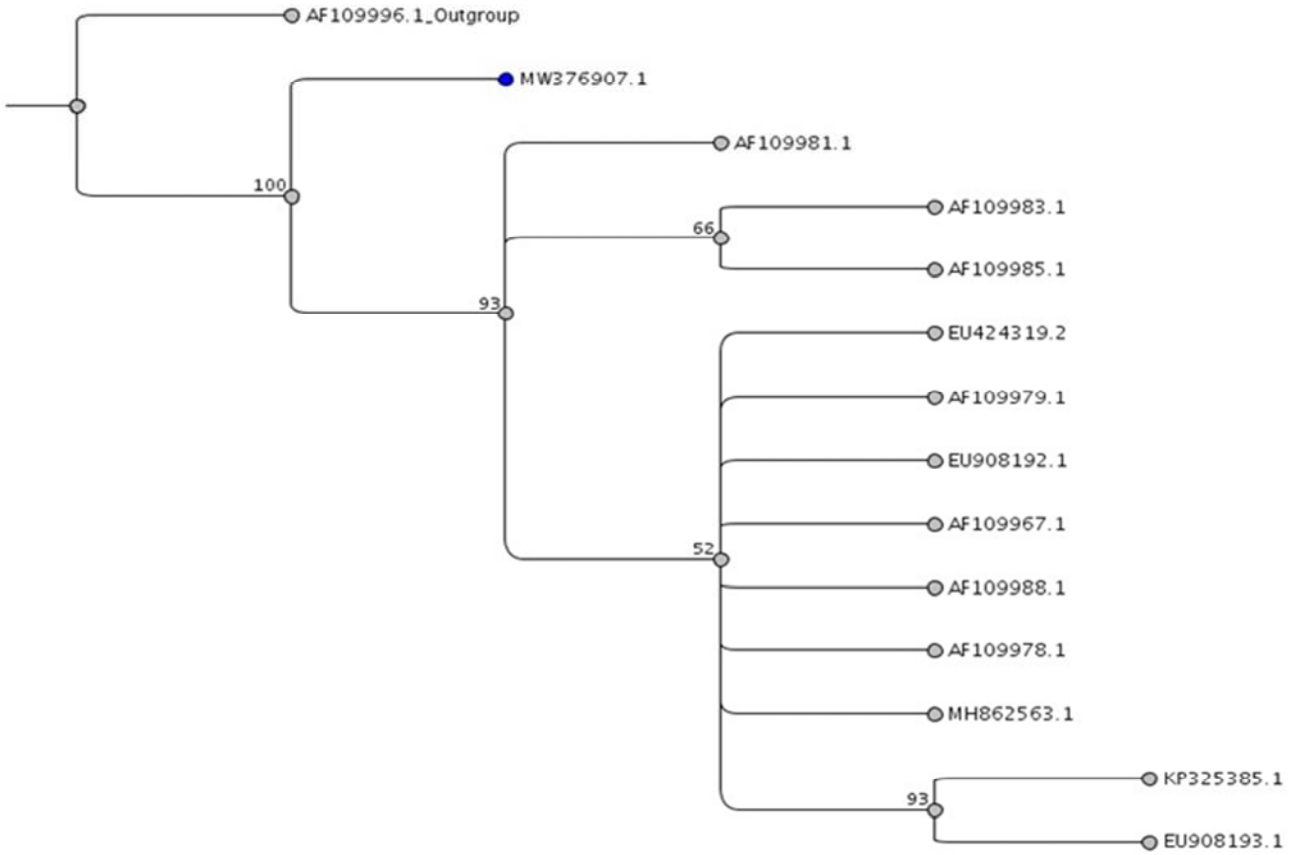

Figure 1. Phylogenetic tree: Sequences were aligned using Geneious Alignment and tree reconstruction using Geneious tree builder version 9.0.5.

The suitability of Sawdust for mycelial growth and fruiting body production has been reported by Kadiri and Fasidi [36]. The rate of ramification of the substrate containing Afra sawdust was twice as fast as those of Teak and Gmalina. The 21 cm length of the bags were fully covered in mycelium in just sixteen days (table 1). This may explain why the mushroom is usually found associated with decaying logs of this tree in the wild. On the other hand, it took substrates of Teak and Gmalina twenty-eight days to be fully colonised by the mushroom. Afra substrates began to produce visible sclerotia after thirty days of inoculation or fourteen days after full colonisation of the bags. On the other hand, it took forty-seven days for sclerotium to be visible in Teak and Gmalina substrates. The first harvesting of sclerotium was done ninety (90) days after inoculation. The yields of the substrates at first harvest are shown in Figure 2 with the afra-based substrates having an average of 140 g/bag while teak and Gmalina substrates gave 105 g/bag and 103 g/bag respectively. However, there was no significant difference in the output of the subtrates incubated at room and high temperature for Afra substrates. The yields of the other two subtrates showed significant differences, with those incubated at high temperature giving better yields. The average biological efficiency of the three substrates at first harvest were Afra (20.05%) *Gmalina* (13.01%) and Teak (12.8%) respectively (Figure 6). At second harvest, the average yields of the substrates were Afra (62.5 g/bag), Teak (80 g/bag) and *Gmalina* (105 g/bag) respectively (Figure 3). The substrates incubated at high temperature showed significantly better yields for all the substrates. At third harvest, the average yields of Afra was 155 g, Gmalina 90 g and teak 30 g (figure 4). The substrates incubated at high temperature also showed better yields except for Afra substrates. The statistical analysis of the overall yields of the substrates (Figure 5) shows that substrates incubated at high temperature gave better yields than the ones at room temperature, with Gmalina HT and Afra HT being the best two. The overall average yields of the subtrates were as follows; Afra 120 g, Gmalina 105 g and Teak 75 g. This shows that Afra substrates gave the best yield of the three substrates and this may explain why Afra is one of the trees of choice for the mushroom to colonise in the wild. These yields are far better than those of Otunla *et al*. [37] who reported sclerotia weight of 35.34 g for cassia sawdust and 37.44 g for mango sawdust, respectively. The difference may be due to the species of tree used or to different method of substrate preparation. Also, the yields of the substrates used in this work are lower compared to those of Chiejina and

Olufokunbi, [10] who reported a maximum yield of 415.48 g for fermented sawdust and minimum yield of 184.44 gm for the mixture of rice bran and fermented sawdust substrate. These high yields may be due to better bio-availability of the lignin, cellulose and hemi-cellulose in the sawdust as a result of the fermentation of the sawdust used. It may also be due to the specie of tree of the sawdust. While sawdust of Teak, Gmalina and Afra were used in this work, Chiejina and Olufokunbi, [10] used sawdust of *Daniela oliveri* (Rolfe), one of the trees with which the mushroom is commonly associated in the wild, and dried Oil Palm Fruit Fibre (OPFF) of *Elaeis guineesis* Jacq. On the other hand, while 60 g of spawn made from fruiting body of germinated sclerotium was used to inoculate the substrates in this work, Chiejina and Olufokunbi used sclerotial cubes (4 x 4 cm^3) as inoculum. Finally, while Chiejina and Olufokunbi, [10] only harvested once, there were three tranches of harvest in this work and a combination of the tree tranches presents figures closer to their results. Overall, and except for teak substrates, substrates incubated at high temperature gave significantly better yields than those incubated at room temperature. This goes further to confirm the already established findings that the production of sclerotium by the mushroom occur as a reaction to harsh environmental conditions of which high temperature is chief. The bio-efficiency of the substrates at second harvest as presented in (Figure 7) shows that *Gmalina* at high temperature (36.37%) was the best while Afra at room temperature has the least (6.67%). This differ significantly from the findings of Chiejina and Olufokunbi, [10] who reported 53.14% as highest for fermented sawdust and 19.30% as least for chopped corn straw and fermented sawdust substrate. Since bio-efficiency is closely linked to bioavailability, and since fermentation improves the bioavailability of the nutrients in the sawdust, it therefore means that substrates formulated with fermented sawdust will have better bioefficiency when compared to those formulated with a non-fermented sawdust. That may explain the difference in the bioefficiency of the substrates used in this work and their effort. At third harvest, the bioefficiency of the substrates (figure 8) showed Gmalina HT, Afra RT and Afra HT in this order, as the best. On the average however, the substrates have the following scores; Afra 40.19%, Gmalina 42.45%, and Teak 18.28% respectively. There is however no significant difference between Afra and Gmalina substrates scores. The overall bio-efficiency of the substrates (Figure 8) showed that Gmalina HT and Afra HT are the best substrates and showed no significance difference in yield. Afra RT also showed no significant difference with Afra RT in yield. The rest of the substrates. That is, Teak RT, Teak HT, and Gmalina RT are all at par. The least bioefficient of the substrates was Teak RT. It also showed that Gmalina has an average bioefficiency of 28.67%, Afra 27.95% and Teak 18.28% respectively. Statistical analysis also showed that substrates incubated at high temperature gave better yields than those incubated at room temperature as follows Afra HT 130 g/bag, Afra RT 110 g/bag; Gmelina HT 130 g/bag, Gmelina RT 60 g/bag; Teak HT 70, Teak RT 60 g/bag. The soil substrates did not produce any sclerotium at all, though it grew what can be described as thick mycelia which were strong enough to pierce the polyethylene bags (figures 10 and 11). This may be due to the inability of the mushroom to overpower other fungi and bacteria present in the soil used. It may also be due to inadequate lignin, cellulose and hemicellulose in the soil and this is in consonance with the findings of Okhuoya and Okogbo [18].

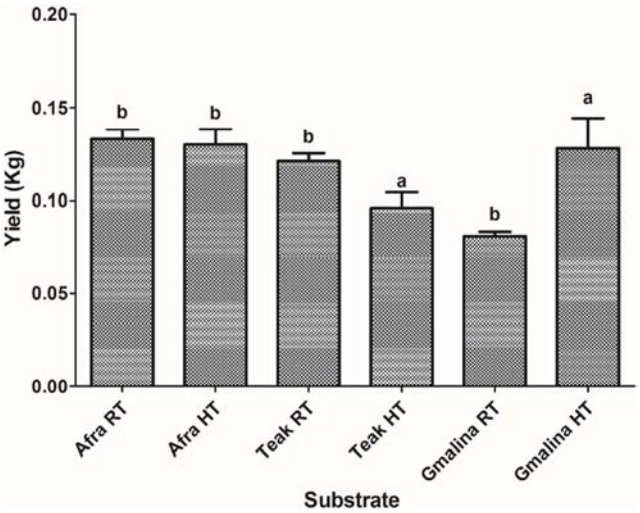

Figure 2. Sclerotium yield of substrates at first harvest.

Key
RT – Room temperature (25±2°C)
HT- High temperature (36±2°C)

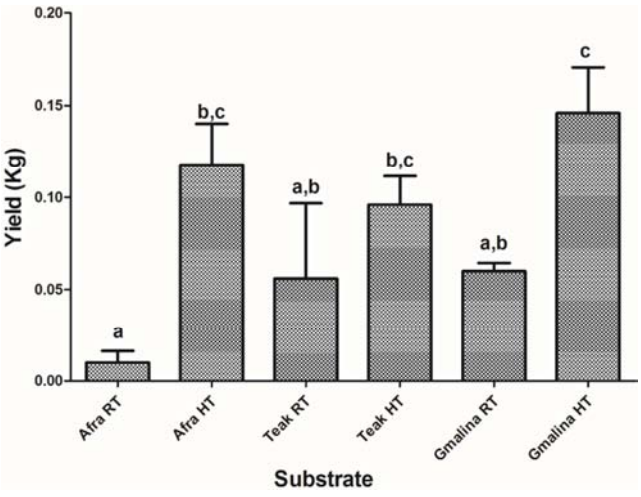

Figure 3. Sclerotium yield of substrates at second harvest.

Key
RT – Room temperature (25±2°C)
HT- High temperature (36±2°C)

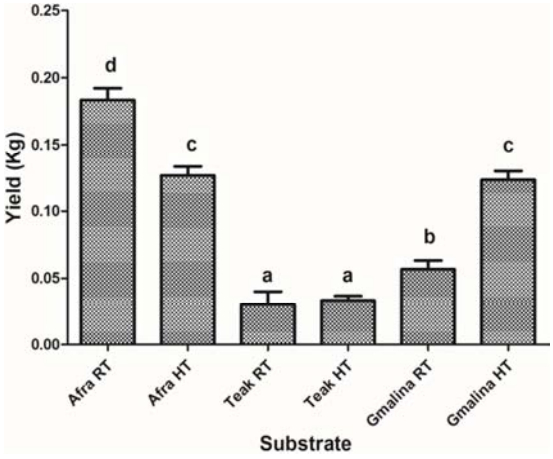

Figure 4. Sclerotium yield of substrates at third harvest.

Key
RT – Room temperature (25±2°C)
HT- High temperature (36±2°C)

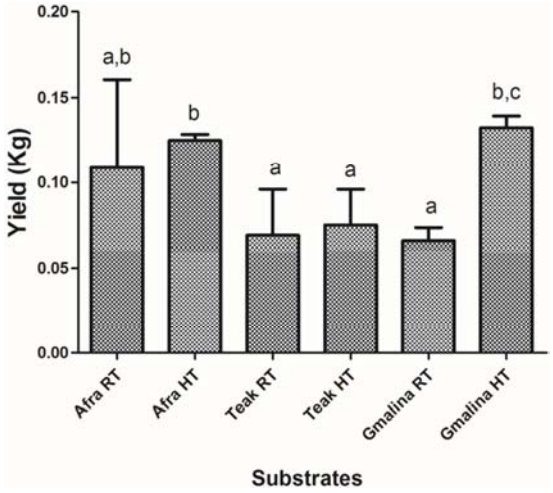

Figure 5. Overall sclerotium yield of substrates.

Key
RT – Room temperature (25±2°C)
HT- High temperature (36±2°C)

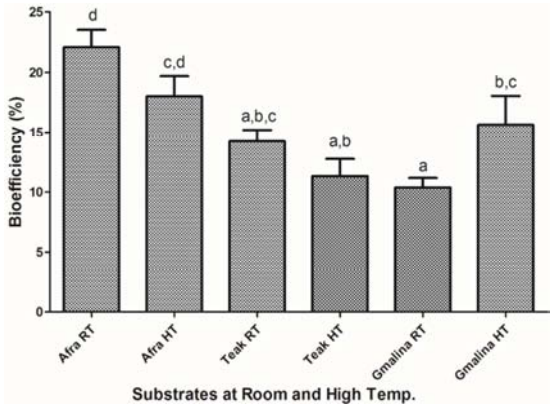

Figure 6. Bio-efficiency of substrates at first harvest.

Key
RT – Room temperature (25±2°C)
HT- High temperature (36±2°C)

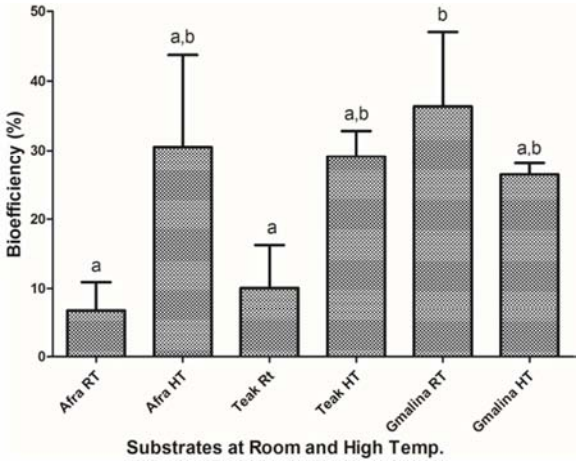

Figure 7. Bio efficiency of the substrates at second harvest.

Key
RT – Room temperature (25±2°C)
HT- High temperature (36±2°C)

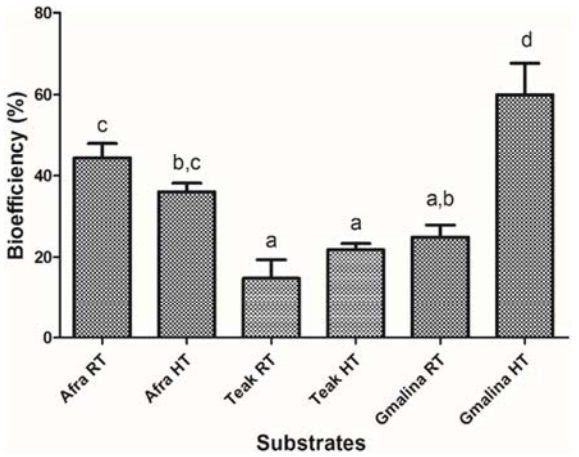

Figure 8. bioefficiency at third harvest.

Key
RT – Room temperature (25±2°C)
HT- High temperature (36±2°C)

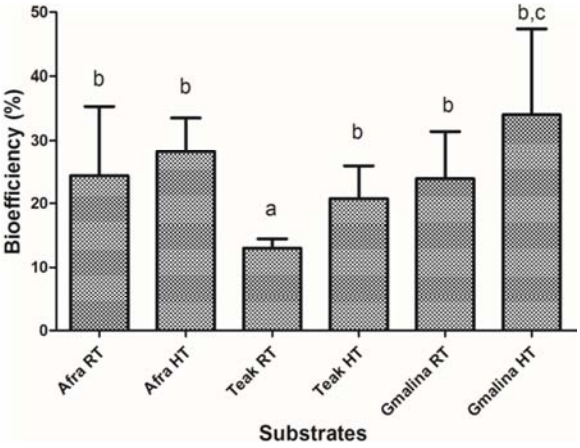

Figure 9. Overall bioefficiency of the substrates.

Key
RT – Room temperature (25±2°C)
HT- High temperature (36±2°C)

Figure 10. *Pleurotus tuber-regium* thick mycelia growing in soil substrate.

Figure 11. *Pleurotus tuber-regium* thick mycelia piercing open polyethylene bags.

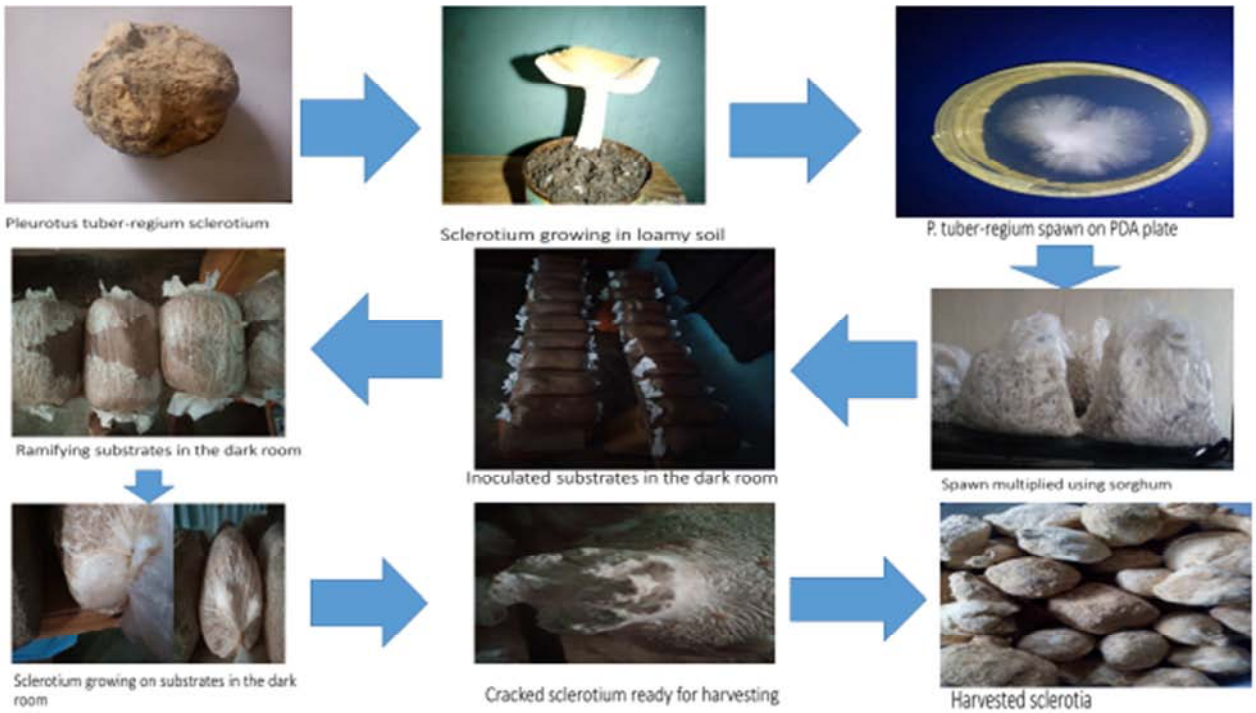

Figure 12. Schematic diagram of Pleurotus tuber-regium cultivation protocol on supplemented lignocellulosic waste.

6. Conclusion

This work has shown that *P. tuber-regium* sclerotia can be easily and profitably cultivated using sawdust of *Terminalia superba* and *Gmalina arborea* by making the growth conditions unconducive for fruiting body production. It has also established the importance of high temperature in encouraging the mushroom to grow sclerotium instead of fruiting bodies. The inability of the soil substrates to produce sclerotium points to the importance of lignin and cellulose in the nutrition of *P. tuber-regium*. It has also proved that the portion of soil from which the sclerotia are exhumed from in the wild are usually very rich in lignin and cellulose. Finally, the work has shown that harvesting can be done more than once with proper management of the substrates which can be a veritable way of converting agricultural wastes to wealth.

Competing Interest

The authors declare that there is no competing interest with respect to this work.

Table 1. Rate of Ramification of the Mushroom in the Substrates.

Substrates	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Afra	5.7±0.19[a]	14.39±0.44[a]	FR	FR	FR	FR
Teak	2.6±0.27[b]	6.72±0.31[b]	10.98±0.29[a]	15.23±0.44[b]	20.07±0.36[b]	FR
Gmelina	2.7±0.29[b]	6.83±0.30[b]	11.04±0.31[a]	15.73±0.43[b]	20.07±0.35[b]	FR

The values are expressed as mean ± SEM, each value followed by the same alphabet across the column are not significantly different at $p < 0.05$
Key FR – fully ramified.

References

[1] Chen AW and Huang N 2004. Production of tuber-like sclerotia of medicinal value by *Pleurotus tuberregium* (Fr.) Sing. (Agaricomycetideae). *International Journal of Medicinal Mushrooms* 5: 313-319.

[2] Oso, B. A. 1977. *Pleurotus tuber-regium* from Nigeria. *Mycologia* 69: 271-279.

[3] Isikhuemhen, SO, LeBauer D S 2004. Growing *Pleurotus tuberregium*. Mushworld Publication 1: 264-274.

[4] Zoberi, MH. 1973. Some edible mushrooms from Nigeria. *Nigerian Field* 38: 81-90.

[5] Stamets P 2001. A novel approach to farm waste management. *Mushroom Journal Winter* 30: 28-32.

[6] Onuoha CI 2007. Cultivation of the mushroom (*Pleurotus tuber-regium*) using some local substrates. *Life Science Journal* 4 (4): 58-61.

[7] Isikhuemhen SO, Moncalvo J, Nerud F, Vilgalys R 2000a. Mating compatibility and phylogeography in *Pleurotus tuberregium*. *Mycology Research*.; 104: 732-737.

[8] Isikhuemhen, OS, Nerud F, Vilgalys R 2000b. Cultivation studies on wild and hybrid strains of Pleurotus tuberregium (Fr.) Sing. on wheat straw substrate. *World Journal of Microbiology and Biotechnology* volume 16, pages 431–435 (2000).

[9] Willetts HJ 1971. Survival of fungal sclerotia under adverse environmental conditions. *Biological Reviews of the Cambridge Philosophical Society* 46 (3), 387-407.

[10] Okhuoya, JA, Okogbo, FO 1990. Induction of edible sclerotia of *Pleurotus tuber-regium* (FR) Sing. in the laboratory. *Annals of Applied Biology* 117: 295-298.

[11] Smith EM, Henkel WT, Rollins AJ 2014. How many fungi make sclerotia? *Fungal Ecology* (2014), http://dx.doi.org/10.1016/j.funeco.2014.08.010

[12] Petersen RH, Hughes KW, Psurtseva N 2009. Biological species in *Pleurotus*. www.bio.utk.edu/mycology/Pleurotus/Species/Ptuber-regium.htm

[13] Akpaja, EO, Isikhuemhen, OS, Okhuoya, JA. 2003. Ethnomycology and uses of edible and medicinal mushrooms among the Igbo people of Nigeria. *International journal of medicinal mushrooms* 5: 313-319.

[14] Kamalebo HM, Malale HN, Ndabaga CM, Degreef J, Kesel AD 2018. Uses and importance of wild fungi: traditional knowledge from the Tshopo province in the Democratic Republic of the Congo. *Journal of Ethnobiology and Ethnomedicine* 14: 13 DOI 10.1186/s13002-017-0203-6.

[15] Akinyele BJ 2020. Fungi and fungal products: Integral Part of Industrialisation in a Developing Economy. Inaugural Lecture Series, Federal University of Technology, Akure, Ondo State, Nigeria Pp 21-22.

[16] Chiejina, N V, Olufokunbi, JO 2010. Effects of different substrates on the yield and protein content of *Pleurotus tuberregium*. *African journal of biotechnology* 9: 1573-1577.

[17] Apetorgbor AK, Dzomeku M, Apetorgbor MM 2013. Growth factors and cultivation of *Pleurotus tuber-regium* on selected plant wastes. *International Food Research Journal* 20 (6): 3387-3393.

[18] Okhuoya, JA and Okogbo, FO 1991. Cultivation of *Pleurotus tuberregium* (Fr.) Sing. on various farm wastes. Proceedings of the Oklahoma Academy of Sciences, 71: 1-3. http://digital.library.okstate.edu/oas/oaspdf/v71/p13.pdf

[19] Cho, S. B. (2004). Oyster mushroom cultivation. Mushworld Publication 1: 1-3.

[20] Ikewuchi, CC, Ikewuchi JC 2009. "Chemical Profile of *Pleurotus tuberregium* (Fr) Sing's Sclerotia". *Pacific Journal of Science and Technology*. 10 (1): 295-299.

[21] Gbolagade J, Ajayi A, Oku I, Wankasi D 2006. Nutritive Value of Common Wild Edible Mushrooms from Southern Nigeria. *Global Journal of Biotechnology & Biochemistry* 1 (1): 16-21.

[22] Jonathan G, Adetolu A, Ikpebivie O, Donbebe W 2006. Nutritive value of common wild edible mushrooms from southern Nigeria. *Global journal of biotechnology and biochemistry* 1: 16-21.

[23] Ohiri RC 2018. Nutriceutical potential of *Pleurotus tuber-regium* sclerotium. *Ukranian. Biochemical Journal.*, 2018, Vol. 90, N 3 doi: https://doi.org/10.15407/ubj90.03.084

[24] Okolo KO, Orisakwe OE, Siminialayi IM 2017. *Pleurotus tuber-regium* Mushrooms in The Diet of Rats Ameliorates Reproductive and Testicular Injury Caused by Carbon Tetrachloride. *Clinical Phytoscience* 3: 14 DOI 10.1186/s40816-017-0051-x.

[25] Adebayo EA, Meneses ME, Martınez-Carrera D, Avila–Nava A, Morales P, Castillo I, Sobal M, Escudero MB 2018. Comparative study of antioxidant and antibacterial properties of the edible mushrooms *Pleurotus levis*, *P. ostreatus*, *P. pulmonarius* and *P. tuber-regium*. *International Journal of Food Science and Technology* 53: 1316- 1330. https:dx.doi:10.1111/ijfs.13712

[26] Bamigboye CO, Oloke JK, Burton M, Dames JF, Agbaje L 2019. Optimization of the Process for Producing Biomass and Exopolysaccharide from the King Tuber Oyster Mushroom, *Pleurotus tuber-regium* (Agaricomycetes), for Biotechnological Applications. *International Journal of Medicinal Mushrooms* 57: 311-322.

[27] Afieroho OE, Olutayo LL, Adedokun M, and Emenyonu, N 2013. Antituberculosis and Phytochemical Investigation of the Dichloromethane Extract *Pleurotus tuber-Regium* (Fries) Singer Sclerotium. *International Research Journal of Pharmacy* 4 (1): 255-257.

[28] Isikhuehmen O, Anoliefo G, Oghale O 2003. Bioremediation of crude oil polluted soil by the white rot fungus *Pleurotus tuberregium* (Fr.) Sing. *Environmental Science. Pollution Research* 10: 108-112.

[29] Hibbett D. S. and Thorn R. G. 1994. Nematode-trapping in *Pleurotus tuberregium*. *Mycologia* 86: 696-699.

[30] Okhuoya, JA and Etugo, JE 1993. Studies of the cultivation of *Pleurotus tuberregium* (Fr.) Sing. an edible mushroom. *Bioresource technology* 44: 1-3.

[31] Zheng A, Ogbadu LJ, Solomon BO 2010. Cultivation of Selected Tropical Edible Mushroom. Marvellous Mike Press Ltd. 1 Ed, Nigeria, pp 10-11.

[32] Adebayo, E. A., Alao, M. B., Olatunbosun, O. O., Omoleye, E. O., Omisakin, O. B., (2014). Yield evaluation of Pleurotus pulmonarius (Oyster Mushroom) on different Agricultural wastes and various grains for spawn production. Ife J. Sci. 16 (3), 475–480.

[33] Vesty, A., Biswas, K., Taylor, M. W., Gear, K., Douglas, R. G., 2017. Evaluating the impactof DNA extraction method on the representation of human oral bacterial and fungal communities. PloS One 12 (1), e0169877.

[34] Gardes, M., Bruns, T. D., 1993. ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizea and rusts. *Mol. Ecol*. 2, 113–118.

[35] Sanger, F., Nicklen, S., Coulson, A. R., 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. Unit. States Am*. 74 (12), 5463–5467.

[36] Kadiri M. and Fasidi, IO. 1990. Variations in chemical composition *Chlorophyllum molybodies* (Mayerex. Fr.) Massres and *Pleurotus tuberregium* (Fries) during fruitbodies development. *Nigerian journal of science* 24: 86-90.

[37] Otunla CA, Gbolagade SJ, Idowu OO, Olawuyi OJ 2019. Mycelial growth and sclerotia production of *Pleurotus tuber - regium* (Fries) singer on four sawdust types at three composting intervals. *Agronomski Glasnik* 80 DOI 10.33128/ag.80.2.3.