

Phytochemical Screening of *Pleurotus tuberregium* (SING) Grown on Different Substrates

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Abstract: The Sclerotia of *Pleurotus tuberregium* (Sing) were grown on four different substrates namely: humus soil, mixture of Sawdust and humus soil, sawdust and shreds of the wood of *Treculia africana*. The quantity and quality of the fruit bodies produced were measured using the following parameters: number of fruit bodies produced, height of fruit bodies, fresh weight, dry weight, diameter of pileus and length of the stipe. Sawdust which served as the control was better growth medium in terms of length of stipe, number of fruit bodies and height of mushroom. The fruit bodies from a mixture of sawdust and humus soil were better in terms of fresh and dry weight while humus soil alone produced fruit bodies with wider pileus diameter. The wood shreds of *Treculia africana* did not support the growth of the mushroom. The phytochemical composition of the sclerotium and fruit bodies from the different substrates showed that the sclerotium had more flavonoids, alkaloids, tannins, saponins and phenols than the fruit bodies from the different substrates. There was no significant difference between the alkaloid, tannin, saponin and phenolic composition of the fruit bodies from the different substrates. There was, however, a significant difference at $P \leq 0.05$ between the flavonoid content of fruit bodies from humus soil, sawdust and those from a mixture of humus soil and sawdust.

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1. Introduction

Mushrooms are a group of fleshy macroscopic fungi, which recently, as other fungi were introduced into the plant kingdom of cell wall and spores. Mushroom has been valued and treated throughout the world as a special kind of food and medicine for thousands of years (Lindquist, et al; 2005, Tribe, et al; 1973).

There are many varieties of mushroom of which *Pleurotus* are characterized by a white spore print, attached to gills, often with an eccentric stipe or no stipe at all. They are commonly known as "Oyster mushroom" (Miles, et al; 1997). *Pleurotus tuberregium* is a tropical sclerotial mushroom which has been gaining some interest in the United States. Being Sclerotial, the mushroom produces sclerotium, or underground tuber, as well as a fruiting body. Both the sclerotium and the fruiting body are edible. The mushroom when matured, the cap curves upwards to form a cup-like shape. The sclerotium is spherical to ovoid and can be quite large-up to 30cm (11.8 inches) or larger in diameter (Oso; 1977). It is dark on the outside and white on the inside. The sclerotium is often formed during uncondusive environmental condition underground in form of a tuber.

Mushrooms are highly nutritious so they contain good quality proteins, vitamins and mineral (Khanna, et al; 1984, Flegg, et al; 1976). Mushrooms are low calorie

food with little fat and are highly suitable for obese persons with no starch and very low sugars, they can serve as medicinal food for diabetic patient (Bano, 1976).

Edible mushroom like *Pleurotus* are known to be among the largest of all fungi and so is said to exist in its natural habitat in a mycorrhizal relationship with a tree, and this is one of the reason why forest are often generous to mushroom hunters. Ogundana, (1978), and Stemets (2001) observed that the fungus is often found growing around the African bread fruit tree (*Treculia africana*). It attacks dead woods, on which it produces globose or ovoid sclerotia (Oso; 1977, Fasidi and Olorunmaiye; 1994).

Despite its nutritional value, mushroom cultivation is not widespread; many mushrooms are considered to be healthy food because they contain large enough protein needs of the rural poor especially during the rains. It is also rich in some essential vitamins (B₁, B₂, C) and essential minerals than most plants. They also have a low fat content and hence high fibre content that enhances digestion of food. They have some medicinal properties as in *Pleurotus tuberregium* (Sing), it is used to treat heart problem in the eastern part of Nigeria especially among the Igbos and Edos, it is used in the treatment of asthma, cough and obesity (Isikhuemhen, et al, 2000).

During the rainy seasons, different species of

both edible and non-edible species usually grow on various natural substrates such as garden soil, decayed wood, termite nest, palm wastes, leaf litters, under the shade provided by cocoa, teak, coffee and rubber plantations. People in the villages (mushroom hunters) usually wake up early in the morning to look for wild edible mushroom. The collected edible species are usually sorted out, cooked or sold in the local markets. This experience of mushroom boom from the wild occurs once or twice a year and disappears within a month. During that period, the price would drop due to increase in supply only for a short period. At the end of this boom, mushroom ceases to be seen any where again despite the continual demand. But this important plant can be grown like any other crop i.e like business thereby being available throughout the year.

Therefore, there is the need to ensure a balanced daily diet through the consumptions of *Pleurotus tuberregium* which is also used in hotels as flavonoids and in preparation of different kinds of delicacies and to improve businesses and income of Nigerians, there is a need to grow and cultivate this mushroom in commercial quantities outside its natural habitat using different substrates. Our forest areas are fast disappearing due to deforestation which has further reduced the quantities of mushrooms produced.

The objectives of this study therefore are to screen some substrates such as sawdust mixture of sawdust and Humus soil, Humus soil and shredded pieces of *Treculia africana* wood on the growth of *Pleurotus tuberregium* and conduct a Phytochemical screening of the grown mushroom from the above mentioned substrates and that of the sclerotium of *Pleurotus tuberregium*.

2. Materials and Methods

This work has two parts. The first part has to do with the growing of the mushroom from its sclerotia on four different substrates while the second part has to do with phytochemical screening of the sclerotia and the cultivated mushrooms from the four different substrates.

The laboratory experiment was carried out at the plant science and biotechnology laboratory in Imo State University, Owerri. *Pleurotus tuberregium* was the specie selected. The sclerotium of this species was sourced from Owerri old market. The materials used as substrates within were sourced within Imo State and they include:

- 1) Saw dust
- 2) Humus soil
- 3) Humus soil and saw dust
- 4) Shreds of *Treculia africana* wood

Preparation of Substrates for Cultivation

The single substrates i.e saw dust, Humus soil and chopped wood of *Treculia africana*, and a mixture of Humus soil and saw dust was mixed on ratio 1:1 bases.

Then the four substrates were sprinkled with distilled water moderately and the water content tested by pressing the substrates with hand to allow water drip. It was tested and it dripped, showing there was moderate water. The substrates were packed in polythene bags and then put into an autoclave for sterilization. After heating for 121^o2 after 15 minutes, after which the substrates were allowed to cool.

1000g (1kg) of each of these substrates was weighed on a weighing balance and poured in three (3) replicates into the Nursery polyrhene, all together were twelve (12) replicates. The sclerotium of *pleurotus tuberregium* was cut into miniset of 4cm² and then planted on each replicate, while the relative humidity was maintained at 75% to 80% required for fructification of the mushroom.

Data Collection

The growth performance of *Pleurotus tuberregium* on the different substrates was determined by recording the number and size of fruiting bodies after sprouting. Measurements from these various replicates were added together and their mean recorded.

Statistical Analysis

At the end of the experiment fruiting bodies of *Pleurotus tuberregium* were harvested at maturity and the following parameters were measured as follows:

1. Total number of fruiting bodies: This was done by counting the number of fruiting bodies on each substrate.
2. Height of fruiting bodies: This was measured in centimeters using a ruler from the base of the stipes to the pileus.
3. Diameter of the pileus: This was measured in centimeters with a ruler from one edge of the pileus across the stipe to the other edge.
4. Fresh weight of fruiting bodies after harvesting: This was weighed using an electronic weighing balance.
5. Dry weight of fruiting bodies: This was weighed using a weighing balance after oven drying, at 121^oC for 15 minutes
6. Length of stipe: This was measured using a ruler in centimeters from the base of the pileus to the point where it was harvested at the base. Results obtained were recorded and subjected to statistical analysis using one-way ANOVA

Phytochemical Screening of Grown Mushroom

This involved phytochemical screening of both the sclerotia and the grown mushroom of *Pleurotus tuberregium* produced from the various substrates of sawdust, humus soil, humus soil and sawdust and shredded wood of *Treculia africana*. The analysis was carried out at the central laboratory unit National crop

and Root Research institute, Umudike, Umuahia in Abia State.

Source of Sample

The sample of the sclerotia of *Pleurotus tuberregium* was bought from Owerri Old Market while the sample of the mushroom of *Pleurotus tuberregium* was grown from the different substrates.

Preparations of Samples

The sclerotia sample bark was shredded and placed in electric oven at 65°C to dry for two hours while the fruit bodies which were already oven dried were grated into powder.

3. Results

Ten days after planting the sclerotia, it was observed that white mycelia had colonized all the substrates. Fruit bodies were first observed on the humus soil, four days later. Two days after it was observed on the mixture of sawdust and humus soil and three days later on sawdust. The fruit bodies never grew on *Treculia africana* wood shreds. Within five days the fruit bodies were finally mature and harvested. The table 1 below shows the values of various parameters measured.

Table 1 – Represents the means and standard deviation valves of the Diameter of the pileus, length of stipe, Number of fruit bodies, Height of the mushroom, fresh weight and dry weight of the mushrooms from the various substrates used.

Substrates	Diameter of the pileus (cm)	length of the stipe(cm)	Number of fruit bodies	Height of the mushroom (cm)	Fresh Weight (g)	Dry Weight (g)
1. Saw Dust	7.74 ±2.18	6.72 ±1.44	2	9.94 ±3.09	7.57 ± 3.79	2.12 ± 1.10
2. Humus Soil	8.65 ± 1.75	5.83 ± 0.47	1	6.92 ±1.13	5.95 ± 3.28	2.12 ± 0.93
Mixture of Humus Soil And Saw Dust	5.23 ± 1.53	4.83 ± 0.77	1	8.12 ± 0.14	8.36 ±0.93	2.38 ±0.18
4. Treluilia africana wood shreds	-	-	-	-	-	-

Results for Phytochemical Analysis

The result of the phytochemical analysis of the various mushroom grow from the various substrate and that of the sclerotium. Where:

Specimen A: represents the mushroom grown from humus soil.

Specimen B: represent the mushroom grown from mixture of humus soil and saw dust.

Specimen C: represents the mushroom grown from sawdust.

Specimen D: represents the sclerotium of *Pleurotus tuberregium*.

Table 2 below shows the mean values and their standard deviation of the percentage presence of

alkaloid, flavonoid, tannin, saponin and phenols of the various specimens. The (sclerotium) contained the highest percentage of alkaloid with 501% while specimen A (mushroom got from the humus soil) contained the lowest yield with 0.55% for the flavonoid, specimen D also had the highest percentage with 2.11% while specimen A and C (mushroom got from humus soil and sawdust) had the lower percentage with 0.08%. In Tannin, specimen D had the highest percentage with 1.84% while specimen C has the lowest percentage of 0.12%. For saponin specimen D still had the highest percentage with 4.98 while specimen A has the lowest percentage with 0.12%. Phenols has specimen D also as the highest percentage with 0.42% while specimen A has the lowest percentage of 0.06%

TABLE 2: Percentage of phytochemicals in the mushrooms investigated.

Specimen	Alkaloid (%)	Flavonoid (%)	Tannin (%)	Saponin (%)	Phenols (%)
A Fruit body from Humus soil	0.55 ±0.01	0.08±0.02	0.14±0.00	0.12±0.02	0.06±0.00
B Fruit body from mixture of Humus soil and saw dust	0.60 ±0.02	0.12±0.01	0.16±0.00	0.14±0.02	0.08±0.00
C Fruit body from saw dust	0.66 ±0.02	0.08±0.03	0.12±0.00	0.18±0.02	0.08±0.00
D The Sclerotium	5.01±0.14	2.11±0.03	1.84±0.00	4.98±0.03	0.42±0.00

3. Discussion

In the work, firstly, the sclerotia of *Pleurotus tuberregium* was grown on four different substrates, namely Humus soil, mixture of sawdust and humus soil, sawdust and *Treculia africana* wood shreds. Only three of the substrates: humus soil, mixture of humus soil and sawdust and then sawdust, were observed to be good substrates for the growth of the mushroom.

Fruiting bodies gotten from the substrates that supported the growth of the mushroom were measured qualitatively using some parameters such as diameter of pileus, length of stipe, number of fruiting body, height of the mushroom, fresh weight of mushroom and its dry weight. All these parameter were subjected to statistical analysis using ANOVA.

Actually out of the six parameters measured in this work for the quality of the fruit bodies produced from the various substrates, Those produced from sawdust had more significance than others in three of the parameters (length of stipe, no of fruit bodies and Height of the mushroom), which shows that fruitbodies from this substrate, could attract high market value as the fruit bodies are usually sizeable and this also agrees with (Candy, 1990), Kadiri and Fasidi (1990) and Okwujiako (1992) that sawdust is a good growth medium for *Pleurotus tuberregium*. Fruit bodies from humus soil produced larger pileus while fruitbodies from the mixture of humus soil and sawdust had higher fresh weight and dry weight. Therefore, fruit bodies from humus soil could also attract high market value because of the size of its pileus.

In the order of fructification, mushrooms from the humus soil fruited first, followed by mixture of humus soil and sawdust and lastly by mushrooms from

sawdust. Fruit bodies from humus soil was able to fruit first perhaps because of the richness of the soil in nutrients and organic matter which are necessary for the formation of fruit bodies as conducted by Chang and Buswel (1996).

Treculia africana wood shreds did not encourage the growth of the mushroom. This could be as a result of low absorption of water by the wood shreds since a humid environment favoured the fructification of the fungus. The mushroom is said to traditionally grow near and on the wood of *Treculia africana* and so it could be that the fungus derives some other nourishment from the soil around the *Treculia* wood which only *Treculia* wood shreds alone could not provide.

Further more, the mushroom produced from the various substrates were compared along side with the sclerotium of *Pleurotus tuberregium* as they were phytochemically analysed and their results subjected to statistical analysis using one-way ANOVA at $P < 0.05$.

In the phytochemical analysis the sclerotium showed high significance in the presence of all the chemicals (flavonoid, alkaloid, Tannins, saponins and phenol) when compared with the mushrooms produced from the three other substrates. These shows that the sclerotium of *pleurotus tuberregium* being very rich in all the phytochemicals mentioned above could serve as a source from which industries needing precursors for the synthesis of complex chemical substances (Moore 2003). This finding also agrees with Moore, (2003) which stated that the phytochemical found in the sclerotium of *Pleurotus tuberregium* promote the function of the immune system, act directly against bacteria and viruses, reduce inflammation and its associated with the treatment and prevention of cancer,

cardiovascular disease and any other malady affecting the health or well being of an individual

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4. References

- Bano Z, (1976) The nutritive values of mushroom. Paper presented at: first symposium on simary And cultivation of edible mushrooms India regional research laboratory, Jammu PP. 148 – 69.
- Candy, C.I. (1990) The cultivation of *pleurotus* is Mauritania proceedings 1st conference on African mycology Mauritania June 1990:1-5.
- Chang, S.T. and Buswell, J.A. (1996) Mushroom nutraceuticals. World Journal of microbiology and biotechnology.
- Fasidi, I.O., Olorunmaiye, Kehinde S. (1994) Studies on the requirements of vegetative growth of *pleurotus tuberregium* (Fr.) Singer a Nigerian mushroom. Food Chem.
- Flegg P.O. and Maw, G.A. (1976) Mushroom and their possible contribution to world protein needs mushrooms Journal 48: 396 – 405.
- Kadiri, M and Fasidi, I.O. (1990) Variations in Chemical composition chlorophyllum molybodies (Mayerex. Fr)
- Massres pleurotus tuberregium (fries) during fruitbody development. Nigerian Journal of Science 24: 86 – 89.
- Khanna, P. and Garcha, H.S. (1984) Pleurotus tuberregium a source of food protein. Mushroom news letter. Topics 4:9-14.
- Lindequist, I.L., Niedermayer, T.H., Julich W.D. (2005) The pharmlological potential of mushroom Evid. Based complement Alternat Med. 2: 285 – 99.
- Moore, D. and Chi, S.W., (2003) Fungi products as food (eds) pointing, S.B. and Hyde, K.O. In Bio-exploitation of filamentous fungi. Fungi Diversity Research lenis 6: 223 – 251.
- Miles, P.G. and Chang, S.T. (1997) Mushroom biology: concise basics and current development (World Scientific Publishing Company, New York).
- Okwujiako, I.A. (1992) Studies on the cultivation of edible mushroom (*pleurotus tuberregium* (fries) singer. Tropical Journal of Applied Sciences 2.
- Ogundana, S.K. (1978) Nigeria and the mushroom. Mushroom Science Part 113: 8 – 32.
- Oso, B.A. (1977) *Pleurotus tuber-regium* from Nigeria. Mycologia LXIX (2): 271 – 9.
- Tribe, I. and Tosco, U. (1973) The world of mushrooms (Orbis Publishing London) Vol. VII

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