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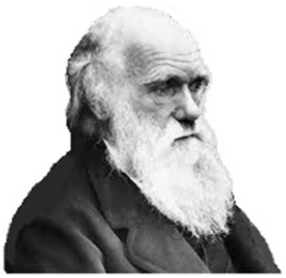

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A Study on Cultivation of Indigenous Mushrooms in South Eastern Nigeria

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ABSTRACT

Studies were carried out to investigate cultivation of some indigenous edible mushrooms in Anambra State. The most suitable substrates for cultivation of *Pleurotus tuberregium* and *Volvariella volvacea* were also investigated. Spawn of *V. volvacea* was obtained from the Department of Life Science, University of Benin, Edo State and sclerotia of *P. Tuberregium* purchased from Eke Awka Market, Anambra State were used for this study. Plantain leaves and oil palm bunch wastes were substrates employed for *V. volvacea* whereas topsoil was used for *P. tuberregium* cultivation. The methods used were site preparation, substrate preparation, soil preparation, sclerotia preparation, cultivation of sclerotia, irrigation, spawn cultivation and harvesting of mushrooms. Means were analyzed statistically using (ANOVA) to test for significance. Means were separated using Duncan's Multiple Range Tests (DMRT).The highest number of fruit bodies of *V. volvacea*, 31±2.68 was obtained from plantain leaves on day 2 after primordial emergence whereas the least, 2±1.00 was recorded from oil palm bunch wastes on days 6 and 14, respectively. The highest number of *P. tuberregium*, 6±1.58 was recorded on day 12 after primordial emergence while the least, 2±0.49 was recorded on days 7, 13 and 14, respectively. All the three substrates utilized for cultivation of mushrooms in this study supported their growth and development. Plantain leaves supported fast colonization and produced high yield fruit bodies of *V. volvacea*. The ability to use agricultural wastes for cultivation of mushrooms will boost food production for ever increasing population.

***Keywords*:** *Pleurotus tuberregium, Volvariella volvacea,* agro wastes, Nigeria, Plantain Leaves

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1. INTRODUCTION

Mushroom can be defined as a macro fungus with a noticeable fruiting body, which may be epigeous (above the ground) or hypogenous (below the ground), large enough to be seen and picked up by hand (Chang and Miles, 1992). It belongs to the kingdom fungi, class Basidiomycetes and order Agaricales (Stanley and Odu, 2012). Chiejina and Olufokunbi (2010), reported that saw dust is a suitable substrate for cultivation of mushroom (*P. tuberregium*), which is dead organic matter. Also, Oyetayo and Ariyo (2013), showed that saw dust is a suitable substrate for cultivation of *P. ostreatus.* Farid *et al.* (2013), reported that mushrooms grow well on dead oak wood found in the forest areas. According to Obire *et al*. (2013), some fungi live and feed on dead animal matter. Moreover, Olufokunbi and Chiejina (2013), demonstrated that oil palm fruit fibre (dead organic substrate) enhances the growth and development of mushroom. Okigbo (2003) revealed that some fungi thrive on the head regions of yam tuber due to the presence of decaying materials found at the level of soil in which head region exists.

Mushrooms are consumed in various parts of the world because of their nutritive value, taste and flavour (Isola, 1993). Abere and Stanley (2012), stated that most rural dwellers are undernourished because of inadequate provision of proteins. The research carried out by Okigbo and Nwatu (2015), demonstrated that edible mushrooms are considered as very vital food delicacy in the Southern Nigeria.

The study carried out by Okhuoya *et al.* (2010), showed that edible mushrooms gathered from forests can be sold in the market or cooked fresh after washing them in warm solution of salt water. Isikheumhen *et al.* (1999), stated that the sporophores of *P. tuberregium* collected from forest or cultivated in the farm can be consumed fresh or dried for future use. According to Yashvant *et al*. (2012) and Olusegun (2011), for centuries, mushrooms have been used as sources of food nutrients and in medicine for treating stomach pain, bareness, heart disease, arthritis, diabetes and liver disease.

Volvariella volvacea (Bull ex. Fries) Singer is a tropical and subtropical edible mushroom, which is widely known (Ukoima *et al*., 2009). It is an edible white mushroom with tough bodies belonging to Division Basidiomycetes (the spore droppers), Class Agariomycetes which genus contains about 50 different species (Ukoima *et al*., 2009). Okigbo *et al.* (2009), revealed that diabetes and cancer can be treated using medicinal plants, but these diseases can also be controlled effectively through consumption of edible and medicinal mushrooms. In Nigeria, *P. tuberregium* is mostly used in preparation of drugs for treatment of fever, headache and stomach upset (Afieroho *et al*., 2013). Isikhuemhen *et al*. (1999), reported that the cultivation of *V. volvacea* is still at its infancy stage in Africa, of which Nigeria is not an exception.

Thapakorn and Thanawan (2013), Gurundevan *et al*. (2012), Markson *et al.* (2012), suggested that banana leaves, rice straw, palm trunk fibre, cotton wastes, water hyacinth and saw dust are suitable substrates for cultivation of mushroom (*Volvariella volvacea*). The aims of this study include: to document the indigenous knowledge of cultivation of mushrooms by people of Anambra State, and to investigate the possibilities of using different substrates for the cultivation of mushrooms.

2. MATERIALS AND METHODS

Sources of Materials

The plant materials used such as dried palm bunches and dried plantain leaves were collected from plantain plantation located in the Nigeria Institute for Oil Palm Research (NIFOR), Benin, Edo State. The sclerotia of *P. tuberregium* used in this study was obtained in lump from Eke Awka market, Anambra State. Spawn of *V. volvacea* was obtained from the Department of Life Science, University of Benin, Edo State, Nigeria.

Preparation of substrates

Dried plantain leaves hanging on their trees were cut using a sterile cutlass. They were chopped into small pieces of 45 cm with the aid of a sterile knife. The oil palm bunches were equally cut into small units of 1 cm using sterile knife, this was followed by sun drying for two weeks to eliminate any insect. The two substrates were soaked in cold water for six hours. At the elapsed of six hours, they were removed from water and allowed to drain before using them to make mushroom beds.

Preparation of soil

A sieve of 1 mm was employed for separating stones and other plant debris from the topsoil. The sieved soil was then poured into the plastic pot of 15 cm diameter and 9 cm deep after perforating each of them at the bottom using electric soldering iron. The essence of making holes at the bottom of each plastic pot was to prevent water-logging of the container. Ten plastic pots were used for the cultivation.

Preparation of sclerotia

The method of Oghenekaro *et al.* (2010), was adopted. The sclerotia were sun dried for 7 days in order to remove insects after which they were soaked in cold water and allowed to stay for 6 hours inside a plastic bucket of 30 cm in diameter and 28cm deep. At the elapsed of 6 hours, they were brought out of water and allowed to drain before cutting them into 20g each using a sterile knife. An electric weighing balance was used to measure each 20 g of sclerotium to ensure accuracy.

Planting of sclerotia

Twenty grammes of sclerotium each was placed into the plastic pots, which were filled to the brim with sieved topsoil. The depth of holes made in each plastic pot was 3 cm. Ten replicates of holes of 3 cm each was dug on bare ground for planting sclerotia. Each hole was covered with soil after planting and allowed for fructification to occur.

Irrigation

The experiment was watered twice daily (7 am and 6 pm) with clean tap water to ensure that the environment was kept humid until it has taken enough water to induce fructification.

Temperature variation

The temperature of the air and soil were recorded daily in the midday throughout the

period of the experiment.

Planting of Spawn

After preparation of substrates into mushroom beds, spawn was manually planted into the substrates. This was followed by covering it (spawn) with thin layers of substrates. Each of the entire substrate was covered completely with white cellophane of 90 cm by 90 cm and allowed for fructification to occur.

Harvesting of mushrooms

The sporophores of *Volvariella volvacea* were harvested when the fruit bodies have fully developed. This was done by holding base of the stipe strongly while twist and pulling them out of the substrates. The small substrates that came off with mushroom were removed using a sterile soft brush. The mushrooms were carefully put into ethylene bag and taken to storage room for preservation. After the first flush (harvest), it would take about 5 to 6 days for the second flush to take place, which is, harvesting of mature fruit bodies. Therefore, harvesting of matured mushrooms can continue as long as the sporophores are still coming out from the substrates.

Data collection

The growth of mushrooms was recorded on weekly basis. Data were collected from different replicates and the mean of each set of data calculated. The yield of fruit bodies were harvested from the different substrates at the end of the experiment and the following parameters were measured: Height (cm), Stipe girth (cm), Number of the fruit bodies for each treatment after sprouting, Diameter of the Pileus (cm), Fresh weight (g), and Dry weight (g).

Statistical Analysis

The data obtained in this study were subjected to Analysis of Variance (ANOVA) to test for significance. The means of sample were calculated and their standard error (S.E) separated using Duncan's Multiple Range Test (DMRT) at $P<0.05$.

3. RESULTS

The first primordial emergence of *Volvariella volvacea* (Bull ex. Fries) Singer was observed on day 11 after planting on both the plantain leaves and oil palm bunch wastes. The primordial were very much on plantain leaves, which developed fully into mature fruit bodies 4 days later (**Table 1**). After the first flush (harvest) of mature fruiting bodies from the substrates, it took 5 to 6 days for next harvest to occur. The time taken by the strains for pinhead formation was very much higher on plantain leaves than on oil palm bunch wastes. The first primordial emergence of *Pleurotus tuberregium* (Fries) Singer was noticed on day 10 after planting it on soil. This was harvested 5 days later when fully developed into mature fruit bodies. There was a significant ($P<0.05$) difference between the yield of *P. tuberregium* on topsoil compared with those planted on plantain leaves and oil palm bunch wastes (Table 1). Topsoil produced the highest fruit bodies of *P. tuberregium*. The highest mean growth rate of *V. volvacea*, 8.71±0.09 cm was obtained from plantain leaves on day 14 whereas the least value,

5.40±0.62 cm was obtained from oil palm bunch wastes on the same day (Table 1). The mean growth rate of *V. volvacea* obtained on day 14 from plantain leaves was significantly (P<0.05) different from that obtained from oil palm bunch wastes. In addition, the highest mean growth rate of *P. tuberregium*, 5.68±1.10 cm was obtained from soil on day 14 (Table 1).

Table 1. Mean growth rate (cm) of *V. volvacea* on plantain leaves, oil palm bunch wastes and *P. tuberregium* on soil

Days	Plantain leaves		Oil palm bunches		Topsoil	
	V. volvacea	*P. tuberregium*	*V. volvacea*	*P. tuberregium*	*V. volvacea*	*P. tuberregium*
1	2.05+0.32^{i}	1.64+ 0.01^{l}	1.90+ 0.01k	1.02+ 0.00^{m}	1.00+ 0.00^{n}	2.01+ 0.03^{i}
2	2.60+0.41	1.67+ 0.03	1.32+ 0.03	1.04+ 0.01	1.01+ 0.02	2.12+ 0.04
3	2.67+ 0.49	1.80+ 0.40	1.90+ 0.31	1.07+ 0.03	1.03+ 0.04	3.05+ 0.42
4	3.41+ 0.31	1.83+ 0.46	2.71+ 0.42	1.09+ 0.02	1.06+ 0.07	3.50+ 0.30
5	4.80+ 0.45	1.90+ 0.52	3.43+ 0.45	1.10+ 0.04	1.10+ 0.09	4.10+ 0.29
6	4.92+ 0.54	1.94+ 0.52	3.92+ 0.47	1.13+ 0.06	1.13+ 0.11	4.60+ 0.46
7	5.82+ 0.71	1.98+ 0.59	4.94+ 0.49	1.15+ 0.07	1.16+ 0.13	4.63+ 0.48
8	6.04+ 0.50	2.00+ 0.61	4.14+ 0.52	2.01+ 0.12	1.20+ 0.17	4.67+ 0.35
9	6.51+ 0.62	2.02+ 0.64	4.58+ 0.60	2.03+ 0.14	1.23+ 0.20	4.81+ 0.60
10	6.83+ 0.25	2.07+ 0.60	4.71+ 0.65	2.06+ 0.18	1.30+ 0.22	4.95+ 0.54
11	7.90+ 1.04	2.10+ 0.67	5.02+ 0.71	2.10+ 0.20	1.42+ 0.26	5.08+ 0.89
12	7.95+ 0.68	2.18+ 0.58	5.09+ 0.76	2.14+ 0.26	1.51+ 0.38	5.10+ 1.07
13	8.61+ 0.97	2.42+ 0.47	5.11+ 0.61	2.18+ 0.30	1.62+ 0.36	5.47+ 1.09
14	8.71+ 0.09a	3.06+ 0.55d	5.40+ 0.62c	2.21+ 0.28e	1.64+ 0.38f	5.68+ 1.10b

Mean ± S.E. values along the same row with different superscripts are significantly different at P<0.05

For the two substrates used for cultivation of *V. volvacea* in this study, the plantain leaves produced the higher yield of sporophores (fruit bodies) whereas the least was obtained from oil palm bunch wastes (**Table 2**). The highest number of fruit bodies of *V. volvacea*, 31±2.68 was obtained from plantain leaves on day 2 after 4 days of primordial emergence while the least, 2±1.00 was recorded from oil palm bunch wastes on days 6 and 14, respectively (Table 2). There was no fruit body of *V. volvacea* harvested on day 4 from plantain leaves as well as oil

palm bunch wastes (Table 2). Daily harvesting of mature fruit bodies of *P. tuberregium* on soil was recorded. The highest number of harvested fruit bodies of *P. tuberregium*, 6±1.58 was observed on day 12 while the least, 2±0.49 was recorded on days 7, 13, and 14, respectively (Table 2). There were no sporophores of *P. tuberregium* harvested on days 2, 3, 5, 8, 9. and 11 (Table 2).

Table 2. Daily harvesting of *V. volvacea* and *P. tuberregium* from different substrates. Plantain leaves and oil palm bunch wastes were used for *V. volvacea* and topsoil for *P. tuberregium*.

Days	Plantain leaves	Oil palm bunches	Topsoil
1	25+ 1.58	3+ 1.03	4+ 1.40
2	31+ 2.68	0+ 0.00	0+ 0.00
3	10+ 1.09	0+ 0.00	0+ 0.00
4	0+0.00	0+ 0.00	4+ 1.40
5	8+ 0.84	0+ 0.00	0+ 0.00
6	6+1.03	2+ 1.00	5+ 1.42
7	7+ 0.66	0+ 0.00	2+ 0.490
8	18+ 1.40	0+ 0.00	0+ 0.00
9	11+ 1.13	3+ 1.03	0+ 0.00
10	9+ 1.06	0+ 0.00	5+ 1.42
11	11+ 1.13	0+ 0.00	0+ 0.00
12	9+ 1.06	0+ 0.00	6+ 1.58
13	8+ 0.84	5+ 1.47	2+ 0.49
14	5+ 0.08	2+ 1.00	2+ 0.49

Mean ± S.E. values along the same row with different superscripts are significantly different at $P<0.05$

Fresh weight of fruit bodies harvested from each substrate was measured immediately using electric weighing balances. A total of 2040g fresh weights of harvested sporophores of *V. volvacea* were obtained from plantain leaves whereas 125g fresh weights were gotten from oil palm bunch wastes (**Table 3**). The fresh weight of *P. tuberregium* harvested from soil was 243 g (Table 3). The highest dry weight of 136g fruit bodies of *V. volvacea* was obtained from plantain leaves but the least, 34g was observed from oil palm bunch wastes (Table 3). The

highest mean height fruit bodies, 5.03±0.05cm of *V. Volvacea* was obtained from plantain leaves while the least, 3.21±0.20cm was obtained from oil palm bunch wastes (Table 3). The mean height fruit bodies of *V. volvacea* obtained from plantain leaves was significantly (P< 0.05) higher when compared with that of oil palm bunch wastes. The mean stipe girth of *V. volvacea*, 2.02±0.12cm was obtained from plantain leaves while the least, 1.10±0.04cm was recorded from oil palm bunch wastes (Table 3). The highest mean pileus diameter of *V. volvacea*, 6.02±0.70cm was obtained from plantain leaves while the least, 4.30±0.41cm was recorded from oil palm bunch wastes (Table 3). The mean height fruit bodies (4.10±0.21cm), stipe girth (1.03±0.01cm) and pileus diameter (5.21±0.42cm) of *P. tuberregium* were also recorded (Table 3).

Table 3. Yield parameters of mushroom fruit bodies from different substrates at harvest after 14 days.

Substrates	**Fruit bodies**	**Fresh weight** (g)	**Dry weight** (g)	**Mean height fruit bodies** (cm)	**Mean stipe girth** (cm)	**Mean pileus diameter** (cm)
Topsoil	*P. tuberregium*	243	96	4.10 ± 0.21^{e}	1.03 ± 0.01^{i}	5.21 ± 0.42^{b}
Plantain leaves	*V. volvacea*	2040	136	5.03 ± 0.05^{c}	2.02 ± 0.12^{g}	6.02 ± 0.70^{a}
Oil palm bunches	*V. volvacea*	125	34	3.21 ± 0.20^{f}	1.10 ± 0.04^{h}	4.30 ± 0.41^{d}

Mean ± S.E. values along the same row with different superscripts are significantly different at P<0.05

4. DISCUSSION

All the three substrates used for cultivation of mushrooms in this study supported their growth. Okigbo and Emoghene (2000) had earlier used orange tree substrate to cultivate *Ganoderma phiippii*. This agrees with the report of Onuoha *et al*. (2009) that *Volvariella volvacea* can be cultivated on agro wastes such as oil palm fibre and saw dust. Thapakorn and Thanawan (2013), observed that rice straw can be used in the commercial cultivation of *V. volvacea*. This shows that *V. volvacea* can be cultivated on different substrates like plantain leaves, oil palm bunch wastes and saw dust. However, findings of Markson *et al*. (2012), revealed that palm trunk fibre and banana leaves are suitable substrates employed for cultivation of the mushroom (*V. volvacea)*. Gurudevan *et al*. (2012), confirmed the use of water hyacinth, maize trash and cotton wastes for production of *V. volvacea*. Oghenekaro *et al*. (2010), observed that soil enhances the growth and development of *Pleurotus tuberregium*. For the two different agro wastes used as substrates for cultivation of *V. volvacea*, plantain leaves supported fast colonization and produced higher yields of fruit bodies. Also, the strain formation was very

high on plantain leaves. This may be attributed to the presence of lignocellulosic enzymes associated with the mushroom as well as accessible food resources contained in the substrates (Osemwegie *et al*., 2009). In addition, high fertility of the substrates increased the yields of the mushroom (Okigbo and Nwatu, 2015). The growth and development of *V. volvacea* was more prominent on plantain leaves than oil palm bunch wastes. This was a lucid indication that the mushroom has capacity to degrade lignocellulose materials in the substrates for enhancement of its growth and development. The observation reported here confirms the work of Belewu and Belewu (2005) on the same species of mushroom using banana leaves as substrate. Uddin *et al.* (2012) and Adesina *et al.* (2011), recorded appreciable yield fruit bodies of the fungus (*V. volvacea* and *Lentinus squarrosulus*) on cow dung, wheat bran, oil palm fibre, corn leaves and chicken manure substrates.

Furthermore, oil palm bunch wastes to a large extent did not produce high yield fruit bodies of *V. volvacea* when compared with plantain leaves. It is reasonable to assume that this was due to inability of the mushroom to produce specialised lignocellulosic enzymes sufficient enough to degrade the substrate. In the similar manner, Akinyele and Adetuyi (2005), observed that rice husk, which is one of the agricultural wastes, enhanced the highest mycelia density of *V. volvacea*. In this work, it was observed that *V. volvacea* did not produce high yield of fruiting bodies on the topsoil substrate. The reason can be attributed to high acidity of the soil and inability of the mushroom (*V. volvacea*) to produce lignocellulosic enzymes to degrade soil nutrients on which they grow.

Be that as it may, it was observed that *P. tuberregium* produced a higher yield of fruit bodies on topsoil than other substrates used for its cultivation. Oghenekaro *et al*. (2010), demonstrated that *P. tuberregium* grow successfully on soil and this may be due to effective utilization of the substrate (soil). Plantain leaves and oil palm bunch wastes gave the least yield fruit bodies of *P. tuberregium* and this can due to inhibitory effects of pathogens present in them. Abere and Stanley (2012), observed that cultivation of *P. tuberregium* on corn cob and rice bran can improves its growth. The high yield fruit body of *P. tuberregium* recorded in this study on topsoil substrate agrees with the findings of Olufokunbi and Chiejina (2013); Abere and Stanley (2012) and Chiejina and Olufokunbi (2010). Also, Olufokunbi and Chiejina (2013), agreed that topsoil and fermented saw dust can improve the yield of *P. tuberregium*. Similarly, Stanley and Odu (2012), demonstrated that corn cob, watermelon pod and plantain peelings have been found to support growth of *P. tuberregium*. The work of Oyetayo and Ariyo (2013), observed that saw dust could be used to grow *P. ostreatus* as well as *P. tuberregium*. According to Stanley and Odu (2012), the various degrees of mycelia growth on substrates employed for cultivation can be attributed to the nature of cultivated mushrooms.

5. CONCLUSION

Since mushrooms have been found to contain higher amount of proteins, vitamins and minerals, they should be used as alternative source of protein and in medicine for treatment of some human diseases. From this study, it was observed that plantain leaves and topsoil contain the important nutrients required for sporophore formation of *Volvariella volvacea* and *Pleurotus tuberregium*, respectively. This conclusion is based on the observation that both substrates (plantain leaves for *V. volvacea* and topsoil for *P. tuberregium*) produced high yield fruit bodies of the mushrooms.

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