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Assessing the Growth Performance of *Volvariella volvaceae* on Local Homemade Substrate

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Abstract: Paddy straw mushroom (*Volvariella volvacea*) is a world famous edible mushroom that has high demand due to its delicious and nutritive value. It is a highly nutritious food source that is widely cultivated. This research is aimed at investigating the growth of *V. volvaceae* on four selected farm waste, as well as the determination of suitable growth factor and the best feasible substrate for the cultivation of this macro fungi on a large scale. Four substrate; rice straw, sawdust, raffia palm trunk and palm bunch wastes were composited and used in cultivating the straw mushroom (*Volvariella volvaceae*). The determination of the nutrient content was done, sawdust had a slight increase in crude protein and nitrogen content of 17.50% and 2.80% respectively over other substrate. Sawdust supported the growth of the mushroom at eight weeks after inoculation of the spawn but rice straw could only show the initiation of mycelial growth with slow sporulation within the given time range. Decaying raffia palm and palm bunch waste also recorded some level of mycelial growth observed to cover four inches of the spawn bag after seven weeks of inoculation but did not support the fruiting of the mushroom.
Keywords: Sawdust, Rice straw, Raffia palm Trunk, Palm bunch, Nutrient composition, *Volvariella volvaceae.*

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Introduction

Mushrooms have become popular throughout the world since they have wonderful nutritional food and medicinal values. No food is so wrapped in mystery as mushrooms with their amazing tiny pinheads on a composting medium growing into buttons rich in protein, vitamins and minerals. Mushrooms are quite unique in taste and have special bonds of nutritive contents. *Volvariella volvacea* was known as “warm mushroom” which grows at a relatively high temperature and is considered as one of the easiest mushrooms for cultivation. The homothallic nature of this mushroom makes its uninucleate haploid self-fertile spores to germinate and produce mycelia (Ahlawat and Tewari, 2007).

It is a fast growing mushroom and under favourable growing conditions of which it total crop cycle is within 4-5 weeks. This mushroom can use a wide range of cellulosic materials with a C:N ratio of 40 to 60, which is high in comparison to other cultivated mushrooms. Compilation of fresh weight data shows that paddy straw mushroom contains about 90% water, 30-43% crude protein, 1-6% fat, 12-48% carbohydrates, 4-10% crude fiber and 5.13% ash. A slightly greater concentration of nitrogen is necessary for the fruiting body formation than the concentration supporting mycelial growth (Jonathan *et al.*, 2009).

Volvariella volvacea can be grown quite quickly and easily on un-composted substrates such as paddy straw and cotton waste or other cellulosic organic waste materials (Ahlawat and Kumar, 2005) such as sawdust, palm bunch waste and raffia palm. This mushroom is known to strive in high cellulose, low lignin-containing a substrate and produces a family of cellulolytic enzymes (Ahlawat *et al.*, 2005; Onuoha, 2008). In addition to rice straw, *V. volvacea* also grows on water-hyacinth, palm oil bunch wastes, pericarp wastes, banana leaves, and cotton waste (Bao *et al.*, 2013). But in recent time, cultivation has been limited due to a low biological efficiency in terms of conversion of growth substrate to mushroom fruit bodies, sensitivity to low temperatures, and an unclear sexuality pattern that has restricted the breeding of improved strains. Nevertheless, the biological effects of this mushroom remains relatively low usually around 20%. By contrast, it is often between 100% and 150% by reference to some other kinds of mushrooms (Bao, 2010).

Environmental conditions play a crucial role in the formation of the fruiting body, the optimal environmental situation for mycelial growth and the subsequent fruiting is usually very distinct; inducing the fruiting body after drastically altering the environmental circumstances (Fan *et al.*, 2008). According to (Scrase and Elliott, 1998) there have not been any universal set of the conditions that lead to fructification in all mushrooms, thereby the conditions for growing and producing fruiting bodies of a given species have to be empirically established putting the natural environment of the fungus in consideration. The research aimed at investigating the growth of *V. volvaceae* on four selected farm waste (rice straw, palm bunch waste, decaying raffia palm and sawdust), determination of suitable growth factor for the cultivation of straw mushroom and determination of the best feasible substrate on cultivating this macro fungus on a large scale.

Materials and method

Materials collection

Rice straw was obtained from kalane village, basang west in Obanliku local government area, in Cross river state, while the palm bunch waste was gotten from oil palm plantation in Akpabuyo local government area also in the same state, sawdust was collected from timber market, Akim in Calabar municipal local government and decaying raffia palm was sourced from the University of Calabar farms in Cross River State. The Mushroom was donated by Zartech farms in Ibadan, Oyo state.

Tissue culture preparation of *V.volvacea:* The medium was prepared according to the manufacturer instruction. 25g of malt extract agar was added to 500ml of distilled water, placed in a boiling water bath to dissolve agar. It was autoclaved at 121^{o}C for 15 min. After cooling, it was dispensed into Petri dishes (85 mm). The fresh fruiting body of *Volvariella volvacea* tissues was cut with a sterile blade (Jonathan, 2009) and aseptically inoculated on the malt extract agar media in the Petri dishes at 32 ± 2^{o}C. The pure culture of the fungus was maintained on Malt Extract Agar (MEA) slants throughout the period of investigation. Sub-culturing of the fungus was done at an interval of two weeks and stored at 25+ 1^{o}C. Seven

days old pure mycelia cultures of the test fungus were used in the preparation of mother spawn (Biswas and Layak, 2014).

Spawn Preparation

V. volvacea spawn was produced using sorghum bicolor, calcium carbonate and water in the ratio of 45:2:55, respectively. The composition was transferred into a drained sterile bottle. The bottles were later autoclaved at 121°C for 20 min after which the bottles were cooled at room temperature. The mycelium from the tissue culture plates were dice into 5cm and inoculated into the spawn bottles. The inoculated spawn bottles were kept at room temperature. The mycelium fully spread through the spawn bottle about 15-20 days of incubation. Daughter spawn was multiplied by sub-inoculating through the mother spawn.

Substrate preparation and composition

Rice straw was chopped into pieces and moistened by soaking them in water and left overnight (12hours) the palm bunch was also chopped into pieces as well as the decaying raffia palm trunk moistened by soaking them in water and left for 12hours. Sawdust was allowed to ferment for about 4weeks and regularly turned for 4days after this period; sawdust was soaked for 24 hours. Substrates were transferred to sack bag and a manual cassava presser was used to drain the water to moisten level of 70%. 45.5kg of rice straw and 50kg sawdust were mixed with 0.3g of limestone, while palm bunch waste and decaying raffia palm were weighed 54.5kg and 51.2kg respectively. A metal drum of about 500litre was used to achieve pasteurization, a wooden platform of about 35cm high was constructed from the bottom, a constant heat was applied for 3hours at 60-80^{o}C (Fasidi, 2006). The substrates were allowed to cool for 12hours before bagging.

Inoculation of spawn into the substrate

After pasteurization the substrate was allowed to cool to a temperature of 35-37^{o}C at room temperature. Spawn bags measuring 30-25cm were opened in a sterilized environment, and each bag was inoculated with a 5g of *Volvoriella volvaceae* spawn which was mixed evenly through the substrate with a sterilized short fork, sterile substrate bags were stopped with a PVC pipe of 3cm long in diameter wrapped with a rubber band and plugged with a sterile cotton wool to prevent pathogenic microorganism from entering the bag, the transparent substrate bags were taken to the mushroom house for proper growth under controlled temperature and relative humidity.

Spawn running /incubation

The substrate bags were hanged using a jute rope tied from the roof down carrying five bags each (Figure 1). A limited light was allowed in the spawn running room and room temperature of about 30^{o}Cand relative humidity between 65%-75% was achieved by spraying the bags and walls of the spawn running room to 2-3 times daily with clean water (Fasidi, 2006; Markson et al., 2012). After the mycelium has colonized the substrates bag were placed side by side in triangular shelves constructed with bamboos and open in a single layer making sure they are not placed directly on the floor or on top of each other as this will generate heat. The room was sprayed with clean water and coarse sand from seabed was used to cover the floor to continuously make it moistened.

Proximate analysis of the substrates

Determination of nitrogen content was done using the Kjeldahl method (TAPPI, 1972), sodium and potassium were determined by the photometric method. Calcium and magnesium were obtained through EDTA extraction method.

Mineral ash was determined by furnace ignition method. The value for crude fiber was obtained by adopting Weeden method. Fat/oil was determined by soxhlet ether extraction methods (A.O.A.C, 1995). About 10ml of distilled water was measured separately and about 0.5g each of the composited substrates. The setups were shaken and filter using four fold cloths to obtain the substrate filtrates. The pH level of the substrate was obtained using Agilant pH meter (Oie, 2003).

Cropping and harvesting

Watering of the substrates was carried out with a hand pump and done three times a day to prevent the mushrooms from drying up. The temperature in cropping room was maintained at 25-30^{o}C by wetting the walls and floors of the mushroom house to achieve a well humid environment, Harvesting was carried out as soon as the fruiting bodies mature (Figure 3).

The mushrooms were harvested by uprooting them neatly to avoid removing the substrates alongside. This was done once or twice a week depending on the growth of the fruiting bodies and time of fruiting.

Assessment of growth and yield of mushroom

Vegetative growth of the *Volvariella volvacea* was assessed by visual observation of the mycelia on the substrates and the time taken for pinheads to form after spawning were recorded. Mushrooms were harvested at the elongation stage, cleaned, weighed and the biological conversion efficiency (Tschierper and Hartman, 1977) was determined using the mathematical relationship. The biological efficiency was calculated by the following formula:

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{The weight of dry substrate}} \times 100$$

$$\text{Average growth rate (mycelium)} = \frac{\text{shortest growth length + longest growth length}}{2}$$

Result and Discussion

After a period of ten days of incubation, whitish mycelia colonized the entire substrates followed by tiny pinhead surfacing from the substrate bags and these grow into full-size mushroom within the next four days. (Figure1-3).

Figure 1. Substrate bags hanged using a jute rope

Figure 2. Full growth mycelium surfacing

Figure 3. Fruiting bodies of *Volvariella volvacea*

Determination of pH and proximate analysis of substrates

The pH levels of the composted were determined. The treated rice straw, sawdust, palm bunch waste, raffia palm trunk was acidic. However, when sawdust was treated with CaCO3 and gypsum, the pH of 8.45 was obtained which indicate been alkaline in nature. The difference in the pH value in each case was slightly above 1. Results obtained from the analysis of the four test substrates are; rice straw moderately alkaline, sawdust moderately acidic, palm bunch waste slightly alkaline and raffia palm trunk slightly acidic.

Proximate analysis of the substrates was carried out for the mushroom cultivation (Figure 4). These substrates, as reported by Stamets (2001), contain essential macro elements for crop production (potassium, calcium, phosphorous, magnesium, nitrogen and sodium). Generally, these essential nutrients combined in various substrates to stimulate fruit body formation and development of *V. volvacea*. There is the likelihood that sawdust must have contained higher levels of antimicrobial constituents which may have slowed down the rate of cellulose solubilization and hence reduced fiber loss.

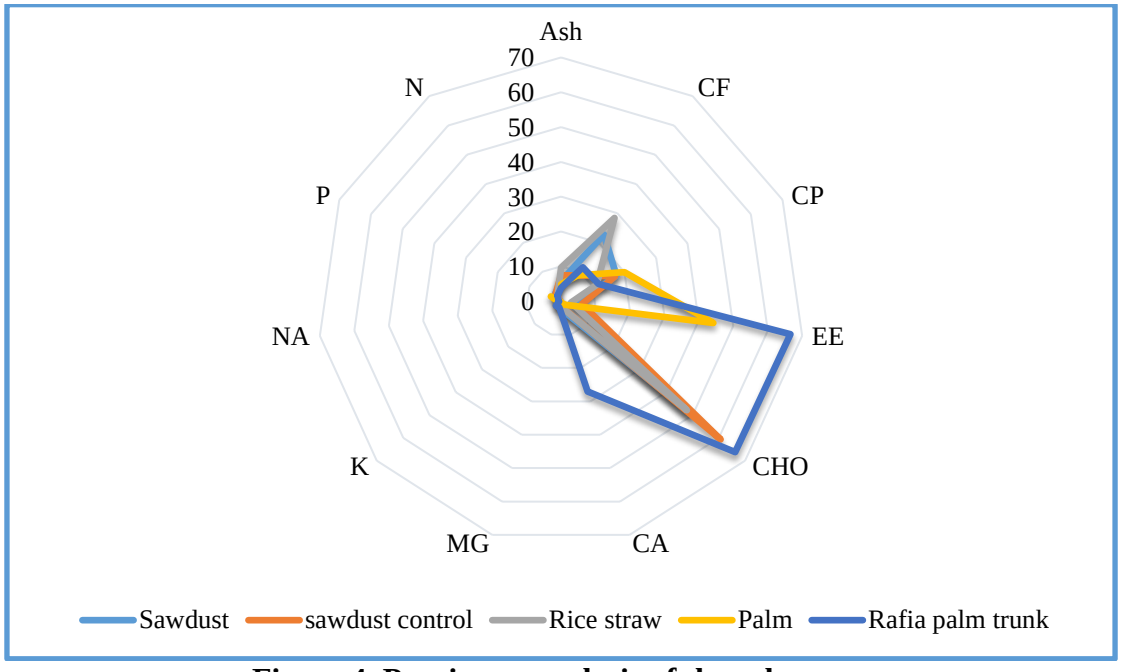

Figure 4. Proximate analysis of the substrates

Spawn running

Paddy straw mushroom is known to strive better in high cellulose, low lining-containing substrate, this could have affected the growth variant of mushroom in each substrate. Of the four substrates tested, only one substrate (sawdust gave a favorable response) others could not support the growth of fruit bodies within the period of study. However, rice straw, palm bunch, raffia trunk recorded some level of mycelial growth which was observed to cover four inches of the spawn bag after 7 weeks following inoculation. Rice straw did not support the growth of the fruit bodies of the mushroom, probably because of the acidity level of these substrates. The slow mycelial growth rate of the test mushroom observed on rice straw may not only result from the low nutrient content of the substrate but may also suggest the likelihood of the presence of high levels of growth inhibitory substances in the substrate. According to (Apetorgbor *et al.*, 2015) rice straw can yield better growth when supplemented with other agricultural waste such as cotton waste and leucaena. However, sawdust was not considered as adequate for the cultivation of *V. volvaceae* when compared with rice straw and banana leaves (Oei, 2003).

Table 1. Observed Characteristics of *Volvariella volvacae* on Sawdust

Parameters	Values
Period of mycelium colonization	14days
Mycelium average growth rate	10.16cm
Total weight of fruit	107.1
Average weight of fruit	53.6g
Biological efficiency	18.46
Banana biomass loss	3.57%

Cropping and harvesting

Pinheads appeared three days after the transfer of the fully colonized substrates to the cropping room. The observation made revealed that shorter and stunted fruit bodies were produced in the first flush, however succeeding harvest recorded fruit bodies with longer stipe, with the longest stipe been 46.0cm recorded after eighteen weeks of harvest. Though there was generally a fluctuation in length of stipe, the stipe becomes longer towards the end of fruit body production than at the beginning. Result obtain for the area of pileus varies at day 14 and day 42 as an area of 378.25cm and two weeks later 56.35cm and on the terminal, recorded 66.69 cm area. This was however not comparable to a size of 221.35cm recorded for the first flush. Fresh and dry weight followed this same trend, with the highest fresh (92.3g) and dry (10.2) weight recorded on the 7th week (day42) which was the 7th flush and the least (fresh 14.9g-dry 1.4g) recorded for week 24(168days).

Table 2. Physical Observation of *Volvariella volvacae*

Parameters	First flush	2 weeks	7 week	18 week	24 week	Terminal
Length of stipe						
Longest stipe recorded	-	-	-	46.0cm	-	-
Area of pileus	221.35 cm	56.35cm	378.25cm	-	-	66.69cm
Weight of mushroom						
Highest Fresh	-	-	92.3g	-	-	-

weight						
Least Fresh weight	-	-	14.9g	-	-	-
Highest Dry weight	-	-	-	-	10.2g	-
Least Dry weight	-	-	-	-	1.4g	-

Conclusion

The advantage with paddy straw mushroom is its shorter life cycle, fast growth, simple cultivation technique. The bottle-neck of lower biological efficiency has almost been sorted out after bringing in sawdust, raffia palm trunk, palm bunches, as the substrate for the straw mushroom; however, much more research work is needed to be done for developing suitable processing technology as well implore scientist in Africa and beyond to explore more about paddy straw mushroom and for better utilization of its nutritional and medicinal contents.

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