International Digital Organization for Scientific Research ISSN:2579-079X
IDOSR JOURNAL OF SCIENCE AND TECHNOLOGY 2(2): 56-64, 2017.

Comparative Study of the Effects of Different Local Agricultural Wastes as Substrates on Growth and Protein Content of *Pleurotus tuber-regium* Mushroom

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ABSTRACT

Mushroom is a greatly unexploited nutritious food resource globally. Its cultivation on lignocellulosewaste is a very economical biotechnology both for reduction of environmental pollution and production of rich and high quality protein food. In this study, the effect of different lignocellulosesubstrates and substrate combinations on the growth and yield components (cap diameters, stipe heights, fresh weights) and protein content of *Pleurotus tuberregium* was determined. The mushroom from grown from sclerotia obtained from Onueke market in Ezza South Local Government Area of Ebonyi State in buckets containing different lignocellulose substrate regimes (T0-T5) with adequate watering forsix weeks in the screenhouse. Growth and yield components and protein content weremeasured. The result showed that the different substrates had significant effects on the mushroom growth and yield but no effect on protein content. Combination of rice husk and saw dust gave the highest performance with a mean fresh weight of 297.20±53.23g, cap diameter of 18.80±1.61cm and stipe height of 15.60±2.20cm.We recommend adoption of this cheap cultivation technology to the rural poor for enhanced mushroom cultivation and utilization to minimize protein deficiency in diet.
Keywords: Mushroom growth, Pleurotus tuberregium, lignocellulose waste, growth, protein content

INTRODUCTION

Mushrooms are fleshly, spore bearing fruiting bodies produced above ground on soil or on lignocellulosic waste materials by sprophytic fungi species. They grow on cheap substrates such as soil, lignocellulose waste materials thereby converting agricultural wastes into valuable food products [1,2,3]. There are many different species of mushroom on earth (about 140,000), but only about 10% are known [4], some of which include button (*Agaricus* species), oyster (*Pleurotous* species), shiitake (*Lentinulaedodes*), straw (*Volvallellavolvacea*) and Chinese mushroom (*Ganoderma* species) [6]. Many of the species are edible and are very nutritious food resources, but not well exploited. In most parts of sub-Sahara Africa, mushrooms serve as cheap alternative sources of high quality protein. Mushrooms are not only rich in protein, but also minerals, vitamins, fibers and essential amino acids [7,8] asserted that the nutritional value of mushroom was found to be comparatively higher than that of other vegetables, fruits, meat and fish.Due to the potential nutritional values of mushrooms, these authors described mushroom as an ideal food supplement. Ethnomycological survey of the utilization of mushroom in north central Nigeria revealed that most people consume mushrooms for their palatability (93.5%) and nutritional values (81.7%) while only 15.1% utilize mushroom based on the medicinal properties [9]. Information on the ethnomedicinal uses of some mushrooms show that *Pleurotus tuber-regium* is used for treatment of headache, stomach pain fever, cold, constipation; *Lentinus*

squarullosus for mumps, heart diseases; *Termitomyces microcarpus* for gonorrhea; *Calvatia cyathiformis* for leucorrhea, barreness; Ganoderma *lucidum* for treating arthritis, neoplasia; *G. resinaceum* for hyperglycemia, liver diseases (hepatoprotector); and *G. applanatum* is used for treatment of diabetes. Studies have attributed the medicinal potentials of some of the mushroom species to their antimicrobial and antioxidant properties [10].

Mushroom cultivation has a long history with over 20 species commerciallycultivated in at least 60 countries of the World, with China, United States, Netherlands, France and Poland being the top producers [11]. Although, mushrooms are also cultivated in South-Eastern and South-Western Nigeria, they are mostly wild grown. Interestingly, mushroom cultivation requires low technology, low investment and can be grown in very little space, suggesting the potential of mushroom as a food security and health food. Cultivation of mushrooms is very essential to make the product available to consumers. Mushroom can be cultivated effectively using agricultural waste which is abundant in African region. These wastes are mostly disposed by incineration which causesenvironmental pollution [12]. There existing management strategies for agricultural wasteis cost intensive and contribute significantly to environment pollution. Mushroom cultivation on agricultural wastes has strong promise to reduce these problems [13,14,15].
Oyster mushroom (*Pleurotus tuber-regium*) is very popular commercially in the world mushroom market, and several species are grown on a commercial scale in many countries [16,17,18]. *Pleurotus* species are outstanding wood decomposers and grow on a wide range of forest and agricultural wastes compared to any other species [19,20]. They thrive on almost all hardwoods, on wood by-products (paper,sawdust, pulp sludge), cereal straws, corn and corn cobs, sugar cane bagasse, coffee residues and on numerous other lignocellulose materials [21,22]. This study was carried out to evaluate the effect of different lignocellulose substrates that are abundant in the study region on growth, yield and protein content of *Pleurotus tuber-rgium.*

Materials and Methods

Plant material and substrates

The plant material used was sclerotia (*osu*in in Igbo language) bought from Eke market in Ezza South Local Government, Ebonyi State. This was the source of the mushroom spores. The substrates were saw dust (collected from Abakaliki timber market), rice husk (obtained from Abakaliki rice mill), humus soil (obtained from PRESCO Campus of Ebonyi State University, Abakaliki), and organic manure (cow dung and poultry droppings).

Preparation of Substrate

The rice husk and saw dust were placed separately on a cemented floor, moist with water at the ratio of 2:1v/v,piled together and covered with a dark polyethylene sheet to undergo fermentation for a period of 3 weeks [16].

Experimental Design

The following substrates; Humus soil (T0), rice husk alone (T1), Rice husk and Cow dung (T2), Rice husk and Chicken droppings (T3), Rice husk and Sawdust (T4), Rice husk and Humus (T5) were used to cultivate mushroom.

Inoculation and Incubation

The sclerotia were cut into sizes ranging from 150 – 200 g and soaked in water for three days after which it was removed from water and tied in a black polyethylene bag and stored for 2 days to induce hyphae growth. The pre-germinated sclerotia were then seeded into perforated buckets containing different lignocellulose substrate regimes (T0-T5) with adequate watering for six weeks.

Data Collection

Mature fruiting bodies were identified by their caps rolling up and inwards like the shape of a funnel. They were harvested at this stage and their fresh weights,cap diameter and

stipe heightwere measured and recorded. After this, the harvested mushroom samples were dried in an oven and macerated into fine powder for protein determination.

Determination of Protein content

The crude protein content of the samples was determined using the Microkjeldahl method of AOAC (1984) [6], which involved protein digestion and distillation. Protein digestion: About 2.0 g of each sample was weighed into a Kjeldahl flask and 4 tablets of Kjeldahl Catalyst were added. This was followed by the addition of 1.0 g copper sulphate and a speck of selenium catalyst into the mixture, and 25 ml concentrated sulphuric acid was introduced. The whole mixture was subjected to heating in the fume cupboard. The heating was done gently at first and increased with occasional shaking till the solution assumed a green colour. (The temperature of digester was above 420^{o}C for about 30 minutes). The solution was cooled and black particles showing at the neck of the flask were washed down with distilled water. The solution was re-heated gently again until the green colour disappeared. Then, it was allowed to cool. After cooling, the digest was transferred into a 250 ml volumetric flask with several washings and made up to a mark with distilled water and then distilled using Markham distillation apparatus. Protein distillation: Before use, the Markham distillation apparatus was steamed through for 15 min. after which a 100 ml conical flask containing 5 ml boric acid /indicator was placed under the condenser such that the condenser tip was under the liquid. About 5.0 ml of the digest was pipetted into the body of the apparatus via a small funnel aperture. The digest was washed down with distilled water followed by addition of 50 ml of 60% NaOH solution. The digest in the condenser was steamed through for about 5-10 min. after which enough ammonium sulphate was collected. The receiving flask was removed and the tip of the condenser washed down into the flask after which the condensed water was removed. The solution in the receiving flask was treated with 0.01M hydrochloric acid. Also, a blank was run through along with the sample. After titration, the % nitrogen was calculated using the formulae below:

$$\% \text{ Nitrogen} = \frac{\text{Vs} - \text{Vb} \times \text{Macid} \times 0.01401 \times 100}{\text{W}}$$

Where, V_s = volume (ml) of acid required to titrate sample; Vb = volume (ml) of acid required to titrate the blank; M acid = molarity of acid, and W = weight of sample in gram.Then, % crude protein was calculated from the % Nitrogen as; % Crude protein = % N xF, where, F (conversion factor), is equivalent to 6.25.

RESULTS

The result showed that the substrates had significant effects on the yield of the *Pleurotus tuber-regium*. The fresh weight of the mushrooms grown in the substrates were significantly ($p<0.05$)higher when compared to the control (T0) with the highest fresh weight observed in the substrate containing rice husk alone (T1) and a mixture of rice husk and saw dust (T4) as shown in Figure 1. The mean valuesof fresh weights ranged from 92.93±16.29g – 297.20±53.23g. Similarly, the substrates significantly ($p<0.05$) increased the stipe height of the mushroom (Fig.1). The substrate combination that had the highest effect on the stipe height is also rice husk alone (T1) with a mean value of 15.60± 2.20cm followed by T4 (a mixture of rice husk and saw dust) which recorded stipe height of 14.00±1.92 cm, while T0 recorded the lowest stipe height (5.80±2.31cm)(Fig. 2). Likewise, the various substrates had significant ($p<0.05$) effects on the cap diameters of the harvested mushrooms withthe least cap diameter observed also in the control (T0; 7.03±2.24 cm) while rice husk and saw dust combination (T4) similarly produced the highest cap diameter with a mean value of 18.80± 1.61cm followed by T5 (15.27±1.82 cm)

and T2 14.23±2.06 cm. Fig.1 and Fig. 2 show the stage of soaking the sclerotia and the mature mushroom ready for harvest, respectively.

The result revealed that there wasno significant difference ($p>0.05$) in the protein content of the matured mushrooms (Fig.4) with respect to the various substrates. T1 and T2 had higher protein content (24.15±0.09% each) followed by T0 (22.82±0.03%) while T3, T4 and T5 showed significantly lower protein contents relative to the control. However, these protein values did not differ statistically.

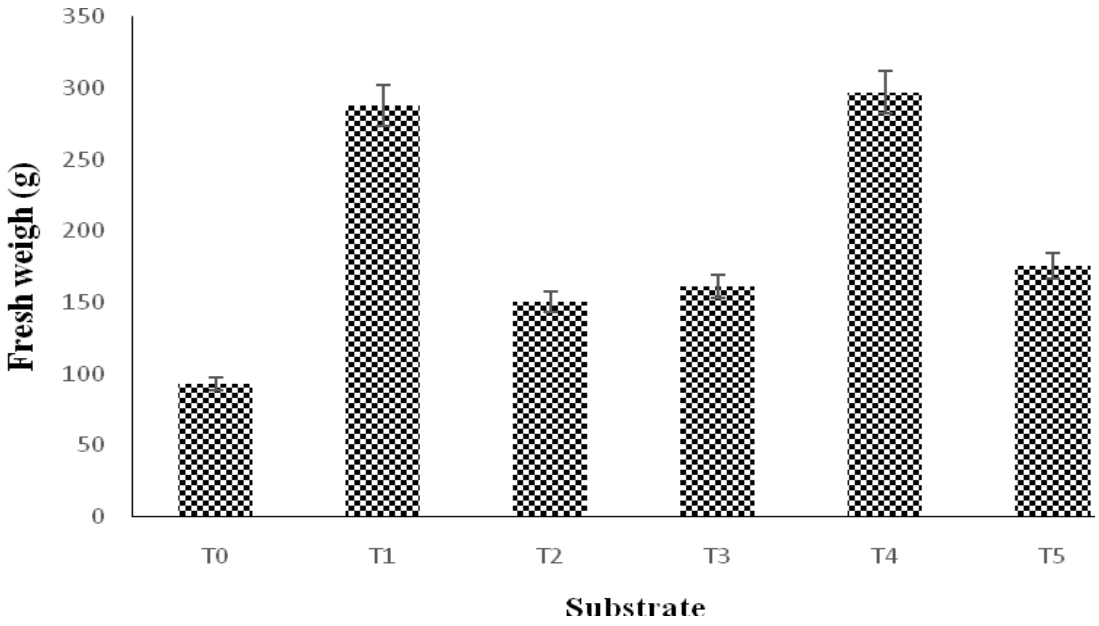

Fig. 1: Effect of different substrates on stripe height of *Pleurotustu berregium* grown for 6 days. T0 = no substrate/soil (control), T1 = rice husk, T2 = rice husk + cow dung, T3 = rice husk + chicken droppings, T4 = rice husk + sawdust (1:1) and T5= rice husk plus Humus.

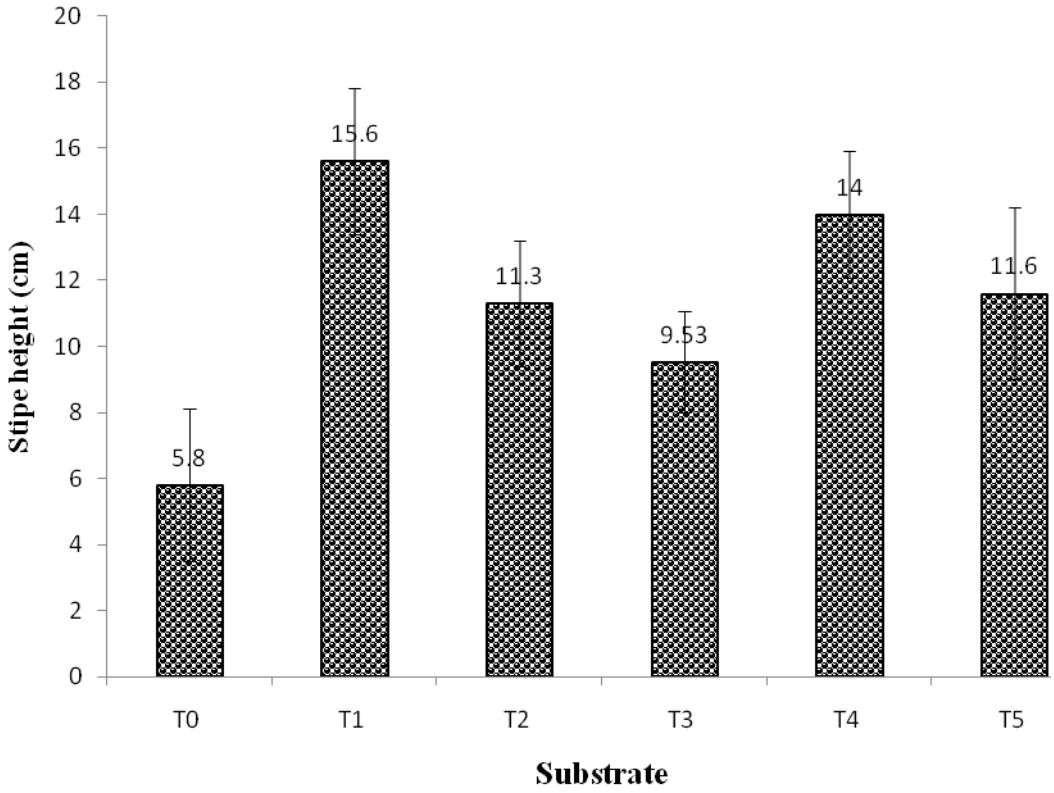

Fig. 2: Effect of different substrates on growth (stripe height) of Pleurotus tuberregium grown for 6 days. T0 = no substrate (control), T1 = rice husk, T2 = rice husk + cow dung, T3 = rice husk + chicken droppings, T4 = rice husk + sawdust (1:1) and T5 =

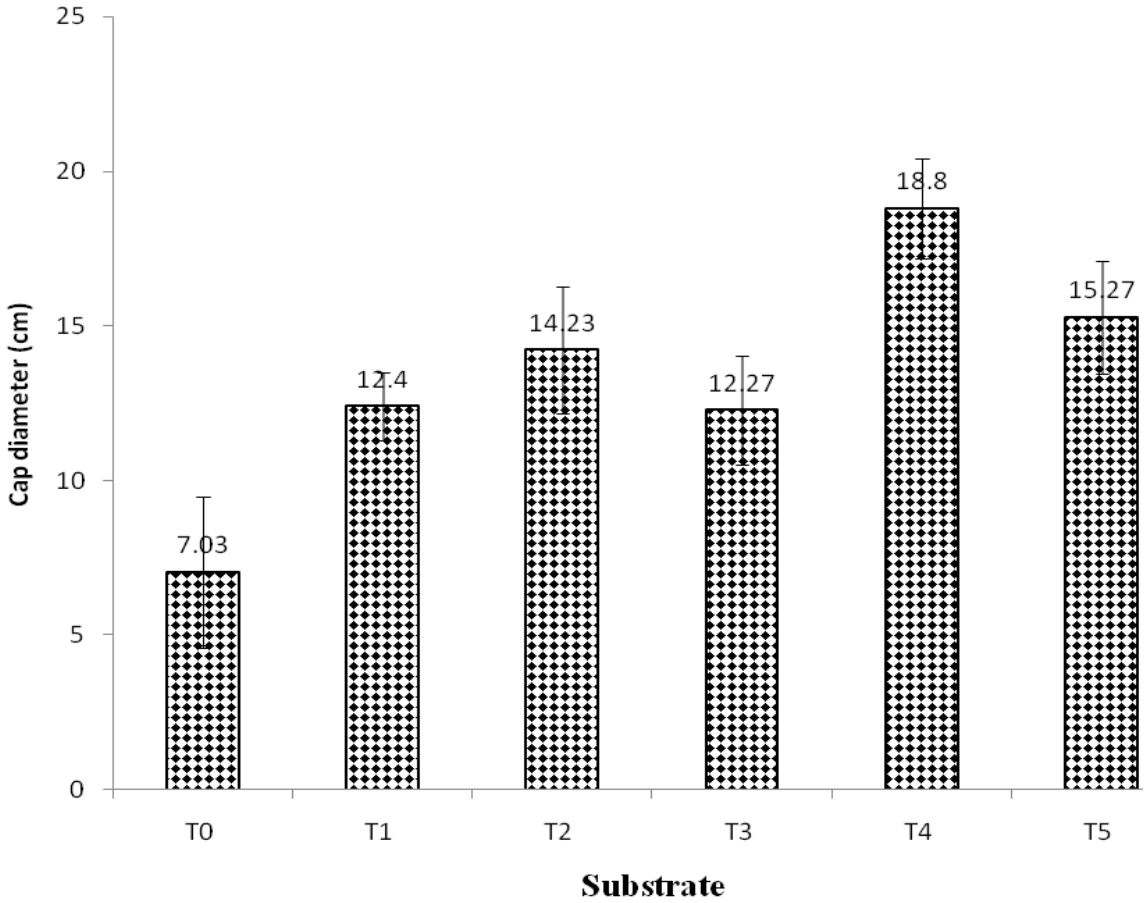

Fig. 3: Effect of different substrates on growth (cap diameter) of Pleurotus tuberregium grown for 6 days. T0 = no substrate (control), T1 = rice husk, T2 = rice husk + cow dung, T3 = rice husk + chicken droppings, T4 = rice husk + sawdust (1:1) and T5 =

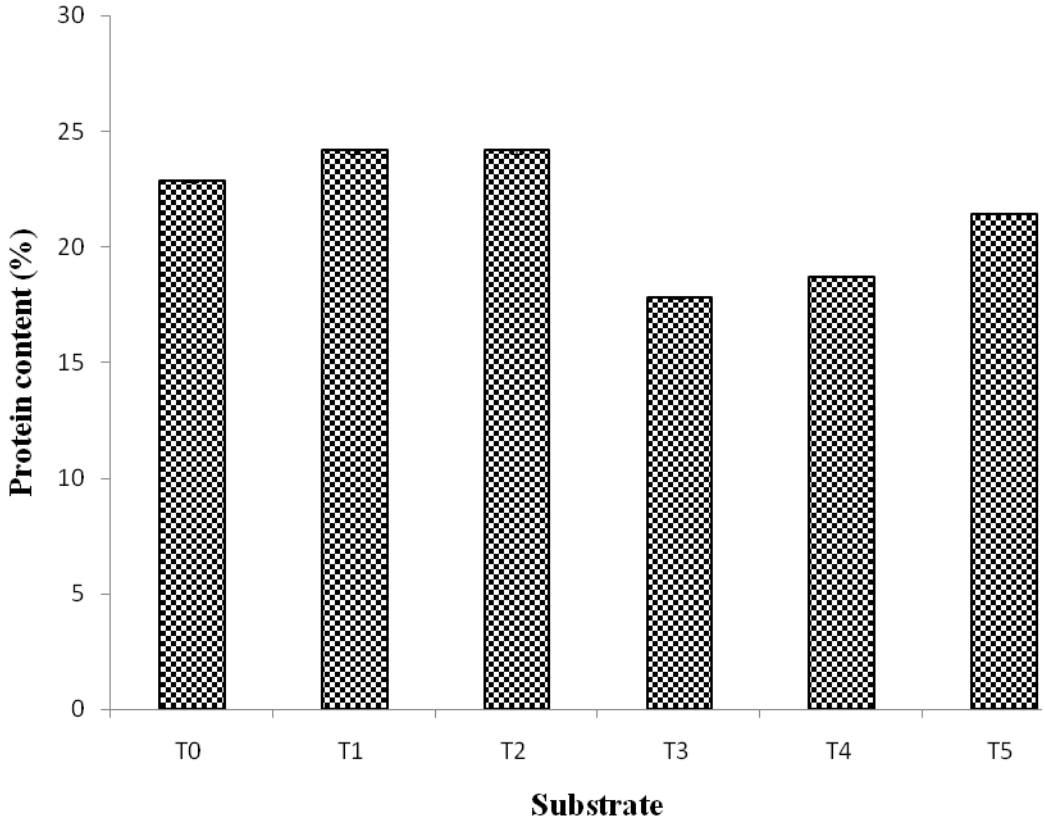

Fig. 4: Effect of different substrates on Protein content of Pleurotus tuberregium grown for 6 days. T0 = no substrate (control), T1 = rice husk, T2 = rice husk + cow dung, T3 = rice husk + chicken droppings, T4 = rice husk + sawdust (1:1) and T5 = rice

DISCUSSION

P. tuber-reguim mushroom in this study was grown from its sclerotia inoculated into various local substrates (agricultural wastes) to investigate the effects of these cheap substrates on growth and yield of the mushroom as well as on its protein content. This study revealed that the different substrates and substrate combinations indeed significantly improved the growth of the mushroom, but the protein content was not significantly altered across the different treatments. Significant increase was observed in all the measured parameters (stipe heights, total fresh weights and cap diameter) with respect to the control (T0), except for protein content.

The fresh weights, the heights, and cap diameters of the mushroom showed that the substrate containing saw dust and rice husk produced the highest yield of the mushroom. This agrees with a study by Kadiri and Fasidi (1990) [14] who reported that saw dust was the best substrate for mycelia growth and fructification. This may be explained by the fact that rice husk and saw dust could produce sclerotia with the higher biological efficiency while the other substrates may have to combat first with the microbial contamination in them [5]. Also, *P. tuberregium* could grow well in saw dust because it is a wood degrading saprophytes which can digest extra cellular lignocelluloses deriving nutrients from them [14]. It was also observed that the mixture of rice husk and chicken droppings had the lowest yield in terms of height, fresh weight and cap diameter. This may be because of microbial contamination from the chicken droppings. [9], reported that chicken manure

should be properly sterilized before use for cultivation of mushrooms to avoid low mushroom growth, because the chicken manure could contain microorganisms harmful to the mushrooms. The variation in the stipe height and cap diameter observed in this study is in line with the report of [5], who stated that the diameter of oyster mushrooms grown on differentsubstrate formulas varied. They also reported that saw dusk favours mushroom growth.The protein contents of the mushroom from the different substrates mixtures showed that rice husk alone and combination of rice husk + cow dung had the same percentage protein and were higher than mushroom from other substrates. It is not clear if the substrates affected the protein contents of the mushroom, since the control group had the same protein content, even higher than some of the mushroom from the substrate treatments. This amounts of protein detected in this study (18 – 24%) werevery close to the values reported by [8] (25.50-26.80%), [12] (19.82-27.78%) and [14] (20.89-23.84%).

CONCLUSION

This study has demonstrated that *P. tuber-regium* can be effectively cultivated using cheap localagricultural wastes such as saw dust and rice husk both of which are highly abundant in Ebonyi State. There is need to promote this simple and cheap technology to enhance protein consumption among the rural poor.

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Fig. 1: Soaking of the Sclerotia

Fig. 2: The Mature Mushroom.