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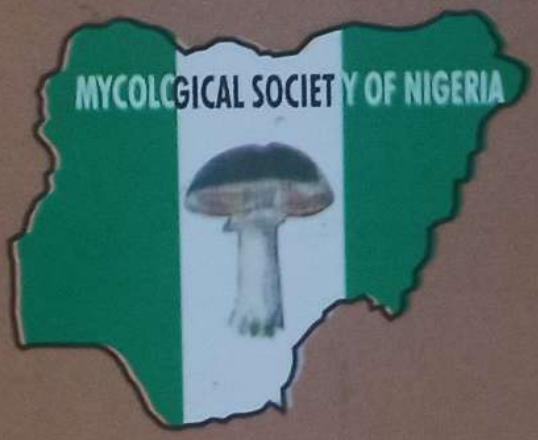

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CONTENT

EFFECTS OF DIFFERENT PLANT SAWDUST SUBSTRATES ON THE CULTIVATION OF *PLEUROTUS OSTREATUS*

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ABSTRACT

Oyster mushroom (*Pleurotus ostreatus*) is an edible mushroom having excellent flavor, tastes and proteins. However, its domestication is rare. On this note, a research was conducted to determine the effects of different sawdust (substrates) from the following trees: *Swietenia mahogani* (Mahogany), *Triplochiton sceroxylon* (Obeche), *Adansonia digitata* (Baobab), *and Gmelina arborea*(Gmelina) supplemented with 7% rice bran and 1% calcium carbonate on its domestication. The diameter and length of stipe and pileus, and proximate composition of Oyster mushroom (*Pleurotus ostreatus*) were determined. The results revealed the highest stipe diameter (0.53cm) and length (1.56cm); and the highest pileus diameter and length (2.43cm and 2.73cm respectively) in *G. arborea* which were significantly different ($P>0.05$) from other substrates used. *P. ostreatus*grown on the *S. mahogani* sawdust gave the highest percentage of carbohydrates (0.30%) but was not significantly different ($P<0.05$) from *G. arborea* sawdust (0.27%) and least in *A. digitata* sawdust (0.18%). Protein content was the highest in mushroom produced on *S. mahogani* sawdust (3.30%), followed by *G. arborea* substrates (3.20%).No significance difference ($P<0.05$) between them, and least in *T. scleroxylon* substrates (2.50%). *Pleurotus ostreatus*fibre content was found to be highest in *S. mahogani* (1.50%) and least in *A. digitata* substrates (1.42%). Fat content was highest in *G. arborea* (0.30%) and the least in *A. digitata* (0.23%). Among all the substrates used, *G. arborea* was found to give the best yield followed by *S. mahogani, A. digitata, and T. scleroxylon*. Therefore, *G. arborea* sawdust and *S. mahogani* sawdust supplemented with 7% rice bran and 1% calcium carbonate are recommended for cultivation of *P. ostreatus*.

Key words: *Pleurotus ostreatus*, Flavour, Domestication, Saw dust, Substrate

INTRODUCTION

Mushrooms are macro-fungi with distinctive fruiting bodies which are either epigenous or hypogenous and sufficiently conspicuous to the naked eye to be hand-picked (Chang and Miles, 2004). Mushrooms are eukaryotic organisms that have a cell containing the polysaccharide, chitin, along with lipids and proteins (Jonathan *et al.*, 2008). Their mode of nutrition is by producing a wide range of enzymes that can break down complex substances, after which they are able to absorb the soluble substances so formed (Cho, 2004).

Mushrooms are important constituents of forest products. They grow on virtually all agro-industrial wastes including most voluminous biomolecules of the biosphere known as cellulose (Chang and Hayes, 1999; Gbolagade, 2006). Edible mushrooms are nutritionally endowed fungi (mostly Basidiomycetes) that grow naturally on tree trunks, leaves and roots as well as decaying woody materials (Chang and Miles, 1992; Gbolagade, 2006). They play an important role in forest ecosystem such as forest flourishing through mycorrhizal association and nutrient cycling through saprobic life (Boa, 2004).

Mushroom cultivation can play an important role in supporting the local economy by contributing to subsistence food security, nutrition, and medicine; generating additional employment and income through local, national and regional trade; and offering opportunities for processing enterprises (such as pickling and drying). Income from mushrooms can supplement cash flow, providing either: a safety netduring critical times, preventing people falling into greater poverty; a gap-filling activity which can help spread income and generally make poverty more bearable through improved nutrition and higher income; or a stepping stones activity to help make people less poor, or even permanently lift them out of poverty.(Akinrele, 1984).

Substrates used in cultivation of mushroom are gotten from agricultural and industrial wastes (Chinda, 2007). The usage of these wastes has a target contribution to resolve the global problem of environmental pollution (Okhuoya, 1991). The substrates for mushroom cultivation, Include waste land and waste materials. The media (substrates) for mushroom cultivation include rice straw, rice bran, wheat straw, pulp, corncobs, cocoa shell wastes, cotton seed bulb, cotton waste from industry, brewers grain, sawdust (a big waste in timber industry), maize husk, and cassava peelings (Stanley, 2011). In view of this, waste dust from different plant sources were used to cultivate mushroom in this study.

MATERIALS AND METHODS

Description of the study area

The study was carried out in Minna in Niger State, Nigeria, metropolis, longitude 6° 34 East and latitude 9° 36 North (Lindequist, 2005).

Collection of Samples

Prior to collection of mushroom cultivated, a reconnaissance survey was carried out in five large and local market in Niger State. The most commonly eating mushroom *Pleurous ostreatus* was collected from wild in May, 2017and identified at the Mushroom Unit of Pathology Section of the Forestry Research Institute of Nigeria. Five different plant sawdust (Obeche - *Triplochiton scleroxylon* K.Schum., Gmelina- *Gmelina arborea* Roxb. and Mahogany – *Swietenia mahogani* Jacq and Baoba – *Adansonia digitata L.* used as substrates were obtained from Maitumbi Sawmill Minna, Niger State between May and June, 2017. The supplements: rice bran, wheat bran and Lime ($CaCO_3$) were obtained from Kure market Minna, Niger State in May, 2017.

Preparation of Substrates

Substrate was prepared with 45%(v/v) sawdust, supplemented rice bran (5%), calcium carbonate (0.02%) and water (50%) thoroughly mixed in a bowl.The substrate was covered with a black plastic polyethylene sheet and left to undergo fermentation for one week(Beyer, 2005). Five treatments were prepared containing different sawdusts.

Bagging and Pasteurization of the Substrates

A polyethylene bag (10cm x 12cm) was filled with 2.5Kg of each substrate. Two replicate bags were prepared for each treatment. The tops of the substrates in the bags were covered with cotton wool and secured with rubber bands. The bagged substrates were sterilized in an autoclave for 30 minutes at 121°C and 15 lb pressure. The pasteurized substrates were later cooled to 27°C (Sarker*et al.*, 2007).

Inoculation and Incubation of Substrate Bags

The substrate bags were allowed to cool down after pasteurization, holes were bored aseptically into the substrate bags and 10grams of *P. Ostreatus* spawn was used to inoculate all the treatments. The bags were kept in a dark inoculating room and the mycelia growth was observed each week for five weeks. Spawn run was observed regulary until fully colonised. The fully ramified bags were exposed and watered daily (Kaul and Dhar, 2007).

Determination of Yield

Harvesting was done by holding the

stipes at the base and twisting lightly with hand. Mushroom were harvested and counted and weighed. At the end of each flush, length and diameter of stipe and length and diameter pileus were determined using ruler.

Proximate Analysis of Harvested Mushroom Samples

The proximate analysis of the samples for crude fibre and fat was carried out in replicate using the methods described by Onwuka (2005). Nitrogen was determined by micro Kjeldah method described by Lowry *et al.* (1951); Onwuka (2005) and the nitrogen content was converted to protein by multiplying by a factor of 6.25. Total carbohydrate content was estimated by 'difference'. All the proximate values were reported in percentage (%).

Determination of Crude Protein

Protein in the sample was determined by kjeldahl method 0.25g of dried samples was taken in digestion flask, with 6ml of concentrated H_2SO_4 and a speck of kjeldahl catalyst (mixture of 10g Na_2SO_4+5g CuSO4+ 0.05g selenium). The flask was swirled in order to mix the contents thoroughly then digested on the digestion block till the mixtures become clear (colourless or greenish in colour). The digest was cooled and transferred to 100ml volumetric flask and volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markham Distillation Apparatus. Ten millilitres of the digestate was introduced in the distillation tube then 10 ml of 40% NaOH was gradually added through the same way. Distillation was continued for at least 10 min and NH_3 produced was collected as NH_4OH in conical flask containing 5ml of 4% boric acid solution with few drops of methyl red indicator. During distillation yellowish colour appears due to NH_4OH. The distillate was then titrated against standard 0.1 N HCI solution till the appearance of pink colour. A blank was also run through all steps as above. Percentage crude protein content of the sample was calculated by following the expression of;

% Crude Protein = 6.25* x %N (*. Correction factor)

$$\%N = \frac{(S-B) \times N \times 0.014 \times D \times 100}{\text{Weight of the sample} \times V}$$

Where

S = Sample titration reading

B = Blank titration reading

N = Normality of HCI

D = Dilution of sample after digestion

V = Volume taken for distillation 0.014 – Milli equivalent weight of Nitrogen

Determination of Crude Fat

Crude fat was determined by ether extract method using Soxhlet apparatus.

A 2g proportion of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. A weighed, cleaned and dried receiving flask was filled with petroleum ether and fitted into the apparatus. The soxhlet apparatus was assembled and allowed refluxing for 6hrs. Thereafter, the extract was transferred into clean glass dish with either washing which was evaporated on water bath. Then the dish was placed in an oven at 105°C-110°C for 1hr and cooled it in desiccators. The percentage crude fat was determined by using the following formula:

$$\% \text{ Crude Fat} = \frac{\text{Weight of either} \times 100}{\text{Weight of sample}}$$

Determination of Crude Fibre

Two (2g) grams of sample was defatted with per ether; boiled under reflux for 30min with 200ml a solution containing 1.25g of H_2SO_4 per 100ml of solution. The solution was filtered through several layers of cheese cloth on fluted funnel, washed with boiling water until the washings are no longer acidic. Then the residue was transferred into a beaker and boiled for 30min with 200ml of solution containing 1.25g of carbonate free NaOH per 100ml, the final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible, then dried in an electric oven and weighed after which it was incinerated, cooled and reweighed. The loss in weight after incineration x 100 is the percentage crude fibre.

Carbohydrate Content Determination:

The nitrogen free method described by A.O.A.C (2015) was used. The carbohydrate is calculated as weight by difference between 100 and the summation of other proximate parameter as Nitrogen free Extract (NFE) percentage carbohydrate (NFE) = 100-(m+p+F+A+F_2).

Where

M = moisture

P = protein

F_1 = Fat

A = ash

F_2 = crude fibre

Data Analysis

All the data obtained from this study were subjected to analysis of variance (ANOVA) procedure using Statistical Analysis System (SAS) and mean separation was by Duncan's New Multiple Range Test (DMRT) at $P < 0.05$.

RESULTS

The results of full ramification of substrate (21 days) with *P. ostreatus* is as shown in Plate a whileits growth on different saw dust substrates are shown in plates b- e. The results on the proximate composition (Table1) showed that the carbohydrate content of

mushroom produced *on S. mahogani* sawdust was the highest (0.30%) and was not significantly different (P<0.05) from *G. arborea* (0.27%) but different from *T. Scleroxylon* and *A. Digitata* with 0.22% and 0.18% respectively.

The protein content of mushroom grown on *S. mahogani* was the highest (3.30%) but was not significantly different from *G. arborea* and *A. digitata* (P<0.05) with 3.20% and 3.10% respectively but the least was in *T. scleroxylon* (2.50%). This trend was also followed by the quantity of fibre got with the highest in *S. mahogani* (1.50%) and the least in *A. Digitata* (1.42%). However, the fat content of mushroom produced on *G. arborea* was highest (0.30%) followed closely by *T. scleroxylon* (0.28%), *S. mahogani* (0.25%) why there was no significance different between them (P<0.05), and the least was in *A. digitata* (0.23%).

The effects of different sawdust substrates on the diameter of mushroom stipe at different week of cultivation (Fig1) showed that at week four, the stipe diameter produced by mushroom grown on *G. arborea* was the highest (0.53cm), followed by *A. mahogani* (0.49cm), *A. digitata* (0.42cm) and the least in *T. scleroxylon* (0.32cm). The same trend was observed at week six where *G. arborea* was the highest (0.57cm) followed by *A. mahogani* (0.52cm), *A. digitata* (0.49) and least in *T. scleroxylon* and at weeks 8 and 10, *G. arborea* was still the highest and the least in *T. sceroxylon*.

The effect of different sawdust substrates on the length of mushrooms stipe at different weeks of cultivation (Fig. 2) showed that at week four, the stipe length produced by mushroom grown on *G. arborea* was recorded to be highest (1.56cm) followed by *S .mahogani* (1.10cm), *A. digitata* substrate (0.98cm) and least in *T. Scleroxylon* (0.73cm). At week six, it was also observed that the *G. arborea* was the highest (2.99cm) followed by *S. mahogani* (2.41cm), *A. Digitata* (2.58cm) and the lowest in *T. scleroxylon* (1.48cm). The same trend was also observed at week eight , it was highest in *G. Arborea* (4.62cm), followed by *A. digitata* (4.10cm), *T. scleroxylon* (3.98cm) and the least in *S. mahogani* (3.63cm). At week ten, the stipe length of mushroom produced was also highest in *G. arborea* substrates (6.20cm), followed by *S. mahogani* (6.05cm), *A. digitata* (5.90cm) and least in *T. scleroxylon* (5.10cm).

The histogram (Fig3) showed the effects of different sawdust substrates on the diameter of mushroom pileus at different weeks of cultivation. At week four, the highest mushroom pileus was observed in *G. arborea* (2.43cm), followed *S. mahogani* (2.20cm), *T. scleroxylon* (1.18cm) and least in *A.*

digitata (1.46cm). It was also observed at week six, it was highest in *G. Arborea* (3.66cm), followed by *S. Mahogani* (3.40cm), *T. scleroxylon* (2.73cm) ,and least in *A. digitata* (2.62cm). At week eight it was highest in *G. arborea* (4.88cm), followed by *S. mahogani* (4.10cm), *T. scleroxylon* (3.62cm) and the least in *A. digitata* (3.33cm).The same trend was observed at week ten, the highest mushroom pileus was *G. aborea* (5.95cm), followed by *S. mahogani* (5.62cm), *T. scleroxylon* (4.92cm) and the least in *A. digitata* substrates (4.78cm).

The effects of different sawdust substrates on the length of mushroom pileus at various weeks of production (Fig 4) showed that at week four of cultivation , the length of the mushroom pileus produced was highest in *G. arborea* (2.73cm), succeeded by *S. mahogani* (2.58cm), *A. Digitata* (2.31cm) and the least in *T. Scleroxylon* (2.10cm). The same trend was also noticed at week six, eight and ten, the highest mushroom pileus was in *G. arborea* (3.89cm, 4.99cm, and 6.45cm) and least in *T. Scleroxylon* (3.23cm, 4.44cm and 5.14cm).

Table1: Proximate Composition of Mushroom Samples Produced on Different Sawdust Substrates

Substrates with supplement (7% Rice bran)	Carbohydrate (%)	Protein (%)	Fibre (%)	Fat (%)
S. mahogani sawdust	0.30^{b}	3.30^{b}	1.50^{c}	0.25^{a}
T. scleroxylon sawdust	0.22^{a}	2.50^{a}	1.46^{b}	0.28^{b}
A. digitata sawdust	0.18^{a}	3.10^{b}	1.42^{a}	0.23^{a}
G. arborea sawdust	0.27^{b}	3.20^{b}	1.47^{b}	0.30^{b}

Values followed by the same superscript on the same column are not significantly Different at $P<0.05$. values are in mean $\pm$ standard error of two determination.

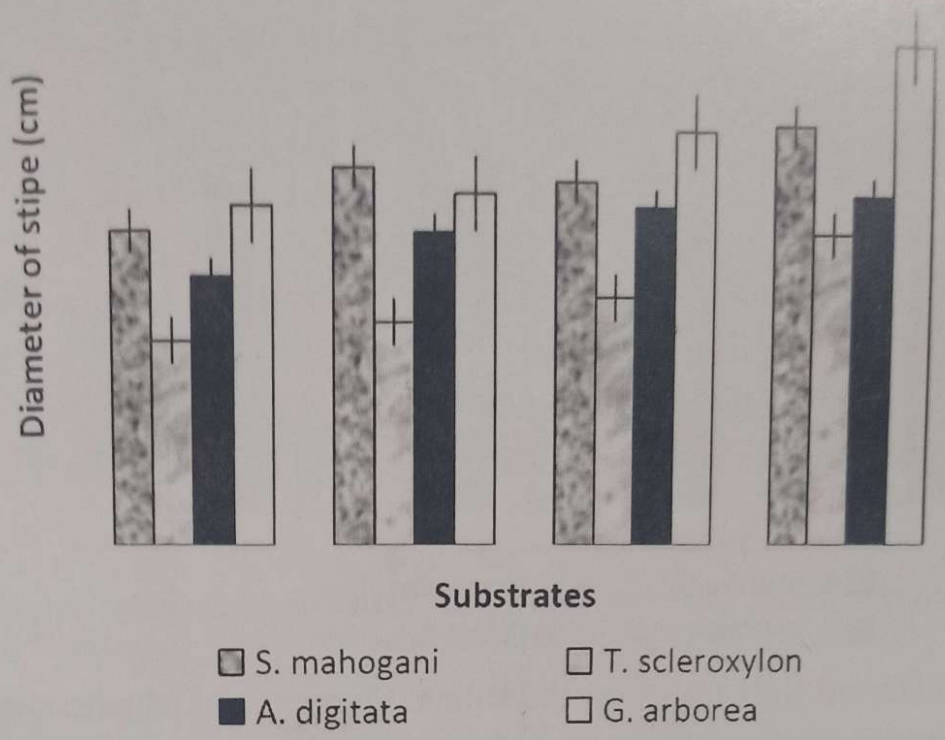


Fig. 1. Effects of Different Substrates on the Stipe Diameter of Mushroom

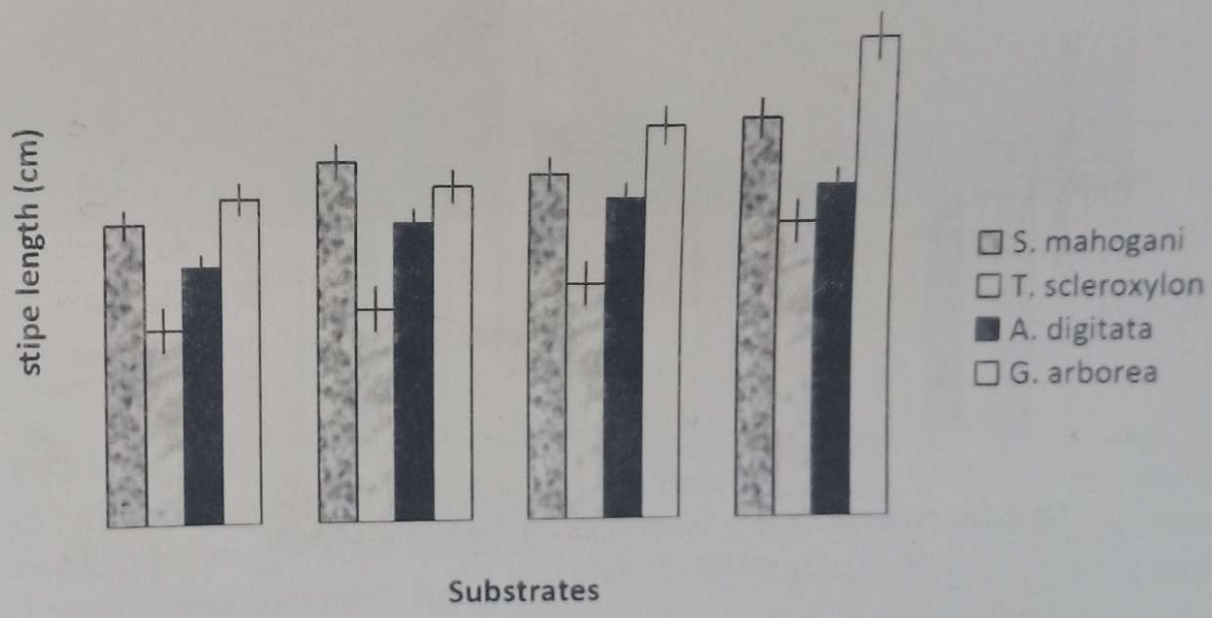


Fig. 2. Effects of Different Substrates on the Stipe Length of Mushroom

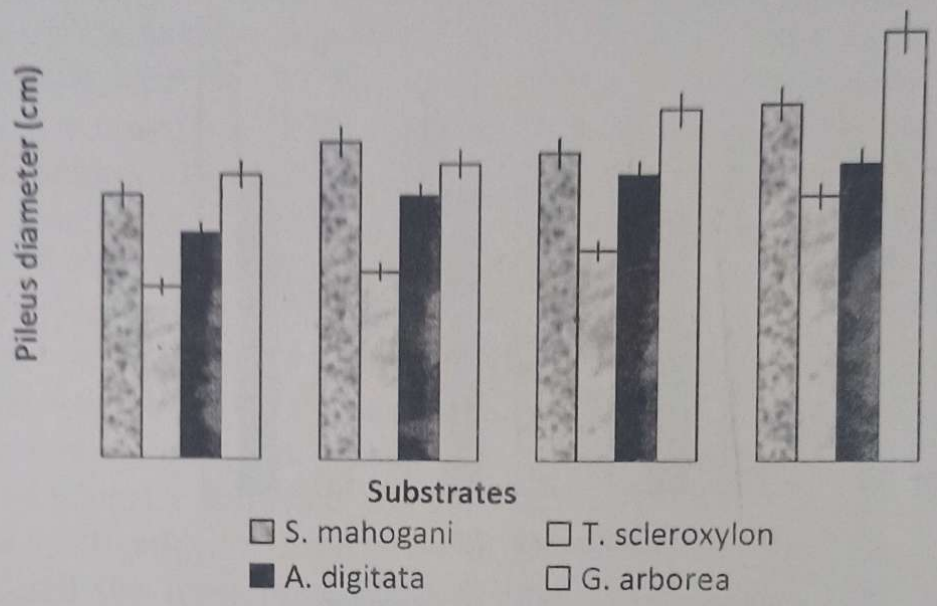


Fig. 3. Effects of Different Substrates on the Pileus Diameter of Mushroom

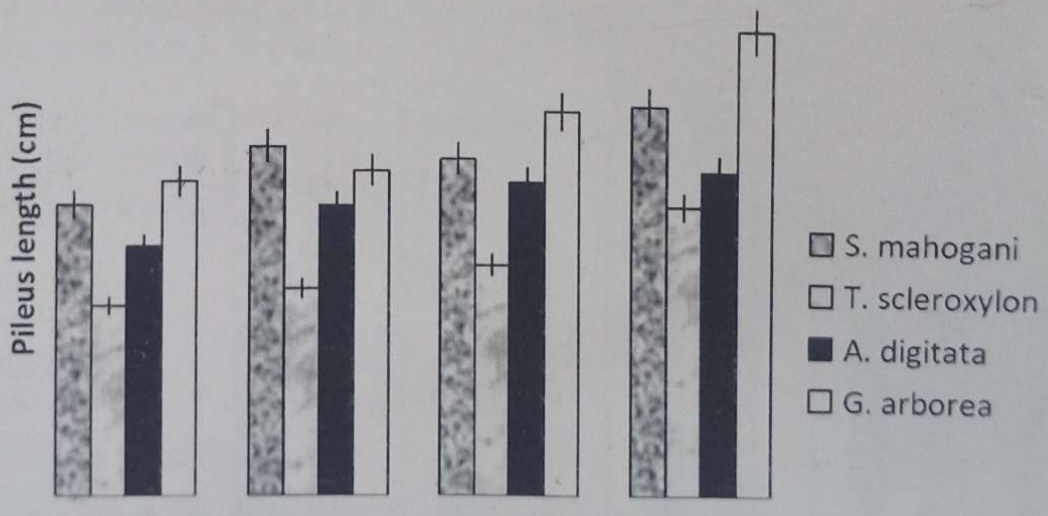
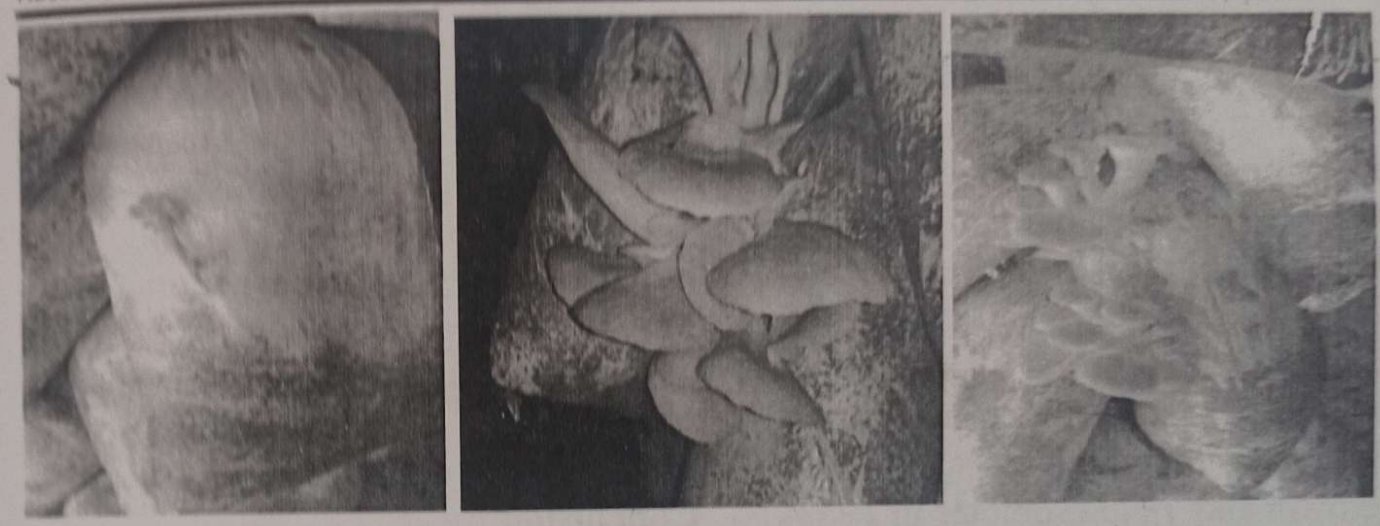
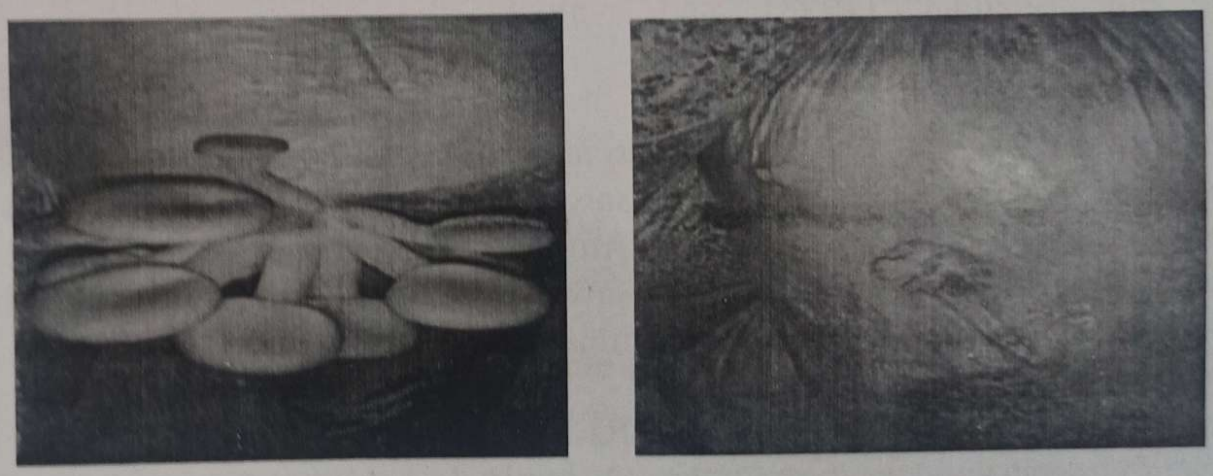


Fig 4 Effects of Different Substrates on the Pileus Length of Mushroom



a b c

Plate 1: Full ramification of substrate (21 days), field source B: *P. Ostreatus* grownon *G. arborea* sawdust substrate Plate C: *P. ostreatus*grownon *S. mahogani* sawdust substrate



d e

P. ostreatus grown on *A. digitata* sawdust substrate Plate E: *P. Ostreatus* grown on *T. scleroxylon* sawdust substrate

DISCUSSION

From all the growth parameters considered, stipe length, stipe diameter, pileus length, pileus diameter, fresh and dry weight of the fruit bodies, the vast growth of *P. ostreatus* on these different supplemented plant sawdust during its cultivation, agreed with the report of Fanadzo *et al.*(2010), where supplement (cotton seed hull) significantly improved the *P. ostreatus*yield on wheat straw during cultivation. The outstanding

mycelia extension observed regularly until it appeared white (colonization) in all substrates supplemented with rice bran suggested that it was the most suitable supplement for growth and yield enhancement in cultivation of *P. ostreatus*. Similarly, Chandra *et al.* (2013) reported that the addition of supplement as rice bran to substrates is beneficial as a nutrient supplement of growth and yield promoter in *P. sajor-caju* cultivation. The ramification was an indication that *P. ostreatus* could perform and utilize substrates, these were in accordance with the finding of Merta and Bhandel (1998) and Gholagade *et al.* (2006) who separately observed complete colonization of mycelia growth of *Pleurotus* sp, and *Lentinus squarrosullus* on different growing media agar plates.

The results obtained in this research revealed that *G. arborea* sawdust produced the highest fresh and dry weights. This could probably be that *G. arborea* provided a good aeration for the germination and fructification of the mushrooms. This was in disagreement with the finding of Yang *et al.* (2013), who reported *M. excels* sawdust as the most suitable substrates for the mushrooms cultivation with the characteristics of relatively large mushroom stalk length, stalk width and cap width. The report of Onyango *et al.* (2011) further explained that large sized mushroom fruit bodies are considered as a good quality in marketable mushroom. The decrease in the mushroom size obtained among each substrates after the first flush, supported the finding of Singh and Singh (2011) where the first flush of *P. citrinopileatus* fruiting bodies gave maximum yield in comparison to second flush.

Conclusion

Oyster mushroom presents a promising potential for treatment of sawdust wastes such as generated in the saw mill. The study was conducted to evaluate the growth of *Pleurotus ostreatus* mushroom on four different substrates i.e. *S. mahogani* sawdust, *T. scleroxylon* sawdust, *A. digitata* sawdust, and G. arborea sawdust. Among all the substrates, *G. arborea* sawdust was found favourable for mushroom cultivation.

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