

Growth Performance and Yield of the Edible White Rot Fungus (*Pleurotus ostreatus*) on Different Agro Waste Materials

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Abstract—A study was carried out to evaluate the growth and yield performance of *Pleurotus ostreatus* spawn on different organic substrates in Lafia, Nasarawa State, Nigeria. 50 g each of four different substrates namely; corncobs, rice straw, sugarcane bagasse and sawdust sourced locally from farmlands and processing sites, were amended with 2% calcium carbonate and calcium sulphide and sterilized using three sterilization methods namely; hot water, steam, and lime. Five grams of *P. ostreatus* spawn were inoculated unto treated substrates, incubated in the dark for 16 days and in light for 19 days at 25 °C for the commencement of pinhead and fruit body formations respectively. Growth and yield parameters such as days to full colonization, days to pinhead formation and days to fruit body formation were recorded. Cap diameter and fresh weight of mature mushrooms were also measured for a total count of four flushes. *P. ostreatus* spawn grown on sugarcane bagasse recorded the highest mean cap diameter (4.69 cm), highest mean fresh weight (34.68 g), highest biological efficiency (69.37%) and highest production rate (2.83 g per day). Spawn grown on rice straw recorded the least number of days to full substrate colonization (11.00). Spawn grown on corn cobs recorded the least mean number of days to pin head (18.75) and fruiting body formations (20.25). There were no significant differences ($P \leq 0.05$) among the evaluated substrates with respect to growth and yield performance of *P. ostreatus*. Substrates sterilized with hot water supported the highest mean cap diameter (5.64 cm), highest biological efficiency (87.04%) and highest production rate (3.43 g per day) of *P. ostreatus*. Significant differences ($P \leq 0.05$) were observed in cap diameter, fresh weight, biological efficiency and production rates among the evaluated sterilization methods. Hot water sterilization of sugarcane bagasse could be adopted for enhanced yield of oyster mushrooms, especially among indigent farming communities in Nigeria and beyond.

Keywords—Agro wastes, growth, *Pleurotus ostreatus*, sterilization methods, yield.

I. INTRODUCTION

MUSHROOMS are macro fungi with distinctive fruit bodies that are large enough to be seen with the naked eye. They include edible and medicinal, as well as poisonous species [1]. Although once termed as “foods for the gods”, several species are currently recognized as food and grown on commercial scale in Nigeria and most parts of the world [2], [3].

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Mushrooms are of enormous nutritional value; they are especially rich in protein, minerals (P, K, Na, and Ca), vitamins (B1, B2, C, niacin and biotin) and abound in essential amino acids [4]. They are low in fats (4%), high in fibre and carbohydrate content [5], [6] and can be in fact considered as a good supplement to cereals and an alternative to meat [1]. Medicinally, mushroom and mushroom products are used mostly for treating chronic catarrh. They also lower the cholesterol level of the blood and improve blood circulation, also serving as remedy for night sweating in tuberculosis, rheumatism, jaundice, dropsy, intestinal worms and have anti-tumour, anti-viral and anticancer properties [7].

Pleurotus ostreatus (Oyster mushroom) is an edible mushroom species commercially produced and sold in markets in Asia, America, Europe and Africa. In Africa, the fungus is often observed in the wild growing around the African breadfruit (*Treculia africana*) and attacking dead wood resulting to varying degrees of white rot [8]. Oyster mushrooms have gained appreciable popularity in Nigerian markets for their exceptional flavor and other nutritional potentials [9]-[11].

This study was aimed at exploring easily available agricultural waste materials and methods for optimal yield and productivity of oyster mushrooms, in attempt to support existing bioremediation efforts and commercial production of oyster mushrooms in the study area.

II. MATERIALS AND METHODS

A. Description of Study Area

Lafia is located on Longitude 8.4916°N and Latitude 8.51667°E in the Guinea Savannah vegetative belt of Nigeria. With a population of about 330,712, the major occupation of the inhabitants is farming.

B. Sources of Organic Substrates and Mushroom Spawn

1. Organic Substrates

Organic substrates namely; corn cobs, saw dust, sugarcane bagasse, and rice straw, were sourced locally from farm lands and processing sites in Lafia.

2. Additives

Substrate additives such as rice bran, calcium sulphide (CaSO_4) and calcium carbonate (CaCO_3) were purchased from Lafia Market.

3. Mushroom Spawn

Three days old spawn of *Pleurotus ostreatus* were supplied by Lauretta ventures Limited Ondo State, Nigeria.

C. Substrate Preparation

The methods reported by [6] and [12] were used as follows:

1. Saw Dust

Six kilograms of sawdust were mixed with two kilograms of rice bran and one kilogram of wood shavings. 2% CaCO₃ was also added to regulate pH and minimize the risk of contamination. The mixture was moistened with water and dispensed into sterile polyethylene bags at the rate of 50 g per bag and labelled appropriately.

2. Sugarcane Bagasse, Corncobs and Rice Straw

Sugarcane bagasse, corncobs and rice straw were chopped into smaller pieces of about 3-5 cm², sun-dried and 90 g of each substrate on dry basis were measured and soaked overnight in about 10 L of tap water. Soaked substrates were removed and allowed to drain for about 3-4 hours. Each substrate was mixed with 2% CaCO₃ and 10% rice bran, bagged and labeled prior to pasteurization.

D. Substrate Sterilization

1. Steam Sterilization

Growth bags containing processed substrates were placed in 55-gallon metal drums containing about 100 litres of water and their lids are tightly sealed to prevent evaporation. The mixture in the polyethylene bags were pasteurized for 5 hours at a constant temperature of 60 °C and allowed to cool.

2. Sterilization in Lime Water

Substrates were separately soaked in fresh tap water mixed with 25 g of lime for 24 hours in a 100 litre volume aluminum can. The pasteurized substrates were dispensed in sieves and allowed to drain for 4 hours.

3. Hot Water Sterilization

Each substrate was separately submerged in a metallic basin and heated up to 60-65 °C for 2 hours. Excess water was drained off and substrates left overnight to cool to about 25°C.

E. Inoculation and Spawn Run

Five grams of *P. ostreatus* spawn were aseptically introduced into various treated substrates and incubated at 20-25 °C in a dark growth room for the commencement of substrate colonization (spawn run). Narrow slits of about 10 cm were made on inoculated bags and sealed with cotton gauze to allow for gaseous exchange in inoculated bags.

F. Fruiting

Upon full colonization of growth substrates, *P. ostreatus* spawn were exposed to light for the commencement of fruiting. The polythene bags were uncovered in order to let in more air and reduce the CO₂ level within the growth bag. The growth room and substrates were kept humid by repeated sprinkling (4-5 times daily) with clean tap water for stimulation of pinhead formation.

G. Harvesting

Mature fruiting bodies were carefully harvested 48 hours after pinhead formation, by hand-picking from the base. Subsequent harvest of fruiting bodies was carried out every 48 hours for four consecutive flushes.

H. Assessment of Growth and Yield Parameters

1. Phenological Data

Phenological data such as Days to Full Substrate Colonization (DTFC), Days to Pinhead Formation (DTPHF), and Days to Fruit Body Formation (DTFBF) were recorded. Cap Diameter (CD) was measured using a meter rule, from the first day of pinhead formation.

2. Total Yield

Fresh weights (g) of mushrooms harvested from separate substrates were measured for four consecutive flushes, and their total weighs considered as the total yield which was also used for calculating biological efficiency as reported by [12].

3. Biological Efficiency

The biological efficiency (BE) of *pleurotus ostreatus* on each substrate was calculated as:

$$BE = \frac{\text{Weight of Fresh Mushrooms}}{\text{Weight of Dry Substrate}} \times 100 \quad (1)$$

4. Production Rate

On the basis of BE on each substrate and the time that was taken (in days) from spawning to harvesting, the production rate (PR) of *pleurotus ostreatus* on each substrate was calculated as:

$$PR = \frac{BE}{\text{TIME}} \quad (2)$$

I. Experimental Design

Treatments were laid out in Randomized Complete Block Design (RCBD) with three replicates.

J. Data Analysis

Data obtained from the study were subjected to the Analysis of Variance (ANOVA) at 5% level of probability using SPSS software version 22.

III. RESULTS

TABLE I
EFFECT OF SUBSTRATE TYPE ON GROWTH AND YIELD PERFORMANCE OF *P. OSTREATUS*

Substrates	DTFC	DTPHF	DTFBF	CD (cm)	FW (g)	BE (%)	PR (g per day)
Sawdust	13.17 ^a	19.75 ^a	21.25 ^a	3.71 ^a	27.43 ^a	53.12 ^a	2.03 ^a
Corn cob	12.33 ^a	18.75 ^a	20.25 ^a	4.41 ^a	29.11 ^a	58.22 ^a	2.33 ^a
Sugarcane bagasse	13.25 ^a	22.83 ^a	24.83 ^a	4.69 ^a	34.68 ^a	69.37 ^a	2.83 ^a
Rice straw	11.00 ^a	23.08 ^a	24.75 ^a	4.42 ^a	34.00 ^a	68.00 ^a	2.35 ^a

Values followed by the same superscripts within the same column are not significantly different (P<0.05).

Values followed by different superscript within the same column are significantly different (P<0.05).

DTFC = Days to Full Colonization; DTFBF = Days to Fruiting Body Formation; FW = fresh weight.

Results of effect of different substrates on growth and yield performance of *P. ostreatus* (Table I) revealed that *P. ostreatus* spawn grown on sugarcane bagasse recorded the highest mean CD (4.69 cm), highest mean FW (34.68g), highest BE (69.37%) and highest PR (2.83 g per day). This was closely followed by spawn grown on rice straw which recorded the least days to full mycelium colonization (11.00). Spawn grown on corn cobs recorded the least mean number of days to pin head (18.75) and fruiting body formations (20.25). There were no significant differences ($P \leq 0.05$) observed among the evaluated substrates.

TABLE II
EFFECT OF METHOD OF SUBSTRATE STERILIZATION ON GROWTH AND YIELD PERFORMANCE OF *P. OSTREATUS*

Treatment Method	DTFC	DTPHF	DTFB F	CD (cm)	FW (g)	BE (%)	PR (g per day)
Lime water	10.67 ^a	17.00 ^a	18.75 ^a	3.65 ^a	18.32 ^a	36.63 ^a	1.34 ^a
Steam	12.00 ^a	24.58 ^a	26.58 ^a	4.64 ^{ab}	41.33 ^b	80.93 ^b	3.06 ^b
Control	13.33 ^a	19.08 ^a	20.42 ^a	3.29 ^a	14.56 ^a	29.12 ^a	1.04 ^a
Hot water	13.75 ^a	23.33 ^a	25.33 ^a	5.64 ^b	51.01 ^b	102.02 ^b	4.09 ^b

Values followed by same superscript within same column are not significantly different.

Values followed by different superscript within same column are significantly different ($P \leq 0.05$).

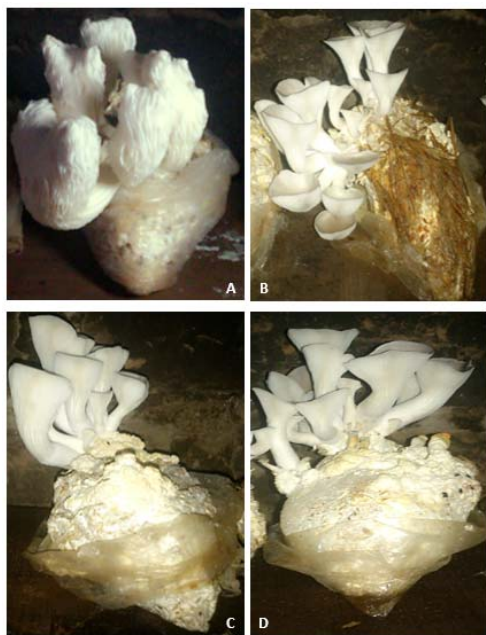


Fig. 1 Mature Fruit Bodies of *Pleurotus ostreatus* growing on different growth substrates: (A) Sugarcane Bagasse; (B) Rice Straw; (C) Corn cobs and (D) Sawdust

Results of effect of method of substrate sterilization on growth and yield performance of *P. ostreatus* (Table II) revealed that substrates sterilized with lime water method recorded the least mean number of DTFC (10.67), least mean number of DTPHF (17.42), and least number of days to *P. ostreatus* fruit body formation (18.75). Substrates sterilized with hot water recorded the highest mean number of DTFC

(13.75), highest mean CD (5.64c m), highest FW (51.01 g), highest BE (102.02%) and highest PR (4.09 g per day). Significant differences ($P \leq 0.05$) were observed in CD, FW, BE and PR among the evaluated sterilization methods.



Fig. 2 Freshly Harvested Fruit Bodies of *P. ostreatus* 26 Days after Spawn Inoculation

IV. DISCUSSION

Growth of *P. ostreatus* mycelia was observed on all evaluated substrates. Similarly, [13] demonstrated that mushrooms can be grown on various organic substrates locally available. References [14] and [15] also reported that *Pleurotus* spp. can be cultivated on different forms of agricultural wastes. The ability of *P. ostreatus* to grow on a vast array of substrates has been attributed to its relatively fast growth and efficient enzyme-assisted breakdown of both simple and complex organic compounds.

Rice straw supported the earliest full mycelial colonization of growth substrates by *P. ostreatus*. Similarly, [16] reported considerable influence of growth substrates on the colonization rates and time needed for complete colonization and fructification of *Pleurotus ostreatus*. Reference [17] also stated that substrate structure is an important determinant of substrate penetration by mushroom mycelia. The ability of rice straw to support faster substrate colonization by the evaluated fungus can be attributed to its high lignocellulosic content and large pore size which are preferences for effective growth and ramification by *P. ostreatus* mycelia. Reference [18] also observed that larger substrate pore sizes supported better mycelium growth rates.

P. ostreatus mycelia grown on shredded corn cobs recorded the shortest pinhead initiation time and number of DTFC. This agrees with the findings of [13] who also obtained the shortest time to pinhead formation of Oyster mushroom (*Pleurotus ostreatus*) on maize cobs. Differences in pinhead formation time observed among the different substrates could be attributed to variations in structural and physiological composition of evaluated substrates. Reference [13] reported that substrates higher in lignin and cellulose content took longer time to initiate pinning compared to substrates with lower contents of lignin and cellulose.

P. ostreatus spawn grown on sugarcane bagasse recorded the highest CD, FW, PR and BE. This could be as a result of

the porous nature of sugarcane bagasse which allows for effective colonization, gas exchange and easy access to free sugars present in the bagasse. Reference [19] also reported the effect of substrate pore space on mushroom yield. Similarly, [20] also explained that the mushrooms grown on sugarcane bagasse receive nutrients and energy from the abundant free sugars present in the bagasse, resulting to increase in CD. Furthermore, [21] also agreed that sugarcane bagasse is rich in cellulose, sucrose and nitrogen which are easily degraded by oyster mushrooms. This however disagrees with the findings of [22] who reported that the complex nature of sugarcane bagasse impedes its efficient conversion by fungal mycelium.

Growth substrates sterilized with hot water enhanced the production of mushrooms with higher CD, BE and PR. This is in agreement with the findings of [23] who reported that the highest yield (95% BE) was recorded in substrates pasteurized with hot water (70 – 80 °C). Reference [24] also reported increased mushroom yield on substrates sterilized by hot water treatment. On the contrary, [25] observed that hot water sterilization of substrate reduced yield by at least 20% when compared to other straw treatments, such as steam, chemical or untreated wheat straw. The enhanced yield and BE of *P. ostreatus* on hot water treated substrates could be due to effective pasteurizing effect which reduced microbial competition for the growing mushrooms and also aided the release and availability of nutrients from treated substrates.

V. CONCLUSION

Hot water sterilization of sugarcane bagasse is an easy, cheap and yet very efficient technique for enhanced yield of oyster mushrooms. It is especially recommended for adoption by indigent farming communities where it can serve as a viable substitute for the often highly expensive high-tech methods used in several developed economies.

REFERENCES

- [1] S.T. Chang, and J.A. Buswell, "Ganoderma lucidum (Curt. Fr.) P. Karst. (Aphyllphoro mycetidease): A mushrooming medicinal mushroom", *International Journal of Medicinal Mushrooms*, vol. 1, 1999, pp. 139–146.
- [2] S.K. Ogunlana, "Studies on the cultivation of the edible mushroom *Pleurotus tuber regium* (Fr) singer", *Tropical Journal of Applied Sciences*, vol. 2, 1992, pp. 56-60.
- [3] P.A. Stamets, "Novel approach to farm waste management", *Mushroom Journal Winter*, vol. 30, 2001, pp. 28-32.
- [4] S.S. Patil, R.M. Kadam, S.L. Shinde, and S.A. Deshmukh, "Effect of different substrates on productivity and proximate composition of *Pleurotus florida*", *International Journal of Plant Sciences*, vol. 3, no. 1, 2008, pp. 151-153.
- [5] O. Peter, *Nutritional aspects and medicinal use Manual on Mushroom Cultivation*, Amsterdam: Tool Foundation, 1991, pp. 23-24.
- [6] E.N. Ekpo, and A.P. Aluko, *Cultivation strategies and nutritive values of edible Mushrooms (Lentinus tuber-regium) as a component of sustainable livelihood*, In *Proceedings of 28th Annual conference of Forestry Association of Nigeria, Akure Ondo State*, J.E. Abu, and L. Popoola, Eds. 2002, pp. 114-118.
- [7] A. Rehana, T. Muhammad, and R. Tahir, "Propagation of *Pleurotus sajor-caju* (Oyster mushroom) through tissue", *Pakistan Journal of Botany*, vol. 39, no. 4, 2007, pp. 1383-1386.
- [8] B.A. Oso, "*Pleurotus tuber-regium* from Nigeria", *Mycologia*, vol. 69, 1977, pp. 271-279.
- [9] C.I. Onuoha, "Cultivation of mushroom (*Pleurotus tuber regium*) using some local substrates", *Life Science Journal*, vol. 4, no. 4, 2007, pp. 58-61.
- [10] I.O. Fasidi, and V. Ekuere, "Studies on *Pleurotus tuberregium* (Fr) Sing: cultivation, proximate composition of stored mushrooms", *Food Chem.*, vol. 55, 1993, pp. 165-168.
- [11] T. Hussain, "Growing mushroom a new horizon in agriculture", *Mushroom Journal*, vol. 21, 2001, pp. 23-6.
- [12] S.T. Chang, O.W. Lau, and K.Y. Cho, "The cultivation and nutritional value of *Pleurotus sajor-caju*", *European Journal of Applied Microbiology and Biotechnology*, vol. 12, 1981, pp. 58–62.
- [13] J.W. Kimenju, G.O.M. Odero, E.W. Mutitu, P.M. Wachira, R.D. Narla, and W.M. Muiru, "Suitability of locally available substrates for oyster mushroom (*Pleurotus ostreatus*) cultivation in Kenya", *Asian Journal of Plant Sciences*, vol. 8, no. 7, 2009, pp. 510-514.
- [14] I.O. Fasidi, and K.S. Olorunmaje, "Studies on the requirements for vegetative growth of *pleurotus tuber regium* (Fr) singer", *Nigerian Mushroom food chemistry*, vol. 50, 1994, pp. 397-401.
- [15] S.M. Ayodele, E.O. Akpaja, and F. Anyiador, "Evaluation of the yield of *Lentinus squarrosulus* (Mont) Singer on selected economic tree species", *Pakistan Journal of Biological Sciences*, vol. 10, no. 23, 2007, pp. 4283-4286.
- [16] A.N. Philippoussis, P.A. Diamantopoulou, and G.I. Zervakis, "Correlation of the properties of several lignocellulosic substrates to the crop performance of the shiitake mushroom *Lentinula edodes*", *World Journal of Microbiology and Biotechnology*, vol. 19, no. 6, 2003, pp. 551-557.
- [17] A. Tripathy, T.K. Sahoo, and S.R. Behera, "Yield evaluation of paddy straw mushrooms (*Volvariella* spp.) on various lignocellulosic wastes", *Botany Research International*, vol. 4, no. 2, 2011, pp. 19-24.
- [18] R. Tinoco, M.A. Pickard, and R. Vazquez-Duhalt, "Kinetic differences of purified laccases from six *Pleurotus ostreatus* strains", *Letters in Applied Microbiology*, vol. 32, no. 5, 2001, pp. 331-335.
- [19] B.O. Onyango, V.A. Palapala, P.F. Arama, S.O. Wagai, and B.M. Gichimu, "Suitability of selected supplemented substrates for cultivation of Kenyan native wood ear mushrooms (*Auricularia auricula*)", *American Journal of Food Technology*, vol. 6, no. 5, 2011, pp. 395-403.
- [20] G. Zervakis, A. Philippoussis, S. Ioannidou, and P. Diamantopoulou, "Mycelium growth kinetics and optimal temperature conditions for the cultivation of edible mushroom species on lignocellulosic substrates", *Folia microbiologica*, vol. 46, no. 3, 2001, pp. 231-234.
- [21] D. Taurachand, *Oyster mushroom cultivation*, Republic of Korea: Mush World, 2004, pp. 118-121.
- [22] G.V. Thomas, S.R. Prabhu, M.Z. Reeny, and B.M. Bopaiah, "Evaluation of lignocellulosic biomass from coconut palm as substrate for cultivation of *Pleurotus sajor-caju* (Fr.) Singer", *World Journal of Microbiology and Biotechnology*, vol. 14, no. 6, 1998, pp. 879-882.
- [23] B.K. Pani, and S.R. Das, "Effect of pre-treatment of substrate on the yield of oyster mushroom *Pleurotus sajor-caju* (Fr. Singer)", *Journal of Mycopathological Research*, vol. 36, no. 2, 1998, pp. 113-114.
- [24] D. Ejigu, and T. Kebede, "Effect of Different Organic Substrates and their Pasteurization Methods on Growth Performance, Yield and Nutritional values of Oyster Mushroom (*Pleurotus ostreatus*)", *Global Journal of Science Frontier Research*, vol. 15, no. 6, 2015, pp. 27-29.
- [25] J.S. Mejia, and E. Alberto, "Heat treatment of wheat straw by immersion in hot water decreases mushroom yield in *Pleurotus ostreatus*", *Revista Iberoamericana de Micología*, vol. 30, no. 2, 2013, pp. 125-129.