YIELD EVALUATION OF *PLEUROTUS PULMONARIUS* (OYSTER MUSHROOM) ON DIFFERENT AGRICULTURAL WASTES AND VARIOUS GRAINS FOR SPAWN PRODUCTION

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ABSTRACT

Six agricultural by-products were used in the cultivation of *Pleurotus pulmonarius*. all of which were supplemented with 2% 15:15:15 N: P: K fertilizer and 3% rice bran to increase the nutritional composition of the substrates. In addition, 1% CaCO₃ was added to minimize bacteria contamination. The ramification rate and yield performance of the substrates were evaluated differently. Palm fruit husk was the best performing substrate with the highest total yield harvest (190.0g), biological efficiency (63.3%) and production rate (6.33%); banana leaves gave the lowest harvest yield (56.6g), biological efficiency (18.9%) and production rate (1.58%). Also, white maize was observed to have the greatest suitability for spawn production out of the five grains evaluated. Utilization of agro-waste as substrate will be of great importance in commercial cultivation of mushroom and bioconversion of agro-wastes.

Keywords: Mushroom, Agrowaste, Spawn, Substrate, Yield

INTRODUCTION

Mushrooms are fruiting bodies of fungi which derive all of their energy and growth materials from their growth media through biochemical decomposition processes (Chang *et al.*, 1991). Mushroom has a high protein content of 25-50% and also contains fat (2-5%), sugars (17-47%), mycocellulose (7-38%), minerals (8-12%) and vitamins such as D, C, B1, B5, B6, niacin and riboflavin. Apart from serving as a protein food for which they are most popular in Nigeria, mushrooms are also medicinal and have some biotechnology–based functions (Taniguchi, 2000).

Prior to the commercial cultivation of mushrooms in Nigeria, a large proportion of the people had relied on collection of wild mushrooms with the occasional dire consequences of morbidity (and less frequently mortality) as a result of consumption of poisonous species of mushrooms. Cultivation of oyster mushrooms is favoured for their preferred organoleptic and medicinal properties, vigorous growth, and undemanding cultivation conditions (Gregori, 2007). Cultivation of *Pleurotus sp.* rates second after Agaricus bisporus in production, in the world (Chang et al., 1991). One of the values of commercial cultivation of mushrooms, especially in a developing economy like Nigeria, is the availability of large quantities of several agro-industrial wastes which can serve as substrates for use as growing media (Banjo et al., 2004). It has been reported that mushrooms can grow on chopped cocoa pods, cotton waste, dried chopped maize straw, oil palm (fibre and bunch) wastes, tobacco straw, used tea leaves, rice straw, sugarcane bagasse, newsprint, old rags and sawdust (Banjo et al., 2004).

The main nutritional sources for oyster mushroom are cellulose, hemicellulose and lignin. Carbon/Nitrogen (C/N) ratio is important factor for optimum substrate composition for oyster mushroom. Oyster mushroom requires much carbon and less nitrogen source than button mushroom (*Agaricus bisporus*). However, most of the main substrate materials such as cereal straw, cotton waste, sawdust need supplementation of nitrogen source such as wheat and rice bran to reach optimum C/N ratio for oyster mushroom

(Chan, 1981).

The availability of good quality spawn and good substrates are major limiting factors in mushroom cultivation, because, they determine the quantity and quality of mushroom produced. The present study was designed to evaluate the suitability of different grains for spawn production and also, cultivate *Pleurotus pulmonarius* mushroom on different nutrient rich agricultural wastes.

MATERIALS AND METHODS

Source of Pleurotus pulmonarius

The strain of *P. pulmonarius* (Jf736658) used in this study was collected from the Department of Ladoke Akintola University of Technology, Pure and Applied Biology (LAUTECH), Ogbomoso, Nigeria. The strain was routinely maintained and preserved on Potato Dextrose Agar (PDA) slant at 4°C.

Preparation of Spawn

Five different types of grains: sorghum, white maize, mustard seeds, millet and wheat were evaluated for suitability in spawn production. Grains (100 g/bottle) were washed four times, boiled for 45 min and sun-dried. Grains were mixed with 1% w/w of Calcium carbonate (CaCO₃), dispensed in bottles and sterilized at 121°C for 15 min. Sterile grains were inoculated with 6 plugs (6 mm) of actively growing culture and incubated at $23\pm2^{\circ}$ C. Mycelia running was recorded at an interval. Four parameters: rate of mycelia ramification, ramification days, weight of ramified mycelia and spawn productivity were determined according to Adebayo *et al.* (2013). The parameters were defined as follow;

- (i) Mycelia Ramification Rate = <u>Average length of mycelia ramification</u> Average number of days
- (ii) Weight of Mycelia Ramification (WMR) = Final weight (Grain + Bottle + Mycelia) – Initial weight (Grain + Bottle)
- (iii) Ramification day = Average number of days for full ramification
- (iv) Productivity Rate = <u>Total weight of mycelia</u> × 100% Substrate weight

Fruit Body Production

Six different agro-wastes evaluated were: sawdust, palm fruit husks, almond leaves, cocoa leaves, mango leaves and banana leaves. Dried shredded substrates were soaked in water (1:3) and drained afterwards for 24 h. Substrates were packed into heat resistant polytene bag (1.4 kg/bag), sterilized at 121°C for 15 min allowed to cool and inoculated with spawn (5-10% w/w). The spawn was carefully mixed with the substrate to allow even spawn distribution in the bag. Bags were incubated in the dark at 25±3°C until they were fully colonized. Fully colonized substrate bags were transferred into mushroom house (dark, humid and between 22°C to 28°C) and watering was carried out twice per day (morning and evening) for fruit body production.

RESULTS AND DISCUSSION

The spawn ramification rate and spawn productivity for sorghum, maize, millet, wheat and mustard seeds are presented in Table 1. Highest ramification rate and spawn productivity were obtained in white maize (ramification rate of 0.378 cm/d and productivity of 9.08%), followed by millet with ramification rate of 0.31 cm/d and productivity of 3.95%, while mustard seeds had the lowest ramification rate of 0.056 cm/d and productivity of 1.0%. Results obtained in this study was better than the previous work of Siddhant et al. (2013), who reported that a faster rate of cropping was noted in Italian and little millet spawn while maize spawn showed delayed cropping. White maize was the best performing grain with a Weight of Mycelia Ramification (WMR) 1.09 g by day 12, followed by millet with WMR 1.07 g on day 16, and mustard seeds had a very poor mycelia ramification of WMR of 0.2 g on day 20.

Cereal grains are generally used as spawn substrate. They act as a reservoir of carbohydrates which offer sufficient nutrition for mycelia growth and provide vehicle for the eventual even distribution of mushroom inoculant. The major disadvantage of small grains is the presence of less food material in their endosperm and greater surface area in a given amount as compared to larger grains (Bahl, 1984). Due to this reason, mycelia of mushroom take more time to establish and run over the surface of small grains resulting in delayed spawn development in mustard seeds. Ramified spawn of *P. pulmonarius* on sorghum, maize, mustard seeds, millet and wheat are shown on Figures 1, 2, 3, 4 and 5 respectively. Most of the grains, especially maize, millet and sorghum show efficient spawn productivity.

 Table 1: The Spawn Ramification Rate and Productivity on Different Grains

 Substrate

Grains	RT (cm/	d) RD (d)	WMR (g)	PDT (%)
Sorghum	0.258	18	1.07	5.94
Maize	0.378	12	1.09	9.08
Mustard	0.056	18	0.20	1.00
Millet	0.310	16	1.17	7.80
Wheat	0.190	20	0.79	3.95

RT = Ramification Rate, RD = Ramification Day, WMR = Weight of Mycelia Ramification, PDT = Productivity

Table 2: The Distribution of Total Fresh Mushroom Production (g) over 3 Harvests on Different
Substrates

Substrates	IP (day)	PP (day)	1 st Harvest (g)	2 ND Harvest (g)	3 RD Harvest (g)	Total Weight (g)
Sawdust	18	10	65.90 (40.70 %)	52.90 (36.70 %)	43.10 (26.62 %)	161.9
Palm fruit husks	18	10	72.2 (38.00 %)	57.60 (30.31 %)	60.20 (31.68 %)	190.0
Almond leaves	18	10	45.6 (44.23 %)	32.3 (31.33 %)	25.2 (24.44 %)	103.1
Cocoa leaves	19	12	32.1 (35.90 %)	27.2 (30.42 %)	30.1 (33.67 %)	89.3
Banana leaves	19	12	25.5 (45.05 %)	11.2 (19. 79 %)	19.9 (35.16 %)	56.6
Mango leaves	18	10	42.1 (39.13 %)	34.3 (31.88 %)	31.2 (29.37 %)	107.6

IP = Incubation Period, PP = Production Period, Figures in parenthesies are percentage of mushroom in each harvest

Table 2 shows the distribution of the total fresh mushroom harvested and primordia formation days for each of the substrates used. Four of the substrates namely sawdust, Palm fruit husk, almond and mango developed primordia in 18 while cocoa leaves and banana leaves developed primordial in 19 days. The results of this study revealed that some of the agrowastes used can be good substrates for the Cultivation of *P. pulmonarius*.

The substrate that gave the best yield was Palm fruit husks (190 g) while the least yield was obtained in banana leaves (56.6 g). Nallathambi and Marimuthu (1993) cultivated *Pleurotus* species on different agrowastes like paddy straw, wheat straw, sorghum stem and they reported maximum yield in paddy straw. Singh (1998) also suggested the use of sugarcane trash for the production of oyster mushroom. This study reveals that the best substrate for cultivation of *P. pulmonarius* was palm fruit husk among the agrowastes tested.



Fig 1: Fully ramified sorghum spawn after 18 days of incubation



Fig 2: Fully ramified maize spawn after 12 days of incubation



Fig 3: Poorly ramified mustard seeds spawn after 20 days of incubation



Fig 4: Fully ramified millet spawn after 16 days of incubation



Fig 5: Ramified wheat spawn after 20 days of incubation

Figure 1-5: Different Mycelia Ramification Rate on Various Grains



Fig 6: Ramified sawdust with fruiting bodies



Fig 9: Ramified banana leaves with fruiting bodies



Fig 7: Ramified palm fruit husks with fruiting bodies



Fig 10: Ramified cocoa leaves with fruiting bodies



Fig 8: Ramified mango leaves with fruiting bodies



Fig 11: Ramified almond leaves with fruiting bodies

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Figure 6-11: Fruiting Bodies on Different Substrates

Substrates	BE (%)	PR (%)
Sawdust	54.0	5.40
Palm fruit husks	63.3	6.33
Almond leaves	34.4	3.44
Cocoa leaves	29.8	2.48
Banana leaves	18.9	1.58
Mango leaves	35.9	3.59

Table 3: The Biological Efficiency (%) and Production Rate (%) of Mushrooms on Different
Substrates

BE = Biological Efficiency, PR = Production Rate

The highest biological efficiency (63.3%) and production rate (6.3%) were obtained in palm fruit husks, followed by sawdust with biological efficiency of 54.0% and production rate of 5.4%, and the least was banana leaves with biological efficiency of 18.9% and production rate of 1.58%. Previous work on using different agrowastes for cultivation of Pleurotus sajor-caju reported maximum biological efficiency on paddy straw plus cotton seeds followed by that obtained on paddy straw, while the lowest biological efficiency was obtained on mango leaves (Ragini et al., 1987). Vyas et al. (2003) reported the Pleurotus flabellatus gave highest biological efficiency on newspaper substrate comparable to paddy straw. The general observation from the literature and in relation with this study is that different species of *Pleurotus* may have affinity for different agrowastes due to the kind of lignocellulose components of the waste or type of lignocellulotic enzymes they produced. Variation in biological efficiency of different substrates may be attributed to low lignolytic and cellulolytic activity of the substrates used (Pathak and Goel., 1988). Matured fruit bodies of P. pulmonarius mushroom on the different substrates is as presented in Figures 6, 7, 8, 9, 10 and 11.

Generally, commercial production of oyster mushroom is largely determined by the availability and utilization of cheap materials especially agricultural waste. In addition, suitability of grains for spawn production determines the prospect of mushroom farming. The grains and substrates used in this study can be considered practically and economically feasible due to their availability in large quantities throughout the year at little or no cost.

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