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Effect of Selected Agro Waste Substrates on the Growth and Yield of Grey Oyster Mushroom (*Pleurotus ostreatus* JACQ.)

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Abstract

A study was conducted on the effects of different local agro waste substrates on the growth and yield of Oyster Mushroom (*Pleurotus ostreatus*). Three sterilized substrates; breadfruit (*Treculia africana*), cassava peel, sterilized sawdust and non-sterilized sawdust were evaluated on mushroom stipe length, pileus diameter, fresh weight, and number of fruiting bodies at four flushes. The substrates significantly ($P \leq 0.05$) affected the number of fruiting bodies, stipe length, fresh weight and pileus diameter of *P. ostreatus* though to varying degrees. The mean number of fruiting bodies of the substrates after four flushes was comparable with bread fruit substrate recording the highest fruiting bodies (14.25) followed by non-sterilized sawdust (12.08), sterilized sawdust (11.16) and cassava peels that gave the least (8.91). Non-sterilized sawdust gave the highest fresh weight (28.31g) followed by breadfruit substrate (19.45 g) whereas sterilized sawdust had the least fresh weight (7.99 g). There was no significant ($p \leq 0.05$) difference in mean pileus diameter of the mushroom with the treatments; non-sterilized cassava peels (3.81 cm), bread fruit substrate (3.73 cm), non-sterilized sawdust (3.23 cm) and sterilized sawdust (3.09).The highest mean stipe length was recorded with cassava peel (2.40 cm) which was not significantly ($p \leq 0.05$) different with those recorded with bread fruit substrate (2.37 cm) and non-sterilized sawdust (2.07 cm). The agro-wastes as substrates enhanced the growth and yield of the Oyster mushroom (*P. ostreatus*) which could be exploited to increase food production in addition to bioremediation and biodegradation of the agricultural residues.

Keywords: P. ostreatus; Agro Wastes; Sterilized; Substrates; Grey Oyster Mushroom

Introduction

Mushrooms are specialized spore-bearing fungi that are saprophytic in feeding with their mycelium in the substrates they feed. They comprise of two major parts, the mycelium and the specialized spore producing structure (sporocarp) [1]. Mushrooms go through two stages, the vegetative stage (hyphae network) that colonize its substrate and the reproductive phase that commences when the hyphae develop primordia that fully mature to form mushroom [2]. Mushrooms produce several lignin, hemicelluloses and cellulose degrading enzymes that enhance the decomposition of lignocellulosic substrates to produce foods rich in protein from various agro-wastes [3].

Substrate is a major part of mushroom cultivation as it forms the nutritional source and the substratum on which the mushroom is tied up [4]. Mushroom-forming fungi are gaining global popularity in both liquid fermentation of industrial effluents and many lingocellulosic wastes such as papers, banana and plantain leaves, and/or peelings, various grasses and leaves, rice and wheat straw combination, wood sawdust and chips, coffee pulp, cotton seed hulls, peanut shells, sunflower seed hulls/stalk, sugarcane bagasses, soybean straw, paddy straw, maize stalks and non--conventional substrates viz, domestic wastes, used tea leaves, bamboo leaves, sawdust of different tree origin, oil palm fruit fibres, bunches and cakes [5,6]. Many of these substrates have been identified as growth media for cultivating oyster mushroom; rice straw, wheat and rice straw, corn cobs, hay, cotton seed hulls, banana leaves, coffee pulps, sawdust, and even paper. Most of these substrates are readily available agricultural wastes containing lignocellulosic substances [7,8] that have been used in the cultivation of several *Pleruotus* species and achieved some level of success in respect to their yield output [9,10]. The ability of mushroom to convert this high percentage of lignocellosic substrates of agricultural byproducts to fruiting bodies increases profitability [11]. Cultivation of edible mushrooms through bioconversion of agro-wastes into valuable human food and some important commercial metabolites has been the best way of managing such wastes and solving environmental pollution-associated challenges and hence affording a cleaner ecosystem [6,8]. Mushroom cultivation is not only the proper way for the management of agro-industrial residues through bioconversion process according to [12] but a profitable agricultural business and source of income generation that can serve as a main support of revenue flow [13], that has improved the socio-economic, nutritional and wellbeing of people in addition to creating employment opportunities with a good environmental impact [14]. Trade in cultivated mushrooms provide a readily available and main source of cash income for all gender and classes of people especially in the tropical less developed countries [15]. Mushroom is used in veterinary practices for treatment of diseases of domestic animals and improve their production [16]. whereas the slurry of spent substrates and compost from mushroom cultivation can be recycled into manure and used as fodder [17].

Pleurotus ostreatus is one of the multipurpose Oyster mushrooms which ispopular for their flavor and sweet smell [18-20] and an excellent source of vitamin B1 (thiamine), B2 (riboflavin), B3 (nicotinic acid), C (ascorbic acid), biotin, devoid of starch and low in calories and other carbohydrates [21-23]. In Nigeria, seasonal hunting of indigenous species of mushroom such as *Pleutotus spp* has over the years contributed greatly as a means of livelihood to some communities [24]. The evaluation of some local agro waste substrates (breadfruit, cassava peel, and *Gmelina arborea* saw dust) on the growth and yield of *P. ostreatus* is presented in this paper.

Materials and Methods

The spawn of *P. ostreatus* were obtained from the Department of Biological Sciences, Federal University of Technology (FUTO) in Owerri, Imo state, Nigeria. *Gmelina arborea* sawdust were collected from Ahiaeke timber market, Breadfruit from Isigate market and cassava peels from Umuariaga, all in Abia State. The study was carried out in the Department of Plant Health Management Laboratory, Michael Okpara University of Agriculture Umudike, Umuahia, Abia State, Nigeria.

The breadfruit seed waste substrate and cassava peels wastes were crushed into pieces and prepared according to modified method of [25]. Each substrate was measured (200 g) for each replicates using a weighing balance and used as substrates. The substrates were pasteurized by

heating up to 100°Cthen steam-sterilized for 2hours after monitoring the temperature with a thermometer and allowed overnight to cool while still in the drum. White sterile transparent buckets that served as experimental units were perforated using a sterile corkborer (5mm diameter) and 200 g of each of the sterilized substrates were separately placed into each of the perforated buckets and were inoculated with the spawn of the fungus.Grain based spawn of *P. ostreatus* (30 g) was aseptically inoculated into each of the treatment. The spawn was sprinkled on the substrates and covered properly then watered every two days to maintain a high relative humidity of between 75-80%. The experiment was laid out in a Completely Randomised Design (CRD) and the treatment replicated three times. The three replicates of each treatment in the sterile buckets were maintained at 30 ± 2°C. The four substrates used were; sterilized *Gmelina arborea* sawdust, unsterilized *Gmelina arborea* sawdust, breadfruit seed wastes, cassava peels. The parameters measured were; the number of fruit bodies, diameter of the pileus, stipe length of the fruit bodies and fresh weight/yield of the fungus after harvest for three flushing periods [26]. The measurements from the various replicates were added and their mean values calculated. The data collected were analyzed using Analysis of Variance (ANOVA) while the different means were separated using Least Significant Difference (LSD) at 5% level of probability.

Results and Discussion

Results

Table 1: Effect of substrates on the number of fruiting bodiesof *Pleurotusostreatus* after four flushes

	Flushes and number of fruiting bodies				
Substrate	1	2	3	4	Mean
Sterilized Bread fruit substrate	16.33	23.30	16.00	1.30	14.25
Sterilized Cassava peel	14.00	14.70	12.30	3.30	8.91
Non-sterilized Sawdust	7.00	6.00	15.70	12.70	12.08
Sterilized Sawdust	14.60	20.70	14.00	3.00	11.16
LSD ($p \leq 0.05$)		3.57	3.63	6.73	7.13

The result of the effect of different substrates on number of fruiting bodies at four flushes of *P. ostreatus* revealed that number of fruiting bodies was highest at the second flush (Table 1). At the first flush, sterilized bread fruit substrate significantly ($P \leq 0.05$) recorded the highest number of fruit bodies (16.33), followed by non-sterilized sawdust (14.00) and sterilized sawdust (7.00) whereas sterilized cassava peel gave the least number of fruiting bodies (5.33). The number of fruiting bodies were not significantly ($P \leq 0.05$) different from each other except with sterilized breadfruit substrate.The substrates recorded significant ($P \leq 0.05$) differences in the number of fruiting bodies in the second and third flushes with sterilized bread fruit substrate recording the highest number of fruiting bodies (23.30 and 16.00), followed by sterilized sawdust substrate (20.70 and 14.00), sterilized cassava peel (14.70 and 12.30) and non-sterilized sawdust (6.00 and 15.70) respectively. In the fourth flush, non-sterilized sawdustsignificantly ($P \leq 0.05$) recorded the highest fruiting bodies (12.70) followed by sterilized cassava peels (3.30),sterilized sawdust (3.00) and sterilized bread fruit substrate gave the least (1.30). The mean number of fruiting bodies of the substrates was comparable after four flushes with the sterilized bread fruit substrate recording the highest fruiting bodies (14.25) followed by non-sterilized sawdust (12.08), sterilized sawdust(11.16) and sterilized cassava peels (8.91) that gave the least.

Table2: Effect of substrate on the fresh weight of *Pleurotus ostreatus* after four flushes

	Flushes and Fresh weight (g)				
Substrates	1	2	3	4	Mean
Sterilized Bread fruit substrate	41.33	32.39	14.23	0.00	19.49
Sterilized Cassava peel	9.33	17.33	8.15	4.75	9.89
Non-sterilized Sawdust	35.95	24.83	27.17	25.30	28.31
Sterilized Sawdust	7.67	11.37	8.90	4.03	7.99
LSD ($p \leq 0.05$)	34.69	36.77	26.48	9.87	26.95

The effect of substrates on fresh weight of *P. ostreatus* (Table 2) showed a reduction on fresh weight of the mushroom across the four flushes assayed except with the sterilized saw dust and non-sterilized saw dust substrates at second and third flushes respectively. The fresh weight of the mushroom was comparable at first and second flushing across the substrates. The sterilized bread fruit substrate gave the highest weight (41.33 g and 32.39 g), followed by non-sterilized sawdust (35.95 g and 24.83 g), sterilized cassava peel substrate (9.33 g and 17.33 g) and sterilized sawdust (7.67 g and 11.37 g) respectively. Though there were no significant ($p \leq 0.05$) differences in the third flush, non-sterilized sawdust gave the highest fresh weight (27.17 g), followed by sterilized bread fruit substrate (14.23 g), sterilized sawdust (8.90 g) and sterilized cassava peel substrate (8.15 g) that had the least. Fresh weight of the mushroom was not significantly ($p \leq 0.05$) different with the treatments in the fourth flush except non-sterilized sawdust substrate that recorded the highest (25.30 g), followed by sterilized cassava peel (4.73 g), sterilized sawdust (4.03 g) and sterilized bread fruit substrate with no fruit body. The mean fresh weight of mushroom with the treatments was comparable ($p \leq 0.05$); non-sterilized sawdust (28.31 g), sterilized bread fruit (19.49 g), sterilized cassava peel (9.89 g), and sterilized sawdust (7.99 g).

There were no significant ($p \leq 0.05$) differences in pileus diameter of *P. ostreatus* with treatments in flush one except with the sterilized sawdust substrate (Table 3). The sterilized bread fruit substrate recorded the highest pileus diameter (6.07 cm), followed by sterilized cassava peel (5.33 cm), non-sterilized sawdust (4.30 cm) and sterilized sawdust that gave the least pileus diameter (2.47). In the second flush, sterilized cassava peel gave the highest pileus diameter (4.47 cm) which was comparable with sterilized bread fruit (3.86 cm), followed by sterilized sawdust (2.95 cm) and non-sterilized sawdust (2.04 cm) that were not significantly ($p \leq 0.05$) different. In the third flush, sterilized sawdust substrate gave the highest pileus diameter (4.66 cm) which was not significantly ($p \leq 0.05$) different with sterilized cassava peel substrate (3.89 cm), followed by non-sterilized sawdust (2.20 cm) and sterilized bread fruit substrate that recorded the lowest (1.27 cm). The non-sterilized sawdust substrate significantly ($p \leq 0.05$) recorded the highest pileus diameter (4.36 cm)in the fourth flush, followed by the sterilized sawdust substrate (2.29 cm), sterilized cassava peel (1.55 cm) and sterilized bread fruit substrate that had no mushroom. There was no significant ($p \leq 0.05$) difference in the mean pileus diameter of the mushroom with the treatments; sterilized cassava peel (3.81 cm), sterilized bread fruit substrate (3.73 cm),non-sterilized sawdust (3.23 cm) and sterilized sawdust (3.09 cm).

Table 3: Effect of substrates on the Pileus diameter of *Pleurotus ostreatus* at four flushes

	Pileus diameter (cm)/Flush				
Substrates	1	2	3	4	Mean
Sterilized Bread fruit substrate	6.07	3.86	1.27	0.00	3.73

Sterilized Cassava peel	5.33	4.47	3.89	1.55	3.81
Non-sterilized Sawdust	4.30	2.04	2.20	4.36	3.23
Sterilized Sawdust	2.47	2.95	4.66	2.29	3.09
LSD ($p \leq 0.05$)	1.73	1.30	1.08	1.09	1.30

There were no significant ($p \leq 0.05$) differences in pileus diameter of *P. ostreatus* with treatments in flush one except with the sterilized sawdust substrate (Table 3). The sterilized bread fruit substrate recorded the highestpileus diameter (6.07 cm), followed by sterilized cassava peel (5.33 cm), non-sterilized sawdust (4.30 cm) and sterilized sawdust that gave the least pileus diameter (2.47). In the second flush, sterilized cassava peel gave the highest pileus diameter (4.47 cm) which was comparable with sterilized bread fruit (3.86 cm),followed by sterilized sawdust (2.95 cm) and non-sterilized sawdust (2.04 cm) that were not significantly ($p \leq 0.05$) different. In the third flush, sterilized sawdust substrate gave the highest pileus diameter (4.66 cm) which was not significantly ($p \leq 0.05$) different with sterilized cassava peel substrate (3.89 cm), followed by non-sterilized sawdust (2.20 cm) and sterilized bread fruit substrate that recorded the lowest (1.27 cm). The non-sterilized sawdust substrate significantly ($p \leq 0.05$) recorded the highest pileus diameter (4.36 cm)in the fourth flush, followed by the sterilized sawdust substrate (2.29 cm), sterilized cassava peel (1.55 cm) and sterilized bread fruit substrate that had no mushroom. There was no significant ($p \leq 0.05$) difference in the mean pileus diameter of the mushroom with the treatments; sterilized cassava peel (3.81 cm), sterilized bread fruit substrate (3.73 cm),non-sterilized sawdust (3.23 cm) and sterilized sawdust (3.09 cm).

Table 4: Effect of substrates on the stipe length of *Pleurotus ostreatus* after four flushes

	Flush and stipe length (cm)				
Substrates	1	2	3	4	Mean
Sterilized Bread fruit substrate	2.72	2.30	2.61	0.00	2.37
Sterilized Cassava peel	2.55	3.67	2.24	0.98	2.40
Non-sterilized Sawdust	1.00	1.41	1.66	2.66	2.07
Sterilized Sawdust	0.67	2.04	2.30	0.67	1.50
LSD ($p \leq 0.05$)		1.39	1.02	0.56	0.09

The result in Table 4 showed reduction in the mushroom stipe length across the four flushes of respective treatments. There was no significant ($p \leq 0.05$) difference in first flush except with the sterilized sawdust treatment that recorded the least stipe length (1.00 cm). The sterilized cassava peel substrate gave the highest stipe length (2.72 cm), followed by non-sterilized sawdust (2.55 cm) and sterilized bread fruit substrate (2.21 cm). Significant ($p \leq 0.05$) differences were observed in the second flush with sterilized cassava peel substrate recording the highest stipe length (3.67 cm), followed by sterilized bread fruit substrate (2.30 cm), sterilized sawdust (2.04 cm) and the least stipe length was recorded with non-sterilized sawdust (1.41 cm). The stipe length was comparable ($p \leq 0.05$) in the third flush with sterilized bread fruit substrate recording the highest (2.61cm) followed by sterilized sawdust (2.30 cm), cassava peel (2.24 cm) and non-sterilized sawdust (1.66 cm). There was no significant($p \leq 0.05$) difference with the treatments in the fourth flush except with non-sterilized sawdust that recorded the highest stipe length (2.66 cm), followed by sterilized cassava peel (0.98 cm), and sterilized sawdust (0.67 cm) whereas sterilized bread fruit substrate had no mushroom growth. The highest mean stipe length was recorded with sterilized cassava peel (2.40 cm) which was not significantly ($p \leq 0.05$) different with those recorded with sterilized bread fruit substrate (2.37 cm) and non-sterilized sawdust

(2.07 cm).

Discussion

Mushroom cultivation requires substrate as nutritional source and the four substrates evaluated; breadfruit, cassava peel, sawdust and non-sterilized sawdust affected significantly ($P \leq 0.05$) the number of fruiting bodies, stipe length, fresh weight and pileus diameter of *P. ostreatus* though to varying degrees with breadfruit substrate as the best. Substrate materials identified as good sources for growing oyster mushroom include; rice straw, coffee pulps, sawdust, paper, bread fruit chaff, cassava peels etc. [27]. Sawdust ranks as one of the best for the cultivation of oyster mushrooms among agricultural wastes used as growth media for cultivating oyster mushroom [6-10]. In this study, breadfruit substrate was the best in the growth and yield of the oyster mushroom probably due to the level of the lignocelluloses materials available in the substrate that supported the mushroom development and growth [6]. The variation in growth, yield and number of flushes of the mushroom recorded with respective substrates in this study could be attributed to the amount of N:C ratio in the substrates and their level of degradation after each flush which in turn affected the quantity and morphological characteristics of the mushroom produced. Flushes from different substrates showed significant differences in their pileus size, one of the contributing characteristics of mushroom yield [10]. There were also disparities in fresh weight of the mushroom at different flushes suggesting that availability of nutrients in the substrates at a particular time influenced the yield of the mushroom which increased as the nutrient content of the substrates decreased [6]. [28,29] in their study observed that the number of fruit bodies produced per flush with different substrates decreased from flush to flush reported and that total mushroom yield did not correspond with the mycelia colonization. The pileus size (area) of *P. ostreatus* was larger in the breadfruit substrate at the first flush in this study. whereas [30] recorded bigger pileus diameter of *P. ostreatus*at the first flush on sterilized sawdust. Sterilized sawdust substrate in this study gave the highest pileus size in the third and fourth flushes with fewer mushroom. The increase in pileus size at the third flush with fewer mushroom could be as a result of the decrease in the nutrient content of the substrate which inversely reduced the quantity of mushroom produced with increased pileus size [31].

Bioconversion of agro-wastes by oyster mushrooms (*Pleurotus* spp.) into valuable human food [21-23] has been suggested as one of the best ways of managing ago-wastes and reducing environmental pollution associated with such challenges [6,8]. Mushroom cultivation using agro-wastes is not only the proper way for the management of agro-industrial residues through bioconversion process [12] but aprofitable agricultural business and source of income generation that could improve the socio-economic, nutritional and wellbeing of people [13,14]. The slurry of spent substrates and compost from mushroom cultivation can be recycled into manure and used as fodder [17]. In Nigeria, seasonal hunting of indigenous species of mushroom such as *Pleutotus spp* has contributed greatly as a means of livelihood [24]. Exploitation of these agro-wastes as substrates in mushroom cultivation will not only increase food production but also reduce environmental pollution associated with agricultural residues.

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