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Effect of Different Nitrogen Sources On Yield And Biological Efficiency Of Oyster Mushroom (*Pleurotus Ostreatus)* Cultivated On Waste Paper

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This study was carried out to investigate the effect of different nitrogen source such as Wheat bran, Urea, and Moringa leaves on the yield of Oyster mushroom and also to estimate the biological efficiency of Oyster mushroom cultivated on the substrate. Grain spawn was prepared using sorghum. Wheat bran, Urea and Moringa leaves were added to the treated waste paper in the following percentage, 2.5 %, 5 %, 7.5 %, 10 % and 12.5 %. The mycelium run, primordia initiation, pilus diameter and stripe length were determined. Also, the average total yield and biological efficiency analyzed using standard methods. The fastest mycelium run (8.80 days), *primordia initiation* (11.80 days), *and highest pilus diameter* (4.30 cm) were observed in *7.5 % moringa, and unsupplemented substrate gave the slowest mycelium run. The stipe length was lowest in substrate supplemented with 12.5 % Urea (1.52 cm), and 5 % moringa gave the longest value* (9.64 *cm). The highest yield (240.00g) and biological efficiency (80.13%) were obtained in 7.5% wheat bran, and the lowest output (90.00g) and biological efficiency (32.60%) observed in 12.5 % urea supplementation. The result predicts the commercial production of good oyster mushroom on waste paper substrate supplemented with 7.5% wheat bran and 7.5% moringa leaves.*

Keywords: Biological efficiency, Oyster mushroom, *Pleurotus ostreatus*, Waste paper, Wheat bran, Moringa leaves.

EFFECT OF DIFFERENT NITROGEN SOURCES ON YIELD AND BIOLOGICAL EFFICIENCY OF OYSTER MUSHROOM (*Pleurotus ostreatus)* CULTIVATED ON WASTE PAPER

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ABSTRACT

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Keywords: biological efficiency, Oyster mushroom, *Pleurotus ostreatus*, waste paper, wheat bran, moringa leaves.

INTRODUCTION

Mushrooms are edible fungi belonging to the genus *Pleurotus* under the class Basidiomycetes, which are fleshy, spore-bearing reproductive structures were grown on organic substrates. (Etich, Nyamangyoku, Rono, Niyokuri and Izamuhaye, 2013). The cultivation of fungi is a current innovation, unlike the farming of higher plants, which started in prehistorical times (Sofi, Ahmad and Khan, 2014). Mushroom is a suitable fungal organism for producing protein-rich, vitamins and minerals food from various composted agro-wastes and popularly called vegetarian meat. Mushroom proteins are considered to be intermediate between that of animals and vegetables (Syed, Kadam, Mane, Patil and Baig, 2009) as it contains all the nine essential amino acids required for the human body.
Oyster mushroom (*Pleurotus ostreatus*) have been cultivated worldwide because of their taste and low maintenance technology. Various substrates such as rice straw, coffee pulps, sawdust and even paper have already been identified as suitable for Oyster Mushroom's cultivation. Most of the substrates are low-value lignocellulosic wastes primarily derived from agricultural practices or the agro-industry (Buswell et al., 1996).

Various factors like substrate source, substrate quality, spawn stripe, compost and complement affect the growth and performance of Oyster mushrooms (Royse, Rhodes, Ohga and Sanchez, 2004, Jafarpour, Jalali, Dehdashtizadeh and Eghbalsaied, 2010). High protein content and nitrogen source have been reported to be effective in shortening the growth period and increasing both yield and biological efficiency (Peksen and Yakupoghu, 2009; Adebayo, Omolara, and Toyin, 2009; Fanadzo, Zireva, Dube, and Mashingaidze, 2010; Jafarpour et al., 2010).
This study's objective was to evaluate the effect of different nitrogen sources like wheat bran, Urea, and Moringa leaves on the yield and the biological efficiency of Oyster mushroom cultivated on waste paper substrate.

MATERIALS AND METHOD

The entirely mystified Oyster Mushroom spawn grain was collected from the biotechnology department of the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria.

Spawn Grain Preparation

Spawn is referred to as the vegetative mycelium of the fungus, which is grown on cereal grains. The sorghum treatment was done according to the method used by Jawad, Muhammad, Waqas, Chaudhry and Jamil (2013).
Untreated sorghum seeds weighing 4kg was soaked overnight in a satisfactory amount of water. The grains were washed and drained three times to remove the dead and floating seeds with water. The following day, the soaked sorghum seeds

were cooked at 96ºC using a cooker for 30minutes and after which the excess water was removed and allowed to cool on a tilted platform. The sorghum grains were now half-filled in bottles, and 2g of $CaCO_3$ and a tablet of vitamin B complex were added to each bottle and then plugged by cotton. The half-filled bottles autoclaved at 121°C and pressure 15 psi for 20 minutes, then left overnight and subsequently inoculated with the mother spawn grain culture. After 3-4 days of inoculation, fungal mycelium started spreading on the grains. The mycelium is a white net web-like in appearance. The bottles were nearly half-filled in 10-12 days, and in 18-21 days, these were filled with white mycelia growth.

Substrate Preparation

Waste paper was collected and cut in 0.5cm width with different lengths.10L of water was taken in a bucket, and 500ml of bleaching solutions (Hypo) were added to this water. Newspaper was added to this bleached water and allowed to hydrate for about an hour, and then it was stirred. After an hour, papers were taken out and were separated under shade to remove the extra water. Lime was added to 1% off the paper's total weight to neutralize the substrate's acidity and mixed thoroughly together until no lime was visible. Then, sugar was added equal to 1% of the mixture to temporarily provide glucose to the mycelia while the cellulose and lignin are being converted into useful forms of carbohydrates. The substrate was then spread on the floor for evaporation of excess moisture, and the squeeze method used to determine the moisture content. The Carbon source (waste paper) was supplemented with each Nitrogen source (wheat bran, urea, and air-dried moringa leaves) at intervals of 2.5% separately in five replicas, as depicted in Table 1**.** Having done that, 300g of the substrate was packed into a well-labelled polypropylene bag, plugged with cotton wool and covered with aluminum foil and tied with a rubber band. The bags were then pasteurized using an oil drum. The pressure and thermometer gauge was attached to the lid of the drum. The bags were loaded into the drum and were pasteurized for about 1hour 30 minutes. The bags were allowed to cool before inoculating with grain spawn.

Table 1: Composition of Mixture Carbon Sources and Nitrogen Sources

Treatments	**Constituents**		
	Carbon sources	**Nitrogen sources**	
0 % supplements	100 % waste paper		Control
2.5 % Urea	97.5 % waste paper	2.5 % Urea	U_1
5. 0 % Urea	95.0 % waste paper	5. 0 % Urea	U_2
7.5 % Urea	92.5 % waste paper	7.5 % Urea	U_3
10.0% Urea	90.0 % waste paper	10.0 % Urea	U_4
12.5 % Urea	87.5 % waste paper	12.5 % Urea	U_5
2.5 % Wheat Bran	97.5 % waste paper	2.5 % Wheat Bran	WB_1
5. 0 % Wheat Bran	95.0 % waste paper	5. 0 % Wheat Bran	WB_2
7.5 % Wheat Bran	92.5 % waste paper	7.5 % Wheat Bran	WB_3
19.5 % Wheat Bran	90.5 % waste paper	19.5 % Wheat Bran	WB_4
12.5 % Wheat Bran	87.5 % waste paper	12.5 % Wheat Bran	WB_5
2.5 % Moringa	97.5 % waste paper	2.5 % Moringa	M_1
5. 0 % Moringa	95.0 % waste paper	5. 0 % Moringa	M_2
7.5 % Moringa	92.5 % waste paper	7.5 % Moringa	M_3
19.5 % Moringa	90.5 % waste paper	19.5 % Moringa	M_4
12.5 % Moringa	87.5 % waste paper	12.5 % Moringa	M_5

Inoculation and Incubation

Inoculation was done under sterile condition. 5g of fully colonized spawn grain added to the bags and where incubated in a dark room at room temperature with relative humidity between 70-80% by sprinkling water on the floor for ramification of mushroom mycelia

Cropping and Watering

Cropping and watering were done according to Jawad et al. (2013) with modification. After completion of spawn running, the temperature of the growing room maintained at room temperature. The fruiting body started to appear as soon as the substrate was fully impregnated. Humidity kept between 80-90% by sprinkling water on the floor, and the moisture requirement of bags accomplished by sprinkling water on them. The mushrooms were harvested at maximum development.

Morphological properties

The total days taken for the colonization, primordial formation and harvest recorded. The weight of fruit bodies recorded. The total weight of all the fruiting bodies harvested from two flushes was measured separately and calculated as total yield. Biological Efficiency (B.E.) determined as the percentage ratio of the fresh weight of harvested mushroom over the substrate's dry weight (Pokhrel and Ohga 2007).

Data Analysis

One-way Analysis of variance (ANOVA) employed to analyze data collected, and significant differences were separated by Duncan Multiple Range Test using the Statistical Package for the social sciences for windows (SPSS2007)

RESULTS AND DISCUSSION

Table 2: Morphological Parameters of P. *ostreatus* Cultivated on Different Concentration of Different Supplements

	SR (Days)	PH (Days)	PD (cm)	SL(cm)	T. Y(g)	B.E (%)
Control	27.20^{f}	32.20^{f}	2.4 4^{cde}	4.6 8^{bcde}	158.00^{bcd}	55.33^{bcd}
U1	27.40^{f}	32.00^{fg}	1.6 4^{bcd}	3.2 4^{bcd}	148.00^{bc}	49.33^{bc}
U2	25.60^{f}	30.60^{efg}	2.2 0^{bcde}	4.3 0^{bcde}	182.00^{bcd}	60.67^{bcd}
U3	20.40^{def}	25.40^{cdefg}	1.3 6^{abc}	3.0 0^{abc}	170.00^{bcd}	56.67^{bcd}
U4	20.60^{def}	25.20^{cdefg}	1.3 4^{abc}	3.1 8^{bcd}	203.60^{cd}	67.87^{cd}
U5	15.80^{cd}	18.80^{bc}	0.7 6^{ab}	1.5 2^{ab}	96.00^{b}	32.60^{b}
WB1	25.00^{ef}	29.60^{defg}	2.2 0^{bcde}	4.6 0^{bcde}	222.00^{cd}	74.00^{cd}
WB2	22.80^{def}	27.60^{cdefg}	3.7 4^{ef}	7.4 8^{ef}	237.00^{cd}	79.00^{cd}
WB3	20.40^{def}	24.80^{cdefg}	3.5 2^{ef}	7.1 8^{ef}	240.20^{d}	80.13^{d}
WB4	17.20^{d}	21.60^{cde}	3.0 8^{def}	6.0 0^{cde}	237.00^{cd}	78.67^{cd}
WB5	10.20^{bc}	13.00^{b}	2.4 0^{cde}	4.9 6^{cde}	149.00^{bc}	49.73^{bc}
M1	17.60^{de}	22.40^{cdef}	2.7 0^{cde}	5.7 4^{cde}	232.00^{cd}	77.33^{cd}
M2	18.40^{d}	23.20^{cdefg}	4.3 0^{f}	9.6 4^{f}	234.80^{cd}	78.27^{cd}
M3	8.80^{b}	11.80^{b}	3.0 0^{def}	6.4 0de	149.00^{bc}	49.67^{bc}
M4	0.00^{a}	0.00^{a}	0.0 0^{a}	0.0 0^{a}	0.00^{a}	0.00^{a}
M5	0.00^{a}	0.00^{a}	0.0 0^{a}	0.0 0^{a}	0.00^{a}	0.00^{a}
SME	1.06	1.26	0.1 8	0.3 7	10.37	3.47

SR= spawn running, PH= Pinhead, PD= Pileus Diameter, SL = Stipe Length, TY= Total Yield, B.E = Biological Efficiency

Spawn run rate was observed for all treatment. The 7.5 % Moringa supplementation gave the fastest mycelium run (8.80 days), then wheat bran 12.5 % (10.20 days) followed by Urea 12.5 % (15.80 days). 5.0 % Moringa and 10.0 % Wheat bran are significantly ($P< 0.05$) similar the lowest spawn run observed in substrate supplemented with 5.0 % urea (25.60 days) which was statically similar to substrate supplemented with 2.5 % of Urea (27.40 days) and control (27.20 days). This result justified the statement made by Mateus et al. (2012) that supplementation of the substrates with various sources of organic nitrogen, such as wheat bran, rice bran, maize wastewater, soya cake powder and rice, has a significant effect on the spread of mycelial growth. Fungi can easily use organic sources of nitrogen because the absorption of these molecules is more energetically efficient than synthesizing the molecules, which allow the fungi to obtain more energy for mycelial growth and mushroom formation.

The time for stimulation of primordial initiation ranged from 3-5 days. The lowest observed in the unsupplemented substrate (32.20 days). The shortest time for primordial initiation was showed in substrate supplemented with 7.5 % moringa leaves and significantly ($P< 0.05$) similar to primordial initiation shown in 12.5 % wheat bran. This result disagreed with Patra and Pani (1995), who found that Oyster mushroom took 4-8days for the initiation of fruiting bodies. The difference among the findings may be due to the cultural environment, substrates or varieties. This study's result is similar to the observation of Jawad et al. (2013) that P. *ostreatus* took a minimum number of days, 3.73 ± 0.32.

The highest pilus diameter is shown in substrate supplemented with 5.0 % moringa leaves and statically different from the rest of the treatments. Substrate supplemented with Wheat bran 7.5% and 5.0 % is numerically different (3.52 cm and 3.74 cm respectively) but statically similar. The lowest pilus diameter observed in substrate supplemented with 12.5% urea (0.76cm) which was followed by 10% urea (1.34cm) and 7.5% urea (1.36) which are significantly similar.

The stipe length of the oyster mushroom was measured in centimeter for all the nitrogen source. The stipe length was lowest in substrate supplemented with 12.5 % of Urea (1.52 cm) followed by substrate supplement with 7.5 % urea (3.00 cm) which is significantly different ($p < 0.05$). Substrate supplemented with 10 % and 2.5 % urea is not significantly different but are numerically distinct with 3.18 cm and 3.24 cm, respectively. The sizes of the mushroom stipe and pileus may be affected by the substrate types. Fasidi, Kadiri, Jonathan, Adenipekun and Kuforiji (2008) reported that very good mushroom pilei were produced on substrates with rice bran (containing 1.3 % N). Also, the mushroom size depends on substrates that were poor in cellulose, hemicellulose, lignin, which constitute physical barrier and are challenging to be broken down without the presence of lignin-degrading enzymes (Jonathan and Adeoyo, 2011). Good growths on agricultural substrates have also been linked with suitable nutrients and adequate environmental conditions (Gbolagade, Fasidi, Ajayi and Sobowale, 2006).

The mushroom's total yield weight observed for the treatments with 12.5 % urea supplementation showed the lowest yield with 90.00 g, and 7.5 % moringa (149.00 g), 12.5 % wheat bran and 2.5 % urea (148.00 g) are statically similar but numerically different. The highest yield was shown by 7.5% wheat bran (240.00 g) which is significantly different ($p<0.05$) from the remaining treatments. Baysal, Peker, Yalinkilic and Temiz (2003) found the highest P. *ostreatus* in the substrate composed of 20% rice husk in weight. Amin et al. (2007) found the highest yield, 247.3 g /packet. He also found that the trend of economic yield corresponds with different supplements at a different level. Oseni, Dube, Wahome, Masarirambi and Earnshaw, (2012) reported that wheat bran supplementation of fermented sawdust substrate above 15% had significant and depressing effects on total yield and B.E. of oyster mushrooms. This decrease in output and B.E. could probably be explained by the fact that 20 % wheat bran might have generated a lot of heat, resulting in the overheating of the substrate, which subsequently affected the mushroom growth negatively, thereby leading to low yield.

The highest biological efficiency observed in 7.5 % wheat bran (80.13 %) followed by 5 % moringa (78.27 %), while the lowest biological efficiency observed in 12.5% urea supplementation of 32.60 g. The biological efficiency was high with wheat bran than with any other supplements used, and this can be attributed to the high moisture retention of the supplement added. Ozcelik and Peksen (2007) has reported that B.E increase can be due to the increased availability of water in substrate added with rice bran since the addition of rice bran decreases the granulometry of substrate, which improve the moisture retention. For mushroom formation, the fungus requires a considerable amount of water due to the high-water content in mushrooms (Tewari, 1986).

An increase in the nitrogen ration on supplement causes contamination. All bags supplemented with Moringa between 10 % to 12.5 % are contaminated and may be due to high protein content in moringa leave than any other supplement used. Though mushroom requires C/N high nitrogen content encouraged the growth of bacteria on the substrate, which affect the spread of mycelium. Also, the heat produced during the decomposition of moringa leaves can lead to hindrance of mycelium growth. All bags supplemented with 10 % and 12.5 % moringa level did not allow the substrate's spread of mycelia. The higher temperature was observed due to the rate of decomposition of this supplement. This explains the report that supplementation causes a rise in substrate temperature, possibly due to faster metabolic activities triggered by extra nitrogen. Therefore, supplements should be cautiously used because excessive bed temperature (more than 35 □C) may kill the mycelium. Higher supplement doses gave even higher temperatures, which were harmful and attracted the growth of competing bacteria.

CONCLUSION AND RECOMMENDATION

This study has revealed that a certain percentage of nitrogen supplementation is required for the oyster mushroom's optimum yield. Moringa can be used as a supplement for mushroom cultivation because its high protein contents will serve as a good nitrogen source for the spread of mycelium growth. It may also add to the medicinal value and nutritional value of the mushroom grown on it. Moringa leaves recommended as a nitrogen source to use for the cultivation of oyster mushroom because it is readily available and cheap so that small scale farmer can use it. Although wheat bran has the highest yield, it is not readily available and can be very expensive.

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