EFFECTS OF NITROGEN SOURCES ON GROWTH PARAMETERS AND PROXIMATE COMPOSITION OF INDIAN OYSTER MUSHROOM (*PLEUROTUS PULMONARIUS*) GROWN ON SAWDUST AND PALM PRESS FIBRE

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ABSTRACT

This study assessed the effect of different nitrogen source inclusion (such as rice husk and rabbit urine) affected the growth performance and proximate compositions of Indian Oyster mushroom. 5% of rice husk and 5% of rabbit urine were added to the prepared substrate composition (sawdust and palm press fibre). The mycelium run, pilus diameter, stripe length, the average total yield, biological efficiency and the proximate composition were analyzed using standard methods. The fastest mycelium run (6.5 days) was observed in 5 % rice husk supplementation. The highest pilus diameter (41.5 cm) and the highest stipe length (18.5 cm) were observed in 5% rice husk supplementation. The highest yield (202.0 g) and biological efficiency (20.2%) were obtained in 5 % rabbit urine supplement and the lowest output (52.0 g) and biological efficiency (5.2%) were observed in 5 % rabbit urine supplementation. The highest moisture (15.61%), crude fibre (8.29%), total ash (9.99%) was observed in 5 % rice husk supplementation and the highest crude fat (3.98%), crude protein (38.15%) and energy (300.04 K/cal) were observed in 5 % rabbit urine supplementation. The result predicts the suitability of combination of palm press fibre and sawdust, supplemented with either 5% rice husk or 5% rabbit urine for commercial cultivation of good oyster mushroom.

Keywords: Biological efficiency; *Pleurotus pulmonarius*; palm press fibre; rice husk; rabbit urine.

INTRODUCTION

Mushrooms is rich in proteins, vitamins (thiamine, riboflavin, folic acid, and niacin) and minerals (Phosphorus, Calcium, Iron, Potassium and Sodium), it also possesses all the essential amino acids compulsory for human being (Reis, Barros, Martins, & Ferreira, 2013). The percent proteins fall in-between that of animals and vegetables hence usually described as the 'vegetarian's meat'. Due to its ability to degrade ligno-cellulosic materials, mushroom grow naturally on diverse wastes called substrates, which are usually by products from domestic, agricultural and industrial activities (Stamets, 2003; Okwulehie, Nwosu and Okoroafor, 2007; Okwulehie and Okwujiako, 2008).

Pleurotus pulmonarius is an edible mushroom (Alexopolous, Mims and Blackwell, 1996; Jonathan, Okon, Oyelakin and Oluranti, 2012) often identified as Indian Oyster and like other *Pleurotus* species, they are economically suitable for bio-conversion of lignocellulosic wastes to proteinous food (Onuoha, Ukaular and Onuoha, 2009).

Pleurotus mushroom are readily cultivated using different wastes of plant origin for example sawdust, paddy straw, bagasse, cornstalks, waste cotton, banana stalks and leaves with cost-friendly process and material requirements (Chang and Miles, 2003). Sawdust is the usually used as as substrate for cultivating *Pleurotus pulmonarius* in Nigeria (Onuoha, Ukaular and Onuoha, 2009). The obtainability, coupled with contamination of the environmental by oil palm waste necessitated the trial of palm press fibre for cultivation of *P. pulmonarius*, because it is a lingo-cellulosic material with a high fiber content (Liasu, Adeeyo, Olaosun and Oyedoyin, 2015).

A large amount carbon and less nitrogen source such as rice and wheat straw. Cotton seed, hills corn cob, sugar cane baggase, sawdust, waste paper, leaves etc. are required for rapid growth of oyster mushroom. Rice husks are waste product generated during rice milling (*Oryza sativa*) in many rice growing localities (Frimpong, Obodai, Dzomeku and Apertorgbor, 2011). Rabbit's urine is one of the by-products of rabbit metabolism that are not utilized. It has high level of nitrogen. Rabbit urine contains ammonia (NH_3) a colourless gas that is lighter than air and possesses strong odour. (Wandita, Darmawan, Triatmojo, & Fitriyanto, 2016).

In this present study, two obtainable carbon-based wastes; Gmelina Sawdust and Palm Press Fibre and two nitrogen sources; Rice Husk and Rabbit Urine were used to cultivate *P. pulmonarius*. Evaluation of the impact of these carbon-based wastes and supplements (i.e., Nitrogen sources), on yield, biological efficiency and proximate compositions of mushroom were done.

MATERIALS AND METHOD

Materials

The entirely mystified Oyster Mushroom spawn grain was collected from the biotechnology department of the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria. Sawdust of melina tree and palm press fibre were collected in Ilaro, Yewa South LGA. Ogun State, Nigeria

Substrate Preparation and Bagging

The substrates were prepared according to the method used by Onyeka, Udeogu, Umelo and Okehie, (2018). The individual substrate combinations as presented in Table 1, was soaked in water for 24 hrs and the moisture content reduced to about 65 %, by allowing the water to drain off on slanted cement floor. The substrates were allowed to ferment for 3 days under polythene sheets cover, then the nitrogen sources (rice husk and rabbit urine) and calcium carbonate were mixed with the substrates until no lumps of were found or visible. The prepared substrates were filled into Polypropylene bags of 5.75" x 10" size and compressed to make bag log weighing 0.5 kg and one-inch polyvinyl chloride (PVC) necks were inserted in their mouths, and plugged with water absorbing cotton wool, wrapped with aluminum foil to inhibit the entry of moisture during pasteurization for 3 – 4 hrs.

Table 3.1: Percentage ratio of substrates in sample mixture

Sample	Substrates' ratio	Substrate s' Composition	Quantity (%)
A	100 : 0	SD : RH : $CaCO_3$	94 : 5 : 1
B	75 : 25	SD : PPF : RH : $CaCO_3$	70.5 : 23.5 : 5 : 1
C	50 : 50	SD : PPF : RH : $CaCO_3$	47 : 47 : 5 : 1
D	25 : 75	SD: PPF : RH : $CaCO_3$	23.5 : 70.5 : 5 : 1
E	100 : 0	PPF : RH : $CaCO_3$	94 : 5 : 1
A_U	100	SD : RU : $CaCO_3$	94 : 5 : 1
B_U	75 : 25	SD : PPF : RU : $CaCO_3$	70.5 : 23.5 : 5 : 1
C_U	50 : 50	SD : PPF : RU : $CaCO_3$	47 : 47 : 5 : 1
D_U	25 : 75	SD: PPF : RU : $CaCO_3$	23.5 : 70.5 : 5 : 1
E_U	100	PPF : RU : $CaCO_3$	94 : 5 : 1

SD; Sawdust, PPF; Palm Press Fibre, RH; Rice husk, RU; Rabbit Urine, $CaCO_3$; calcium carbonate, A; 100 SD : 0 PPF with RH, B; 75 SD : 25 PPF with RH, C; 50 SD : 50 PPF with RH, D; 25 SD : 7 PPF with RH, E; 100 PPF : 0 with RH, A_U; 100 SD : 0 PPF with RU, B_U; 75 SD : 25 PPF with RU, C_U; 50 SD : 50 PPF with RU, D_U; 25 SD : 7 PPF with RU, E_U; 100 PPF : 0 with RU.

Inoculation and Incubation

The pasteurised substrate bags (0.5 kg) were inoculated with 50 g spawn grain at room temperature under aseptic condition. the inoculated bags were incubated in a dark room at room temperature, and the relative humidity kept between 70-80 % by wetting the floor with water (Onyeka and Okehie, 2018).

Watering and Harvesting

Watering was done according to Jawad et al. (2013) with modification. The spawn run was observed as the bags are impregnated with white mycelium, and after the bags have fully colonized, they were moved to the fruiting room. Once the tiny pinhead appears on the surface of the substrates, the top of the bags was opened to produce fruiting, while the room humidity and the bags' moisture requirement were kept by spraying the floor and the bags with water (Sarkar, Hossain, Sultana and Mian 2007). Harvest the fruit at the moment the mushroom pileus edge began turning upwards, and kept for Proximate analysis.

Proximate Analysis

The proximate analysis was done using official methods (AOAC, 2005). Carbohydrate content was determined by difference [100 – (sum of moisture, protein, fat, ash, fibre)]. The total energy value was calculated according to the following equation (Barros et al., 2007), as shown below:

Energy value (kcal/100 g) = [(4 x Crude protein) + (9 x Crude fibre) + (4 x Nitrogen Free Extract)]

Yield and biological efficiency

The total spawn run time (days), stipe length, pileus diameter and weight of fruit bodies harvested from four flushes were determined separately, and the total yield calculated. 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).

Statistical Analysis

The data obtained from the experiment were subjected to One-way Analysis of variance (ANOVA), and significant differences were separated by Duncan Multiple Range Test using the Statistical Package for the social sciences for windows (SPSS, 2007)

RESULTS AND DISCUSSION

Table 2: The spawn run, pileus diameter and stripe length of *Pleurotus pulmonarius* mushroom

Sample	Spawn Run (days)	Pileus Diameter (mm)	Stripe Length (mm)
A	6.50±.50[a]	16.00±2.00[a]	8.00±1.00[a]
B	7.50±.50[b]	18.00±3.00[a]	8.50±1.00[a]
C	7.67±.29[b]	29.00±2.00[b]	14.50±2.00[b,c]
D	7.83±.29[b]	33.00±3.00[b,c,d]	16.00±3.00[c,d]
E	8.33±.29[b,c]	36.30±2.00[d]	18.50±1.00[d]
A_U	9.00±1.00[c,d]	28.60±2.65[b]	12.00±1.00[b]
B_U	9.33±.58[d,e]	29.40±2.00[b,c]	13.00±2.00[b]
C_U	9.50±.50[d,e]	33.50±2.00[c,d]	13.50±1.00[b,c]
D_U	10.00±.00[e]	37.90±3.00[d]	13.50±1.00[b,c]
E_U	10.00±.00[e]	41.50±2.00[e]	17.30±1.00[d]

Values are means ± SD, the means reported with the same superscript in each column indicated no significant variation ($p < 0.05$).

A; 100 SD : 0 PPF with RH, B; 75 SD : 25 PPF with RH, C; 50 SD : 50 PPF with RH, D; 25 SD : 7 PPF with RH, E; 100 PPF : 0 with RH, A_U; 100 SD : 0 PPF with RU, B_U; 75 SD : 25 PPF with RU, C_U; 50 SD : 50 PPF with RU, D_U; 25 SD : 7 PPF with RU, E_U; 100 PPF : 0 with RU.

Spawn run rate measured for all substrate composition as depicted in Table 2, revealed that substrate A gave the fastest mycelium run (6.50 days), while the slowest mycelium run was observed in Substrate D_u and E_u(10.00 days). Hence rice husk supported faster significant (P<0.05) mycelium run than rabbit urine which corroborated the findings of Saxena and Rai, (1992) and Salmones, (1999) that the apparent attribute, high permeability and the air circulation in the rice husk are responsible for fast colonization of the substrate. Addition of palm pressed fiber with sawdust retarded the mycelium run, but the results of this study is in contrast with Onuoha, et al, (2009) who reported that oil palm fiber alone did not support mycelium growth thus did not produce any fruit body.

The results in Table 2 shows that sole palm pressed fiber (PPF) supplemented with rabbit urine gave the highest pileus diameter (41.50 mm), while sawdust supplemented with rice husk produced mushroom with smallest pileus diameter (16.00 mm). The mushroom pileus diameter is the favored quality index of mushroom, and European Community Commission (ECC) Regulation tagged mushrooms pileus diameter (15-45 mmm) as small; medium (30-65 mm); and large when the size is greater than 70 mm (ECC, 2002). Therefore, according to the above EC Regulation, the average pileus diameter of all the mushrooms cultivated in this study, falls within the small category. The stipe length of the cultivated Indian oyster varied significantly ($p < 0.05$) with the shortest stipe length observed in substrate A (8.00 mm) and the longest in substrate E (18.50 mm). Hence PPF as substrate and rice husk as nitrogen source supported high stipe length. Mushroom stipe and pileus sizes are may be affected by the type of substrates and nitrogen sources used for the cultivation This result contradicts the report of Stanley, Umolo and Stanley (2011) that sole palm press fibre substrate did not support mushroom growth and agrees with Akinyele, Ajibade and Fatoye (2013) that reported Oil palm fibre improves C:N ratio which support the growth of *P. pulmonarius*. Also, agrees with the report of Wandita, Darmawan, Triatmojo, and Fitriyanto (2016) that rabbit urine promotes the growth of the mushroom because it improves the percent nitrogen of the substrate. Nonetheless, mushroom's stripe length and pileus diameter are influenced by the environment temperature and relative humidity (AMGA, 2004).

Hypothetically, the yield showed gradual decrease over the flushes as shown in Table 3 below. First flush gave more mushrooms yields than other flushes, and substrate D_u produced the highest first flush (72 g) and the lowest was produced by substrate A (17 g). The highest total yield was shown by substrate E_u (202 g), which confirmed the report of Chiejina and Osibe (2015) that oil palm fruit fiber produced the highest total yield because left over oil in the waste probably supported higher mushroom yield. Also, results of this current study revealed that substrate supplemented with rabbit urine improved mushroom yield in each substrate mixture. This is because rabbit urine high nitrogen content and thus improve mushroom yield (Chiejina and Osibe, 2015).

Table 3: Yield, total yield and biological efficiency of different substrates

Substrate	Yield/Flush (g)				Total	Biological
	First	Second	Third	Fourth	Yield	Efficiency
A	17	14	11	10	52	10.4
B	20	15	14	10	59	11.8
C	26	20	18	15	79	15.8
D	45	32	20	12	109	21.8
E	63	43	36	22	152	30.4
A_U	36	28	23	11	98	19.6
B_U	47	39	25	12	123	24.6
C_U	50	41	36	25	164	32.8
D_U	72	46	39	34	191	38.2
E_U	63	55	49	35	202	40.4

Values are means ± SD, the means reported with the same superscript in each column indicated no significant variation ($p < 0.05$).
A; 100 SD : 0 PPF with RH, B; 75 SD : 25 PPF with RH, C; 50 SD : 50 PPF with RH, D; 25 SD : 7 PPF with RH, E; 100 PPF : 0 with RH, A_U; 100 SD : 0 PPF with RU, B_U; 75 SD : 25 PPF with RU, C_U; 50 SD : 50 PPF with RU, D_U; 25 SD : 7 PPF with RU, E_U; 100 PPF : 0 with RU.

The highest biological efficiency was observed in substrate E_u (40.4 %) and the lowest in substrate A (10.4 %). These results are in accordance with the report of Peksen and Yakupoglu (2009) that there is a positive correlation among yield, nitrogen content of substrate and biological efficiency.

Proximate Composition

Table 4: Proximate composition of *Pleurotus pulmonarius* mushroom

Substrate	Moisture %	Crude protein %	Crude Fat %	Crude Fibre %	Total Ash %	NFE %	Energy k/cal

A	15.34±.16^{e}	36.24±.40^{d}	2.84±.06^{d}	6.42±.04^{a}	7.89±.24^{b,}	31.27±1.03^{b,c}	295.60±.1.41^{d}
B	14.28±.25^{c}	35.96±.15^{d}	2.88±.03^{d,}	6.89±.04^{a,b}	8.47±.07^{d}	31.52±.74^{b,c}	295.84±23^{d}
C	14.17±.11^{c}	31.91±.21^{c}	2.97±.02^{d,}	7.05±.08^{b,c}	8.59±.05^{d}	35.31±.98 ^{f}	295.61±30 ^{d}
D	15.61±.42^{e}	30.80±.04^{b}	2.98±.00^{e}	7.45±.11^{c,d}	9.08±.05^{e}	34.08±.06^{e,f}	283.46±65^{a}
E	14.54±.18^{c,}	30.13±.47^{a}	3.50±.02^{f}	8.29±.04^{e}	9.99±.01^{f}	33.55±.64^{d,e,f}	286.22±.1.41^{b}
A_u	13.99±.46^{c}	38.15±.06^{g}	2.04±.08^{a}	6.90±.04^{a,b}	7.15±.05^{a}	31.77±.33^{c,d}	298.04±1.36^{d,e}
B_u	14.99±.15^{c,}	37.00±.18^{f}	2.40±.01^{b}	7.02±.13^{b,c}	7.82±.06^{b}	30.77±1.09^{b,c}	292.68±.96^{c}
C_u	12.59±.50^{a}	36.92±.05^{e,f}	2.56±.04^{c}	7.45±.48^{c,d}	8.15±.26^{c}	32.33±.95^{c,d,e}	300.04±1.47^{e}
D_u	13.23±.04^{b}	36.30±.47^{d,}	2.66±.03^{c}	7.89±.48^{d,e}	9.00±.12^{e}	29.68±1.41^{a,b}	296.86±1.22^{d}
E_u	13.97±.09^{c}	35.92±.29^{d}	2.98±.13^{e}	8.01±.06^{e}	9.68±.18^{f}	28.14±.20 ^{a}	292.06±1.33^{c}

Values are means ± SD, the means reported with the same superscript in each column indicated no significant variation ($p < 0.05$). NFE – Nitrogen free extract

A; 100 SD : 0 PPF with RH, B; 75 SD : 25 PPF with RH, C; 50 SD : 50 PPF with RH, D; 25 SD : 7 PPF with RH, E; 100 PPF : 0 with RH, A_U; 100 SD : 0 PPF with RU, B_U; 75 SD : 25 PPF with RU, C_U; 50 SD : 50 PPF with RU, D_U; 25 SD : 7 PPF with RU, E_U; 100 PPF : 0 with RU.

The results of proximate analysis of *P. pulmonarius* obtained from each substrate presented in Table 4, varied significantly ($P \leq 0.05$). Numerically the moisture varied significantly ranging from substrate C_u (12.59 %) to substrate (D 15.61 %). The protein content ranges from substrate E (30.13 %) to substrate A_u (38.15 %). In this present study the protein content obtained in *P. pulmonarius* is higher than 20.03 % protein content that was reported by Liasu, Adeeyo, Olaosun and Oyedoyin, (2015). Usha and Suguna (2014) who investigated the nutrient content *Auricularia polytricha* and *Pleurotus ostreatus* in India reported similar moisture value (36.0 % and 33.3 % respectively).

Substrate A_u had the least percent crude fat (2.04 %), while substrate E_u had highest crude fat content (3.50 %). The results obtained in this study were higher than those (0.15-1.83 %) reported by Chinda (2007) and Onyeka et al., (2018). Highest crude fibre content was observed in substrate E (8.29 %) and substrate E_u (8.01 %), substrate A had the least fibre content of 6.42 %. this result is similar to the report of Ahmed, Kadam, Mane, Patil and Baig, (2009) who worked on paddy straw (8.10 %) and soybean straw (8.02 %) and disagreed with the report of Shah, Ashraf and Ishtiaq (2004) that crude fibre value ranges from 2.5 to 3.5 %.

The highest ash composition was obtained from substrate E (9.99 %), and the lowest from Substrate A_u (7.15 %). This result is higher than percent ash of *P. florida* cultivated on soybean straw (8.00 %) and paddy straw (6.60 %) respectively (Ahmed, Kadam, Mane, Patil and Baig 2009). The nitrogen free extract (carbohydrate content) of *P. pulmonarius* obtained from this study ranges from substrate E_u (28.14 %) to substrate C (35.31 %). The carbohydrate contents obtained in this study was similar to that of *Auricularia polytricha* and *Pleurotus ostreatus* (28.5% and 44.7%) respectively (Usha and Suguna, 2014) those and less than the values (39.82- 42.83 %) recorded by Alam et al., (2008) those for carbohydrate in *Pleurotus* spp respectively. Substrate highest energy value C_u (300.04 Kcal/100 g), while the lowest energy value (283.46 Kcal/100 g) was observed in substrate D. These results slightly agreed with the findings of those Hoa, Chun-Li Wang and Chong-Ho Wang (2015) on *Pleurotus ostreatus* total energy (265.95 to 295 kcal/100 g) and *Pleurotus cystidiosus* (287 to 304.85 kcal/ 100 g).

CONCLUSIONS

This study has revealed that a certain percentage of substrate composition and nitrogen source supplementation is required for the optimum yield of Indian oyster mushroom and selection of substrate and supplement hinge on their obtainability and cost. The use of palm pressed fiber as substrate decreased mycelium run time, and caused increased stipe length, pileus diameter, yield and biological efficiency. The inclusion of nitrogen sources in the substrates influence the physical characteristic of mushroom. Also, each substrate and nitrogen source affected the proximate composition and energy value of mushroom differently. Palm press fibre combined with sawdust can serve as substrate for mushroom cultivation. Rice husk can serve as good nitrogen source for cultivating mushrooms, due to its physical characteristic, high porosity and the aeration attributes. Rabbit urine can also be used as a supplement because it contains high level of nitrogen and can increase the substrate nutrient.

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