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EFFICACY OF DIFFERENT SUBSTRATES ON THE GROWTH AND BIOACTIVE COMPONENT OF TWO OYSTER MUSHROOM *(PLEUROTUS PULMONARIUS* AND *PLEUROTUS FLORIDA)*

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ABSTRACT
Agricultural waste can be harnessed to improve food safety and security. This study was carried out with the aim of investigating the efficacy of substrate on the growth and bioactive component of two oyster mushroom (Pleurotus pulmonarius and Pleurotus florida). Three different substrates namely, i.e. sawdust (SD), rice husk (RH) and sawdust + rice husk (SD+RH) were used. The substrates were pasteurized before inoculation of the spawns and later kept in a controlled environment to allow for the development of fruiting bodies. The substrate media were found to influence pin head formation, number of fruiting bodies and maturity period in days. The number of fruiting bodies for P. florida was higher in sawdust (17.50) than in rice husk (16.17). The increase in the number of fruiting bodies for sawdust substrate could be suggestive of production of various enzymes during the vegetative and reproductive phases which helped to solubilize the lignin and degrade the cellulose which were later absorbed by the mushroom mycelium for the production of fruiting bodies. The pin heads appeared fastest on rice husk (53.67 days) for P. pulmonarius followed by sawdust (74.50 days) and sawdust + rice husk (77.83 days). The variation in the number of days taken for a spawn to complete colonization of a given substrate is a function of the fungal strain, growth condition and substrate type. The development of oyster mushroom is therefore predicted on these important factors; nutrition, genetics, environment, and the species of interest.
KEY WORDS: *agricultural waste; fruiting bodies; mycelium; pin head; substrate*

INTRODUCTION

Mushrooms had hitherto being touted as important staples in rural areas because of inadequate food supply, malnutrition, attendant health challenges and alarming environmental deterioration (Kinge *et al.* 2014). Oyster mushroom is rich in abundant metabolites of pharmacological and nutraceutical interest. Its role in biodegradation, bioremediation and production of extracellular enzymes cannot be overemphasized. The medicinal importance of mushrooms is predicated on their nutritional and chemical composition. Apart from consumption, the industrial and pharmaceutical sector of the economy had recorded tremendous boost (Jayakumar *et al.* 2010). *Pleurotus* spp is mostly cultivated on woody materials rich in cellulose and lignin; however, they are capable of degrading these complex substances under the

enzymatic influence of polyphenol oxidases. Mycelia growth and fructification is best achieved under sawdust substrate. In order to achieve maximum yield of oyster mushroom, an optimal temperature of 25^{o}c is needed but performs better at a temperature range of 17-23^{o}c during fructification (Ravi *et al.* 2010). Mycelia growth is also dependent on adequate light intensity to allow the inoculated substrates achieve full ramification (Sarangi *et al*. 2006) as excessive light could result into photosynthetic damage. Oyster mushroom is a very unique and distinctive macro-fungus. It is an organoleptic fungus which enzymatically degrades complex organic substances. to simpler compounds for its nutrition (Chang & Miles, 1991). The objectives of this study are therefore to determine the efficacy of substrate on the growth of two oyster mushroom (*Pleurotus pulmonarius and Pleurotus florida*) and to investigate the efficacy of the substrate on the bioactive components of *P. pulmonarius and P. florida.*

MATERIALS AND METHODS

Study location. This study was conducted in research laboratory of the Department of Biology, Federal University of Technology, Akure.

Collection of samples. Agro-industrial residues namely saw dusts were collected from sawmill and rice husk were collected from Mami market rice mill, Ondo road, Akure, Ondo state. The spawn were purchased from Mushroom farm of Afe Babalola University, Ado Ekiti (ABUAD).

Substrate. The substrate used for the cultivation of the oyster mushroom (*Pleurotus pulmonarius* and *Pleurotus florida)* were 100% Rice husk (RH)-control, 100% Sawdust (SD) and 70% Sawdust + 30% rice husk (SD+RH)-combined substrate.

Preparation and formulation of substrates. The individual substrates (Sawdust and Rice husk) and the combined substrates (Sawdust + Rice husk) were thoroughly mixed and moistened with water for 24 h. They were later stalked on sterilized floor containing 1% calcium carbonate to remove microbial contaminants and to get rid of excess moisture in order to achieve optimal moisture level of 65%.

Spawn preparation. The substrates were filled into polythene bags, homogenized and later sterilized in an autoclave for 2 h. The openings of the polythene bags were air tight and plugged with an adsorbent (cotton wool). The substrates were later sterilized at about 100^{o}c for 4 h. The bags were cooled to about 30^{o}c before inoculation.

Spawning. Spawn of oyster mushroom (*Pleurotus pulmonarius* and *Pleurotus florida*) were used to inoculate the substrates. The inoculated substrates were later incubated in a darkroom for about 21 days to allow colonization.

During this period, temperature and relative humidity of the incubation room were maintained.

Fructification. After the incubation period, the mushroom substrates were transferred to the fruiting room where adequate moisture, optimal temperature and relative humidity were maintained. The first primordial (pin heads) appeared 7 to 10 days after opening depending upon the nature and compactness of the substrates.

Harvesting of mushroom. Mature fruiting bodies were carefully harvested without destroying the substrates by holding the stipe and gradually pulled out from the substrates. Each bag is then moisturized to enable fruiting after harvest.

Determination of morphological and yield parameters

***Biological yield*:** This was determined by the average of the total fresh weight of the fruiting bodies with respect to the substrate and the flushes.

Biological efficiency (%): This was calculated by taken the percentage weight of the cluster of the fruiting body to the initial weight of the substrate.

$$\textit{Biological efficiency (\%)} = \frac{\text{Total yield (kg)}}{\text{Weight of substrate used}} \times 100$$

Pin head formation: The total number of primordial in each of the flush in a particular substrate was calculated and recorded.

Maturity period: The time taken for primordial initiation to maturity of the fruiting bodies were calculated and recorded.

Number of fruiting bodies: The total number of fruiting bodies at harvest in each of the substrate was calculated and recorded.

Statistical analysis: Data collected were subjected to analysis of variance and significant means were separated using Duncan Multiple Range Test.

RESULTS AND DISCUSSIONS

There were significant differences between the mean weight of sawdust (SD), rice husk (RH) and the combined substrates (SD+RH). The mean weight of RH was significantly higher than the mean weight of SD and the combined substrates, respectively (Table 1). However, the mean weight of the fruiting bodies of *Pleurotus pulmonarius* was significantly higher on the RH while the mean weight of *Pleurotus florida* was significantly higher on the combined substrates (SD+RH).

TABLE 1. Effect of different substrates on the biological yield of *Pleurotus pulmonarius* and *Pleurotus florida* .

Growth Parameters/ Substrates	*Pleurotus pulmonarius*				*Pleurotus florida*			
	Flush 1	**Flush 2**	**Flush 3**	**Mean±S.E of Flush**	**Flush 1**	**Flush 2**	**Flush 3**	**Mean±S.E of Flush**
Mean weight on sawdust	59.1	35.6	32.8	67±13.074a	27.9	21.9	28.4	22.33±6.21a
Mean weight on rice husk	64.2	45.8	36.4	947.17±13.074a	43.3	37.8	20.7	32.30±6.21a
Mean weight on sawdust + rice husk	39.5	50.58	49.5	83±13.074a	57.6	47.6	34.5	42.12±6.21a

Mean pin head formation, maturity period and number of fruiting bodies of *Pleurotus pulmonarius* and *Pleurotus florida* is shown in Table (2). Mean number of flushes in days for the substrate ranged from 53.6-77.83 for *P. pulmonarius* and 69.17-134.16 for *P. florida.* There were significant differences on the pin head formation for *P. florida* cultivated on the different substrates. The highest mean number of flushes in days for *P. florida* based on the pin head formation was adjudged to be higher than *P. pulmonarius* which was recorded in the sawdust substrate. Also, there were significant differences in the maturity periods of both *P. pulmonarius* and *P. florida*. Significant differences were recorded in the number of fruiting bodies which sprouted from the combined substrates (sawdust + rice husk) compared with the solitary substrates.

Biological efficiency and mean moisture content of the substrate is shown in Table 3. *Pleurotus pulmonarius* was observed to have the highest biological efficiency on the rice husk (84.5%) while; the least was recorded in sawdust (36.7%). However in *P. florida*, the combined substrates (sawdust + rice husk) recorded the biological efficiency of 56.8% and moisture content of 90% as compared to 84% that was recorded for *Pleurotus pulmonarius* in the combined substrates.

TABLE 2. Mean pin head formation, maturity period and number of fruiting bodies

Growth Parameters	Species	Flush 1 on S	Flush 2 on S	Flush 3 on S	Flush 1 on R	Flush 2 on R	Flush 3 on R	Flush 1 on S+R	Flush 2 on S+R	Flush 3 on S+R	Mean±S.E of Flush on S	Mean±S.E of Flush on R	Mean±S.E of Flush on S+R
Pin head formation (Days)	*P. pulmonarius*	63.5	75.0	85.0	45.5	48.5	67.0	67.0	74.0	92.5	74.50±3.22^{b}	53.67±3.22^{a}	77.83±3.22^{b}
	P. florida	127.5	137.0	138.0	53.5	70.0	91.5	55.0	64.5	88.0	134.17±4.12^{b}	71.67±4.12^{a}	69.17±4.12^{a}
Maturity Period (Days)	*P. pulmonarius*	65.0	77.0	87.0	47.5	50.5	69.0	69.0	76.0	94.5	76.33±3.24^{b}	55.67±3.24^{a}	76.83±3.24^{b}
	P. florida	129.5	140.0	140.5	56.0	72.0	93.5	57.0	66.5	91.5	136.67±4.20^{b}	73.83±4.20^{a}	71.67±4.20^{a}
Number of Fruiting body	*P. pulmonarius*	14.5	5.5	10.0	16.5	11.5	7.5	11.0	14.0	9.0	10.00±3.46^{a}	11.83±3.46^{a}	11.33±3.46^{a}
	P. florida	11.5	8.0	5.0	24.0	16.0	8.5	22.5	17.0	13.0a	8.17±1.630^{a}	16.17±1.63^{a}	17.50±1.63^{b}

Whereas, S = Sawdust, R= Rice husk, S+R= Saw dust + rice husk

TABLE 3. Biological efficiency and mean moisture contents of *Pleurotus pulmonarius* and *Pleurotus florida* on the substrates.

Species/substrates	Biological efficiency (%)	Mean moisture content (%)
Pleurotus pulmonarius		
Sawdust	36.7	80
Rice husk	84.5	92
Sawdust + Rice husk	58.2	84
Pleurotus florida		
Sawdust	28.6	80
Rice husk	37.6	84
Sawdust + Rice husk	68.8	90

There were great variation in the growth and yield of *P. florida* and *P. pulmonarius* depending on the substrate (Table 1). Findings from this study revealed that rice husk had the greatest influence on both growth and total yield. It demonstrated clearly excellent biological yield, greater height and pileus length (Shah et al. 2004; Mane et al. 2007; Patil et al. 2010). The variations in the effect of different substrates on the growth, yield and quality of mushroom had been extensively studied (Zhang et al. 2002; Belewu, 2003; Alam et al. 2007; Iwalokun et al. 2007). The popular trend in the cultivation of oyster mushroom globally is orchestrated by their ability to grow on a wide range of agricultural wastes. The development of oyster mushroom notwithstanding is predicated upon four important factors; nutrition, genetics, environment and the species of interest. The observed variation in the biological efficiency of *P. pulmonarius* and *P. florida* could be attributed to the effect of different on the yield (flushes). *P. pulmonarius* and *P. florida* growth was enhanced in rice husk than in sawdust and the combined substrates respectively (Table 3). This study is not in conformity with the study of Shah et al. (2004) who reported that sawdust produced maximum yield of *P. ostreatus.* Also, pin head formation, maturity periods and the number of fruiting bodies were achieved within few weeks of complete ramification in rice husk substrates than in other substrates (Table 2). However, for sawdust, longer period of pinhead formation, maturity period and the number of fruiting bodies were achieved (Kues et al., 2000**;** Kinge et al., 2014). The observed variability in pin head formation, maturity period and in the number of fruiting bodies had earlier being reported by Shah et al. (2004) due to the presence of different composition of the substrates. Bughio, 2001, also supported that the number of days for pin head formation was 25-50 days and maturation of fruiting bodies was achieved within 5-6 days after pin head formation. The substrates were found to enhance pin head formation, number of fruiting bodies and maturity period in days. The pin head formation was observed following the invasion of substrates by mycelia growth (Kinge et al. 2014**)**. The pin heads appeared fastest on rice husk (53.67 days) for *P. pulmonarius* followed by sawdust (74.50 days) and sawdust + rice husk (77.83 days). There was difference in the appearance of pinheads of different substrate. The time required for the formation of pin heads is comparable with other similar studies. Ahmed et al. (2009) reported pin head formation of oyster mushroom cultivated in different substrates to be between 23 and 27 days after spawning while Tesfaw et al. (2015) reported it to be 20-23 days. Badu et al. (2011) recorded 23-26 days for the appearance of pin heads while, Behnam et al. (2008) recorded 20-24 days on paddy straw.

CONCLUSIONS

Findings in this study revealed that the increase in the number of fruiting bodies for sawdust substrate could be suggestive of production of various enzymes during the

vegetative and reproductive phases which helped to solubilize the lignin and degrade the cellulose which were later absorbed by the mushroom mycelium for the production of fruiting bodies.

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