Evaluation of Yield and Spoilage Moulds of *Pleurotus Ostreatus* Grown on Sawdust, Wood Ash and Cassava Bran

Agbagwa, S. S.[1], Chuku, E. C.[1] & Mbah, C. G.[2]

[1]*Department of Plant Science and Biotechnology, Rivers State University, Rivers State, Nigeria*
[2]*Department of Microbiology, Rivers State University, Rivers State, Nigeria*

Abstract: **Evaluation of yield and spoilage moulds of *Pleurotus ostreatus* grown on sawdust, wood ash and cassava bran were conducted in the Department of Plant Science and Biotechnology and Dilomat Farms and Services Limited, Rivers State University. The three substrate materials were subjected to different concentrations and mixtures. A total of ten treatments were formulated including the control containing only sawdust and a combined effect containing all the substrate materials. Yield parameters assessed were height of fruiting body, length of stipe, width of stipe, length of pileus, diameter of pileus and weight of harvest. Sawdust and cassava bran (SCB) treatment had highest height of fruiting body (11.50±1.41). Equal diameter of pileus (6.25±1.06) was recorded for both combined effect (CE) and SWA treatments. However, equal values of pileus length were recorded for both CE and sawdust and wood ash (SWA) treatments. The control treatment had highest values for length of stipe, width of stipe and weight of harvest. Four fungal organisms viz: *Rhizopus oryzae, Aspergillus flavus, Fusarium oxysporium* and *Trichoderma harzianum* were found to contaminate the experiment bags. *R. oryzae* and *T. harzianum* had equal incidence of 27%. This was followed by 24% incidence recorded for *A. flavus*. Lowest incidence of 22% was observed for *F. oxysporium*. The deteriorative activities of these isolates affected the development of higher concentration levels of sawdust and cassava bran treatments as there was no harvest recorded for these treatments due to contamination. Generally, the control treatment performed better than other treatments used.**

Keywords: **Yield, *Pleurotus ostreatus*, sawdust, wood ash and cassava bran**

I. INTRODUCTION

Pleurotus ostreatus are known to be found in the wild where they are collected. Its cultivation arose in order to compensate for food shortage in the World War II. Although, the first account of cultivation was recorded in China which still records the highest production capacity [1]. *P. ostreatus* names originated from its nature and shape. *Pleurotus* being the Latin interpretation of its sideways growth and *ostreatus* for its oyster shape; hence commonly known as Oyster mushroom [2].

P. ostreatus is described by its macroscopic and microscopic characters. Macroscopically, oyster mushrooms are known to possess pileus measuring about 40 to 250mm in width. Shaped liked an oyster, the pileus is variable in colour with a smooth margin that later become serated when older and mature [3]. Beneath the pileus are found the gills that bear the spores. *P. ostreatus* also possesses a stipe that serves as a stem bearing the pileus [4]. The microscopic portion of the oyster mushroom includes the spores, basidium sterigmata and pileipellis. The fruiting body may be white or grey in colour, fleshy and could be thick or thin [5].

Studies have shown the relevance of Oyster mushroom in the food industry as it serves as a source of food, especially to the vegetarians [6]. The availability of mushroom also helps in poverty alleviation as it is easily assessed by the poor [7], [8]. Mushroom cultivation has also served as a source of income to mushroom growers [9].

Pests and diseases have always been a major problem in agriculture and mushroom cultivation is also faced with the same challenge. Several authorities have implicated different organisms to cause disease and menace to cultivated mushroom [10]. Different fungal organisms including *Aspergillus spp, Trichoderma spp, Cladobotryum spp, Penicillum spp, Gliodadium spp* and *Verticiluium spp* have been reported to be associated with *Pleurotus* contamination [11]. In addition, several species of insect have been reported to be associated to *Pleurotus* as well as other forms of mushroom. Literature has shown that larvae of *Megaselia spp, Scaria fenestralis, Lycriella spp* and *Lepidocyrtus spp* feed on the mushroom mycelium [12]. The activity of these organisms does not only affect the marketability of the mushroom but also the income of the producer [13].

There scanty literature on the cultivation of P. ostreatus using wood ash, cassava bran and sawdust which motivated this research in order to evaluate the yield and possible spoilage moulds of oyster mushroom on these substrate materials.

II. MATERIALS AND METHODS

A. Sample Collection

Cassava peels were collected from Omagwa community in Ikwerre Local Government Area, Rivers state. The peels were dried for one month and immediately ground into powder for further use. Sawdust and *Rhizophora racemosa* wood were both obtained from Timber Market Mile II Diobu in Rivers State. *R. racemosa* woods were later burnt to collect the ash.

Healthy spawns of *Pleurotus ostreatus* were bought from Dilomat Farms and Services Limited, Rivers State University for the study. The above materials were all conveyed to the experimental site at Dilomat farms.

B. Substrate Compositions

The materials used for the cultivation were cassava bran, wood ash and sawdust and were subjected to various mixtures leading to their respective compositions for the experiment. Three concentrations of cassava bran (100, 150 and 200g) and wood ash (0.30, 0.60 and 1.0g) were varied against a constant quantity of sawdust (1000g). Combined and control treatments were also set up. A total of ten treatments were used for the cultivation of *P. ostreatus*:

Sawdust and wood ash (SWA): SWA1, SWA2, SWA3

Sawdust and cassava bran (SCB): SCB1, SCB2, SCB3

Combined effect (CE): CE1, CE2, CE3

Control (C)

The numbers attached to the abbreviated formulation indicate the concentration levels of wood ash and cassava bran in an increasing order.

C. Cultivation Studies

The cultivation methods of Chinda & Chinda [14] were adopted for this research. The different substrate compositions were composted for 40days. At the end of composting, the substrates were immediately bagged and sterilized through pasteurization at 100^0C for 6hours. After the sterilized bags cooled, they were inoculated with 74.99±21.66g of spawn and incubated at room temperature (25±3^0C) for 38days. Fully colonized bags were immediately cropped and watered for 3days. Fruiting bodies were later harvested and subjected to various studies.

D. Isolation of Fungi

Spoilage fungi associated with the substrate bags were isolated using Sabouraud Dextrose Agar (SDA). The medium was prepared in accordance with the manufacturer's standard of 65g of SDA to 1000ml of distilled water. The direct plating method described by Mehrotra & Aggarwal [15] was used for this study, where 1g of the inoculum was placed on the prepared plate in an aseptic manner. The plates where then incubated at room temperature of 25±3C for five days and further culturing was done to obtain pure cultures [16].

E. Characterization and Identification Of Isolates

Fungal isolates were characterized and identified using the outlined microscopic and macroscopic examination processes described by Barnett & Hunter [17] and Cheesbrough [18]. This was aided by the use of cotton blue in lacto phenol and microscope power of x10 and x40.

F. Isolate Percentage Incidence

The occurrence and incidence of the various isolates in percentage were also evaluated using the methods of Chuku *et al*., [19] and Nnaji & Rao, [20]. This was done using the formula below:

% incidence = X/Y x 100

Where;

X = total number of each organism in a variety

Y = total number of all identified organisms in a variety

G. Statistical Analysis

Data obtained from the above studies were subjected to the analysis of variance (ANOVA). Duncan multiple range test was also used for mean separation with the aid of SPSS

III. RESULTS AND DISCUSSION

Table 1: Effect of different substrate compositions on the yield and growth parameters of *P. ostreatus* (cm)

Sub. Comp.	HFB	LS	WS	LP	DP	WH (g)
C	10.90±0.98^a	5.50±0.00^a	1.90±0.14^c	8.00±3.53^a	5.25±1.06^{ab}	48.71±50.13^a
CE1	10.15±0.49^a	3.75±1.06^a	1.00±0.28^a	8.25±0.35^a	4.35±0.49^a	27.67±0.82^a
CE2	9.45±1.34^a	4.65±0.49^a	1.10±0.14^{ab}	6.90±0.14^a	4.40±0.28^a	16.41±1.66^a
CE3	10.65±0.91^a	4.90±0.14^a	1.10±0.14^{ab}	6.85±1.20^a	4.15±0.49^a	25.90±1.19^a
SCB1	11.50±1.41^a	4.75±0.35^a	1.50±0.14^b	7.55±0.63^a	6.25±1.06^b	32.24±13.40^a
SCB2	-	-	-	-	-	-
SCB3	-	-	-	-	-	-
SWA1	9.20±1.41^a	3.30±0.28^a	1.60±0.14^b	6.35±0.63^a	4.40±0.14^a	15.80±1.73^a
SWA2	9.50±3.53^a	3.75±1.76^a	1.15±0.21^{ab}	6.00±0.70^a	3.75±0.35^a	15.28±2.07^a
SWA3	11.40±4.10^a	5.25±3.18^a	1.80±0.56^c	8.25±1.76^a	6.25±1.06^b	44.99±47.27^a

*Means with the same superscript across the column are not significantly different ($p \leq 0.05$)

HFB=height of fruiting body, LS=length of stipe, WS=width of stipe, LP= length of pileus, DP=diameter of pileus, WH=weight of harvest, Sub. Comp.=Substrate compositions and - = no harvest.

Table 2: Fungal isolates and percentage incidence

Fungal isoates	Percentage incidence (%)
Rhizopus oryzae	27
Fusarium oxysporium	22
Aspergillus flavus	24
Trichoderma harzianum	27

The result of effect of different substrate compositions on the yield and growth parameters of *P. ostreatus* outlined in Table 1, revealed that the highest and lowest heights of fruiting body were 11.50±1.41 and 9.20±1.41 for SCB1 and SWA1 respectively while the control and SWA1 recorded the highest and lowest stipe lengths (5.50±0.00 and 3.30±0.28) respectively. It was also observed that the control had the highest width of 1.90±0.14 and CE1 had the lowest width of 1.00±0.28 for stipe width. Highest length of pileus was observed for both CE1 and SWA3 while the lowest length of 6.00±0.70 was recorded for SWA2. In addition, SCB1 and SWA3 had equal values for pileus diameter 6.25±1.06 whereas SWA2 had the lowest diameter (3.75±0.35). The weight of harvest examined revealed that the control recorded highest harvest weight (48.71±50.13) while SWA2 had the lowest weight (15.28±2.07). SCB2 and SCB3 had no harvest which was due mainly to contamination as at the 5th week of incubation.

The fungal characterization and percentage incidence data presented in Table 2, indicated four fungal organisms viz: *Fusarium oxysporium, Aspergillus flavus, Rhizopus oryzae and Trichoderma harzianum* to be responsible for the contamination and spoilage of the incubated substrate bags. Highest percentage incidence of 27 was observed for both *R. oryzae* and *T. harzianum*. This was followed by *A. flavus* which had a percentage incidence of 24. The least incidence of 22% was recorded for *F. oxysporium*.

The result of the present study has shown that there is no significant difference on the effect of the different substrate compositions for the height of fruiting body, length of stipe, length of pileus and weight of harvest of the cultivated *P. ostreatus* as revealed by the ANOVA test at ($p \leq 0.05$). Generally, the control treatment performed better for growth and yield studies than every other treatment.

The results of length of stipe, diameter of pileus and weight of harvest presented in this current study agrees with those earlier reported by Jonathan *et al*., [21] for *P. pulmonarius* cultivated on coir fibre, oil palm waste, sawdust and rice straw. Although, they reported lower height of fruiting body compared to those reported in this study. The findings of the present study are in line with the report of Onouha *et al*., [22] as they implicated higher number of fruiting body, height of fruiting body, diameter of fruiting body and weight of fruiting body for the treatment containing only sawdust compared to the oil palm fibre, dry cassava peel, cassava peels and oil palm fibre treatments they tested as well on *P. pulmonarius*. However, the values they reported are lower than their equivalents in the present study.

The data of the present study are similar to those reported by Das *et al*., [23] for length of stipe, stipe width, pileus diameter of five *Pleurotus* species cultured in warm temperature on non-sterilized rice straw. Nevertheless, they also reported a lower weight of harvest. Furthermore, the findings of the present study disagree with the report of Liasu *et al*., [24] as they reported lower length of stipe for *P. pulmonarius* cultivated on amended palm press fibre waste. Sobowale *et al*., [11] implicated lower length and diameter of pileus and length of stipe for *P. ostreatus* cultivated on sawdust from two different woods. The growth parameter results of the present study is in agreement with the earlier report of Chukwurah *et al*., [25] for *P. ostreatus* cultivated on different local agrowastes supplemented with lime. However, they did not experiment *P. ostreatus* on cassava and wood ash.

Due to much availability of carbohydrate in SCB2 and SCB3, the treatments faced greater losses to spoilage fungi leading to their contamination and inability to fruit. Akinmusire *et al*., [26] also reported similar situation as they showed that *P. pulmonarius* did not fruit on rice straw substrate and sawdust substrate in Maiduguri, Nigeria.

The present study implicated four fungal organisms to be responsible for the contamination and spoilage of the prepared substrate bags. This finding is in line with those reported by early researchers as they were shown to be able to grow and develop within the same nutrient requirement of *P. ostreatus* [10]. Sobowale *et al*., [11] reported *A. flavus* and *Trichoderma* species to be responsible for the contamination of *P. ostreatus* and *P. pulmonarius* cultivated in *Ceiba pentandra* and *Ficus mucuso* woods. More so, they reported lower incidences for both organisms compared to those recorded for their equivalents in this study.

The microbial contamination recorded in this work during the cultivation processes could be due to several reasons such as unhealthy spawn inoculation and improper sterilization. Chuku & Barber [27] revealed the potential of *F. oxysporium, A. niger* and *Penicillium frequentans* to contaminate the spwans used for the cultivation of *P. ostreatus*. They further reported higher incidence for *F. oxysporium* compared to that recorded in this study. The research of Shah *et al*., [28] further implicated *Trichoderma* species to be associated with *Pleurotus spp* green mould disease. They also revealed that *T. harzianum* had the highest incidence of 38.7% and was responsible for most cases of the *Pleurotus* cultivation green mould disease. Nevertheless, there is dearth of information about the contamination of mushroom by *Rhizopus* implicated in this study.

In addition, the economic importance of these isolates cannot be overlooked as they readily caused spoilage of the experiment bags. Devastating impact of these isolates was observed more in the treatments containing cassava bran which suffered severe contamination that led to lost of

harvest. This could be attributed to the greater source of carbon, which is a major nutrient for fungal organisms, provided by cassava bran within the treatments. This observation agrees with the report of Okpako *et al*., [29] as they revealed the extensive support of cassava peels for the growth and development of *A. niger* and *Lactobacillus rhamnosus*.

IV. CONCLUSION

The substrate materials and their various mixtures employed in the study had different influence on the yield and yield components of the cultivated *P. ostreatus*. However, the control treatment performed better. Other fungal organisms were also able to utilize these substrates and their activities caused loss of harvest in treatments with higher concentrations of sawdust and cassava bran.

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