

Effect of Different Substrate Compositions on Mycelia Ramification Parameters of *Pleurotus Ostreatus*

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ABSTRACT: Studies on the effect of different substrate compositions on mycelia ramification parameters of *Pleurotus ostreatus* were carried out in Dilomat Farms and Services Limited, Rivers State University. Three substrates viz: sawdust cassava bran and *Rhizophoraceosa* wood ash were utilized. Three concentrations of wood ash and cassava bran were mixed with a constant concentration of sawdust. Ten treatments were obtained including the combined and control treatments. Ramification parameters assessed were rate, length, productivity and weight. Highest productivity ($3.09 \pm 0.23\%$) and weight of mycelia ($30 \pm 0.23\text{g}$) ramification were observed for the sawdust and wood ash treatments (SWA). Highest lengths of mycelia ramification were recorded for SWA treatments for weeks 1, 2 and 3 of incubation. However, the control had highest length of mycelia ($16.5 \pm 0.00\text{cm}$) at the 4th week of incubation. Similar observations were also made for the rate of mycelia ramification as the SWA treatments had highest rates for weeks 1, 2 and 3 while highest rate of 0.59 ± 0.00 was recorded for the control treatment. Generally, the sawdust and wood ash treatment performed better than every other treatments on mycelia ramification of *P. ostreatus*.

Key word: Mycelia, ramification, *Pleurotus ostreatus* and substrate compositions

I. INTRODUCTION

Mycology as a branch of science focuses on the study of fungi which encompasses their morphology, physiology, ecology, reproduction, taxonomy and their relative economic importance [1]. However, fungi are eukaryotic organisms that do not possess chlorophyll, grow on dead and decaying substances as saprophytes and mostly reproduce by spores. They possess cell wall and hyphae that branches into several networks to form mycelium [2]. Fungi can be grouped into macroscopic and microscopic forms in relation to their size. Filamentous fungi such as *Aspergillus*,

Penicillium, *Rhizopus* and many others are of the microscopic form whereas mushrooms which are larger in size are regarded to be the macroscopic forms of fungi [3].

Pleurotus species commonly known as Oyster mushroom are typical examples of edible mushroom and belongs to the Agaricales Order under the Basidiomycota division [4], [5]. Several studies conducted by early researchers have shown the genus not to be only edible but also medically important as well as having the ability to remediate polluted environment [6],[7],[8]. Like every other mushroom, *Pleurotus* also requires a carbon and nitrogen source for growth and development. More so, its cultivation further depends on other abiotic factors such as pH, temperature, humidity and others that must be present in their optimum levels [9],[10],[11].

Several substrate materials such as bagasse, corn cobs, palm wastes, wood wastes, rice bran and wheat bran have been used to provide *Pleurotus* as well as other mushrooms their necessary nutrition for growth and development. These materials are properly mixed, composted and pasteurized before used for cultivation [12],[13],[14]. Furthermore, the use of different kind of agrowaste materials in the cultivation of mushroom play an important role in the general production process [15],[16].

II. MATERIALS AND METHODS

SAMPLE COLLECTION

Sawdust and *Rhizophoraceosa* wood were both obtained from Timber market Mile II Diobu (LAT 4.78902/LON 6.98781; $4^{\circ}47'20.49''\text{N}/6^{\circ}59'16.11''\text{E}$). *R. racemosa* woods were later burnt to collect the ash. Cassava peels were collected from Omagwa community in Ikwerre Local Government Area, Rivers state (LAT 4.99937/LON 6.90741; $4^{\circ}59'57.7726''\text{N}/6^{\circ}54'26.67''\text{E}$). The peels were dried for one month and immediately ground into powder for further

use. Healthy spawns of *Pleurotusostreatus* 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 (LAT 4.80611/LON 6.98046; 4° 48' 22"N/6° 58'49.668"E).

SUBSTRATE COMPOSITIONS

The substrate materials made up of cassava bran, wood ash and sawdust were subjected to various mixtures leading to their respective compositions for the experiment. The concentrations of cassava bran and wood ash were varied against a constant quantity of sawdust. The summary of the various substrate compositions are seen in the table below.

Table 1. Different substrate compositions and treatment levels in grams (g).

S/N	Wood ash (WA)	Cassava bran (CB)	Sawdust (S)	Sub. Comp.
1	0.30	-	1000	SWA1
2	0.60	-	1000	SWA2
3	1.0	-	1000	SWA3
4	-	100	1000	SCB1
5	-	150	1000	SCB2
6	-	200	1000	SCB3
7	0.30	100	1000	CE1
8	0.60	150	1000	CE2
9	1.0	200	1000	CE3
10	-	-	1000	C
Total	3.80	900	10,000	

SWA=Sawdust and wood ash, SCB=Sawdust and cassava bran, CE=Combine effect, C=Control and Sub. Comp.=Substrate compositions

CULTIVATION STUDIES

The cultivation methods of ChindaandChinda [17] 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°C for 6hours. After the sterilized bags cooled, they were inoculated with 74.99±21.66g of spawn and incubated at room temperature (25±3°C).

RAMIFICATION STUDIES

The method of Adebayo et al[18] was adopted for the estimation of ramification parameters using the following formula:

Rate of mycelia ramification (RMR) = length of mycelia ramification/number of days
 Mycelia ramification weight (MRW) = final weight after complete colonization – weight of substrate immediately after inoculation
 Productivity rate (PR) = total weight of mycelia/substrate weight x100

III. RESULTS

Table 2: Effect of different substrate compositions on the ramification weight and productivity of *P. ostreatus*

Sub. Comp.	WS (g)	WSAI (g)	WSEI (g)	MRW (g)	PR (%)
SWA1	970±28.28	1023.33±12.47	1053.33±18.85	30±6.38	3.09±0.23
SWA2	948.33±2.36	1010±8.16	1036.67±4.71	26.67±3.45	2.81±1.46
SWA3	950±0.00	1010±8.16	1026.67±18.86	16.67±10.70	1.75±10.70
SCB1	900±0.00	996.66±4.71	1003.33±4.71	6.67±0.00	0.74±0.00
SCB2	916.66±23.57	1003.33±4.71	1013.33±18.86	10.33±14.15	1.13±0.60
SCB3	936.66±26.25	995±10.80	1003.33±12.47	8.33±1.67	0.89±0.06
CE1	916.66±23.57	993.33±4.71	1000±0.00	6.67±4.71	0.73±0.20
CE2	951.66±2.36	1003.33±4.71	1008.33±6.24	5±1.53	0.53±0.65
CE3	956.66±4.71	1013.33±9.43	1021.67±18.40	8.34±8.97	0.87±1.90
C	923.33±20.55	1010±10.80	1030±14.14	20±3.34	2.17±0.16

Sub. Comp.=Substrate compositions, WS=Weight of substrate, WSAI=Weight of substrate after inoculation, WSEI=Weight of substrate at the 4th week of incubation, MRW=Mycelia ramification weight and PR=Productivity rate

Table 3: Effect of different substrate compositions on the ramification length (cm) of *P. ostreatus*

Sub. Comp.	Week1	Week2	Week3	Week4
SWA1	10±1.22	12.83±0.62	15±0.82	15.66±0.47
SWA2	5.66±2.70	9.5±1.87	12.83±1.55	14.66±0.94
SWA3	6.66±0.20	9.66±0.24	13±0.42	15±0.00
SCB1	6.33±0.89	8.33±0.62	11.5±0.71	14.33±1.18
SCB2	4±0.35	5.5±0.71	7.16±1.03	9.16±1.84
SCB3	4±0.00	4.66±0.24	6±0.41	7.33±0.62
CE1	6.5±0.94	9.5±1.08	12.16±1.03	15.33±1.25
CE2	3.33±1.08	5.16±2.25	6.33±3.06	7.66±4.29
CE3	4±1.27	5.16±1.69	7±2.04	8.66±2.05
C	8.5±0.94	11.5±0.82	14.16±0.24	16.5±0.00

Sub. Comp.=Substrate compositions

Table 4: Effect of different substrate compositions on the rate of ramification (cm/days) of *P. ostreatus*

Sub. Comp.	Week1	Week2	Week3	Week4
SWA1	1.43±1.22	0.92±0.62	0.71±0.82	0.56±0.47
SWA2	0.81±2.70	0.68±1.87	0.61±1.55	0.52±0.94
SWA3	0.95±0.20	0.69±0.24	0.62±0.42	0.54±0.00
SCB1	0.90±0.89	0.60±0.62	0.55±0.71	0.51±1.18
SCB2	0.57±0.35	0.39±0.71	0.34±1.03	0.33±1.84
SCB3	0.57±0.00	0.33±0.24	0.29±0.41	0.26±0.62
CE1	0.93±0.94	0.68±1.08	0.58±1.03	0.55±1.25
CE2	0.48±1.08	0.37±2.25	0.30±3.06	0.27±4.29
CE3	0.57±1.27	0.37±1.69	0.33±2.04	0.31±2.05
C	1.21±0.94	0.82±0.82	0.67±0.24	0.59±0.00

Sub. Comp.=Substrate compositions

The result of ramification parameters for the different substrate compositions as presented in Table 2, showed the various weight of substrates after inoculation, weight of substrate at the end of incubation, mycelia ramification weight and the productivity rate for the ten treatments. The highest mycelia ramification weight (30±0.23) and

productivity rate (3.09±0.23) were recorded for SWA1 while the lowest values of 5±1.53 and 0.53±0.65 were recorded for mycelium ramification weight and productivity rate respectively for CE2 treatment.

Length of mycelia ramification after four weeks of incubation as presented in Table 3, revealed that

SWA1 and CE2 had the highest and lowest lengths of 10 ± 1.22 and 3.3 ± 1.08 cm respectively for week1. At week2 of the incubation period, SWA1 still gave the highest length of 12.83 ± 0.62 while SCB3 recorded the lowest length of 4.66 ± 0.25 cm. Furthermore, SWA1 at week 3 also recorded the highest length of mycelia 15 ± 0.82 cm while SCB3 recorded the lowest length (6 ± 0.41). At the 4th week of incubation, the control treatment recorded the highest mycelia length measuring 16.5 ± 0.00 cm and SCB3 recorded the least length (7.33 ± 0.62). The result of rate of ramification after four weeks of incubation presented in Table 4, showed that SWA1 treatment had the highest rates (1.43 ± 1.22 , 0.92 ± 0.62 and 0.71 ± 0.82) for weeks 1, 2 and 3 respectively while the control recorded the highest rate (0.59 ± 0.00) for week4. However, CE2 recorded the least rate of ramification (0.48 ± 1.08) at week1 while SCB3 had the least rates of ramification (0.33 ± 0.24 , 0.29 ± 0.41 and 0.26 ± 0.62) respectively for weeks 2, 3 and 4.

IV. DISCUSSION

The present study has shown that SWA treatments performed better in terms of mycelia ramification weight and productivity rate than other treatment combinations. However, the combined effect (CE) treatments recorded the least performance. The findings from this study showed that increase in the levels of substrate composition resulted in reduced colonization and hence lowered the rate of mycelia ramification. On the other hand, lower levels of substrate combination increased colonization of spores and increased mycelia ramification [19].

The efficient performance of the SWA treatment could be as a result of the presence of wood ash which has been implicated by early researchers to stabilize pH [20]. On the contrary, cassava bran treatments had the least performance which further decreased with increase in concentration. This condition could be as a result of the presence of cyanide in the cassava bran, which limited and slowed down mycelia development [21].

The productivity values recorded in this study were lower than those reported by Ahmed et al[22] and Adebayo et al [18] for different *Pleurotus* species including *P. ostreatus*. Nevertheless, the mycelia weight result of this current study is higher than that reported by Adebayo et al [18] for the spawns of *P. pulmonarius*.

The result for mycelia length in this study has shown the progressive increase in length as the weeks of incubation increased, while the rate of

ramification which is a product of the length of mycelia and the number of days decreased as the weeks of incubation increased. These results agree with the report of Adebayo et al[18].

SWA treatments performed better for the length of mycelia and rate of ramification for weeks 1, 2 and 3 whereas the control was highest at week 4 for both results. Wood ash could be said to be responsible for the better performance of SWA treatments as its presence have been reportedly shown to balance pH level [23]. The lower length and rate of ramification of all the substrate compositions containing cassava bran could be attributed to the presence of cyanide as the research of Gomez et al[24] pointed out the abundance of cyanide in the leaves and peels of cassava. Fungi hypha are able to branch and increase in length based on their enzymatic ability to degrade the substrate and the rate at which this occurs is associated with the substrate composition and nature [25].

The rate of ramification presented in this study disagrees with those reported by Abdulganiet al[26] for *P. pulmonarius* and *P. citrinopileatus* as they reported higher rates (8.2 ± 0.1 and 7.7 ± 0.1) respectively. Adebayo et al[18] also researched on the rate and length of *Pleurotus* ramification but the values of the current study are higher than those they reported. Nevertheless, the result obtained from the current study agrees with the report of Liasuet al[27] as they showed that the nature of substrate combination affected and influenced mycelia growth. Interestingly, the rate of mycelia ramification in this study conforms to that reported by Thongklang and Luanharn[28] for different substrate combinations such as sorghum and rice straw, sorghum and sawdust and only sorghum. The current findings are in line with the report of Mkhize et al.,[29] for maize stalk residues supplemented with maize flour and wheat bran for the cultivation of *P. pulmonarius*.

V. CONCLUSION

The various utilized substrates affected mycelia ramification rate, length, weight and productivity of *Pleurotus ostreatus*. The sawdust and wood ash treatments generally performed better than every other treatment utilized.

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