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Growth Influence of Some Additives on the Mycelial Growth and Fruit Body Development of Pleurotus Ostreatus (Jacq. Et. Fr.) Kummer

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Abstract
Studies were conducted to establish the response of a wood – rotting fungus, *Pleurotus ostreatus* to different growth substrates (sawdust (SD), dry banana leaves (BL) and a combination of both) and additives [groundnut cake (GC) and palm kernel cake (PKC)]. Spawning (Mycelia running rate) and fruit body development (area of pileus, length of stipe and fresh weight) were assessed. Initiation of mycelial growth took place in both treated and untreated substrate samples within 7 days post inoculation (dpi). Spawn run was delayed in SD substrates treated to PKC beyond 2% and in BL substrates treated with 4% PKC and above. In BL+SD, spawn run was delayed at all levels of PKC treatments. Spawn run in the BL, BL+SD and SD controls were completed in 21, 28 and 35 days respectively, following inoculation. Primordia production occurred in all the samples within 7 days of transfer of bags to the cropping room. Treatment of substrates with additives (GC and PKC) negatively affected the performance of the substrates. Addition of 4% of the additives on BL and BL+SD substrates produced mushrooms with smaller pileus size with the smallest (13.08cm^2) observed on 4% PKC-treated BL substrates. Similar trends were observed in length of stipe, fresh weight and biological efficiency of the mushroom. All substrates evaluated supported production of fruit bodies for 70 days making a total of 14 flushes from the inception of cropping.

Key words: Additives, Mycelial growth, fruit body production, *Pleurotus ostreatus.*

1. INTRODUCTION

Agro-industry and the wood industry generate enormous amount of wastes yearly which are either burnt or dumped in places that constitute hazard to humans and the environment. The wise use and recycling of waste will not only minimize health hazards in the environment but can serve to generate wealth. Chang and Miles (1989) believe that using only 25% of the yearly volume of burnt cereal straws in the world for mushroom production could result in a mushroom yield of 317 million metric tons (317 billion kg) of fresh mushrooms per year. It is also the belief of Poppe (2004) that about 360 billion kg of fresh mushrooms would have been produced from the estimated 600 billion kg of dry wastes generated from agriculture (500 billion kg) and forestry (100 billion kg) within a year. Such level of mushroom production will directly translate to 60kg of mushroom per head per year which will automatically provide the 4% protein content of mushroom to them. *Pleurotus ostreatus* is a species of the mushrooms belonging to the group commonly called oyster mushrooms. Like many of the members of the Pleurotaceae, *P. ostreatus* is a primary decomposer of hardwood trees and are distributed worldwide (Kong, 2004).

Of the over 70 species of Pleurotus, *P. ostreatus* is the most important and most commercially viable because of the compatibility of its growth requirements with the prevailing environmental factors of most regions of the world. However, different *Pleurotus species* require varying environmental conditions for optimal growth. For *P. ostreatus*, the optimum temperature range for spawn run is put at between 25-35^{0}C, primordia formation occurs at temperature between 10 and 15^{0}C while production of fruit bodies is accomplished between 10 to17^{0}C.

Kong (2004) reported that *P. ostreatus* requires a sharp drop in temperature usually referred to as 'cold shock' for primordia formation to be initiated. Co_2 requirement is put at < 1,000 and the suitable methods of cultivation ranges from shelf cultivation through cultivation in boxes, in bottles and in bags (Kong, 2004). Substrate is a major component of mushroom cultivation as it forms the nutritional source and the substratum on which the mushroom is anchored. Among the commercially viable mushrooms known, only

Pleurotus spp. have been reported to be capable of utilizing the largest variety of wastes with its fast mycelial growth and multilateral enzyme system capable of degrading nearly all types of wastes (Poppe, 2004). Worldwide survey on oyster mushroom substrates revealed about 200 different types of substrates reported to effectively support the growth of *Pleurotus spp.* Among the listed substrate materials, are dry banana leaves and sawdust. These materials do not produce optimum mushroom growth in isolation; other substances are usually added to them to boost their growth-support potentials. Such materials are called additives. Most additives are organic materials such as flours of maize, wheat, rice, cowpea, horse gram (Renganathan *et al.*, 2008) and, poultry dung, rice bran, palm kernel cake (PKC), Groundnut cake (GC) and cow dung (Fasidi, 2006). This study is therefore undertaken to assess two additives for growth performance of *Pleurotus ostreatus* on sawdust and banana leaves substrates.

2. MATERIALS AND METHODS

2.1 Collection of materials

Dry banana leaves were collected from the University of Calabar farms. Sawdust was obtained from Government-owned timber market at Marian Hill, Calabar. Palm kernel cake was obtained from Federal Institute of Industrial Research (FIIRO), Oshodi, Lagos State, Nigeria and groundnut cake collected from hausa traders at Bogobiri, Calabar Municipality, Calabar, Cross River State. The spawn was donated by Zartech farms, Ibadan, Oyo State, Nigeria. Rice bran was collected from private-owned rice mill in Itu Mbonuso, Ini L. G. A., Akwa Ibom State, all in Nigeria.

2.2 Substrate composition

Three substrates were composted namely: sawdust, dry banana leaves and sawdust + dry banana leaves. Sawdust was allowed to ferment for about 30 days. During this period it was turned regularly at 7 days interval. The fermented sawdust was soaked for 24 hrs. The banana leaves were shredded into bits of about $2cm^2$ and soaked for 12 hrs. These substrates were transferred to sac bags and drained of water to a moisture level of 70% using cassava press. Fifty kilograms (50kg) of each of banana and sawdust substrates were mixed with 0.4kg of $CaCO_3$ and 10kg of rice bran. Pasteurization was achieved in sac bags using metal drums of about 500litres capacity with a wooden platform of about 35cm high within at $60\text{-}80^0$C for 6 hrs (Fasidi, 2006). Initial water level in the drum was 30cm from the bottom of the drum. The pasteurized substrates were allowed to cool for 12 hrs, and then were divided into 3.5kg portions representing the content of each mushroom bag. Each of these portions was mixed with 2%, 4% or 6% of either GC or PKC separately before bagging. Bagging was done in an axenic condition. Plastic bags measuring 30x25cm were used. Each bag was inoculated with 5g of *Pleurotus ostreatus*. The open end of each bag was secured with a PVC pipe 3cm long and 3cm in diameter wrapped with a rubber band and plugged with cotton wool.

2.3 Spawn running

The bags were hanged with ropes serially from the roof down with each line carrying a maximum of five bags. Spawn running room was allowed limited light. Room temperature was about 30^0C and relative humidity between 65 and 75% achieved by spraying the compost bags and walls of the spawn running room 2 to 3 times daily with clean water (Fasidi, 2006).

2.4 Cropping and harvesting

At the end of spawn run, the bags of mycelial colonized substrates were transferred to the cropping room stacked on bamboo shelves and opened. This set up was sprayed with clean water after every 6hrs. The floor of the cropping room was covered with coarse sand from the sea bed and continuously moistened. The walls of the mushroom house were wetted regularly to humidify the environment to about 70%. On fruit bodies' formation and maturity, data were taken on area of pileus, length of stipe using conventional

methods. Fresh weight was taken using Agilent electronic balance. Data were analyzed using SPSS version 14.0.

$$\text{Biological efficiency (\%)} = \frac{\text{Total weight of the fruit bodies}}{\text{Total weight of substrate (compost)}} \times \frac{100}{1}$$

3. RESULTS

3.1 Spawn run

Result from the study revealed that initiation of mycelial growth took place in both treated and untreated substrate samples within 7 days post inoculation (dpi). However, completion of spawn run was delayed in SD substrates treated to PKC beyond 2% and in BL substrates treated with 4% PKC and above and in BL+SD at all levels of PKC treatments. Spawn run in the BL, BL+SD and SD controls were completed in 21, 28 and 35 days respectively, following inoculation (data not shown). The uniformity in the initiation of mycelial growth in all the substrates may stem from the low hydrolase activity reported of *P. ostreatus* during substrate colonization (Elisashvili *et al.*, 2008). The implication is that the level of breakdown of substrate materials for the release of nutrients is low or non-existent within this short period of 7 days, hence nutrients in the additives may have not been released and consequently not available to the mycelia and hence had no influence on the initial growth of the mycelia. The variation in the spawn run rate between the treated substrate samples may have resulted from the differences in the chemical composition of both the substrates and additives and the rate at which these materials were hydrolyzed. Guillen-Navarro *et al.* (1998) linked the production of mycelial biomass to the hydrolytic enzymes and availability of glucose in the substrate of cultivation. Variations in the speed of colonization (spawn run) by mycelia of different species of Pleurotus cultivated on different substrates supplemented with various additives have been reported (Stanley and Awi-Waadu, (2010), Renganathan *et al.*, 2008, Pathmashini *et al.*, 2008). However, the negative correlation observed between additive supplementation of substrates and the mycelial growth of *P. ostreatus* is in contrast with an earlier report (Renganathan *et al.*, 2008) on impact of additives on the growth of mushrooms. The contrast noted may be a function of the type and availability as well as quantity-dependent interactions between the nutrient content of the additives and that of the substrates. Chemical content of palm kernel cake (PKC) revealed the presence low amounts of nutrients and particularly, proteins reported to be lowest in PKC among the seed cakes in Sub Saharan Africa (FAO, 1984). This may have contributed to the reduced growth performance on PKC supplemented substrates.

3.2 Cropping

Primordia production in all the substrates took place within the first seven days of transfer of the bags to the cropping room, however, the earliest (4 days) was in the controls of the three substrates and the last (7 days) to produce primordial was in PKC- treated SD substrates. There were a total of 14 flushes within 10 weeks with a significant decline in growth parameters from the 8^{th} week of harvest, especially in mushrooms cultivated on PKC-treated SD substrates.

Assessment of the influence of substrates and additives on the pileus (cap) area of the test mushroom revealed that areas of mushroom cap were larger in untreated substrate samples compared with those produced on treated units (Table 1). The largest pileus area ($40.64cm^2$) was recorded in mushrooms produced on BL substrates (control), while SD substrates (control) supported mushrooms with the least pileus area ($24.82cm^2$). The disparity observed in the size of pileus produced on BL and SD substrates is likely a function of nutrient availability from the materials of the two substrates. Sawdust contains high amounts of lignin (a complex carbon source highly resistant to enzymatic degradation) than cellulose (a compound easily broken down to glucose which is the form taken in by the fungus as nutrient) and vice versa (Choi, 2004), hence glucose may have been readily available to the fungus grown on banana leaves substrates than those on sawdust. However, there were no significant difference ($P<0.05$) in pileus areas on SD substrates treated to either additives at all levels of concentration. Generally, addition of 4% of the additives on BL and BL+SD substrates produced mushrooms with smaller pileus size with the smallest

($13.08cm^2$) observed on 4% PKC-treated BL substrates. This negative impact of additive on pileus size is probably consequent upon its influence on the mycelial growth (Baysal *et al.,* 2006). When the performance of the additives were considered irrespective of the level of concentration, it was discovered that PKC supplemented sawdust substrates recorded a slightly significantly ($P<0.05$) larger pileus area than those grown on other substrates. This suggests that, concentration of PKC was the major determinant of the vigor of growth when added to sawdust substrates. BL and BL+SD substrates performed better when they were supplemented with GC+PKC. This result points to the fact that the presence of GC may have improved on the nutrient status of the substrate. Groundnut has been reported to be high in nutrients such as protein (25.2%), oil (4.8%) and 4.5% soluble sugars (Jambunathan, 1991). The additives did not have any significant effect on the length of stipe, irrespective of the substrate used except marginal differences observed in mushrooms produced on PKC supplemented SD substrates and in BL substrates supplemented with zero and 2% GC+PKC (Table 2).

There were no significant differences observed in the length of stipe produced on the test substrates supplemented with different levels of concentrations of the two additives. However, the length of stipe produced on all the treated substrates were significantly ($P<0.05$) lower than those produced on untreated substrate samples (Table 4). This suggests that the growth and development of stipe was not influenced by the concentration levels of the additives, but by the very presence of the additives in the substrates.

The influence of additives and substrates on the fresh weight of the test mushroom was assessed. A significant ($P<0.05$) decline in the fresh weight of *P. ostreatus* was observed when PKC was added to sawdust and when the same substrate was supplemented with GC+PKC beyond 2%. The decline in growth observed seems to be influenced by the introduction of PKC. The low protein status of this supplement may be the determining factor. FAO (1984) reported PKC to contain the lowest protein level among the oil seed cakes in Sub-Saharan Africa. On banana leaves substrates, the highest fresh weight of the test mushroom was recorded on the untreated samples. Treatment with additives did not encourage fresh weight accumulation in the mushroom cultivated on BL alone. However, when this substrate (BL) was supplemented with sawdust (BL+SD) and treated with PKC or GC+PKC, the mushroom recorded weight increases at all levels of the additives concentration (Table 5). The banana leaves sawdust composite (BL+SD) seems to be compatible with the additives when compared with sawdust or banana leaves alone. The reason for positive growth support compatibility of the additives with BL+SD substrates is likely resultant from the high cellulose content of banana leaves (which makes up for the highly lignolytic nature of sawdust) (Choi, 2004), and the high nutritional content of groundnut cake (FAO, 1984) which makes up for the low nutrient status of PKC. The difference between the mean fresh weight of mushroom produced on untreated (no additives) samples and those obtained from additive-supplemented substrates were in the range of 1.12 to 14.35g (Table 6). The weight differences were smaller in samples containing BL and GC but higher in sawdust-containing samples treated to PKC alone as the additives. The result obtained for fresh weight is as expected since the fruit body yield is largely dependent on the performance of the fungus at mycelial stage and primordia formation (Baysal *et al.,* 2006).

Irrespective of the concentration of the additives, the mean fresh weight of mushroom produced on sawdust treated to PKC (21.74g) and GC+PKC (22.21g) at the end of 10 weeks of harvest were not significantly different ($P<0.05$). However, the mean fresh weight produced on banana leaves substrate treated to GC+PKC was significantly ($P<0.05$) higher than the value obtained for samples treated with only PKC. Similar trend was observed in banana leaves + sawdust substrates (Table 7). The highest mean fresh weight (29.87g) of mushroom was recorded on banana leaves + sawdust substrates treated with GC+PKC while the lowest mushroom weight was seen on the sawdust substrate treated to GC+PKC. Biological efficiency (BE) of the three substrates treated with PKC and GC+PKC were assessed. Results showed that mushrooms grown on substrate samples without additives treatment recorded higher mean values of biological efficiency compared with values obtained on treated samples except for BL+SD substrates which recorded comparable BE values at all levels of additives treatment . Sawdust substrates treated with 2% GC+PKC and 6% PKC still recorded higher BE values. Generally, sawdust or banana leaves substrates supplemented with 4% and above of PKC were not efficient in the cultivation of *P. ostreatus* (Table 8).

All growth parameters of *Pleurotus ostreatus* negatively correlated with PKC and GC + PKC supplemented sawdust substrates. Sawdust substrates were more biologically efficient when they were supplemented with 2% GC+PKC. Sawdust or banana leaves substrates supplemented with 4% PKC and above were not efficient in supporting the growth of *P. ostreatus*. Though, substrates were not supplemented with GC only, it is likely that supplementation of sawdust and banana leaves substrates alone or BL+ SD composite with GC alone may produce better results. Substrate supplementation with organic and/or inorganic substances often boosts mushroom production (Quimio *et al.*, 1990, Renganathan *et al.*, 2008, Adejoye and Mesewonrun, 2009). This is, however, true but with some reservations. The findings of this study revealed that substrate supplementation can only be result-oriented when the nutrient status of both the substrates and the supplement (additive) is considered complementary, and the additives' concentrations adequate. This fact should be borne in mind by mushroom farmers, especially when PKC is the additive and sawdust or banana leaves the growth substrates.

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Table 1: Influence of substrates and additives on area of pileus of *Pleurotus ostreatus.*

Concentration (g/kg)	Substrate (kg) /Additives (g) and Area of Pileus (cm^2)					
	SD		BL		BL+SD	
	PKC	GC+PKC	PKC	GC+PKC	PKC	GC+PKC
0	24.82	24.82	40.64	40.64	35.58	35.58
2	17.48	24.49	28.36	27.12	16.61	23.73
4	27.27	27.86	13.08	23.99	19.72	19.70
6	28.32	19.48	14.32	23.67	18.77	23.44
LSD	1.24		1.17		1.34	

SD = sawdust, BL = banana leaves, PKC = palm kernel cake, GC = Groundnut cake.

Table 2: Comparative influence of PKC and GC+PKC supplemented Sawdust, Banana leaves and Banana leaves + Sawdust substrates on the area of pileus of *Pleurotus ostreatus.*

Substrates	**Substrate (kg) and Area of Pileus (cm^2)**		
	SD	BL	BL+SD
PKC	24.38*	22.78	22.13
GC + PKC	24.16	28.86*	25.61*
t- Value	0.33	0.41	0.36

*Significantly different at P<0.05.
SD = sawdust, BL = banana leaves, PKC = palm kernel cake, GC = Groundnut cake.

Table 3: Influence of substrates and additives on length of stipe of *Pleurotus ostreatus.*

	Substrate (kg) /Additives (g) and Length of Stipe (cm)					
	SD		BL		BL+SD	
Concentration (g/kg)	PKC	GC+PKC	PKC	GC+PKC	PKC	GC+PKC
0 (untreated)	2.66	2.66	3.33	3.33	3.27	3.27
2	2.10	2.74	1.84	3.30	2.45	2.66
4	2.00	2.01	1.73	2.38	2.53	2.72
6	2.00	1.89	2.41	1.97	2.44	2.29
LSD	1.21		1.03		1.15	

SD = sawdust, BL = banana leaves, PKC = palm kernel cake, GC = Groundnut cake.

Table 4: Comparative influence of PKC and GC+PKC supplemented Sawdust (SD), Banana leaves (BL) and Banana leaves + Sawdust (BL+SD) substrates on the length stipe of *Pleurotus ostreatus.*

	Substrate (kg) and Length of Stipe (cm)		
Substrate	SD	BL	BL+SD
PKC	2.19Ns	2.33	2.67Ns
GC + PKC	2.33	2.75Ns	2.74
t- Value	0.21	0.43	0.11

SD = sawdust, BL = banana leaves, PKC = palm kernel cake, GC = Groundnut cake. Ns= no significant difference between treatments.

Table 5: Influence of substrates and additives on fresh weight of *Pleurotus ostreatus.*

	*Substrate (kg) /Additives (g) and Fresh weight (g)					
	SD		BL		BL+SD	
Concentration (g/kg)	PKC	GC+PKC	PKC	GC+PKC	PKC	GC+PKC
0	27.87	27.87	33.75	33.75	31.17	31.17
2	13.91	30.69	15.80	24.12	30.34	35.12
4	21.08	16.67	19.92	15.86	37.38	21.30
6	15.74	13.59	12.46	26.69	25.30	31.89
LSD	1.36					

*values are means of three determinations
SD = sawdust, BL = banana leaves, PKC = palm kernel cake, GC = Groundnut cake.

Table 6: difference in mean fresh weight of *P. ostreatus* produced on additive-treated and untreated mushroom substrates.

	Fresh weight (g)		
Substrates	Untreated	Treated	Difference
SD alone	27.87	-	
SD+PKC	-	22.07	5.8
SD+GC+PKC	-	22.25	5.62
BL alone	33.75	-	
BL+PKC	-	19.40	14.35
BL+GC+PKC	-	25.11	8.64
BL+SD alone	31.17	-	
BL+SD+PKC	-	25.72	5.45
BL+SD+GC+PKC	-	30.05	1.12

SD = sawdust, BL = banana leaves, PKC = palm kernel cake, GC = Groundnut cake.

Table 7: Comparative influence of PKC and GC+PKC supplemented Sawdust (SD), Banana leaves (SD) and Banana leaves + Sawdust (BL+SD) substrates on the fresh weight of *Pleurotus ostreatus.*

	Substrate (kg) and Fresh weight (g)		
Substrate	SD	BL	BL+SD
PKC	19.65ns	20.48	31.03
GC + PKC	18.461	25.02*	29.87*
t- Value	1.22	1.31	1.14

*significantly different at P<0.05; ns – not significantly different at P<0.05.
SD = sawdust, BL = banana leaves, PKC = palm kernel cake, GC = Groundnut cake.

Table 8: Biological efficiency of *Pleurotus ostreatus* as influenced by different substrates and additives

	*Biological efficiency (%)					
	SD		BL		BL+SD	
Concentration (g/kg)	PKC	GC+PKC	PKC	GC+PKC	PKC	GC+PKC
0	7.47	7.96	9.12	9.64	7.71	8.91
2	3.97	8.77	4.51	6.89	8.67	10.03
4	6.02	4.76	5.69	4.53	10.68	6.09
6	3.88	7.35	3.56	7.63	7.23	9.11

*Values are computed from means of 14 flushes.
SD = sawdust, BL = banana leaves, PKC = palm kernel cake, GC = Groundnut cake.