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GROWTH PERFORMANCE OF *Pleurotus ostreatus* (Jacq. et. Fr.) Kummer ON DIFFERENT SUBSTRATES TREATED WITH USED AUTOMOBILE ENGINE OIL

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ABSTRACT

The successful cultivation of edible mushroom is, among other factors, dependent on the type and composition of the growth substrate. An investigation on the growth response of *P. ostreatus* to different substrates treated with used automobile oil was conducted in Calabar, Cross River State, Nigeria. The test mushroom was cultured on three substrates [Dry banana leaves (BL), sawdust (SD) and Dry banana leaves/sawdust composite (BL/SD)] treated to six levels of concentration (0ml, 20ml, 40ml, 60ml, 80ml, 100ml per 3.5kg of substrates) of used automobile engine oil. Initiation of mycelial growth was within 7 days post inoculation (dpi) in all the tested substrates. Total colonization of untreated substrates was achieved in BL, BL/SD and SD in 21, 28 and 35 days post inoculations. There was a negative correlation between growth response of *P. ostreatus* and concentration of used automobile oil. Of the three substrates, banana leaves/sawdust composite showed greatest resistance to the effect of oil treatment. Generally, the length of mycelia added to the substrate during colonization was highest in the 2^{nd} and 3^{rd} weeks of spawn run. Higher concentrations of used oil produced reduced area of pileus. Pileus areas of $3.37cm^2$ and $45.51\ cm^2$ were produced on banana leaves substrates treated with 100ml and 20ml of spent oil respectively. The number of fruit bodies produced by the oil- treated substrates progressively declined with higher concentrations. Mean number of fruit bodies recorded for SD, BL/SD and BL were 2.60, 5.09, 6.10 and 0.95, 1.04, 0.3 for 20ml and 100ml oil treatment respectively. The result for thickness of pileus and length of stipe followed similar pattern. The fresh weight obtained for the three substrates were significantly different ($P<0.05$) irrespective of the level of oil concentration except at 100ml where the fresh weights of the fruit bodies obtained from BL/SD and SD were comparable ($P<0.05$). All samples treated to oil concentrations beyond 20ml were comparable ($P<0.05$) in the dry weight of *P. ostreatus* obtained.

INTRODUCTION

Pleurotus ostreatus commonly called oyster mushroom is an edible mushroom with high nutritional profile. Cultivated on different lignocellulosic agro-wastes, *P. ostreatus* contains hig amounts of proteins, minerals (Ca, P, Fe, K and Na), vitamins C, B-complex (thiamine, riboflavin, folic acid and niacin) (Patil *et al.,* 2010). They are eaten for their nutritive and medicinal values (Agrahar –Murugkar and Subbulakshmi, 2005). Proteins contained in mushrooms are known to be superior in quality as they contain all essential amino acids (Purkayastha and Nayak, 1981). *P. ostreatus* possesses anti-tumour activity (Yoshioka *et al.,* 1985). Oyster mushrooms are effective against cardiovascular and artery-related disorders through its effective reduction of plasma cholesterol and triglyceride level (Alam *et al.,* 2007) exhibits hypoglycaemic effects in experimentally induced diabetic rats (Chorvathoba *et al.,* 1993) .However, successful cultivation of this mushroom is hampered by some environmental factors, one of which is contamination of substrates (Oei, 2003). Among the contaminants is used automobile engine oil. Following the era of oil boom in Nigeria in the early 1970s, economy of the country improved. This unilaterally translated to a higher standard of living for individuals, especially those in government. This period also experienced a corresponding increase in car ownership. And in the early 1990s where used cars were introduced into the country, a lot of people patronized because of its low cost. This actually flooded the country with cars. Though there was a positive impact on the transportation sector of the economy, the environment suffered the burden of indiscriminate disposal of used automobile oil by local mechanics. Since then, this has become a common phenomenon in our environment. Used automobile oil is the spent oil drained

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from vehicle engines after a certain period of routine operation. This oil is a serious environmental pollutant. Motor oil undergoes a range of chemical and physical transformations during routine engine operations. During this period, a lot of new and poisonous compounds develop. A comparison of chemical characterization of used and fresh engine oils revealed higher levels and new aliphatic and aromatic hydrocarbon compounds (1, 3, 5-trimethylbenzene, *p*-xylene and methyl ester undecanoic acid) and polycyclic aromatic hydrocarbon (PAHs) in used oil than in the fresh oil. Used oil also contains pollutants and heavy metals like lead (Pb), and zinc (Zn) (Dominguez-Rosado and Pichtel, 2003) which is considered as threats to the environment and public health. The impact of crude oil and heavy metal contamination on the growth and development of mushroom have been reported (Ogbo and Okhuoya, 2009, Oghenekaro *et al.*, 2008). Mushrooms possess enzymes that breakdown complex substances (like crude oil, engine oil and heavy metals) into simpler molecules which they ingest. Bioaccumulation of heavy metals in mushrooms is known to be higher than in agricultural crop plants, vegetables and fruits (Oghenekaro *et al.*, 2008) because of the network structure of their mycelia (Turkekul *et al.*, 2004) and the mode of nutrient absorption mechanism directly across the hyphal cell wall. This is the basis of the effective mycoremediation ability of many mushrooms (Okparanma *et al.*, 2011, Adenipekun and Fasidi, 2005, Oghenekaro *et al.*, 2008). However, mycoremediation studies place fewer premiums on the mycelial and fruit bodies' yield of the mushroom under consideration. This paper aims at evaluating the impact of used automobile oil on the growth (mycelial growth and fruit body production) of *Pleurotus ostreatus.*

MATERIALS AND METHODS

Collection of materials

Dry banana leaves were collected from the University of Calabar farms. Sawdust was obtained from timber market; spawn purchased from Royal farms Ikot Effanga Mkpa and spent engine oil was obtained from mechanic shop along Etta Agbor road both in Calabar Municipal, Government Area. Rice bran was collected from private-owned rice mill in Itu Mbonuso, Ini Local Government Area (L. G. A), Akwa Ibom State. Substrate composition Three substrates were composted namely: sawdust, dry banana leaves and sawdust/banana leaves composite. Sawdust was allowed to ferment for about 28 days. During this period it was turned regularly. 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 composted with 0.4kg of $CaCO_3$ and 10kg of rice bran. For sawdust/banana leaves composite, 25kg each of sawdust and banana leaves was thoroughly mixed with 0.1kg of $CaCO_3$ and 5kg of rice bran added. Pasteurization was achieved using metal drums of about 500litres capacity with a wooden platform of about 35cm high within at 60-80^{0}C for 48 hrs. Initial water level in the drum was 30cm from the bottom. The pasteurized substrates were allowed to cool for 12 hrs before bagging. Bagging was done in an axenic condition. Plastic bags measuring 30x25cm were used. Each bag was inoculated with 5g of *P. ostreatus.* The open end of each bag was secured with 3cm diameter PVC pipe 3cm long, wrapped with a rubber band and plugged with cotton wool.

Spawn run

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 28^{0}C and relative humidity between 60 and 75% was maintained by spraying the compost bags and the spawn running room environment 2 to 3 times daily with cold clean water (Oei, 2003).

Cropping

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 (Madunagu, 1988). 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, girth of stipe and thickness of pileus using thread and meter rule. Number of fruit bodies was obtained by count; fresh weight was taken using Agilent electronic balance. Dry weight was obtained by drying the mushroom to a constant weight in Agilent oven and weighed with Agilent electronic weighing balance. Data were analyzed using SPSS version 14.0.

RESULTS AND DISCUSSION

The initiation of mycelial growth was observed in all the experimental units within seven (7) days post inoculation (dpi). Spawn running was completed in untreated samples (control) of banana leaves/sawdust composite substrate within 21days of inoculation (Fig 1.). The banana leaves substrate was fully colonized in about 7 days later while sawdust substrate was fully covered with mycelia within 35 days post inoculation (dpi) (data not shown).

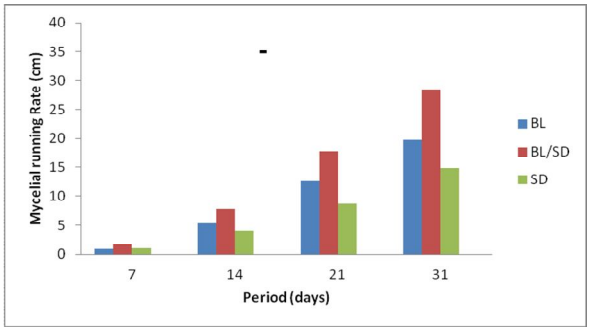

Fig. 1 Mycelial Running Rate (MMR) of *Pleurotus ostreatus* on untreated sawdust (SD), banana leaves (BL) and sawdust/banana leaves composite (BL/SD) substrates after 31 days of incubation. Bar represents LSD (P<0.05).

Spawn run

There was a negative correlation between growth response of *P. ostreatus* and concentration of spent automobile oil during spawn run (Fig. 2). The length of mycelia added was consistently reduced with increase in concentration of spent oil. Mycelia grown on banana leaves/sawdust composite showed significant (P<0.05) capacity to resist the growth reduction effect of the spent oil when compared with the other two substrates. There were only marginal differences in the mycelial growth value recorded for banana leaves substrate with increase in oil concentration

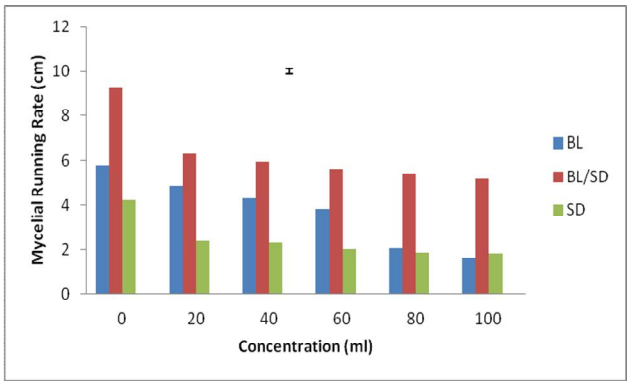

Fig. 2 Mycelial Running Rate (MMR) of *Pleurotus ostreatus* on sawdust (SD), banana leaves (BL) and sawdust/banana leaves composite (BL/SD) substrates treated with various concentrations of spent automobile oil after 5 weeks of incubation. Bar represents LSD (P<0.05).

Assessment of the weekly colonization of the substrate by the mycelia of the fungus showed that colonization was fastest on banana leaves/sawdust composite substrate and slowest on sawdust. The rate of substrate colonization was however comparable (P<0.05) between banana leaves and sawdust substrates only on the first week of incubation (Table 1). Generally, the length of mycelia added to the substrate was greater in the 2nd and 3rd weeks of colonization than in the last (4th) week where there was a significant decline.

Table 1 Weekly assessment of Mycelial Running Rate (MRR) of *Pleurotus ostreatus* cultivated on banana leaves, sawdust and banana leaves /sawdust composite substrates

Substrates	Mycelial Running Rate (MRR) (cm) / Period (weeks)			
	1	2	3	4
BL	8.21	5.21	3.63	1.57
BL/SD	9.65	7.56	4.69	1.09
SD	5.11	4.47	0.28	0.00
LSD	0.14			

Values are means of triplicates

The number of fruit bodies produced by the oil- treated substrates progressively declined with higher concentrations. At 100ml concentration level, banana leaves substrate recorded 12 times less the number of fruit bodies obtained in the untreated samples. Though sawdust substrate produced 7 times less the number of fruit bodies at the same concentration level, the total number of fruit

Table 3 Effect of various concentrations of spent engine oil on the area of pileus of *Pleurotus ostreatus* cultivated on banana leaves, sawdust and banana leaves /sawdust composite substrates.

Substrates	* Area of Pileus (cm^2)/ Concentration (ml)					
	0	20	40	60	80	100
BL	68.51	45.51	22.94	19.84	7.29	3.37
BL/SD	98.42	40.24	43.16	49.68	41.3	27.17
SD	38.23	13.41	17.79	13.15	18.01	7.33
LSD	3.80					

*Values are means of triplicates

Table 4 Effect of various concentrations of spent engine oil on the thickness of pileus of *Pleurotus ostreatus* cultivated on banana leaves, sawdust and banana leaves /sawdust composite substrates.

Substrates	*Thickness of Pileus (cm)/ Concentration (ml)					
	0	20	40	60	80	100
BL	0.91	0.63	0.42	0.45	0.25	0.12
BL/SD	1.78	0.66	0.64	0.64	0.55	0.30
SD	1.27	0.40	0.30	0.29	0.29	0.18
LSD	0.05					

Cropping

Higher concentrations of spent oil produced reduced area of pileus. Banana leaves substrate samples treated to 100ml of spent oil produced mushrooms with pileus area of 3.37cm compared to 45.51cm (15 times the size) recorded in mushrooms treated to 20ml (Table 3). However, the impact was not as great in banana/sawdust composite substrate. Similar results was recorded when the impact of oil on the thickness of pileus (Table4) and length of stipe (Table 5) were assessed. However, spent oil beyond 20ml concentration did not record any significant difference (P<0.05) in the length of stipe of mushrooms produced on the test substrates (Table 5).

Table 5 Effect of various concentrations of spent engine oil on the length of stipe of *Pleurotus ostreatus* cultivated on banana leaves, sawdust and banana leaves /sawdust composite substrates.

Substrates	*Length of stipe (cm)/ Concentration (ml)					
	0	20	40	60	80	100
BL	3.11	2.90	1.65	2.14	0.96	0.39
BL/SD	4.23	3.06	2.30	1.94	1.72	1.00
SD	2.02	1.17	0.81	1.28	1.01	0.65
LSD	2.05					

*Values are means of triplicates

bodies that emerged from the untreated sawdust sample was the least in comparison with other substrates (Table 6). The fresh weight obtained for the three substrates were significantly different irrespective of the level of oil concentration except at 100ml where the fresh weights of the fruit bodies obtained from BL/SD and SD were comparable (P<0.05) (Table 7). Except for samples treated to 20ml oil concentration and the controls, dry weight of *P. ostreatus* obtained by samples treated to other levels of concentration were comparable (P<0.05)

Table 6 Effect of various concentrations of spent engine oil on the number of fruit bodies of *Pleurotus ostreatus* cultivated on banana leaves, sawdust and banana leaves /sawdust composite substrates.

Substrates	*Number of fruit bodies (cm)/ Concentration (ml)					
	0	20	40	60	80	100
BL	12.02	6.10	4.49	4.35	1.95	0.30
BL/SD	16.33	5.09	5.84	3.70	2.20	1.04
SD	7.25	2.60	1.60	1.55	1.45	0.95
LSD	0.48					

*Values are means of triplicates

Table 7 Effect of various concentrations of spent engine oil on the fresh weight of *Pleurotus ostreatus* cultivated on banana leaves, sawdust and banana leaves /sawdust composite substrates.

Substrates	*Fresh weight (gm)/ Concentration (ml)					
	0	20	40	60	80	100
BL	15.12	11.35	11.59	13.14	6.16	2.31
BL/SD	30.38	23.81	20.13	18.06	13.20	7.14
SD	9.97	5.93	3.21	3.76	3.72	2.81
LSD	7.32					

*Values are means of triplicates

Table 8 Effect of various concentrations of spent engine oil on the dry weight of *Pleurotus ostreatus* cultivated on banana leaves, sawdust and banana leaves /sawdust composite substrates.

Substrates	*Dry weight (gm)/ Concentration (ml)					
	0	20	40	60	80	100
BL	5.94	2.85	2.29	2.08	1.27	0.41
BL/SD	9.37	4.77	3.20	3.85	2.68	1.34
SD	3.20	1.41	0.99	0.81	0.79	0.54
LSD	3.10					

*Values are means of triplicates

DISCUSSION

Studies have been undertaken to assess the impact of petroleum products and heavy metals on the growth and development of plants and fungi (Kayode *et al.,* 2009, Ogbo and Okhuoya, 2009, Oghenekaro *et al.,* 2008, Adedokun *et al.,* 2007, Adedokun and Ataga, 2005,). In this study, all the levels of used engine oil concentrations recorded varying growth inhibitory effects on mycelial running rate and on fruit bodies' production of *P. ostreatus* cultivated on the three growth substrates tested. Our results also showed that the growth inhibitory impact of used engine oil was concentration-dependent. Adedokun and Ataga (2005) reported the sensitivity of three *Pleurotus* species and *Lentinus squarrosulus* to different concentrations of Crude Oil (COIL), Automotive Gasoline Oil (AGO), Fresh Engine Oil (ENGOIL) and Spent Engine Oil (SENGOIL) *in vitro.* Their results showed that mycelial growth of *Pleurotus pulmonarius* and *Lentinus squarrosulus* were completely inhibited by ENGOIL and SENGOIL at concentrations beyond 10%. They asserted that the mycelial growth inhibitory effect increased with higher oil concentrations. Oghenekaro *et al.* (2008) showed similar results when they cultivated *P. tuber regium* on heavy metal polluted sawdust substrates. They recorded the lowest mycelial density on copper-contaminated substrates that were inoculated with sclerotia. They noted formation of fruit bodies only on copper treated substrates at 1.0 and 2.0g/250g concentrations which were observed to shrink within seven days of formation. Lead and zinc completely inhibited the growth of the mushroom. However, Adenipekun (2008) reported that *P. tuber regium* caused increased levels of nutrients in soils contamination with 1-40% engine oil within six months of incubation. She recorded increases of 0.78, 0.43 and 0.31% for organic matter, carbon and potassium respectively and observed bioaccumulation of zinc and nickel in mushrooms grown in samples treated to 20% engine oil concentrations. Improvement in the nutrient status of the treated soils did not, however, translate to higher mushroom yield. There is likelihood that the added nutrients may not have been available to the mushroom or may have been above the required level for optimal growth (Oei, 2003). Generally, mushrooms are decomposers. They thrive through extracellular enzymatic degradation of complex organic compounds to release simpler molecules which diffuse across the permeable hyphal wall into the lumen of the hyphae (Nkang *et al.*, 2005). Absorption of poisonous and harmful substances rather than support the growth and development of mycelia and sporophore (fruit body), interfere with the physiology of the fungus hence results in growth inhibition. In addition, oil has the ability to interfere with the permeability of the hyphal wall and hence may reduce the level of diffusion of valuable nutrients from the growth substrate into the hyphal lumen, leading to reduced growth. Studies on the effect of crude oil and engine oil in plants revealed that oil penetrates pore spaces and subsequently impedes photosynthesis and other physiological processes including respiration and water relations causing asphyxiation resulting from exclusion of air (Odejimi and Ogbalu, 2006). The exhaustion of oxygen in the substrate increases the activity of microbes competing for nutrients. This affects growth of the plant. Mushrooms may be similarly affected by the engine oil treatment. The results of this study consistently revealed the negative impact of engine oil on the growth of *P. ostreatus.* Proper disposal or recycling of used engine oil will save the ecosystem from loss of biodiversity. *Pleurotus ostreatus,* as many other mushrooms, is a valuable asset of our ecosystem. It is highly nutritive (rich proteins containing all the essential amino acids and vitamins) and medicinal (with anti-tumour and antioxidant effects) (Patil *et al,* 2010, Alam *et al,* 2008). The protection and preservation of this mushroom species will provide a ready and affordable source of natural protein, vitamins and medicine that could serve as drug-free therapy for nutrition and cardiac related problems currently assuming an alarming proportion worldwide

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