Influence of Different Percentages of Crude Oil Pollution and Substrate Quantities on Primordial Formation, Yield and Biological Efficiency of *Pleurotus ostreatus* (Jacq.Ex.Fr) P. Kumm and *Pleurotus pulmonarius* (Fries) Quel.

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ABSTRACT
The study was conducted to determine the effect of crude oil pollutant and substrate quantity on primordial formation, yield and biological efficiency (B.E%) of P. ostreatus and P. pulmonarius. Crude oil as pollutant was used at 3 levels of 2%, 4% and 6% and control against 2500g of loamy soil while Andropogon gayanus straw was used as the substrate at 150g/kg (4cm) and 300g/kg (8cm) thick layer respectively stuffed into 5lit. transparent plastic buckets perforated with two lateral holes of 5mm diameter from middle to the top. Results showed that different percentage crude oil pollution affected spawn duration by apparently delaying fruit body formation of both oyster mushroom species when compared to control. 300g/kg quantity of substrate supported high yield of P. ostreatus than that of P. Pulmonarius and same trend observed at 150g/kg. Therefore, both oyster species could be adopted in mycoremediation process if substrate is used in right quantity.

Key Words: *Crude Oil, Substrate, Primordial and Pleurotus Spp.*

INTRODUCTION

Mushrooms are the reproductive structures of fleshy macro fungi. They are a unique biota which assembles their food by the secretion of degrading enzymes and decompose the complex food materials present in the biomass where they grow (Chang, 2004). These substrate materials are usually by-products from industries, households, agriculture etc, and are usually considered as wastes (Okwulehie *et al*., 2008). However, these wastes are actually resources in the wrong place at a particular time and mushroom cultivation can harness them for its own benefit (Chang, 2013).

The presence of right proportion of alpha-cellulose, hemicellulose and lignin was the probable cause of higher rate of mycelium running in corncobs and palm cones. Buswell *et al.,* (1993) suggested that the utilization of insoluble ligno-cellulosic substrates by edible mushrooms depends on the production of the enzymes such as cellulases, hemicellulases, ligninases which bring about hydrolysis of the macro molecules of cellulose, hemicellulose and lignin components of the substrate, thereby liberating the low molecular weight nutrients essential for mushroom growth. In another study, saw dust acting as a substrate gave the lowest mycelium running rate, which might be due to presence of different kinds of

polyphenolic substances in them as suggested by Wang (1982) and low content of cellulose (Gohl, 1993). Suitable C:N ratio might be responsible for the higher mycelia growth in corncobs and palm cones. Quimio and Sardsud (1981) reported similar results, whereby the optimum days to complete mycelium running in spawn bags ranged 21.00 days to 24.06 days on different substrates. Groundnut hull had the least oyster mushroom yields which is contrary to observation by Poppe (1995) who reported that legume straws, mostly rich in N, was suitable as *Pleurotus* substrates. Maize stalk had the least biological efficiency of 39.88± 4.59, which was lower than maize straw biological efficiency of 52% for *Pleurotus sajor-caju* (Pani *et al.*, 1997). Cereal straw has 0.5% total N, 38% cellulose, 15% lignin, C/N = 90 (Kaul *et al.*, 1981), which suggest that basic substrate for oyster mushroom may need to be enriched with different additive to maximize production. Royse *et al.*, (2004) suggested that physical processing of substrates' material.

The beneficial impacts of mushrooms on the environment have attracted the attention of many scientists in recent time. Crude oil spillage on the environment constitutes a serious pollution problem. This can threaten human health as well as beneficial organisms in the environment (Aboribo, 2001). Increasing petroleum exploration, refining and operation petroleum companies in the Niger Delta region of Nigeria have led to the wide scale contamination of most of its soils, creeks, swamps, rivers and streams.

Petroleum hydrocarbon has been shown to improve the growth of white rot fungi in contaminated soil by increasing their size and yield indicating that the pollutant has a fertilizer effect (Isikhuemhen *et al*., 2003; Stamets, 2005).

The success of white rot fungi in crude oil contaminated site is well documented but the effect on the chemical composition of the edible mushroom with reference to its nutritional status and consumption safety should also be considered.

This research therefore aims at understanding the effect of crude oil pollutant and substrate layer on the primordial formation, yield and biological efficiency of *P. ostreatus* and *P. pulmonarius* species.

MATERIALS AND METHODS

STUDY AREA

The study was conducted at the screen house of the Michael Okpara University of Agriculture Umudike, Abia State. Umudike is located between longitude 7^0 and 70^005^0E and latitude 5^0 and 5^025^0N; with humid tropical climate. Rainfall is bi-modally distributed with peaks between July and September of each year. Annual rainfall is approximately 170mm, spread between April and November each year (Achufusi, 2016)

SOURCE OF CULTURE AND SPAWN MULTIPLICATION

Pure mycelia culture of *P. ostreatus* and *P. pulmonarius* were obtained and multiplied at the department of biotechnology, Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos State. Spawns were produced using sorghum grains. Sorghum grains were washed and soaked in tap water overnight. Grains were further boiled in same tap water in the ratio of 1:1 (sorghum grain: water) for 15mins, using the industrial cooking gas as a local heat source. 4% (w/w) $CaCo_3$ and 2 % (w/w) $CaSO_4$ were added to optimize pH and prevent clumping of grains respectively as described by Muhammad *et al.*, (2007). Completely drained Sorghum grains were stuffed in glass Bama bottles tightly sealed with Aluminium foil and before being sterilized in an autoclave at 121^oc for 30mins. After sterilization, the bottles were allowed to cool, before they were inoculated with actively growing mycelia of *P. ostreatus* and *P. pulmonarius* by grain-to- grain transfer and incubated in the dark at (27 ± 2^oc) for 10-15days until the grains were fully colonized by mycelia. (Shyam *et al.*, 2010).

SOURCE OF POLLUTANT (CRUDE OIL)

The Bonny Light crude oil was obtained from the Nigerian National Petroleum Co-operation. (N.N.P.C.), Port Harcourt.

BUCKET PREPERATION

Five litre (5lits.) transparent plastic buckets were used during this investigation. The upper half of each 5-liter plastic bucket was perforated, with two lateral holes of 5mm diameter Okulehie and Okwujiako (2008).

SOURCES OF SOIL AND SUBSTRATE (PREPARATION)

Source and Soil Preparation

Loamy soil sample was collected from a farmland within the vicinity of Government College Umuahia, Abia State. Soil sample was collected from the 'A' horizon (0-25 cm). To obtain a semi-sterile soil, the soil was treated in three successive pasteurization periods of 2hrs each, for two consecutive days, following the modified method of Kristanti *et al* (2011). The moisture content of the soil was adjusted to 60% of maximum water-holding capacity (WHC; 0.2ml/g dry soil) after each pasteurization.

Source and Substrate Preparation

Andropogon gayanus, a locally available straw substrate was obtained from a farmland in Umudike. The grass was dried and chopped into about 1 - 2cm lengths before steeping it in tap water overnight to ensure adequate moisture content (Sharma, 2003).

The soaked substrate was drained of excess water before being transferred into a metallic drum for pasteurization at 80^0C for 2hrs and was allowed to cool overnight as recommended by (Muhammad *et al.*, 2007).

CRUDE OIL POLLUTION

Each perforated plastic bucket contained 2.5kg of pasteurized soil polluted with 50g, 100g and 150g w/v of crude oil to make 2%, 4% and 6% crude oil pollution of the soil.

EXPERIMENTAL PROCEDURE

The experiment was conducted using 2.5lit plastic bowels. Three levels of crude oil pollutant at 50g, 100g and 150g w/v was used to homogenized each 2500g of the prepared soil sample to make 2%, 4% and 6% respectively. The various levels of crude oil polluted soil (%PS+M) were poured into each perforated transparent plastic bowel while the control (–PS+M) was not polluted with crude oil. All the crude oil treatment levels including control were made up of two groups. The first group consist of 4cm (150g) thick layer of prepared *A. gayanus* substrate placed on the surface of the crude oil polluted soil and inoculated with 30g of grain based spawn of actively growing mycelia of *P. ostreatus* and *P. pulmonarius* while the second has 8cm (300g) thick layer of same substrate and was inoculated with 60g grain based spawn of same mushrooms according to a modified method of Okwulehie and Okwujiako (2008).

The spawn-inoculated substrate served as a mycelia mat on the surface of the crude oil polluted soil, contained in the buckets according to the method of Fatuyi (2014).

After spawn inoculation, spawn run was completed in the dark by covering the inoculated buckets with thick non-transparent polythene mat, until the substrate is fully colonized by the mycelia. Primordial initiation was preceded by fruit body maturity before mushrooms were finally harvested.

DETERMINATION OF SPAWN RUN DURATION

Spawn run duration of each experimental group was determined by counting the number of days taken for mycelia to completely colonize the substrate, which was preceded by primordial initiation.

DETERMINATION OF YIELD AND BIOLOGICAL EFFICIENCY

Total fresh weight (g/kg) of all the fruit bodies harvested from each of the 5 replications was measured as the total yield of each mushroom species. The biological efficiency (BE) ie, the percentage yield of fresh mushroom fruit bodies per dry weight (g/kg) substrate was calculated using the formula recommended by Chang and Milles (2004b), viz:

$$B.E = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times \frac{100}{1}$$

STATISTICAL ANALYSIS

The results obtained were statistically analysed using analysis of variance (ANOVA), and tests of significance carried out by Duncan's multiple range test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Table 1: Effect of percentage crude oil pollution and 4cm substrate layer on the spawn run duration of *P. ostreatus* and *P. pulmonarius* fruit bodies

% PS + M	*P. ostreatus*/days	*P. pulmonarius/days*
2% PS + M	14^{b}	16^{b}
4% PS + M	20^{a}	17^{a}
6% PS + M	14^{b}	15^{c}
-PS + M	11^{c}	15^{c}

PS + M = Polluted Soil and Mushroom, values are yields from 5 replicates.

Table 2: Effect of percentage crude oil pollution and 8cm substrate layer on the spawn run duration of *P. ostreatus* and *P. pulmonarius* fruit bodies

% PS + M	*P. ostreatus*/days	*P. pulmonarius*/days
2% PS + M	13^{b}	15^{c}
4% PS + M	15^{a}	16^{b}
6% PS + M	12^{c}	18^{a}
-PS + M	11^{d}	14^{d}

PS + M = Polluted Soil and Mushroom, values are yields from 5 replicates.

Table 3: Effects Of Percentage Crude Oil Pollution And 4cm Substrate Layer (g/Kg) On The Yield (g/Kg) And Biological Efficiency (BE%) of *P. ostreatus*.

% PS + M	D/Wt substrate (g/kg)	Yield (g/kg)	BE (%)
2% PS + M	750	214.95^{b}	28.66^{b}
4% PS + M	750	33.40^{c}	4.45^{d}
6% PS + M	750	52.51^{c}	7.00^{c}
-PS + M	750	363.32^{a}	48.44^{a}

PS + M = Polluted Soil and Mushroom, BE = Biological Efficiency values are yields from 5 replicates.

Table 4: Effect Of Percentage Crude Oil Pollution and 8cm Substrate Layer (g/Kg) On Yield (g/Kg) And Biological Efficiency (BE%) Of *P. Ostreatus.*

S/N	% PS + M	D/Wt substrate (g/kg)	Yield (g/kg)	BE (%)

1	2% PS + M	1500	390.51[b]	26.03[b]
2	4% PS + M	1500	219.37[d]	14.62[d]
3	6% PS + M	1500	298.46[c]	19.87[c]
4	-PS + M	1500	819.12[a]	54.61[a]

PS + M = Polluted Soil and Mushroom, BE = Biological Efficiency values are yields from 5 replicates.

Table 5: Effect of Percentage crude oil pollution and 4cm Substrate layer (g/kg) on yield (g/kg) and Biological Efficiency (BE%) of *P. pulmonarius* fruit bodies.

% PS + M	D/Wt substrate (g/kg)	Yield (g/kg)	BE (%)
2% PS + M	750	22.52[d]	3.00[d]
4% PS + M	750	28.95[c]	3.86[c]
6% PS + M	750	39.52[b]	5.27[b]
-PS + M	750	46.86[a]	6.25[a]

PS + M = Polluted Soil and Mushroom, BE = Biological Efficiency values are yields from 5 replicates.

Table 6: Effect of Percentage crude oil pollution and 8cm Substrate layer (g/kg) on yield (g/kg) and Biological Efficiency (BE%) of *P. pulmonarius* fruit bodies

% PS + M	D/Wt substrate (g/kg)	Yield (g/kg)	BE (%)
2% PS + M	1500	220.60[b]	14.71[b]
4% PS + M	1500	190.02[c]	12.67[c]
6% PS + M	1500	27.58[d]	1.84[d]
-PS + M	1500	304.14[a]	20.28[a]

PS + M = Polluted Soil and Mushroom, BE = Biological Efficiency values are yields from 5 replicates.

Results of the experiment conducted to determine the influence of crude and substrate quantity on the spawn run duration, productivity and biological efficiency of *P. ostreatus* and *P. pulmonarius* is presented and discussed as follows.

Table 1 represents the effect of crude oil and 150g/kg dry weight substrate on the run duration of both p ostreatus and p. pulmonarius. Effect of 2% crude oil concentrations differs significantly on *P. ostreatus* (14 days) and *P. pulmonarius* (16 days) ability to initiate mushroom primordial on 150g/kg substrate. At 4% level of crude oil pollution, *P. ostreatus* and *P. pulmonarius* initiated primordial within 20 days and 17 days respectively while at 6% level, there seem to be quicker response to pinhead formation compared to 4%. –PS+M i.e., control also produced mushroom primordial within 11 days of spawn inoculation may workers have argued that the interference of chemical compound soil such as crude oil, could delay fruit body formation while others believe that rather than delay, crude oil could enhance early fruit body formation hence the use of mushroom in cleaning polluted environment (Adenipekun, 2008).

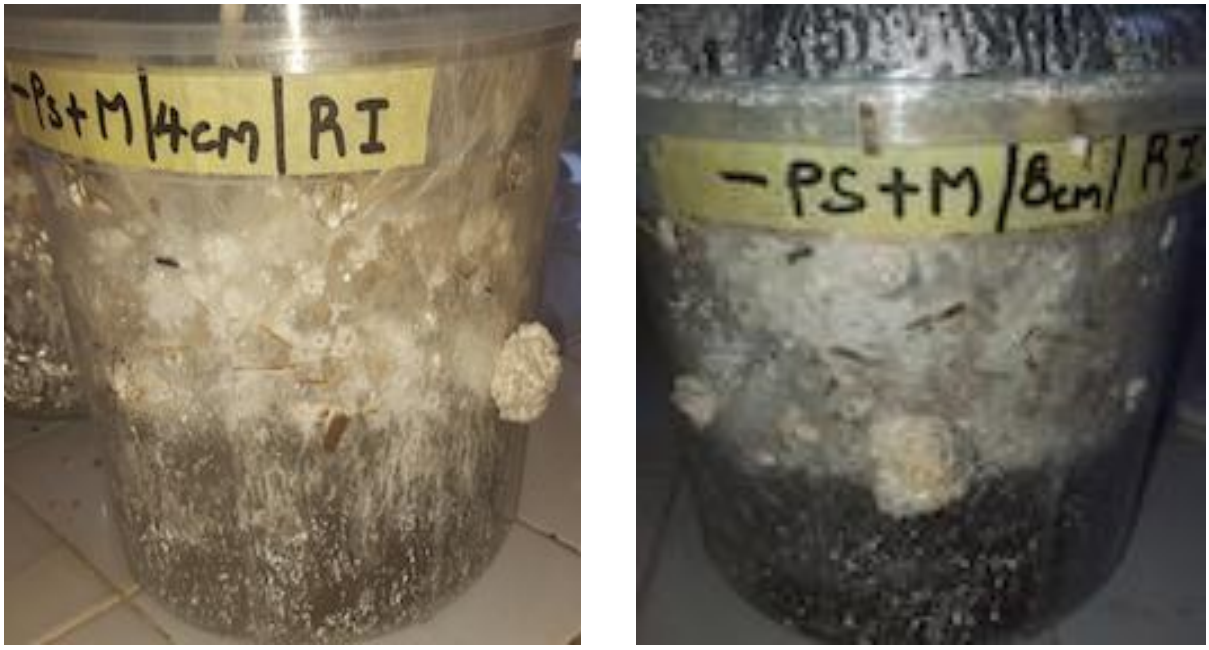

Plate 1: Fruit body Primordial formation of *P. ostreatus* visible within 14 days on non-polluted Soil.

Shah *et al* (2004) recorded minimum and maximum spawn run duration of 16 and 25 days. Tan (1981) observed primordial formation within 2 and 3 weeks, Ahmad (1986) observed pinhead formation within 17-20 days when there was no crude oil interference.

Plate 2: Fruit body Primordial formation of *P. ostreatus* visible within 14 days on polluted Soil.

These are clear indications that oyster mushrooms thrive on and derive nutrients from crude oil there by conforming to the works of Okwujiako *et al* (2013). However, the control of this experiment could not justify their claims, but shows that crude oil inference delayed pinhead formation in *p. ostreatus* and *p. pulmonarius*.

Table 2 shows the effect of crude oil pollution and 300g/kg of substrate on both oyster mushroom species. At 2% level of crude oil pollution, primordial of both p. ostreatus and p. pulmonarius visible within 13 and 15 days respectively. 4% level of crude oil was higher at 15 and 16 days for p ostreatus and p. pulmonarius respectively like in table 1, 6% crude oil level favoured p. ostreatus within 12days, but delayed *P. pulmonarius* up to 18 days before pinhead could emerge control was the first among other treatment groups to the treatment groups to produce *p. ostreatus* and *P. pulmonarius* primordial after 11 and 14 days respectively. Comparing all the treatment groups with their control allies in table one. One

could observe that substrate quantity greatly affected spawn run duration on the decreasing trend and agrees with the findings of Assan and Mpofu (2014). However, environmental factors such as humidity, temperature, air etc can also affect primordial formation in oyster mushrooms (Mohammad *et al*, 2010).

Table 3 represents the effect of crude oil pollution and substrate quantity on the yield and biological efficiency of P. ostreatus control gave the highest(363.32) yield and B.E (48.445) for p. ostreatus followed by 2% at 214.95g/kg and 28.66% for yield and B.E respectively. At 4% level of crude oil pollution, P. ostreatus gave the lowest yield (33.40 g/kg) and BE (4.45%). 6% crude oil pollution encouraged fruit body production compared to 4%. The reason for decrease in sporophore production has not been well understood as the treatments were given under the same condition. Considering the control, one clear observation in the result here is that crude oil pollution may have contributed to reduction in yield indicating that mycelia colonization may have been more vigorous in the experiment with no crude oil treatment, hence the reason for earlier fruiting (table 1and 2) and higher yield which does not conform with the works of Okwujiako *et al* (2013), Adenipekun and Isikhumhen (2008) who recorded higher mycelia colonization as well as increased fruit body yield with mushroom grown on higher levels of hydrocarbon pollutants.

Result representing the effect of percentage crude oil pollution and 300g/kg substrate quantity on p. ostreatus is shown in table 4. According to the result, substrate quantity significantly affected yield and biological efficiency of p. ostreatus fruit bodies when compared to the results in table 3 (Assan and Mpofu, 2014). In confirmation of the observation that crude could have probably, contributed to the reduction in mycelia colonization (table 3), it is equally clear that control supported the highest fruit body yield and BE of 819.12g/kg fresh weight and 54.61% respectively. For further confirmation, the lowest crude oil concentration (%) gave second to the highest fruit body yield and BE (390.5g/1kg and 26.03%) respectively and the trend in yield continued to reduce with increase percentage crude oil concentration and disagrees with the reports of Ukoima, (2017), Adenipekun (2008), Okwujiako, *et al* (2013) who in their separate investigations maintained that hydrocarbon compounds may have contributed significantly to the growth and yield of oyster mushrooms mycelia and sporophores respectively.

Therefore the increase in fruit body production with it attendant increase in substrate quantity observed in the comparison of both tables 3 and 4 may suggest more efficient utilization of insoluble lingo-cellulosic materials which hinges on the production of more enzymes such as cellulose, hemicellulase and lignase which cause hydrolysis of their respective macro molecules which they act on (Assan and Mpofu, 2014).

Table 5 shows the effect of percentage crude oil pollution and 150g/kg on yield and BE of p. pulmonarius with respect to the substrate. The result shows that control still maintains the highest yield compared to other treatment levels (tables 3 and 4), but show a contrasting trend in yield and BE at 2%, 4% and 6% compared to table 4. As percentage crude oil pollution increased from 2%- 6%, yield and BE of p. pulmonarius sporophores increased from 22.52g/kg, 3.00% and which gave the highest (46.86g/kg and 6.25%) yield and BE. This implies that p. pulmonarius grown on 150g/kg quantity of substrate produces more fruit bodies with high concentration of crude oil and this attribute suggests it a suitable fungus the remediation of polluted environment (Okwujiako *et al*, 2013 and Onyeizu, *et al*, 2017).

Considering the high effect of percentage crude oil pollution and 300g/kg substrate, which forms a layer of 8cm after stuffing it in the plastic buckets? Control still maintains the highest as observed tables 3-5. Other treatments levels (2%-6%) show a complete reverse trend in yield and BE of p. pulmonarius in comparison with table 5. The result indicates that increase in fruit body production together with BE were favoured by decrease in crude oil pollution

suggesting that p. pulmonarius cultivated on 300g/kg quantity of substrate may not be affectively used in mycoremediation process. High concentration of crude does not support good colonization of its mycelium and consequently its fruit body production. This conforms to the work of Eggen and Vaclav (2002) who reported that one of the major factors influencing the effectiveness of fungal soil remediation is the concentration of the respective pollutant in the soil.

CONCLUSION

In this investigation, levels of crude oil pollution and substrate quantities are the main treatment factors involved. Increased crude oil concentration delayed primordial formation in both mushroom species compared to their various control, while increase in substrate quantity from 150g/kg – 300g/kg enhanced earlier primordial formation by reducing spawn run duration (**Tables 1 and 2**).

Increase in crude oil concentration from 2% to 6% significantly decreased yield and B.E of *P. ostreatus* from 214.95g/kg-52.51g/kg at 150g/kg quantity of substrate. But increase in the quantity of *A. gayanus* substrate from 150g/kg- 300g/kg boosted its yield and BE (**Tables 3 and 4**)

Yield of *P. pulmonarius* was high in 150g/kg quantity of substrate as crude oil concentration increased from 2%- 6%. This shows that this mushroom may be used for remediation of polluted soil at that level of substrate, but may not be effective once the quantity of substrate is increased to 300g/kg or 8cm thick layer. It is therefore patient to know that high yield of *P. ostreatus* was enhanced by increase in the quantity of substrate to 300g/kg while that of *P. pulmonarius* was higher at substrate level of 150g/kg or 4cm thick layer. Therefore in an attempt to adopt any of these oyster mushroom species in mycoremediation processes, substrate quantity should be put into consideration.

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