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Effects of Crude Oil on the Growth of Oyster Mushroom; *Pleurotus ostreatus* (Jacaum ex.fr. Kummer)

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ABSTRACT: The effect of crude oil on polluted sawdust on the growth of *Pleurotus ostreatus* was evaluated. The experiment was laid in a completely randomized design (CRD) with five replicates. Data collected were subjected to analysis of variance (ANVOA) and the mean separation was done using Duncan Multiple Range test (DMRT) at the probability of 5%. Result showed that micro-morphological characteristics of *P. ostreatus* grown on sawdust polluted with crude oil at varying concentrations of 50 ml, 100ml and 150ml and allowed to be contaminated for 8, 10 and 12 day respectively before sowing the mycelia in the polluted sawdust. Marco-Morphological at 10ml level of crude oil polluted sawdust significantly reduced the marco-morphological features of the *P. ostreatus* mushroom harvest at different days compared to 50ml crude oil polluted sawdust, in 8 days, the cap length measurement are (4.43cm±1.10 – 6.55cm±1.10), for stipe length (3.31cm ± 0.01 - 7.41cm ± 0.02) while the fresh fruit body weight (115.2g ± 2.01 - 175.3g ± 2.01). It is observed that the growing of *P. ostreatus* using wastes will help to reduce the huge agricultural wastes within our environment that sometimes when not properly managed caused serious environmental pollution. The research therefore recommended for the use of sawdust in the growing of *Pleurotus ostreatus* mushroom species, since sawdust and mushroom are good remediators of crude oil pollution. The summary of the result indicated that increase in crude oil pollution significantly ($P \leq 0.5$) affected the macro morphological features of the oyster mushroom production.

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The ability of mushrooms to utilize various lignocellulosic substrates. Substrate type is one of the major factors that affects the yield and quality of oyster mushroom (Chitamba *et al.,* 2012). The nutrient present in the substrate determine the extent of saprobiotic colonization of mushroom (Tshinyangu and Hennebert, 1995). However, crude oil contamination in the Niger Delta, Nigeria have become a regular occurrence (Ogbo and Okhuoya, 2009), therefore the biological approach to clean up oil spills through the use of waste materials and macrofungi (Ogbo and Okhuoya, 2018). Several fungi like *Pleurotus ostreatus, Lentinus subnudus, Pleurotus tuber-regium* etc have been grown in crude oil contaminated soils (Isikhuemhen *et al.,* 2003; Adenipekun and Fasidi, 2005). *P. tuber-regium* was able to the contaminated crude oil thereby reducing the amount of the hydrocarbon and other polycyclic aromatic hydrocarbon (PAH). The ability of the mushrooms to yield a desired product depend on the utilization of the hydrocarbon (Achufuisi, 2016). Mushrooms are a nutritious food source being rich in protein, vitamins and minerals. Earlier reports have shown that mushrooms are rich in ascorbic, amino acids, and protein is the most abundant nutrient (Fasidi and Kadiri. 1990: Aletor, 1995). The food and agricultural organization, mushrooms as food contributing to the protein nutrition of the countries which depend largely on cereals because of their high protein quality (Kuforiji and Fasidi, 2009). They are also known to contain substances that enhance the immune system, light infectious diseases, and lower blood pressure and cholesterol levels. The fact that mushrooms is a novel source of protein offer a promising way of alleviating protein malnutrition in developing countries. In addition, Nigeria by virtue of its vantage tropical location is one of the world's potential hotspots for various forms of biological resources including mushroom (Akpaja *et al.,* 2003). Currently, the exploitation of indigenous Nigerian mycoresources is still over-shadowed by the preponderance of green plants (Okhuoya *et al.,* 2010). Vigorous researches on these easily over, looked forest members might evolve an accidental source of drugs that would resolve the world's cancer, AIDS and leukemia problems (Okhuoya *et al*., 2010). Chang and Miles (1992) defined mushroom as a macrofungus with a distinctive fruiting body, which can be either epigenous growing on or close to ground or hypogenous (growing under the ground) and large enough to be visible to the naked eye and to be picked up by hand. Mushrooms can be ascomycetes, growing underground, have a non-fleshy texture and need not be edible (Chang, 2008). Mushrooms are widespread in nature and since earliest recorded history; humans have viewed them as a special kind of food, acknowledging the nutritional value of this special group of fungi (Chang and Buswell, 1996).

It is now known that mushrooms are rich in high quality protein, unsaturated fatty acids and have a nucleic acid content low enough to allow daily use as a form of vegetable (Chang, 1996). Modern analytical techniques has provided a scientific basis for assigning medicinal value through the identification of various mushroom derived compounds including anti-cancer, anti-viral, immune potentiating, hypocholesterolaemic and hepatoprotective agents (Liu *et al.,* 1995). Pharmacological activities of *Ganoderina lucidum* have been attributed mainly to triterpenes and polysaceharicics produced by the mushroom. Several polysaccharides and protein-bound polysaccharides with immune-modulating and anti- tumour activities have also been isolated from a variety of mushrooms (Chang, 1996). Agricultural production and the agro-food industry furnish large volumes of solid wastes, residues and by-products, produced either in the primary agro-forestry sector by secondary processing industries (processing-based) with the major part being lignocellulosic biomass (Philippoussis and Diamantopoulou, 2011). Zhang (2008), reviewing the global world information about lignocellulose availability estimated the production of lignocellulosic biomass to be more than 200x 109 tonnes per year. In nature, mushrooms have not only been a source of food for man and other animals, but also have played an important role in the cycling of carbon and other elements through the breakdown of lignocellulosic plant residues and animal dung, which serve as the substrates for these saprophytic fungi (Chang, 1996). In this way, mushroom species, as agents of decay help keep the environment from being overwhelmed by the dead organic debris of plants and animals. Mushroom forming fungi are therefore, amongst nature's most powerful decomposers, secreting strong extracellular enzymes due to their aggressive growth and biomass production (Adenipekun, 2009). They have the capability to produce a wide range of enzymes that can break down complex substrates into simpler soluble substances and absorb them for their growth and development (Oei, 1991). Strong consumer demands and threats of depletion of mushrooms have stimulated increased worldwide production in the past few decades (Chang and Miles, 2004). The increased demand for mushrooms is due to their unique culinary and medicinal properties (Yan *et al.,* 2003). Commercial cultivation of mushrooms as a source of food, nutriceutical and medicine is now a worldwide industry with over 120 countries contributing to a crop which, in 1999 totaled 4.3 million tonnes (Chang and Miles, 1991). Several reports indicate that commercial production of fresh edible mushrooms is a rapidly growing industrial activity. In 2002, world production of cultivated mushrooms was estimated to be 12,250 thousand tonnes and was valued at about US$ 32 billion, whereas mushroom products used mainly for dietary supplements were assessed to have generated about US$ 11 billion (Chang, 2006). Mushroom cultivation is an efficient and relatively short biological process of food protein recovery from lignocellulosic materials (Martinez-Carrera *et al.,* 2 000). The cultivation of edible mushrooms has become an increasingly important practice in modern society due to the biotechnological process of bioconversion of various residues into edible mushrooms or in dietary supplements of high nutritional value, enabling a more efficient utilization of waste materials. Interestingly, the spent compost that remains after harvesting mushrooms may still be recycled for use as animal feeds and soil conditioner. Earlier studies have demonstrated that spent compost of both *Volvariella* and *Pleurotus* had increased crude protein content compared with raw straw (Quimio, 2004). Mushroom production can be a lucrative cottage industry for low-income rural households in developing countries (Ferchak and Croucher, 1996). The activity is labour intensive and can provide full or part-time employment. A small mushroom production business can be established with low capital investment and with minimal requirements for space and equipment. Mushroom production represents an important opportunity for developing countries, particularly Nigeria, since innovations in cultivation and post-harvest processing make possible new opportunities (Ferchak and Croucher, 1996). A mushroom is a fleshly fungi, spore-bearing fruiting bodies of typically produced above ground on soil or on their food sources fruiting body of fungi (Alexopolus and Mims, 2003). Mushrooms often refer to fruiting body of the gill fungi. They do not contain chlorophyll like green plants and as a result cannot manufacture their own food. In this respect they are like animals because they feed themselves by digesting other organic matter. The context of the usage usually forms deviating from the standard form have more specific names, such as puffballs, stinkhorn, morels etc and gilled mushrooms themselves are often called agances in reference to their similarity to Agaricus or placement in the order Agaricales (Gbolagade *et al*., 2015). By extension, mushroom can also designate to the entire fungus when in culture or when referring to the whole thallus called a mycelium of species forming fruit bodies called mushrooms (Oci, 2003).

Identifying mushrooms requires a basic understanding of their macroscopic structure. Most are Basidiomycetes and gilled. Their spores, called Basidiospores are produced on the gills and fall in a fine rain of powder from under the caps as a result. At the microscopic level the Basidiospores are shot off of Basidia but then fall between the gills in the dead air space. As a result, for most mushrooms if the cap is cut off and placed gill-side down, usually overnight a powdery impression reflecting the shape of the gills, or pore, or spines. etc is found when the fruit body is sprouting. The colour of the powdery print which is called a spore print has been used to help classify mushrooms, hence is used to help identify & them (Shah *et al*., 2004). Mushrooms have continued to generate a lot of interest particularly in its consumption as food, in the cure of diseases, in bioremediation as important items of commerce all over the world (Oei. 1999). The world of fungi is large and diverse. They are not only one of the most primitive life forms but it intrigue mycologists with their diversity their unique richness of biologically active ingredients (Adhiraj *et al*., 2015). Though, mushrooms are just a small part of the world fungi that arc presently the most extensively studied organism (Patra *et al*., 2013). The operations of the oil and gas industry in Niger Delta of Nigeria have led to widespread Petroleum hydrocarbon pollution in the area, alongside with huge quantities of agricultural wastes. Therefore, there is considerable pressure nowadays to develop biotechnological processes for the rational treatment and/or disposal of these vast quantities of agricultural materials generated annually of cultivation of edible mushrooms. However, Agricultural wastes, such as oil palm and timber wastes among others, can provide the mushroom with the nutrients required for spawn run and fructification which under controlled conditions and procedures will result in an optimum yield. The aim of this research are to determine the macro-morphological characteristics of *Pleurotus ostreatus* grown on sawdust polluted with different levels of crude oil and to evaluate the yield of *Pleurotus ostreatus* grown on different levels of crude oil polluted sawdust.

MATERIALS AND METHODS

Study Area: The study was carried out at the Screen House of Diplomat Farm Nigeria Limited, Rivers State University, Nkpolu Oroworukwo, Port Harcourt. Located between Longitude and latitude with humid tropical climate (Chukunda, 2014).

Source of Culture and Spawn Multiplication: Pure mycelial culture of *Pleurotus ostreatus* was obtained from Diplomat Farm Nigeria Limited, Rivers State University, Nkpolu-Oroworukwo and multiplied at the Department of Forestry and Wildlife Management, Rivers State University. The spawn was produced using sorghum grains washed and soaked in tap water over night and later boiled with tap water in ratio of 1:1 (Sorghum grain: Water) for 15 mins. Four percent (4%) of $CaCO_3$ and 2% of CaS_{O4} were added to optimize pH and prevent champing of grains (Muhammad, *et al*., 2007). After sterilization, the bottles were allowed to cool before they were inoculated with actively growing mycelia of *P. ostreatus* by grains – to – grain transfer and incubated in the dark at (27± 2^0C) for 10-15 days (Shyam *et al.,* 2010; Obinna-Echem and Chukunda, 2018).

Source of Crude Oil: The Bonny light crude oil was obtained from the Nigerian National Petroleum Co-operation (NNPC) Port Harcourt, Nigeria (Onyeizu *et al*., 2017).

Collection and Preparation of Substrates/ Crude oil: The sawdust was collected from the local wood industry at Elabuchi Port Harcourt. However, the sun-dried substrates were packed separately bagged in black polyethene bags and labelled (Sales- Campos. 2018). Two hundred gram (200g) of the substrate was humidified with 50% of water and added with 5g of calcium carbonate ($CaCO_3$) in relation to the dry sample weight of the substrate with pH of 6.5 (Okhuoya and Okogbo, 1990; Onyeizu *et al*., 2017).

Pasteurization and Inoculation: Two hundred grams of sawdust, substrate was bagged in 12 x 24 x 15 cm^3 polypropylene bags with one hundred grams of rice bran thoroughly mixed together and sterilized using autoclave at 121^0C for one hour to kill microbes or contaminates associated with the substrates. After inoculation in aseptic conditions, incubation was performed .inside a humid chamber for one month at the temperature of 28 $\pm$ 2°C and relative humidity of 85-90% with maintained regular sprinkling of water until primordial emergence (Chukunda and Abere, 2017).

Crude oil Pollution on Sawdust Waste for Mushroom Growth: The agricultural waste used to grow the *Pleurotus ostreatus* was polluted with 50m1, 100ml and 150m1 V/V of crude oil to make 50ml, 100ml and 150ml non-polluted substrate (Okwulchie and Okwujiako 2008; Onyeizu *et al.,* 2018).

Determination of Macro-Morphological Parameter of Mushroom Production

(i) *Cap Length (Pileus):* This was determine by placing a transparent rule across the center of the Pileus of each harvested mushroom fruit body and

reading off the diameter. (Chukunda *et al*., 201 7; Ukoima *et al*., 2009b; Ukoima *et al*., 2018).

(ii) Stipe Length: This was determined by placing transparent ruler along the length of each fruit body stipe. (Chukunda and Abere, 2017).

(iii) Weight of fresh fruit bodies: This was determined by weighing each fruit body immediately after harvest using a portable digital balance (Chukunda and Abere, 2018).

Experimental Design and Analysis of Data: The experiment was laid out in a Completely Randomized Design (CRD). The treatment was replicated five times. Data was statistically analyzed using the method of Steel and Torrie (1980) and the means were separated using Duncan Multiple Range Test (DMRT) at a probability of 5%.

RESULTS AND DISCUSSION

The results on the morphological parameters of *Pleurotus ostreatus* grown on sawdust polluted with crude oil arc shown in Table 1. The results revealed that *Pleurotus ostreatus* significantly (P 0.05) grew better at 50ml of crude oil pollution which resulted into good cap length, stipe length and fruit body fresh weights at different days interval. However, in 50ml of crude oil polluted sawdust at different days interval performed better than other levels of pollution indicated that after 8 days resulted the number of spout per bag (3), cap length (6.55cm ± 2.0l) stipe length (7.41cm ± 0.2) and fresh fruit body weight (175.3 ± 2.01). it is important to note that sawdust polluted with 50ml of crude oil performed better than 100 and 150ml crude oil pollution..

Table 1: Macro-Morphological parameters of *Pleurotus ostreatus* grown on sawdust polluted with crude oil after 8 days

Sawdust polluted with crude oil (ML)	Primodal formation (days)	Sprout per bag	Cap length (cm)	Stipe Length (cm)	Fresh fruit body weight (g)
150ml	8	2	4.34 ± 1.10^{c}	3.31 ± 0.1^{c}	115.2 ± 2.01^{c}
100ml	8	2	5.33 ± 2.01^{b}	6.30 ± 0.2^{b}	145.2 ± 1.02^{b}
50ml	8	3	6.55 ± 2.01^{a}	7.41 ± 0.2^{a}	175.3 ± 2.01^{a}

Means followed by the same letters are not significantly (p≤ 0.05) different at DMRT (5%) probability.

Table 2 Morphological parameters of *Pleurotus ostreatus* grown on sawdust polluted with crude oil after 10 days

Sawdust polluted with crude oil (ML)	Primodal formation days)	Sprout per bag	Cap length (cm)	Stipe Length (cm)	Fresh fruit body weight (g)
150ml	10	2	4.44 ± 1.05^{c}	5.43 ± 0.12^{c}	115.2 ± 2.01^{c}
100ml	10	2	9.31 ± 3.04^{b}	6.32 ± 2.0^{b}	232.2 ± 4.13^{b}
50ml	10	3	11.09 ± 2.05^{a}	7.42 ± 2.4^{a}	$272. \pm 2.05^{a}$

Means followed by the same letters are not significantly (p ≤ 0.05) different at DMRT (5%) probability.

Table 3 Morphological parameter of *Pleurotus ostreatus* grown on Sawdust polluted with crude oil after 14 days (Mean ± SD)

Sawdust polluted with crude oil (ML)	Primodal formation (days)	Sprout per bag	Cap length (cm)	Stipe Length (cm)	Fresh fruit body weight (g)
150ml	14	2	9.55 ± 2.05^{c}	7.50 ± 0.25^{c}	272.5 ± 3.05^{c}
110ml	14	2	11.25 ± 3.04^{b}	8.00 ± 0.12^{b}	312.3 ± 6.12^{b}
50ml	14	3	15.08 ± 1.25^{a}	8.60 ± 0.08^{a}	320.6 ± 6.80^{a}

Means followed by the same letters are not significantly (p ≤ 0.05) different at DMRT (5%) probability

Besides, there is a positive relation with the time of pollution and remediation process to reduce the effect of the crude on the performance of mushroom The result indicated that increase in the concentrations of crude oil polluted sawdust significantly (P≤ 0.05) reduced the cap diameter of *P. ostreatus* (4.34±1.25 – 15.08cm ±1.25) when compared to the control (0%) unpolluted sawdust. However, the increase in the stipe length of the test mushroom showed that *P. ostreatus* at 50ml and 150ml concentrations of the crude oil pollution caused the highest stipe length when compared to the control (0%). Similarly, the weights of the test mushrooms significantly (P≤ 0.05) reduced with increase in percentage level of pollution. These results conform to that of Okwujiako *et al.,* (2013) which indicated that oyster mushrooms have the ability of growing on a wide range of recalcitrant pollutants such as polyaromatic hydrocarbon, chlorophenols and other pesticides. Okwulchie and Okwujiako (2008) and Bumpus *et al.,* (1988) reported that increased crude oil pollution affected the mushroom production.

The use of wood sawdust as a good substrate gave a better yield performance in *Pleurotus ostreatus* (Okwujiako *et al.,* (2008). It is reported that crude oil has some physiological effect on the moisture content of mushroom fruit bodies which has been attributed for its increase in fresh weight (Chang and Miles, 2004). Increase in stipe length may not necessarily imply the ability of mushrooms to degrade pollutants, but can be determined first by the species involved (Stamets, 2000). The higher amount of substrate in a crude oil polluted environment could reduce the higher amount of fruit bodies harvested because of the attendant increase in lignocellulases activities (Chang and Miles, 2004). These conform to the findings of Staments (2000) who reported that this reduction does not rule out the fact that oyster mushrooms are good remediators of polluted environment but agrees with the works of Eggen and Sasek (2002). There was a gradual decrease in cap diameter of the mushrooms in all the levels of crude oil concentrations 50ml, 100ml and 150ml compared to their control. At 50ml crude oil polluted sawdust there was an increase in cap diameter of the mushrooms when compared to controls. This is as a result of abundant nutrient supply, which played active role in the growth of the fungi. (Eggan and Vaclcar, 2002). The toxicity of the pollutant did not strongly affect both the oyster mushrooms probably because the abundant substrate may have formed a thick barrier between the mushrooms and the crude oil pollutants (Assan and Mpofu, 2014). According to Nelson-Smith (1973), crude oil is a naturally occurring complex mixture of hydrocarbon and non-hydrocarbon compounds, which possess a measurable toxicity towards living things.

At all levels of crude oil concentrations, the yield and biological efficiency decreased as this conforms with the report of Eggan and Vaclar, (2002) and Onyeizu *et al*., (2018) who maintained that factors such as increase in the concentration of pollutant can hinder vegetative and reproductive yield of mushrooms. This confirms the reports of Olanipekun (2014) and Okwujiako *et al.,* (2013). Eggan and Vaclav (2002), who in their separate investigations reported that white rot fungi have a high potential for degradation and removal of recalcitrant pollutants such as polycylic aromatic hydrocarbons, chlorophenols and pesticides. This is simple because, white rot fungi take advantage of their mycelia, which can penetrate contaminated soil to reach the PAHs that spread beyond the top layer of the soil (Bumpus *et al*., 1985).

*Conclusion***:** Nowadays, the research on conversion of agro-industrial wastes into some other useful forms is ongoing. Mushroom is said to be the most important bioconversion product. Therefore any lingo-cellulosic was generated around our environment can be used for mushroom production. It is therefore recommended that 1. *Pleurotus ostreatus* production using agro-industrial wastes will help in solving environmental pollution problems created by unmanaged agro-industrial wastes around Port Harcourt. 2. Measures should be taken to determine the amount of metal content in the fruit bodies of mushrooms growing on pullulated substrate, due to absorption process and 3. Further information is needed on the level of toxicity of *Pleurolus ostreatus* mushrooms, grown on sawdust polluted with crude oil which may cause some health related problem if mistaken consumed.

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