

BIODEGRADATION OF PETROLEUM OILS BY FUNGI ISOLATED FROM OIL PALM FRUIT AND MECHANIC VILLAGE

^{1*}Adekunle, A. A.; ¹Ani, E and ²Kanife, U.C.

¹Department of Botany, University of Lagos. Akoka, Lagos. Nigeria.

²Department of Biological Sciences, Yaba College of Technology, Yaba, Lagos. Nigeria

*Corresponding authors email: aaded63@yahoo.com or aaded63@hotmail.com

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ABSTRACT

This study was done to determine the relative ability of fungi isolated from auto-work shop and diseased fruit of oil palm (*Elaeis guineensis*) to degrade petroleum products. Nine fungi were isolated altogether over a six months period. *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. wentii*, *Mortierella* sp, *Rhizopus* sp, *Penicilium* sp *Candia* sp and *Mucor* sp were isolated from the oil contaminated soil while *Rhizopus* sp was isolated from diseased *Elaeis guineensis* fruit. An assessment of the relative ability of seven of the isolated fungi to biodegrade petroleum oils and extracted oil of *Elaeis guineensis* on minimal salt solution was done by measuring the change in optical density read on spectrophotometer as well as gas chromatographic analysis. From the result obtained, all the fungi tested were capable of biodegrading petroleum oils, though at different rates. Both *Rhizopus* sp and *A. niger* used up completely C_{6, 8} and C₂₄₋₂₈ in diesel and C_{6,13,19} and C₂₃ upwards in spent engine oil after the 40 days of incubation. *Aspergillus niger* had the highest ability to degrade diesel and spent engine oil with efficiency of 84% and 95% in the two oils respectively; *Rhizopus* sp had the highest ability to degrade kerosene; unspent engine oil and extracted oil of *Elaeis guineensis* with the efficiency of over 83% in the three oils while *Penicilium* sp had the highest ability to degrade crude oil.

Key words: Myco-degradation, *Elaeis guineensis*, *Mortierella* sp, Hydrocarbon, Pollution

INTRODUCTION

Crude oil is a naturally-occurring complex mixture of hydrocarbon and non-hydrocarbon compounds which possesses a measurable toxicity towards living systems (Nelson-Smith, 1973). The increase in demand for crude oil as a source of energy and as a primary raw material for industries has resulted in an increase in its production, transportation and refining which, in turn, has resulted in gross pollution of the environment. (Bartha, 1986; Obire, 2003).

Methods for restoring oil-polluted sites vary from complete removal of the affected soil to doing nothing at all and "letting nature take its course". Physical methods such as incineration may destroy indigenous organisms, including oil-degrading microbes, and increase the toxicity of the petroleum residue. Mechanical removal of stranded oil from sand dunes or salt marshes is far more damaging than leaving it alone; not only is the ecological balance disturbed, but the aesthetic effect may also be irreparable (Nwangwu and Okoye, 1981). Chemical methods for removing or dispersing spilled oil from the environment were condemned by Nelson-Smith (1973) because of

their side-effects on the ecosystem and their toxicity, which is sometimes more pronounced than that of the oil itself.

Mycoremediation, an aspect of bioremediation is a term used when fungi are used to decontaminate an area. The term *mycoremediation* was coined by Paul Stamets and refers specifically to the use of fungal mycelia in bioremediation. One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fiber. These are organic compounds composed of long chains of carbon and hydrogen, structurally similar to many organic pollutants. Since the molecules found in hydrocarbons (oil and petrochemicals) are the same as those found in carbohydrates (plant-based material), many fungi possess the ability to break down both. The key to mycoremediation is determining the right fungal species to target a specific pollutant. (Obire, *et al.*, 2008).

The aim of this work is to investigate and document the ability of fungal species isolated from oil palm fruits and mechanic village to utilize and degrade petroleum hydrocarbons in crude petroleum oil and some petroleum products such as diesel, kerosene, spent and unspent engine oil and palm oil. The biodegradation abilities of the fungal species isolated from the two sources (palm fruits and oil polluted soil) is also compared.

MATERIALS AND METHOD

Collection of Fruit Samples

Palm fruits (*Elaeis guineensis*) were collected from three different markets (Oyingbo, Yaba and Mushin) in Lagos State while soil sample was collected from the Mechanic Village in Ladipo Market, Mushin, Lagos. The visually-diseased fruits were used for this experiment. Sampling was done thrice every month for six months. Over three hundred fruits were collected from each market at every sampling period while crude oil and petroleum products were collected at different filling stations across the country.

Isolation of Fungi from Diseased *Elaeis guineensis* in the Absence of Petroleum Fume

About 15 to 20 diseased fruits from each market per sampling day was surface sterilized. This was done by first preparing a solution of Sodium hypochloride (bleach) and distilled water in the ratio 3:2. A non absorbent cotton wool was dipped into the solution and used to properly wipe the surface of the palm fruit while holding it. A sterile blade/knife was used to cut out the sterilized mesocarp and placed on the surface of a solidified agar in a Petri dish. The culture plate was then sealed and incubated at room temperature.

Isolation of Fungi from Diseased *Elaeis guineensis* in the Presence of Petroleum Fume

To isolate fungi from *E. guineensis* in the presence of petroleum fumes, a modified method of Adekunle and Oluyode (2005) was adopted. Twenty (20) filter papers (Whatman No 1001125) were sterilized in the Autoclave and allowed to dry in the oven at 40 °C for 15 minutes. The dried filter papers were dipped in 250 ml crude oil contained in 500 ml beaker for about 15 to 20 seconds with

the help of sterile forceps and drained.

Each of the crude oil-treated papers was placed on the cover of a Petri dish containing solidified Potato Dextrose Agar (PDA) and the sterilized and cut mesocarp of *E. guineensis* under sterile condition. The aim of the petroleum fume was to supply the fungi with hydrocarbons through vapour transfer by supplying petroleum fumes.

Isolation of Fungi from Soil

Twenty grammes of the collected soil sample was dissolved in 10 ml of distil water. The solution was decanted into a test tube. A pipette was used to transfer 1ml of the decanted solution to another test tube containing 9ml of distilled water to give 10⁻¹ml. Then 1ml was also transferred from the 10⁻¹ml solution to another test tube containing 9ml of distilled water to give 10⁻²ml. This procedure continued until the solution reached 10⁻⁵ml; 0.1ml of each of the dilutions was pipetted on solidified Potato Dextrose Agar (PDA) plates.

All the plates were incubated at room temperature. The culture plates were observed daily for fungal growth. This process was repeated three times per sample from the three markets and from the soil sample. The developing fungal colonies were sub-cultured into fresh PDA plates to get pure culture of isolates.

Identification

To identify the fungi, microscopic slides were prepared in a sterilized Ultra Violet (UV) room to prevent air-borne fungal spores from getting unto the slides. The prepared slides were stained with Lactophenol blue. Light microscopic examinations were carried out. Also cultural characteristics such as colour of the fungal colony, number of days taken for the fungus to reach maximum diameter of the Petri dish were noted. The morphological and cultural features of each fungus was compared with the descriptions given by Talbot (1971), Deacon (1980) and Bryce (1992) for identification.

Confirmatory Test for Hydrocarbon Utilization Potential of the Fungi

To test for hydrocarbon utilization of the fungi, a modified enrichment medium described by Adekunle *et al* (2007), were used. A minimal salt solution (MSS) was prepared using 1.25 g of Na_2HPO_4 ; 0.29 g of KCl; 10.0 g of NaCl; 0.42 g of NaN_3 ; 0.83 g of KH_2PO_4 ; 0.42 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 2.0 g of Agar-Agar in 1000 ml of distilled water. The test tubes to be used were sterilized in an autoclave between 121 °C and 126 °C at a pressure range of 0.1 mPa and 0.14 mPa and were arranged in a test tube rack on cooling.

In each test tube, 8 ml of minimal salt solution was measured using a measuring cylinder. The first five racks consist of eight (8) test tubes because there are 7 fungi to be tested and one test tube is for control. The last rack consists of seven (7) test tubes having the seven fungi inside and the last tube was without a fungus; it was used to calibrate the spectrophotometer. For all, 2 ml of the various oils were measured using a sterilized measuring cylinder and added to the 8 ml of minimal salt solution already present in the test tubes respectively, making it 10ml in each test tube. All the test tubes were incubated at room temperature for 40 days. The test tubes were also regularly shaken to ensure there is proper contact of oil/ cell phase.

The ability of the fungi to degrade kerosene, diesel, Spent engine oil, unspent engine oil, palm oil, and crude oil (based on the growth rate of the organism on minimal salt medium) were measured at an interval of 5 days using the visual method which is based on turbidity of the salt medium. These were measured using a spectrophotometer at wavelength of 530 nm and 620 nm respectively (Amund, and Akangbou, 1993). The result from the test were organized and plotted. The statistical analysis was also done.

Gas Chromatography Analysis of Some Oil Samples

Gas chromatography analysis was carried out to assess the ability of *Aspergillus niger* and *Rhizopus* sp to each degrade spent engine oil and diesel. The method of Song and Bartha (1990), Kampfler and Steoif (1991) and Salminen, *et al.* (2004) was used for extraction of samples and GC analysis. For the extraction process, about 20ml hexane was used. Each sample was poured into a separating funnel and 20 ml of hexane was added and the different oils fraction collected were well shaken. Column was prepared to get pure extracts. This was done using a 150 ml burette; the base of the burette was blocked with cotton wool to provide a base for the silica gel. About 20 ml of hexane was poured into a beaker and about 5 g of Na_2CO_3 was added, which was the drying agent. The drying agent was poured into the burette. The silica gel helped to remove impurities.

The extract was poured into the burette and 20 ml hexane was added. The extract diffused down the column and collected in a 14 ml McCartney bottle. All traces of hexane in the extract were allowed to evaporate by leaving the McCartney bottle opened, and the final extract was used subsequently. Gas chromatographic analysis was carried out using Perkin Elmer Auto-system GC equipped with flame ionization detector. A 30 m fused capillary column with internal diameter 0.25 mm and 0.25 mm thickness was used, and the peak areas were analyzed with a SRT model 203 peak simple chromatography data system. About 1-2 ml of extracted sample was injected. The column temperature was 60°C for 2 minutes to 300°C programmed at a rate increase of 120°C/min. Nitrogen was used as carrier gas with pressure of 30 ml. Hydrogen and air flow rates were 30ml/min respectively

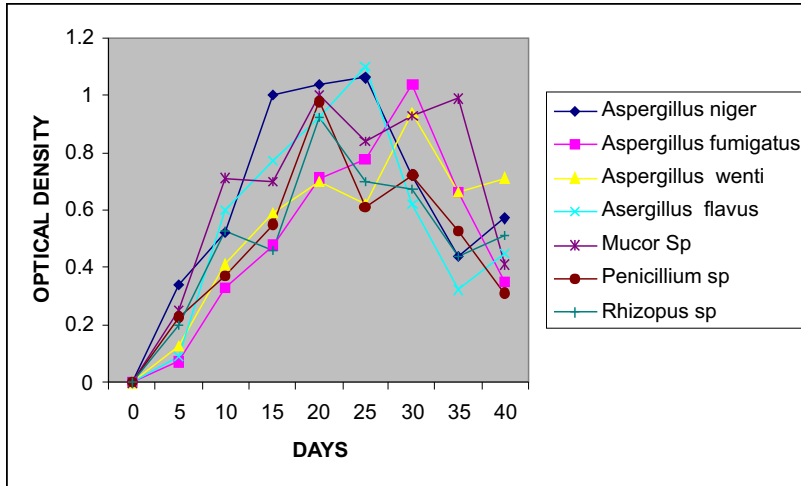


Fig. 1a: The Growth Pattern of Fungi in Minimal Salt Solution at 530 nm Wavelength

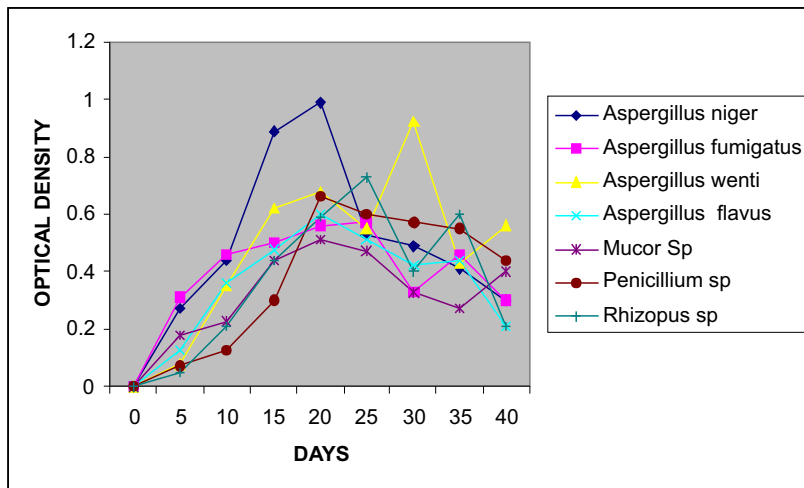


Fig. 1b: The Growth Pattern of Fungi in Minimal Salt Solution at 620 nm Wavelength

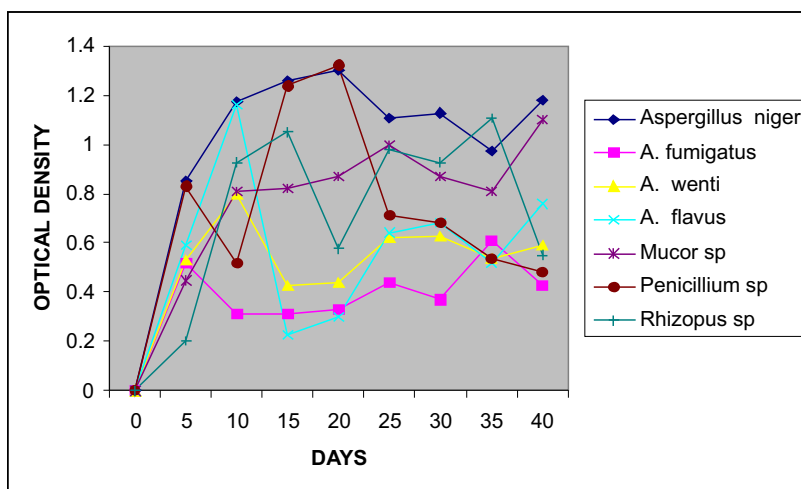


Fig. 2a: The Growth Pattern of Fungi in Minimal Salt Solution with Kerosene at 530 nm Wavelength

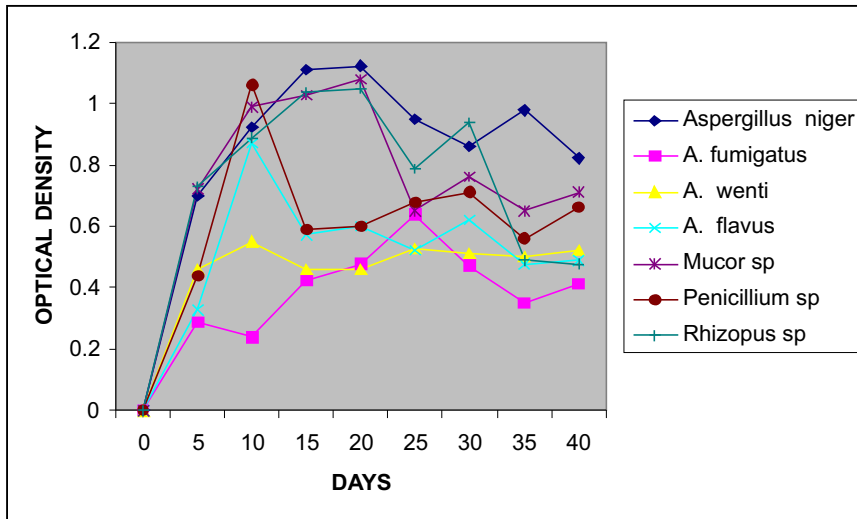


Fig. 2b: The Growth Pattern of Fungi in Minimal Salt Solution with Kerosene at 620 nm Wavelength

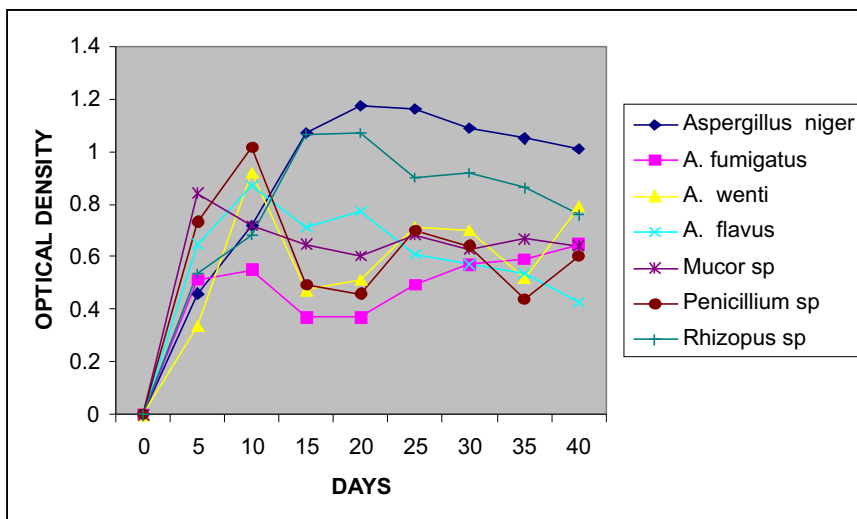


Fig. 3a: The Growth Pattern of Fungi in Minimal Salt Solution with Diesel at 530 nm Wavelength

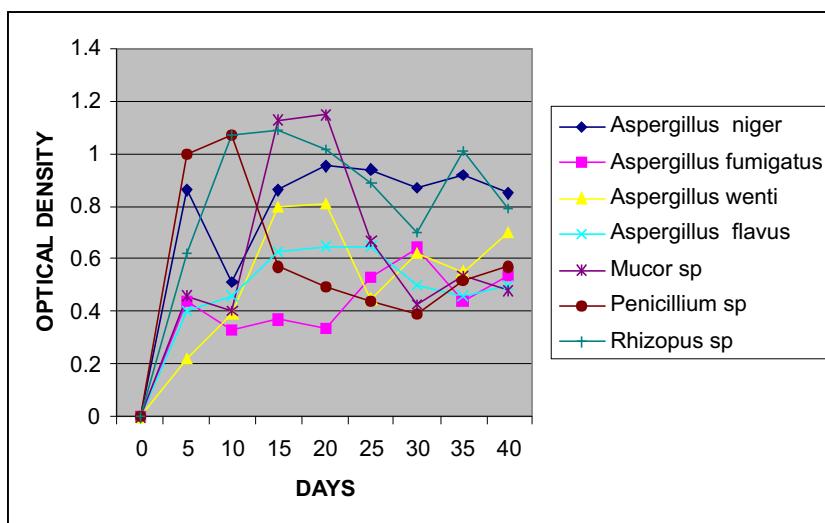


Fig. 3b: The Growth Pattern of Fungi in Minimal Salt Solution with Diesel at 620 nm Wavelength

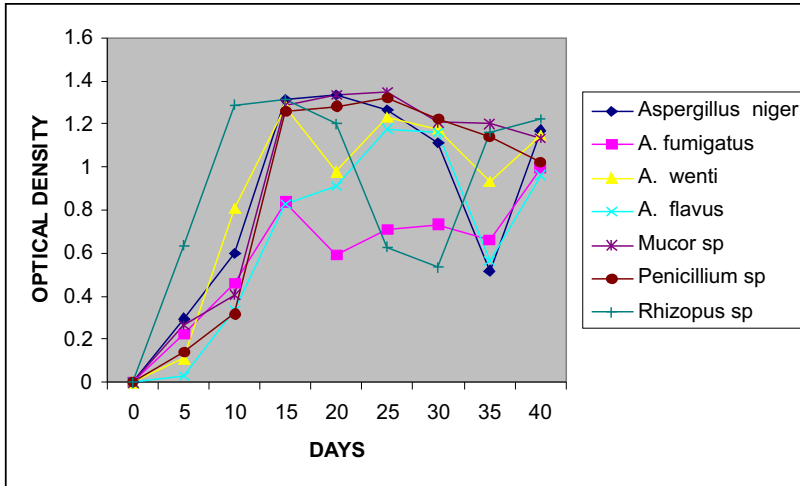


Fig. 4a: The Growth Pattern of Fungi in Minimal Salt Solution with Unspent Engine Oil at 530 nm Wavelength

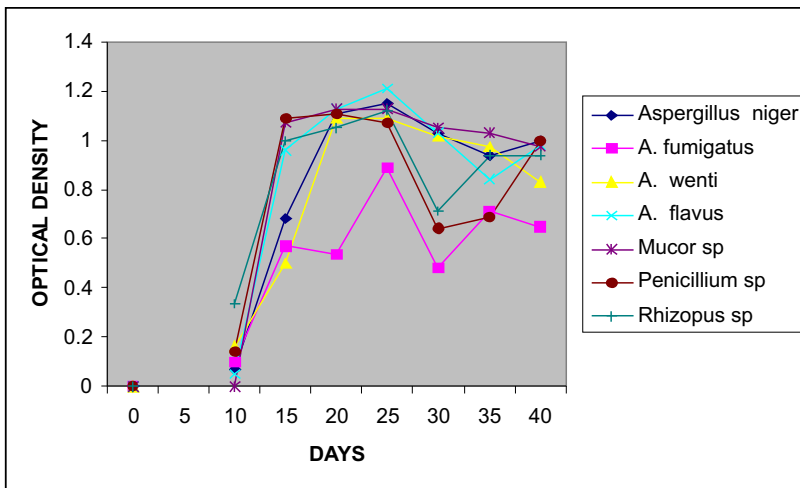


Fig. 4b: The Growth Pattern of Fungi in Minimal Salt Solution with Unspent Engine Oil at 620 nm Wavelength

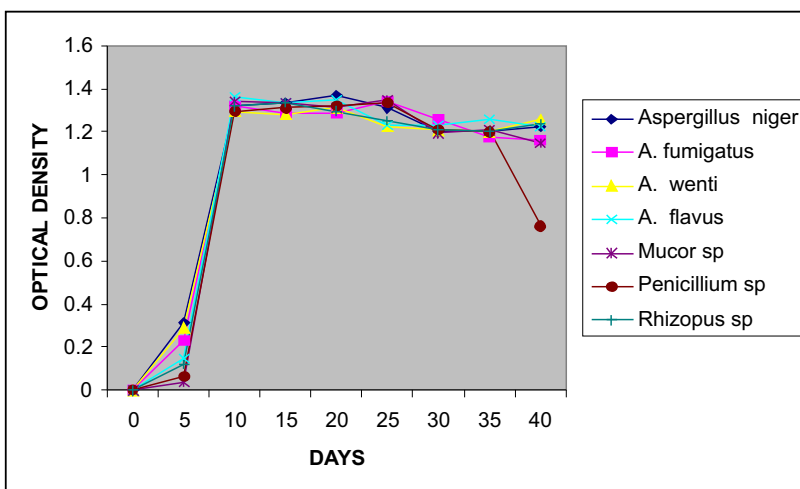


Fig. 5a: The Growth Pattern of Fungi in Minimal Salt Solution with Spent Engine Oil at 530 nm Wavelength

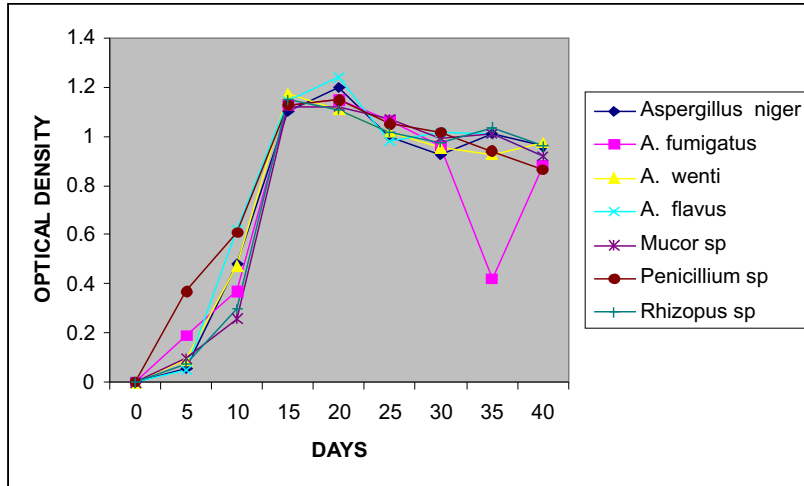


Fig. 5b: The Growth Pattern of Fungi in Minimal Salt Solution with Spent Engine Oil at 620 nm Wavelength

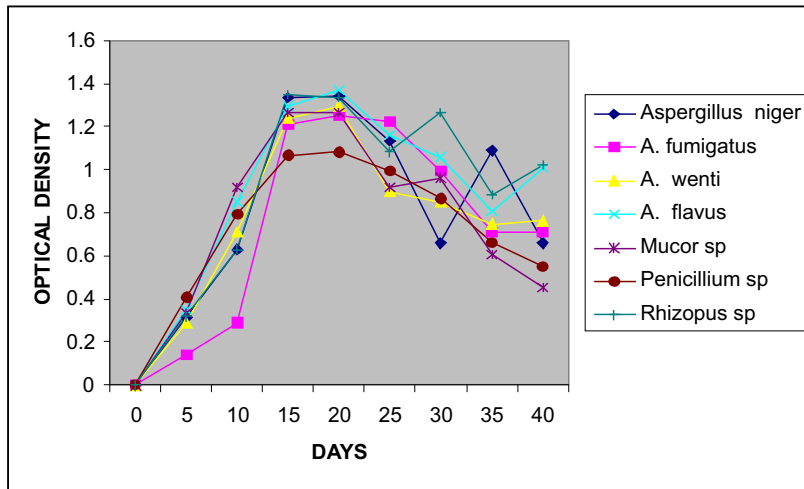


Fig. 6a: The Growth Pattern of Fungi in Minimal Salt Solution with Palm Oil at 530 nm Wavelength

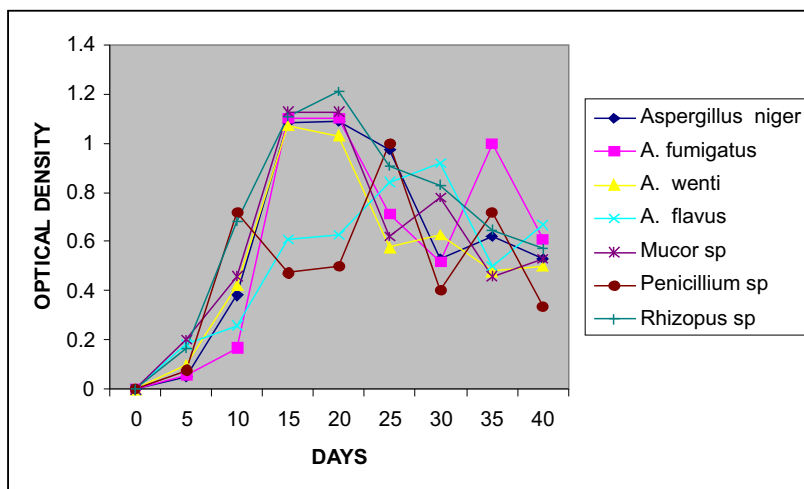


Fig. 6b: The Growth Pattern of Fungi in Minimal Salt Solution with Palm Oil at 620 nm Wavelength

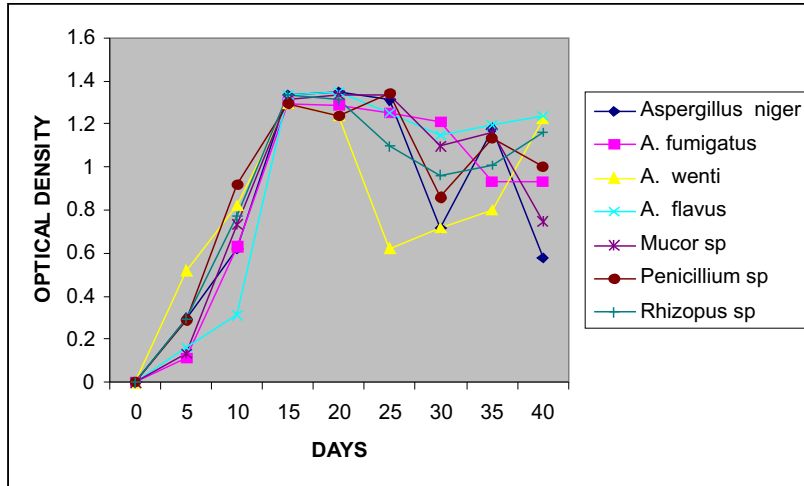


Fig. 7a: The Growth Pattern of Fungi in Minimal Salt Solution with Crude Oil at 530 nm Wavelength

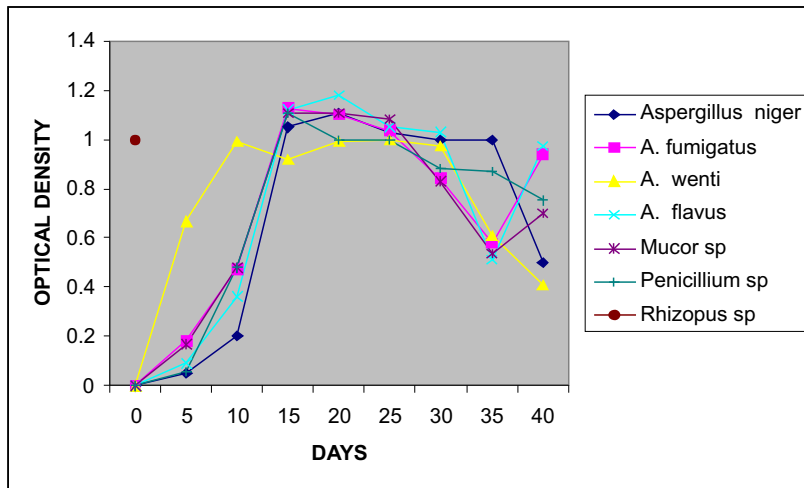


Fig. 7b: The Growth Pattern of Fungi in Minimal Salt Solution with Crude Oil at 620 nm Wavelength

Table 1: The Order of Biodegradation of the Various Fungi in the Different Petroleum Products after 40 Days of Incubation

OILS/ ORGANISMS	<i>A. niger</i>	<i>A. fumigates</i>	<i>A. wenti</i>	<i>A. flavus</i>	<i>Mucor sp</i>	<i>Penicillium sp</i>	<i>Rhizopus sp</i>
Kerosene	2	7	6	5	3	5	1
Diesel	1	7	3	5	4	6	2
Unspent Engine oil	5	7	4	6	2	3	1
Spent Engine oil	1	4	4	3	2	6	5
Palm oil	3	6	5	2	4	7	1
Crude oil	6	5	7	2	4	1	3

Key note

Ranking 1 to 7 represents the order of biodegradation from the highest to the lowest

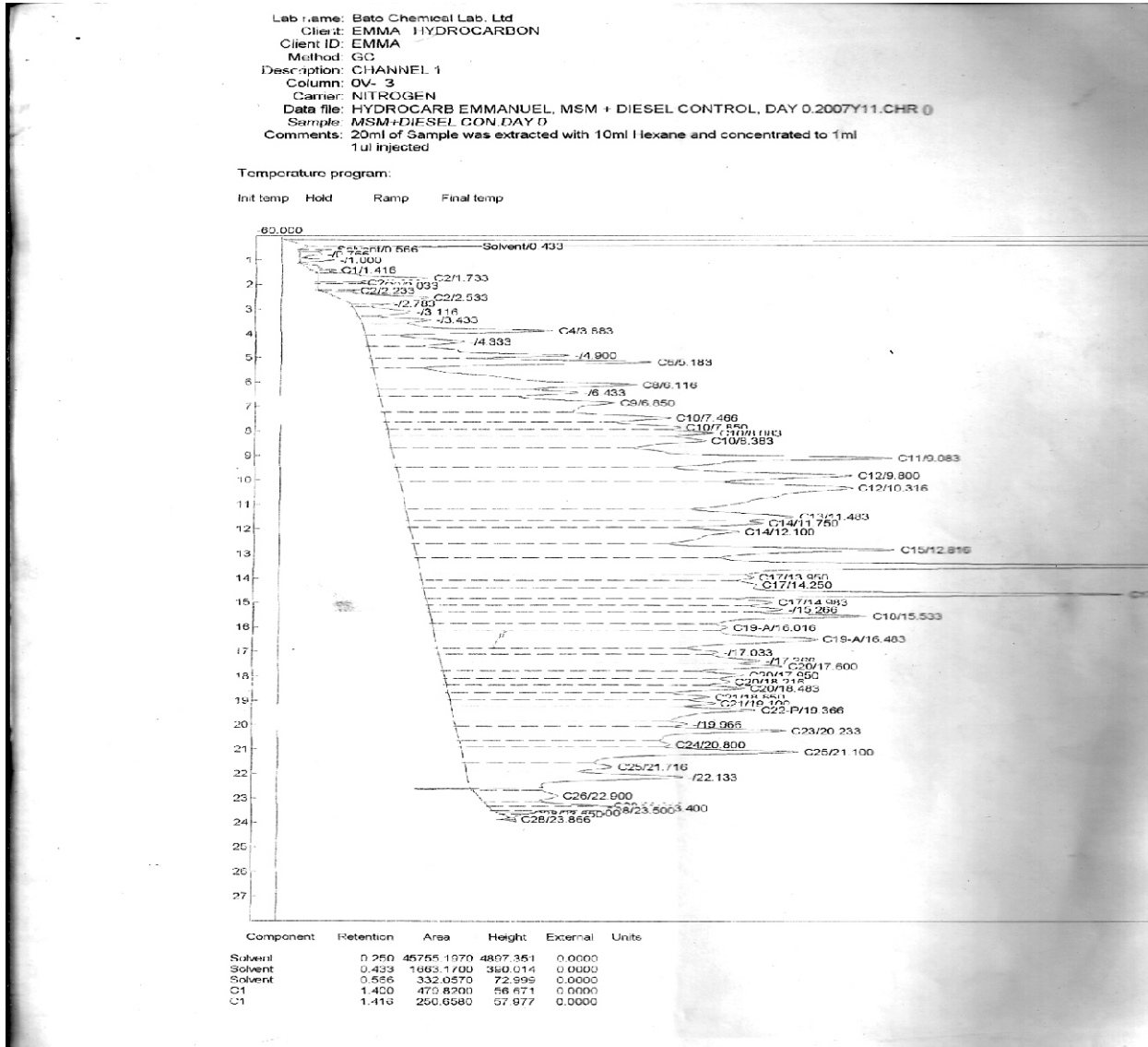


Fig. 8: G.C Analysis of Minimal Salt Solution and Diesel (Control)

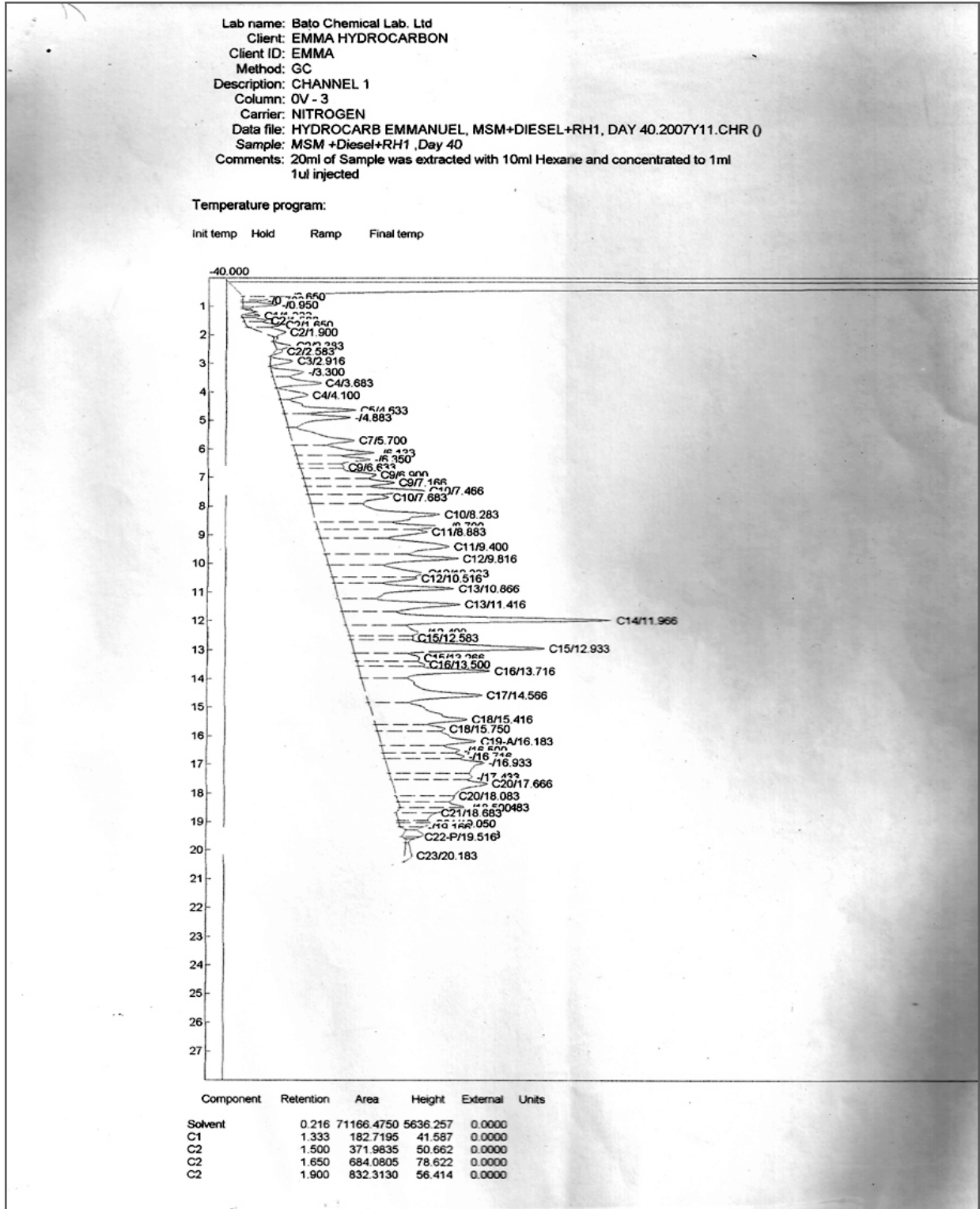


Fig. 9: G.C Analysis of *Rhizopus* Sp in Minimal Salt Solution and Diesel

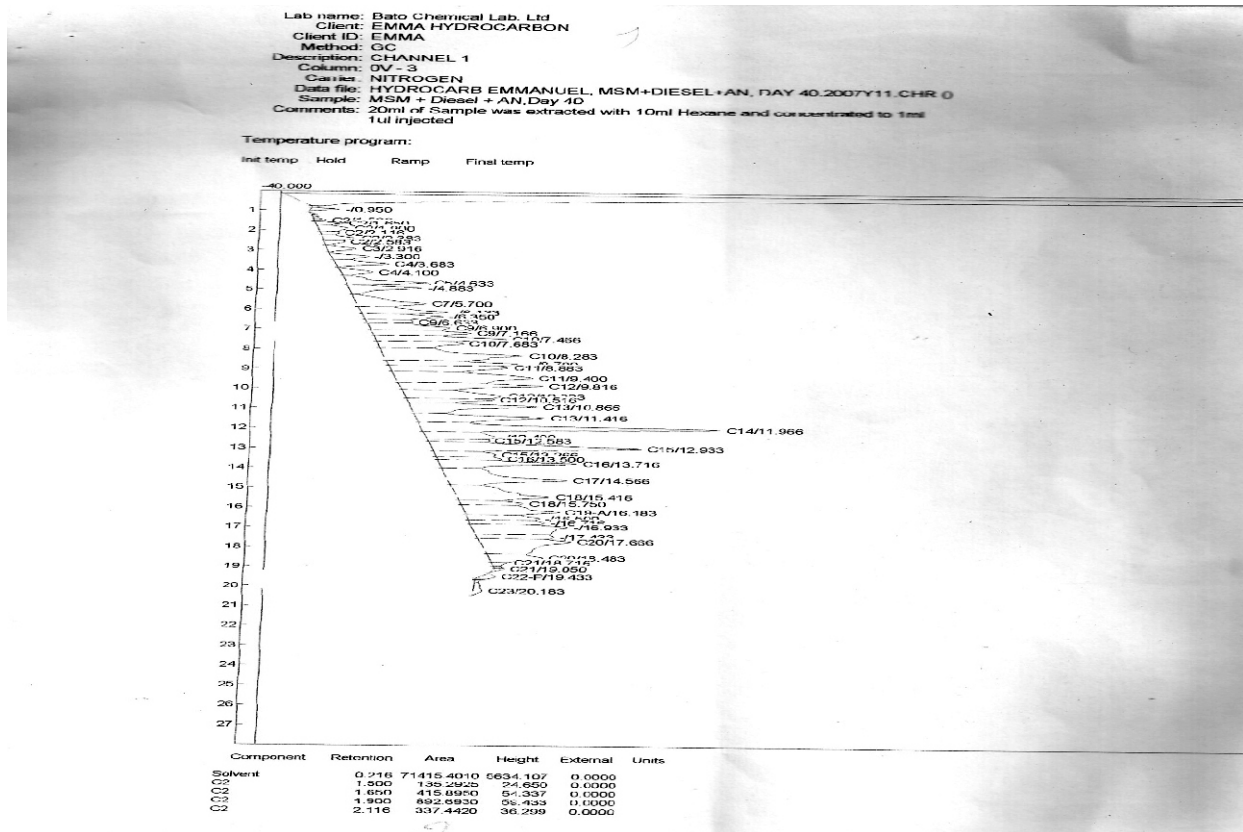


Fig. 10: G.C Analysis of *Aspergillus niger* in Minimal Salt Solution and Diesel Minimal Salt Solution and Spent Engine Oil

RESULTS AND DISCUSSION

In all, nine (9) fungi were isolated; eight (8) fungi (*Aspergillus niger*, *Aspergillus wentii*, *Aspergillus fumigatus*, *Rhizopus* sp, *Mortierella* sp, *Mucor* sp, *Candida* sp, *Penicillium* sp, were isolated from the soil samples collected from mechanic village while only one fungus (*Rhizopus* sp) was isolated from the diseased *Elaeis guineensis* fruits during six months of sampling and were identified according to the descriptions of Talbot (1971), Deacon (1980), and Bryce (1992). *A. niger* had the highest frequency of occurrence in soil and was shown to be the best in biodegrading diesel and spent engine oil while *Rhizopus* sp was best in biodegrading kerosene, unspent engine oil and extracted oil of *Elaeis guineensis* (Fig.1-7; Table 1). This is similar to the findings of Adekunle and Adebambo (2007) but with fungi from *Detarium senegalense* seeds.

Result of the Chromatographic analysis shows that both *Rhizopus* sp and *A. niger* in the media (MSS and diesel) decreased the peaks of all the detected carbons though at different rates and a complete disappearance of C_{6,8} and C₂₄₋₂₈ for the

two organisms after the 40 days of incubation (Fig. 8-10). Also the Chromatogram of the growth of *Rhizopus* sp and *A. niger* in MSS and spent engine oil showed complete disappearance of C_{6,13,19} and C₂₃ and above for *Rhizopus* sp while *A. niger* degraded completely C_{5-7,19} and C₂₂ and above.

Several works have implicated several species of fungi isolated from soil to be capable of biodegrading petroleum hydrocarbon. Gesinde *et al.*, (2008) showed that *Aspergillus* sp, *Penicillium* sp and *Rhizopus* sp isolated from soils could degrade petroleum hydrocarbon. Ogbo *et al.*, (2010) showed *Pleurotus tuberregium* to be capable of remediating soil contaminated with diesel oil. Shaw (1995) found that organisms break down hydrocarbons and use the energy to synthesize cellular components. Vida, *et al.* (2015) implicated species of *Aspergillus*, *Penicillium*, *Fusarium*, *Acremonium*, *Candida*, *Mucor*, *Aureobasidium*, *Rhizopus*, *Alternaria*, in the of utilization petroleum hydrocarbons.

After being completely broken down, the reaction releases Carbon (IV) oxide, water and energy used to create cellular biomass (Keeler, 1996, April *et al.*, 2000). El-shafie *et al.* (2007) showed that *Aspergillus niger* and *Penicillium chrysophyllum* could biodegrade n-alkanes (C₁₃ – C₁₈). Several other fungi including *candida* sp, *Mucor* spp, *Mortierella* sp, *Polyporus* sp isolated from soil have been shown to be capable of biodegrading petroleum hydrocarbon (Batha and Atlas 1977; Sood and Lai 2008; Nakagawa *et al.* 2006; Hadibarata and Tachibana 2009). The ability of fungi isolated from oil seeds to degrade petroleum hydrocarbon has been demonstrated by Adekunle and Oluyode (2002).

Aspergillus niger was shown by the result to be the best in biodegrading both diesel and spent engine oil with efficiency of 95% in spent engine oil and 84% in diesel respectively after the 40 days of incubation (Table 3). This is similar to the findings of Okafor (2009) and El-morsy (2005) which showed *Aspergillus niger* to have efficiency of up to 98% in biodegrading petroleum hydrocarbon. *Rhizopus* sp- which was isolated from oil palm (*Elaeis guineensis*) fruits was also shown to be good in biodegrading petroleum hydrocarbon with efficiency of 94% in spent engine oil and 82% in diesel respectively. *Rhizopus* had earlier been shown to be very potent in biodegrading kerosene and extracted oil of *Detarium senegalense* seed (Adekunle and Adebambo 2007), and *Treculia africana* (Decene) seeds (Adekunle and Adeniyi, 2015). This is a strong indication that fungi which could biodegrade oils of *Elaeis guineensis* kernels can do same with petroleum hydrocarbon. Chimezie (2015) also showed *Mucor* sp- and *Penicillium* sp- to be capable of degrading petroleum hydrocarbons in the aquatic environment. The abilities of these fungi in degrading petroleum hydrocarbon can be attributed to the non-specific nature of their enzymes especially the peroxidases (Okafor, 2009).

CONCLUSION

In conclusion the result here shows that fungi isolated from *Elaeis guineensis* fruit kernels can be exploited in the biodegradation of crude petroleum oil spill and bioremediation of the environment. This will provide alternative in sourcing for fungi needed for mycoremediation

and restoration of polluted environment.

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