# POTENTIALS FOR MYCOREMEDIATION OF PETROLEUM PRODUCTS BY FUNGI ISOLATED FROM *IRVINGIA GABONENSIS* SEEDS


**BY**

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## CERTIFICATION

This work titled "Potentials for mycoremediation of petroleum products by fungi isolated from *Irvingia gabonensis* seeds" submitted to the School of Postgraduate Studies, University of Lagos, Lagos, Nigeria for the award of Doctor of Philosophy (Ph.D) in Botany is an original research carried out by SANYAOLU, Adeniyi Adetola Akeem in the Department of Botany, University of Lagos, under the supervision of Professor A.A. Adekunle and Dr. A.A. Osuntoki.

This work has not been previously submitted either wholly or in part for the award of any other academic degree or Professional qualification.

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# DEDICATION

This work is dedicated to the triune God for being my all in all, and to my lovely wife, Victoria, for placing her own Ph.D studies on hold for me to complete mine.

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## ABSTRACT

The Petroleum Industry has a huge potential for the pollution of the environment at every stage of its operation, hence the need to constantly fashion out new strategies for maintaining the sanctity of our environment. Fungi associated with diseased *Irvingia gabonensis* (Baill) seeds found in the open markets were suspected to possess the ability to degrade the hydrocarbon substances contained in these seeds. These fungi were isolated bimonthly from the diseased seeds of *I.gabonensis* from four open markets in the Lagos metropolis over a two year period. Simultaneously, fungi were also isolated from the soil from five other places, namely two mechanic villages, one public dump site, and two agricultural sites. Three important concepts, namely, mycodeterioration, mycodegradation and mycoremediation were investigated with regards to the hydrocarbon utilizing potentials of these fungal isolates. A preliminary screening of these fungal isolates for their petroleum hydrocarbon utilizing abilities was carried out by culturing the fungal isolates under fume from crude oil. A total of twenty five fungal isolates from the genus *Aspergillus* were able to grow under the crude oil fume. These isolates were selected for their ability to biodegrade Crude oil (Co) and four other petroleum products namely Spent Engine oil (SEO), kerosene (kero), diesel and Fresh engine oil (FEO) as well as one vegetable hydrocarbon (oil extracted from *I.gabonensis* seed- I.O.) using spectrophotometric techniques. The results from this mycodegradation studies showed that the fungi had a significant ($p \leq 0.05$) effect on the optical densities of each of the six different hydrocarbon sources at the wavelengths of 530 and 620 nm. The results of the confirmatory studies for the mycodegradation of the different hydrocarbon sources by the fungal isolates conducted on the 40$^{th}$ day showed a Gas Chromatographic (GC) value that indicated a reduction in the Total Petroleum Hydrocarbon (TPH) value for each of the petroleum hydrocarbon compounds thus: Co from $9.84 \times 10^5$ to $7.85 \times 10^5$ mg/l, SEO from $9.56 \times 10^5$ to $8.5 \times 10^5$ mg/l, kero from $9.88 \times 10^5$ to $8.74 \times 10^5$ mg/l, diesel from $9.73 \times 10^5$ to $8.35 \times 10^5$ mg/l and FEO from $9.8 \times 10^5$ to $8.57 \times 10^5$ mg/l. There was also a change in the fatty acid methyl ester (FAME) level of the vegetable hydrocarbon compound at day forty when compared to what was available at day zero. This result therefore confirms the ability of these organisms to utilize (mycodegrade) petroleum and vegetable hydrocarbon compounds. The results of the field mycoremediation studies show that the *Aspergillus oryzae* deployed to remediate the SEO polluted field had by the 6th month after the SEO spillage achieved between 98.9 - 99.1% reduction

in the level of the initial TPH present on the spillage site. In addition, this fungus was also able to effect a steady rise in the level of the total nitrogen (from 0.07 – 0.12%), available potassium (from 0.81 – 2.95 Meq/100g), phosphorus (from 1.23 – 11.94 mg/kg) and magnesium (from 0.55 – 1.03 Meq/100g) present in the SEO polluted soil to levels significantly ($p \leq 0.05$) higher than the control values by the 3rd and the 6th months after the SEO spillage. Also, the addition of *A. oryzae* as a mycoremediation agent on a SEO polluted field further caused a significant ($p \leq 0.05$) increase in the vegetation cover of the field to levels higher than the controls at the 2nd, 4th and 6th months after the SEO spillage. The results from the mycodeterioration studies show that the *A. oryzae* (isolated from the diseased seeds of *I.gabonensis* from Ajegunle market), caused some significant changes in the physico-chemical and the nutritional qualities of the oil and the flour respectively from *I.gabonensis* seed. A Gas Chromatographic confirmatory analysis of the oils from both the healthy and diseased seeds of *I.gabonensis* further confirmed some significant differences in the free fatty acid profiles of both oils. The results of the DNA studies showed a noticeable diversity in the genetic base of the fungal isolates. This phenomenon, coupled with the obvious variation in their utilization of the different nutrient sources (hydrocarbons) and some variation in their growth pattern in the plates may eventually lead to a renaming of some of the fungal isolates that were encountered in this research. This research has thus contributed to fulfilling the need for the much desired efficient and ecologically sound strategies for neutralizing in our environment the inadvertent or the deliberate spillage of hazardous pollutants such as petroleum and its refined products.

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background of study

Man and the environment relate to each other dialectically. In his own submission, Mellanby (1971) noted that man has existed for hundreds of thousands of years, but it is only within the last ten thousand years that he has had any considerable ecological effect on the biosphere, and only within the last millennium that he has really began to change the appearance and composition of the biosphere. Until this time therefore, man has had little effect on his environment. Population explosion and the quest for development are the two most important factors that have contributed in no small measure to man’s desecration of the environment. Population explosion in the world has resulted in an increase in Environmental pollution; this is because it brings with it a growing pressure on the exploitation of natural resources (biotic, abiotic and edaphic resources in man's environment). As a direct consequence of the above, new and varied industries have evolved. It must be noted however, that in the absence of proper management, maintaining the quality of life will be a herculean task, especially in the face of these new developments, most of which are not compatible with our natural environment.

One of the most powerful machines driving our modern world (and unarguably one of the newest to evolve) is the Petroleum Industry. Man’s quest for development (and consequently an increased quality of life, which is provided by the petroleum Industry) has indeed have an inverse relationship with the overall quality of the environment. In their respective opinions, Hallier-Soulier *et al*.(1999), Margesin and Schinner (2000) and Adekunle and Adebambo (2007) all noted that the dominance of petroleum products in the world economy has created a platform for the distribution

of a large amount of the toxins into populated areas and ecosystems around the globe. Today, the consumption of petroleum, its refined constituents and by-products as fuel and its dominance in the world market as a source of chemicals has diversified tremendously. In spite of the colossal blessings from the Petroleum Industry, there are various negative impacts on the human environment. Some of these include hydrocarbon contamination of the soil, air, surface and underground aquifer. This observed phenomenon of hydrocarbon contamination emanates from multiple sources and activities of this important Industry. Right from its prospecting and exploration activities, to drilling, transportation and storage, refining and even to the consumption of its product(s), this industry has the potential for enormous environmental pollution. Of these, the accidental or unregulated disposal of petroleum, petroleum products and petroleum product wastes into the environment are of serious concern. This phenomenon has therefore compelled global attention on ways to sustain and preserve the environment. One way of making this endeavour possible – sustaining and preserving our environment - is through the practice of sustainable development in our entire endeavour, the use of Biotechnology inclusive. In Nigeria today, sustainable development is an increasing concern of governments, communities, non-governmental organizations (NGOs), and the ordinary environment conscious citizen. Basically, the tenets of sustainable development are: economic development, sound environmental/natural resource stewardship, population management and social equity (NEST, 1991). As a component strategy of sound environmental stewardship, natural Justice and social equity in our drive for sustainable development, the Nigerian society of today is increasingly concerned about some of the negative consequences of the operations of the Oil and Gas Industry such as soil degradation, lack of the sustainability of soil productivity and erosion of biodiversity.

Petroleum is an extremely complex mixture of hydrocarbon and non hydrocarbon compounds resulting from anaerobic conversion of biomass under high temperature and pressure and it is an essential element of life (Margesin and Schinner, 2000). The contamination of the environment with petroleum hydrocarbons provides serious problems for many countries (Margesin and Schinner, 2000). Natural resources in need of restoration from petroleum hydrocarbon contamination include forest habitats, mangroves, lowland rainforests, swamp forests, agricultural sites, and barrier Island forests (Imoobe and Iroro, 2009). On the terrestrial environment, oil spills cause extensive damage ranging from the destruction of terrestrial flora and fauna to biomagnifications of the toxic components of the petroleum, conversion of arable land to barren soils and the destruction of the aesthetic quality of the environment. Other environmental consequences of oil pollution include the adverse effects on the soil microflora and microfauna and ground water pollution (Obire and Anyanwu, 2009).

Some hydrocarbon components have been known to belong to a family of carcinogenic and neurotoxic organopollutants (Hallier-Soulier *et al.*, 1999). The processes leading to the eventual removal of hydrocarbon pollutants from the environment has been extensively documented and involves the trio of physical, chemical and biological alternatives.

The currently accepted disposal methods of incineration or burial in secure landfills (USEPA 2001; ITOPF as cited by Okoh, 2006) can become very expensive, especially when the amounts of contaminants are large. This often results in cleanup delays while the contaminated soil continues to pollute the environment, thus necessitating speedy removal of the contaminants. Corroborating this, Imoobe and Iroro (2009) noted that although most of petroleum components are subject to

biodegradation, this occurs at relatively slow rates, and that sometimes, Petroleum hydrocarbons are poorly degraded and thus become contaminants.

According to Adekunle and Uma (1996), Adekunle and Oluyode (2005) and Adekunle and Adebambo (2007), pathogenic fungi isolated from melon- *Cucumeropsis mannii* (Naud), soybean- *Gycine max* (L.) Merr. and *Detarium senegalense* (G.F. Gmel.) seeds were found to contain some enzymes (lipases) that possess the ability to degrade hydrocarbons from the components of these oil seeds, and subsequently, some petroleum hydrocarbon products such as crude oil, kerosene and diesel. Adekunle and Adebambo (2007) therefore observed that the most rational way of decontaminating an environment polluted with petroleum hydrocarbon derivatives is the application of methods that is based mainly on the metabolic activity of microorganisms- biodegradation, especially since these microorganisms possess the innate ability to degrade hydrocarbons present in the oils contained in the seeds on which they are pathogenic. The effectiveness of bioremediation often is a function of the microbial population, and how they can be enriched and maintained in an environment (Marques *et al*., 2000; Prasad *et al*., 2012). The immense diversity of soil-living microbes remains to an extent under utilized. This is not surprising since one gram of soil may harbor up to 1,010 microorganisms (MacNaughton *et al*., 1999). Microorganisms with the ability to degrade oils have been severally reported in literatures as been ubiquitously distributed in different environments such as water (Ilori *et al.,* 2005; Adebusoye *et al*., 2007; Ilori *et al*., 2007; Ilori *et al.,* 2008), in association with some plants (Ilori *et al.,* 2006) and in soils (Ilori and Amund, 2000; Ilori and Amund, 2001; Igwo-Ezikpe *et al*., 2006; Oboh *et al*., 2006; Amund *et al.*, 2006; Adebusoye et al., 2007a; Adebusoye et al., 2007b; Adebusoye *et al*., 2008a; Adebusoye *et al*., 2008b; Ilori *et al*., 2008; Obayori *et al.,* 2008). To assess soil

quality and fertility, however, indicators reflecting the physical, chemical and biochemical components of the soil are needed. Microorganisms being in intimate contact with soil micro-environment are in many ways ideal as test organisms to monitor soil pollution (Bento *et al*., 2005).

Successful application of bioremediation technology to contaminated systems also requires the knowledge of the characteristics of the site and the parameters that affect the microbial biodegradation of pollutants (Sabate *et al.,* 2004).

Biodegradation has been intensively studied in controlled conditions (Sugiura *et al*., 1997; Chaillan *et al.,* 2004) and in open field experiments (Chaıneau *et al.,* 2003; Gogoi *et al.,* 2003), and has acquired a new significance as an increasingly effective and potentially inexpensive cleanup technology. Biodegradation is the use of naturally occurring microorganisms or genetically-engineered microorganisms (bacteria and fungi) by man, to detoxify man- made pollutants (Okoh, 2006). Its potential contribution as a countermeasure biotechnology for decontamination of oil polluted systems could be enormous. The composition and inherent biodegradability of the petroleum hydrocarbon pollutant, therefore, is the first and most important consideration when the suitability of a cleanup approach is to be evaluated (Okoh, 2006).

Microorganisms (primarily bacteria and fungi) are nature’s original recyclers. Their capabilities to utilize natural and synthetic chemicals as sources of energy and raw materials for their own growth suggests that expensive chemical or physical remediation processes of polluted environments might be replaced with biological processes that are less expensive and more environment friendly (Sasikumar and Papinazath, 2003). Petroleum contains a wide range of organic compounds that can

serve as nutrients for microorganisms. Fungi and bacteria can be genetically modified to detoxify man-made pollutants (Ogden and Adams, 1989). The oil service industry already utilizes genetically manipulated microorganisms as producers of surfactants and polysaccharide polymers (Chakrabarty, 1985; 1986).

Microorganisms, particularly fungi, represent a promising, largely untapped resource for environmental biotechnologies. Research continues to verify the mycoremediation potential of fungi. For example, in their own report, Davies and Westlake (1979), reported that about forty species of fungi were found to grow on petroleum and or petroleum products, while Adekunle and Oluyode (2005) in their own work found twenty one species of fungi growing on petroleum and its refined constituents. AI-Awadhi *et al.* (1998), noted that some of the white rot fungi like *Phanerochaete sp, Pleurolus sp* and *Coriolus sp* were found to have the fastest hydrocarbon degrading rates. Other fungi known to play important roles in hydrocarbon utilization according to Cerninglia and Perry (1973) are *Cumingamella sp* and *Cladosporium sp*. The ease of transportation, genetic engineering, and multiplication makes fungi the organisms of choice in bioremediation.

As a natural consequence of the law of thermodynamics, in nature, all substance must ultimately succumb to decay (Zeyaullah *et al*., 2009). Bioremediation is therefore based on the idea that all organisms (including micro organisms) remove substances from the environment to carry out their own growth and metabolism, and in the process effect the decay of these substances. Fungi are particularly good at digesting complex organic compounds that are normally not degraded by other organisms (Zeyaullah *et al*., 2009). According to Covino *et al*. (2010), an important characteristic which distinguishes filamentous fungi from bacteria and makes them excellent candidates for bioremediation strategies, is the penetration of the fungal

hyphae into the polluted matrix and the excretion of oxidative enzymes. These oxidases, mainly laccase, lignin peroxidase and manganese peroxidase present very low substrate specificity and, being active in the extracellular environment, are able to attack scarcely bioavailable contaminants. Furthermore, Covino *et al.* (2010), reiterated the well known fact that fungi are involved in soil humification process; in this respect, the use of these organisms in soil remediation could lead, not only to the decontamination, but also to the re-use of the soil for agricultural purposes. In addition, low molecular weight poly aromatic hydrocarbons (PAH) are known to be readily degraded by bacteria, whereas, high molecular weights PAH resist extensive bacteria degradation in soil and sediment media, (Aust, 1990; Mueller *et al*, 1992). Some fungi however are believed to have a greater access to poorly bioavailable substrates such as these heavy fractions of PAH, since they secrete extracellular enzymes involved in the oxidation of complex aromatic compounds. Fungi can also grow under environmentally stressed conditions such as low pH and poor nutrient status, where bacteria growth might be limited (Davis and Westlake, 1979). In addition to degrading hydrocarbons directly, fungal mycelia can penetrate oil, thereby increasing the surface area available for biodegradation than bacterial attack (Covino *et al*., 2010).

In a taxonomic study of fungi, Nyns *et al.* (1969), found that hydrocarbon assimilation is most common in the orders Mucorales and Monilales, as well as in the genera *Aspergillus* and *Penicillium* (order Eurotiales). Furthermore, in comparison with eight other genera, *Aspergillus* and *Penicillium* species were the most efficient metabolizers of hydrocarbons (Obire *et al.*, 2008). Hydrocarbon assimilation was, however, relatively rare, and was a property of individual strains, not of species or higher taxa (Nyns *et al*., 1969). Nevertheless, diverse fungi have been isolated from

oil-contaminated environments, and/or shown to degrade hydrocarbons in the laboratories. It was also reported by Covino *et al.* (2010) that although bacteria initiated the degradation of a synthetic petroleum mixture, twice as much was degraded when both bacteria and fungi were present.

Species in many fungal genera are known to metabolize hydrocarbons/ and or thrive in oil-contaminated sites. They include:

- ✓ *Acremonium* (Llanos and Kjoller, 1976)
- ✓ *Aspergillus* (Bartha and Atlas, 1977; Obire *et al.*, 2008)
- ✓ *Aureobasidium* (Bartha and Atlas, 1977)
- ✓ *Candida* (Bartha and Atlas, 1977; Obire *et al*., 2008)
- ✓ *Lentinus subnudus* (Adenipekun and Fasidi, 2005)
- ✓ *Pleurotus tuber-regium* (Ogbo and Okhuoya, 2008; Adenipekun, 2008; Ogbo and Okhuoya, 2011)
- ✓ *Pluerotus ostreatus* (Okparanma *et al*, 2011)
- ✓ *Cephalosporium* (Bartha and Atlas, 1977; Obire *et al*., 2008)
- ✓ *Cladosporium* (Bartha and Atlas, 1977; Obire *et al.,* 2008)
- ✓ *Cunninghamella* (Bartha and Atlas, 1977)
- ✓ *Fusarium* (Llanos and Kjoller, 1976; Obire *et al*., 2008)
- ✓ *Geotrichum* (Obire *et al*., 2008)
- ✓ *Giiocladium* (Llanos and Kjoller, 1976)
- ✓ *Graphium* (Llanos and Kjoller, 1976)
- ✓ *Hansenula* (Bartha and Atlas, 1977)
- ✓ *Mortierella* (Llanos and Kjoller, 1976)
- ✓ *Mucor* (Obire *et al*., 2008)
- ✓ *Paecilomyce*s (Llanos and Kjoller, 1976)

- ✓ *Penicillium* (Llanos and Kjoller, 1976; Bartha and Atlas, 1977; Obire *et al*., 2008)
- ✓ *Rhodosporidium* (Ahearn *et al*., 1976; Bartha and Atlas, 1977)
- ✓ *Rhodotorula* (Bartha and Atlas, 1977; Obire *et al*., 2008)
- ✓ *Saccharomyces* (Bartha and Atlas, 1977)
- ✓ *Sphaeropsidales* (Llanos and Kjoller, 1976)
- ✓ *Sporobolomyces* (Bartha and Atlas, 1977)
- ✓ *Torulopsis* (Bartha and Atlas, 1977)
- ✓ *Trichoderma* (Llanos and Kjoller, 1976; Obire *et al*., 2008)
- ✓ *Trichosporon* (Ahearn *et al*., 1976; Bartha and Atlas, 1977)

Although there have been reports of commercial production of fungal and bacteria inocula for treatment of oil spills in developed countries (Bartha and Atlas, 1977), there has been none in Nigeria. In petroleum-producing regions of Nigeria, Obire (1988) found several species of oil-degrading aquatic fungi in the genera *Candida, Rhodotorula, Saccharomyces* and *Sporobolomyces* (yeasts) and, among filamentous fungi, *Aspergillus niger* (Tiegh.) Speg., *Aspergillus terreus* (Thom.), *Blastomyces sp., Botryodiplodia theobromae* (Pat.), *Fusarium sp., Nigrospora sp., Penicillium chrysogenum* (Thom.), *Penicillium glabrum* (Whemer) Westling, *Pleurofragmium sp., and Trichoderma harzianum* (Rifai.).

## 1.2 Statement of problem

In Nigeria, and most other countries of the world, the current methods of cleaning up petroleum hydrocarbon contamination in the environment include the following: doing nothing- letting nature take its course; the use of physical/mechanical methods and the use of chemicals to disperse the spilled oil. All the above mentioned methods

are known to have varied forms of ecologically undesirable effects. However, in terms of compatibility with the natural environment, the use of higher plants (phytoremediation), the use of fungi (mycoremediation) and the use of bacteria in cleaning up pollutants have all been found to be very useful. Over 20 years ago Amund *et al.* (1987 and 1993) established the many dangers associated with the indiscriminate disposal of spent engine oils in the Nigerian environment. Pollution from petroleum products and it's wastes as one of the environmental problems in Nigeria is more wide spread than crude oil pollution (Nwaogu *et al.*, 2008). The ever increasing frequency of occurrence of lubricating oil usage over a wide geographical spread in Nigeria (especially on account of huge population and non functional public power supply) may constitute a severe environmental problem now and in the future due to waste oil disposal problem if not properly controlled.

No report is yet available on the mycoremediation of petroleum hydrocarbons using pathogenic fungal species isolated from the seed of *Irvingia gabonensis* (Baill.), nor has there been a comparative study of the relative efficiency of fungi from this source and other sources such as soils from a public dump site, automobile mechanics' workshop, and agricultural fields. This research may fulfill the need for the much desired efficient and ecologically acceptable strategies for neutralizing hazardous pollutants such as petroleum and its refined products.

## 1.3 Aims and objectives of the study

The aim of this research is to investigate the fungal species associated with diseased conditions in the *I.gabonensis* oilseeds in the open market. This work is to confirm that fungi suspected to be capable of utilizing the vegetable hydrocarbons present in the *I.gabonensis* oilseeds are also capable of utilizing hydrocarbons of petroleum

origin. It is also to compare the efficiency of fungi isolated from *I. gabonensis* with that of some other fungal species isolated from different soil environments in utilizing hydrocarcobon in the petroleum and petroleum products.

The specific objectives of this research therefore are to:

(1) isolate pathogenic fungal species from an oilseed - *Irvingia gabonensis*- and from soils collected from automobile mechanic workshops, agricultural farmlands and a public dumpsite.

(2) determine the exact identities (race or strain) of some of the choice fungal species in objective (1) above through a molecular study-DNA sequencing.

(3) compare the abilities of the isolated fungal species in objective (1) above at utilizing different petroleum hydrocarbon compounds and hydrocarbon of plant origin through studies in the laboratory.

(4) evaluate the effectiveness of one of the fungi (found to be amongst the most promising in the laboratory studies) at mycoremediating petroleum hydrocarbon pollution under field conditions.

(5) investigate the biodeterioration potentials of the pathogenic fungal species (associated with *I. gabonensis* seed) on the nutritional composition of the flour and the physico-chemical characteristics of the oils extracted from the *I.gabonensis* seed.

## 1.4 Significance of Study

Species of micro organisms are known to be environment specific (Adekunle and Adebambo, 2007, Obire *et al*., 2008), hence the need to test fungi that are indigenous to our specific environment in bioremediating oil pollution in our environment. It should be noted that in Nigeria, no information is available regarding the commercial

production of fungi inocula for use in bioremediation of oil polluted environments. This effort therefore may probably represent the necessary step in this regard. Besides, there is the need for putting to a constructive use (such as in cleaning up oil pollution) some fungal species that have been implicated in the pathogenicity of a useful oilseed such as *I. gabonensis* , thereby providing a platform for reversing some losses in our national economy due to the activities of these pathogenic fungal species. Most reports on biodegradation or bioremediation using fungi have been mainly on fungi isolated from the soil or aquatic environment (Cavalca *et al.*, 2000 and Adekunle and Oluyode, 2005). However, no report has been published on the comparative efficiency of fungi from different kinds of soil environment (such as a dump site, mechanic workshop and agricultural farm) and fungi from an oilseed at degrading petroleum hydrocarbon and bioremediating petroleum hydrocarbon pollution. It must be noted that this work probably represents some pioneering attempt at comparing the relative efficiency of fungi species associated with an oilseed (*I. gabonensis*) and other sources such as dumpsite and auto mechanic workshops at remediating petroleum hydrocarbon pollution.

Studies have shown that bioremediation agents may be effective in the laboratory but significantly less so in the field (Lee *et al.*, 2002, Mearns, 1997; Venosa *et al.*, 1996 and 2002). This is because laboratory studies cannot always simulate complicated field conditions such as spatial heterogeneity, biological interactions, climatic effects, and nutrient mass transport limitations. Therefore, field studies (which is generally regarded as the ultimate test or the most convincing demonstration of the effectiveness of any bioremediation agent), was used in addition to laboratory studies in this research.

## 1.5 Research questions

(1) Can it be demonstrated through experiments in the laboratory that there are fungal species found associated with diseased seeds of *I.gabonensis* and from soils in automobile mechanic villages, a public dump site and from agric farms that are capable of utilizing petroleum hydrocarbon and vegetable hydrocarbon compounds?

(2) Are the fungal species associated with the diseased seeds of *I.gabonensis* more efficient at utilizing petroleum and vegetable hydrocarbon compounds than the fungal species from these other soil environments?

(3) Can any of the fungal species in (1) above have potential to mycoremediate an environment that is contaminated with petroleum hydrocarbon in a timely and environment friendly manner?

(4) Is there any variation in the genetic constitution of the fungal isolates from different places that were found to be capable of biodegrading petroleum products?

(5) Can any of the fungal species found associated with *I.gabonensis* seed cause a deterioration in the seed's nutritional value or in the physico-chemical integrity of the oil extracted from these seeds?

## 1.6 Operational Definition of Terms

**Biodegradation**: Biodegradation or biotic degradation or biotic decomposition is the chemical dissolution of materials mainly by microorganisms or other biological means. The term is often used in relation to ecology, waste management, biomedicine, and the natural environment.

**Biodeterioration**: the breakdown of materials by microbial action, often with a resultant decrease in the value of the original material.

**Bioremediation**: environmental cleanup by biological agents to restore contaminated land, especially the addition of microorganisms such as fungi, bacteria and other organisms that consume or neutralize contaminants in the soil, water, gases.

**Mycoremediation**: a form of bioremediation, which involves the use fungi to degrade or sequester contaminants in the environment essentially through the enzyme activity and mycelia action of the fungus, this can be achieved in-situ or ex-situ.

**Phytoremediation**: the use of plants to remove, degrade, or contain soil pollutants such as heavy metals, pesticides, solvents, crude oil, polyaromatic hydrocarbons, and landfill leacheates.

**Inoculation**: the process of introducing a microorganism into a medium suitable for its growth.

**Optical Density/ Optical activity**: a measure of how dense a medium is, based on the amount of visible light in the visible spectra that it is able to absorb. The denser the medium, the more the amount of light that it will absorb (or the less it will transmit). This property helps determine how microorganisms are able to "proliferate" or flourish in a Minimal Salt Solution (MSS). The more they flourish in a MSS medium, the denser the medium becomes.

**Total Petroleum Hydrocarbon (TPH):** a family of organic compounds composed predominantly of carbon and hydrogen, the basic constituent of all organic compounds, including Petroleum and all Petroleum Products.

**DNA Sequence:** the relative order of base pairs in a fragment of DNA, a gene, a chromosome, or an entire genome. This helps in confirming the exact identity of any organism.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

Petroleum hydrocarbon pollution and it's clean up: a bird's eye-view

Environmental pollution has become unacceptable for modern living, especially as public awareness of its effects on the environment has increased. Unfortunately, it is not possible within a short time to replace all the industrial processes generating polluting waste streams with cleaner alternatives. Therefore, treatment both at source and after release must be considered as alternatives in many cases for these pollutants (Betts, 1991). Conventional oil spill countermeasures include various physical, chemical, and biological methods. Commonly used physical methods include booming and skimming, manual removal (wiping), mechanical removal, water flushing, sediment relocation, and tilling. Physical containment and recovery of bulk or free oil is the primary response option of choice in the United States for the cleanup of oil spills especially in marine and freshwater shoreline environments. Chemical methods, particularly dispersants, have been routinely used in many countries, including Nigeria, as a response option. However, chemical methods have not been extensively used in a number of countries due to the disagreement about their effectiveness and the concerns of their toxicity and long-term environmental effects (U.S.EPA, 1999).

Although conventional methods, such as physical removal, often are the first response option, they rarely achieve complete cleanup of oil spills. Bioremediation is beginning to emerge as a promising technology, particularly as a secondary treatment option for oil cleanup. This technology is based on the premise that a large percentage of oil components are readily biodegradable in nature (Atlas, 1981, 1984; Prince, 1993).

The search for cheaper and environment friendly options of enhancing petroleum hydrocarbon degradation has continued to stimulate research interest. The

involvement of microorganisms in the degradation of petroleum hydrocarbons in the environment has been established as an economic, efficient, versatile, and environment friendly treatment method (Margesin and Schinner, 2000; Yakubu, 2007; George-Okafor *et al.*, 2009; Agarry *et al.*, 2010). Furthermore, Amund *et al.*, 1987; Amund and Igiri, 1990, Amund *et al.,* 1993; Wang *et al*, 1996 and Adekunle and Adebambo, 2007 all observed that the soil ecosystem is endowed with an immense versatility with regards to degradation and humification of organic substances, and that many species of bacteria, fungi and algae species possess the enzymatic ability to use petroleum hydrocarbon as food. The soil environment is the most dynamic site of interactions in nature and it is also the region in which many of the biochemical reactions concerned in the decomposition of organic matter and nutrition of plants occur (Torstensson *et al.,* 1998). Microbial community carries out the majority of decomposition processes in the soil and is irreplaceable in the transformation and degradation of synthetic organic compounds and natural waste materials (Atlas, 1991; Atlas and Bartha, 1992; Torstensson *et al*., 1998).

Intimate relationship exist between physicochemical properties of soil, soil organisms and soil biological properties. The use of microorganisms and their activities in tests of effects of a specific chemical substance in soil, as well as in studies of soil pollution has often been recommended. Microorganisms, more so than any other class of organisms, have a unique ability to interact chemically and physically with a huge range of both man-made and naturally occurring compounds leading to a structural change to, or the complete degradation of the target molecule (Head and Swannell, 1999). For bioremediation to be successful, the bioremediation methods depend on having the right microbes in the right place with the right environmental factors for

degradation to occur. The right microbes are fungi and bacteria, which have the physiological and metabolic capabilities to degrade the pollutants.

### 2.1.0 Mycoremediation of petroleum hydrocarbon pollution

The term mycoremediation refers specifically to the use of fungi to degrade or remove toxins including heavy metals from the environment (Drennan, 2008). The advantages associated with fungal bioremediation lay primarily in the versatility of the technology and its cost efficiency compared to other remediation technologies {(such as incineration, thermal desorption, extraction) (Aust, 1990)}. The use of fungi is expected to be relatively economical as they can be grown on a number of inexpensive agricultural or forest wastes such as corncobs and sawdust. More so, their utilization is a gentle non-aggressive approach. The application of the bioremediation capabilities of indigenous fungi species especially to clean up pollutants is viable and has economic values (Bijofp, 2003). Fungi are especially good at digesting complex organic compounds that are normally not degraded by other organisms (Zeyaullah *et al.*, 2009). In addition to degrading hydrocarbons directly, fungal mycelia can penetrate oil, thereby increasing the surface area, vertically and horizontally that is available for biodegradation. Fungi can also grow under environmentally stressed conditions such as low pH and poor nutrient status, where bacteria growth might be limited (Davis and Westlake, 1979).

In a study reported by George-Okafor *et al.* (2009), twelve fungal isolates recovered from oil-contaminated soils were screened for crude oil biodegradation in a shake-flask culture. Among the twelve fungal isolates, only eight demonstrated the ability to biodegrade the oil; two of them, identified as *Aspergillus versicolor and Aspergillus niger* exhibited the fastest onset and highest extent of biodegradation. When selected

for further study on specific polycyclic aromatic hydrocarbon (PAH) biodegradation, both isolates exhibited above 98% degradation efficiency for polycyclic aromatic hydrocarbon moieties when grown in a culture medium incorporated with 1% crude oil (hydrocarbon) and 0.1% Tween 80 for 7 days.

Based on previous work some bacteria such as *Pseudomonas, Bacillus and Acinetobacter species* were rated (70.6% - 82%) in their ability to degrade hydrocarbon. However, it was observed by George-Okafor *et al* (2009) that a higher biodegradation efficiency (>98%) was exhibited by *A.versicolor and A. niger,* thus proving these fungi to be better hydrocarbon degraders. Additional reports has confirmed they can be effectively utilized for the degradation of oil polluted farm lands especially those located within the vicinity of the isolation soil sites, especially as fungi are important agents in biogeochemical cycling and mineralization.

The use of fungi for the treatment of petroleum hydrocarbon and heavy-metal containing effluents has been well established due to their ability to accumulate metals from their external environment (Siegel *et al*., 1990; Gadd, 1993; Kalac *et al*., 1999; Adenipekun, 2008; Adenipekun and Isikhuemhen,2008; Ogbo and Okhuoya, 2011). Fungi are increasingly being investigated and used in the remediation of contaminated environments because of their ability to degrade an extremely diverse range of very toxic environmental pollutants (Isikhuemhen *et al*., 2003).

Atagana *et al*. (2006) reported that *Pleurotus sp*. performed best as a biodegrader of creosote in soil compared to other types of fungi. Stamets (2006) reported the successful use of a *Pleurotus sp*. to reduce more than 95% of toxic PAH inoculated into a test soil into non-toxic components. Another fungus species- a mushroom- *Pleurotus tuber-regium,* indeginous to Nigeria and distributed across sub Saharan Africa (Isikhuemhen *et al*., 2000; Ogbo and Okhuoya, 2008), has also been shown to

have the capability to ameliorate crude oil polluted soils. Once remediated, the soil was demonstrated to support seed germination and seedling growth of *Vigna unguiculata* at levels which in some cases were better than the control (Isikhuemhen *et al*., 2003).

Similarly, *Lentinus squarrosulus* has been found to mineralize soil contaminated with various concentration of crude oil, resulting in increased nutrient content in treated soils after 6 months of incubation (Adenipekun and Fasidi, 2005).

Fungi grow on hydrocarbon and non hydrocarbon contaminated soils, secretes enzymes luccase, manganese dependent peroxidase and lignin peroxidase which are used for remediation (Barr and Aust, 1994; Aust *et al*, 2003; Mansur *et al*, 2005). Similary, Oudot (1999) and Stamets (2005), reported that some mushrooms will grow optimally in harmful contaminants. Lau *et al* (2003), reported the use of mushroom compost to degrade PAH contaminated soil. Fungi exhibits extra ordinary abilities to transform recalcitrant pollutants and also degrade broad spectrum of structurally diverse toxic environmental pollutants (Lang *et al*, 1995). Their extra cellular ability allows them to degrade insoluable toxic compounds and non-popular compounds (Levin *et al*, 2003). Cajthaml *et al.* (2002), reported that enzymes of fungi degrade several PAH (anthracene, flouranthrene, phanathrene and pyrene) after 50 days of incubation to less than 1% anthracene, less than 5% of phanathrene and less than 7% of pyrene. In the same vein, Levin *et al* (2003) reported that *Trametes trogii* metabolized and degraded 90 – 97% highly concentrated nitrobenzene and anthracaene. Fungi utilizes different substrates and degrades them during the period of incubation as food (Wang, 1984; Barr and Aust, 1994). They exhibit different range of growth in remediation of petroleum contaminated soils (Nicolotti and Eglis, 1988; Emuh, 2010). This show the abilities of different fungi species and strains to

breakdown and absorb or mineralize the pollutants. Thomas *et al*, (1999) reported that Oyster mushroom mineralized and metabolized 97% of oil similar to oil spilled at Exxon Valdez tanker of Alaska, USA after 8 weeks of incubation. Similarly, Bhatt *et al.* (2002), Sasek (2003), reported the use of some mushroom to degrade hydrocarbons and its by-produts such as diesel, gasoline, oil and tar while Kondo *et al.* (2003), Gadd (2004) reported their use in degrading even pesticides and preservatives.

Fungi generally are particularly proficient in breaking down many recalcitrant compounds, dissembles long chain molecules and harmful toxins to less toxic but simpler chains (Stamets, 2005). Eggen and Sasek (2002) reported that Oyster mushroom compost significantly and effectively reduce toxin in oil polluted soils.

### 2.1.1 Action of some fungi on heavy metals

Fungi breaks down and absorbs or mineralizes environmental pollutants into non-toxic form (Hamman, 2004). The presence of heavy metals and other harmful contaminants, which they attack extra-cellularly, and digests is known to have lead to an increase in their number as opposed to an inhibition and the subsequent removal of toxic metal in the environment as demonstrated by the shiitake mushroom (Hitivani and Mecs, 2003; Stamets, 2005). The scavenging of metals from polluted sites by fungi (Malik, 2004) are due to remediation and purifying abilities of these species. Oudot (1999), Barr and Aust (1994), Stamets (2005), Emuh (2009), reported that mushroom grows in the presence of heavy metals, secretes enzymes and detoxified such contaminants. Similarly, Mulligan and Galver-Cloutier (2003) reported that fungi species degraded metal contaminated soil. Stamets (2005) reported that mushroom channels heavy metals from land to fruity bodies for removal from the

soil/environment. The mechanism as reported by Stamets (2005) is first by denaturing the toxins and finally absorbing such heavy metals.

Fungi species are hyper accumulators of heavy metals and radioactive metals that are toxic to consume and are thus eliminated from the environment. These are bio-concentrated in solid forms in their biomass (Wasser *et al,* 2003; Sasek, 2003). Similarly, Arica *et al*. (2003) reported, the use of turkey tail mushroom and phoenix oyster mushroom mycelia to eliminate 97% mercury ion from water. Furthermore, Humer *et al.* (2004), reported that some fungi species identified as mushrooms degraded copper and chromium in treated woods.

### 2.2.0 Fungi and petroleum hydrocarbon degradation in Nigeria

In 2005, Adenipekun and Fasidi investigated the ability of *Lentinus subnudus* to mineralize soil contaminated with various concentrations of crude oil. The results obtained from this work showed that organic matter and carbon levels in the soil were higher than that of the control- soils without *L. subnudus-* at all concentrations of crude oil contamination in the soils after 3 months. Nutrient contents were generally higher after 6 months of incubations except potassium levels which were not significantly different from the control. As for the total petroleum hydrocarbon (TPH) in crude – oil contaminated soils, *L. subnudus* was found to achieve the highest rate of biodegradation at 20% concentration after 3 months and 40% after 6 months of incubation.

In line with observations made with crude oil, Adenipekun (2008), found with used engine oil that *Pleurotus tuber-regium* has the ability to increase nutrient contents in soils polluted with 1 - 40% engine-oil concentration after six months of incubation. *P. tuber-regium* increased organic matter, carbon and available potassium to 5.19%,

2.99% and 0.97 meq/100 g respectively compared to 4.41%, 2.56% carbon and 0.66 meq/100 g, respectively in the control.

Ogbo and Okhuoya (2008) were able to demonstrate the importance of biostimulation, particularly the use of nutrient enhancement in making mushrooms more effective in cleaning up pollutants. They investigated the degradation of Total Petroleum Hydrocarbonsn (TPH), aliphatic, aromatic, ashphaltenes and resinic hydrocarbon compounds using the mushroom *Pleurotus tuber-regium* in combination with different substrates. They also conducted the same experiment using the synthetic fertilizer NPK.

In this work Ogbo and Okhuoya (2008) reported that the reduction of TPH was higher in Treatments with a combination of fertilizers and co-substrates and also that the degradation of TPH was higher in poultry litter Treatment than the NPK Treatments.. Reporting on the other Treatments, they noted that the degradation of aliphatics in substrates with cosubstrates was higher than those without co-substrates. Reduction of aromatics was appreciable in all Treatments with the least being 40% in banana leaf blades + contaminated soil and contaminated soil only substrates.

Degradation of resins was higher in contaminated soils with co-substrates only than those with fertilizers + co-substrates. The reduction of asphaltenes was low and some substrates instead of a decrease, recorded increase in the asphaltic fraction. Phytoasssessment tests show that the addition of poultry litter and sawdust and banana leaf blades to fungal remediation restored the contaminated soil. Concluding, Ogbo and Okhuoya (2008) noted that *P. tuber-regium* is better able to remove hydrocarbons from soil with the aid of poultry litter and co-substrates than with NPK.

Edema *et al.(*2011), recorded that the levels of PAHs associated with crude oil was found, using the gas chromatographic method, to have been significantly reduced within 3 weeks of introducing this mushroom into the contaminated soil.

Okparanma *et al*. (2011) used the substrate from spent *Pluerotus ostreatus* to biotreat oil based drill cuttings from an oil field, focusing on the PAH component of this drill cutting. In this work, *P. ostreatus* was found to have reduced the total amount of residual PAHs in the drill cuttings after 56 days of composting to between 19.75 and 7.62%, while the overall degradation of PAHs increased to between 80.25 and 92.38% with increasing substrate addition. Individual PAH degradation ranged from 97.98% in acenaphthene to 100% in fluorene, phenanthrene and anthracene, thus confirming the ability of this mushroom to degrade even the most toxic and recalcitrant components of petroleum hydrocarbon.

Veryfying the effects of *Pleurotus tuber-regium* on the bioavalability of metallic elements in crude oil contaminated soil, Ogbo and Okhuoya (2011) suggested the use of *P.tuber-regium* for the harvesting of some metals from heavy metal contaminated sites as the concentration of metals tested for such as Iron (Fe), manganese (Mn), nickel (Ni), zinc (zn), copper (Cu), lead (Pb), chromium (Cr), cadmium (Cd), and arsenic (As) in their work were found to be reduced. However, they noted that *P.tuber-regium* has the potentials for increasing metal toxicity in soils as they were found to increase the bioavalability of some heavy metals such as cobalt that are needed only in trace amount.

2.3.0 The attributes of fungi in bioactivities

It must be stated that the act of degrading pollutants in the environment by fungi species (and indeed any other organism) is not an act of benevolence, but rather, it is a

strategy for survival. Fungi feed by secreting enzymes and digests food externally and absorb the nutrients in net like chain called hypha. The net like chain (hypha) is exposed to stimuli in their ecological niche and act as a conscious intellect and respond to stimuli. Dense and regular branching of hypha endows fungi with potentials to pervade any substrate thoroughly (Hudson, 1986). The higher the mycelium thickness, the higher the rate of mechanical penetration and breaking down of substrate; thus culminating in the higher rate of digestion of substrate through the secretion of extra-cellular enzymes. This shows the potentials of bioremediation capabilities of fungi species (Hudson, 1986; Juhaz and Naidu, 2002; Hamman, 2004).

These hyphae (mycelia) penetrates contaminated soils, thus placing a mat on them, is the process of breaking down and absorption of toxic products or pollutants. Generally the bonds in hydro-carbon and petroleum products such as premium motor spirit (PMS) and automotive gas oil (AGO) are similar to bonds that hold the plant materials together (Schliphake *et al,* 2003). The enzymes produced by them which are lignin perioxidase, manganese perioxidase and luccase penetrate, break and digest or mineralizes these hydrocarbon, petroleum products and pesticides to primary non-solid products and are liberated in the forms of water and carbon (iv) oxide (Schliphake *et al,* 2003; Stamets, 2005). These enzymes act singly or collectively in aiding mycelium to break down nature or human made resistant materials (Stamets, 2005). Similarly, Hitivani and Mecs (2003), reported that the mycelium of shiitake mushroom exposed to heavy metals of cadmium, copper, lead, mercury and zinc increased the production of enzymes luccase, decolourized them and subsequently absorbed the heavy metals.

The white-rot fungus- basidiomycetes- are strong decomposers of wastes due to their capability to synthesize the relevant and unique oxidative network of extracellular

enzymes (Maganhotto de Souza Silva *et al*., 2005; Eichlerova *et al*., 2006). The genus Pleurotus includes species that have unique ability to produce extra cellular lignocellulolytic enzymes including laccase and Manganese peroxidase (Stajic *et al*., 2006), xylanase (Elisashvili *et al*., 2008), CMCase, b-glucosidase and b-xylosidase. These enzymes have shown enormous biotechnological potential as they can be used in the degradation of a whole array of xenobiotics (Ren and Buschle- Diller, 2007) and detoxification of industrial residuals with high phenolic content (Mata *et al*., 2005).

Fungal mats are now known as the largest biological entities on the planet, with some individual mats covering more than 20,000 acres. The momentum of mycelial mass from a single mushroom species, growing outwards at one-quarter to two inches per day, staggers the imagination! (Stamets, 2006). These silent but aggressive mycellia affect all biological systems upon which they are dependent. As one fungus matures and dies back, a panoply of other fungi quickly comes into play. Every gramme of soil hosts not just one species, but thousands of species of fungi. The genetic diversity of fungi is vast by design, and apparently crucial for life to continue. It must be emphasized that these adaptive mechanisms are the very foundation of ecological stability and vitality in a rapidly changing environment. We should learn from our elders and the native peoples worldwide who for centuries have viewed fungi as spiritual allies. In the words of stamets (2006), they are not only the guardians of the forest. They are the guardians of our future.

### 2.4.0 Bioremediation

Bioremediation offers several advantages over conventional techniques such as landfilling or incineration. Bioremediation that can be done on site is often less

expensive and site disruption is minimal, it eliminates waste permanently, eliminates long-term liability, and has greater public acceptance, with regulatory encouragement, and it can be combined with other physical or chemical treatment methods. Other potential advantages over conventional technologies are being less costly, less intrusive to the contaminated site, and more environmentally benign in terms of its end products. The success of oil spill bioremediation depends on one's ability to establish and maintain conditions that favor enhanced oil biodegradation rates in the contaminated environment. Numerous scientific review articles have covered various factors that influence the rate of oil biodegradation (Zobell 1946; Atlas, 1981 & 1984; NAS, 1985; Focht and Westlake, 1987; Leahy and Colwell, 1990; Atlas and Bartha, 1992). One important requirement is the presence of microorganisms with the appropriate metabolic capabilities. If these microorganisms are present, then optimal rates of growth and hydrocarbon biodegradation can be sustained by ensuring that adequate concentrations of nutrients and oxygen are present and that the pH is between 6 and 9. The physical and chemical characteristics of the oil and oil surface area are also important determinants of bioremediation success. There are two main approaches to oil spill bioremediation:

• *Bioaugmentation,* in which known oil-degrading bacteria are added to supplement the existing microbial population, and

• *Biostimulation*, in which the growth of indigenous oil degraders is stimulated by the addition of nutrients or other growth stimulating co-substrates.

Compared to laboratory investigations, few tests have been carried out to evaluate the effectiveness of bioremediation agents in the field because such trials are both difficult and expensive to conduct (Zhu *et. al*, 2004). One of the most difficult tasks in field studies is the proper evaluation of oil biodegradation. Oil contaminated sites are

often highly heterogeneous, where oil concentrations can vary greatly within a small area. Physical and chemical weathering may also significantly affect the composition and concentration of oil contamination. Consequently, variability associated with field studies can be so high as to preclude or interfere with one's ability to discern significant treatment differences. Nevertheless, the efficacy of bioremediation in the field can be verified through well-designed monitoring programmes and proper data interpretation.

### 2.4.1 The basis for bioremediation

Bioremediation is a means of cleaning up contaminated environment by exploiting the diverse metabolic abilities of microorganisms to convert contaminants to harmless products by mineralization, generation of carbon (iv) oxide and water, or by conversion into microbial biomass (Baggott, 1993; Mentzer and Ebere, 1996). Bioremediation is based on the idea that all organisms remove substances from the environment to carry out growth and metabolism (Zeyaullah *et al.*, 2009). According to Alexander (1994), they use the molecules from the pollutants as a source of carbon, energy, nitrogen, phosphorus, sulphur or any other element needed by their cell. To these microorganisms, the molecules presented by these pollutants are simply another organic substrate from which the population can obtain the needed elements or energy required for their day to day existence. In other words, the fact that these microorganisms eat up pollutants from their environment must not be misconstrued for a benevolent gesture, rather it is a necessary act of survival for them. A common research procedure that relies on the ability of microorganisms to use organic compounds as sources of carbon and energy for growth is known as the enrichment culture technique. Under this condition, a species that is able to grow by utilizing the

chemical will multiply, few other fungi and bacteria will proliferate in this medium. However, species that use products excreted by the populations acting on the added organic nutrient will equally flourish. Therefore, the final isolation of a microorganism in pure culture requires plating on an agar medium so that individual colonies can be selected.

Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer *et al.*, 1993; Ilori and Amund, 2000). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms. Fungi are especially good at digesting complex organic compounds that are not generally degraded by other organisms (Zeyaullah *et al.*, 2009; Covino *et al*. 2010). At times, bioremediation removes the pollutant from the environment without degrading it (Broad, 1992). For example, plants like locoweed remove large amounts of selenium, a toxic element. This selenium is stored up in plant tissues where it poses no harm until the plant is eaten (Caplan, 1993). In addition, many bacteria and algae are known to produce secretions that attract metals that are toxic in high levels. In essence, these metals are removed from the food chain by being bound to these secretions, without them being degraded (Zeyaullah *et al.*, 2009). Glaser *et al.* (1991) noted that throughout the world, over 70 genera of microorganisms are known to be capable of degrading hydrocarbons. This number which accounts for less than 1% of the natural population of microorganism, may however account for as much as 10% of microorganism in polluted ecosystems (Vidalli, 2001). Ilori and Amund (2001), reported that the growth of microorganisms on petroleum hydrocarbon is often associated with the emulsification of the insoluble carbon source, and that this spontaneous release of biosurfactants is often associated with hydrocarbon uptake. According to Ilori *et*

*al*.(2005), biosurfactants are veritable agents in crude oil recovery, environmental protection and management, health care and food processing activities clearly has some distinct advantages such as lower toxicity, biodegradability, specific activity at some extreme conditions of pH and temperature etc over their synthetic counterparts. Where these microorganisms are not present in a system, they can be added to promote bioremediation. This process of adding a microorganism which is not naturally present in an environment or a medium with the intent for them to clean up pollutants is called *bioagumentation*. Where the required microororganisms are present, but not in sufficient number, there may be the need to boost their population, usually through the addition of nutrients; this practice is called *biostimulation*. The biostimulation and bioagumentation processes are all variant techniques of bioremediation.

### 2.4.2 The conditions necessary for bioremediation to take place

The control and optimization of bioremediation processes is a complex system of many factors, thus, it is imperative that many conditions be satisfied for bioremediation to take place in a given environment (Alexander, 1994 and Zeyaullah *et al*., 2009). According to Alexander (1994) and Zeyaullah *et al*. (2009) these conditions include the following:

- The existence of microbial population(s) that have the necessary enzyme(s) to degrade the pollutant.
- The microorganism must be present (or otherwise introduced) in the environment containing the pollutant.
- The pollutant must be accessible to these microbes possessing the requisite enzymes.

- The initial enzymes bringing about the degradation of the pollutants if extracellular, the bonds acted upon by that enzyme must be exposed for the catalyst to function.
- Should the enzyme(s) in question be intracellular, the molecules of the pollutant must penetrate the surface of the cell to the internal sites where the enzyme(s) act.
- Conditions in the environment must be conducive to allow the proliferation of the potentially active microorganisms. These conditions include the type of soil or water, temperature, pH, presence of oxygen or other electron acceptors and nutrients.

### 2.4.3 The limitations of bioremediation technology

As microorganisms are frequently the major and occasionally the sole means for degrading particular compounds, one important limitation therefore borders on the presence or the absence and the nature of the microorganisms present at the pollution site (Alexander, 1994). Where the required microbes are absent from a polluted site, or are otherwise unable to function either due to its nature or in conducive environmental condition, it therefore means that the pollutant will disappear very slowly, or may not disappear at all where they are the sole agents of degradation. A number of microorganism employed in bioremediation often require some form of modification in their environmental condition to better suit them so as to be able to do their job optimally. In some instances, the microorganisms needs to be biologically engineered *ab-initio* by first presenting them with a low level of the pollutant over time so as to induce them to produce the requiste metabolic pathway needed to degrade the pollutant (Cavalca *et. al*., 2000).

A number of studies involving the use of microorganisms such as fungi and bacteria have demonstrated the need to add some form of nutrient source or oxygen source to the polluted matrix so as to enhance the innate abilities of these microbes to degrade the pollutant in question. This practice is usually referred to as biostimulation. Although biostimulation has often been indicted for its capability of causing a disruption in the ecological balance of the affected area especially when done in situ as they confer some advantages on the target species by encouraging their proliferation over and above other resident species (Vidalli, 2001; Zeyaullah etal., 2009). The ability of some pollutants to persist have only confirmed the reality that microorganisms are not omnipotent and that suitable ones are not omnipresent. In a nut shell, microbial successes are evident because the molecules of the pollutants are destroyed, and their failings also evident because the chemical persists.

#### 2.4.4 A comparison of the key *dramatis personae* employed in the bioremediation of petroleum hydrocarbons

At present, various microbial genera have been isolated from petroleum hydrocarbon contaminated environments- soil and water. Mancera-Lopez *et al.*(2007) reported that individual microorganisms can mineralize only a limited range of hydrocarbon substrates. Hence a consortium of mixed populations with a broad range of enzymatic capabilities are required to increase the rate and the extent of petroleum biodegradation. In this respect, the most frequently found microorganisms are fungi and bacteria, with fungi assuming the dominant role in terrestrial ecosystems and bacteria playing the dominant role in aquatic ecosystems (Atlas and Cerniglia, 1995). In a recent studies involving the use of a consortium of petroleum hydrocarbon degrading microorganisms, Llado *et al* (2012) reported between 30-50 percent Total petroleum hydrocarbon (TPH) removal, with the white rot fungus *Trametes vesicolor*

being the most effective degrader, in addition to stimulating the growth of a number of some bacteria groups such as the *Brevundimonas* genus concurrently with other α-proteobacteria, β-proteobacteria and Cytophaga-Flexibacter-Bacteroides (CFB) as well as Actinobacteria groups. Furthermore, Leahy and Colwell (1990) and Alexander (1994) reported that adapted communities previously exposed to hydrocarbons exhibit higher biodegradation rates than communities with no history of hydrocarbon contamination.

Although bacteria in particular are reputed to be capable of taking up large amounts of metals and minerals so as to ensure adequate resources for their binary fission (Zeyaullah et al., 2009), yet fungi are particularly of interest because of their ability to synthesize relatively unspecific enzymes involved in cellulose and lignin decay and which at the same time are capable of degrading high molecular weight, complex and more recalcitrant toxic compounds, including aromatic structures (Blanques and Guieysse, 2008; Covino *et al*., 2010). Unlike fungi, bacteria are known to produce substrate specific enzymes (Ilori and Amund, 2000), hence their inability to degrade constituents of petroleum hydrocarbon such as hexane, xylene, phenol, benzene, toluene and kerosene (Ilori and Amund, 2000). Bacteria and yeast are also known to show decreasing abilities to degrade alkanes with increasing chain length, while fungi are not known to exhibit preferential degradation for particular chain lengths (Walker *et al*., 1973). Ilori *et al*.(1999) affirmed that there is no evidence to prove that some types of bacteria, precisely the sulphate reducing bacteria are able to utilize hydrocarbons. Furthermore, the ease of transportation, the ease of genetic engineering, and scaling-up makes fungi the organisms of choice in bioremediation.

## 2.5.0 The plant *Irvingia gabonensis*

*Irvingia gabonensis* (family Irvingiaceae) is a tree that grows naturally in Central and Western Africa (Harris, 1996). It is commonly called African Mango tree, bread-tree, bush mango or wild mango in English, Bobo, 'manguier sauvage' (French). 'Biri, goron' (Hausa), 'Oro' (Yoruba), 'Ogbonno' (Ibo) and its trade name is dika nut (Okafor, 1974**).** The tree grows up to 15 – 40 m in height, its bole is slightly buttressed (Leakey *et al*, 2000). The outer bark is smooth to scaly, grey to yellow-grey, leaves are alternate, simple and entire, stipules up to 4cm long, unequal, forming a cone protecting the bud, caduceus, leaving an annular scar on the branches, petiole up to 5mm long; blade elliptical 4.5 – 8 cm x 2 – 4cm, base cuneate, apex acute or indistinctly acuminate, thinly leathery, pinnately veined (Harris, 1999). Inflorescence is an axillary panicle up to 9 cm long. Flowers are bisexual, regular, 5-merous, small; pedicel up to 5 mm long; sepal free, 1-1.5 mm long; disk 1.5 mm in diameter, bright yellow nectariferous ovary superior, 2-celled, style 1-2 mm long (Shiembo *et al.*, 1996). Fruit is an ellipsoid to cylindrical drupe, occasionally nearly spherical, slightly laterally compressed, 4-6.5 cm x 4 – 6.5 cm x 3.5 – 6cm, smooth, green when ripe, pulp bright orange, soft, juicy, sweet to slightly bitter, with a few weak fibres, stone woody, 1 – seeded. Seed 2.5 – 4 cm x 1.5 – 2.5 cm seedling with epigeal germination (Harris, 1999).

The sap wood is light brown and heart wood is slightly dark/greenish brown (Okolo *et al*, 1995). The wood is very hard and is immune to termite attack (Sallenave, 1971 as cited by Richter and Dallwitz, 2000). The tree is valued for its dika nut in addition to producing a yellowed fruit. According to reports from Nigeria, it has played a major role in the nutrition, economy and traditional medicine in western and south-western

tropical Africa from Nigeria to Angola through several wars during the early 1800s (Burkill, 1994).

*Irvingia gabonensis* seed is high in fat, similar to other nuts and seeds and contain an extraordinary fibre content of 14% (Giami *et al*, 1994). It has been prized for its healing properties and now become one of its most exciting discoveries in weight loss industry by United States department of Agriculture products fact sheet (Tabuna, 1999).

### 2.5.1 The geographical distribution of *Irvingia gabonensis*

*I.gabonensis* is commonly found in Nigeria, Angola, Cameroon, Central African Republic, Congo, Cote d'ivore, Democratic Republic of Congo, Equitorial Guinea, Gabon, Ghana, Guinea Bissau, Liberia, Senegal, Sierra Leone, Sudan, and Uganda (Harris, 1996).

### 2.5.2 The ecology and management of *Irvingia gabonensis*

The preferred habitat of *I. gabonensis* is moist low kind tropical forest below 1000m in altitude and with annual rainfall of 1500 – 3000mm. It is better adapted to acid ultisols in high rainfall areas than to less acid Alfisols (Lowe *et al*., 2000), and prefers well-drained sites. Often 2 – 3 trees grow together and in some areas it is reported to be gregarious (Lowe *et al*., 2000). Although in most areas, *I. gabonensis* occurs in a wild stand, or is retained in plantations of cocoa, coffee or annual food crops or in home gardens, it is commonly planted in some regions. Management tasks mostly include pruning, harvesting (gathering and picking) and fertilization. Maintenance operations such as watering and weeding are required only in the nursery (Richter and Dallwitz., 2000).

### 2.5.3 The reproductive biology of *Irvingia gabonensis*

*Irvingia gabonensis* is haemaphroditic, with flowers being pollinated by Coleoptera, Diptera, Hymenoptera and Lepidoptera. In Nigeria, flowering is from March to June. *Irvingia gabonensis* is mainly propagated by seed (Lowe *et al*., 2000). Criteria for the selection of seeds to be planted are large fruit size, good taste, high yield, regular production (every year), early maturity, good sliminess and drawability of kernels and easy kernel extraction. Transplanting of wildings and retainment and protection of wildlings when clearing land for Agriculture are common (Atangana *et al*., 2002). Germination of *Irvingia gabonensis* seeds takes more than 14 days and they should be extracted from the fruit and dried for at least 2 days. A germination rate of 80% can be reached in this way (Atangana *et al*., 2002). Methods of vegetative propagation through rooting of leafy stem cuttings under mist have developed, and micro propagation, grafting and marcoting experiments are also considered (Okafor and Okolo, 1974). Preliminary results show that plants from bush mango marcotts can fruit 2-.5 years after transplanting (Harris, 1999).

### 2.5.4 Diseases and Pests of *Irvingia gabonensis*

No disease or pest of *Irvingia gabonensis* tree have been recorded. Seeds however are known to be infested by the larvae of the merchant grain beetle (*Oryzaephilus mercatus)*. Eggs are laid between the testa and cotyledons of the seed or in the cracks in the cotyledon. Prevention of crack prevents infestation (Dudu *et al*., 1998).

### 2.5.5 Biophysical limits of *Irvingia gabonensis*

Altitude: 200 – 500 m

Mean Annual Temperature: 25 - 32°C

Mean Annual Rainfall: 1500 – 3000 mm

Soil Type: Does not have a particular soil preference, it however grows very well in well drained and acidic soils (Harris, 1999).

2.5.6 Uses of *Irvingia gabonensis*

- Kernels of *Irvingia gabonensis* are grinded and crushed to thicken soups and stews (Tchoundjeu, 2005).
- The oil extracted from the seed of *Irvingia gabonensis* is used in cooking and also used as a substance for producing cocoa butter and also for making soaps (Ayuk *et al.*, 1999).
- The pulp of *Irvingia gabonensis* is used for preparing juice, jelly, jam and wine and also to prepare dyes for clothes (Akabor, 1996).
- The bark is rubbed on the body to relieve pains and are applied to sores and wounds and also used against toothache.
- *Irvingia gabonensis* wax is used as adjunct in making medicinal tablets (Burkill, 1994).
- Its wood is used locally for construction and also to build ship.
- It aids weight loss (Vido and Antonello, 1993).
- It delays stomach emptying which leads to absorption of dietary sugar and by so doing, it reduces blood sugar levels after a meal. (Wu and Peng, 1997).
- It helps to lower blood cholesterol as well as other blood lipids (Arvill and Bodin, 1995).
- The seed fibre can bite acids in the guts and carry them out of the body in the faeces, which requires the body to convert more cholesterol into bile acids (Hamann and Matthaei, 1996).

- It relieves dysentery and is also used as a purgative in gastro-intestinal and liver conditions, hernias and urethral discharge (Pischon *et al*., 2008).
- The seed is a valuable source of cash income (Tabuna, 1999).
- Its timber is used for making canoes, pestles for yam mortars and also suitable for boards, planking, ship decking and paving blocks (Okolo *et. al*., 1995).
- Young *Irvingia gabonensis* trees are used for making poles and stakes while its branches are made into walking sticks or thatched roof supports and dead branches are used as fire wood (Ejifor *et al*., 1987).

2.5.7 Nutritional composition of *Irvingia gabonensis*

The nutritive value of the kernels per 100 g edible portion is: 4 g energy {2918 KJ (697 k cal)}, protein 8.5 g, fat 67 g, carbohydrate 15 g, Ca 120 mg, Fe 3.4 mg, Thiamin 0.22 mg, riboflavin 0.08 mg, niacon 0.5 mg (Platt, 1962). Drawability (sliminess) and viscosity of soups imparted by the kernels varies between kernels from different trees. Fat content of kernels also varies between trees and is 37.5 – 75 g / 100g; the approximate fatty acid composition is:

| | |
|---|---|
| Lauric acid | 20 – 59% |
| Myristic acid | 33 – 70% |
| Palmitic acid | 2% |
| Stearic acid | 1% |
| Oleic acid | 1 – 11% |

The residue obtained after separation from the fat has good properties for processing in the food industry (Dudu *et al*., 1998). The nutritive value of the fruit pulp of *I. gabonensis* are zingiberne and ∝ - curcumene, ethyl and methyl esters of cinnamic acid, dodecanal and decanol imparting spicy-earthy, fruity and wine-yeast flavour

(Burkill, 1994). The pulp yield about 75% juice (Hong *et al.*, 1996). Wine produced from it was found to be of good colour, mouth feel, flavour and general acceptability (Akabor, 1996**).** The nutritive value of the fruit pulp per 100g edible portion is: water 81g energy {255 kg (61Kcal)}, protein 0.9 g, Fat 0.2 g, carbohydrate 15.7 g, Ca 20 mg, P 40 mg, Fe 1.8 mg, Ascorbic acid 7.4 mg (Van- Dijk, 1997).

### 2.5.8 Chemical composition and functional properties of *Irvingia gabonensis* seed flour

The seed of *Irvingia gabonensis* (g/100 g) contain protein (12.75), fibre (5.87), fat (40.26) and carbohydrate (37.47). The predominant metal in the seed is sodium (840mg/100g). Water absorption and oil emulsion capacities are relatively high, while foaming capacity and least gelatin concentration are low. According to Abdullahi (2002), the seed is useful in some food formulations.

### 2.5.9 Relationship between leptin and *Irvingia gabonensis*

Leptin is a hormone produced by adipocytes (Fat cells) that functions to maintain a lean body composition by at least two distinct mechanisms. First, it modulates appetites by binding to a specific area of the brain, known as the hypothalamus where if signals satiety (Sahu, 2003). Normally, a well-nourished state is reflected by an increase in leptin production and in turn, the elevated serum leptin signals the hypothalamus to limit hunger and second, leptin enhances the body's inability to access and utilize fat stores as an energy source (Wang *et al.*, 2005). Researchers believed that the apparent paradox could be explained by an acquired resistance to leptin (Wang *et al.*, 2005**).** Since being overweight leads to chronically elevated levels of the hormone, it is hypothesized that prolonged exposure to this leptin overload

could eventually cause target tissues to become immune to the effects of leptin, loosing the normal capacity to respond to it (Hamann *et al*., 1996).

*Irvingia gabonensis* shows tremendous promise in correcting leptin resistance promoting weight loss, and combating components of metabolic syndrome. *Irvingia gabonensis* facilitates the breakdown of body fat by reducing an enzyme (glycerol-3-phosphate dehydrogenase) that enables glucose to be stored as triglycerides in fat cells. Further, *Irvingia gabonensis* increases the insulin-senitizing hormone adiponectin and inhibits the digestive enzyme amylase digestion (Pischon *et al*., 2008).

It has been suggested that elevated leptin provokes the growth of certain malignancies, including many form of breast cancer, which helps explain the higher breast cancer risk observed in overweight women and obeisity which is also known to increase stroke risk (Soderberg *et al.*, 2003) and promote cardiac hypertrophy - enlargement of the heart (Ren, 2005).

*Irvingia* reverses leptin resistance and also increases insulin-sensitizing hormone and inhibits the digestive enzyme (amylase) that allows ingested carbohydrate to be broken down and absorbed into the blood stream (Hamann, 2004).

2.6.0 The proximate and physico-chemical composition of some vegetable oils

The terms fat, oil and lipid are often used interchangeably by food scientists. Although sometimes the term *fat* is used to describe those lipids that are solid at the specified temperature, whereas the term *oil* is used to describe those lipids that are liquid at the specified temperature. Lipids- fat and oil- are usually defined as those components that are soluble in organic solvents (such as ether, hexane or chloroform),

but are insoluble in water. This group of substances includes triacylglycercols, diacylglycercols, monoacylglycercols, free fatty acids, phospholipids, sterols, caretonoids and vitamins A and D. The lipid fraction of a fatty food therefore contains a complex mixture of different types of molecule. Even so, triacylglycercols are the major component of most foods, typically making up more than 95 to 99% of the total lipids present. Triacylglycerols are esters of three fatty acids and a glycerol molecule. The fatty acids normally found in foods vary in chain length, degree of unsaturation and position on the glycerol molecule. Consequently, the triacylglycerol fraction itself consists of a complex mixture of different types of molecules. Each type of fat has a different profile of lipids present which determines the precise nature of its nutritional and physiochemical properties.

Lipids are one of the major constituents of foods, and are important in our diets for a number of reasons. They are a major source of energy and provide essential lipid nutrients. Nevertheless, over-consumption of certain lipid components can be detrimental to our health, e.g. cholesterol and saturated fats. In many foods, the lipid component plays a major role in determining the overall physical characteristics, such as flavor, texture, mouthfeel and appearance. For this reason, it is difficult to develop low-fat alternatives of many foods, because once the fat is removed some of the most important physical characteristics are lost. Finally, many fats are prone to lipid oxidation, which leads to the formation of off-flavors and potentially harmful products. Some of the most important properties of concern to the food analyst are*:*

- Total lipid concentration
- Type of lipids present
- Physicochemical properties of lipids, *e.g.,* crystallization, melting point, smoke point, rheology, density and color

- Structural organization of lipids within a food.

In a study involving the characterization of the seed oil from *Monechma ciliatum and Prunus mahaleb* Seeds, Mariod *et al*. (2009) reported the following values for the physico-chemical and proximate parameters of the oils for both seeds, the oil content was found to be 30.95 and 13.15% in white and black mahlab seeds, respectively. The refractive indices of white mahlab oil (WMO) and black mahlab oil (BMO) were 1.475 and 1.470, and specific gravities were 0.8511 and 0.8167 g/cm$^3$, respectively. Saponification values were 184.23 and 180.3 mg KOH/g, peroxide values were 2.54 and 4.43 meq/kg, and unsaponifiable matter was 0.92 and 0.66%, respectively. The major fatty acids were palmitic 4.5%, stearic 16.0%, oleic 47.3%, and linoleic 31.4% in BMO, while in WMO they were palmitic 5.7%, oleic 45.0%, and linoleic acid 47.0%. A moderate amount of tocopherols were found at 45.2 and 28.5 mg/100 g in BMO and WMO, respectively. Protein content was found to be 21% in black and 28% in white mahlab seeds. The total amount of amino acids in black and white mahlab seeds was found to be 783.3 and 1,223.2 mg/g N, respectively.

Adeleke and Abiodun (2010) noted that the protein and fat content of *B. glabrum* seeds were 10.23% and 58.23% respectively. Carbohydrate content of the seed was low at 16.60%, when compared to other oil seeds like soybeans and groundnut. The relative density of the oil was 1.125. The value was above the recommended codex standard for edible vegetable oils. The refractive index for the *Bombax glabrum* seed oil was 0.628 which was lower than the standard value obtained for cotton seed oil which was 1.458-1.466 and groundnut oil at 1.460-1.465. Saponification and iodine value obtained were 42.93 mgKOH/g and 3.38 Wij's. The unsaponifiable matter was 4.20 g/kg and was lower than the values reported for soybeans, cotton seeds etc. Acid value was 0.71 mgKOH/g. The peroxide value (3.64 meq/kg) was lower than the

codex standard for edible vegetable oils (10 meq/kg). Moisture content and free fatty acid of the oil were low. Concluding, Adeleke and Abiodun (2010), recommended the seed as a good source of protein and fat.

In another report involving the proximate and the physico-chemical analysis of the oil from the seed of *Arachis hypogaea,* Atasiel *et al*. (2009), reported that the groundnut oil contained 47.00% fat, 38.61% protein, 5.80% moisture, 1.81% carbohydrate, 3.70% crude fibre and 3.08% ash. Minerals (mg/100g) included: Na (42.00±0.71), K (705.11±0.86), Mg (3.98±0.04), Ca (2.28±1.94), Fe (6.97±1.62), Zn (3.20±0.11), P (10.55±0.68). The physico-chemical characteristics showed saponification value to be 193.20 mgKOH/g, iodine value 38.71 (g/100g), acid value 5.99 (mgKOH/g), free fatty acid (mgKOH/g) 3.01 peroxide value 1.50 (meq/kg) and refractive index 1.449. The predominant fatty acid was found to be oleic acid (41.11%). In concluding, Atasiel *et al*. (2009) that groundnut oil can thus be considered as a good source of protein with a high nutritional value.

Butternut- *Jugulans cinerea-* oil was investigated for some of its physico-chemical properties. Reporting this work, Essien and Amadi (2009) observed that the oil was found to be deep amber in color and slightly thick. The oil melts at $26^0$C, has specific gravity of 0.99 and a refractive index of 1.44 at $40^0$C. The chemical characteristics showed the total oil content to be 46.15%. Unsaponifiable matter was 0.69%, saponification value was 248.02mg/KOH/g oil. The iodine value was 40.45 while the acid and peroxide values were 3.96mg/KOH/g oil and 7.2Meq/100g oil respectively. The free fatty acid content of the oil was 3.16%. Concluding, Essien and Amadi (2009) observed that the oil can be said to have some advantageous physicochemical properties and could be regarded as seed oil due its high fat content.

In another studies, Oladimeji and Kolapo (2007), focusing on fungi and bacteria induced changes in the proximate composition of 5 common oilseeds in Nigeria - Melon *(Colocynthis citrullus),* Soybean *(Glycine max*), Cashew *(Anacardium occidentale),* Groundnut *(Arachis hypogeae*) and Coconut (*Cocos nucifera*) – reported a general degradation in the nutritional composition of these oilseeds over time. They found that the proximate parameters such as % protein, % ash, % ether extract, % carbohydrate and % moisture decreased in all the stored residue, with melon residue recording the highest decrease(protein: 48.1 - 42.1%; Ether extract: 19.2 - 18.0%; Carbohydrate: 10.2 - 9.6%) while coconut residue had the lowest decrease(protein: 19.9 - 19.2%; Ether extract: 16.2 - 15.8%; Carbohydrate: 28.6 - 26.7%). According to Oladimeji and Kolapo (2007), prominent organisms isolated on these oilseeds include *Aspergillus niger*, *Rhizopus spp*, *Bacillus subtilis*, *Bacillus licheniformis* and *Proteus mirabilis* .The effect of proliferation of the isolated organisms on the storage qualities of these oil residues they noted may have been responsible for the reduction in the nutritive value of the stored oilseed residues. Results from these studies have revealed that the storage qualities of oilseeds are time dependent.

2.7.0 Spent Engine Oil (SEO), the ubiquitos xenobiotic of our clime and time

Spent engine oil (SEO) is also called used mineral-based engine or crankcase oil. It is the brown – to – black, oily liquid removed from the engine of a motor vehicle or power plant engine when the oil is changed. It is a petroleum distillate product composed of 75% mineral oil, 20% oxidation inhibitors and detergents and 5% poor depressant and viscosity (US EPA, 2001). SEO is similar to unused oil except that it contains additional chemicals that are produced or that build up in the oil when it is used as an engine lubricant.

The US Environmental protection Agency defines used oil as follows "Used oily is any oil that has been refined from crude oil or any synthetic oil that has been used and as a result of such use is contaminated by physical or chemical impurities". During normal use, impurities such as dirt metal scrapings, water, or chemicals can get mixed with the oil, so that in time the oil no longer performs well. Eventually, this oil must be replaced with virgin or re-refined oil to do the job at hand. EPA's used oil management standards include a three-pronged approach to determine if a substance meets the definition of used oil. To meet EPA's definition of used oil, a substance must meet each of the following three criteria:

(1) **Origin** – the first criterion for identifying the used oil is based on the origin of the oil. Used oil must have been refined from crude oil or from synthetic materials. Animal and vegetable oils are excluded from EPA's definition of used oil.

(2) **Use** – the second criterion is based on whether and how the oil is used. Oil used as lubricants, hydraulic fluids, heat transfer fluids, buoyant, and for other similar purposes are considered used oil. Unused oil such as bottom clean-out waste from virgin fuel oil spillage tanks or virgin fuel oil recovered from a spill, do not meet EPA's definition of used oil because these oils have been "used". EPA's definition also excludes products used as cleaning agents or solely for their solvent properties, as well as certain petroleum – derived products like antifreeze and kerosene

(3) **Contaminants** – the third criterion is based on whether or not the oil is contaminated with either physical or chemical impurities. In other words, to meet EPA's definition, used oil must become contaminated as a result of being used. This aspect of EPA's definition includes residues and contaminants

generated from handling, storing, and processing used oil. Physical contaminants could include metal shavings, sawdust, or dirt. Chemical contaminants could include solvents, halogens, or saltwater.

Many investigations have verified the effect of Spent Engine Oil (SEO) on soil physical, chemical and biological properties (Amund *et al*., 1993; Sztompka, 2000) etc as well as its effect on a number of plants such as *Amaranthus hybridus* Linn. (Edeoga and Otoide, 2001; Odjegba and Sadiq, 2002; Omosun *et al*., 2008), *Ricinus communis* L. (Vwioko and Fashemi, 2005, Vwioko *et al*., 2006) and on Okra (Udo and Fayemi, 1975).

SEO contamination of the soil is known affect the soil and plant growth in the following general ways:

(A) **Growth inhibition**

Toxins from SEO can build up in plants, causing them to show reduced growth level. Processes such as photosynthesis which is essential for plant growth are often incapacitated by SEO. It causes poor soil conditions as a result of the exhaustion of minerals and oxygen which inhibit plant growth (Mason, 1991). Chemicals and additives within spent engine oil can cause malignant growth in plants (Edebiri and Nwakwole, 1983).

(B) **Stress**

The physical pressure of motor oil in the soil can make the soil much harder for plants to function normally. The greater stress on the plant resulting in physical symptoms of drought even when the plants are properly irrigated (Mason, 1991) Contaminated soil

is also darker, and may cause plant to suffer from stress due to heat damage, since darker soil absorbs more heat from the sun; these effects can persist for months or years after the initial contamination. (Edebiri and Nwakwole, 1983).

**(C) Germination**

As SEO reduces oxygen and minerals levels in soil it makes it difficult for a seed to germinate. Likewise, it can suffocate the emerging seedling or radical before it is able to take root. (Okonkwo, 1983)

**(D) Suffocation**

When spent engine oil is dropped into soil, it not only kills off microbial life but can also make the soil impossible for worms and other small organisms. This activity leads to lack of aeration in the soil that can literally suffocate soil until the affected area is little more than dust. Soil polluted in this way is unsuitably for any growths and contaminated areas may require years and specialized treatment to recover fertility (Mason, 1991).

**(E) Composition**

SEO also contains chemical by- product from engine use and may also contain elements of antifreeze and gasoline. These chemicals are hazardous to the environment and negatively affect plants (Odu, 1979). SEO can also present a threat to human health through skin contact, skin absorption, inhalation or ingestion. Many of the problems associated with used engine oil are due to exposure to heavy metals. These health problems are cumulative with each exposure to used engine oil and the amount of heavy metals added to the body's system increase (Okonkwo, 1983).

**(F) Yield**

Heavy metals present in spent lubricant can accumulate in plant and affect metabolic process (Prasad and Prasad, 1987). This suggest why there are reduction in the growth of plant sown on contaminated soil. Some leaves of the plants in the contaminated soil experienced chlorosis, necrosiss, and curled upwards from their tops. Some of these symptoms such as stunted growth and chlorosis have been attributed to zinc toxicity by Lepp (1981). There was also a significant decrease in leaf area, plant height and root length. This agrees with the findings of Anoliefo and Vwoiko (1995) who reported that spent engine oil in soil creates unsatisfactory conditions for plant growth ranging from heavy metal toxicity to insufficiency in aeration of the soil.

### 2.7.1 Hydrocarbons present in engine oil

Hydrocarbons are present in all oils but especially present in used engine oil, where the hydrocarbons have been distilled and concentrated by combustion engine (Wang *et.al.,* 2000). Hydrocarbon such as the ones found in used engine oil can be present in trace amounts in healthy, non polluted soils. These trace amounts are not enough to interrupt the fertility of the soil (Yong *et.al*., 1992). However, even a small amount of SEO pollution can saturate the soil with hydrocarbons, making it too toxic to support normal plant growth (Odu, 1979).

SEO has many of the characteristics of unused oil. These include straight chain (alphatic) hydrocarbons and aromatic or polycyclic aromatic hydrocarbons (PAH), which are distilled from crude oil, and various additives that improves the performance of the oil in the engine. SEO smells like unused oil and contain the chemical found in unused oil (USEPA, 2001).

There are relatively large amount of hydrocarbon in the spent oil, including the highly toxic polycyclic aromatic hydrocarbons (Wang *et al*., 2000). Also most heavy metals such as Vanadium (V), Lead (Pb), Aluminum (Al), Nickel (Ni), and Iron (Fe), which were below detection in unused lubricating oil have been reported by Whisman *et al.* (1974) to give high value (ppm) in SEO. These heavy metals may be retained in soils in the form of oxides, hydroxides, carbonates, exchangeable cations, and or bonds to organic matter in the soil (Yong, *et al*., 1992).

The disposal of SEO in open vacant plots and farms is a common practice in Nigeria, especially by motor mechanics. SEO is usually obtained after servicing and subsequently draining from automobile and generator engines (Anoliefo and Vwioko 2001) and much of this oil is poured into the soil.

Oil pollution of soil leads to build up of essential (organic carbon, phosphorus, calcium, magnesium) and non essential (magnesium, zinc, iron, cobalt, copper) element in soil and the eventual translocation in plant tissue (Vwioko *et al*., 2006). Although some heavy metals at low concentrations are essential micronutrients for plants, but at high concentration they may cause metabolic disorders, and growth inhibition for most of the plant species (Fernandes and Henriques, 1991).

In addition to the chemicals found in lubricating oil, SEO also contains chemicals that are found when the oil is exposed to the high temperatures and pressures insides an engine as it runs, and also contains small amounts of water, gasoline, antifreeze and chemicals that come from gasoline when it burns inside the engine (Rowell, 1977).

Plants however respond differently to these pollutants (Fernandes and Henriques, 1991). Anoliefo and Vwoko (1995) reported that the contamination of soil with SEO caused growth retardation in plants. Previous studies revealed that the oils penetrate

the pore space of terrestrial vegetation (Bossert and Bartha, 1984), and subsequently impedes photosynthesis and the physiological process of the plant (Odu, 1981). Plant on such soils becomes suffocated due to the exclusion of air by the oil (Udo and Fayemi, 1975). Earlier studies had revealed that oil pollution often results in insufficient aeration of the soil due to displacement of air from the space between the soil particles, retard growth of plant, result in chlorosis of leaves and dehydration of plant (Rowell, 1977).

2.8.0 The use of Polymerase Chain Reaction (PCR) in fungi identification

Although vast knowledge about fungi has been gathered to date, yet, most of the about 6 million species of these organisms remain uncharacterized (Hawksworth and Rossman, 1997; Stamets, 2006, Thieman and Palladino, 2009). The reasons for this deficiency include habitats that have not been well investigated, organisms that are difficult to culture axenically, such as obligately associated fungi, and inaccurate identification of catalogued samples. Strategies used to rectify this deficit should include the use of comparative sequence analysis of rRNA and rRNA genes, which has led to the discovery of many new fungal, bacterial and archael phylotypes in different environments (Barns *et al*, 1994).

Several PCR primers that amplify fungal rDNA from a wide range of taxonomic groups have been described (White *et al,* 1990), but few of these were designed for use with environmental samples. Such a tool must have high specificity, as fungal DNA may be rare compared to DNA in other sources, such as bacterial, plants, or other eukaryotes (Harris, 1994). The ITSL-F and ITS4-B primers have been used to amplify basidiomycete ITSI, ITS2 and 5.8S rRNA sequences from plant tissues containing fungi (Gardes and Bruns, 1993). Similarly, the VANSI primer has been used in combination with other primers to amplify DNA from vesicular arbuscular

endomycorrhizal fungi (Simon *et al.,* 1992). To identify disease causing fungi, PCR primers have been designed to specifically amplify both human and plant pathogens (Kappe. *et al.,* 1996). In addition three PCR primer pairs described by Smith *et al.,* (1999) have been used to amplify fungal DNA from wheat rhizoshpere samples

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### 2.8.1 Denaturing Gradient Gel Electrophoresis (DGGE) - how it works in identification

The application of DGGE to study microbial population is a multi-step procedure relying on several techniques. Firstly, DNA is extracted from the environmental sample (for example, soil, plant, tissue or insect gut). The efficient, routine extraction of total DNA from environments such as soil in a relatively recent development, but this step is critical as the DNA recovered must be representative of the habitat sampled. The second technique is polymerase chain reaction (PCR) which allows the specific amplification of a segment of DNA round 1 billion times the original concentration (Mullis *et al.,* 1994). After the total microbial DNA is extracted from the sample bacterial 16S rRNA gene or fungal genes are amplified by PCR with universal primers. The double stranded PCR products are then subjected to DGGE.

DGGE enables PCR amplification products of the same length but with different internal sequence composition to be separated in gradient gels according to the melting behaviour of the DNA. In electrophoresis, DNA fragments are separated on the basis of size as they move through the gel. In DGGE, the gels contain a linearly increasing gradient of denaturing chemicals (formamide and urea). As the double stranded DNA fragments begin to move through the gel, they reach higher levels of denaturing chemicals and begin to melt at a point in the gel that is determined by the sequence of the fragment, which is different from species to species. Branching of the

molecule then sharply decreases the mobility of the fragment through the gel. To stabilize the melting of the DNA fragment and to ensure that they do not denature completely during electrophoresis, a GC clamp, consisting of a long stretch of mostly GC base pairs is incorporated into one of the primers and subsequent into the PCR products. By this method, PCR mixtures containing numerous species will be derived from a single species.

Various statistics are applied to compare banding patterns between lanes and gels. One of the attractive features of this technique is that individual bands can be excised for subsequent sequence determination and sequence comparison with databases. Hence the species present in the community can be identified if similar species are in the database. Alternatively, PCR products from the initial amplification can be cloned into plasmid vectors, run on DGGE and sequenced to determined species. The scope of the analysis depends on the specificity of the primers used in the PCR. The technique has been used extensively for analysis of PCR products amplified from 16S rRNA; genes in complex bacterial communities. Subgro-primers designed for particular groups, x - and b proteobacteria, as well as various groups of Gram negative bacteria and fungal populations (Van Elsas *et al.,* 2000).

### 2.8.2 Limitations of DGGE analysis of PCR products

As with any technique used to study microbial communities in the environment, there are limitations to DGGE analysis that need to be taken into account some of these limitations are not specific only to this technique but still require consideration.

As the analysis of microbial communities is based on extraction of community DNA from environmental samples, it is important that the DNA recovered is representative of the habitat. To achieve this, consideration must be given to several factors,

including an appropriate sampling strategy, efficient extraction of cells from the environmental sample and efficient lysis of cells.

Previously, lack of efficient techniques for extraction of DNA from soil has limited the analysis of microbial populations in soil but a number of rapid and flexible techniques are now available (Niemi *et al.,* 2001) DNA extraction efficiency with respect to both the quantity and quality of DNA recovered can vary between techniques and soil types as found in a study of New Zealand soils (Lloyd – Jones and Hunter, 2001).

Assuming efficient extraction of DNA which represents the microbial community is achieved, it is important to note that biases may be introduced by the PCR amplification step. These can occur for a number of reasons, for example preferential because of inefficient primer annealing. PCR - introduced biases have been reviewed by Wintzingerode *et al.* (1997).

There are some limitations that are specific to DGGE analysis of microbial communities. Ideally one species yields one band but in some strains two or more bands have been detected. On the other hand, it has sometimes been observed that closely related or even phylogentically unrelated strains have similar electropheretic mobilities, resulting in the band from one species hidden behind the band of another species. For ecosystems containing large numbers of equally abundant micro-organisms, highly complex banding patterns will be obtained, which can be difficult to interpret (Cole *et al.,* 2003).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1.0 Collection of samples

Diseased seeds of *I. gabonensis* from 4 sources, and soil samples from 5 sources were sampled for fungal species over a 30 month period (once in 2 months) between December 2007 and May 2010. All the sampling sites were in Lagos metropolis, Lagos State, Nigeria.

Seeds of *I. gabonensis* from which fungi were isolated were sampled from the following places:

(a) Oyingbo market.

(b) Ajegunle market.

(c) Bariga market.

(d) Agege market.

Soils also from which fungi were isolated in the laboratory were aseptically collected from the following places into sterile petri dishes:

(a)Mechanic workshop in Sabo, Yaba ( **N 06$^0$30.353$^/$, E 003$^0$22.833$^/$.**
**ELEVATION: 22.9 metres**).

(b) Mechanic workshop in Onitiri, Akoka.

(c) Biological Gardens, University of Lagos (**N 06$^0$31.119$^/$, E 003$^0$24.O47$^/$**
**ELEVATION: 49.9 metres).**

(d) Shodex Horticultural Gardens, Anthony-Village (**N 06$^0$ 33.543$^/$, E 003$^0$ 21.992$^/$**
**ELEVATION: 93.6 metres)**

(e) Olusosun landfill site, Ojota ( **N 06$^0$ 35.774$^/$, E 003$^0$ 22.637$^/$ ELEVATION: 117 metres ).**

The following sources of hydrocarbon were used in this work:

- Crude Oil (CO).
- Diesel (Automotive Gas Oil- AGO).
- Kerosine (Dual Purpose Kerosine- DPK).
- Fresh lubricating oil (FEO).
- Spent engine Oil (SEO).
- Oil extracted from both healthy and diseased *I. gabonensis* seed(I.O).

The sources, collector and dates of collection of the different petroleum hydrocarbon sources are as shown in Table 1.

Table 1: Collection of the samples for the different hydrocarbon sources

| Petroleum hydrocarbon source. | Collection site | Collector | Date of collection |
|---|---|---|---|
| CRUDE OIL. | Escravos, Warri-Delta state | Ojadeni, T.E.(Miss). | May, 2008 |
| AGO (DIESEL). | MRS (formerly Texaco) Filling Station, Akoka- Lagos. | Sanyaolu, A.A.A. | May, 2008 |
| DPK (KEROSINE) | African Petroleum (AP) Filling Station, University Of Lagos. | Sanyaolu, A.A.A. | May, 2008 |
| FRESH ENGINE OIL (FEO). | African Petroleum (AP) Filling Station, University Of Lagos. | Sanyaolu, A.A.A. | May, 2008 |
| SPENT ENGINE OIL (SEO). | AutoMechanic's workshop, African Petroleum (AP) Filling Station, University Of Lagos. | Sanyaolu, A.A.A. | May, 2008 |
| OIL EXTRACTED FROM *I. GABONENSIS* SEEDS (I.O). | An admixture of seeds from all the 4 markets above. | Sanyaolu, A.A.A. | May, 2008-June,2010 |

3.2.0 Isolation of fungi from *I.gabonensis* seed and soil samples

Isolation of fungi intended for use in the further studies were carried out from diseased seeds of *I. gabonensis* from 4 open markets in Lagos metropolis, namely Oyingbo, Bariga, Alayabiagba in Ajegunle and Agege markets. In addition, soil samples from 5 sources namely Olushosun dump site, Ojota; Shodex Beautification Gardens, Anthony village; Biological Gardens, University Of Lagos; Mechanic village, Sabo-Yaba and Mechanic village, Onitiri - Akoka were all sampled for fungal species. This sampling was done over a 30 month period (once in 2 months) between December 2007 and May 2010.

The modified method of Adekunle and Oluyode (2005) was employed in isolating fungi from both the seed and soil samples. Diseased seeds of *I. gabonensis* (from each of the sites) that had been cut into discs were surface sterilized by leaving them in a solution of common bleach (sodium hypochloride - NaOCl) and sterile distilled water in a ratio of 3:2 for one min, then rinsed in three changes of distilled water, and were thereafter placed (using sterile forceps) into the already prepared sterile LabM$^R$ PDA plates.

Serial dilution was carried out on the soil samples by weighing out 1g of soil sample (from each of the sites) into a test tube. 10 ml of sterile distilled water was thereafter added. This served as the stock solution. From the stock solution, 1ml was transferred into a fresh test-tube, and 9ml of sterile distilled water was added to have a concentration of $10^{-1}$ dilution strength. This process was repeated till a dilution of $10^{-4}$ was achieved. Subsequently, 0.1ml of each of these dilution strengths was aseptically transferred into sterile Petri dishes, and freshly prepared LabM PDA was poured into the plates, and the plates were allowed to solidify and thereafter incubated at room temperature (28-31$^o$C) in the incubator and observed daily for fungal growth. To

obtain a pure culture, resultant fungal cultures were repeatedly sub cultured into fresh PDA plates until each plate contains only one type of fungal isolate.

### 3.3.0 Identification of fungi

The first step in the identification of the fungal species was the morphological studies which are hinged on observing the shape, colour, size and texture of fungal species in plates and time taken for each of the fungus to reach the maximum diameter (9cm) in plates. After this, slides of each isolate were prepared by teasing out a little portion of the growth in the plate on to the slide, this was then teased out and stained with lactophenol in cotton- blue, and observed under a compound light microscope. The photomicrographs were then compared with the descriptions given by Talbot (1971), Deacon (1980), Domoschet *et al.* (1980) and Bryce (1992) for identification. Further confirmation of the identities of most of the isolate was done by Professor A.A. Adekunle in the department of Botany, Faculty of Science, University of Lagos. The final confirmation of the identities of the key fungal species, and a comparison between these key isolates was done using molecular techniques such as PCR and electrophoresis gel analysis and DNA sequencing at the Biotechnology Centre, University Of Agriculture, Abeokuta (UNAAB) and Macrogene Inc., USA.

### 3.4.0 Pathogenicity test on the diseased *I.gabonensis* seeds

This was done using Koch's postulate. Here freshly prepared sterile plates of Lab M PDA were inoculated with between 2-3 pieces of freshly cut, surface sterilized, diseased *I.gabonensis* seeds. This was left to grow at room temperature and observed daily for fungal growth. Fungal growth at between 4-5 days after inoculation showed multiple growth per plate. After this, each of the distinct fungal pathogen were

reapeatedly (asceptically) subcultured into freshly prepared sterile LabM PDA until each plate contained only a single fungus.

Spores of about 1 X $10^5$ from each Pure plate were inoculated into plates containing freshly prepared sterile LabM PDA. Between 1-2, surface sterilized, visually healthy whole seeds of *I.gabonensis* were dropped in each of these plates that were inoculated with spores. These were incubated for 5 – 7 days. Following this, a re-isolation was made on the *I.gabonensis* seed, which by now had become infected by the newly introduced spores. The similarities found in the characterization of the new isolates compared to the initial isolates, as well as the symptoms produced on both the initial and the new *I.gabonensis* seed proved the pathogenicity of the initial fungal isolates on *I. gabonensis*.

3.5.0 Mycodeterioration studies

To compare the ability of *Aspergillus oryzae* to cause a deterioration in *I. gabonensis* seeds, both the visually healthy and *A.oryzae* infected (diseased) seeds of *I. gabonenesis* (Plates 1a and 1b respectively) were subjected to a proximate analysis, and the oil extracted from these seeds were also subjected to a complete physico-chemical characterization. In addition, a Gas chromatographic profiling of the fatty acids from both the oils extracted from both types of seed were also done. Results obtained in all cases were the mean for 3 replicates. The data so obtained were subjected to statistical analysis using the soft ware statistical package SAS (2005) at 5% level of significance, and mean separation was done using the Duncan's multiple range test

**PLATE 1A: HEALTHY SEEDS** OF ***I.gabonensis.*** X 0.5

**PLATE 1B: DISEASED SEED OF *I.gabonensis*** X 0.5

**Procedure:**

Visually healthy seeds were inoculated with one of the pathogenic fungal species (*A.oryzae*) isolated from the diseased seeds, and left for between 8-10 days after the inoculation before their oils were extracted. The oils were extracted using the soxhlet extraction method. Oil was extracted from both the healthy and diseased seeds of *I. gabonensis.* The oils extracted from both the healthy and diseased seeds were subjected to a complete physico-chemical characterization, where parameters such as the pH, refractive index, melting point, yield, saponification value, unsaponifiable matter, iodine value, peroxide value, acid value, percentage free-fatty acid composition, cholesterol value, relative density, percentage yield, specific gravity and TBA value were all determined.

In addition, the extracted oils from both the diseased and healthy seeds were analyzed to determine and compare their fatty-acid methyl ester profiles, using the Gas Chromatographic (GC) method- GC model HP 6890 powered with HP Chemstation Rev. A 09.01{1206} software.

Also the effect of these fungal isolates on the nutritional value of *I. gabonensis* was investigated. Proximate analysis was carried out on both the healthy and diseased seeds of *I. gabonensis*, considering such entities as moisture, fats, ash, protein, crude fibre, carbohydrate and energy.

3.5.1a Extraction of oil from *Irvingia gabonensis* seeds

Lipids are soluble in organic solvents, but insoluble in water. They can be separated from water soluble components such as carbohydrates, minerals etc using the solvent extraction method such as the soxhlet method of oil extraction as described by AOAC (2000). Soxhlet extraction method is an example of a semi continuos solvent

extraction technique. The principle employed here is based on the evaporation and condensation of the extracting solvent.

3.5.1b Sample preparation of the extracted oil from *I.gabonensis* seed

In using this method, the preparation of samples involved the following steps:

Samples were air dried, and thereafter oven dried at 120°C for 10 hrs to a moisture content of less than 1%. Dried samples were grounded to a fine powder using a blender. The sample which had been dried and ground was placed in a porous thimble, which was then placed in all glass soxhlet extractor.The extracting solvent used was petroleum ether (60°C-80°C). About 40ml of this extracting solvent was placed into an attached flat bottom flask and heated using an electric hot plate.

The solvent was heated to a vapour which ascends and condenses directly into the thimble. As it condenses, it helps extract the oils from the solute in the thimble. After enough volume of oil has accumulated in the section where the thimble was placed, the oil flowed back into the flat bottom flask. This process was repeated for 6 hours, after which all the oil had been extracted.

The now turbid solvent which contains the oil is removed and heated in a beaker so as to recover the oil alone by vapourizing the solvent. This was confirmed by placing a finger over the beaker after heating, in which no mist was formed on the finger.

The percentage oil was calculated thus:

Initial weight of ground sample (before extraction) = $W_1$

Final weight of sample (after extraction) =$W_2$

$$\text{Percentage content} = \frac{W_1 - W_2}{W_1} \times 100$$

### 3.5.2 Physico- chemical characterization of the oil extracted from *I.gabonensis* seed

The oil extracted was characterized for the parameters earlier stated.

#### 3.5.2a Acid value

This is defined as the quantity of KOH i.e. potassium hydroxide (in milligrams) required to neutralize the free fatty acids released during the hydrolysis of a given oil sample. This is also a measure of the free fatty acids present in a given amount of fat/oil.

**Procedure**

Diethyl ether (25ml) was mixed with ethanol (25 ml). Phenolpthalein indicator (2 drops) was also added and neutralized with 0.1M NaOH solution. The oil sample (2.0 g) was dissolved in the neutralized solvent mixture and titrated with 0.01M KOH until a faint pink colour, which persists for 20 – 30 seconds, was obtained. The value of its acid value was derived using the formular below:

$$\text{Acid value} = \frac{\text{Titre (ml) x 5.61}}{\text{Weight of sample used}}$$

#### 3.5.2b Saponification value

This is defined as quantity of KOH (Potassium hydroxide) in milligrams needed to saponify one gram of fat. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali.

This is the measure of the average molecular weight of the triacylglycerol in a given sample of oil. The higher the saponification number, the smaller, the average weight of the triacylglycerol present and also the chain length.

**Method/Procedure**

Twenty milliliters (20 ml) of 0.5 N ethanolic KOH was pipetted into two separate conical flasks of 250 ml capacity and labeled A & B (A is the reaction vessel, B is the control vessel). 2 g of oil sample was added into A only, a reflux condenser was fitted to each flask and refluxed for 45 minutes with occasional shaking until the solution was clear. It was then cooled to room temperature and phenolphthalein (indicator 2 – 3 drops) was added and titrated against 0.5 N $H_2SO_4$ until the pink colouration just disappears.

The saponification value was then calculated using the formula given below:

$$\text{Saponification value} = \frac{Vb - Vs}{\text{Weight of oil}} \times 28.05$$

Where

Vb = titre value for the blank

Vs = titre value for the sample

3.5.2c Iodine number

This is defined as the amount of iodine (g) absorbed by 100 g of fat. It gives an indication of the level of unsaturation of the oil. One of the commonly used methods for determining the iodine number of value of lipids is Wigs method (AOAC, 2000). This same method was used.

**Procedure**

Two boiling tubes were labeled A (reaction vessel) & B (control vessel). Into A the lipid solution ($2cm^3$) was added while chloroform (2.0g) was added into B. From a

burette, Wijjs solution ($5cm^3$) was added into each tube. The content of the tube was mixed thoroughly and the tubes were then left to stand for 5 minutes in the dark. After this, 7.5% KI ($5cm^3$) was added to each tube and titrated to a straw colour using 0.1N $Na_2S_2O_3$ solution. The starch indicator (3drops) was then added at this point and the titration was continued till a milky solution was gotten. Iodine number was then calculated using the formula below:

$$\text{Iodine number} = \frac{(Tb - Ts)}{\text{Weight of sample}} \times 1.269$$

Where

Tb = Titre value for the blank

Ts = Titre value for the sample

### 3.5.3 Confirmation Studies for the mycodeterioration of the extracted oil from *I.gabonensis* seed using the GC technique

### 3.5.3a Sample preparation (Derivatization) of the extracted oil from *I.gabonensis* seed prior to GC reading

Fat (from the previously extracted oil) was re-extracted with redistilled n-hexane for the recorvery of the undiluted oil. The crude oil extract was made to be free of water by filtering through the anhydrous sodium sulphate salt. The hexane was removed from the oil/hexane mixture by using rotatory evaporator.

The fatty acid profile i.e. Fatty Acid Methyl Ester (FAME) analysis consisting of the saturated, monounsaturated and the poly unsaturated fatty acids were carried out by following the modified AOAC 965.49 and AOAC 996.06 official methods. Here, fifty milligramme of the oil was saponified (esterified) for five minutes at $95^oC$ with 3.4 ml of the 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCl.

In addition, 3 ml of 14% boron triflouride in methanol was added. The mixture was heated for 5 minutes at the temperature of 90$^{o}$C to achieve complete methylation process. The FAMEs were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated with 1ml for GC analysis and 1µl was injected into the injection port of the GC machine.

The GC model and its condition for the FAMEs analysis are as detailed below:

GC: HP 6890 powered with HP Chemstation Rev. A 09.01{1206} software.

Injection temperature : Split Injection.

Split Ratio: 20:1

Carrier Gas: Nitrogen.

Inlet temperature: 250$^{o}$C.

Column type: HP INNOW ax

Column dimensions: 30m X 0.25m X 0.25µm

Oven programme: Initial temperature @ 60$^{o}$C

First Ramping @ 12$^{o}$C/min for 20min, maintained for 2 min.

Second Ramping @ 15$^{o}$C/min for 3 min, maintained for 8 min.

Detector: Flame Ionization Detector- FID.

Detector temperature: 320$^{o}$ C.

Hydrogen pressure: 22 psi.

Compressed air: 35 psi.

### 3.5.4 Proximate analysis of the *I.gabonensis* seed flour

Proximate analysis is a system of analysis of nutrients in which the gross components (proteins, fat, carbohydrate, ash etc) of a food material are determined.

#### 3.5.4a Crude fibre content determination

**Principle**

Crude fibre is the organic residue which remains after the material has been treated under standardized conditions with light petroleum, boiling dilute $H_2SO_4$, boiling dilute NaOH solution, dilute HCI, alcohol and ether.

**PROCEDURE.**

Three gramme (3.00 g) of the defatted sample was put into a 250 ml beaker, thereafter, two hundred milliliters (200ml) of boiling water was added with 1.25% $H_2SO_4$ into the 250ml beaker containing the sample. The content in the beaker was heated and kept boiling for 30 minutes during which the mixture was stirred with the glass stirring rod to remove all particles from the sides and top up with boiling water and went down. After this, the mixture was filtered hot with buckner funnel fixed with ashless filter paper. The beaker was rinsed with 50ml boiling water and washed through Buchner funnel to collect the precipitate. The precipitate in the funnel was washed until it was neutral to litmus paper. The residue was scrapped off from the ashless filter paper back into the 250 ml beaker and 200ml boiling water with 2.5% NaOH and was boiled for 30 minutes and during this period, boiling water was added from time to time to make up the 200 ml level and rewashed through Buchner funnel to collect the precipitate, after which the residue was washed with twice with 95% ethanol. This final residue was thereafter dried in an oven to a constant weight and thereafter cooled in a dessicator.

The crude fibre content was calculated using the formula:

$$\% \text{ crude fibre} = \frac{X_1 - X_2}{X_3} \times 100$$

Where

$X_1$ = weight before.

$X_2$ = weight after.

$X_3$ = weight of sample

3.5.4b Moisture content

**Principle**

This involves the measurement of the weight loss due to the evaporation of water. 3.00 g of the sample was weighed and placed in a crucible of constant weight. The crucible containing the sample was placed in the oven at 105°C for about 14 hours. After drying, the weights were measured every one hour until a constant weight was obtained. The loss in weight represents the moisture content.

**Calculation:**

$$\% \text{ moisture} = \frac{A - B}{C} \times 100$$

Where A = weight of sample before drying

B = weight of sample after drying

C = weight of sample used

3.5.4c Ash content determination

**Principle**

The ash of a foodstuff is the inorganic residue remaining after the organic matter has been burnt away.

**PROCEDURE**

Exactly three gramme of the sample was weighed into a crucible of known weight and placed in a furnace which was ignited for about 24 hours until grey ash was obtained. The crucible containing the ash sample was removed from the furnace and cooled in the dessicator and then weighed using the analytical balance.

**Calculation**

$$\% \text{ ash} = \frac{\text{weight of ash}}{\text{Weight of sample}} \times 100$$

3.5.4d Protein content determination

**(Total nitrogen)**

**Principle**

The kjeldahl method for determining total Nitrogen involves first heating with concentrated $H_2SO_4$ in a long-necked digestion flask. The reaction rate is accelerated by adding sodium or potassium sulphate which serves as catalyst to raise the boiling point. The oxidation causes the Nitrogen to be converted to ammonia sulfate. After making alkaline with concentrated NaOH solution, the ammonia is distilled into either excess boric acid or standard acid (such as $H_2SO_4$) and is estimated by titration.

**Procedure**

(A) Digestion of sample

Approximately two gramme of the sample was weighed into the kjeldah's flask and thereafter, 0.1g of $CuSO_4$ and 1.0g $Na_2SO_4$ was added into the kjeldah's flask containing the sample. Afterwards, 20 ml of concentrated $H_2SO_4$ was also added. The flask was then set up in a slanting position on the kjeldah's digestion heating mantle and then in the fume cupboard until the colour changes from black to bluish green. The digest on the flask was then removed from the fume cupboard and allowed to cool. This digest was diluted with distilled water and made up to 200 ml. The blank was also prepared using the same procedure above with exception of the sample.

(B) Distillation and titration.

Fifty ml of the aliquot of the digest was poured into a distillation flask, and 50ml of 50% NaOH was carefully layered into the solution to make it strongly alkaline. Also, 50ml of 0.1M $H_2SO_4$ was measured into the receiving flask and two drops of methyl red indicator was added. The flask containing the digest was heated by using the Bunsen burner and the content was distilled into the receiving flask containing the 0.1M $H_2SO_4$. The distillation was stopped by removing the solution in the receiving flask before the heat was put off. This was done to avoid a drop in pressure. Excess acid was then titrated with 0.1M NaOH from the burette. The blank was set up using the procedure above. Protein content was obtained by multiplying percentage nitrogen by 5.18 the conversion factor for almonds.

$$\% \text{ Nitrogen} = \frac{(B - A) \text{ X Molarity of NaOH x } 0.014}{W}$$

Where B = titre value of blank

A = titre value of sample

W = weight of sample used

% protein = % Nitrogen x conversion factor

**Standardization of 0.1m sodium hydroxide**

**Procedure**

About $10cm^3$ of the primary standard of potassium hydrogen phthalate was weighed into a conical flask. Sodium hydroxide was then placed into a burette and two drops of phenolphthalein indicator was added to the evolution in the beaker. The sodium hydroxide was titrated against the primary standard.

3.5.4e Carbohydrate content

Carbohydrate content was obtained by subtracting the sum of total crude fibre, total lipid content, total protein, ash and moisture from 100.

If A = % protein

B = % fat

C = % fibre

D = % ash

E = % moisture

% carbohydrate

100 – (A + B + C + D + E).

### 3.6.0 Hydrocarbon utilization studies

In this aspect of the work, the following were done:

(i) A preliminary test for petroleum hydrocarbon utilizing ability of the fungal isolates via the growth test under a petroleum hydrocarbon source- crude oil fume.

(ii) Measuring of the optical activity (density) of the different fungal isolates in the different petroleum hydrocarbon sources over a 40day period using the spectrophotometric test.

(iii) A confirmatory studies of the petroleum hydrocarbon utilizing abilities of the different fungal isolates using the Gas chromatographic technique.

### **3**.6.1 Preliminary experiment for petroleum hydrocarbon utilising ability of the isolated fungal samples

The modified method of Amund *et al*. (1987) as described by Adekunle and Oluyode (2005) was adopted for this. Sixty filter papers (Whatman No. 1001125) that had been previously sterilized were dipped in about 400mls of crude oil contained in 1000 ml beaker for about 25 sec. using a sterile forcep. Using the same forcep, these filter papers were allowed to drain for another 60 sec. Each of the crude oil treated paper was then placed on the cover of Petri dishes containing freshly prepared solidified sterile PDA, and the diseased cotyledons of *I. gabonensis*. All plates were incubated at room temperature and observed regularly for fungal growth.

This was carried out in order to determine the best samples out of the many isolates, that posses the most ability to degrade petroleum hydrocarbon. Consequently, a total of twenty five (25) fungal isolates from the different sources that showed active growth under the crude oil fume were the ones that were used for the subsequent biodegradation experiments.

### 3.6.2 Advanced preliminary experiment for petroleum hydrocarbon utilising ability of the isolated fungal samples using uv/vis spectrophotometric machine

The biodegradation potentials of 25 fungal isolates from the 9 sources on 5 different petroleum hydrocarbon sources namely, Spent engine Oil (SEO), Crude Oil (CO), Kerosene (kero), Diesel (diesel) and Fresh engine Oil (LUBE) and a hydrocarbon source of plant origin- oil extracted from the seed of *Irvingia gabonenesis* (IO) – were verified. The biodegradation potentials of the different fungal isolates were tested by measuring their different optical activities in these different hydrocarbon sources over a 40 day period, at 5days intervals using the Ultraviolet/visible (uv/vis) Spectrophotometer Model T80, PG Instuments Ltd (plate 7) at 2 photosynthetically active wave lengths of 530 and 620 nms. Also the initial (at day 0) and the final levels (day 40) of the TPH present in each of these medium of petroleum origin, and the free fatty acid profile of the media containing the oil of plant origin were evaluated using the Gas Chromatographic (Gc) technique, so as to determine the level and the extent to which the biodegradation of these oils have been effected by the fungal isolates. Essentially, the uv/vis spectrophotometric technique provided a very good basis to determine the efficacy of the different fungal isolates against one another, while the Gc technique was adopted as a confirmatory (or otherwise), as well as a comparative measure of the ability of one of the most promising fungal isolates (as determined by the spectrophotometric experiment) to degrade these 2 hydrocarbon sources - petroleum hydrocarbon and vegetable hydrocarbon sources.

Song and Bartha (1990), Kampfler and Steoif (1991) both described the technique of enumerating hydrocarbon degraders using Most Probable Number (MPN). This method involves culturing microbes in liquid enrichment medium to which contaminants has been added. The modified methods of Amund *et al.*(1987),

Nwachukwu (2000) and Adekunle and Oluyode (2005) were used in preparing a Minimal Salt Solution (MSS) containing 2.13g of $Na_2HPO_4$, 1.3 g of $KH_2PO_4$, 0.5 g of $NH_4Cl$, 0.2 g of $MgSO_4.7H_2O$ and 0.055 g 0f yeast extract all dissolved in 1000ml of distilled water; because the medium was to be used for petroleum hydrocarbon degrading fungi, the pH was adjusted to 4.5 using a pH buffer powder dissolved in 250ml of deionised water at $50^o$ C. pH reading was taken using the pH meter Gallenkomp PHS-3C, serial number 600410069047. The entire mixture was then autoclaved at $121^o$ C for 15 minutes. Exactly 200 test tubes of 20ml capacity each were sterilized and plugged with non-absorbent cotton wool and thereafter placed in test tube racks at the rate of seven (7) test tubes per rack, thus making a total of 28 test tube racks.

In each of the 200 test tubes were 10ml of MSS. Out of this number, thirty three (33) test tubes each had an additional 2mls of either Crude Oil (CO), Diesel, Kerosene, Fresh Engine Oil (FEO), Spent Engine Oil (SEO) or the Oil extracted from *I.gabonensis* seeds (I.O).

The remaining two test tubes left of the 200 contained MSS only, these were used as blank in calibrating the spectrophotometer. The model of the spectrophotometer used was **T80 UV/VIS -by PG Instruments Limited**. Of the 33 test tubes for each of the oils, each contained only 1 of the 25 fungal isolates, making a total of 25 test tubes per oil for all the isolates. Of the remaining 8 test tubes per hydrocarbon source (oil), 2 test tubes each had no fungus in them, thus serving as control, while of the remaining, some were taken for GC analysis, the remaining were kept as reserves.

The fungi were asceptically dropped each time into the test tubes using a 2 mm cork-borer. All the test tubes were thereafter kept at room temperature in an incubator for a 40 day period, during which time, the ability of each of the fungus to degrade each of

the oils were measured by taking the optical density of each of the mixture in each of the test-tubes. This was done using a spectrophotometer at two wavelengths of 530 nm and 620 nm every 5 day interval from day zero to day 40. Constant shaking of the test tube was done to facilitate oil/cell interphase (contact). This experiment was replicated three times, and the results were statistically analyzed using the soft ware statistical package SAS.

### 3.6.3 Confirmatory experiment of the petroleum hydrocarbon utilizing ability of the fungal samples using Gas Chromatograhic (GC) methods

Following the 40 day optical density experiment, a representative fungal isolate of the 25 fungal isolates was taken for each of the six oils for a Gas Chromatograhic (GC) analysis to determine the Total Petroleum Hydrocarbon (TPH) content that remained undegraded at day 40. This was compared with the GC analysis earlier conducted on the same fungus (for each of the oils) at Day zero. For the I.O. however, fatty acid determination was done (instead of TPH) using the GC at days 0 and 40. In confirming the level and the extent that the fungal isolates have biodegraded these hydrocarbon sources, the initial TPH (at day 0) and the final TPHs (at day 40) that was present in each of these medium of petroleum origin, and the free fatty acid profile of the medium containing the oil of plant origin i.e. oil extracted from the seed of *Irvingia gabonensis* was evaluated using the Gas Chromatographic (Gc) technique. Three levels of GC profiling were done per oil. These were: Media + *Aspergillus oryzae* at day 0, media + *Aspergillus oryzae* at day 40 and media only (no *Aspergillus oryzae* added).

### 3.6.4 Extraction of the Total Petroleum Hydrocarbon (TPH) for GC reading

The liquid sample from each test tube was transferred to a 50ml separatory funnel, and 3ml of the redistilled mixture of hexane: dichloromethane in ratio 3:1 was added.

The separatory funnel was shaken vigorously for about 2 minutes with periodic venting to release vapour pressure. The organic layer was allowed to separate for 10 minutes and was recovered into the 50 ml beaker. The aqueous layer was re-extracted twice with 3 ml of the extractant. The combined extract was dried by passing through the funnel containing anhydrous sodium sulphate. The dried extract was concentrated with a stream of nitrogen gas. The concentrated extract was cleaned by passing the extractant through the silica gel packed column after rinsing severally with redistilled hexane.

The mixture was concentrated to about 1.0 ml by stream of the nitrogen gas before the Gas Chromatographic analysis.

The GC model and GC conditions for determining the TPH are as outlined below:

GC: HP 6890 powered with HP Chemstation Rev. A 09.01{1206} software.

| | |
|---|---|
| Injection temperature : | Split Injection. |
| Split Ratio: | 20:1 |
| Carrier Gas: | Nitrogen. |
| Inlet temperature: | 250°C. |
| Column type: | HP 5. |
| Column dimensions: | 30m X 0.25m X 0.25μm |
| Oven programme: | Initial temperature @ 60°C<br>First Ramping @ 10°C/min for 20min, maintained for 2min. Second Ramping @ 15°C/min for 4min, maintained for 4min. |
| Detector: | Flame Ionization Detector- FID. |
| Detector temperature: | 320° C. |
| Hydrogen pressure: | 28 psi. |
| Compressed air: | 40 psi. |

### 3.7.0 Field trial of mycoremediation experiment

The next stage of this experiment was to take one of the promising fungal isolates from the preceding mycodegradation studies (that was conducted in the laboratory) for an actual field trial. On the field, the evaluation of the effectiveness of this fungus (*A.oryzae*) as a mycoremediation agent on a petroleum hydrocarbon contaminated soil was done using the Gas Chromatographic (GC) technique. Gc reading was taken on 2 occasions namely, immediately after the application of the Treatments ($1^{st}$ T1- $1^{st}$ T8) and at 6 months after the application of Treatments (T1 – T8) to compare the differences in the Total Petroleum Hydrocarbon (TPH) level in the soil for all the 8 Treatments administered.

Following a modified approach of Egunjobi and Onweluzo (1979), Amund *et al.* (1993) and Egberongbe *et al*. (2010), a single experimental plot of land measuring $80.0m^2$ was mapped out behind the Department of Computer Science, Faculty of Science, University of Lagos. This plot was deliberately polluted with spent engine oil (SEO) at the rate of 2 $L/2m^2$, and/or fungal spores in different combinations called Treatments. For some of the Treatments, weeding was done while for others weeding was not done. The Treatment was applied only once during the entire duration of the experiment. The fungal spores and SEO were applied to the soil at the same time using different pre-sterilized stainless steel, fine nozzle watering cans. The SEO spilled on the Treatment cells was not worked into the soil; the essence of which is to provide a scenario as close to real life as possible. Using the Randomnised Complete Block Design (RCBD), the plot was divided into 5 blocks called Block 1 ($B_1$), Block 2 ($B_2$)….. Block 5 ($B_5$). Within each of the blocks, the eight Treatments were randomly assigned using the random number table. Each of these Treatments were

replicated 5 times. The eight Treatments outlined below were assigned to this plot as shown in Figure1.

Treatment 1($T_1$) = not weeded (natural vegetation) with nothing added.
Treatment 2 ($T_2$) = weeded with nothing.
Treatment 3 ($T_3$) = weeded + seo + *aspergillus oryzae*.
Treatment 4 ($T_4$) = not weeded + seo + *aspergillus oryzae.*
Treatment 5 ($T_5$) = weeded + seo – *aspergillus oryzae*.
Treatment 6 ($T_6$) = not weeded + seo – *aspergillus oryzae.*
Treatment 7 ($T_7$) = weeded – seo + *aspergillus oryzae.*
Treatment 8 ($T_8$) = not weeded – seo + *aspergillus oryzae.*
It is important to note that that the 8 Treatments above can be grouped into 4 pairs (with 1 of the pair acting as a control for the other Treatment in the pair) thus:

**PAIR A**: $T_1$ AND $T_2$ general control pair (where $T_1$ is acting as a control for $T_4$, $T_6$ and $T_8$ and as a baseline for all the other treatments and as well as for $T_2$).

**PAIR B**: $T_3$ and $T_5$ (this Treatment pair is the only pair that is verifying in an exclusive manner i.e. without the encumbrances of vegetation cover, the ability of *A.oryzae* to mycoremediate a petroleum hydrocarbon polluted field. Here $T_5$ is acting as a control to $T_3$).

**PAIR C**: $T_4$ and $T_6$ (this Treatment pair seeks to verify how efficient *A.oryzae* can mycoremediate a petroleum hydrocarbon polluted field that has some vegetation cover. Here, $T_6$ is acting as control to $T_4$).

**PAIR D**: $T_7$ and $T_8$ (this Treatment pair seeks to verify how *A.oryzae* can affect a terrestrial environment (with and without vegetation cover) in the absence of petroleum hydrocarbon pollution. This treatment pair in addition further seeks to address the ethical considerations that are involved in introducing microorganisms from one environment to another.

| BLOCK 5 | BLOCK 4 | BLOCK 3 | BLOCK 2 | BLOCK 1 |
| --- | --- | --- | --- | --- |
| $T_1$ | $T_3$ | $T_7$ | $T_8$ | $T_6$ |
| $T_7$ | $T_8$ | $T_4$ | $T_1$ | $T_3$ |
| $T_4$ | $T_6$ | $T_8$ | $T_6$ | $T_7$ |
| $T_3$ | $T_5$ | $T_2$ | $T_3$ | $T_4$ |
| $T_8$ | $T_7$ | $T_6$ | $T_2$ | $T_5$ |
| $T_2$ | $T_4$ | $T_3$ | $T_5$ | $T_1$ |
| $T_5$ | $T_2$ | $T_1$ | $T_7$ | $T_8$ |
| $T_6$ | $T_1$ | $T_5$ | $T_4$ | $T_2$ |

Figure 1: Visual presentation of field Layout

On the field, data were taken on the following parameters:

(1) Weed species before the application of treatment (baseline weed data), at 2 months, 4 months and 6 months after the application of the Treatments.

(2) Soil nutrient namely total nitrogen (%N), available phosphorus (Mg/Kg), potassium (meq/100g) and magnesium (meq/100g) were all analysed for in each Treatment. Data on the effect of *A.oryzae* as an agent to mycoremediate a SEO polluted soil in this experiment was collected 3 times thus: at baseline point, at the 3$^{rd}$ month and the 6$^{th}$ month after the application of Treatments. Soil for each Treatment was taken from each block, and this was thereafter bulked into one composite sample. Each composite sample was analysed in 3 replicates, thus giving us the mean concentration of element in the soil for each treatment.

(3) Amount of Total Petroleum Hydrocarbon (TPH) in the soil (using GC method) both as a baseline data (day 0) and at 6 months after the application of Treatment were determined for each of the Treatments. As above, sample for each Treatment was taken per block. The values of TPH in the soils of the different Treatments were obtained after triplicate analysis from electronic integration measurements using flame ionization detector.

### 3.7.1 Weed study

The effects of the Treatments applied were investigated on the natural flora found on the experimental plot. The weeds were studied by noting their abundance over time. A semi systematic method of sampling using a quadrat size of 0.5 m X 1 m size was used in the sampling of these weed species. A single quadrat was thrown per experimental cell of 2 m X 1m.

### 3.7.2 Soil nutrient analyses

### 3.7.2a Digestion of soil samples

A total of 5.0g of the soil sample was weighed into the pre cleaned borosilicate 250ml capacity beaker for digestion. Thereafter, 30ml of the mixture of hydrochloric acid and nitric acid in the ratio 3: 1 was added into the weighed sample in the beaker. The sample with the digesting solution was placed on the hot plate digestion in the fume cupboard. The beaker and its content after the digestion was allowed to cool. Another 20ml of the digesting solution was added and digested further in the fume cupboard, and the mixture was allowed to cool to the room temperature. The mixture was filtered into 250ml volumetric capacity borosilicate container. The filterate was made up to the mark with de-ionised water. All the digested samples were sub-sampled into pre-cleaned borosilicate glass containers for Atomic Absorption Spectrophotometer analysis. In all cases, data analyzed and presented were the mean values from three replicates. The SAS statistical software was used in the analysis of the data, and the means were separated using the Duncan's multiple range test.

**(i) Magnesium (Mg)**

Standards of Magnesium solution of 0.2, 0.4, 0.6, 0.8 and 1.0mg/l were made from heavy metal solution of 1000mg/l stock solution of the analyte. The set of standard solutions and the filterate of the digested samples were read by an Atomic Absorption Spectrophotometer (AAS). The detection limit of the metal in the sample was 0.0001mg/l. **The model of the AAS is UNICAM 929 London, powered by SOLAAR soft ware.** Magnesium cathode lamp was used for the analysis of the heavy metal ion in the standards and the filterate of the samples. Gas mixtures were used in the generation of the flame.

Callibration curve is as shown and the correlation coefficients are also included in the calibration curve.

**(ii) Potassium (K)**

All the digested samples from the AAS analysis were sub-sampled into pre-cleaned borosilicate glass containers for the for Flame Photometeric analysis of potassium in soil samples.Standards of potassium solutions of 0.2, 0.4, 0.6, 0.8 and 1.0mg/l were made from the metal solution of 1000mg/l stock solutions of the analyte. The set of standard solutions and the filterate of the digested samples were analysed by Flame Photometer. The model of the Flame Photometer is **JENWAY PFP 7**. Gas mixture of air and LPG was used in the generation of the flame, while the detection limit of the metal in the sample was 0.0001mg/l

**(iii) Nitrogen forms (nitrate and total nitrogen)**

Measurement of nitrogen in the soil samples was carried out as total nitrogen. The analyses of the various forms relied on the chemical reaction of the samples with the catalyzing agents. The extract now reacted with color forming agents (for example sulfanic acid in nitrate analysis) to form specifically coloured complexes and/or compounds.

Measurement of the concentrations of the nitrogen form is then made spectrophotometrically at specific wavelengths against standard concentration curves.

**(iv) Phosphorus forms (phosphate and total phosphorus)**

Soil samples were analyzed for forms of phosphorus as phosphate. Phosphorus analysis APHA 4500-PE (STD Methods, $19^{th}$ ed.) consists of two general procedural steps:

(a+) conversion of the phosphorus form of interest to dissolved orthophosphate, and

(b) spectrophotometric determination of dissolved orthophosphate.

The primary forms determined in this analysis were reactive phosphorus and total phosphorus. Phosphates that respond to colometric tests without preliminary hydrolysis or oxidative digestion of the samples are termed “reactive phosphorus”. While reactive phosphorus is largely a measure of orthophosphate, a small fraction of any condensed phosphate present usually was hydrolyzed unavoidably in the procedure. Reactive phosphorus occurs in both dissolved and suspended forms. Total phosphorus (orthophosphate, condensed, and organically bound) was determined by acid oxidation with persulfate, followed by the reactive phosphorus test. Organically bound phosphate was then determined by subtracting the acid-hydrolzable phosphorus.

### 3.8.0 DNA extraction and gel electrophoresis of fungal isolates

#### 3.8.1 Extraction of DNA from the fungal samples

The CTAB protocol of DNA extraction as described by Thottappilly *et al*. (1999) was adopted for this work. Here, 2ml of fungal cultures were added to the eppendorf tubes and spinned at the rate of 10,000 rpm for 10 minutes. After this, the supernatant was gently decanted into fresh tubes. To this decant, 400µl of CTAB buffer and 75µl of 10% SDS were added. Thereafter, it was incubated at 65$^{o}$C for 15 minutes. Afterwards, 500µl of chloroform was added, and this was mixed for another 5 minutes, after which it was spinned at 10,000rpm for 10 minutes using the eppendorf centrifuge model 5415C. Following the spinning, the supernatant was collected into fresh eppendorf tubes, after which 700µl isopropanol was added to the freshly decanted supernatant. This was kept at -20$^{o}$C for 1 hour in the Ultra freezer. Afterwards, this supernatant was spinned at 1000rpm for another 10 minutes. The

supernatant was gently decanted and washed in 200µl 70% ethanol. Afterwards, this was air dried for 50 minutes and re-suspended in 200µl sterile water.

### 3.8.2 Gel preparation and Gel electrophoresis

After the DNA has been extracted, a 1% gel electrophoresis of the extracted DNA samples was prepared thus: 1.0g agarose was weighed in 100µl of 1xTAE and microwaved at medium temperature for 3minutes, after which it was allowed to cool. After cooling, 3µl of ethidium bromide was added. This was poured and allowed to solidify. Before the loading of the samples, 3µl of loading dye was added to each 10µl of sample. The samples and the markers were thereafter loaded and allowed to run at between 120V for approximately 1 hour in the Cleaver UV Transilluminator.

After confirming the presence of DNA in the fungal samples through the Gel electrophoresis, the extracted DNA from the fungal samples were then sent to Macrogene Inc, USA for sequencing.

The alignment of the raw results for the DNA sequencing, the construction of the phylogenetic tree of the aligned base pairs and the identification of the fungal isolates based on the results from the DNA sequence were all done using the computer software CLC Sequencer viewer 6.5.2 at the International Institute for Tropical Agriculture (IITA), Ibadan.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1.0 Isolation studies

The results of the sampling studies are as summarized in Table 2 and Tables 3A - 3F. Plates 1A and 1B – 4A and 4B show the pictures and the photomicrographs of some of the different fungal species encountered in this research. On a general note however, in terms of number and species abundance, the wetter and more humid months appeared to have more fungal isolates than the drier, less humid months as corroborated by the meteorological data (appendix 52). In addition, the results fromTables 3A – 3F also show that in general, *Aspergillus niger* has a higher percentage occurrence than the other fungal species encountered, and also, *A.niger* was at all times found in the different soil environments. In addition, these Tables show that the percentage occurrence of all the fungal species was higher in the soil than from the *I.gabonensis* seed sampled from the different open markets.

Table 2: Percentage frequency of occurrence of fungal isolates from each of the experimental site over a 30 month period

| Fungal Isolate | Site ID and percentage frequency of occurrence per site | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Average frequency of occurrence of each fungal species in all the sites. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Aspergillus niger* | | 100 | 100 | 100 | 100 | 100 | 53.3 | 60.0 | 73.3 | 66.6 | 82.88 |
| *Aspergillus oryzae* | | 93.3 | 60.0 | 46.6 | 73.3 | 86.6 | 46.6 | 6.6 | 40.0 | 26.6 | 53.28 |
| *Aspergillus tubingensis* | | 40.0 | 20.0 | 26.6 | 20.0 | 20.0 | 6.6 | 6.6 | 40.0 | 6.6 | 20.71 |
| *Mucor sp* | | 40.0 | 13.3 | 13.3 | 20.0 | 6.6 | 26.6 | 13.3 | 26.6 | 33.3 | 21.44 |
| *Rhizopus sp* | | 46.6 | 6.6 | 20.0 | 13.3 | 20.0 | 26.6 | 20.0 | 26.6 | 13.3 | 21.44 |
| *Penicillum sp* | | 46.6 | 33.3 | 26.6 | 26.6 | 20.0 | 13.3 | 13.3 | 26.6 | 26.6 | 25.87 |
| Average fungal occurrence per site | | 61.8 | 38.9 | 38.9 | 42.2 | 42.2 | 28.8 | 20.0 | 38.9 | 28.8 | |

**Key**

1 = Soil from Olushosun Dump Site, Ojota
2 = Soil from Shodex Beautification Gardens, Anthony village
3 = Soil from Biological Gardens, University Of Lagos
4 = Soil from Mechanic village, Sabo-Yaba
5 = Soil from Mechanic village, Onitiri Akoka
6 = *Irvingia gabonensis* seed from Oyingbo market
7 = *Irvingia gabonensis* seed from Bariga market
8 = *Irvingia gabonensis* seed from Ajegunle
9 = *Irvingia gabonensis* seed from Agege market

TABLE 3A: *Aspergillus niger* isolated from the different sites/sources and the frequency of occurrence

| Fungal species | *Aspergillus niger* | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site ID | Month Isolated | Dec. 2007 | Feb. 2008 | Apr. 2008 | Jun. 2008 | Aug. 2008 | Oct. 2008 | Dec. 2008 | Feb. 2009 | Apr. 2009 | Jun. 2009 | Aug. 2009 | Oct. 2009 | Dec. 2009 | Feb. 2010 | April. 2010 | Total Frequency Of Occurrence (%) |
| 1 | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| 2 | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| 3 | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| 4 | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| 5 | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| 6 | | + | + | - | + | + | - | - | + | + | - | + | - | + | - | - | 53.3 |
| 7 | | - | + | - | + | + | - | + | + | + | + | + | - | - | + | - | 60.0 |
| 8 | | + | + | + | + | + | + | - | + | + | + | + | + | - | - | - | 73.3 |
| 9 | | + | + | + | + | + | - | - | + | + | + | + | - | + | + | - | 66.6 |
| Monthly frequency of occurrence (%) | | 77 | 100 | 55.5 | 100 | 100 | 66.6 | 66.6 | 100 | 100 | 100 | 100 | 66.6 | 77.7 | 77.7 | 77.7 | |

**Key:**

+ = Present.
- = Absent.
.

1 = Soil from Olushosun Dump Site, Ojota
2 = Soil from Shodex Beautification Gardens, Anthony village
3 = Soil from Biological Gardens, University Of Lagos
4 = Soil from Mechanic village, Sabo-Yaba
5 = Soil from Mechanic village, Onitiri Akoka
6 = *Irvingia gabonensis* from Oyingbo market
7 = *Irvingia gabonensis* from Bariga market
8 = *Irvingia gabonensis* from Ajegunle
9 = *Irvingia gabonensis* from Agege market.

TABLE 3B: *Aspergillus oryzae* isolated from the different sites/sources and the frequency of occurrence

| Fungal species | *Aspergillus oryzae* | | | | | | | | | | | | | | | | Total % frequency Of occurence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site ID | Month Isolated | Dec. 2007 | Feb. 2008 | Apr. 2008 | Jun. 2008 | Aug. 2008 | Oct. 2008 | Dec. 2008 | Feb. 2009 | Apr. 2009 | Jun. 2009 | Aug. 2009 | Oct. 2009 | Dec. 2009 | Feb. 2010 | April. 2010 | |
| 1 | | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | 93.3 |
| 2 | | + | + | - | + | + | - | - | - | + | - | + | + | - | + | + | 60.0 |
| 3 | | - | + | - | - | + | - | - | + | - | - | + | + | + | - | + | 46.6 |
| 4 | | + | + | + | + | + | - | + | + | - | - | - | + | + | + | + | 73.3 |
| 5 | | + | + | + | + | + | + | + | + | - | + | + | + | - | + | + | 86.6 |
| 6 | | - | + | - | - | + | + | - | + | - | + | - | - | + | - | + | 46.6 |
| 7 | | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 06.6 |
| 8 | | - | - | - | + | + | + | - | - | + | + | - | - | - | - | + | 40.0 |
| 9 | | + | - | - | - | + | - | - | + | + | - | - | - | - | - | - | 26.6 |
| Monthly frequency of occurrence (%) | | 66.6 | 66.6 | 33.3 | 55.5 | 88.8 | 44.4 | 33.3 | 66.6 | 44.4 | 44.4 | 44.4 | 55.5 | 44.4 | 33.3 | 77.7 | |

**Key:**

\+ = Present.

\- = Absent.

1 = Soil from Olushosun Dump Site, Ojota

2 = Soil from Shodex Beautification Gardens, Anthony village

3 = Soil from Biological Gardens, University Of Lagos

4 = Soil from Mechanic village, Sabo-Yaba

5 = Soil from Mechanic village, Onitiri Akoka

6 = *Irvingia gabonensis* from Oyingbo market

7 = *Irvingia gabonensis* from Bariga market

8 = *Irvingia gabonensis* from Ajegunle

9 = *Irvingia gabonensis* from Agege market.

TABLE 3C: *Aspergillus tubingensis* isolated from the different sites/sources and the frequency of occurrence

| Site ID | *Aspergillus tubingensis* Month Isolated | Dec. 2007 | Feb. 2008 | Apr. 2008 | Jun. 2008 | Aug. 2008 | Oct. 2008 | Dec. 2008 | Feb. 2009 | Apr. 2009 | Jun. 2009 | Aug. 2009 | Oct. 2009 | Dec. 2009 | Feb. 2010 | April. 2010 | Total % Frequency Of Occurence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | - | - | - | - | + | + | + | - | + | - | - | + | + | - | - | 40.0 |
| 2 | | - | - | - | - | + | - | + | - | - | - | - | - | - | + | - | 20.0 |
| 3 | | - | - | - | - | + | - | + | - | - | + | - | - | - | + | - | 26.6 |
| 4 | | - | - | - | - | + | - | - | - | + | - | - | - | - | + | - | 20.0 |
| 5 | | - | - | - | - | - | - | - | - | + | - | - | - | - | + | - | 20.0 |
| 6 | | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | 6.6 |
| 7 | | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | 6.6 |
| 8 | | - | - | - | - | + | + | + | - | + | - | - | + | - | + | - | 40.0 |
| 9 | | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | 6.6 |
| Monthly frequency of occurrence (%) | | 0 | 0 | 0 | 0 | 77.7 | 22.2 | 33.3 | 0 | 44.4 | 11.1 | 0 | 33.3 | 11.1 | 66.6 | 0 | |

**Key:**

+ = Present.

- = Absent.

1 = Soil from Olushosun Dump Site, Ojota

2 = Soil from Shodex Beautification Gardens, Anthony village

3 = Soil from Biological Gardens, University Of Lagos

4 = Soil from Mechanic village, Sabo-Yaba

5 = Soil from Mechanic village, Onitiri Akoka

6 = *Irvingia gabonensis* from Oyingbo market

7 = *Irvingia gabonensis* from Bariga market

8 = *Irvingia gabonensis* from Ajegunle

9 = *Irvingia gabonensis* from Agege market.

TABLE 3D: *Mucor species* isolated from the different sites/sources and the frequency of occurrence

| Site ID | *Mucor spp* Month Isolated | Dec. 2007 | Feb. 2008 | Apr. 2008 | Jun. 2008 | Aug. 2008 | Oct. 2008 | Dec. 2008 | Feb. 2009 | Apr. 2009 | Jun. 2009 | Aug. 2009 | Oct. 2009 | Dec. 2009 | Feb. 2010 | April. 2010 | Total % Frequency Of Occurence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | - | - | - | - | + | + | + | + | - | - | - | + | - | - | + | 40.0 |
| 2 | | - | - | - | - | - | - | + | - | - | + | - | - | - | - | - | 13.3 |
| 3 | | - | - | - | - | - | - | - | - | - | + | - | - | - | + | - | 13.3 |
| 4 | | - | - | - | - | - | - | - | - | - | + | - | + | - | + | - | 20.0 |
| 5 | | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | 6.6 |
| 6 | | - | - | - | - | - | - | + | - | - | - | - | - | + | + | + | 26.6 |
| 7 | | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | 13.3 |
| 8 | | - | - | - | - | - | - | - | - | + | + | - | + | - | + | - | 26.6 |
| 9 | | - | - | - | - | - | - | + | - | - | - | + | + | + | + | - | 33.3 |
| Monthly frequency of occurrence (%) | | 0 | 0 | 0 | 0 | 11.1 | 11.1 | 44.4 | 11.1 | 11.1 | 55.5 | 11.1 | 44.4 | 33.3 | 55.5 | 22.2 | |

**Key:**

\+ = Present.

\- = Absent.

1 = Soil from Olushosun Dump Site, Ojota

2 = Soil from Shodex Beautification Gardens, Anthony village

3 = Soil from Biological Gardens, University Of Lagos

4 = Soil from Mechanic village, Sabo-Yaba

5 = Soil from Mechanic village, Onitiri Akoka

6 = *Irvingia gabonensis* from Oyingbo market

7 = *Irvingia gabonensis* from Bariga market

8 = *Irvingia gabonensis* from Ajegunle

9 = *Irvingia gabonensis* from Agege market.

TABLE 3E: *Rhizopus species* isolated from the different sites/sources and the frequency of occurrence

| Site ID | *Rhizopus sp.* Month Isolated | Dec. 2007 | Feb. 2008 | Apr. 2008 | Jun. 2008 | Aug. 2008 | Oct. 2008 | Dec. 2008 | Feb. 2009 | Apr. 2009 | Jun. 2009 | Aug. 2009 | Oct. 2009 | Dec. 2009 | Feb. 2010 | April. 2010 | Total % Frequency Of Occurence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | - | - | - | - | - | - | + | - | + | + | - | + | - | - | + | 46.6 |
| 2 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 |
| 3 | | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | 6.6 |
| 4 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | 6.6 |
| 5 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | 6.6 |
| 6 | | - | - | - | - | - | - | + | - | - | - | - | + | - | - | - | 13.3 |
| 7 | | - | - | - | - | - | - | - | + | - | - | - | + | - | - | - | 13.3 |
| 8 | | - | - | - | - | - | - | - | + | - | - | - | + | - | - | - | 13.3 |
| 9 | | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | 6.6 |
| Monthly frequency of occurrence (%) | | 0 | 0 | 0 | 0 | 0 | 0 | 22.2 | 22.2 | 11.1 | 22.2 | 0 | 55.5 | 0 | 0 | 33.3 | |

**Key:**

+ = Present.
- = Absent.

1 = Soil from Olushosun Dump Site, Ojota
2 = Soil from Shodex Beautification Gardens, Anthony village
3 = Soil from Biological Gardens, University Of Lagos
4 = Soil from Mechanic village, Sabo-Yaba
5 = Soil from Mechanic village, Onitiri Akoka
6 = *Irvingia gabonensis* from Oyingbo market
7 = *Irvingia gabonensis* from Bariga market
8 = *Irvingia gabonensis* from Ajegunle
9 = *Irvingia gabonensis* from Agege market.

TABLE 3F: *Penicillium species* isolated from the different sites/sources and the frequency of occurrence

| Site ID | *Penicillium sp.* Month Isolated | Dec. 2007 | Feb. 2008 | Apr. 2008 | Jun. 2008 | Aug. 2008 | Oct. 2008 | Dec. 2008 | Feb. 2009 | Apr. 2009 | Jun. 2009 | Aug. 2009 | Oct. 2009 | Dec. 2009 | Feb. 2010 | April. 2010 | Total % Frequency Of Occurence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | - | - | - | - | - | - | + | + | + | + | - | + | - | - | + | 40 |
| 2 | | - | - | - | - | - | - | + | + | - | - | - | + | - | - | - | 20 |
| 3 | | - | - | - | - | - | - | + | + | - | - | - | + | - | - | - | 20 |
| 4 | | - | - | - | - | - | - | + | - | - | - | - | - | - | - | + | 13.3 |
| 5 | | - | - | - | - | - | - | + | - | - | - | - | - | - | - | + | 13.3 |
| 6 | | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | 6.6 |
| 7 | | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 6.6 |
| 8 | | - | - | - | - | - | - | - | - | + | - | - | - | + | - | + | 20 |
| 9 | | - | - | - | - | - | - | - | + | + | - | - | + | - | + | - | 26.6 |
| Monthly frequency of occurrence (%) | | 0 | 0 | 0 | 0 | 0 | 0 | 55.5 | 55.5 | 33.3 | 11.1 | 0 | 44.4 | 22.2 | 11.1 | 44.4 | |

**Key:**

+ = Present.
- = Absent.

1 = Soil from Olushosun Dump Site, Ojota
2 = Soil from Shodex Beautification Gardens, Anthony village
3 = Soil from Biological Gardens, University Of Lagos
4 = Soil from Mechanic village, Sabo-Yaba
5 = Soil from Mechanic village, Onitiri Akoka
6 = *Irvingia gabonensis* from Oyingbo market
7 = *Irvingia gabonensis* from Bariga market
8 = *Irvingia gabonensis* from Ajegunle
9 = *Irvingia gabonensis* from Agege market.

### 4.2.0 Mycodeterioration studies on *Irvingia gabonensis* seeds by *Aspergillus oryzae*

Plates 1A and 1B show the pictures of the representative healthy and diseased seeds respectively of *Irvingia gabonensis* from which oil was extracted, and some of which was ground into flour from which the proximate composition was determined. Compared to the healthy seeds, the diseased (*A.oryzae* infected) seeds showed symptoms of dry, hard rot, with the seeds becoming mummified as well as a black discolouration (compared to the normal straw brown colour found in healthy seeds).

### 4.2.1 Physico-chemical characterization of the oil extracted from both the healthy and diseased ( *A.oryzae* infected) seeds of *I.gabonensis*

The results obtained (Table 4) showed that the mean value for some parameters such as pH, refractive index and percentage yield were significantly ($p \leq 0.05$) higher in the oils from the healthy seeds than the oils from the diseased seeds. In terms of all the parameters that are indicative of deterioration such as saponification value, unsaponifiable matter, acid value, peroxide value and free fatty acid (FFA), the mean values from the oils of the diseased seeds however were significantly ($p \leq 0.05$) higher than those of the healthy seeds. For such parameters as iodine value, cholesterol value and relative density, there was no significant difference in the mean values for both oils from the healthy and the diseased seeds; even though the oils from the healthy seeds had a higher mean value for such parameters as iodine value, cholesterol and relative density compared to the oils from the diseased seeds (Table 4).

TABLE 4: A comparison of the mean values of some physico-chemical parameters of the oils extracted from healthy and diseased seeds of *I.gabonensis*

| s/n | parameter | Healthy seeds. | Diseased seeds. |
|---|---|---|---|
| 1 | pH | $6.200_b$ | $5.000_a$ |
| 2 | refractive index (g/100g). | $1.516_b$ | $1.458_a$ |
| 3 | melting point | 30.067 | 30.067 |
| 4 | saponification value(mgkoh/g) | $224.040_a$ | $236.173_b$ |
| 5 | unsaponifiable matter (g/kg). | $20.963_a$ | $23.327_b$ |
| 6 | iodine value (g/100g). | $39.667_a$ | $37.013_a$ |
| 7 | peroxide value (meq/kg). | $9.333_a$ | $12.667_b$ |
| 8 | acid value (mg/g) | $11.967_a$ | $19.450_b$ |
| 9 | percentage free fatty acid (%ffa). | $6.013_a$ | $9.773_b$ |
| 10 | cholesterol (mg/100g). | $12.310_a$ | $12.190_a$ |
| 11 | relative density | $0.901_a$ | $0.895_a$ |
| 12 | % oil yield | $58.020_b$ | $55.287_a$ |
| 13 | TBA (µg/g) | $16.243_a$ | $18.727_b$ |

*Means in the same row carrying different subscripts are significantly different at $p \leq 0.05$.

### 4.2.2 A comparison of the proximate (nutrient) composition of the flour from healthy and the diseased (*A.oryzae* infected) seeds of *I.gabonensis*

A statistical analysis of the overall mean for each of the following proximate parameter of moisture, ash, fats, proteins, fibre, carbohydrate and energy in the flour of both the healthy and the diseased seeds of *I.gabonensis* showed that the Treatment had a significant effect ($p \leq 0.05$) on each of the nutritional parameter examined with the exception of fibre on which the Treatment did not exert a significant effect. A comparison of the individual means (Table 5) for both flours however reveal that for each of the parameters mentioned above, with the exception of fibre, where there was no significant ($p \leq 0.05$) difference between the flour from both the healthy and diseased seeds, the values of the nutritional composition for parameters such as moisture, ash, protein, carbohydrate and energy from the diseased seeds were significantly ($p \leq 0.05$) lower than what was obtained for the flours from the healthy seeds (Table 5). The only exception to this was for fat, where the diseased seeds have a significantly ($p \leq 0.05$) higher value than the healthy seeds (Table 5).

TABLE 5: A comparison of the individual means for the proximate compostion of the flour from healthy and diseased seeds of *i. gabonensis*

| s/n | nutrient | Healthy seeds | Diseased seeds |
|---|---|---|---|
| 1 | moisture | $5.0733_b$ | $4.4700_a$ |
| 2 | ash | $2.6500_b$ | $2.2700_a$ |
| 3 | fats | $55.4533_a$ | $56.2700_b$ |
| 4 | protein | $10.0300_b$ | $8.8600_a$ |
| 5 | fibre | $3.8567_a$ | $3.3233_a$ |
| 6 | carbohydrate | $24.8000_b$ | $22.9367_a$ |
| 7 | energy | $641.0700_b$ | $631.0670_a$ |

*Mean values in each row showing different subscripts are significantly different at $P \leq 0.05$.

### 4.2.3 Confirmation of the indices and the extent of mycodeterioiration of *I. gabonensis* oil by *A.oryzae* using Gas Chromatographic (GC) technique

The oil from the diseased seeds were shown to have a higher percentage composition of saturated fatty acids than the oil from the healthy seeds, while the oils from the healthy seeds contained a higher proportion of the unsaturated (both the monounsaturated and the polyunsaturated) fatty-acids (Table 6).

The results as shown in Table 7 however indicate the percentage composition of the individual fatty acids of the oils extracted from the healthy and diseased seeds of *I.gabonensis* (as determined by the GC machine) and their corresponding degree of saturation or unsaturation. From Table 7, it can be seen that the oil from *I.gabonensis* seeds (either healthy or diseased seed) are composed of a total of 12 fatty acids namely Myristic, Palmitic, Palmitoleic, Stearic, Oleic, Linoleic, Linolenic, Arachidic, Arachidonic, Behenic, Erucic and Lignoceric acids in different proportion and that *I.gabonensis* seed are lacking in some types of fatty acids namely caprylic, capric, lauric and margaric acids as their percentage composition in the oils was less than 0.000001. From Table 7, it can be seen that the most abundant fatty acid present in both the healthy and diseased (*A.oryzae* infected) seeds of *I.gabonensis* is the polyunsaturated Linoleic acid with a percentage composition of 25.03 and 23.82 respectively, while the least abundant fatty acid in both the healthy and *A.oryzae* infected seeds was the saturated myristic acid with a percentage composition of 0.33 and 0.86 respectively.

Figures 2a and 2b show the GC profile of the oils extracted from the seeds of healthy and diseased *I. gabonensis* respectively.

Table 6: Gc determined types of fatty acid and percentage composition in oil extracted from both healthy and diseased seeds of *I.gabonensis*

| S/N | Type of fatty acid | % composition in oil from healthy seeds | % composition in oil from diseased seeds |
|---|---|---|---|
| 1 | Saturated | 19.821944 | 24.67064 |
| 2 | monounsaturated | 30.234518 | 28.152946 |
| 3 | polyunsaturated | 49.943539 | 47.176414 |

TABLE 7: GC determined individual percentage composition and saturation level of fatty acids in oils from healthy and diseased seeds of *I.gabonensis*

| S/N | Fatty acid and their corresponding degree of saturation or unsaturation. | percentage fatty acid composition in healthy seeds. | percentage fatty acid composition in diseased seeds. |
|---|---|---|---|
| 1 | Myristic acid: **C14:0** | 0.336766 | 0.860036 |
| 2 | Palmitic acid: **C16:0** | 11.392863 | 13.401511 |
| 3 | Palmitoleic acid: **C16:1** | 6.291373 | 5.582964 |
| 4 | Stearic acid: **C18:0** | 5.954527 | 8.281806 |
| 5 | Oleic acid: **C18:1** | 21.477837 | 20.708873 |
| 6 | Linoleic acid: **C18:2** | 25.036503 | 23.822111 |
| 7 | Linolenic acid: **C18:3** | 23.014590 | 21.598781 |
| 8 | Arachidic acid: **C20:0** | 0.529693 | 0.540171 |
| 9 | Arachidonic acid: **C20:4** | 1.892446 | 1.755522 |
| 10 | Behenic acid: **C22:0** | 0.426655 | 0.595662 |
| 11 | Erucic acid: **C22:1** | 2.465308 | 1.861109 |
| 12 | Lignoceric acid: **C24:0** | 1.012433 | 1.160461 |

The value of oil components were obtained after triplicate analysis from electronic integration measurements using flame ionization detector

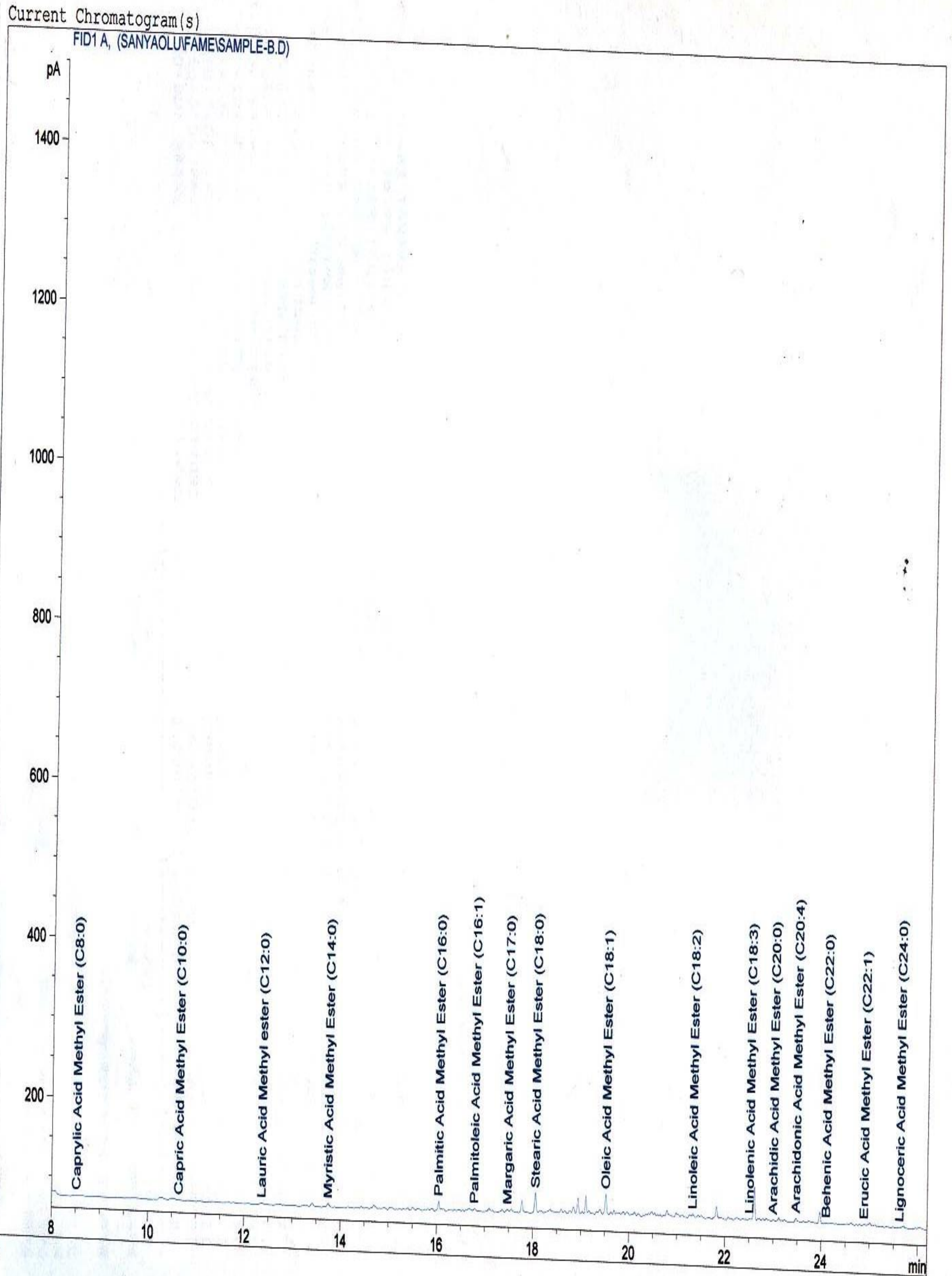


Figure 2a: GC chromatograms of the oils from healthy seeds

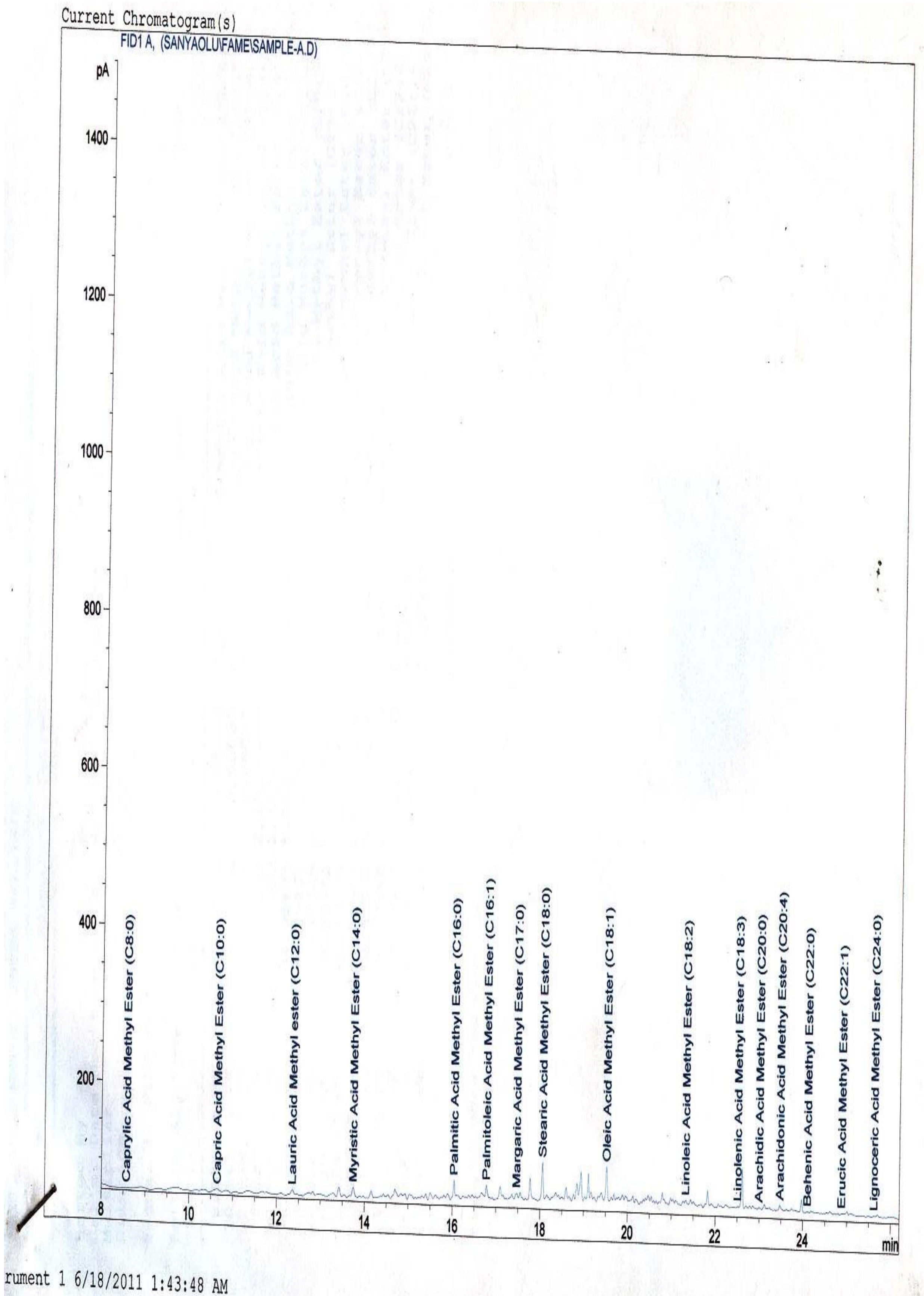


Figure 2b: GC chromatograms of the oils from diseased seeds

## 4.3.0 Mycodegradation studies

### 4.3.1 Preliminary screening of fungal isolates for petroleum hydrocarbon utilizing ability

The results for the fungal isolates that 'passed' (i.e. those that were able to grow within 5 days under crude oil fume) the preliminary hydrocarbon utilizing test and their sources are as summarized in Table 8. There were 25 isolates which were obtained from the different sources (as indicated in Table 8).These isolates include such species as *Aspergillus niger, A.oryzae* and *A.tubingensis* from each of the different locations and sources and at the different months.

TABLE 8: Fungal isolates suspected to be capable of utilizing petroleum hydrocarbon, their location and sources

| S/n | Location | Source | Identity | Tag name |
|---|---|---|---|---|
| 1 | Olushosun dump site, Ojota | SOIL | *A. niger** | ojt1F*AN* |
| 2 | " | " | *A. oryzae**** | ojt2*AO* |
| 3 | " | " | *A. oryzae.**** | ojt2*AO* |
| 4 | " | " | *A. oryzae**** | ojt3*AO* |
| 5 | " | " | *A. niger.** | ojt3*AN* |
| 6 | " | " | *A. niger** | ojt4*AN* |
| 7 | Mechanic village, Onitiri- Akoka | " | *A. niger**** | ont1*AN* |
| 8 | " | " | *A. oryzae**** | ont3*AN* |
| 9 | Mechanic village, Sabo-Yaba. | " | *A. tubingensis**** | *sab1AT* |
| 10 | " | " | *A. niger** | sab1F*AN* |
| 11 | " | " | *A. tubingensis**** | sab2*AT* |
| 12 | " | " | *A. niger** | sab2F*AN* |
| 13 | " | " | *A. oryzae**** | sab3*AO* |
| 14 | " | " | *A. niger**** | sab3F*AN* |
| 15 | " | " | *A. niger** | sab4*AN* |
| 16 | " | " | *A. niger**** | sab4F*AN* |
| 17 | Biological Gardens, Unilag. | Soil | *A. niger**** | bg1*AN* |
| 18 | " | " | *A. niger** | bg2*AN* |
| 19 | Shodex Gardens, Anthony- village. | " | *A. oryzae**** | Sho3*AO* |
| 20 | Bariga market | *I. gabonensis* seed | *A. niger** | barIv1*AN* |
| 21 | Oyingbo market. | " | *A. niger.** | oygIv*AN* |
| 22 | " | " | *A. oryzae**** | oygIv2*AO* |
| 23 | Alayabiagba market, Ajegunle. | " | *A. oryzae**** | ajgIv1*AO* |
| 24 | " | " | *A. niger** | ajgIv2*AN* |
| 25 | Agege market | " | *A. niger** | aggIvF*AN* |

***** identity confirmed using DNA sequence**
***identity not yet confirmed by DNA sequence**

### 4.3.2. Optical activity of each of the fungal species (over a 40 day period) in each of the petroleum hydrocarbon oils and the vegetable hydrocarbon oil at wavelenghts 530 and 620 nm using the spectrophotometric technique.

The values for the means separation of the OD for each of the hydrocarbon source shows that the optical activity of most of the individual fungal species in each of the hydrocarbon compound was significantly higher than in the control samples for most of the days under consideration (Figures 3A – 14C and Appendix 18A and B – 23A and B).

Another trend that was evident was the fact that the pattern of growth of the fungus in the control 1 samples for each of the hydrocarbon source at each of the wavelengths generally conformed to the sigmoidal growth pattern. From these figures, it can be generally observed that the *A.oryzae* isolated from *I.gabonensis* seed from Ajegunle market was the most optically active species especially in the Spent Engine Oil (SEO) medium. For most of the other petroleum hydrocarbon media, this particular fungus also generally retained its impressive growth as reflected by its generally high optical activity as reflected in the high OD value for each of the hydrocarbon media containing this fungus. Another observation from these Figures is the fact that exactly similar species of fungus from different environments showed dissimilar growth pattern in most of the hydrocarbon compounds.

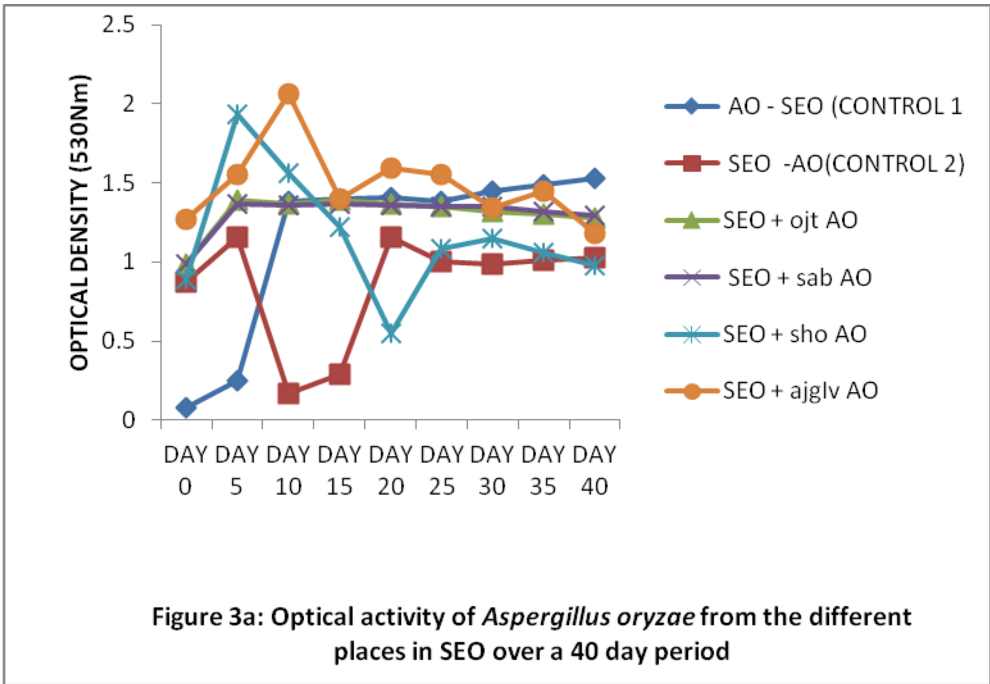


**Figure 3a: Optical activity of *Aspergillus oryzae* from the different places in SEO over a 40 day period**

Key

SEO - SPENT ENGINE OIL

AO - *ASPERGILLUS ORYZAE*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

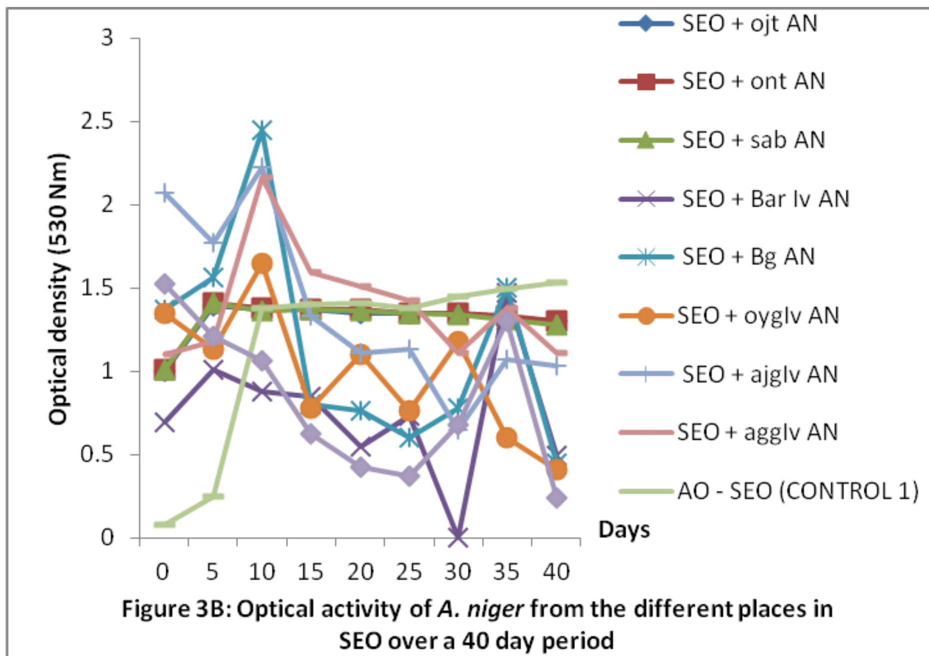

**Figure 3B: Optical activity of *A. niger* from the different places in SEO over a 40 day period**

Key

SEO - SPENT ENGINE OIL

AN - *ASPERGILLUS NIGER*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Bg - Soil from Biological Gardens, Unilag

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

Agg Iv – *Irvingia gabonensis* seed from Agege market

Bar Iv - *Irvingia gabonenesis* seed from Bariga market

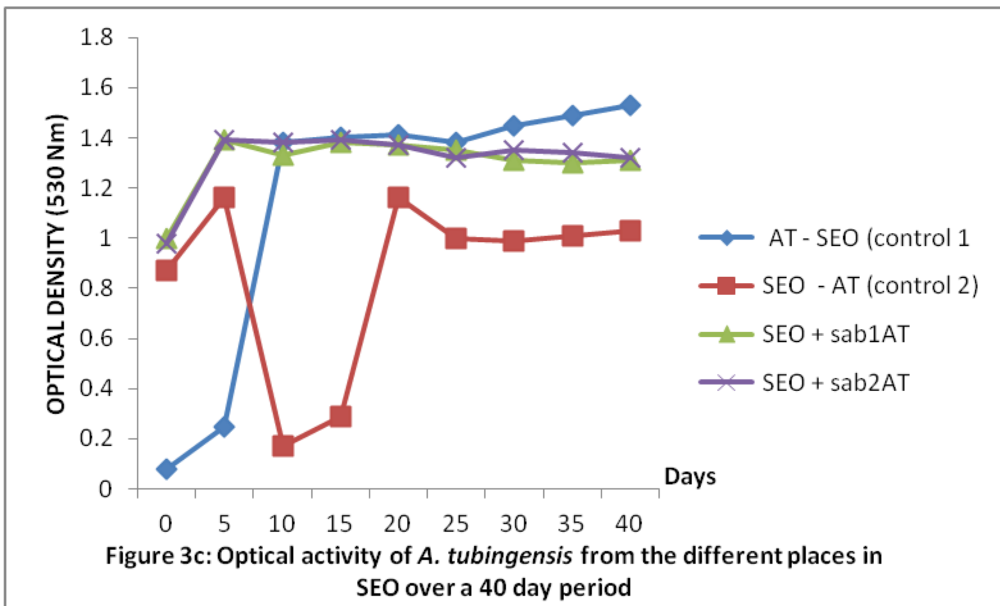


**Figure 3c: Optical activity of *A. tubingensis* from the different places in SEO over a 40 day period**

Key

SEO - SPENT ENGINE OIL

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

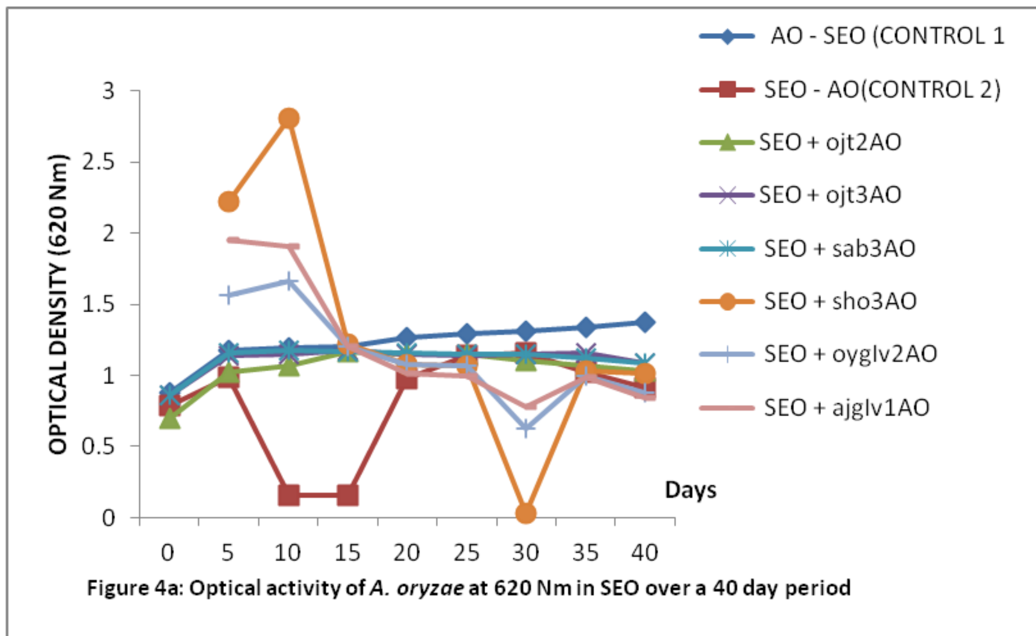


Figure 4a: Optical activity of *A. oryzae* at 620 Nm in SEO over a 40 day period

Key

SEO - SPENT ENGINE OIL

AO - *ASPERGILLUS ORYZAE*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

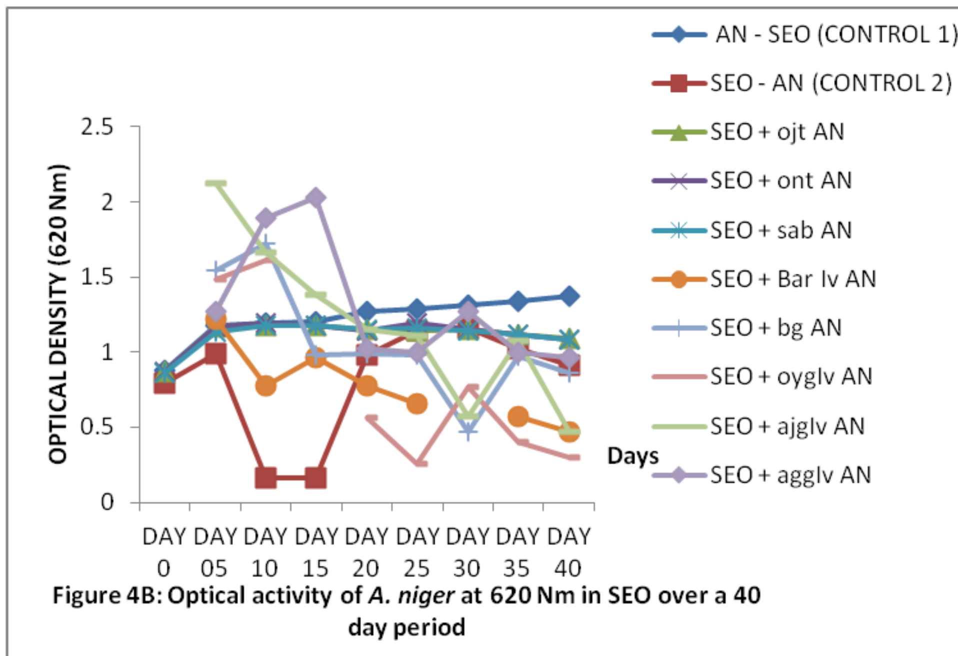


**Figure 4B: Optical activity of *A. niger* at 620 Nm in SEO over a 40 day period**

Key

SEO - SPENT ENGINE OIL

AN - *ASPERGILLUS NIGER*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Bg - Soil from Biological Gardens, Unilag

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

Agg Iv – *Irvingia gabonensis* seed from Agege market

Bar Iv - *Irvingia gabonenesis* seed from Bariga market

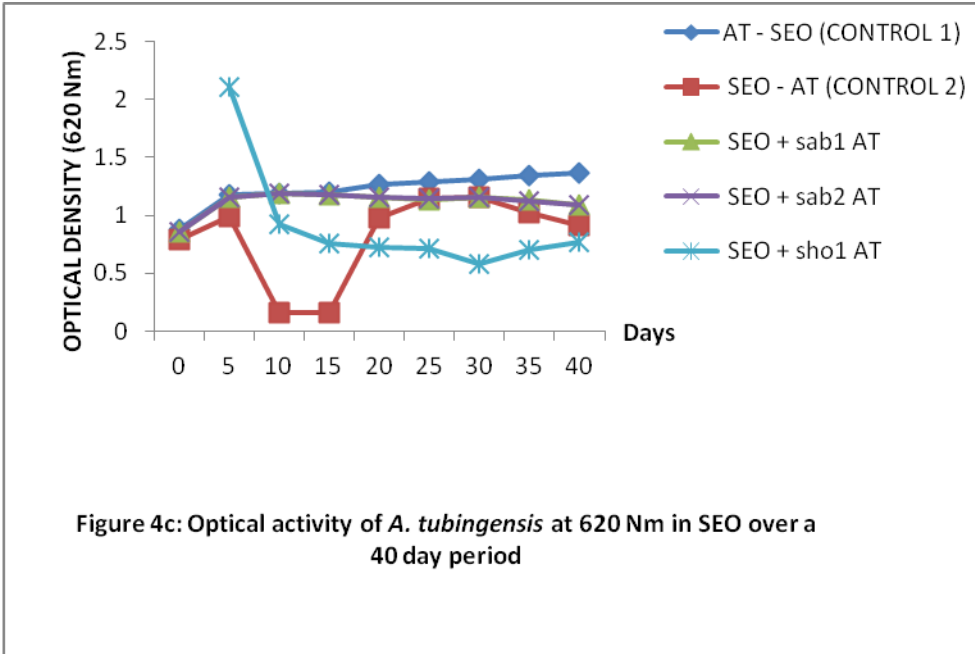


**Figure 4c: Optical activity of *A. tubingensis* at 620 Nm in SEO over a 40 day period**

Key

SEO - spent engine oil

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

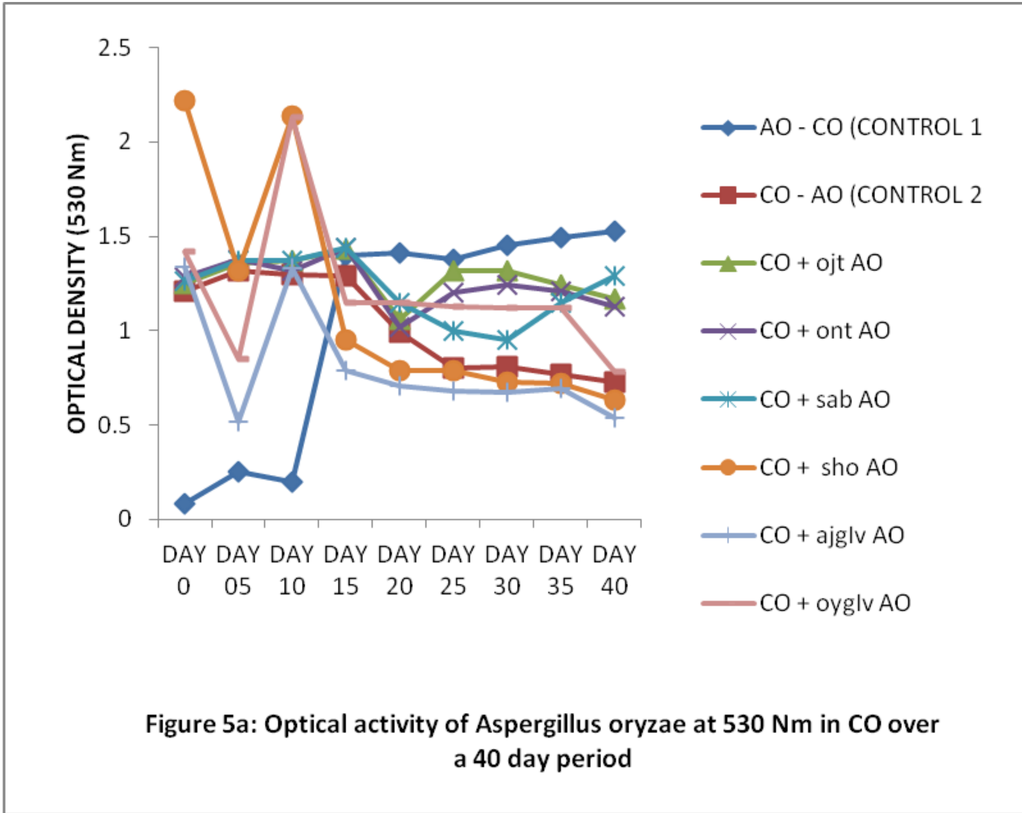


**Figure 5a: Optical activity of Aspergillus oryzae at 530 Nm in CO over a 40 day period**

Key

CO - CRUDE OIL

AO - *ASPERGILLUS ORYZAE*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

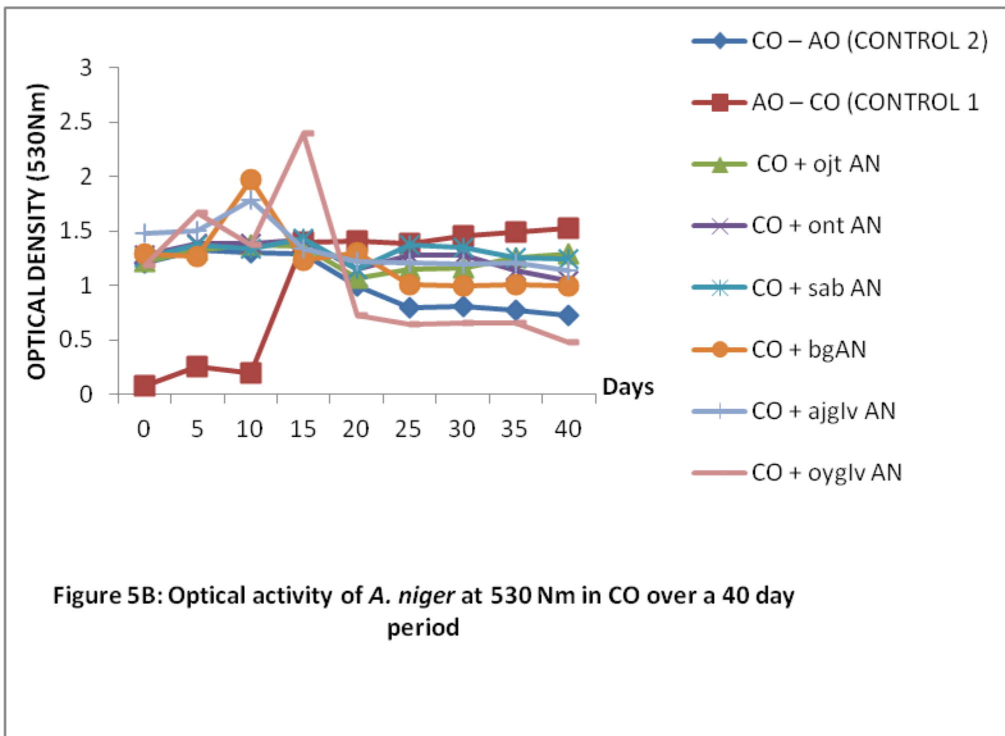


**Figure 5B: Optical activity of *A. niger* at 530 Nm in CO over a 40 day period**

Key

CO - CRUDE OIL

AN - *ASPERGILLUS NIGER*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Bg - Soil from Biological Gardens, Unilag

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

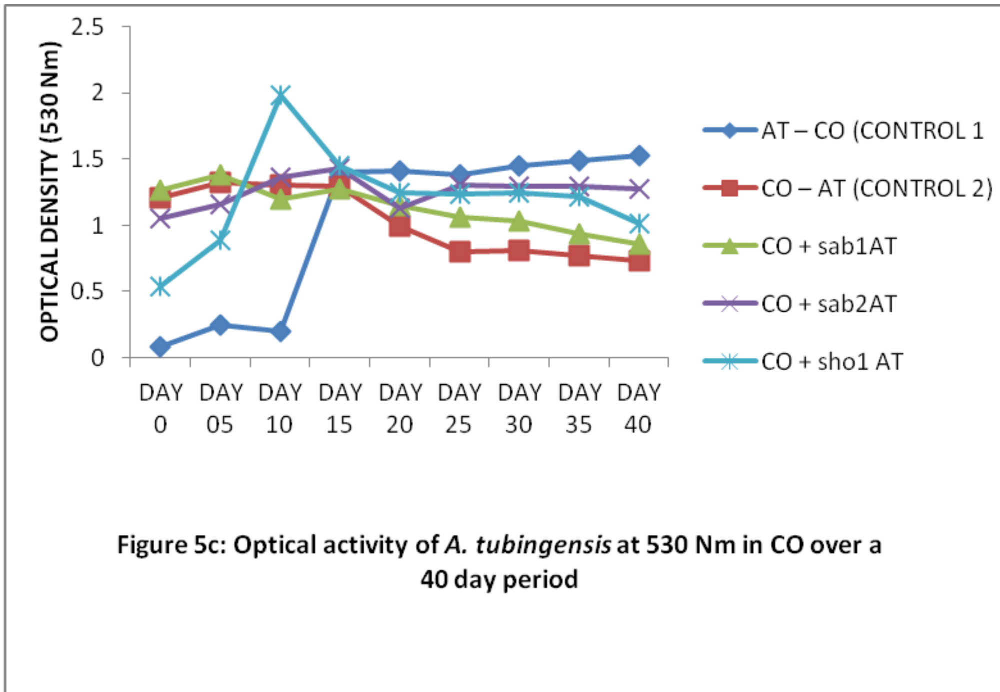


**Figure 5c: Optical activity of *A. tubingensis* at 530 Nm in CO over a 40 day period**

Key

CO - CRUDE OIL

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

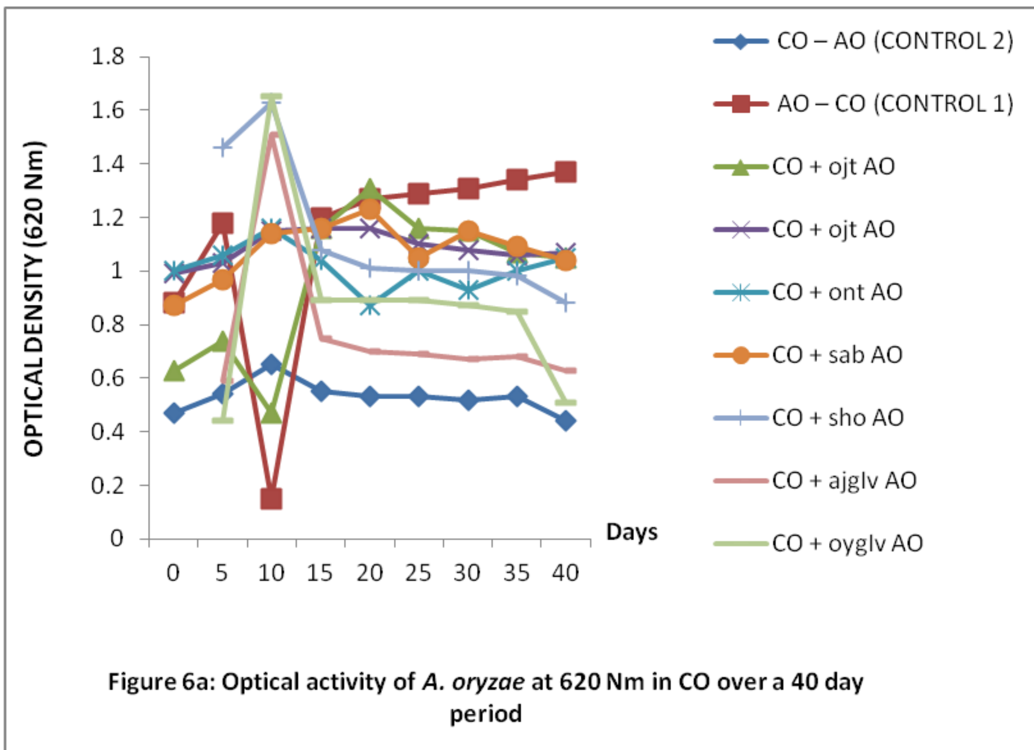


**Figure 6a: Optical activity of *A. oryzae* at 620 Nm in CO over a 40 day period**

Key

CO - CRUDE OIL

AO - *ASPERGILLUS ORYZAE*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

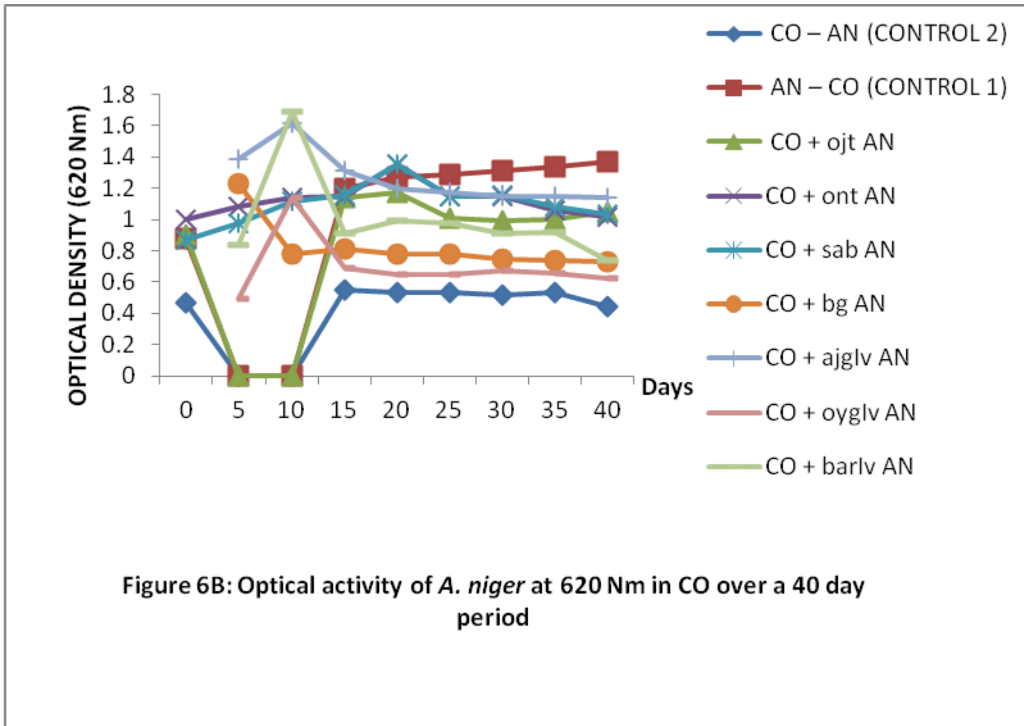


**Figure 6B: Optical activity of *A. niger* at 620 Nm in CO over a 40 day period**

Key

CO - CRUDE OIL

AN - *ASPERGILLUS NIGER*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Bg - Soil from Biological Gardens, Unilag

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

Agg Iv – *Irvingia gabonensis* seed from Agege market

Bar Iv - *Irvingia gabonenesis* seed from Bariga market

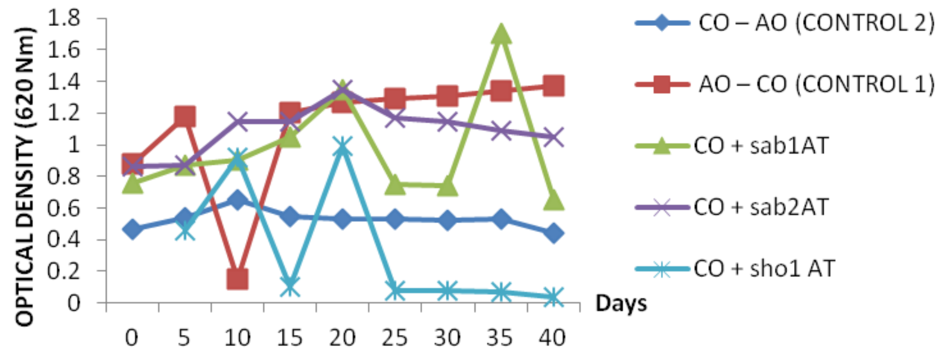


**Figure 6C: Optical activity of *A. tubingensis* at 620 Nm in CO over a 40 day period**

Key

CO - CRUDE OIL

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

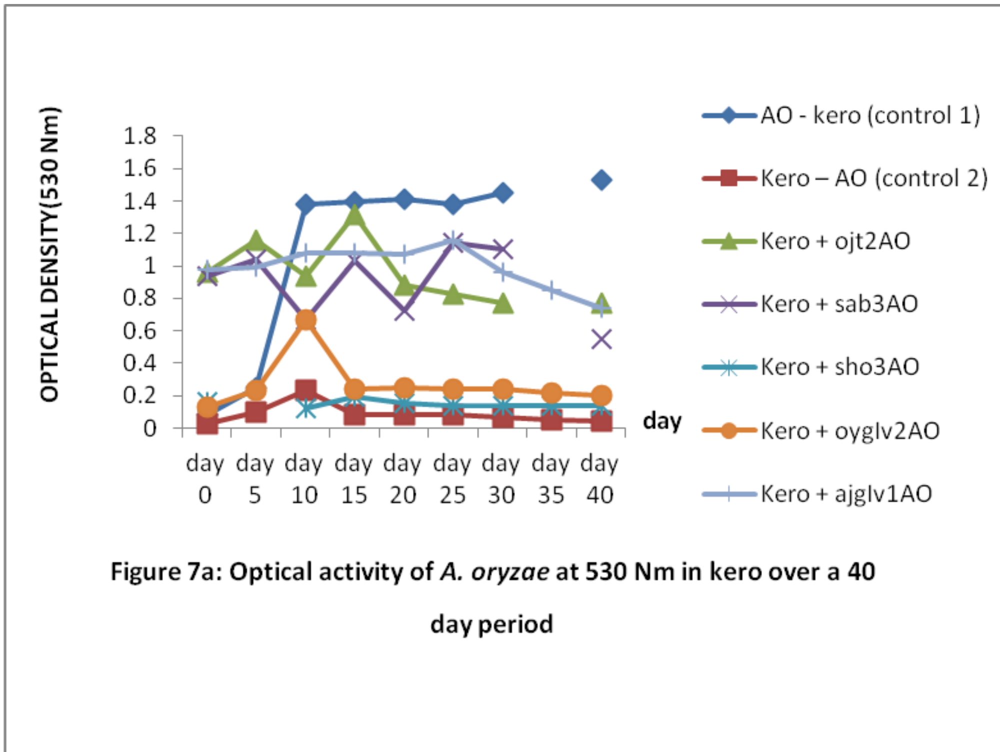


**Figure 7a: Optical activity of *A. oryzae* at 530 Nm in kero over a 40 day period**

**KEY**

KERO - DUAL PURPOSE KEROSINE (DPK)

AO - *ASPERGILLUS ORYZAE*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

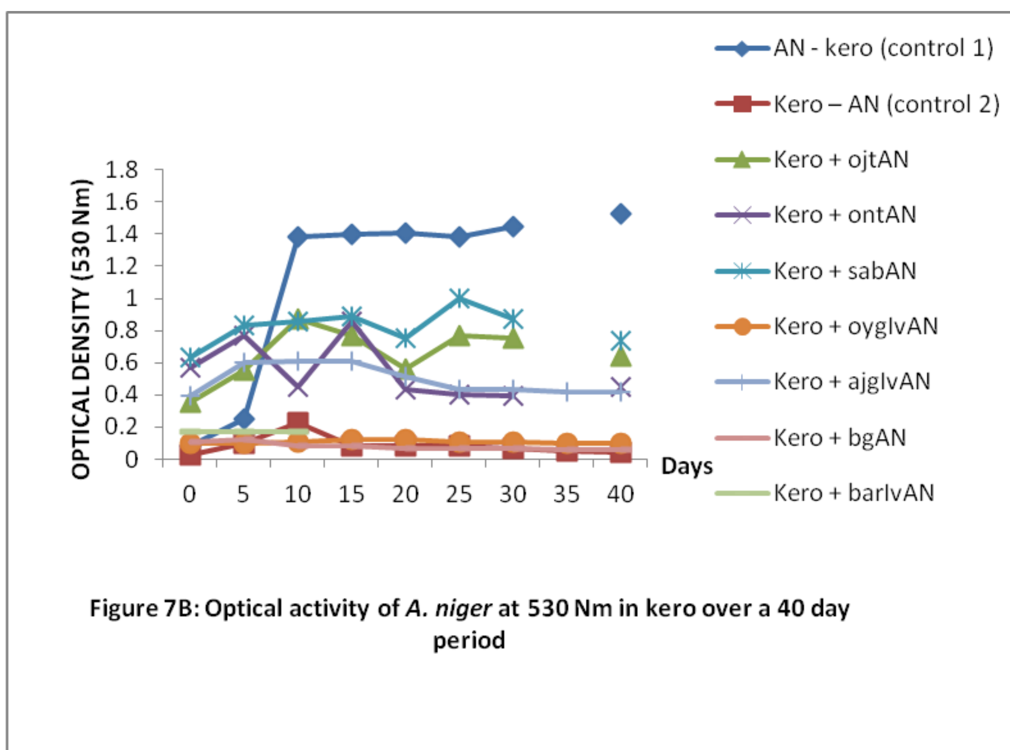


**Figure 7B: Optical activity of *A. niger* at 530 Nm in kero over a 40 day period**

**KEY**

KERO - DUAL PURPOSE KEROSINE (DPK)

AN - *ASPERGILLUS NIGER*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Bg - Soil from Biological Gardens, Unilag

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

Agg Iv – *Irvingia gabonensis* seed from Agege market

Bar Iv - *Irvingia gabonenesis* seed from Bariga market

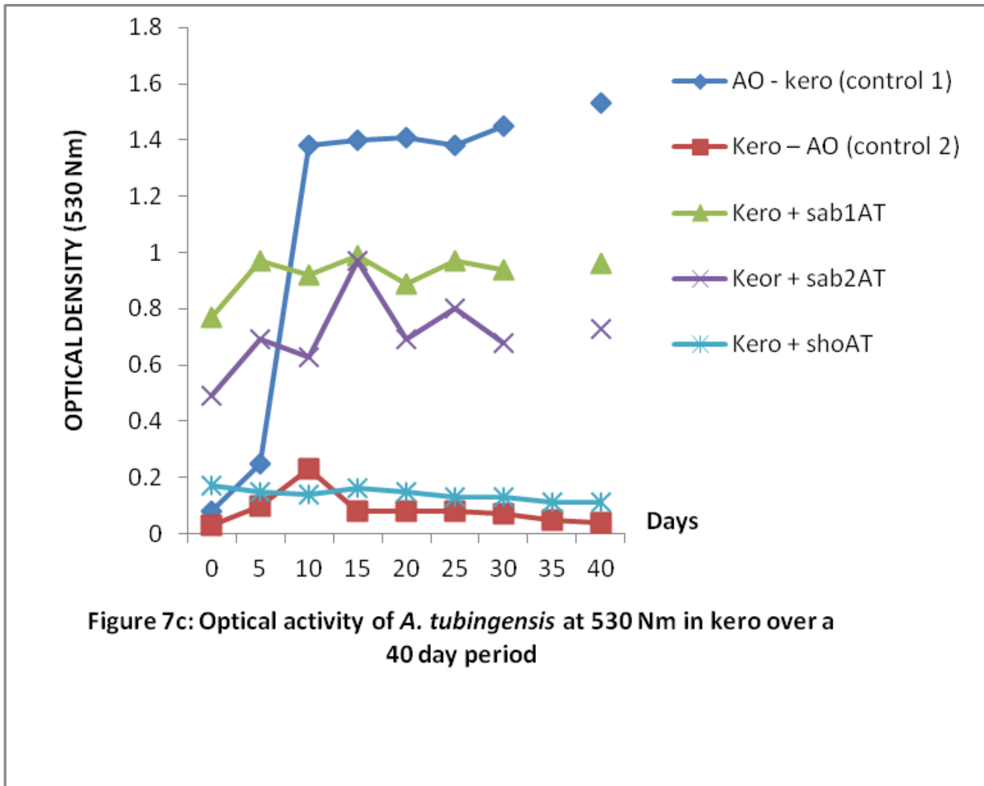


Figure 7c: Optical activity of *A. tubingensis* at 530 Nm in kero over a 40 day period

**KEY**

KERO - DUAL PURPOSE KEROSINE (DPK)

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

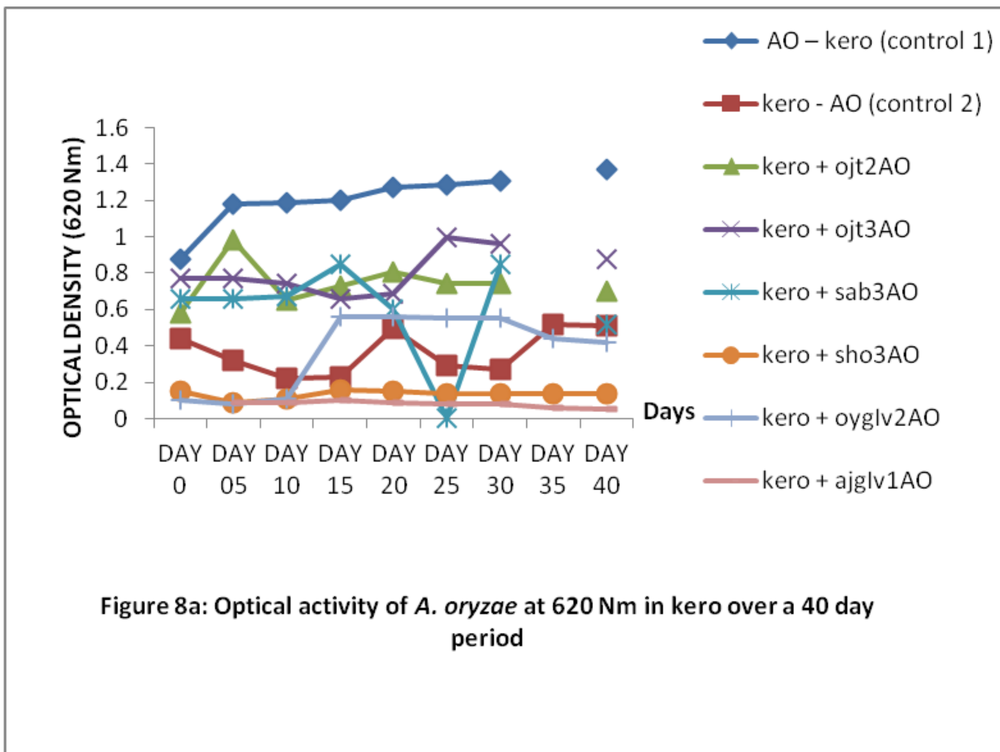


**Figure 8a: Optical activity of *A. oryzae* at 620 Nm in kero over a 40 day period**

**KEY**

KERO - DUAL PURPOSE KEROSINE (DPK)

AO - *ASPERGILLUS ORYZAE*

Sab – Soil from mechanic workshop, Sabo

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Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

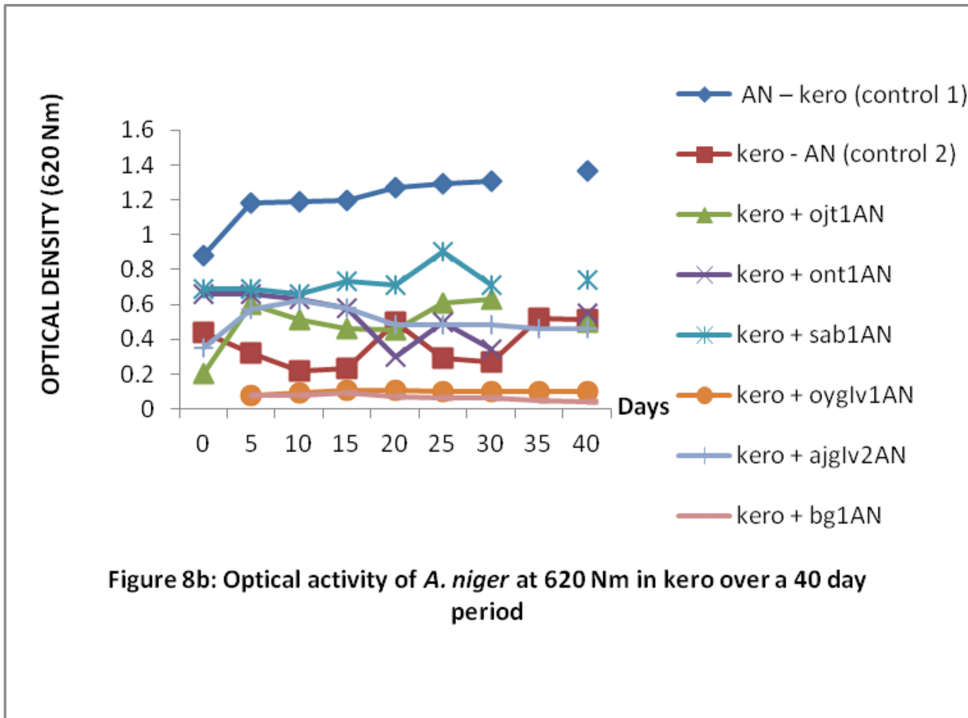



**Figure 8b: Optical activity of *A. niger* at 620 Nm in kero over a 40 day period**

**KEY**

KERO - DUAL PURPOSE KEROSINE (DPK)

AN - *ASPERGILLUS NIGER*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Bg - Soil from Biological Gardens, Unilag

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

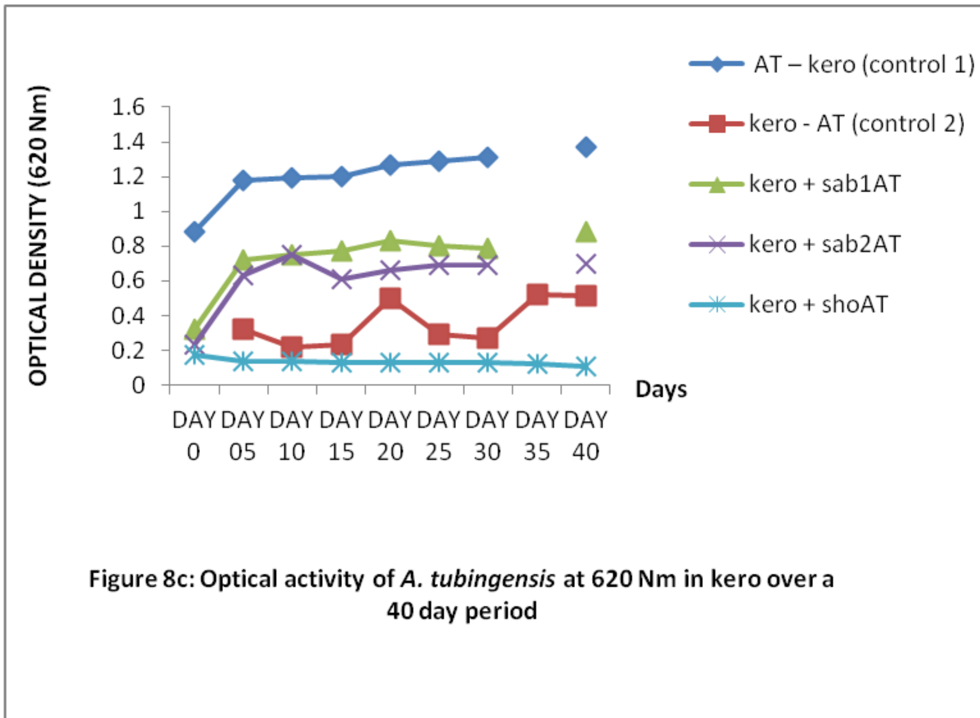



**Figure 8c: Optical activity of *A. tubingensis* at 620 Nm in kero over a 40 day period**

**KEY**

KERO - DUAL PURPOSE KEROSINE (DPK)

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

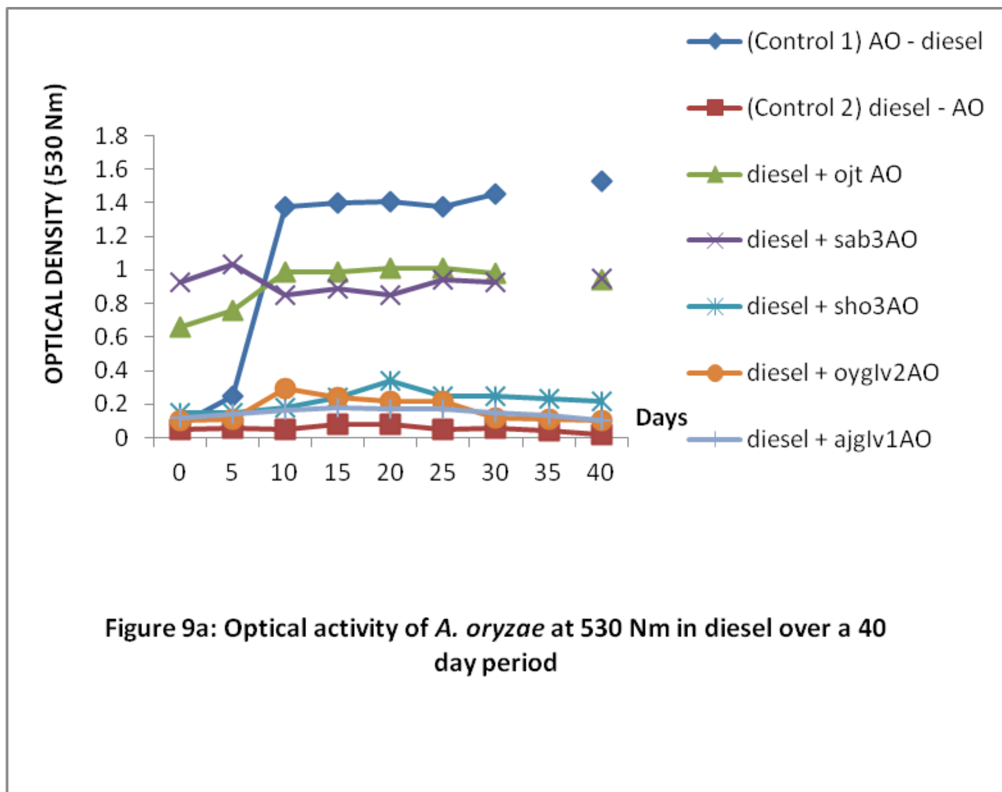



**Figure 9a: Optical activity of *A. oryzae* at 530 Nm in diesel over a 40 day period**

**KEY**

DIESEL – AUTOMOTIVE GAS OIL (AGO)

AO - *ASPERGILLUS ORYZAE*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

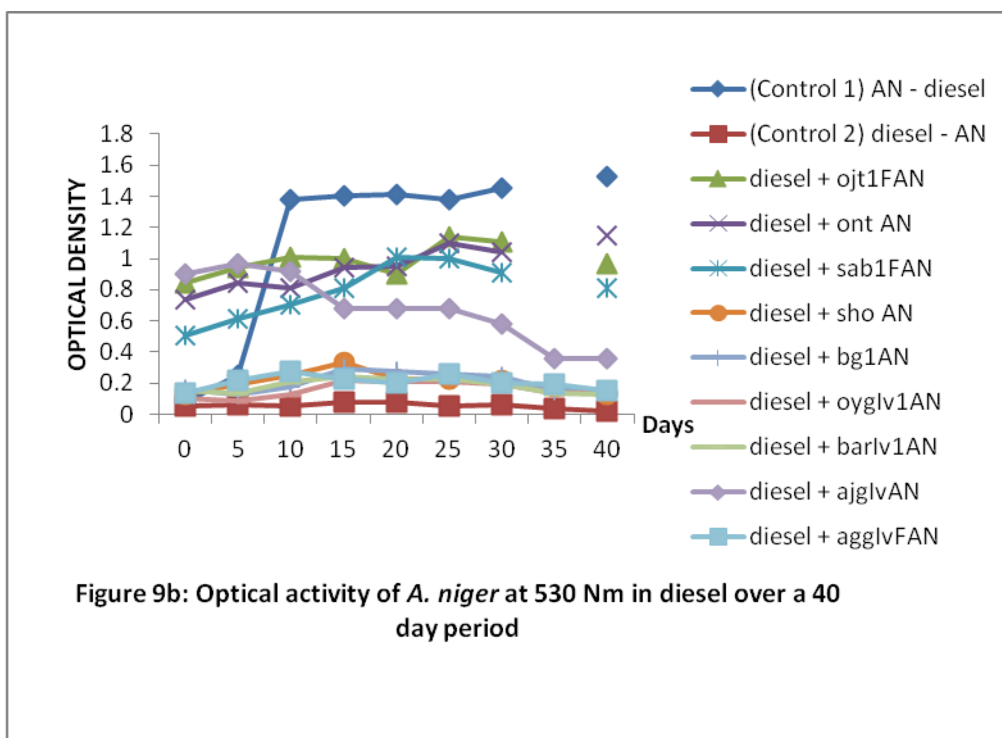



**Figure 9b: Optical activity of *A. niger* at 530 Nm in diesel over a 40 day period**

**KEY**

DIESEL – AUTOMOTIVE GAS OIL (AGO)

AN - *ASPERGILLUS NIGER*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Bg - Soil from Biological Gardens, Unilag

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

Agg Iv – *Irvingia gabonensis* seed from Agege market

Bar Iv - *Irvingia gabonenesis* seed from Bariga market

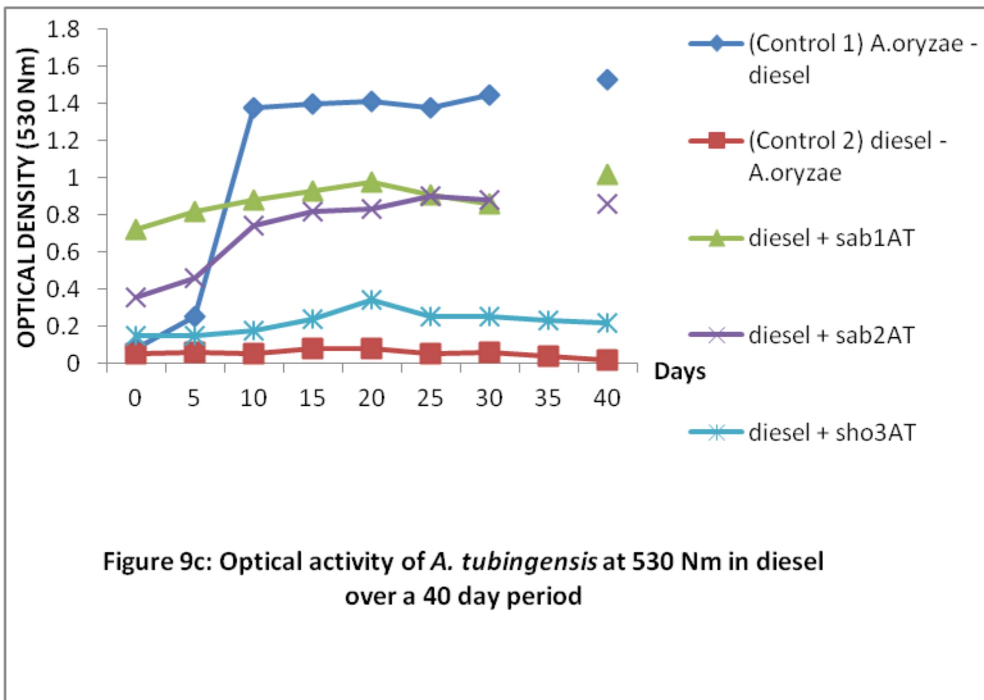



**Figure 9c: Optical activity of *A. tubingensis* at 530 Nm in diesel over a 40 day period**

**KEY**

DIESEL – AUTOMOTIVE GAS OIL (AGO)

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

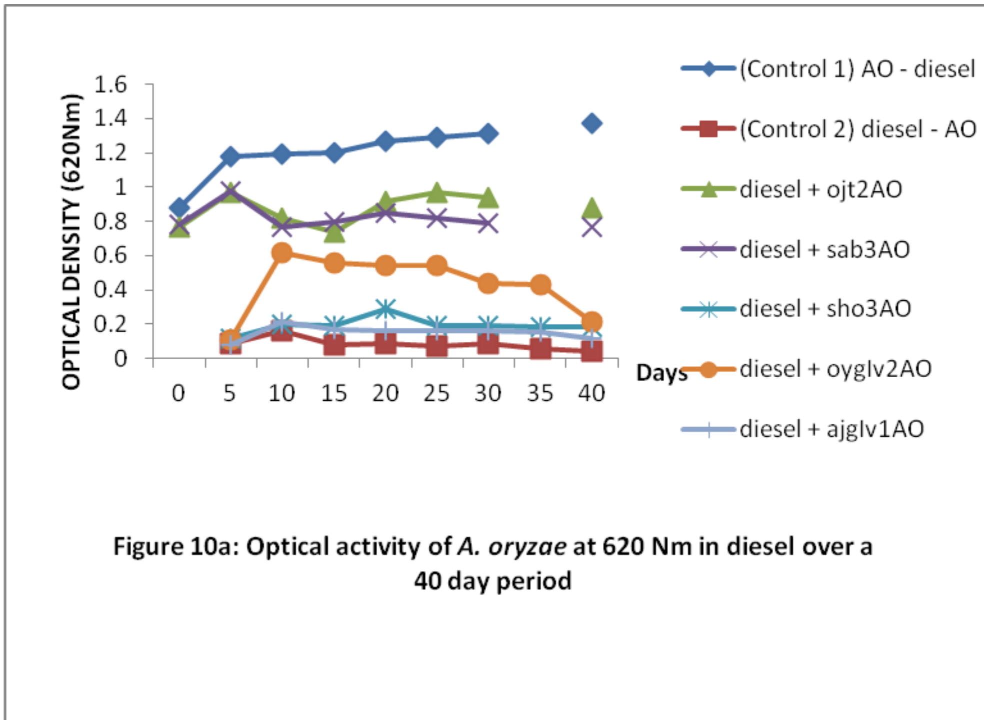



**Figure 10a: Optical activity of *A. oryzae* at 620 Nm in diesel over a 40 day period**

**KEY**

DIESEL – AUTOMOTIVE GAS OIL (AGO)

AO - *ASPERGILLUS ORYZAE*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

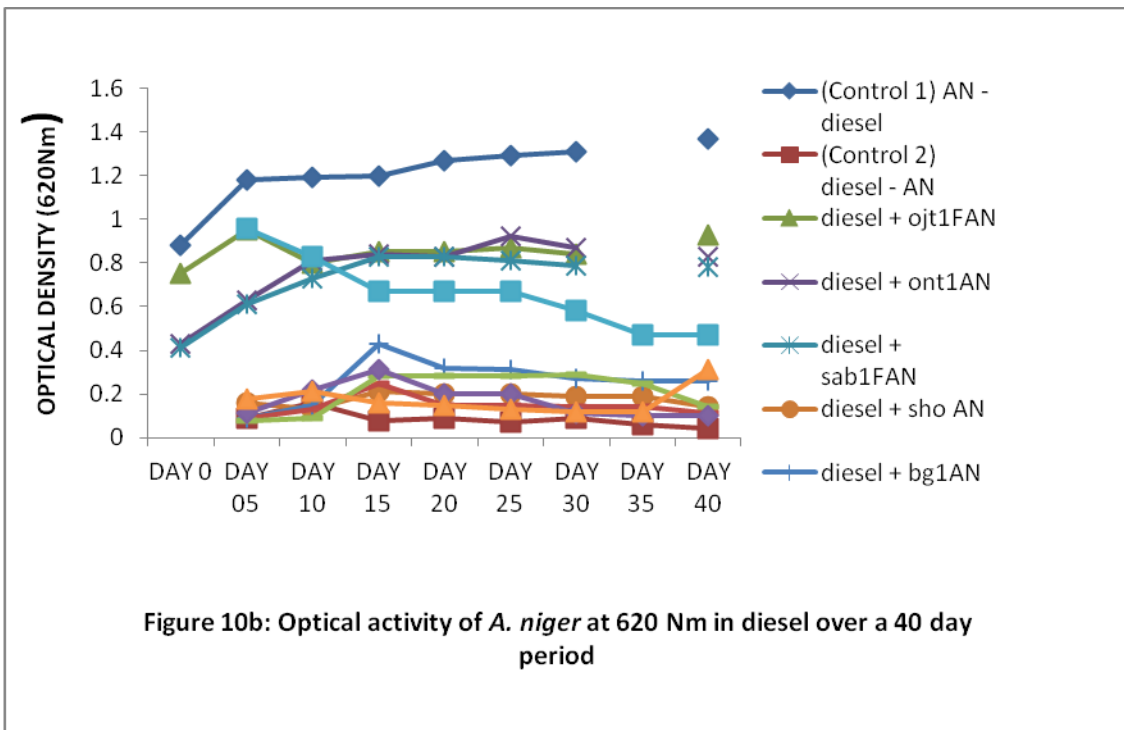



**Figure 10b: Optical activity of *A. niger* at 620 Nm in diesel over a 40 day period**

**KEY**

DIESEL – AUTOMOTIVE GAS OIL (AGO)

AN - *ASPERGILLUS NIGER*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Bg - Soil from Biological Gardens, Unilag

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

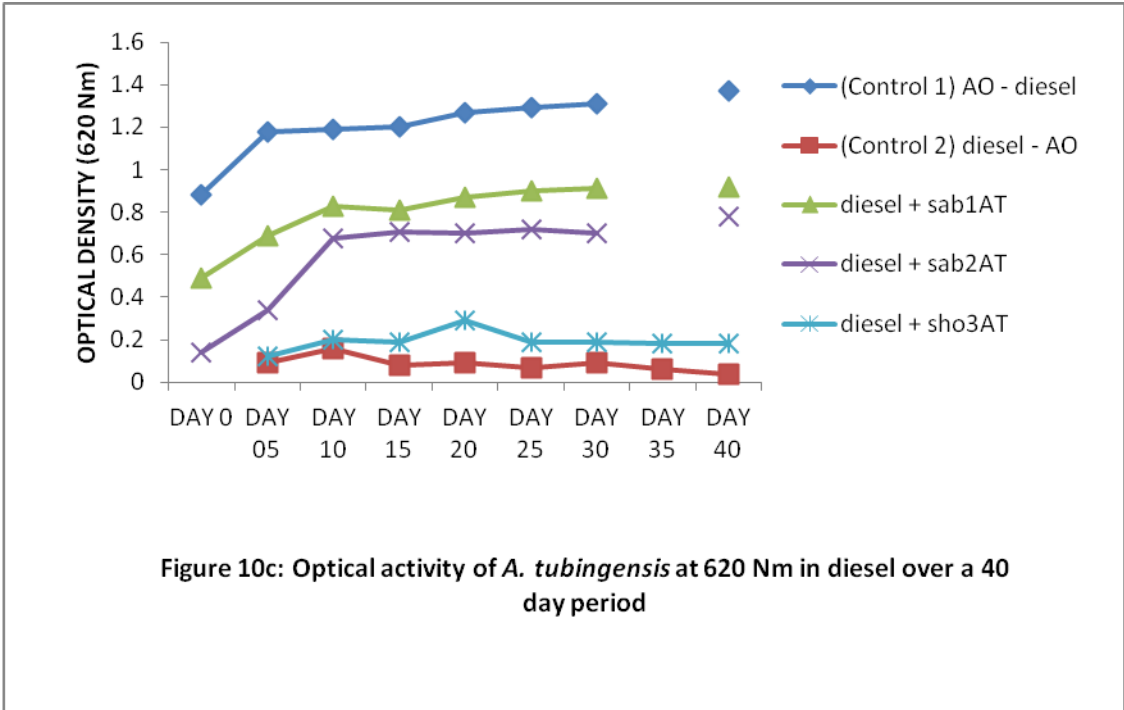



**Figure 10c: Optical activity of *A. tubingensis* at 620 Nm in diesel over a 40 day period**

**KEY**

DIESEL – AUTOMOTIVE GAS OIL (AGO)

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

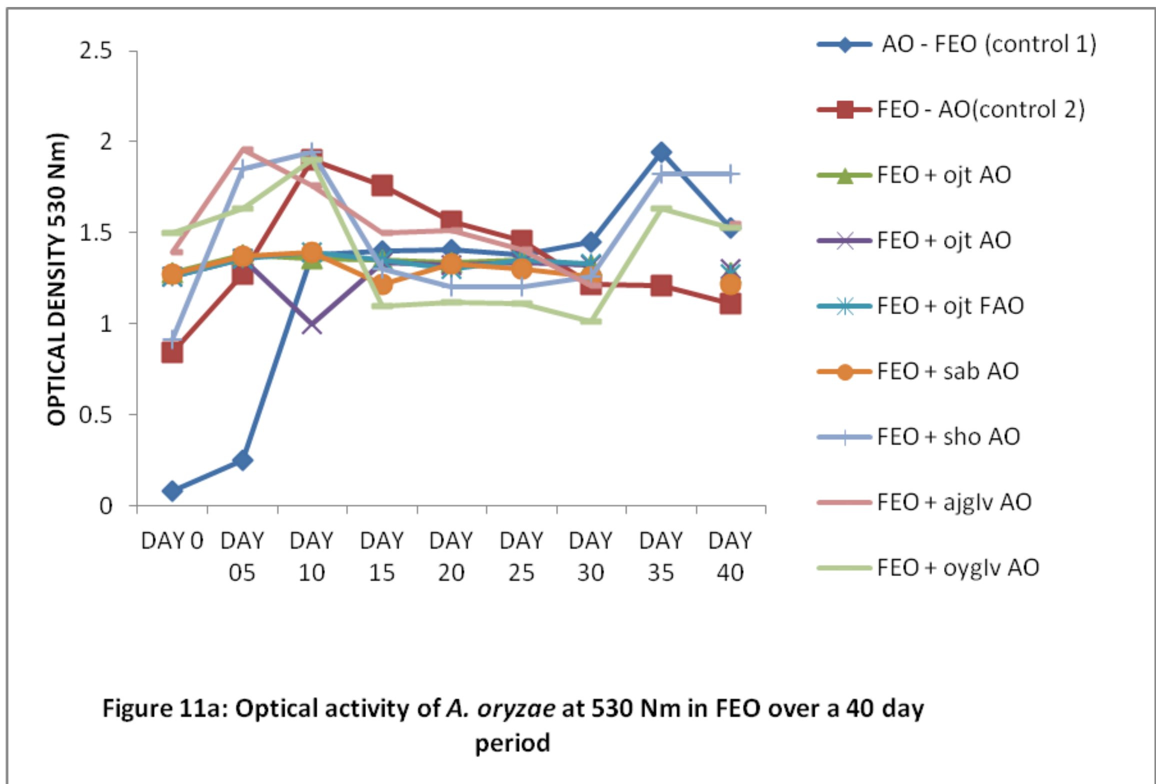



**Figure 11a: Optical activity of *A. oryzae* at 530 Nm in FEO over a 40 day period**

**KEY**

FEO - FRESH ENGINE OIL

AO - *ASPERGILLUS ORYZAE*

AN - *ASPERGILLUS NIGER*

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

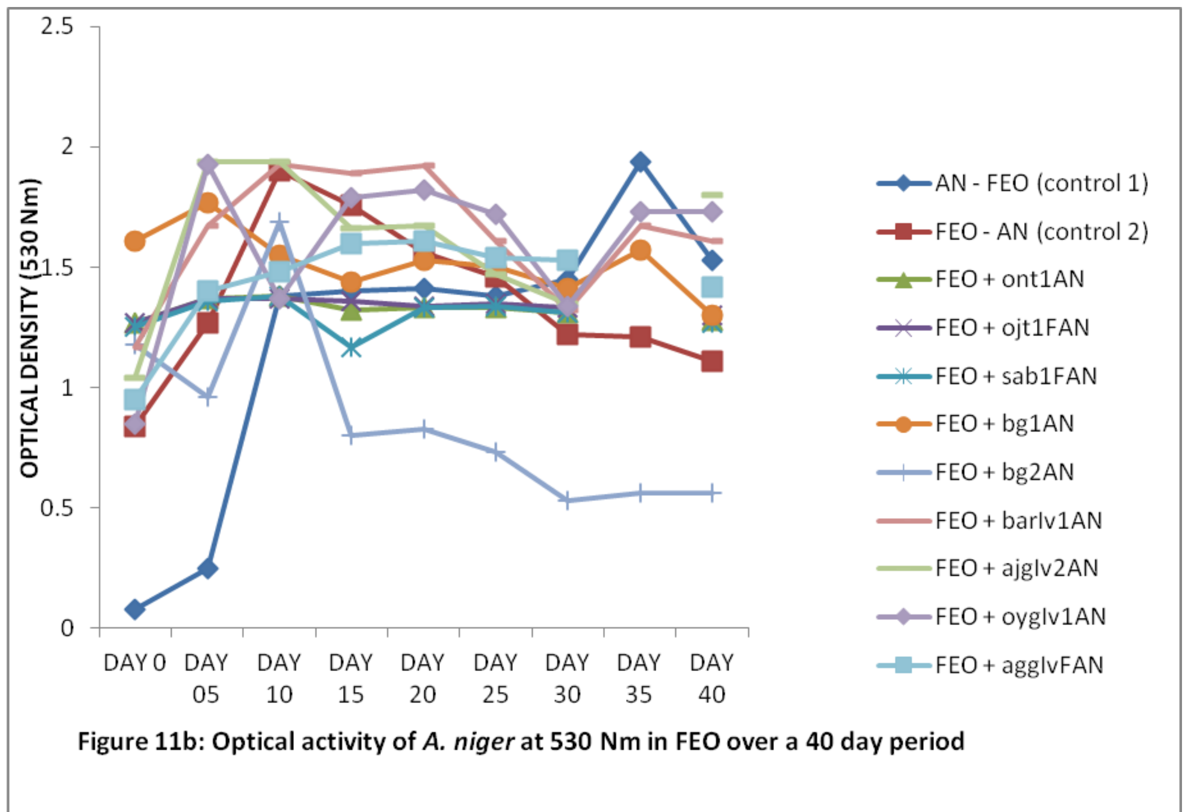


**Figure 11b: Optical activity of *A. niger* at 530 Nm in FEO over a 40 day period**

**KEY**

FEO - FRESH ENGINE OIL

AN - *ASPERGILLUS NIGER*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Bg - Soil from Biological Gardens, Unilag

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

Agg Iv – *Irvingia gabonensis* seed from Agege market

Bar Iv - *Irvingia gabonenesis* seed from Bariga market

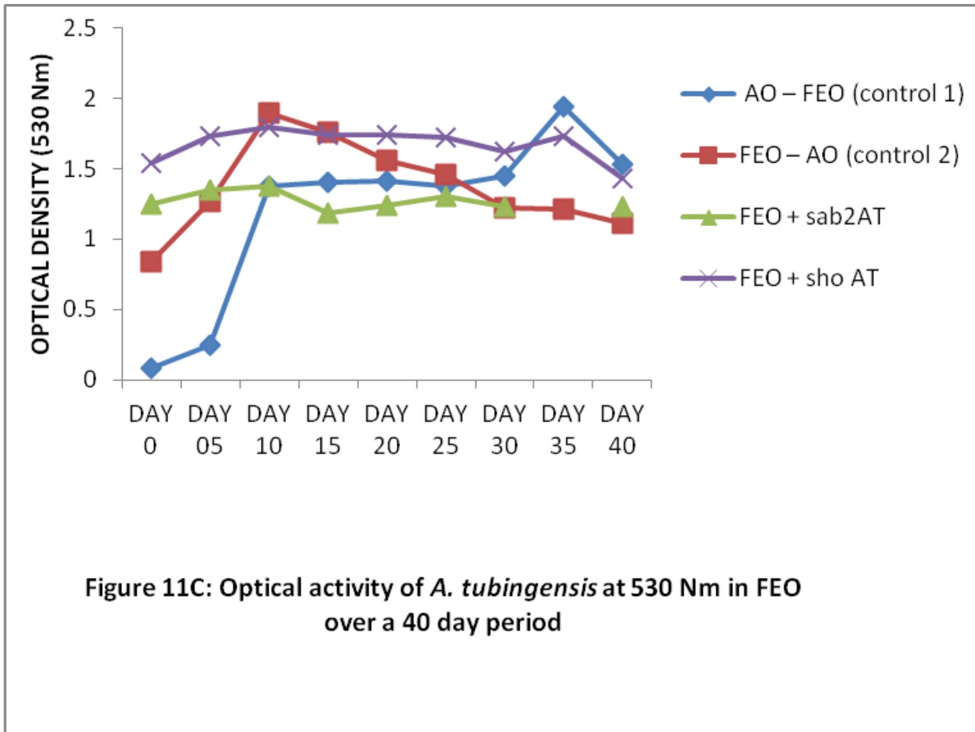



**Figure 11C: Optical activity of *A. tubingensis* at 530 Nm in FEO over a 40 day period**

**KEY**

FEO - FRESH ENGINE OIL

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

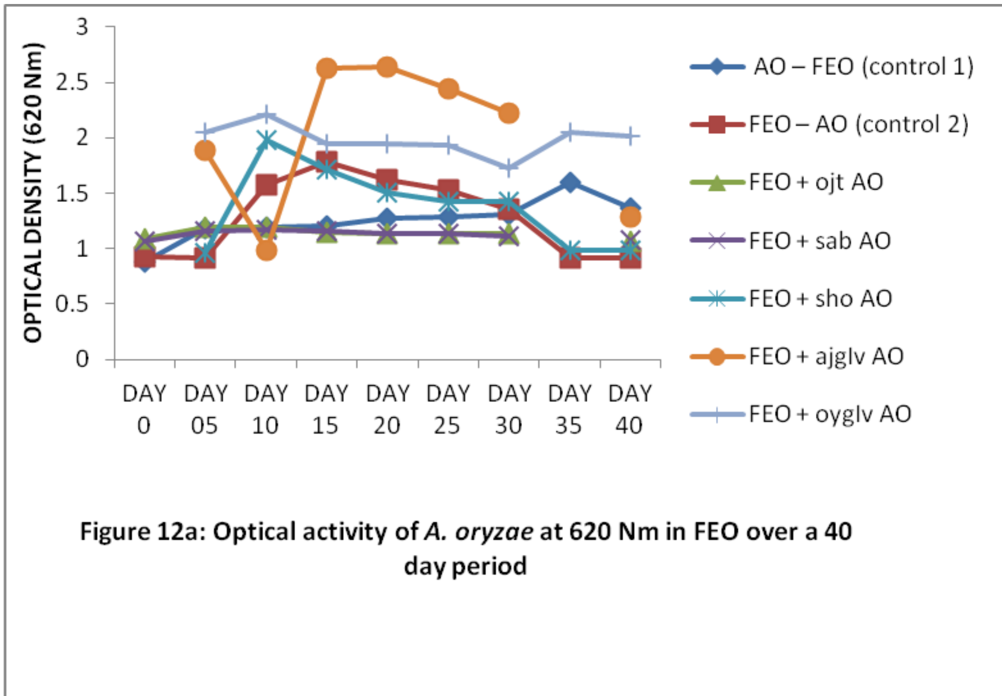



**Figure 12a: Optical activity of *A. oryzae* at 620 Nm in FEO over a 40 day period**

**KEY**

FEO - FRESH ENGINE OIL

AO - *ASPERGILLUS ORYZAE*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

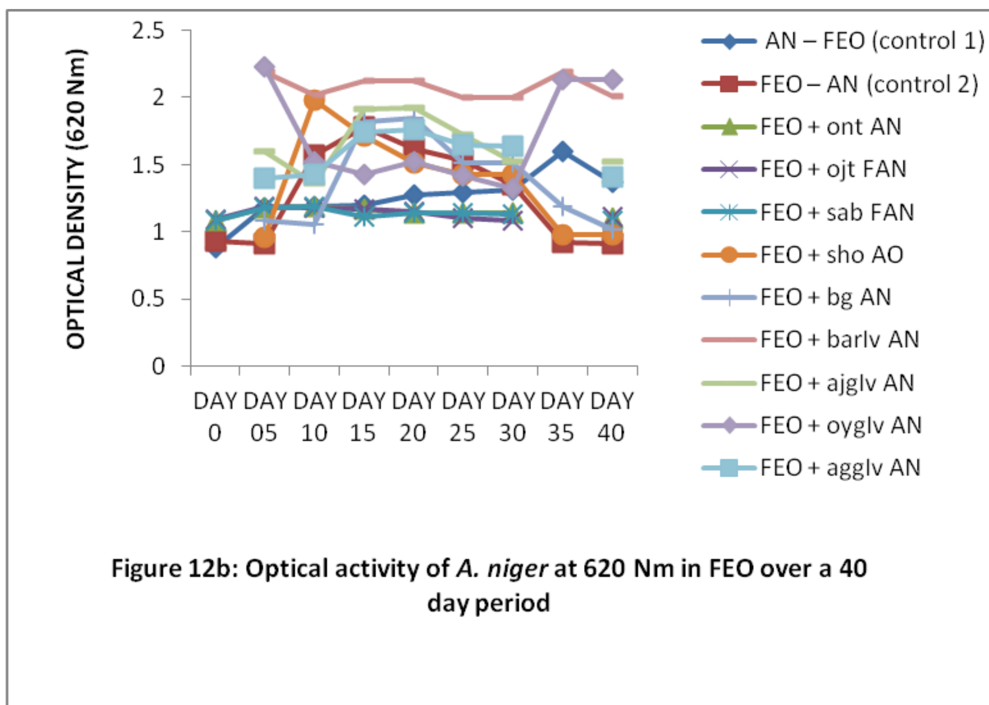



**Figure 12b: Optical activity of *A. niger* at 620 Nm in FEO over a 40 day period**

**KEY**

FEO - FRESH ENGINE OIL

AN - *ASPERGILLUS NIGER*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Bg - Soil from Biological Gardens, Unilag

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

Agg Iv – *Irvingia gabonensis* seed from Agege market

Bar Iv - *Irvingia gabonenesis* seed from Bariga market

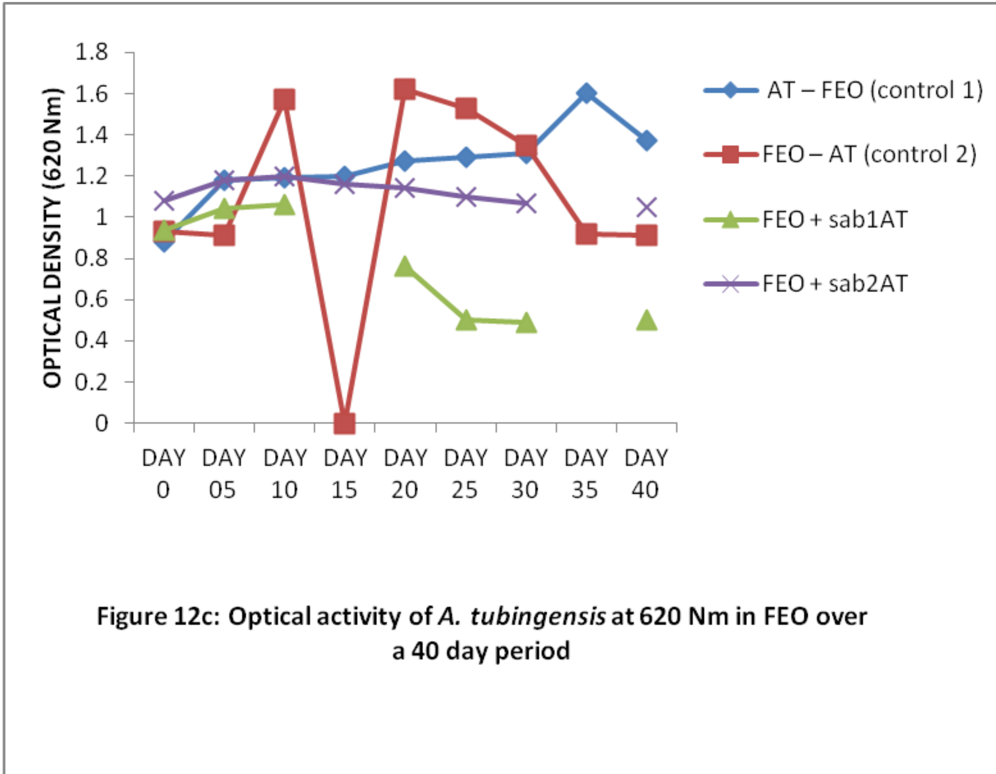



**Figure 12c: Optical activity of *A. tubingensis* at 620 Nm in FEO over a 40 day period**

**KEYS**

FEO - FRESH ENGINE OIL

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

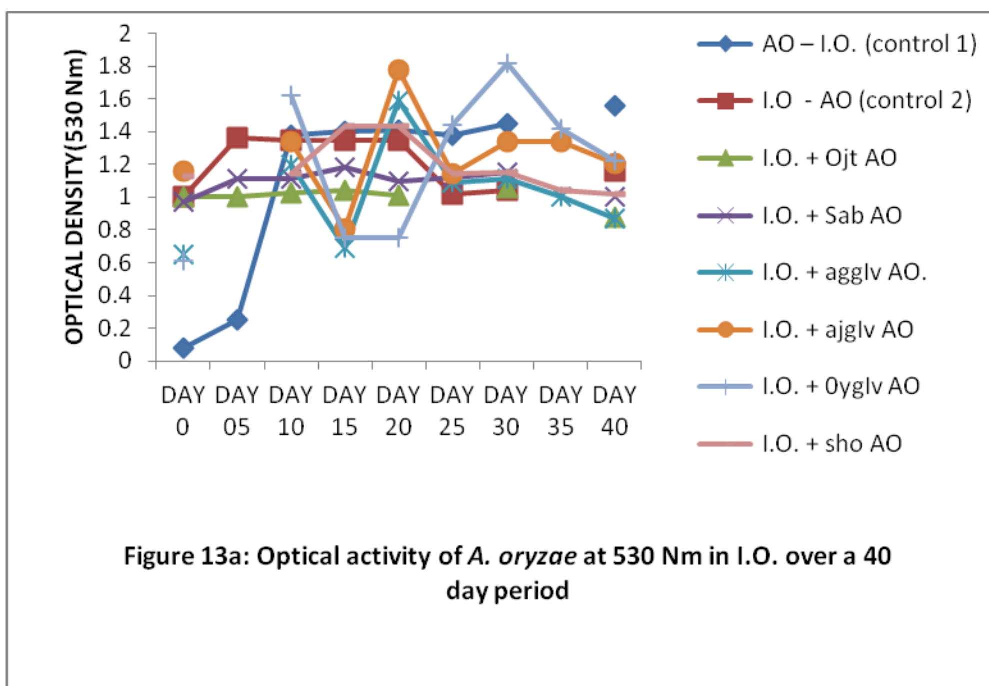



**Figure 13a: Optical activity of *A. oryzae* at 530 Nm in I.O. over a 40 day period**

**KEY**

I.O. - OIL EXTRACTED FROM *IRVINGIA GABONENSIS* SEED

AO - *ASPERGILLUS ORYZAE*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

Agg Iv – *Irvingia gabonensis* seed from Agege market

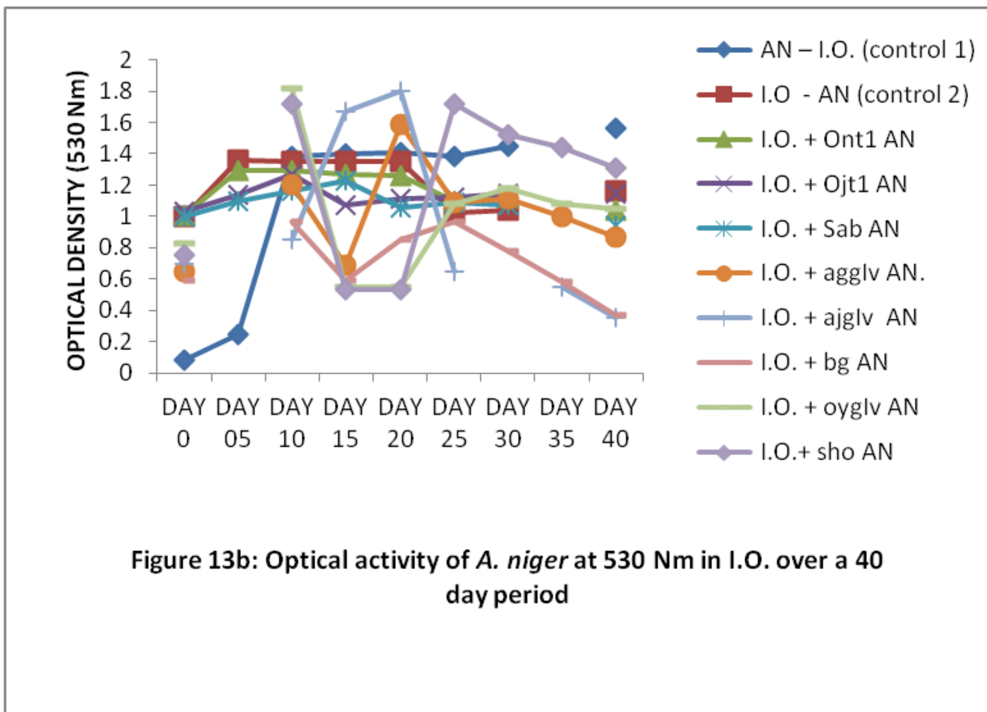



**Figure 13b: Optical activity of *A. niger* at 530 Nm in I.O. over a 40 day period**

**KEY**

I.O. - OIL EXTRACTED FROM *IRVINGIA GABONENSIS* SEED

AN - *ASPERGILLUS NIGER*

Sab – Soil from mechanic workshop, Sabo

Ont - Soil from mechanic village, Onitiri – Akoka

Bg - Soil from Biological Gardens, Unilag

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

Agg Iv – *Irvingia gabonensis* seed from Agege market

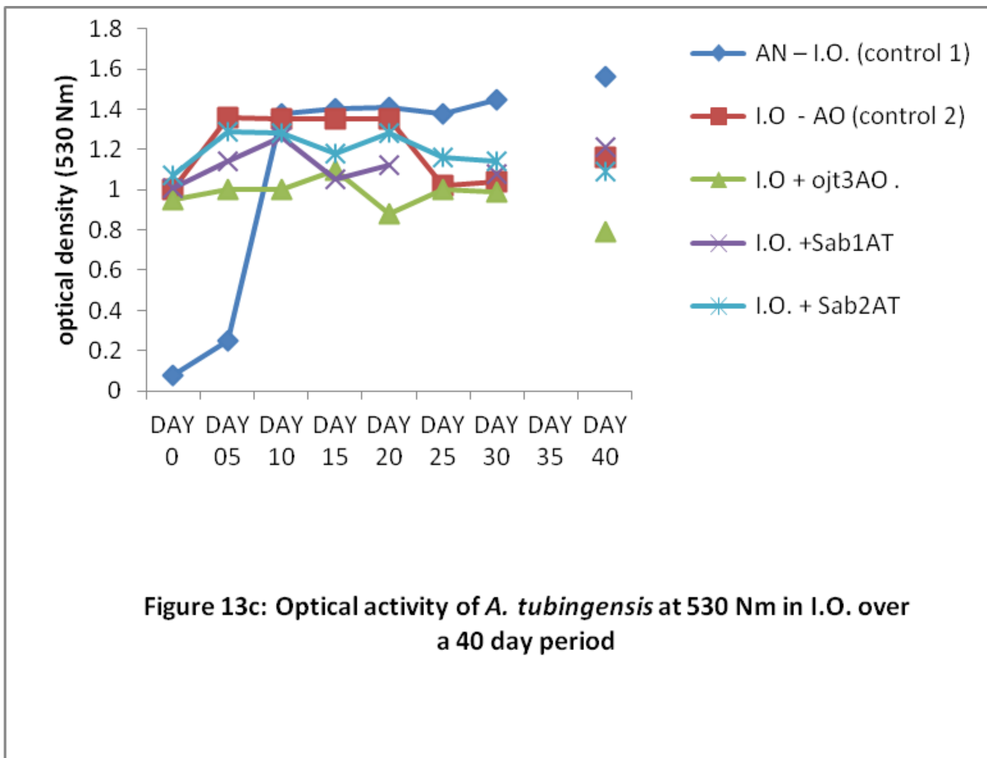



**Figure 13c: Optical activity of *A. tubingensis* at 530 Nm in I.O. over a 40 day period**

**KEY**

I.O. - OIL EXTRACTED FROM *IRVINGIA GABONENSIS* SEED

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

Ojt - Soil from Olusosun dump site, Ojota

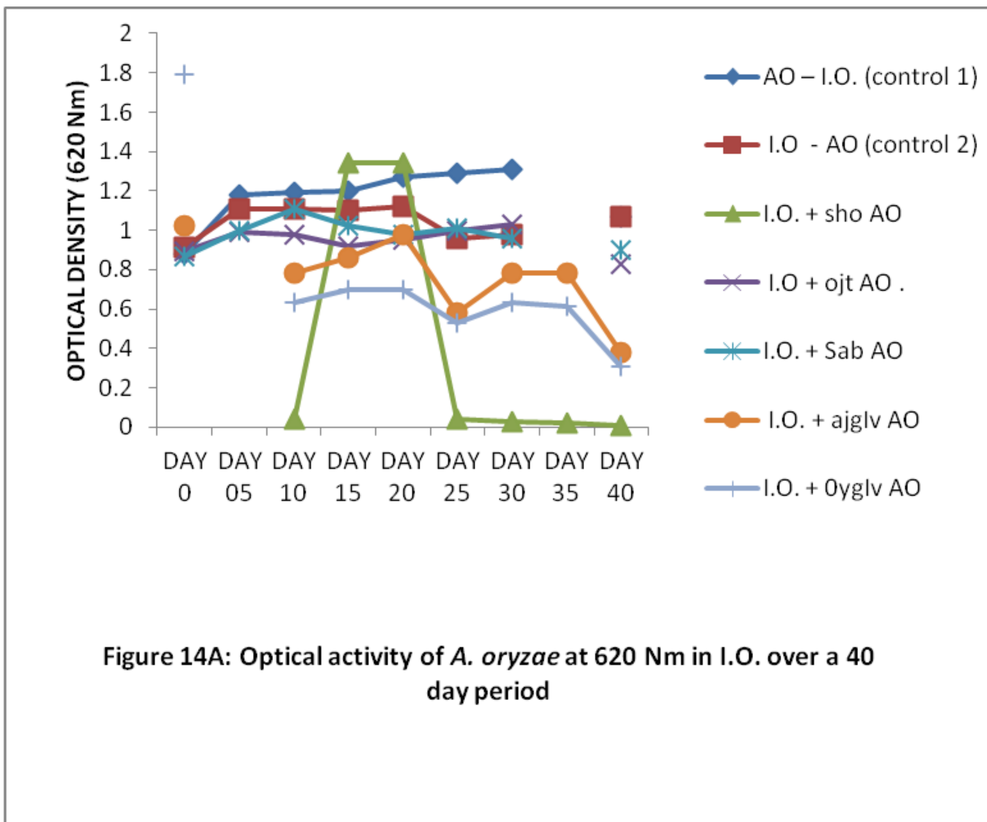



**Figure 14A: Optical activity of *A. oryzae* at 620 Nm in I.O. over a 40 day period**

**KEY**

I.O. - OIL EXTRACTED FROM *IRVINGIA GABONENSIS* SEED

AO - *ASPERGILLUS ORYZAE*

Sab – Soil from mechanic workshop, Sabo

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

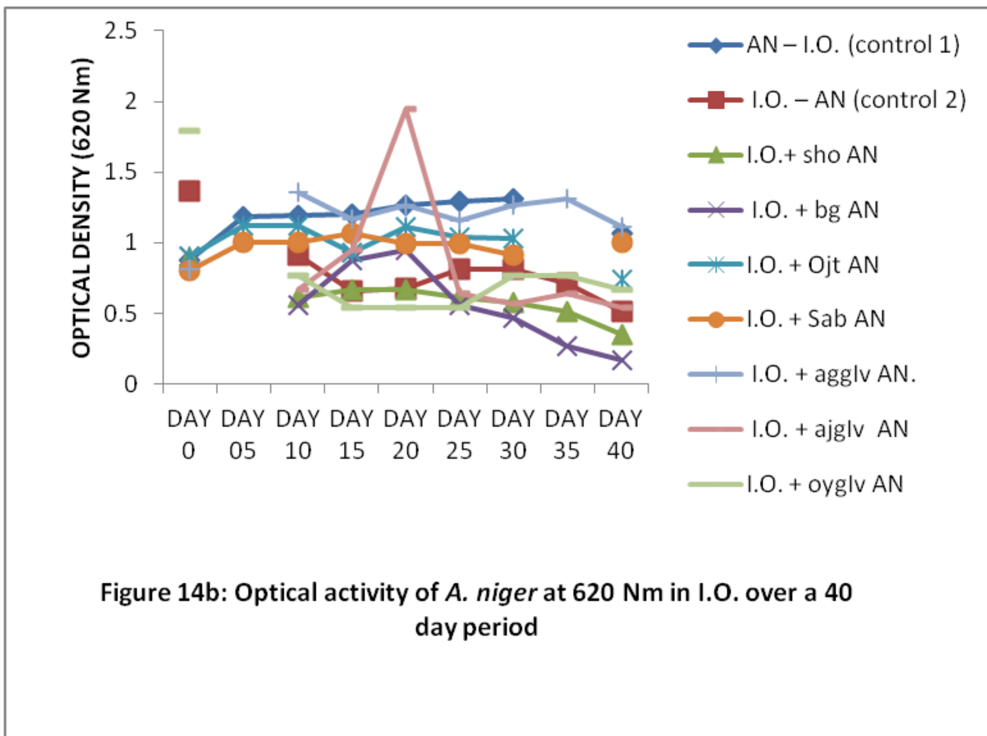



**Figure 14b: Optical activity of *A. niger* at 620 Nm in I.O. over a 40 day period**

**KEY**

I.O. - OIL EXTRACTED FROM *IRVINGIA GABONENSIS* SEED

AN - *ASPERGILLUS NIGER*

Sab – Soil from mechanic workshop, Sabo

Bg - Soil from Biological Gardens, Unilag

Sho - Soil from from Shodex Gardens, Anthony - Village

Ojt - Soil from Olusosun dump site, Ojota

Ajg Iv - *Irvingia gabonensis* seed from Ajegunle market

Oyg Iv – *Irvingia gabonensis* seed from Oyingbo market

Agg Iv – *Irvingia gabonensis* seed from Agege market

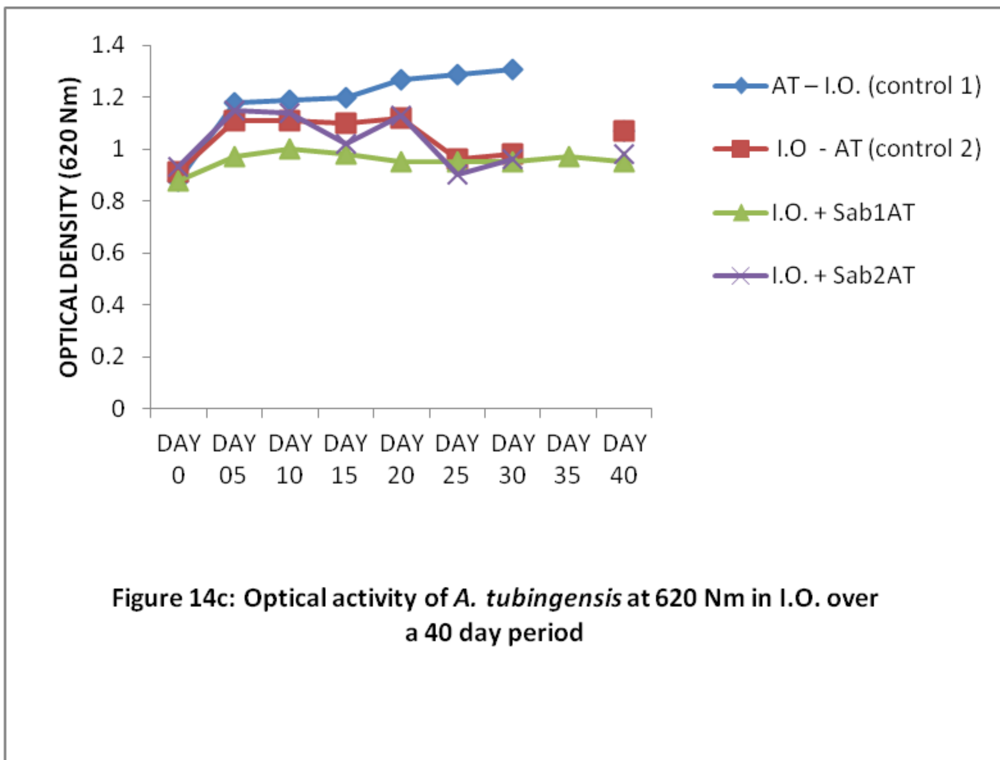



**Figure 14c: Optical activity of *A. tubingensis* at 620 Nm in I.O. over a 40 day period**

**KEY**

I.O. - OIL EXTRACTED FROM *IRVINGIA GABONENSIS* SEED

AT - *ASPERGILLUS TUBINGENSIS*

Sab – Soil from mechanic workshop, Sabo

#### 4.3.2.2 Confirmation of the degradation of petroleum hydrocarbons (by the fungal Samples) in the OD experiment using GC technique

In confirming the mycodegradation of the petroleum hydrocarbon and the vegetable hydrocarbon compounds as shown in the OD spectrophotometric experiment, one day 40 and two day zero samples (day zero without the fungus and day zero with the fungus) for each of the hydrocarbon samples were subjected to a GC determination of their Total Petroleum Hydrocarbon (TPH) content. In all cases, the values for TPH were the means of triplicate analysis from electronic integration measurements using flame ionization detector

*A.oryzae* (from *I.gabonensis* seed from Ajegunle Market) was picked as a representative sample of the most promising species in the OD experiment for a confirmation evaluation of the Total Petroleum Hydrocarbon. The results as obtained for each of the petroleum hydrocarbon sources are summarized in Figures 15 - 19.

On a general note, there was a reduction in the TPH levels of the petroleum hydrocarbon compounds at day 40 when compared to what was available at day 0, thus confirming the ability of this fungus to degrade petroleum hydrocarbon compounds. However, there was no significant difference in the TPH levels between the 2 day 0s i.e. day 0 with *Aspergillus oryzae* and the day 0 without *Aspergillus oryzae* for each of the 5 oils containing Petroleum hydrocarbon (Figures 15 - 19).

Another observation in this aspect - the mycodegradation experiment - is with respect to the GC confirmatory studies of the extracted oil from *Irvingia gabonensis.* Here, there was an increase in all the values of the saturated free fatty acid components, and a corresponding decrease in all the values of the unsaturated free fatty acid components of the oil at day 40, when compared to the 2 day zero samples (Table 9).

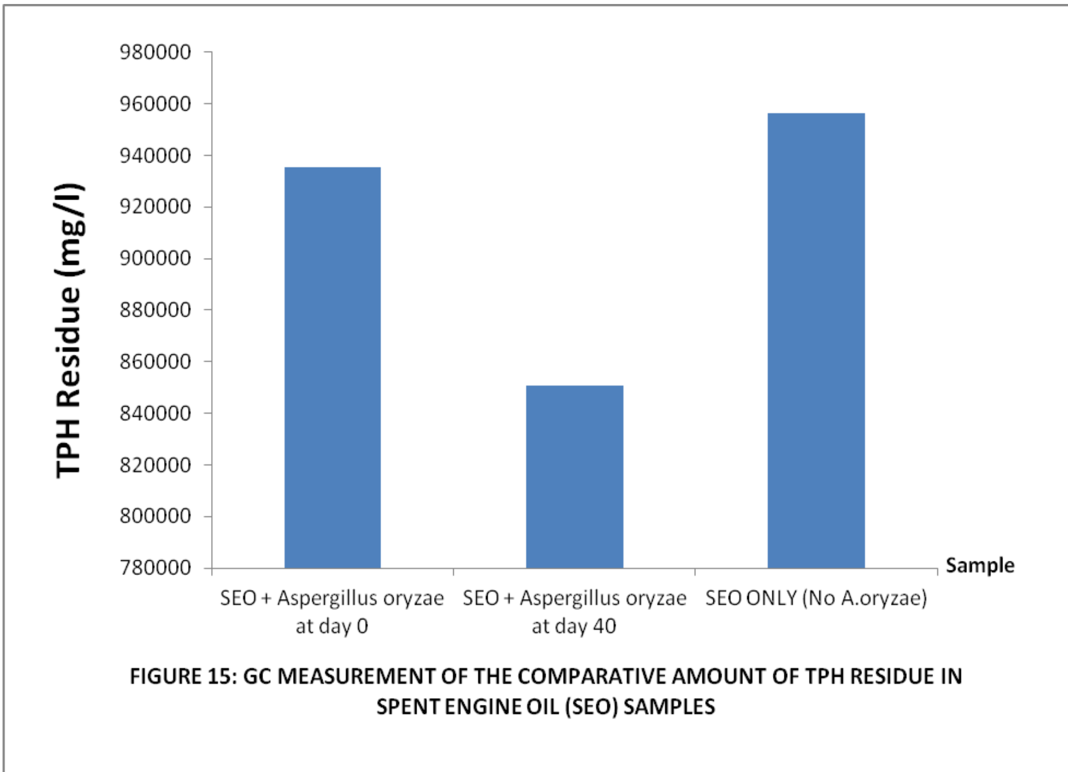



FIGURE 15: GC MEASUREMENT OF THE COMPARATIVE AMOUNT OF TPH RESIDUE IN SPENT ENGINE OIL (SEO) SAMPLES

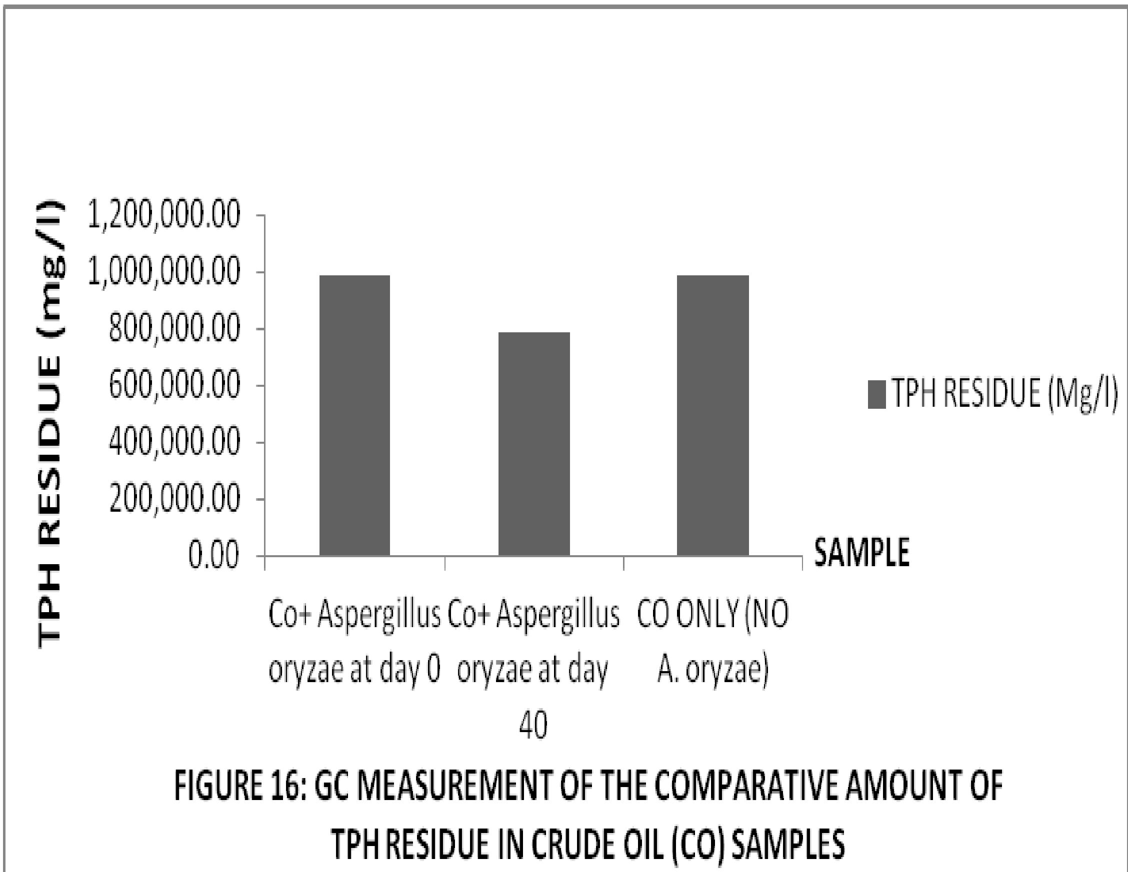



FIGURE 16: GC MEASUREMENT OF THE COMPARATIVE AMOUNT OF TPH RESIDUE IN CRUDE OIL (CO) SAMPLES

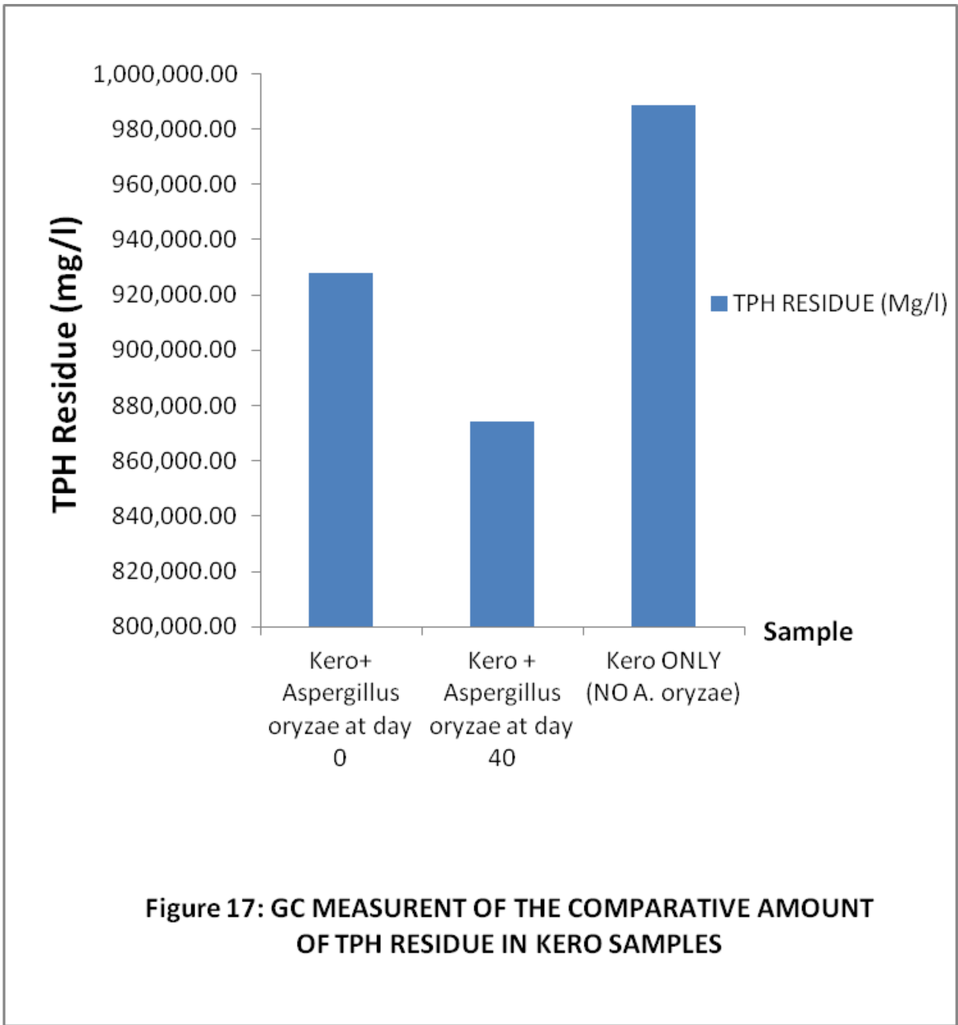



Figure 17: GC MEASURENT OF THE COMPARATIVE AMOUNT OF TPH RESIDUE IN KERO SAMPLES

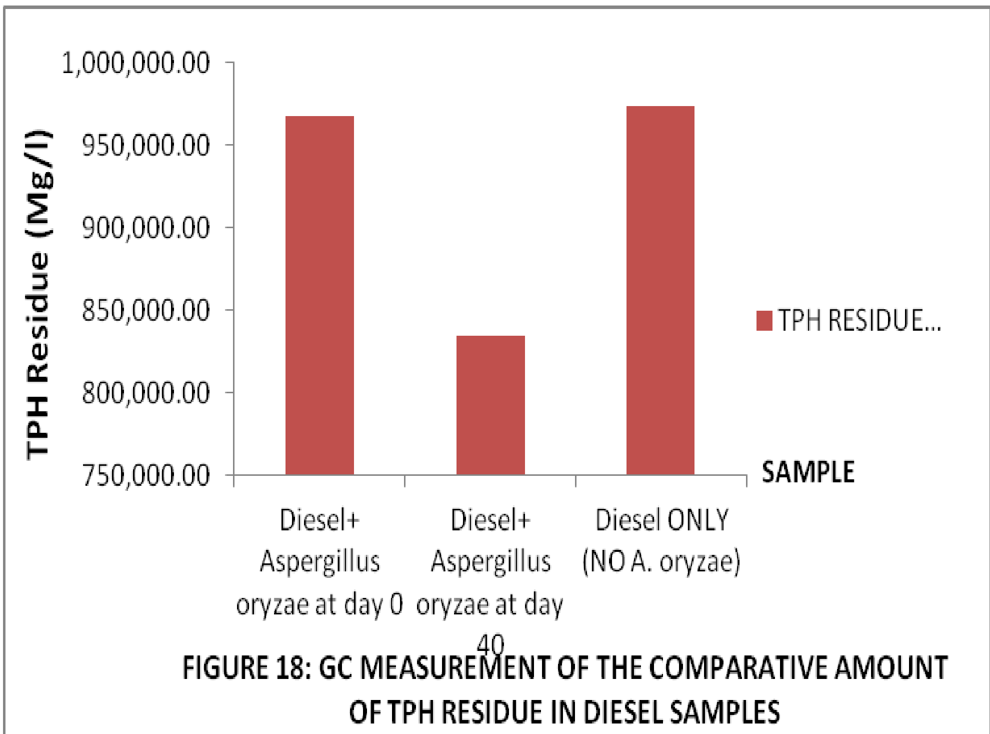



FIGURE 18: GC MEASUREMENT OF THE COMPARATIVE AMOUNT OF TPH RESIDUE IN DIESEL SAMPLES

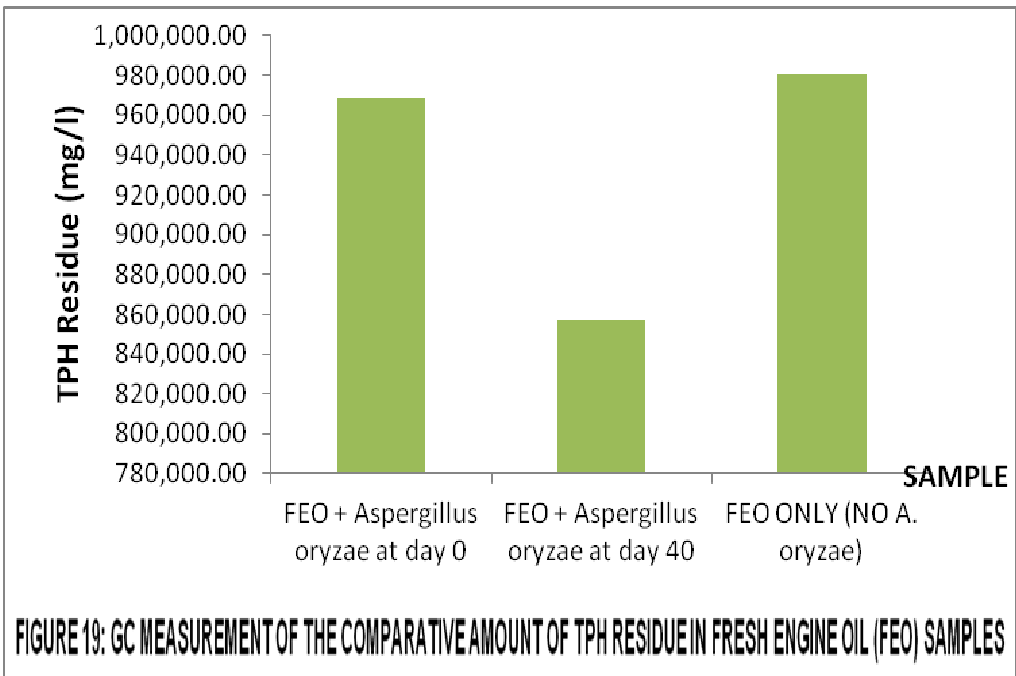



FIGURE 19: GC MEASUREMENT OF THE COMPARATIVE AMOUNT OF TPH RESIDUE IN FRESH ENGINE OIL (FEO) SAMPLES

TABLE 9: GC measurement of the comparative rate of mycodegradation of extracted *Irvingia* Oil (I.O.)

| S/N | Fatty acid parameter | Level of Saturation/Unsaturation of fatty acid in i.o. | % conc. of FFA. in oil containing *A.oryzae* on day 0 | % conc. of FFA. in oil containing *A.oryzae* on day 40 | % conc. of FFA. in oil without *A.oryzae* |
|---|---|---|---|---|---|
| 1 | Myristic acid | C14:0 | 1.287384* | 1.674678* | 0.983125* |
| 2 | Palmitic acid | C16:0 | 14.693561 | 16.068987 | 12.466371 |
| 3 | Palmitoleic acid | C16:1 | 5.709499 | 5.114147 | 6.583224 |
| 4 | Stearic acid | C18:0 | 9.997257 | 14.100507 | 9.441241 |
| 5 | Oleic acid | C18:1 | 19.850643 | 17.370984 | 19.946531 |
| 6 | Linoleic acid | C18:2 | 20.943899 | 20.081964 | 21.184452 |
| 7 | Linolenic acid | C18:3 | 24.230244 | 21.820027 | 24.784376 |
| 8 | Arachidic acid | C20:0 | 0.409376 | 0.436645 | 0.378047 |
| 9 | Arachidonic acid | C20:4 | 1.424217 | 1.274306 | 1.808686 |
| 10 | Behenic acid | C22:0 | 0.443065 | 0.640777 | 0.289867 |
| 11 | Erucic acid | C22:1 | 0.876731 | 0.687040 | 1.621775 |
| 12 | Lignoceric acid | C24:0 | 0.477014 | 0.540238 | 0.359107 |

*The percentages of oil components were obtained as the mean value of triplicate analysis from electronic integration measurements using flame ionization detector

### 4.4.0 Mycoremediation studies

### 4.4.1 Confirmation studies for the effectiveness of *Aspergillus oryzae* as a mycoremediation agent on a petroleum hydrocarbon contaminated soil

The summary of the Gas Chromatographic (GC) reading for the Total Petroleum Hydrocarbon (TPH) in the soil at the initial point (on application of Treatments) and at the final point (6 months after the application of Treatments) are presented on Table 10.

The results from Table 10 show a very high initial level of TPH for all the Treatment soils that were polluted with Spent Engine oil (SEO) irrespective of the presence or the absence of *A.oryzae* and or vegetation cover ($T_3$, $T_4$, $T_5$ and $T_6$) when compared to those Treatment soils that were not polluted ($T_1$, $T_2$, $T_7$ and $T_8$). However, those Treatments that had the mycoremediation agent- *Aspergillus oryzae* - applied to the SEO pollution ($T_3$ and $T_4$) showed the most remarkable rate of reduction of 99.100% and 98.919% respectively in their final TPH levels as compared to those treatments that had only SEO pollution and no *A. oryzae* ($T_5$ and $T_6$) which on their own had a 92.083% and 93.011% reduction in the TPH left in the soil 6 months after the application of the Treatments. A further comparison of the amount of TPH that was removed from the soil after 6 months, using Treatments 3 and 4 as a comparison to the remaining other Treatments i.e $T_1$, $T_2$, $T_7$ and $T_8$ as seen from Table 10 further shows the level of efficiency of *A.oryzae* at remediating a petroleum hydrocarbon polluted soil as the percentage removal of TPH from the soils that had neither *A.oryzae* nor petroleum hydrocarbon contamination ($T_1$ and $T_2$) ranged from between 2.700% and 6.287% respectively when compared to the between over 99% and 98% reduction in the TPH achieved for the soils that had *A.oryzae* as a mycoremediation

agent in the oil pollution ($T_3$ and $T_4$). Furthermore, Table 10 shows that the presence of vegetation in $T_4$ and $T_6$ (as of the time when pollution occurred) compared to the absence of vegetation as of the same time ($T_3$ and $T_5$) caused a slight reduction in the amount of SEO (hence the amount of TPH) that was able to get to the soil, and that remained in the soil 6 months after, the only exception with regards to this trend after 6 months being the Treatments ($T_3$ and $T_4$) that had *A.oryzae* added to the SEO pollution.

Figures 20A and 20B – 27A and 27B show the GC chromatograms for the initial and the final TPH left in the soil i.e. $1^{st}$ $T_1$ and $T_1$ – $1^{st}$ $T_8$ and $T_8$ respectively.

Table 10: GC reading of the total petroleum hydrocarbon (TPH) level in the soil at months 0 and 6

| S/N | Sample ID | Initial TPH in the soil (mg/kg) | Final TPH in soil (mg/kg) | Percentage TPH removed from the soil at 6 months |
|---|---|---|---|---|
| 1 | **T1** (NOT WEEDED, NOT POLLUTED AND NO A.ORYZAE ADDED) | 19,867.13* | 18, 618.09 | 6.287 |
| 2 | **T2** (WEEDED, NOT POLLUTED AND NO A.ORYZAE ADDED) | 19,955.20 | 19, 402.44 | 2.770 |
| 3 | **T3** (WEEDED, POLLUTED AND A.ORYZAE ADDED) | 2, 013,070 | 18, 156.87 | 99.100 |
| 4 | **T4** (NOT WEEDED, POLLUTED AND A.ORYZAE ADDED) | 1,749,360 | 18,909.03 | 98.919 |
| 5 | **T5** (WEEDED, POLLUTED,NO A.ORYZAE ADDED) | 1,820,700 | 120,748.59 | 92.983 |
| 6 | **T6** ( NOT WEEDED, POLLUTED,NO A.ORYZAE ADDED) | 1,756,400 | 122,759.95 | 93.011 |
| 7 | **T7** (WEEDED, NO POLLUTION, A.ORYZAE ADDED) | 20,131.38 | 19,444.88 | 3.410 |
| **8** | **T8** (NOT WEEDED, NO POLLUTION, A.ORYZAE ADDED) | 20,129.00 | 19,585.54 | 2.700 |

*The values for TPH were the means of triplicate analysis from electronic integration measurements using flame ionization detector

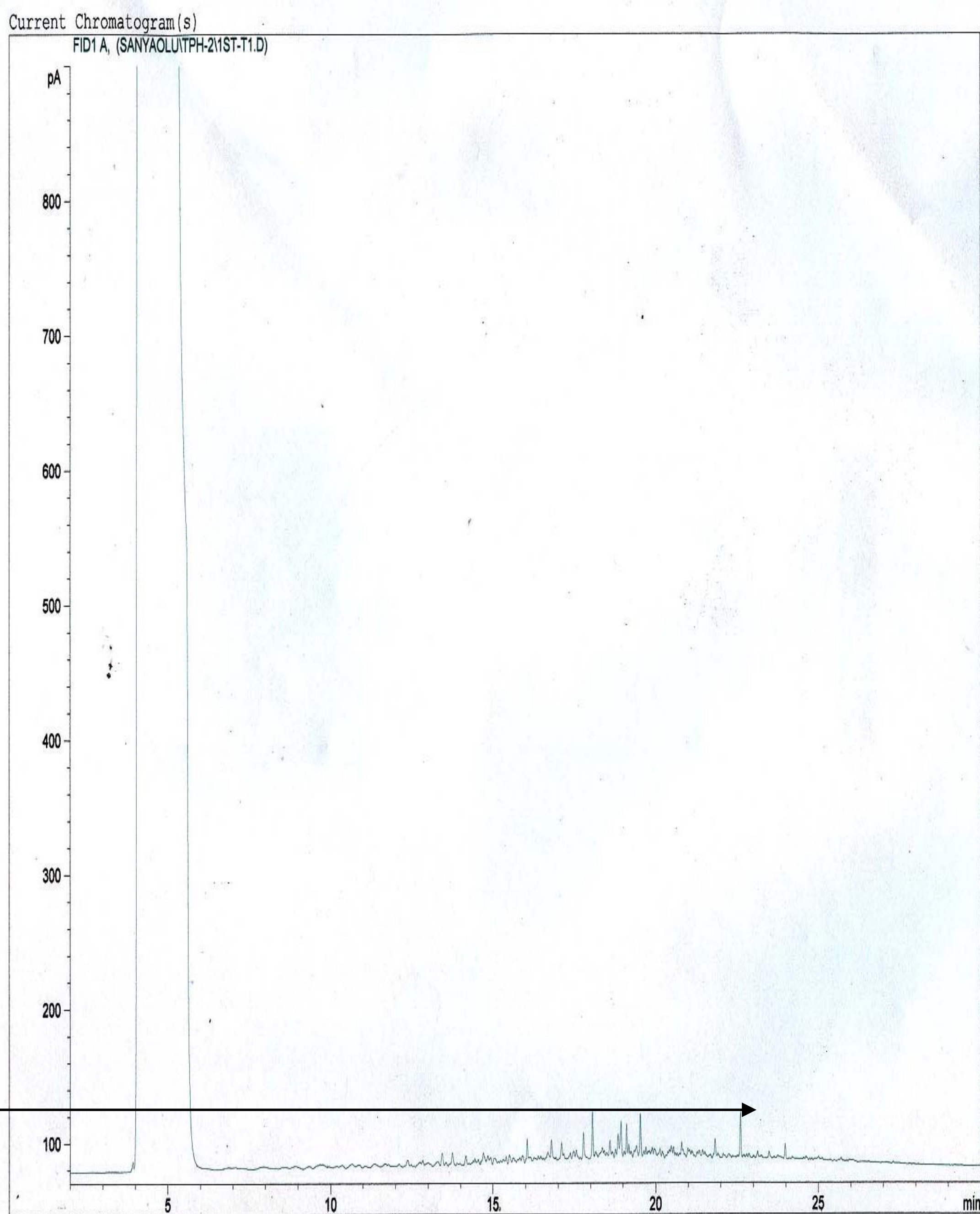



Figure 20A: GC chromatogram showing the level of TPH in the soil on day 0 for $T_1$

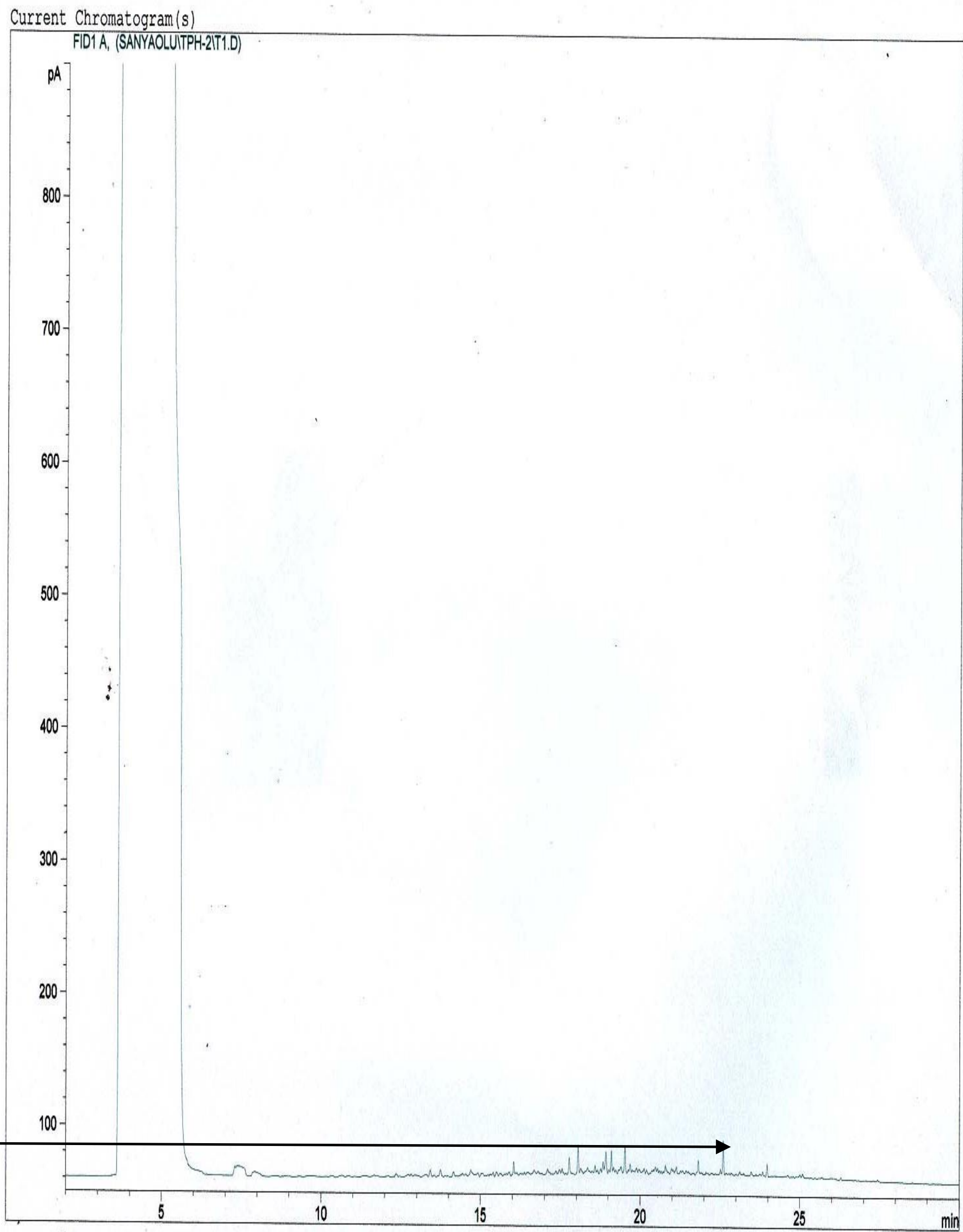



Figure 20B: GC chromatogram showing the level of TPH in the soil at the 6th month for $T_1$

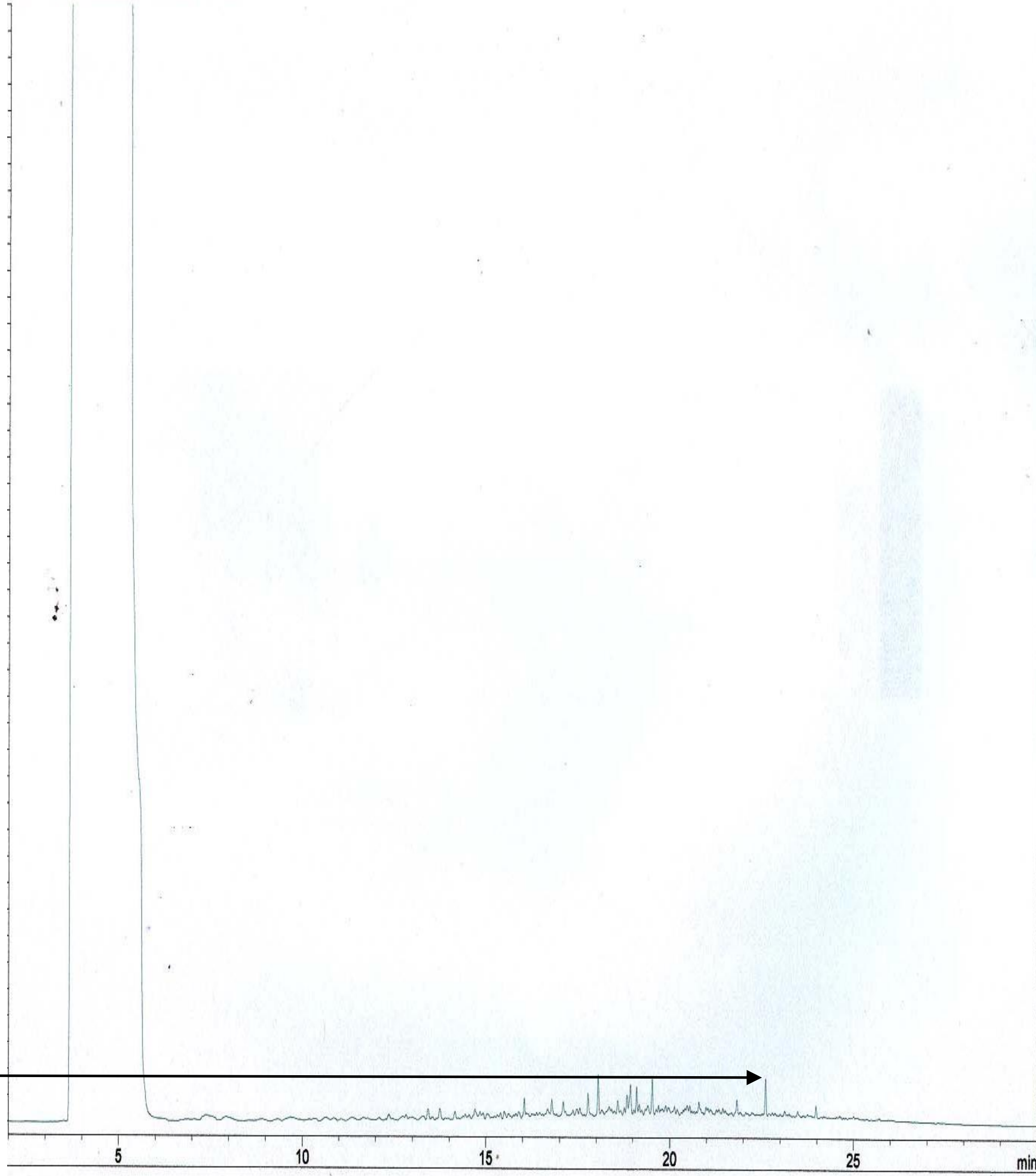
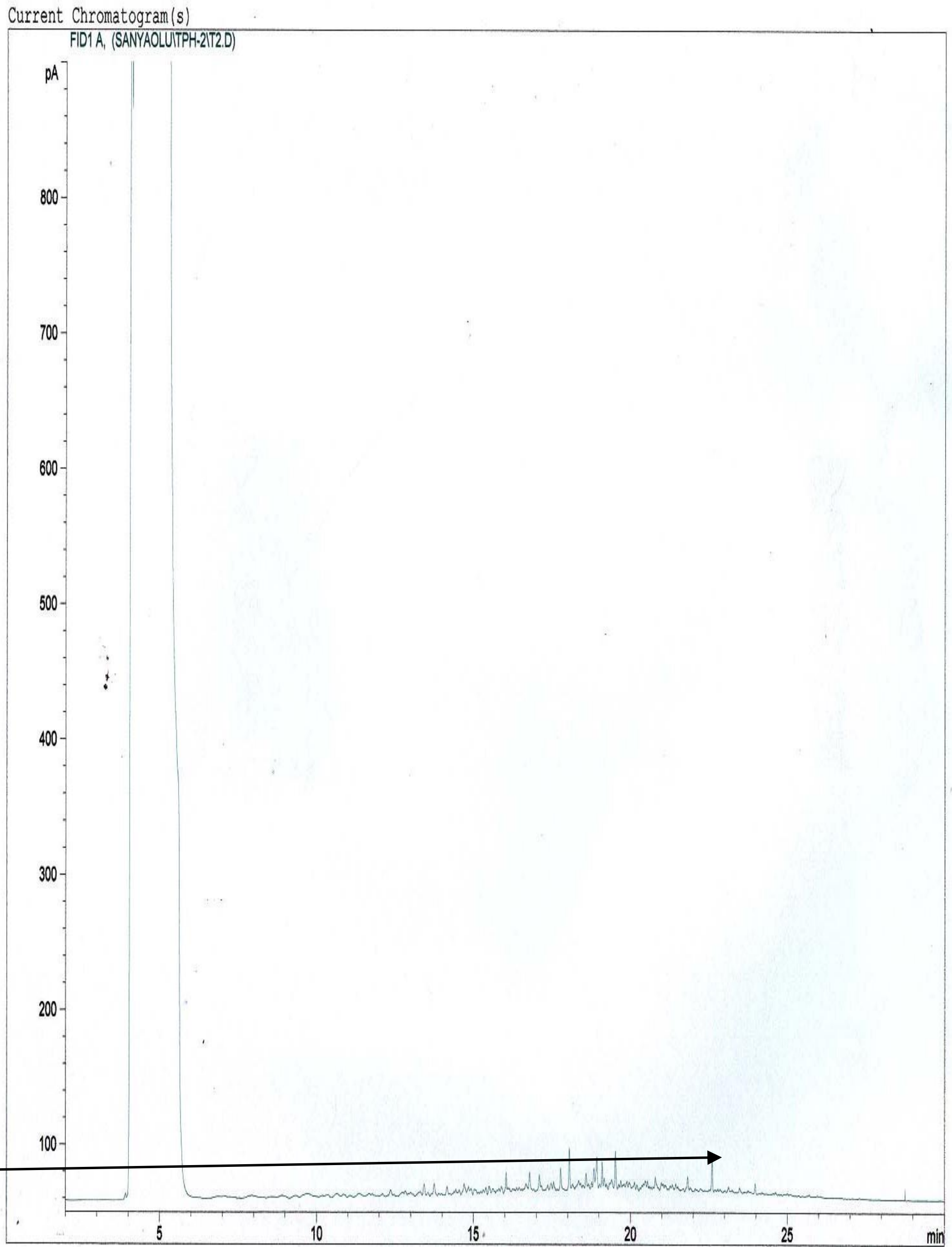



Figure 21A: GC chromatogram showing the level of TPH in the soil on day 0 for $T_2$

Print of window 38: Current Chromatogram(s)




TPH peaks at the 6th month for $T_2$ not visually less prominent than at day 0

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Figure 21B: GC chromatogram showing the level of TPH in the soil at the 6th month for $T_2$

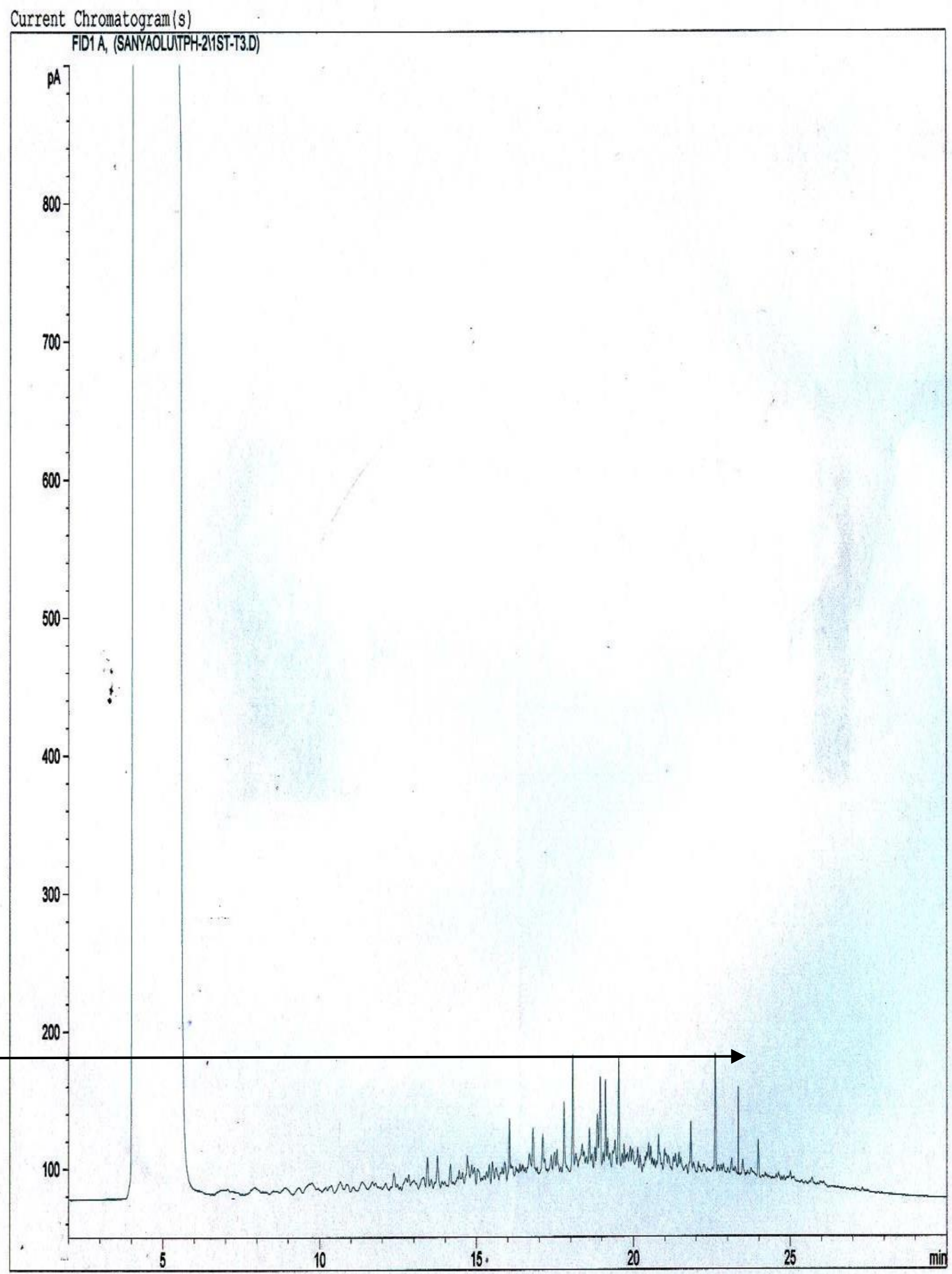
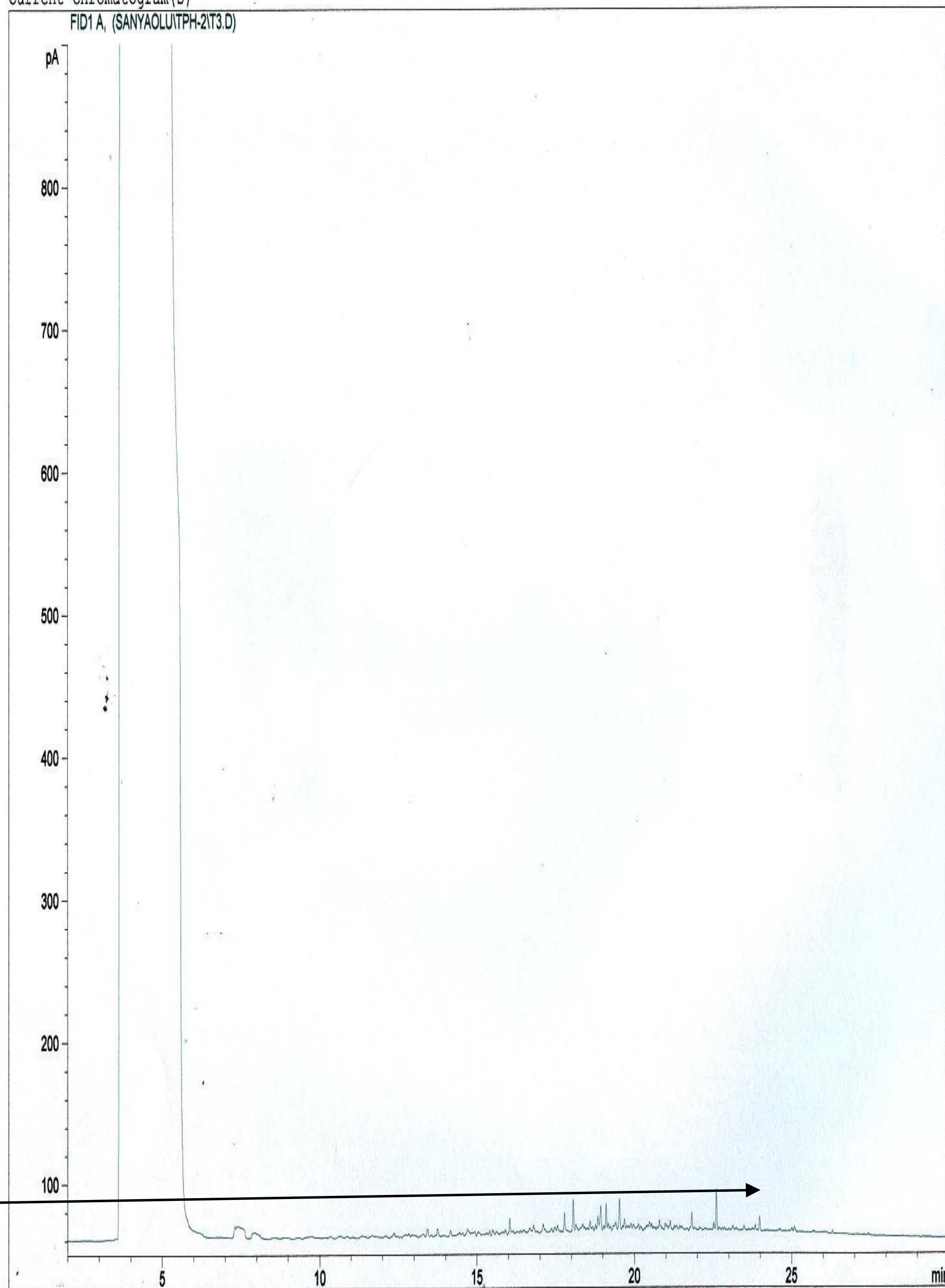



Figure 22A: GC chromatogram showing the level of TPH in the soil on day 0 for $T_3$

Print of window 38: Current Chromatogram(s)




Reduced TPH peaks for $T_3$ at the 6th month

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✍⑥⑨ ☒

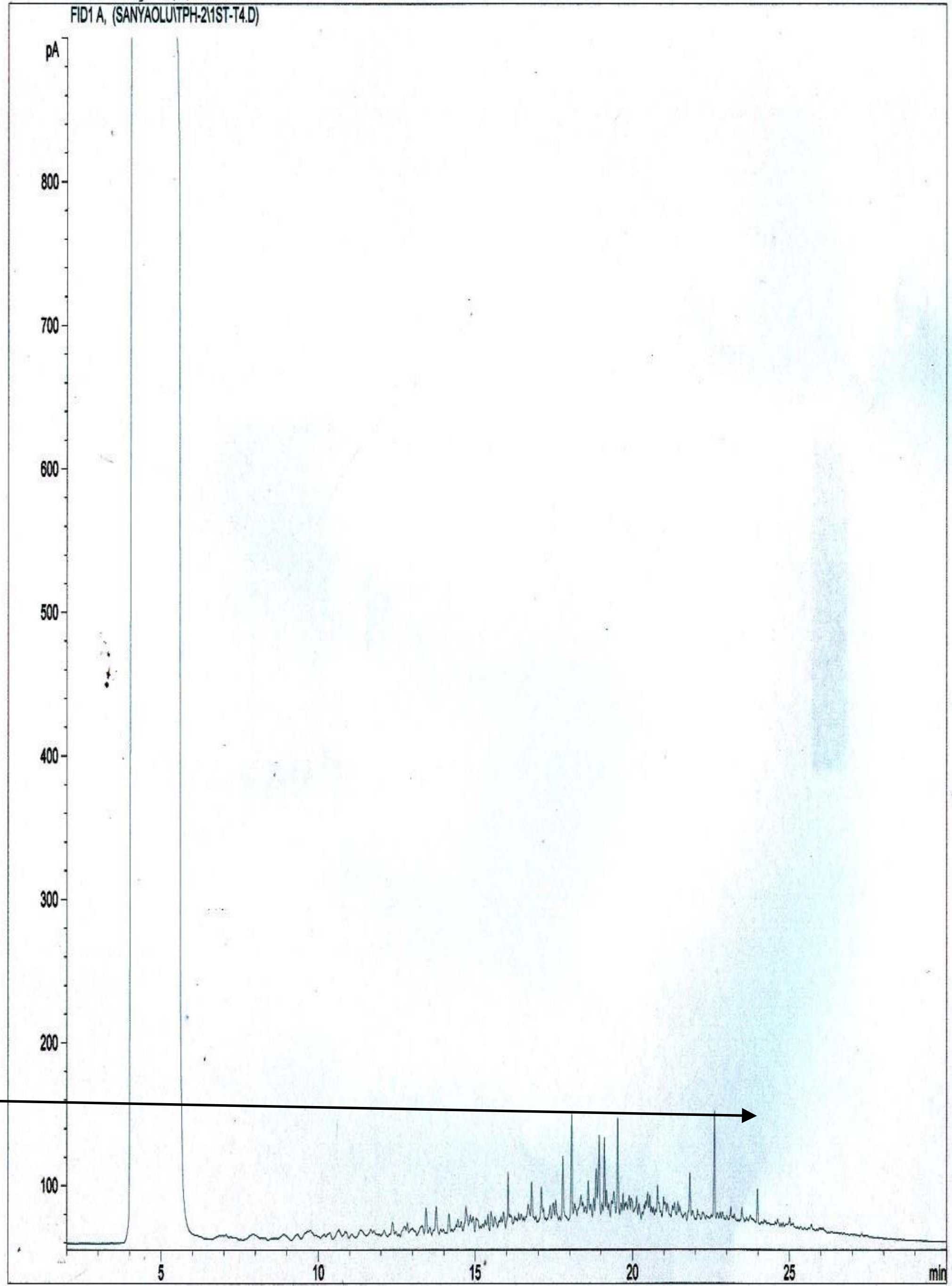

Figure 23A: GC chromatogram showing the TPH level in the soil at day 0 for $T_4$

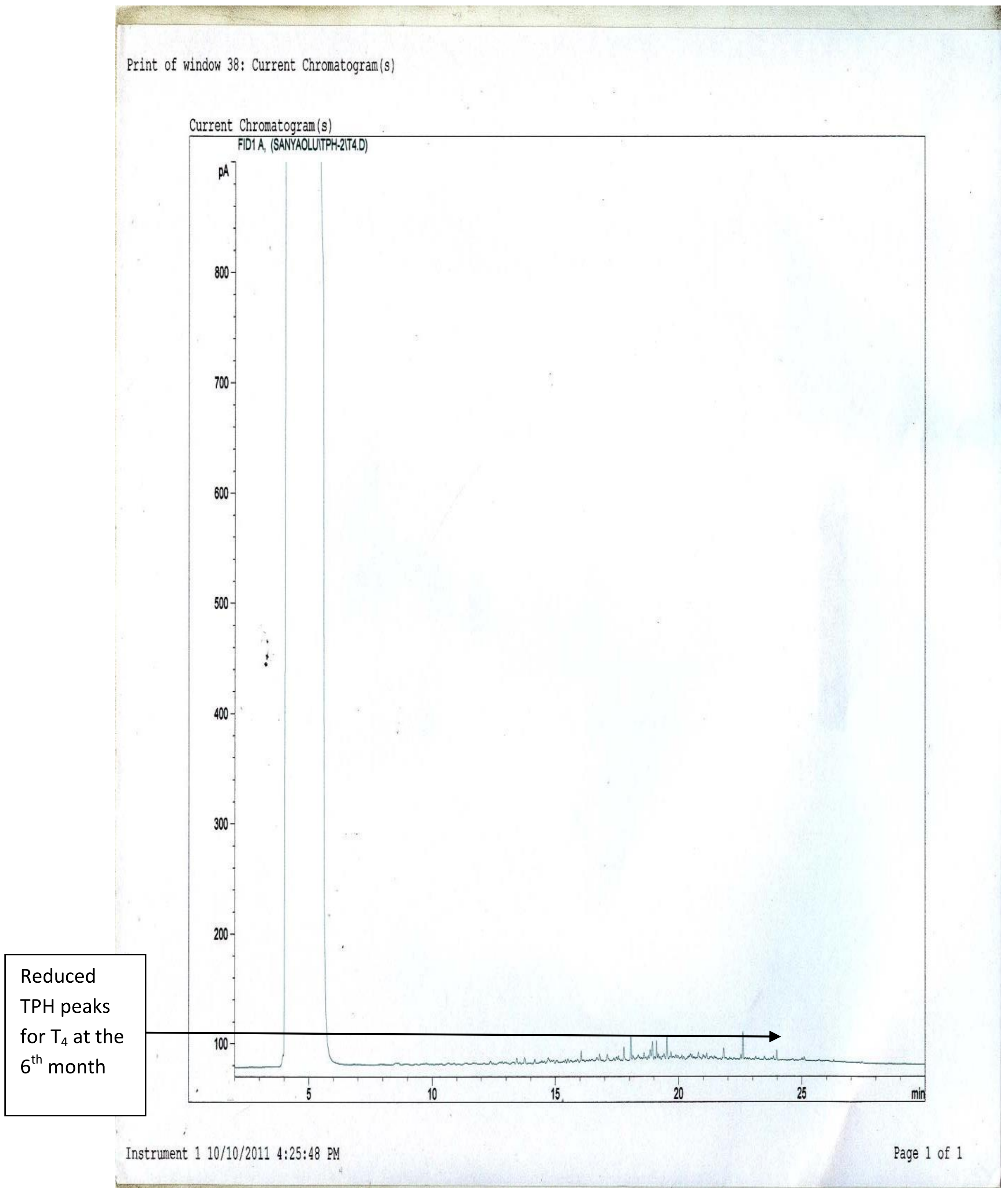


Figure 23B: GC chromatogram showing the level of TPH in the soil after 6 months for $T_4$

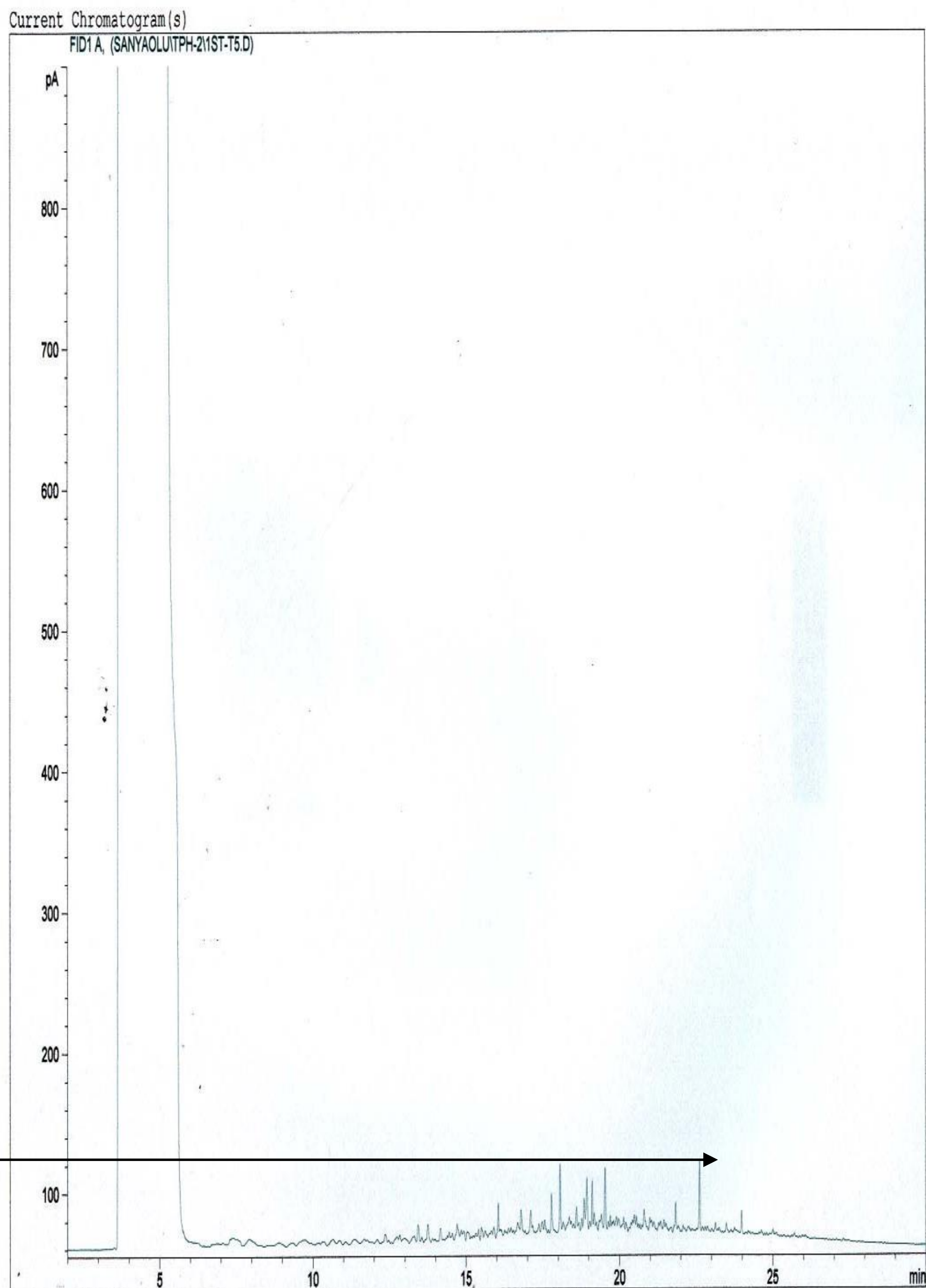


Figure 24A: GC chromatogram showing the level of TPH in the soil at day 0 for $T_5$

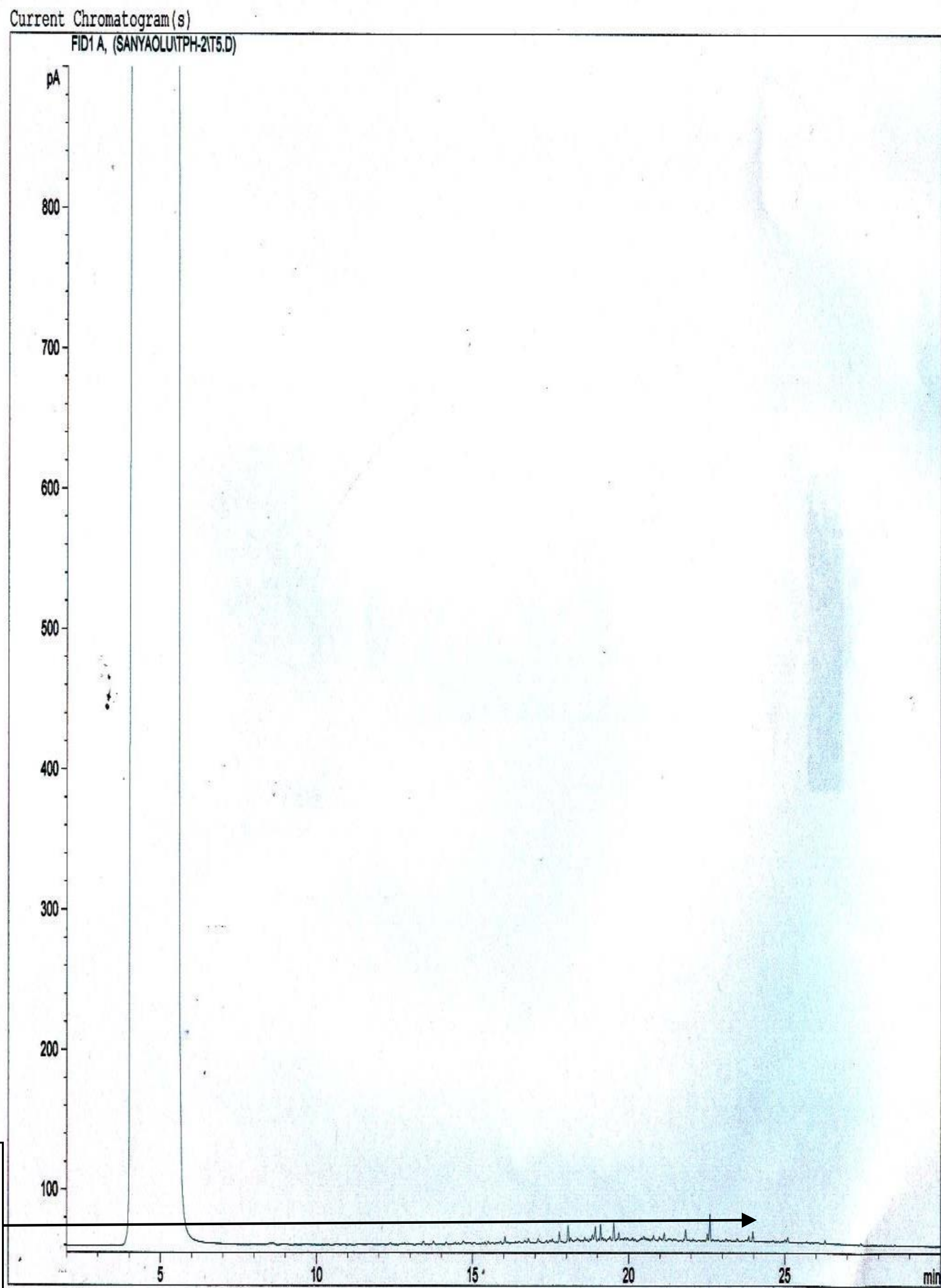


Figure 24B: GC Chromatograph showing the level of TPH in the soil after 6 months for $T_5$

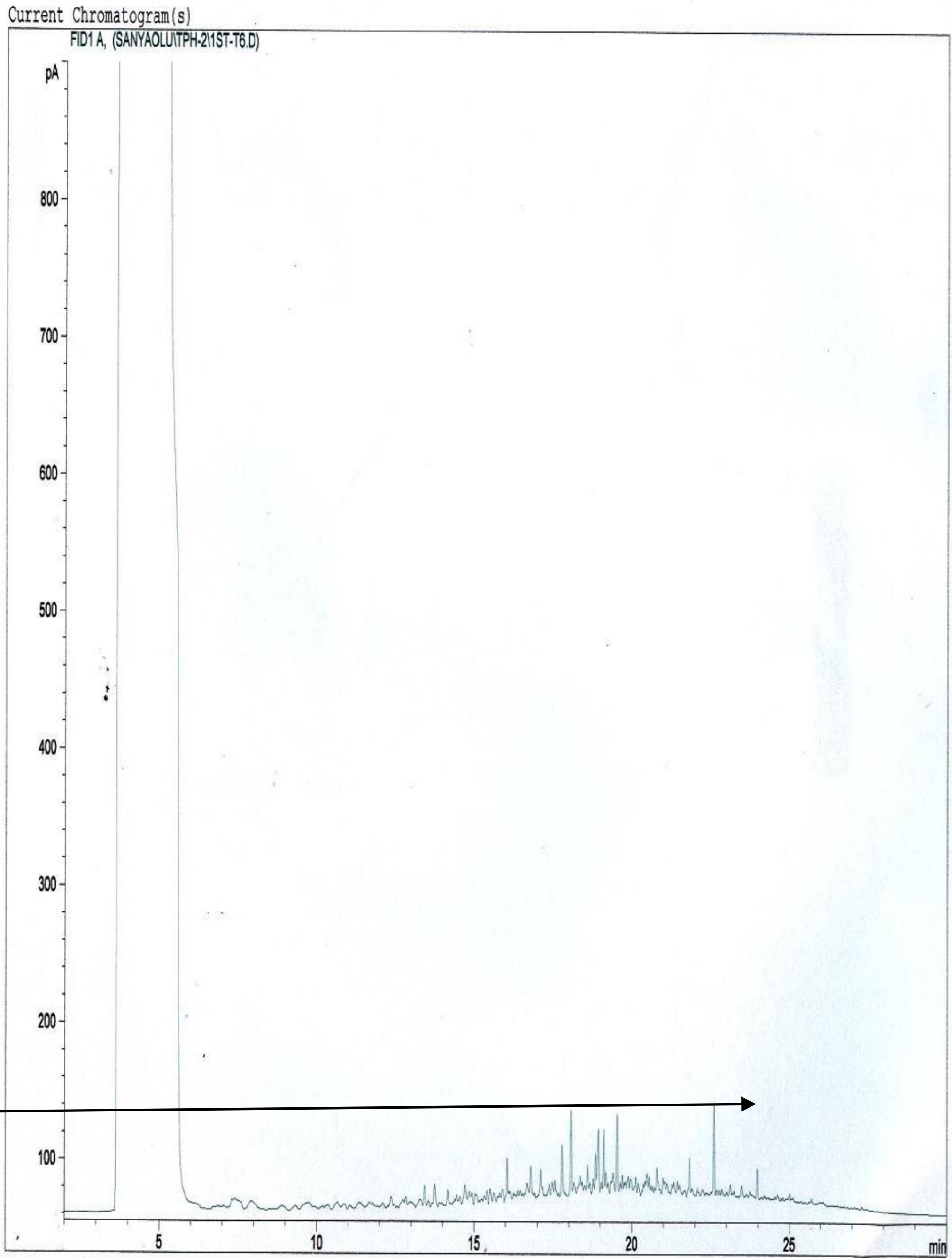


Figure 25A: GC chromatogram showing the level of TPH in the soil at day 0 for $T_6$

Print of window 38: Current Chromatogram(s)

Current Chromatogram(s)

FID1 A, (SANYAOLU\TPH-2\T6.D)

pA

800

700

600

500

400

300

200

100

5 10 15 20 25 min

Reduced peaks for $T_6$ at the 6th month

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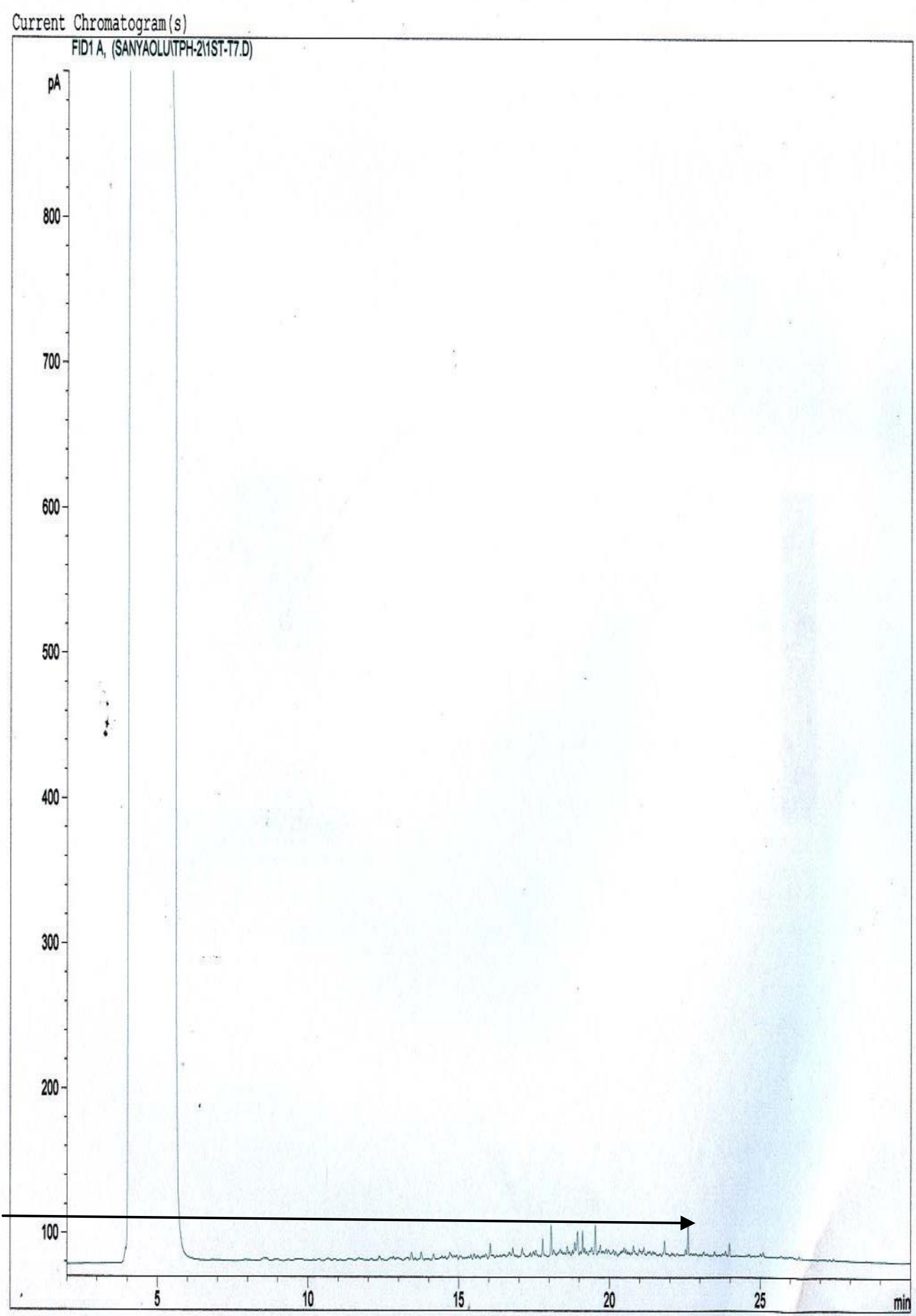
Print of window 38: Current Chromatogram(s)




TPH peaks for $T_7$ at day 0 slightly more prominent than at the 6th month

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Figure 26A: GC chromatograph showing the level of TPH in the soil at day 0 for $T_7$

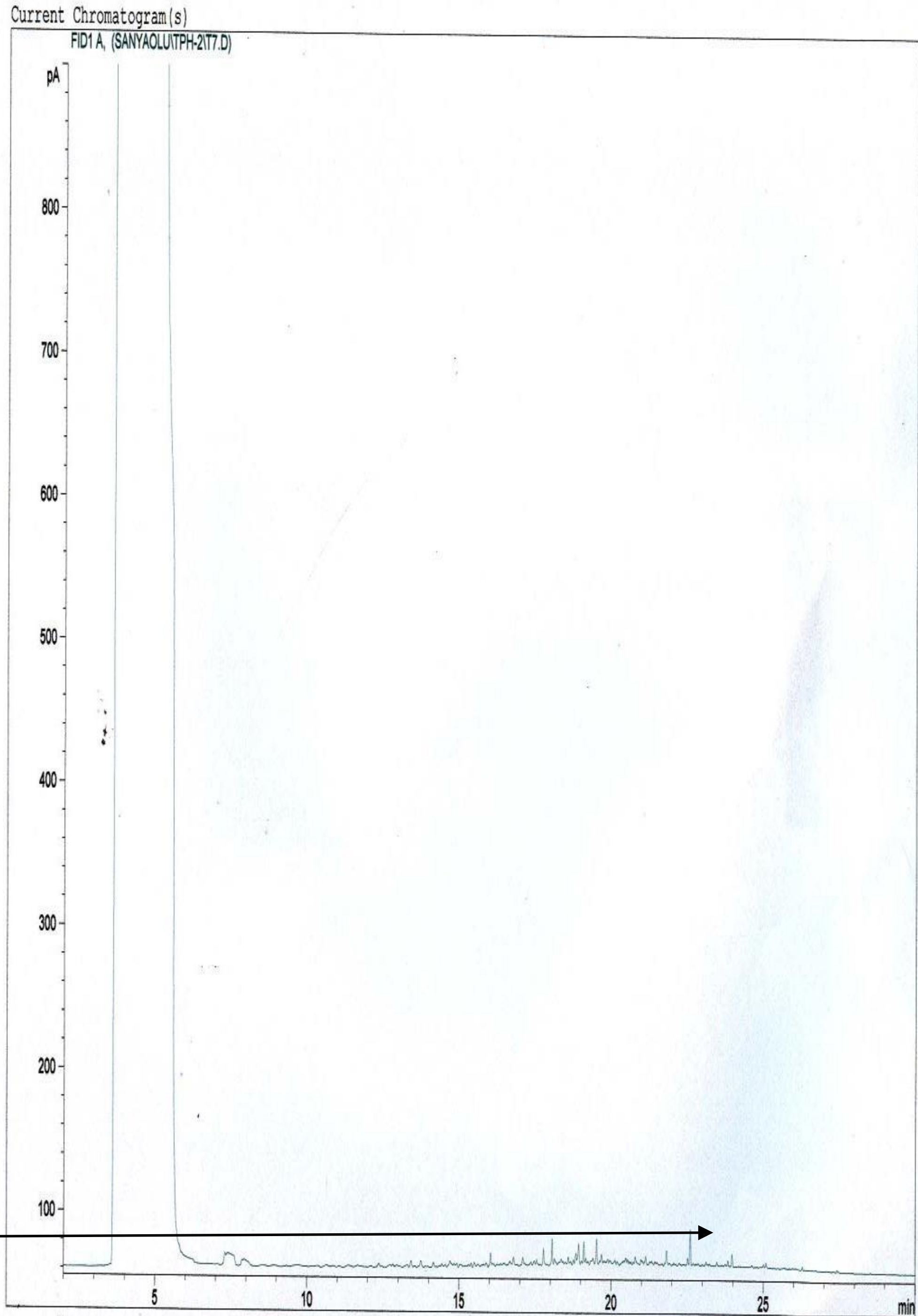
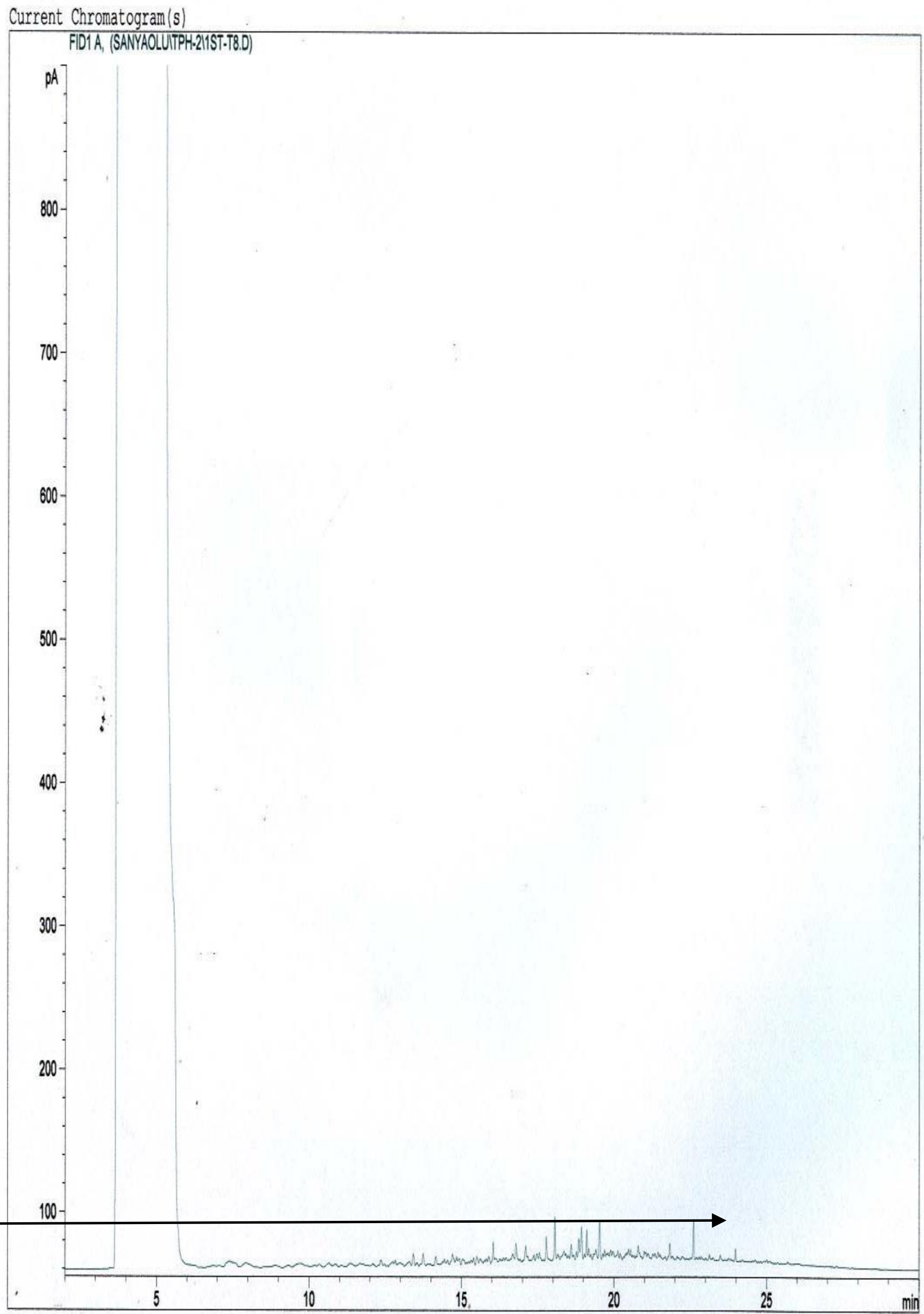



Figure 26B: GC chromatograph showing the level of TPH in the soil after 6 months for $T_7$

Print of window 38: Current Chromatogram(s)




TPH peaks for $T_8$ at day 0 slightly more prominent than at the 6th month

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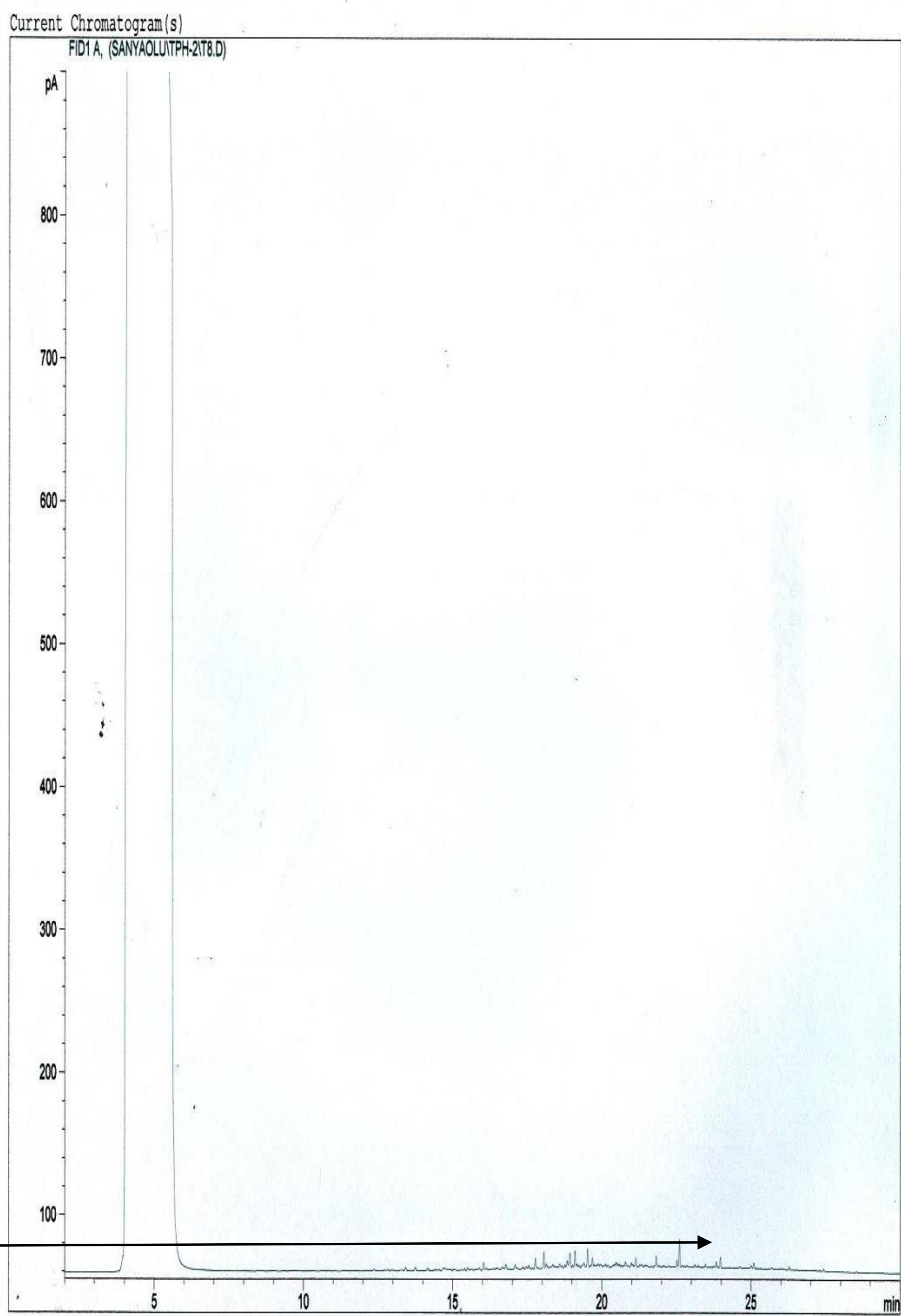
Figure 27A: GC chromatograph showing the level of TPH in the soil at day 0 for $T_8$




TPH peaks for $T_8$ at the $6^{th}$ month slightly less prominent than at day 0

Figure 27B: GC Chromatograph showing the level of TPH in the soil after 6 months for $T_8$

### 4.4.2 Effect of the mycorememdiation agent on the nutrient status of the soil

The following results were obtained for the following macro nutrients:

#### 4.4.2.1 Total nitrogen (%)

The results on Table 11 show the effect of the Treatments on the amount of total nitrogen (N) in the soil. The statistical analysis of the data for N show that the Treatments at each of the time of sampling had a significant ($P \leq 0.05$) effect on the N level. This results further show that the spillage of SEO (irrespective of any of the other factors such as the presence or the absence of *A.oryzae* and or weed) caused a significant reduction in the total N in the soil at the baseline point (Table 11 Treatments 3, 4, 5 and 6). It can also be seen from Table 11 that the addition of *A.oryzae* (irrespective of the absence or the presence of vegetation- $T_3$ and $T_4$ respectively) to the SEO polluted field by the $2^{nd}$ and $3^{rd}$ sampling (at the $3^{rd}$ and $6^{th}$ months respectively) has caused a significant increase in the amount of total N in the soil form 0.07 to 0.11 and 0.12% at baseline point, month 2 and month 6 respectively for Treatment 3. These values obtained for $T_3$ especially at the $6^{th}$ month was significantly higher than the values obtained from the 2 control treatments ($T_1$ and $T_2$), and indeed all the other treatments at this time. Comparing $T_3$ and $T_4$ at the $2^{nd}$ sampling, it can be seen that the presence of vegetation appears to have caused a reduction in the ability of *A.oryzae* to increase the amount of total N in the SEO polluted soil as the amount of total N in $T_4$ was significantly lower than in $T_3$ (Table 11). By $3^{rd}$ sampling at the $6^{th}$ month however, this trend had been reversed (Table 11), as both soils that had A.oryzae added as a mycoremediation agent to the SEO polluted soil had a total N content that was significantly higher than all the other treatments.

Comparing Treatment 3 with Treatment 1, it is evident from Table 11 that by the 3$^{rd}$ month after the application of the Treatment, *A.oryzae* had effectively restored the N level in the soil to a normal (comparable to the control) level, and by the 6$^{th}$ month after the application of the treatments, *A.oryzae* had left the soil better off in terms of its N content.

TABLE 11: Mean separation (using Duncan's multiple range test) for the effect of the Treatments on the total nitrogen (%) in the soil at the different sampling times

| S/N | TREATMENT | BASELINE | 3RD MONTH | 6TH MONTH |
|---|---|---|---|---|
| 1 | **T1**(NOTWEEDED, NOT POLLUTED AND NO A.ORYZAE ADDED) | $0.11^{a}$ | $0.11^{a}$ | $0.10^{bc}$ |
| 2 | **T2** (WEEDED, NOT POLLUTED AND NO A.ORYZAE ADDED) | $0.11^{a}$ | $0.11^{a}$ | $0.11^{b}$ |
| 3 | **T3** (WEEDED, POLLUTED AND A.ORYZAE ADDED) | $0.07^{c}$ | $0.11^{a}$ | $0.12^{a}$ |
| 4 | **T4**(NOTWEEDED, POLLUTED AND A.ORYZAE ADDED) | $0.07^{c}$ | $0.10^{b}$ | $0.12^{a}$ |
| 5 | **T5** (WEEDED, POLLUTED,NO A.ORYZAE ADDED) | $0.07^{c}$ | $0.10^{bc}$ | $0.11^{b}$ |
| 6 | **T6**(NOT WEEDED, POLLUTED,NO A.ORYZAE ADDED) | $0.07^{c}$ | $0.10^{bc}$ | $0.10^{bc}$ |
| 7 | **T7** (WEEDED, NO POLLUTION, A.ORYZAE ADDED) | $0.10^{b}$ | $0.09^{d}$ | $0.11^{b}$ |
| 8 | **T8**(NOTWEEDED, NO POLLUTION, A.ORYZAE ADDED) | $0.11^{a}$ | $0.10^{b}$ | $0.10^{bc}$ |

Mean values along the same column that are carrying different superscripts are significantly different at $P \leq 0.05$.

4.4.2.2 Available Phosphorus (Mg/Kg)

The results from Table 12 show that the Treatments had a significant ($P \leq 0.05$) effect on the Phosphorus level in the soil at the baseline point and at the 3rd month but not at the 6th month. Also, the results in Table 12 (baseline values for $T_3$, $T_4$, $T_5$ and $T_6$) show that SEO pollution (irrespective of the presence or absence of the *A.oryzae* and or vegetation cover) in the soil caused an initial significant reduction in the level of available phosphorus. The addition of *A.oryzae* as a mycoremediation agent however by the 3rd month caused a significant increase in the level of available phosphorus (Table 12, $T_3$ and $T_4$) over and above the control plots (Table 12, $T_3$ compared with $T_1$ and $T_2$ ), a trend which continued until the 6th month. Generally however, it appears that the presence of vegetation cover on the treatment plots significantly reduced the efficiency of this mycoremediation agent (*A.oryzae*) at increasing the amount of available phosphorus in the soil both at the 3rd and 6th months after the application of the treatments (Table 12, comparism between $T_3$ and $T_4$).

TABLE 12: Mean separation (using Duncan's multiple range test) for the effect of the Treatments on the available phosphorus (mg/kg) in the soil at the different sampling times

| S/N | TREATMENT | BASELINE | 3RD MONTH | 6TH MONTH |
|---|---|---|---|---|
| 1 | **T1** (NOT WEEDED, NOT POLLUTED AND NO A.ORYZAE ADDED) | 16.93[a] | 13.00[bc] | 11.87[a] |
| 2 | **T2** (WEEDED, NOT POLLUTED AND NO A.ORYZAE ADDED) | 15.85[b] | 14.73[a] | 12.45[a] |
| 3 | **T3** (WEEDED, POLLUTED AND A.ORYZAE ADDED) | 1.23[e] | 14.75[a] | 11.94[a] |
| 4 | **T4** (NOT WEEDED, POLLUTED AND A.ORYZAE ADDED) | 1.16[f] | 13.89[b] | 10.84[ab] |
| 5 | **T5** (WEEDED, POLLUTED,NO A.ORYZAE ADDED) | 1.13[g] | 12.48[c] | 10.04[ab] |
| 6 | **T6** (NOT WEEDED, POLLUTED,NO A.ORYZAE ADDED) | 1.12[h] | 14.11[b] | 11.76[a] |
| 7 | **T7** (WEEDED, NO POLLUTION, A.ORYZAE ADDED) | 14.04[d] | 12.42[c] | 10.62[ab] |
| 8 | **T8** (NOT WEEDED, NO POLLUTION, A.ORYZAE ADDED) | 15.42[c] | 13.16[bc] | 10.04[ab] |

Mean values along the same column that are carrying different superscripts are significantly different at $P \leq 0.05$

### 4.4.2.3 Available Potassium (Meq/100g).

As observed for the 2 preceeding elements, Table 13 shows that at all the sampling times under consideration, the Treatments had a significant ($P \leq 0.05$) effect on the level of potassium in the soil. The results from this research also show that SEO pollution caused a significant reduction in the level of potassium in the soil at the baseline point (Table 13, baseline values for $T_3$, $T_4$, $T_5$ and $T_6$ compared with $T_1$, $T_2$, $T_7$ and $T_8$). The addition of *A.oryzae* to a SEO polluted soil (with or without a vegetation cover) clearly caused a significant increase in the level of available potassium in the soil by the 3$^{rd}$ and 6$^{th}$ months (Table 13, $T_3$ and $T_4$ compared with $T_1$, $T_2$, $T_7$ and $T_8$).

4.4.2.4 Available Magnesium (Meq/100g)

Results from this work showed that all the Treatment had a significant ($P \leq 0.05$) effect on the amount of magnesium found in the soil at all the periods in which sampling was done (Table 14). Results from this research also showed that the pollution of a soil with SEO (irrespective of the presence or the absence of a mycoremediation agent and or vegetation cover) caused a significant reduction in the amount of soil magnesium at the baseline point (Table 14, baseline values for $T_3$, $T_4$, $T_5$ and $T_6$ compared with $T_1$, $T_2$, $T_7$ and $T_8$). The effect of the addition of *A.oryzae* (on available soil magnesium) as a mycoremediation agent to the SEO polluted soil however shows clearly that by the 3$^{rd}$ and the 6$^{th}$ months after the application of Treatments, this fungus caused a significant increase in the level of magnesium in the soil (Table 21B, $T_3$ compared with $T_1$ and $T_2$). The presence of vegetation cover in a SEO polluted soil however appears to have caused a significant reduction in the ability of *A.oryzae* to increase the level of magnesium in the soil (Table 14, $T_4$ compared with $T_3$).

TABLE 13: Mean separation (using Duncan's multiple range test) for the effect of the Treatments on the available potassium (meq/100g) in the soil at the different sampling times

| S/N | TREATMENT | BASELINE | 3RD MONTH | 6TH MONTH |
|---|---|---|---|---|
| 1 | **T1** (NOT WEEDED, NOT POLLUTED AND NO A.ORYZAE ADDED) | 3.13[a] | 3.15[a] | 2.92[c] |
| 2 | **T2** (WEEDED, NOT POLLUTED AND NO A.ORYZAE ADDED) | 3.11[a] | 2.99[cd] | 2.72[f] |
| 3 | **T3** (WEEDED, POLLUTED AND A.ORYZAE ADDED) | 0.81[e] | 3.14[a] | 2.85[e] |
| 4 | **T4** (NOT WEEDED, POLLUTED AND A.ORYZAE ADDED) | 0.86[d] | 3.17[a] | 2.95[b] |
| 5 | **T5** (WEEDED, POLLUTED,NO A.ORYZAE ADDED) | 0.74[f] | 2.99[cd] | 2.69[g] |
| 6 | **T6** (NOT WEEDED, POLLUTED,NO A.ORYZAE ADDED) | 0.70[g] | 3.11[ab] | 2.99[a] |
| 7 | **T7** (WEEDED, NO POLLUTION, A.ORYZAE ADDED) | 2.96[c] | 2.95[d] | 2.56[h] |
| 8 | **T8** (NOT WEEDED, NO POLLUTION, A.ORYZAE ADDED) | 3.01[b] | 3.05[bc] | 2.86[d] |

**Mean values along the same column that are carrying different superscripts are significantly different at $P \leq 0.05$.**

TABLE 14: Mean separation (using Duncan's multiple range test) for the effect of the Treatments on the available magnesium (meq/100g) in the soil at the different sampling times

| S/N | TREATMENT | BASELINE | 3RD MONTH | 6TH MONTH |
|---|---|---|---|---|
| 1 | **T1** (NOT WEEDED, NOT POLLUTED AND NO *A.ORYZAE* ADDED) | 1.58[a] | 1.07[b] | 1.03[a] |
| 2 | **T2** (WEEDED, NOT POLLUTED AND NO *A.ORYZAE* ADDED) | 1.53[a] | 1.07[b] | 1.02[b] |
| 3 | **T3** (WEEDED, POLLUTED AND *A.ORYZAE* ADDED) | 0.55[c] | 1.07[b] | 1.03[a] |
| 4 | **T4** (NOT WEEDED, POLLUTED AND *A.ORYZAE* ADDED) | 0.47[d] | 1.05[c] | 1.01[c] |
| 5 | **T5** (WEEDED, POLLUTED,NO *A.ORYZAE* ADDED) | 0.45[d] | 0.93[f] | 0.79[f] |
| 6 | **T6**(NOT WEEDED, POLLUTED,NO *A.ORYZAE* ADDED) | 0.45[d] | 1.13[a] | 1.00[d] |
| 7 | **T7** (WEEDED, NO POLLUTION, *A.ORYZAE* ADDED) | 1.11[b] | 0.97[e] | 0.72[g] |
| 8 | **T8** (NOT WEEDED, NO POLLUTION, *A.ORYZAE* ADDED) | 1.08[b] | 1.03[d] | 0.96[e] |

Mean values along the same column that are carrying different superscripts are significantly different at $P \leq 0.05$

### 4.4.3 Effect of the mycorememdiation agent on the density of the vegetation cover on the experimental site

A total of 30 weed species, which belongs to 16 families were encountered altogether on the field (Table 15)**.** The results in Table 16 show that each of the 8 Treatments had a significant ($P \leq 0.05$) effect on the vegetation cover (as a whole) of the experimental site at each of the different times of baseline, 2$^{nd}$ month, 4$^{th}$ month and 6$^{th}$ month after the application of the Treatments. Another general trend that is evident from Table 16 is the gradual increase in the overall population density of the vegetation on the experimental site from one sampling period to the next up till the final sampling at the 6$^{th}$ month after the application of the Treatments.

Also, it can be observed from Table 16 that at the baseline point, the mean vegetation density of the Treatment plots that has *A.oryzae* added to the SEO pollution ($T_3$ and $T_4$) was lower than what was obtained for the other Treatment plots. By the 2$^{nd}$ month after the application of the treatments (Table 16) however, there has been an increase in the mean vegetation density of these two Treatments ($T_3$ and $T_4$), at levels which was comparable to those of the control Treatment1 ($T_1$) and even better than for the control Treatment 2 ($T_2$). Also evident from Table 16 is the fact that the mean vegetation density for the Treatment plots that received only SEO pollution with no *A.oryzae* added ($T_5$ and $T_6$) had declined from 0.7276 and 0.7733 at the baseline point to 0.4933 and 0.6800 respectively at the 2$^{nd}$ month. The results from Table 16 also show that the addition of *A.oryzae* alone (to the exclusion of any SEO pollution) to the soil ($T_7$ and $T_8$) also caused an increase in the density of the vegetation to a level comparable to control Treatment1 ($T_1$) and higher than control Treatment 2 ($T_2$). Another trend from Table 16 is found in the comparison of the presence of vegetation cover on the soil before the application of *A.oryzae* ($T_4$ and $T_8$), and the removal of vegetation cover from the soil before the addition of *A.oryzae* ($T_3$ and $T_7$). In the

former case, the presence of vegetation cover caused a reduction in the ability of this fungus to cause an increase in the vegetation cover of the affected plots when compared to their counterparts that had no vegetation cover.

Results from Table 16 also show that by the $4^{th}$ month after the application of the Treatments, the addition of *A.oryzae* as a mycoremediation agent in the absence of vegetation cover on the soil ($T_{3,}$ and $T_7$) had caused an increase in the vegetation density of the soil to a level that was equal to the control Treatment 1, and to a level better than the control Treatment 2.

By the $6^{th}$ month however (Table 16), the results produced by the addition of *A.oryzae* as a mycoremediation agent to the soil on the vegetation cover shows that the addition of this fungus to the soil either in the presence or the absence of SEO pollution ($T_3$, $T_4$, $T_7$ and $T_8$) had resulted in an increase in the vegetation cover to a level higher than what was obtained for the two control Treatments ($T_1$ and $T_2$).

In all cases however, except at the baseline point, the addition of SEO to the soil without the mycoremediation agent *A.oryzae* ($T_5$ and $T_6$) consistently resulted in a low vegetation density of the affected plots.

For some individual weed species such as *A.gangentica* for example, the results of the statistical analysis show that the different Treatments had a significant ($P \leq 0.05$) effect on this weed at the different times of baseline, $4^{th}$ month and $6^{th}$ month after the application of Treatments (Table 17). The result of the mean separation for this weed further shows that the Treatment plots that had the mycoremediation agent i.e. *A.oryzae* added ($T_3$, $T_4$, $T_7$ and $T_8$) had by the $2^{nd}$ month through to the $6^{th}$ month shown an increase in the density of *A.gangetica* that was comparable by the $2^{nd}$ month, and higher by the $4^{th}$ and $6^{th}$ months after the application of the Treatments than the two control Treatment plots $T_1$ and $T_2$. For those Treatment plots that

received only SEO pollution and no *A.oryzae,* the results of the separation of means (Table 17) for this weed show that *A.gangentica* on these plots from the 2nd month after the application of Treatment consistently recorded the least density relative to all the other Treatment plots.

TABLE 15: Weed species encountered on the experimental site

| Family | Weed species |
| --- | --- |
| Asteraceae | *Assystasia gangentica, Emilia praetermissa, Synedrella nodiflora, Tridax procumbens, Lantana camara,* |
| Poaceae | *Axonopus compressus, Oplismenus burmannii, Cynodon dactylon, Setaria barbata, Panicum maximum, Paspalum scrobitulatum,* |
| Commelinaceae | *Commelina erecta* |
| Leguminosae | *Desmodium scorpiurus, Senna obtusifolia* |
| Euphorbiaceae | *Euphorbia hirta, Phylanthus amarus* |
| Amaranthaceae | *Gomphrena celosoides* |
| Melastomataceae | *Heterotis rotundifolia* |
| Convolvulaceae | *Ipomea involucrate* |
| Malvaceae | *Malvastrum coromandelianum, Sida acuta* |
| Cyperaceae | *Mariscus alternifolius, Cyperus haspan* |
| Rubiacea | *Mitracarpus villosus, Oldenlandia corymbosa* |
| Solanaceae | *Schwenckia Americana* |
| Portulacaceae | *Talinum triangulare* |
| Urticaceae | *Laportea aestuans* |
| Lamiaceae | *Solenostemon monostachyus* |
| Loganiaceae | *Spigelia anthelmia* |

TABLE 16: Effect of the Treatments on the overall mean of the vegetation cover (weed species) on the experimental site at the different times

| s/n | Treatment | Baseline point | 2nd Month | 4th Month | 6th Month |
|---|---|---|---|---|---|
| 1 | **T1**(NOTWEEDED, NOT POLLUTED AND NO *A.ORYZAE* ADDED) | 0.7800 | 0.9333 | 1.3867 | 1.5533 |
| 2 | **T2** (WEEDED, NOT POLLUTED AND NO *A.ORYZAE* ADDED) | 0.7267 | 0.7733 | 0.9133 | 1.6267 |
| 3 | **T3** (WEEDED, POLLUTED AND *A.ORYZAE* ADDED) | 0.5333 | 0.8800 | 1.3867 | 1.6400 |
| 4 | **T4**(NOT WEEDED, POLLUTED AND *A.ORYZAE* ADDED) | 0.4000 | 0.7800 | 1.1467 | 1.6267 |
| 5 | **T5** (WEEDED, POLLUTED,NO *A.ORYZAE* ADDED) | 0.7267 | 0.4933 | 0.6400 | 0.8533 |
| 6 | **T6**(NOT WEEDED, POLLUTED,NO *A.ORYZAE* ADDED) | 0.7733 | 0.6800 | 0.5133 | 1.2933 |
| 7 | **T7** (WEEDED, NO POLLUTION, *A.ORYZAE* ADDED) | 0.7800 | 0.8733 | 1.3867 | 1.8467 |
| 8 | **T8**(NOT WEEDED, NO POLLUTION, *A.ORYZAE* ADDED) | 0.8267 | 0.8667 | 0.9467 | 1.6733 |

TABLE 17: Mean separation (using Duncan's multiple range test) for the Treatment effect on *A.gangentica* at the different times

| s/n | Treatment | Baseline Mean values | $2^{nd}$ Month Mean values | $4^{th}$ Month Mean values | $6^{th}$ Month Mean values |
|---|---|---|---|---|---|
| 1 | **T1** (NOT WEEDED, NOT POLLUTED AND NO A.ORYZAE ADDED) | $1.40^{b}$ | $1.60^{b}$ | $4.20^{ab}$ | $10.40^{b}$ |
| 2 | **T2** (WEEDED, NOT POLLUTED AND NO A.ORYZAE ADDED) | $2.80^{a}$ | $2.80^{a}$ | $4.20^{ab}$ | $10.20^{b}$ |
| 3 | **T3** (WEEDED, POLLUTED AND A.ORYZAE ADDED) | $2.60^{a}$ | $2.80^{a}$ | $5.10^{a}$ | $12.40^{a}$ |
| 4 | **T4** (NOT WEEDED, POLLUTED AND A.ORYZAE ADDED) | $2.00^{ab}$ | $2.80^{a}$ | $5.30^{a}$ | $10.50^{b}$ |
| 5 | **T5** (WEEDED, POLLUTED,NO A.ORYZAE ADDED) | $2.50^{a}$ | $1.80^{ab}$ | $2.40^{c}$ | $5.60^{cd}$ |
| 6 | **T6** (NOT WEEDED, POLLUTED,NO A.ORYZAE ADDED) | $2.20^{ab}$ | $1.40^{b}$ | $2.00^{cd}$ | $6.80^{c}$ |
| 7 | **T7** (WEEDED, NO POLLUTION, A.ORYZAE ADDED) | $1.60^{b}$ | $2.80^{a}$ | $4.20^{ab}$ | $10.81^{b}$ |
| 8 | **T8** (NOT WEEDED, NO POLLUTION, A.ORYZAE ADDED) | $0.00^{c}$ | $2.20^{ab}$ | $4.80^{a}$ | $10.60^{b}$ |

Mean values carrying different superscripts along the same column are significantly different at $P<0.05$

### 4.5.0 Extraction and sequencing of the DNA of some of the fungal isolates

#### 4.5.1 Extraction of DNA

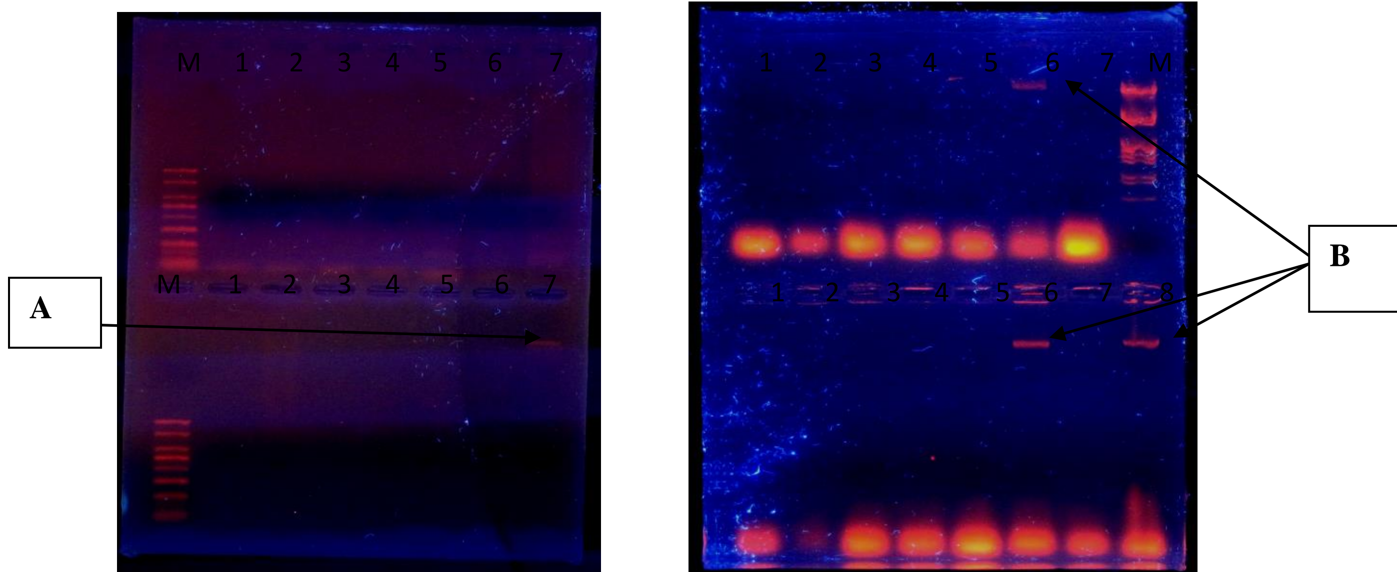
Plates 3A and 3B show some representative gel electrophoresis plates for the DNA extraction of the fungal isolates. Out of a total of 25 fungal samples presented for DNA extraction, DNA was found only in 14 of these fungal samples.

#### 4.5.2 DNA sequence of fungal isolates

The DNA sequence alignment and the phylogenetic tree for all the isolates are as presented in Figures 28A – I and Figure 29 respectively. The results of the DNA sequence show that the sequencing of the bases was done at between 10kbp - 270kpb (Appendixes 54A – 54Z and 55A – 55B). The result from the sequence alignment of the base pairs show much variation in this sequence at between 10 – 30kbp and 243 – 256kbp only (figures 28A and 28 I), at other points, the sequence of the base pairs for the DNA of all the fungal isolates were the same.

Based on the sequence of the base pairs of the DNA, the phylogenetic tree (figure 29) recognizes branching along 2 major divisions among the 14 fungal isolates. The 1$^{st}$ major grouping recognizes 100% consensus between *A*.niger (Unilag isolate), *A.tubingensis* (Sabo isolate 1), *A.tubingensis* (Sabo isolate 2), *A.oryzae* (Sabo isolate 3), *A.niger* (Sabo isolate 3F) and *A.niger* (Sabo isolate 4F). On its own, the 2$^{nd}$ grouping however recognizes just 87% consensus among the remaining 8 isolates, except however for IRS_fun 10F (*A.oryzae*, Shodex isolate) which is quite distinct from all the other isolates (Figure 29). In the 1$^{st}$ group, the 1$^{st}$ 2 species i.e. *A.niger* (Unilag isolate) and *A.tubingensis* (Sabo 1 isolate) are 100% homologous to each other. These 1$^{st}$ 2 isolates (figure 29) are however 54% homologous to the others (as

identified in the key to figure 29). In the 2nd major division, the 1st 2 isolates are 36% homologous to each other, while the extent of similarity amongst the other isolates are as shown in Figure 29, and the identities and the sources of the isolates are as shown in the key to Figure 29.




Plates 3A and B: Representative electrophorogram of the extracted fungal DNA Samples: A and B, Chromosomal DNA; M, marker (Lambda DNA Hind III), 1,2, 3, *A.niger*, 4 and 5, *A.oryzae*; 6 and 7, *A.tubingensis.*

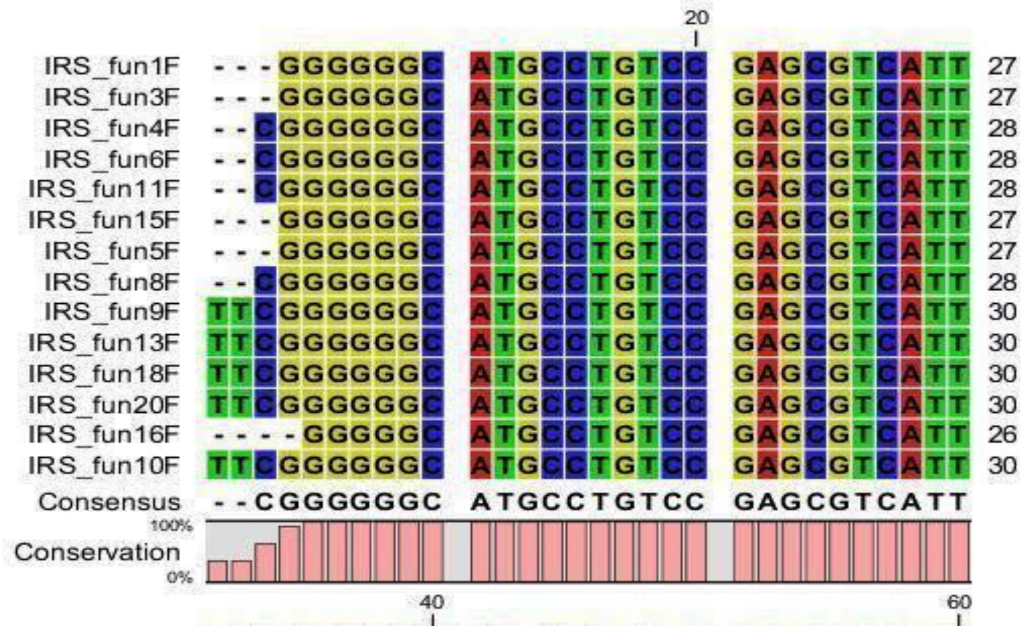



Figure 28 A: Sequence alignment for the 14 fungal isolates at between 10 -30kbp

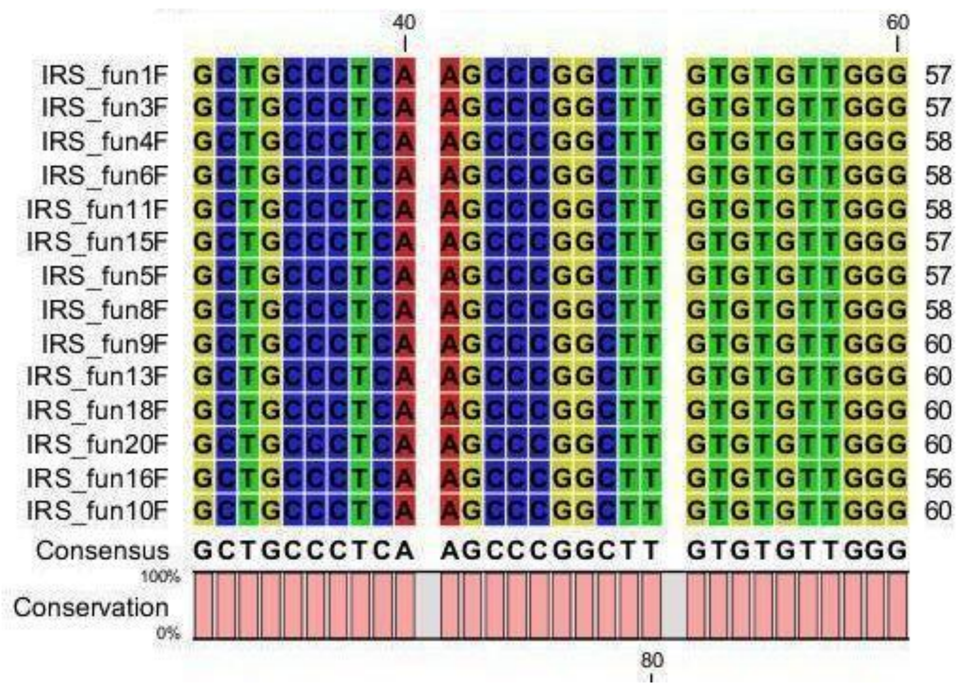



Figure 28 B: Sequence alignment for the 14 fungal isolates at between 31 -60kbp

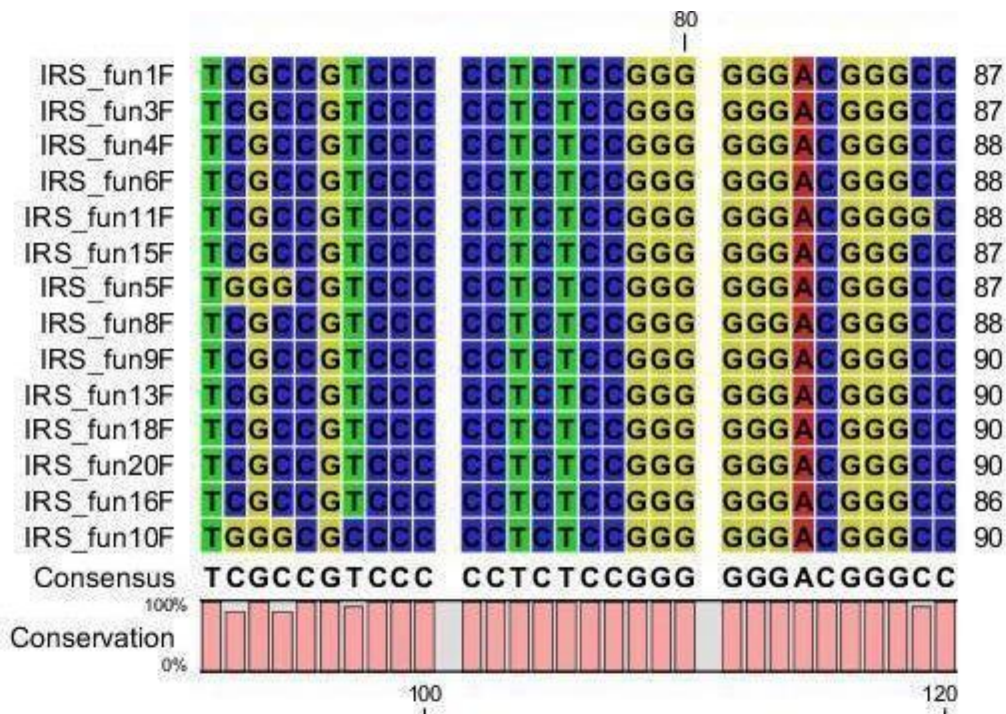



Figure 28 C: Sequence alignment for the 14 fungal isolates at between 61 -80kbp

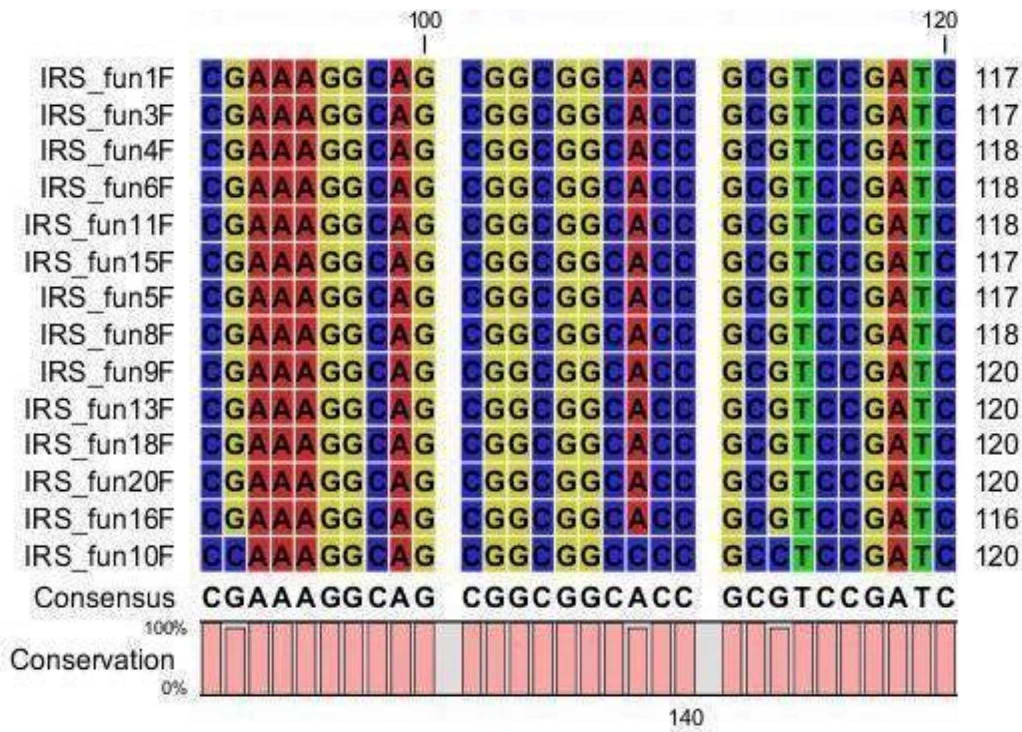



Figure 28 D: Sequence alignment for the 14 fungal isolates at between 91 -120kbp

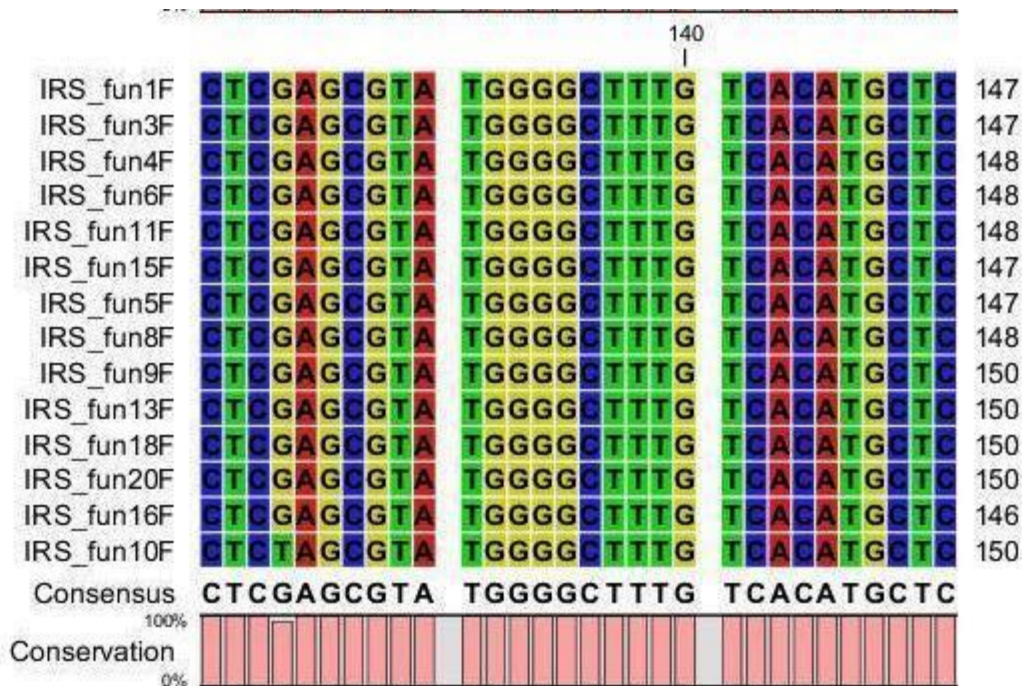



Figure 28 E: Sequence alignment for the 14 fungal isolates at between 121 -150kbp

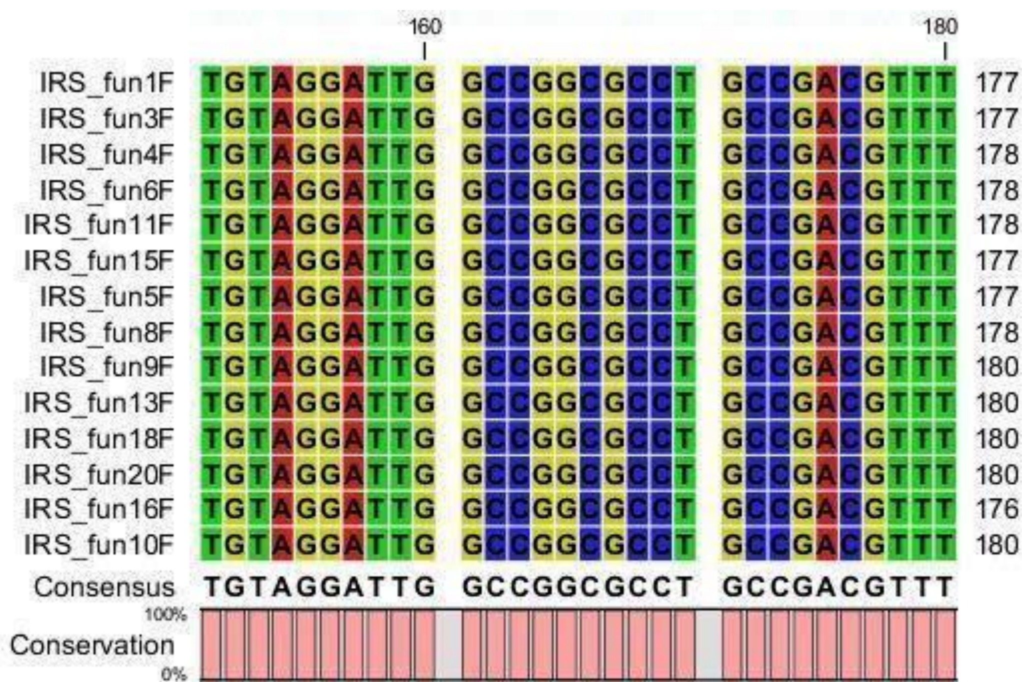



Figure 28 F: Sequence alignment for the 14 fungal isolates at between 151 -180kbp

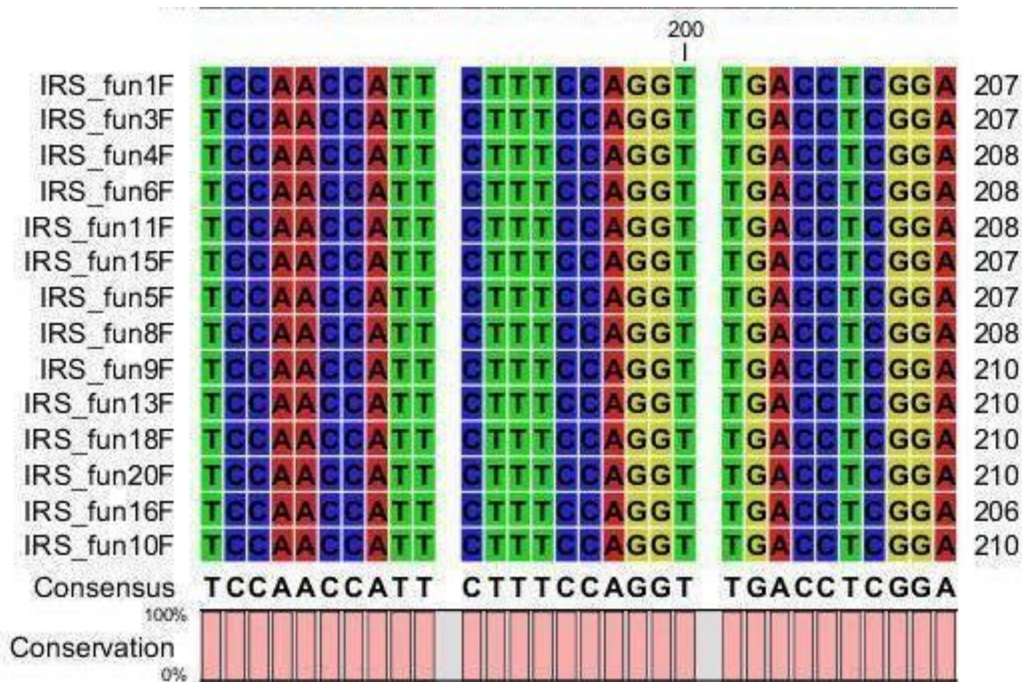



Figure 28 G: Sequence alignment for the 14 fungal isolates at between 181 -210kbp

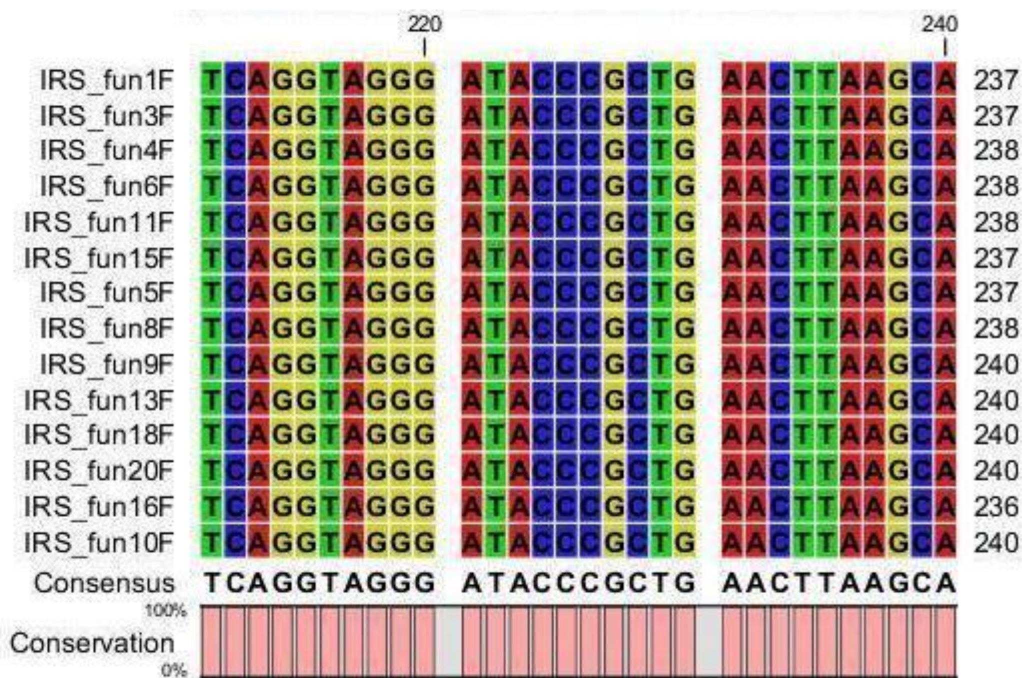



Figure 28 H: Sequence alignment for the 14 fungal isolates at between 211 -240kbp

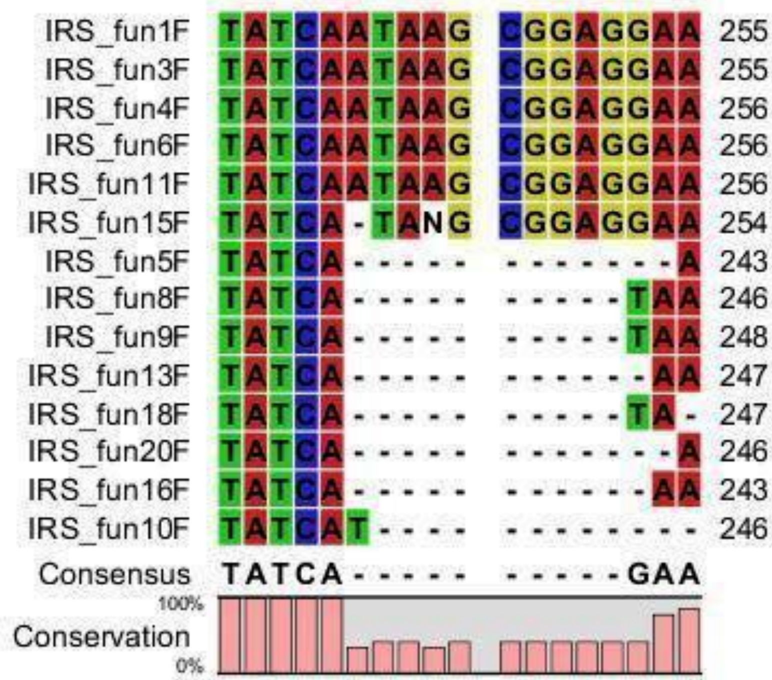



Figure 28 I: Sequence alignment for the 14 fungal isolates at between 241 -256kbp

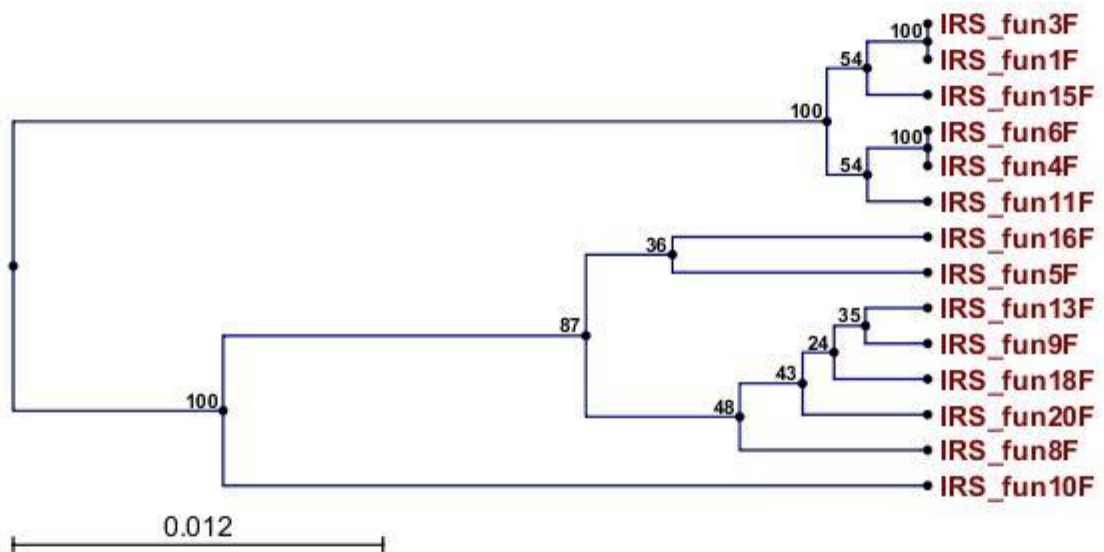



Figure 29: THE PHYLOGENETIC TREE FOR SOME OF THE FUNGAL ISOLATES

**KEY TO THE PHYLOGENETIC TREE OF FUNGAL ISOLATES.**

IRS_fun3F = *Aspergillus niger* (Biological Gardens, Unilag).

IRS_fun1F = *Aspergillus tubingensis* (Mechanic Village, Sabo 1).

1RS_fun15F = *Aspergillus tubingensis* (Mechanic Village, Sabo 2).

IRS_fun6F = *Aspergillus oryzae* (Mechanic Village, Sabo 3).

IRS_fun4F = *Aspergillus niger* (Mechanic Village, Sabo 3F).

IRS_fun11F = Aspergillus *niger* (Mechanic Village, Sabo 4F).

IRS_fun16F = *Aspergillus oryzae* ( Dump Site, Ojota 2).

IRS_fun5F = *Aspergillus oryzae* (Dump site, Ojota 2).

IRS_fun13F = *Aspergillus niger* (Dump Site, Ojota 3).

IRS_fun9F= *Aspergillus oryzae (I.gabonensis,* Ajegunle 1).

IRS_fun18F = *Aspergillus niger* (Mechanic Village, Onitiri 1).

IRS_fun20F = *Aspergillus oryzae* (Mechanic Village, Onitiri 3).

IRS_fun8F = *Aspergillus oryzae (I.gabonensis,* Oyingbo 2).

IRS_fun10F = *Aspergillus oryzae* (Shodex Gardens 3F).

### 4.5.3 Identification and naming of fungal isolates

Based on the nucleotide arrangements, and using the software package CLC sequencer, the sequenced DNA data were identified as outlined below. The results below appears to be suggesting that *A.orzae* and *A.tubingensis* are both strains of *A.niger* which have wxperienced some changes in their nucleotide sequence possibly on account of differences in their environments.

BG1 (Biological Gardens, Unilag).

NNNNNNNNNNNNNNNNNN

GGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCC
CCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGG
CTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTGA
CCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA 255

*Aspergillus niger* strain 100%

SAB1 (Mechanic Village, Sabo).

NNNGTNNNNNNNGGNNTNNN

GGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCC
CCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGG
CTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTGA
CCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA 255

*Aspergillus niger strain* *100%*

*Aspergillus tubingensis* 100%

SAB2 (Mechanic Village, Sabo).

NNNNNNNNNNNGNNNN

CGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCC
CCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGG
GCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTG
ACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA 256

*Aspergillus niger strain* *100%*

*Aspergillus tubingensis* 100%

SAB3 (Mechanic Village, Sabo).

NNNNNNNNCNNNNNNTNN

GGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTGGGCGTCCC
CCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGG
CTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTGA
CCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAA 243

*Aspergillus oryzae* *100%*

*Aspergillus niger* strain 100%

SAB3F (Mechanic Village, Sabo).

NNNNNNNNNCNNNNNNTN

CGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCC
CCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGG
GCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTG
ACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA 256

*Aspergillus niger strain* 100%

SAB4F (Mechanic Village, Sabo).

NNNGNNNNCNNNNNNTN

CGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCC
CCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGG
GCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTG
ACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCATAA 246

*Aspergillus niger strain* 100%

OJT2 (Olushosun Dumpsite, Ojota).

NNNNNNNNCNNGGNN

TTCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGT
CCCCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGG
GGCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTT
GACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCATAA 248

*Aspergillus oryzae* 100%

*Aspergillus niger* strain 100%

OJT2F (Olushosun Dumpsite, Ojota).

NNNNNNCCNGGNN

TTCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTGGGCGC
CCCCCTCTCCGGGGGGACGGGCCCCAAAGGCAGCGGCGGCCCCGCCTCCGATCCTCTAGCGTATGG
GGCTTTGTCACATGCTCTGTAGGAT

TGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCC
GCTGAACTTAAGCATATCAT 246

*Aspergillus oryzae* *100%*

*Aspergillus niger* strain 100%

OJT3 (Olushosun Dumpsite, Ojota).

NNNNNNNNNCNNNNNNTN

CGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCC
CCCTCTCCGGGGGGACGGGGCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGG
GCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTG
ACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA 256

*Aspergillus niger strain* 100%

AJG Iv1 (*I.gabonensis*, Ajegunle Market)

NNNNNNNCNNNNNN

TTCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGT
CCCCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGG
GGCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTT
GACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAA 247

*Aspergillus oryzae* *98%*

*Aspergillus niger* strain 98%

ONT1 (Mechanic Village, Onitiri-Akoka).

NNNNNNNNGNNTTCN

GGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCC
CCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGG
CTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTGA
CCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCATANGCGGAGGAA 254

*Aspergillus niger strain* 100%

ONT3 (Mechanic Village, Onitiri-Akoka).

NNNNNN

GGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCCC
CTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGC
TTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTGAC
CTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAA 243

*Aspergillus oryzae* *99%*

*Aspergillus niger* strain 99%

OYGIv2 (*I.gabonensis*, Oyingbo Market)

NNNNNNCNNGNN

TTCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGT
CCCCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGG
GGCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTT
GACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCATA 247

*Aspergillus oryzae* 98%

*Aspergillus niger* strain 98%

SHO3F (Shodex Gardens, Anthony-Village).

NNNNNNNNNGGNN

TTCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGT
CCCCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGG
GGCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTT
GACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAA 246

*Aspergillus oryzae* 99%

*Aspergillus niger* strain 99%

# CHAPTER FIVE.

## 5.0 DISCUSSION

With respect to the hydrocarbon utilizing ability of fungi species, the results from this work show that the fungal species associated with pathogenic conditions in the seed of *I.gabonensis,* and the fungal species from different soils are all capable of degrading petroleum hydrocarbon and vegetable hydrocarbon. This finding is in agreement with many previous reports, where filamentous fungi in particular were severally reported to have degraded a whole array of hydrocarbon containing compounds (Zobell, 1946; Adekunle and Oluyode, 2005; Saratale *et al.,* 2007, George-Okafor *et al*., 2009, Reuben *et al*., 2011) by producing capable enzymes. On account of their aggressive growth, greater biomass production and extensive hyphal growth in soil, fungi offer potential for mycoremediation technology (Kenneth, 1995; Saadoun 2002, Obire and Anyanwu, 2009). It is of interest that 2 strains of *Aspergillus niger* i.e. *A.oryzae* and *A.tubingensis* from the results obtained from this work are shown probably for the first time in any report to have the ability to utilize different types of petroleum and vegetable hydrocarbon compounds, and for *A.oryzae* in particular, to be capable of efficiently mycoremediating a spent engine oil (SEO) polluted soil.

The results obtained from this study indicate that all the 25 different fungal isolates that were employed in the mycodegradation test (the measurement of the optical density of the different hydrocarbon compounds in the laboratory) for hydrocarbon utilization were all capable of degrading and utilizing all the 6 different hydrocarbon sources that were used in this research. This is so because the optical density for most of the oils containing each of the different fungal species were consistently higher than what was obtained for the controls that do not contain any fungus. In this respect

however, the *A.oryzae* isolated from the *I.gabonenesis* seed from Ajegunle market in particular appears to be more efficient at utilizing the different hydrocarbon sources, especially the SEO. This is because this fungus showed consistently rank within the first five (out of 25 fungal isolates) most optically active fungi during the entire sampling period of 40 days.

It is known that communities of microrganism have been shown as petroleum hydrocarbon utilizers. Amund *et al.* (1987) commented that soil pollution either due to oil spillage or indiscriminate dumping of refuse is a common phenomenon in many places in Nigeria, hence the wide spread presence in our environment of many species of bacteria, fungi and algae that have the enzymatic capability to utilize petroleum hydrocarbons as food. A report by Ekundayo and Obire (1987) have shown that a marked change in properties occur in soils polluted with petroleum hydrocarbons, thus affecting the physical, chemical, biological and microbiological properties of the soil. According to Vwioto *et al*. (2006) oil pollution of soil leads to build up of essential (organic carbon, phosphorus, calcium, magnesium) and non essential (magnesium, zinc, iron, cobalt, copper) elements in soil and the eventual translocation in plant tissue. This submission probably explains the trend seen in the results obtained in this work where after the baseline, there was a general increase in the level of all the elements that were tested for in the soils that were polluted with SEO over and above the control soils that were not polluted with SEO. Furthermore, even between the soils polluted with SEO, those Treatments that had *A.oryzae* added to the SEO pollution generally showed a higher nutrient level than those Treatments that did not have *A.oryzae* added to their SEO pollution. These results seem to be highlighting the role of fungi in the soil mineralization process as reported by Covino *et al*. (2010).

With respect to the vegetation and the nutrient studies, the ability of *A.oryzae* to mycoremediate a spent engine oil polluted soil was evident when the mean count of the weed *A.gangentica* and the level of all the macronutrients investigated are compared, particularly for Treatments 3 and 5. The exclusive effect of the mycoremediation agent (*A.oryzae*) on spent engine oil (SEO) polluted field can be illustrated by contrasting Treatments 3 and 5 ($T_3$ and $T_{5)}$. This contrast shows that this fungus on a general note appears to have aided the recovery of some of the weed species after exposure to spent oil pollution by causing some varying degree of increase in the density of the different weed species especially at the 2$^{nd}$ month after the application of the Treatments. From the foregoing (i.e. the results for Treatments 3 and 5 for the macronutrient and weed studies), it can be seen that the application of *A.oryzae* to a spent engine oil polluted soil caused a general elevation in the soil macronutrients that were tested for and in the weed population density. At 2$^{nd}$ month after the application of the Treatments (when some of the oil had probably been degraded), this elevation in the soil macronutrient probably caused the corresponding increase in the density of most weed species as seen from the results. The observed increase in the level of some soil macro nutrients that were found in this work following the degradation of the spent engine oil (as from the 2nd month after the application of the Treatments) corroborates the position of earlier workers such as Udo and Fayemi (1975) and Odu (1979). These workers all reported an increase in the organic matter content, total carbon and nitrogen in petroleum contaminated soils when compared with normal soils. Udo and Fayemi (1975) also reported increases in carbon: nitrogen ratio (C:N ratio) in oil contaminated soils. Adams and Ellis (1960) reported increases in the phosphorus content of oil contaminated soil, a position which agrees with the findings in this work. The general increases that was observed

in the level of the investigated soil macronutrients especially on those spent engine oil polluted plots that had *A.oryzae* added might be attributable to the large changes in the redox (oxidation and reduction) potential of oil contaminated soils as was first reported by Adams and Ellis (1960). Another evidence shown in this work as to the ability of *A.oryzae* to mycoremediate a petroleum hydrocarbon polluted environment is a comparison of the results of the Gas Chromatography (GC) determined initial and final TPH levels in the soil between Treatments 3 and 5, and Treatments 4 and 6. To the exclusion of any other factor, the extent of reduction achieved in the final level of TPH in the soil can be adduced directly to the action of the *A.oryzae* that was added to the spent engine oil (SEO) polluted soil. This results agrees with the findings of Thangarajan *et al.* (2011) where the reduction in the TPH level as a result of bioaugementation of a hydrocarbon polluted soil with the fungus *Scedosporium apiospermum* consistently produced a lower TPH value (at least marginally) from day 0 – day 35 than the naturally attenuated soils.

In addition, the action of *A.oryzae* in this work appears to further reinforce the belief in the role of fungi in the soil humification process (Covino *et al.*, 2010). Furthermore, it appears that in this studies, the presence of vegetation appears to have caused a reduction in the efficiency of this fungus to mycoremediate the spent engine oil pollution as evident when the results for Treatments 4 and 6 are compared with those of treatments 3 and 5 for most of the weed species, the final TPH levels in the soil and the level of the macro nutrients that were studied in this work.

It should be noted that the response of terrestrial plants to oil pollution differ and are highly species specific (Mason, 1991). This varied reaction may be influenced by factors such as the thickness of the cuticle, permeability of the leaves, species environmental condition, the root system, the level of the food reserve in the plant and

the type of oil spilled (Moore and Ramamoorthy, 1984). In view of all these, it should not be surprising therefore that the results of this work showed weed species such as *Assystasia gangentica* and *Axonopus compressus* with thick cuticle, prolific root system and large food reserve surviving theSEO pollution, while such species as *Commelina erecta, Synedrella nodiflora, Setaria barbata* and other less hardy and persistent species encountered in this studies disappeared after the pollution.

In terms of the ethical issues surrounding the spread of microorganisms, the results from this research show that the application of *A.oryzae* to the soil (either in the presence or absence of SEO) does not have any adverse effect on vegetation growth. This is because for most of the weed species encountered in this study, the application of *A.oryzae* to the soil (as compared to those soils that does not have *A.oryzae* added) did not cause any significant change in the population density of most of the weed species.

The results from this study indicate measurable differences in the hydrocarbon utilization potential of the fungal isolates. This is due perhaps to the differences in their habitats. The result from the growth studies of the fungal isolates in crude oil and spent e was indicated by a relatively lower optical density (OD). This might be due to the fact that these two hydrocarbon sources have darker colours, thus higher ODs were obtained in the early stages of the experiment, and this reduced with time. On the contrary, optical activity in fresh engine oil, diesel and kerosene were indicated by lower ODs due to their lighter colours. The *A.oryzae* isolate from *I.gabonensis* seed (from Ajegunle market) distinguished itself in its growth ability in the media SEO media as indicated by the results obtained for the means separation for the different fungal isolates in this media. Another group of fungal isolates with a commendable rate of growth in most of the petroleum hydrocarbon sources were those isolates from

the soils from the mechanic villages, especially the *A.oryzae* from the mechanic village in Sabo. As explained by Atlas and Bartha (1972), this could probably be due to the fact that when an environment is contaminated with petroleum hydrocarbon, the indigenous microbial population that are found in this type of environment are likely to contain populations that have a higher affinity for the degradation of petroleum hydrocarbon. For the growth rate of all the fungal isolates in the other hydrocarbon sources, the results for the means separation of the OD shows a highly variable result, thus indicating some differences in the ability of each of the different fungal isolate to grow in each of the different hydrocarbon sources. On a general note however, it was observed that the fungal isolates had a higher growth rate in the media containing hydrocarbons when compared to the media having only the minimal salt solution (MSS) i.e. the control. A probable reason for this may be as a result of the higher concentration of the nutrient source (notably carbon) which is more abundant in those media that contain hydrocarbon; and these hydrocarbons are required for their growth (Shaw, 1995). The result obtained from this study shows that fungi responses to oil pollution through the emulsification of the oil is generally indicated by the reduced viscosity of the oil as time progresses. An increase or a decrease in the turbidity of the media is reflected by an increase or a decrease in the OD of the media, which in turn reflects as a rise or a decline in the number of the cells of the fungal isolate in the media. The different fungal isolates showed a marked fluctuation in their growth i.e. a sinusoidal growth curve, with occasional maximum and minimum values obtained at different times during the experiment. The reason for the fluctuations as observed in this work may be due to the release of some organic acids (on account of the metabolic activities of these organisms) into these media. These organic acids (and some other metabolites) probably causes lysis and death of the cells hence the

attendant fluctuations in the growth pattern ( Okpokwasili and James, 1995; Adebusoye *et al*., 2007a).

The differences recorded in the growth pattern of the different fungi in the minimal salt solution (MSS) might be due to the fact that each fungus utilized each of the different hydrocarbon sources at different rates. This observed differences in their rate of utilizing these different hydrocarbons might be due to each of these fungi secreting different types of enzymes, or if the same or similar types of enzymes, then at different rates (Keeler, 1991). From the foregoing, there is therefore some suspicion that some of the fungi obtained in this work might actually be entirely new species as suggested by the DNA sequence and the consistent differences in their rates of utilizing the six different hydrocarbon sources.

Lipids are one of the major constituents of foods, and are important in our diet. They are a major source of energy and provide essential fatty acids (EFAs). EFAs are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them. The term "essential fatty acid" refers to fatty acids required for biological processes, and not those that only act as fuel (Ellie and Rolfes, 2008). Notwithstanding, the over-consumption of certain fat components can be detrimental to our health, e.g. cholesterol and saturated fats. The results of the free fatty acid profiles of the oil expressed from *I.gabonensis* seeds shows this oil to be rich in a complex of fatty acids, particularly the unsaturated fatty acids which have been generally acclaimed as being good for human health (Mozaffarian *et. al*., 2004). The oils extracted from healthy seeds of *I.gabonensis* had a lower amount of saturated fatty acids of when compared to the oil expressed from *A.oryzae* infected seeds of *I.gabonensis*.

This reduction in the amount of the unsaturated fatty acids observed in the *A.oryzae* infected seeds of *I.gabonensis* may be because these group of fatty acids are believed to be more vulnerable to oxidative degradation than their saturated counterparts (Leibovitz *et al*., 1990), a phenomenon which *A.oryzae* appears to have contributed to. The values obtained for the different fatty acid components of the oils extracted from both the healthy and the diseased *I.gabonensis* seeds generally suggests that the oil from the healthy seeds of *I.gabonensis* is of higher nutritional value when compared to the oil from the *A.oryzae* infected seeds of *I.gabonenesis*. This is because the former had a higher proportion of unsaturated fatty acids and a corresponding lower proportion of saturated fatty acids (Leibovitz *et al*., 1990). Polyunsaturated fatty acids have been indicated in the protection of mammals against cardiac arrhythmias, and a lowered resistance to insulin resistance while the monounsaturated fatty acids have also been reported as not having a positive correlation with coronary atherosclerosis (Storlien *et al*., 1996). When compared to the values provided by Gebhardt and Thomas (2002), in terms of fatty acid composition, the oil expressed from the seed of *I.gabonensis* is higher in quality when compared to butter (66.1% saturated fatty acid, 30.3% monounsaturated fatty acid and 3.7% polyunsaturated fatty acid) and margarine (20.4% saturated fatty acid, 43.6% monounsaturated fatty acid and 33.3% polyunsaturated fatty acid).

The physico-chemical indices of the expressed oil from the healthy seed of *I.gabonensis* showed that this oil is of comparable quality when compared to the oil from many other oil seeds such as *Bombax glabrum*, *Arachis hypogaea*, *Glycine max* and cotton seeds. In *B.glabrum* a relative density of 1.125 was reported by Adeleke and Abiodun (2010). This value falls above the Codex standard of between 0.891-0.926 recommended for most edible oils, whereas a relative density of 0.901 and

0.895 obtained for the oil from the healthy and the diseased seeds respectively of *I.gabonensis* falls within this Codex recommended range. In addition, the oil expressed from the seeds of *I.gabonensis* also showed a higher saponification value (224.040mgKOH/g and 236.173mgKOH/g for the healthy and diseased seeds respectively) than most oil seeds indexed by Codex except palm kernel oil (230-254mgKOH/g), babassu oil (245-256mgKOH/g) and coconut oil (248-265mgKOH/g). This high saponification value of the oil from the seeds of *I.gabonensis* thus makes it a potentially useful material for soap making business. The iodine value of 39.667 and 37.013g/100g for the oils from the healthy and the diseased respectively of *I.gabonensis* was also within the Codex recommended range (6.3-150g/100g) of iodine value for edible oils. The iodine value however has implications for unsaturation and rancidity. The lower the iodine value, the higher the level of unsaturation of the oil, and thus the less likely the possibility of oxidative degradation (rancidity) of the oil. In this respect, the oil from the seed of *I.gabonensis* appears to be satisfactory. Also, the level of the unsaponifiable matter found in the oils extracted from the seed of *I.gabonensis* (20.963 and 23.327g/kg for the healthy and the diseased seeds respectively) falls within the Codex recommended range of 12-28g/kg for edible oils. Acid value is the measure of the extent to which the glycerides in oil have been decomposed by lipase or other action (Ihekoronye and Ngoddy, 1985). In this regard therefore, comparing the acid values of the oil from the healthy and the *A.oryzae* infected seeds of *I.gabonensis*, the ability of this fungus -*A.oryzae*- to also secrete the lipase enzyme is suspected (which in this case is suspected of causing a deterioration in the quality of the oil). It has previously reported that some other pathogenic fungi which were found in association with some other oil seeds such as melon, soybean and *Detarium senegalense* by Adekunle and Uma (1996),

Adekunle and Oluyode (2005) and Adekunle and Adebambo (2007) produced this lipase enzyme. According to Adeleke and Abiodun (2010), the maximum recommended peroxide level in edible vegetable oil by Codex is 10meq/kg. The perioxide value in the oil from the healthy seeds of *I.gabonensis* falls within the Codex limit. However, the peroxide value of the oil from the diseased seeds of *I.gabonensis* is above the Codex limit for the peroxide values in edible vegetable oils. This is important because peroxide level is an indication of the deterioration of fats; meaning therefore that the action of *A.oryzae* on the seed of *I.gabonensis* clearly resulted into the spoilage of the seeds. Another frightening dimension in this regard was the peroxide value of 19.54meq/kg recorded by Ebuehi and Avwobobe (2006) for the oils from some melon seed in Nigeria. Another indicator of deterioration of the oil from the diseased seed of *I.gabonensis* is the higher level of free fatty acid (9.773%) when compared with that of the oil from the healthy seeds (6.013%). Free fatty acid as a parameter indicates the presence of fatty acids in the oil, and the higher the free fatty acid, the higher the fatty acid content of the oil, and thus the higher the possibility of oxidative deterioration (Okpokwasili and Molokwu, 1996; Atasie *et al.,* 2009). In other words, the higher the fatty acid composition of an oil, the lower the stability and the lower the shelf life of the oil (Enemuor *et al.,* 2012). Another important indices of deterioration attributable to the action of *A.oryzae* on the seed of *I.gabonensis* is the significant increase in the TBA value of the *A.oryzae* infected seed oil (18.727µg/g) when compared to (16.243µg/g) recorded for the oil from the healthy seeds. A higher TBA value is a clear indication of deterioration. Although the action of *A.oryzae* significantly reduced the oil yield in the seed of *I.gabonensis*. This notwithstanding, *I.gabonensis* seed can be said to be a very rich source of oil, as the percentage oil yield from this seed was higher than what was obtained for many other

oil seeds such as *Jugulans cinerea,* 46.15% (Essien and Amadi, 2009), Moringa oleifera, 34.80% (Anwar and Rashid, 2007), *Monechma ciliatum (black mahlab* seeds*),* 13.5% *and Prunus mahaleb (white mahlab seeds),* 30.95% (Mariod *et al.,* 2009).

In terms of its nutritional value, the protein level recorded for the healthy seed of *I.gabonensis* falls within the range 10.23 recorded for *B.glabrum* (Adeleke and Abiodun, 2010), but lower than recorded for *M.oliefera* seeds, 29.36% (Anwar and Rashid, 2007). Furthermore, *I.gabonensis* can be said to be a rich source of carbohydrate as the carbohydrate level in both the healthy and diseased seeds of *I.gabonensis* was higher than what was obtained for other oilseeds such as *B.glabrum*, 16.60% (Adeleke and Abiodun, 2010) and *Arachis hypogeae*, 1.81% (Atasie *et al*., 2009).

Fat is important in diets as it promotes the absorption of fat soluble vitamins. Also, fat is a high energy nutrient and does not add to the bulk of the diet (Atasie *et al*., 2009). The fat and fiber content obtained for both the healthy and diseased seeds of *I.gabonensis* in this work falls within the range of what was obtained for some other oilseeds such as *A.hypogeae* (Atasie *et al*., 2009) and *B.glabrum* (Adeleke and Abiodun, 2010). The crude fibre in this result indicates the ability of *I.gabonensis* seed to maintain a healthy physiological grip for a normal peristaltic movement of the intestinal tract. Diets low in crude fibre is undesirable as it could cause constipation and such diets have been associated with diseases of colon like piles, appendicitis and cancer (Atasie *et al*., 2009). Generally, in terms of the changes in the nutritional value of this oilseed-I.gabonensis-, the findings in this work generally agrees with the findings of Oladimeji and Kolapo (2008), where different microorganisms that were

inclusive of fungi were shown to cause a reduction in the nutritional worth of some oilseeds in Nigeria.

## 5.1 CONCLUSION

From the findings of this study, the fungus (*A.oryzae*) isolated from the diseased seeds of *I.gabonensis* from Ajegunle market appears to be more efficient at utilizing most petroleum hydrocarbon compounds, particularly the spent engine oil (SEO) as seen from the mycodegradation studies conducted in the laboratory. It is nevertheless evident that all the other fungi, irrespective of their habitat were capable of utilizing both petroleum and vegetable hydrocarbons (though at different rates) for their growth. The findings from the mycoremediation field trial showed that as a result of the addition of *A.oryzae* to the spent engine oil polluted soil, Gas Chromatographic (GC) analysis of the Total Petroleum Hydrocarbon (TPH) showed that there was a significant reduction in the TPH level in the soil 6 months after the application of the Treatment. In addition, mycoremediating a SEO polluted soil with this *A.oryzae* also resulted in a significant increase in the level of the following macronutrients N, P, K and Mg from the 3$^{rd}$ month up to the 6$^{th}$ month into the mycoremediation studies. Also, the addition of *A.oryzae* as a mycoremediation agent in a SEO polluted soil did not adversely affect the vegetation cover that was present on the field.

The findings from the physico-chemical characterization of the expressed oil from the seeds of *I.gabonensis* and from the seed flour itself showed that the oil and the seed of *I.gabonensis* are of a high nutritional value. This is because this oil showed a high value that falls within the recommended limits for all the parameters indicated by Codex for edible oils. These parameters include the concentration of the saturated and

unsaturated fatty acids, iodine value, cholesterol level, percentage yield and relative density. When compared with other oilseeds such as groundnut, melon seed, cotton seed and cashew nut seed, the seeds of *I.gabonensis* showed a higher level of carbohydrate, fats, protein and fiber content. In addition, the oil expressed from the *A.oryzae* infected seeds in particular show much promise for industrial application in soap making because of its high saponification value.

In terms of their ability to cause a deterioration however, fungal species (*A.oryzae*) found associated with diseased seeds of *I.gabonensis* caused a deterioration in the quality of the oil, and the nutritional value of the seeds as parameters such as the peroxide value, TBA value, acid value and other parameters that are indicative of deterioration in oils were significantly higher in the oil from the diseased seeds as compared to the oil from the healthy seeds. Also, the corresponding parameters that are indicative of nutritional quality such as protein, fats, fiber, carbohydrate, moisture and energy were significantly lower in the diseased seeds than in the healthy seeds.

## 5.2 SUMMARY OF FINDINGS

- This research is probably the first to report on pathogenic fungal species associated with the diseased seeds of *I.gabonensis* in the open market and to conduct a DNA sequence on these fungal isolates.
- DNA sequence identified the following fungi from diseased *I.gabonensis* seeds: 1 strain each of *Aspergillus oryzae* from Oyingbo and Ajegunle Markets. From the soil of the following places, DNA sequence equally identified the following: 3 strains of *A.oryzae* from Olushosun dumpsite,

Ojota; 1 strain each of *A.niger* and *A.oryzae* from Mechanic Village, Onitiri; 2 strains each of *A.tubingensis* and *A.niger* and 1 strain of *A.oryzae* from Mechanic Village, Sabo and 1 strain each of *A.niger* from Biological Gardens, Unilag and Shodex Gardens, Anthony Village.

- DNA studies suggest that differences in environmental conditions may have caused some changes in the genetic composition of the same species of fungus, and that this may have caused a significant difference in their abilities to utilize petroleum and plant hydrocarbon compounds.
- Some fungi that were isolated from diseased *I.gabonensis* seeds and the different soils (such as the soils from the automechanic workshops, the public dump site and from the two agricultural sites) were all found to be capable of utilizing hydrocarbons both of petroleum and vegetable origin.
- *A.oryzae* isolated from the *I.gabonensis* seeds from Ajegunle market from studies in the laboratory appeared to be more efficient at utilizing petroleum and vegetable hydrocarbon compounds compared to those from the other sources.
- The addition of *A.oryzae* (isolated from the diseased seeds of *I.gabonensis*) to a spent engine oil (SEO) polluted soil caused a significant reduction in the level of Total Petroleum Hydrocarbon (TPH) burden left in the soil 6 months after the pollution. This Treatment also resulted in a significant rise in the level of some soil macro nutrients 3 months and 6 months after the pollution with SEO and the remediation with this fungus.

- The addition of *A.oryzae* (isolated from the diseased seeds of *I.gabonensis*) to a SEO polluted soil also caused a significant increase in the abundance of some weeds. These weeds are thus perceived to be showing some promise for exploitation as a phytoremediation agent.
- The presence of vegetation cover on a soil prior to the occurrence of oil pollution caused a reduction in the amount of the pollutant, and consequently, the amount of pollution (TPH) in the soil.
- *Aspergillus oryzae* (isolated from diseased *I.gabonensis* seeds) caused some significant changes in the physicochemical and the nutritional qualities of *I.gabonensis* seed as well as the oil expressed from these seeds.
- The fatty acid composition of the oils from both the healthy and *A.oryzae* infected seeds of *I.gabonensis* were determined and their respective concentrations in these seeds were also documented.

## 5.3 CONTRIBUTIONS TO KNOWLEDGE

(1) To the best of my knowledge, this work is a first report on the isolation and DNA sequencing of fungi associated with disease conditions in *I.gabonensis* seeds.

(2) The genetic information of the different fungal isolates from various environments has been deposited in a Gene Bank, thus contributing to information on the genetic diversity of fungal species.

(3) This work is most likely a first report at comparing the efficiency of pathogenic fungal species from an oilseed and fungal species from different soil environments at utilizing petroleum and vegetable hydrocarbon compounds.

(4) *A.oryzae* isolated from diseased seeds of *I.gabonensis* in this study has the potential to mycoremediate spent engine oil polluted soil effectively

(5) *A.oryzae* (isolated from the diseased seeds of *I.gabonensis*) caused deterioration in the nutritional component of *I.gabonensis* seed flour, the free fatty acid profile and changes in the physico-chemical integrity of the oil found in *I.gabonensi*s seed.

### 5.4 SUGGESTIONS FOR FUTURE WORK

To elucidate the specific genes and enzymes of the fungi that are responsible for the degradation of each hydrocarbon source and to further understand their mechanism of action in situ and ex situ in a pollution site.

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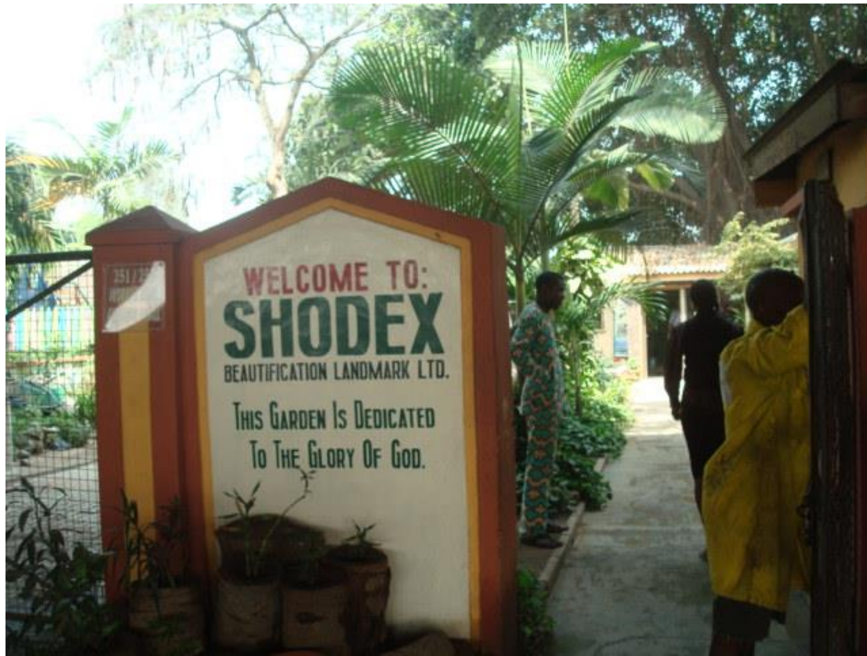
# APPENDICES

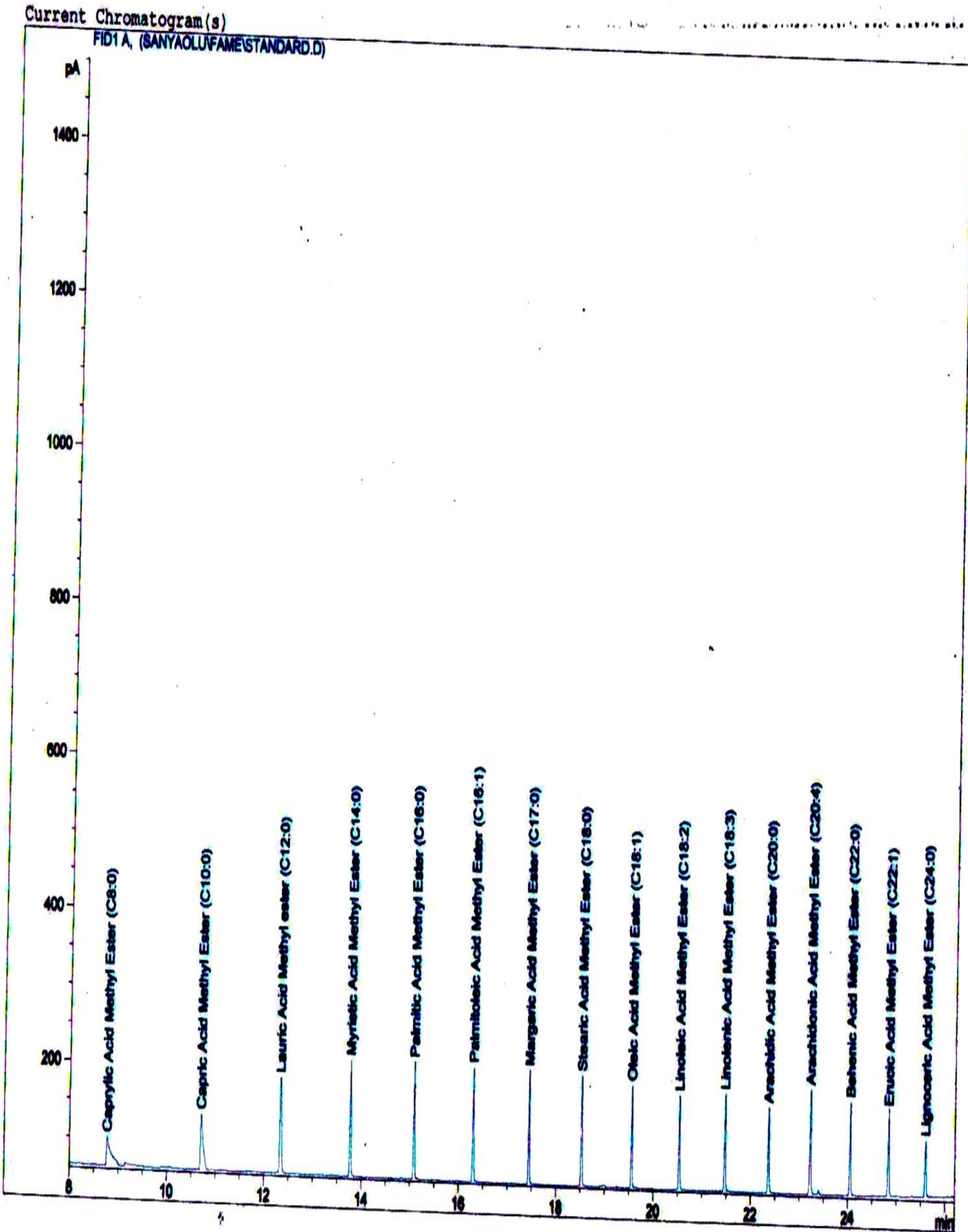
APPENDIX 1A: OLUSOSUN DUMP SITE, OJOTA-LAGOS.

APPENDIX 1B: OLUSOSUN DUMP SITE, OJOTA-LAGOS




APPENDIX 1C: SHODEX GARDENS, ANTHOHY VILLAGE, LAGOS.

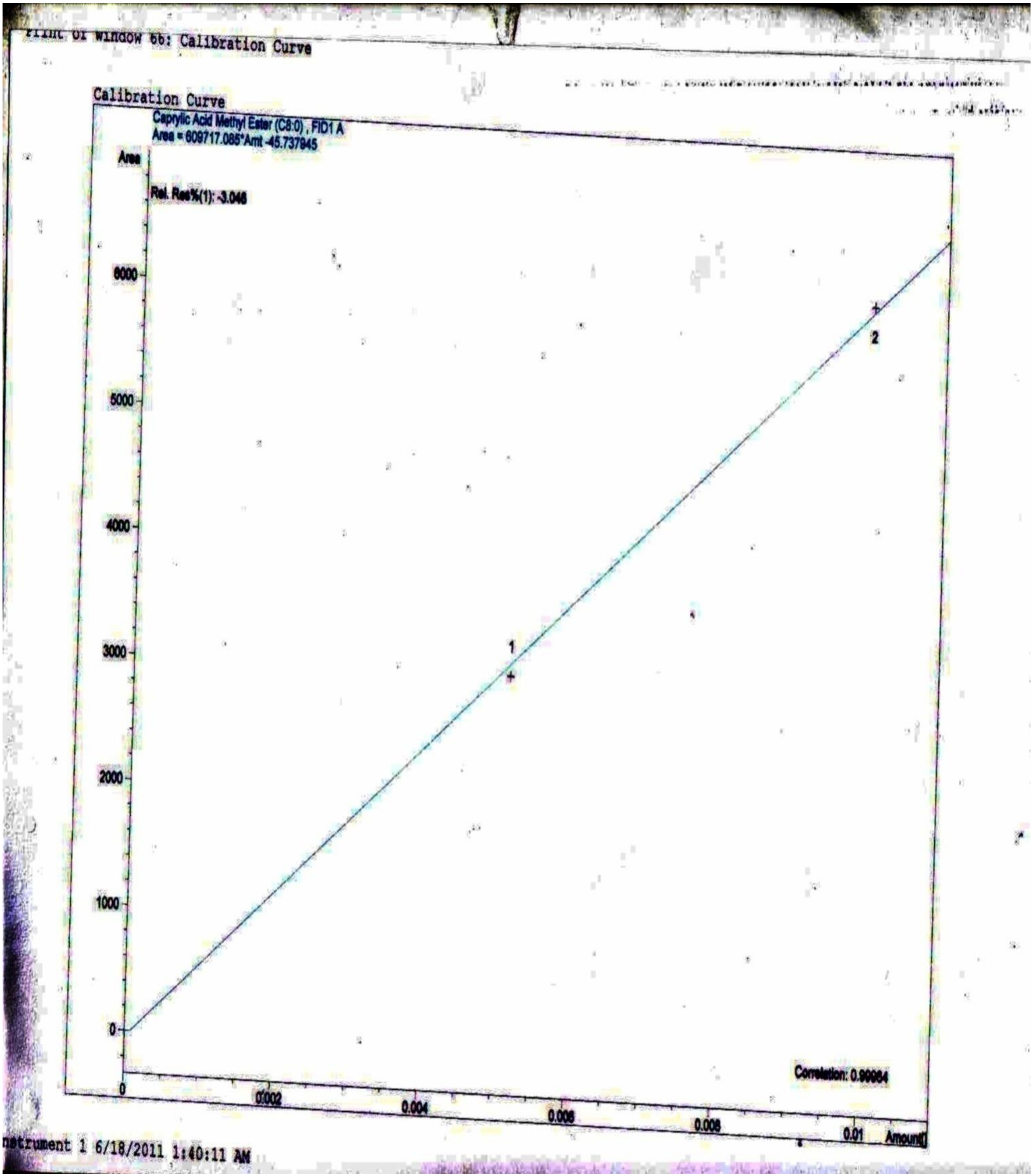
APPENDIX 1 D: SHODEX GARDENS, ANTHOHY VILLAGE, LAGOS.



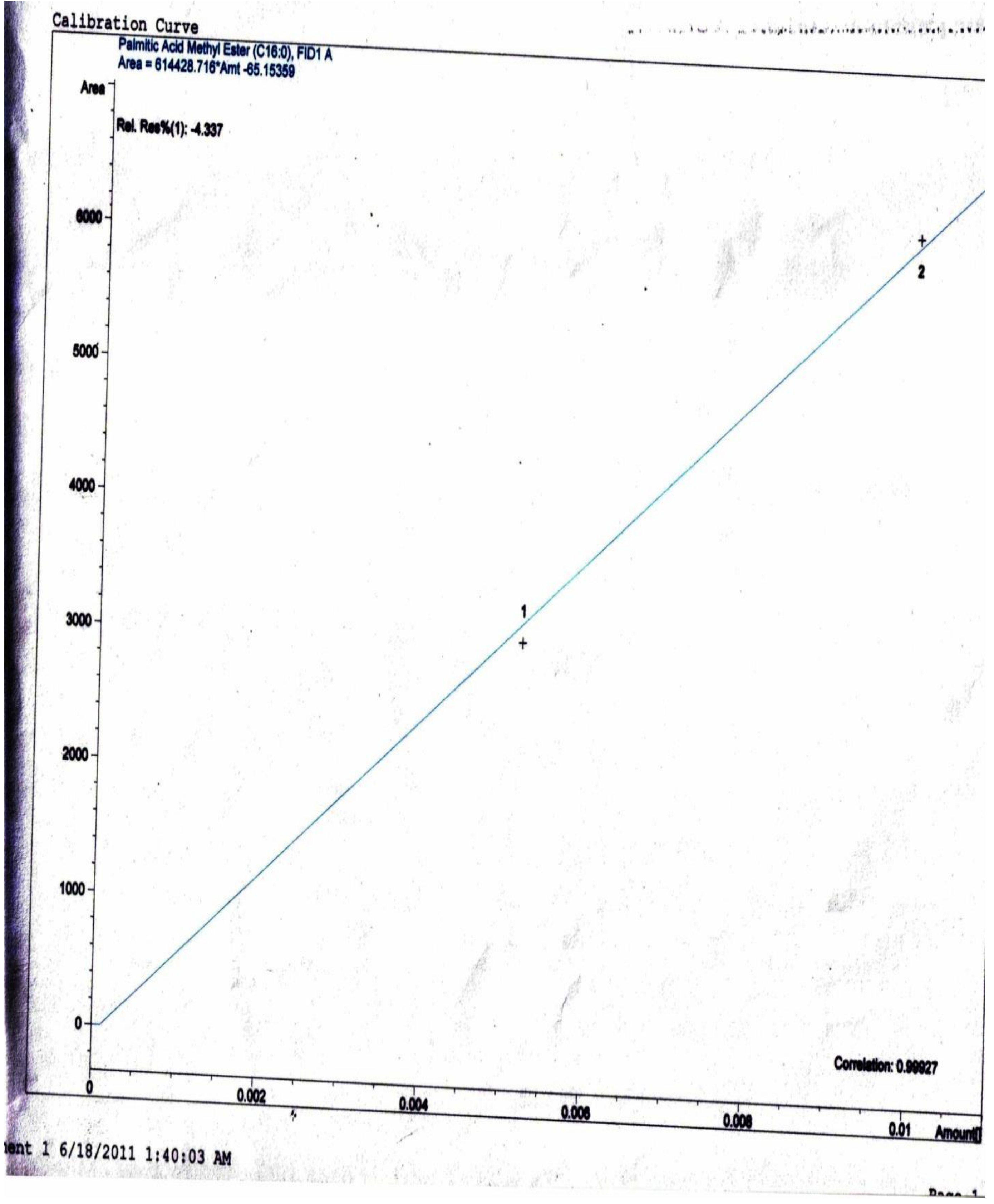

Appendix 2: fatty acid standards

## APPENDIX 3: CORRELATION COEFFICIENTS OF DIFFERENT FATTY ACID METHYL ESTERS AS DETERMINED BY THE GC MACHINE

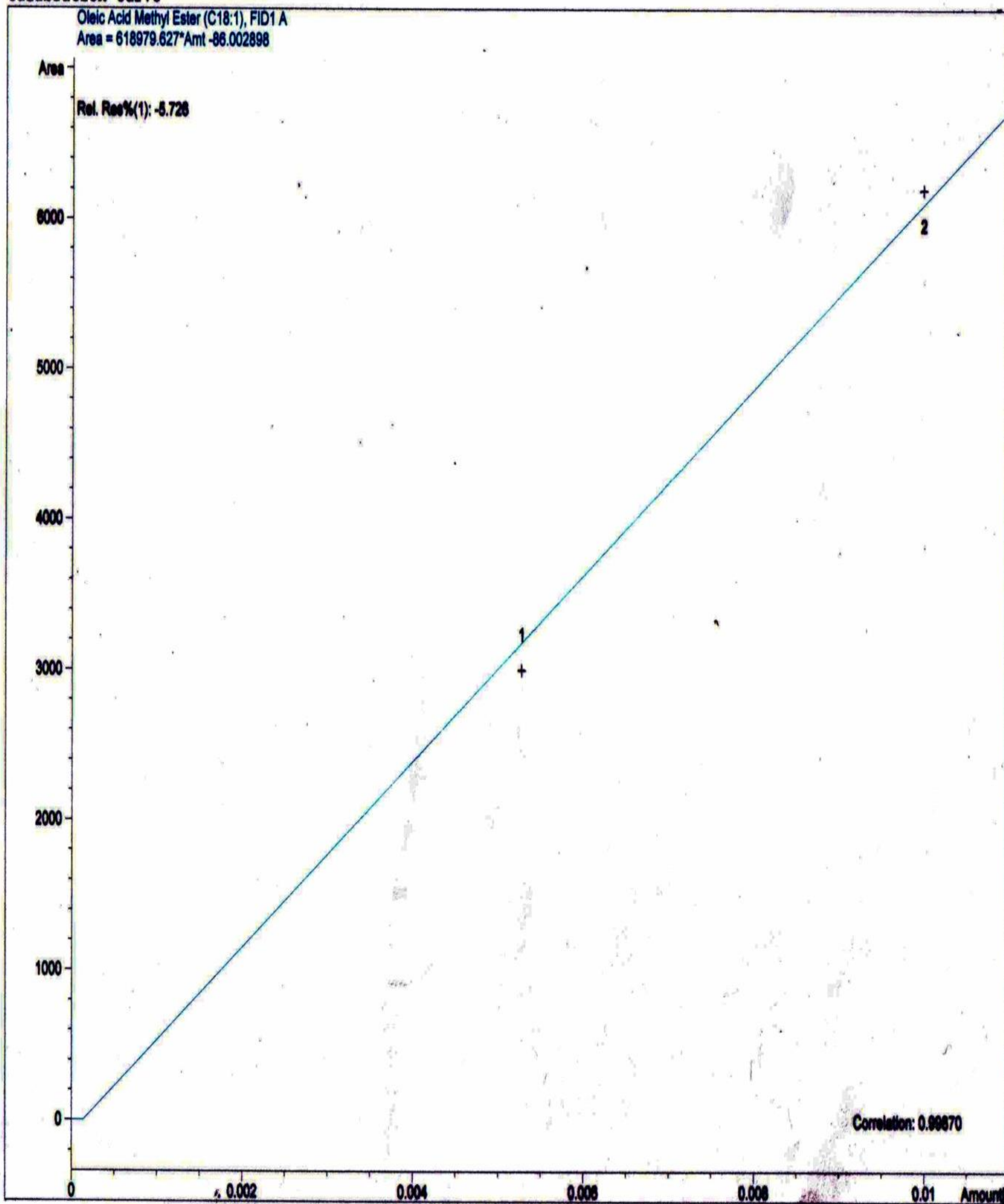
| S/N | Fatty acids | Correlation coefficient |
|---|---|---|
| 1. | Caprylic acid | 0.99964 |
| 2. | Palmitic acid | 0.99927 |
| 3. | Oleic acid | 0.99870 |
| 4. | Erucic acid | 0.99953 |
| 5. | Lignoceric acid | 0.99899 |



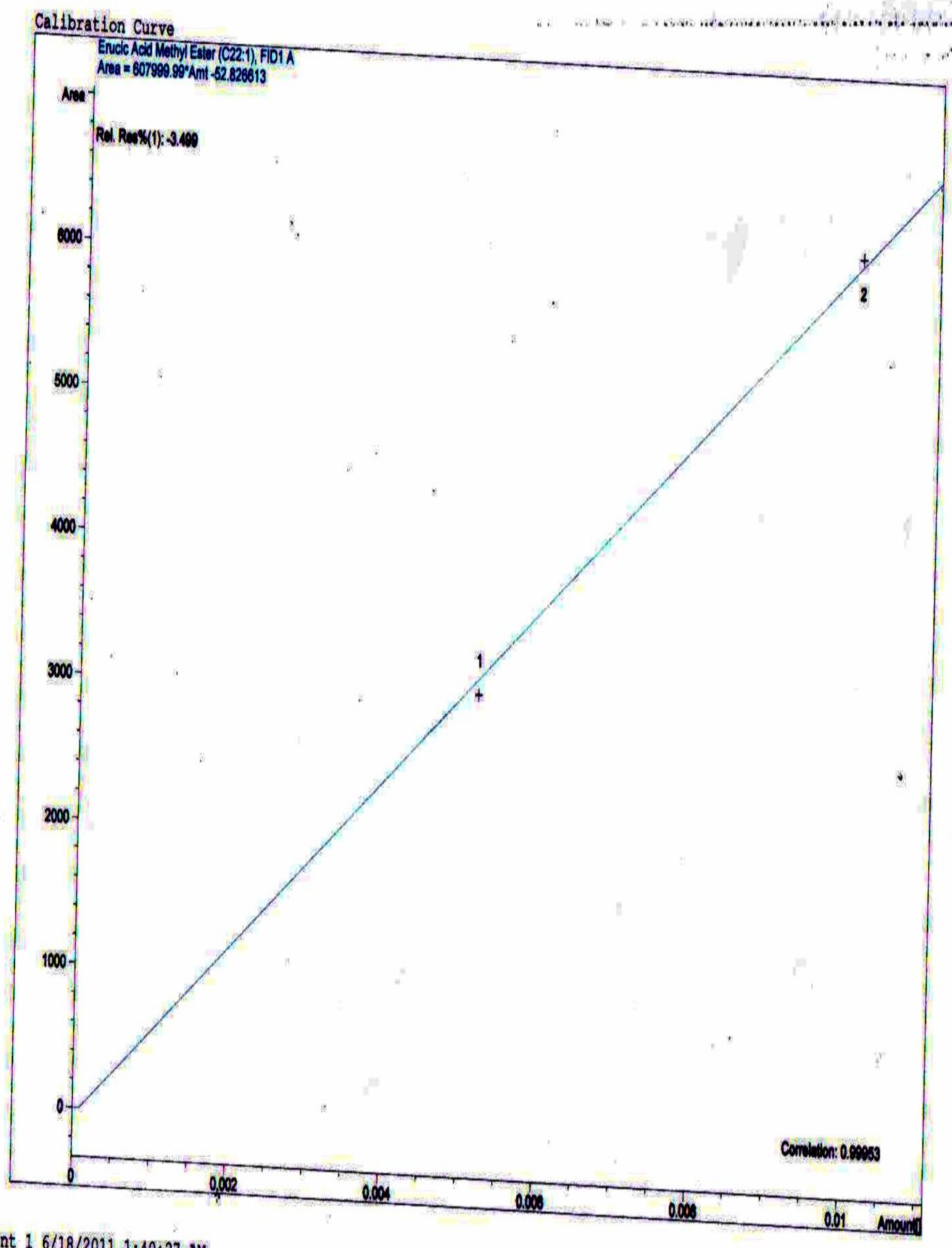

Appendix 4: Calibration curve and the correlation coefficient for caprylic acid



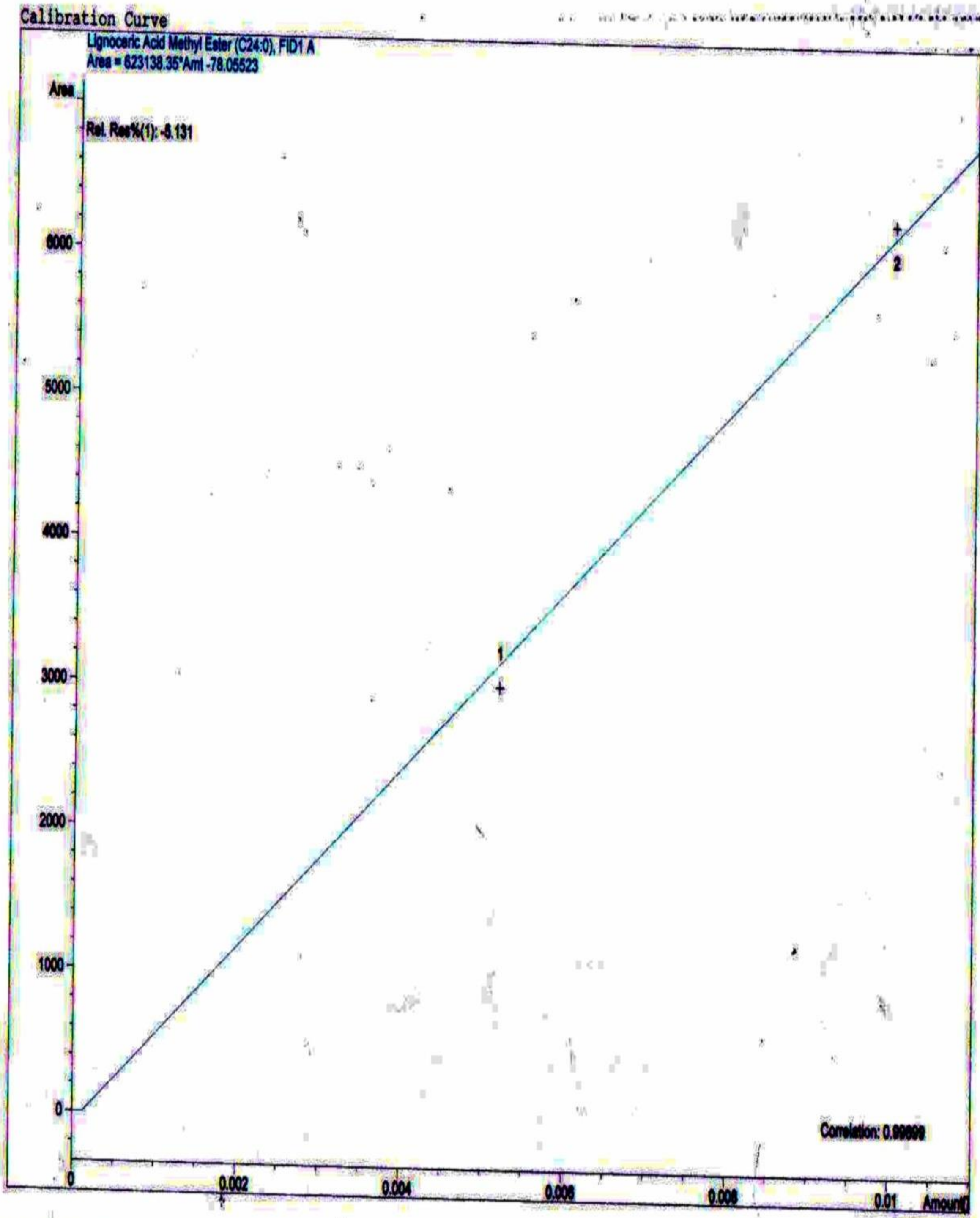

Appendix 5: Calibration curve and the corellation coefficient for palmitic acid.



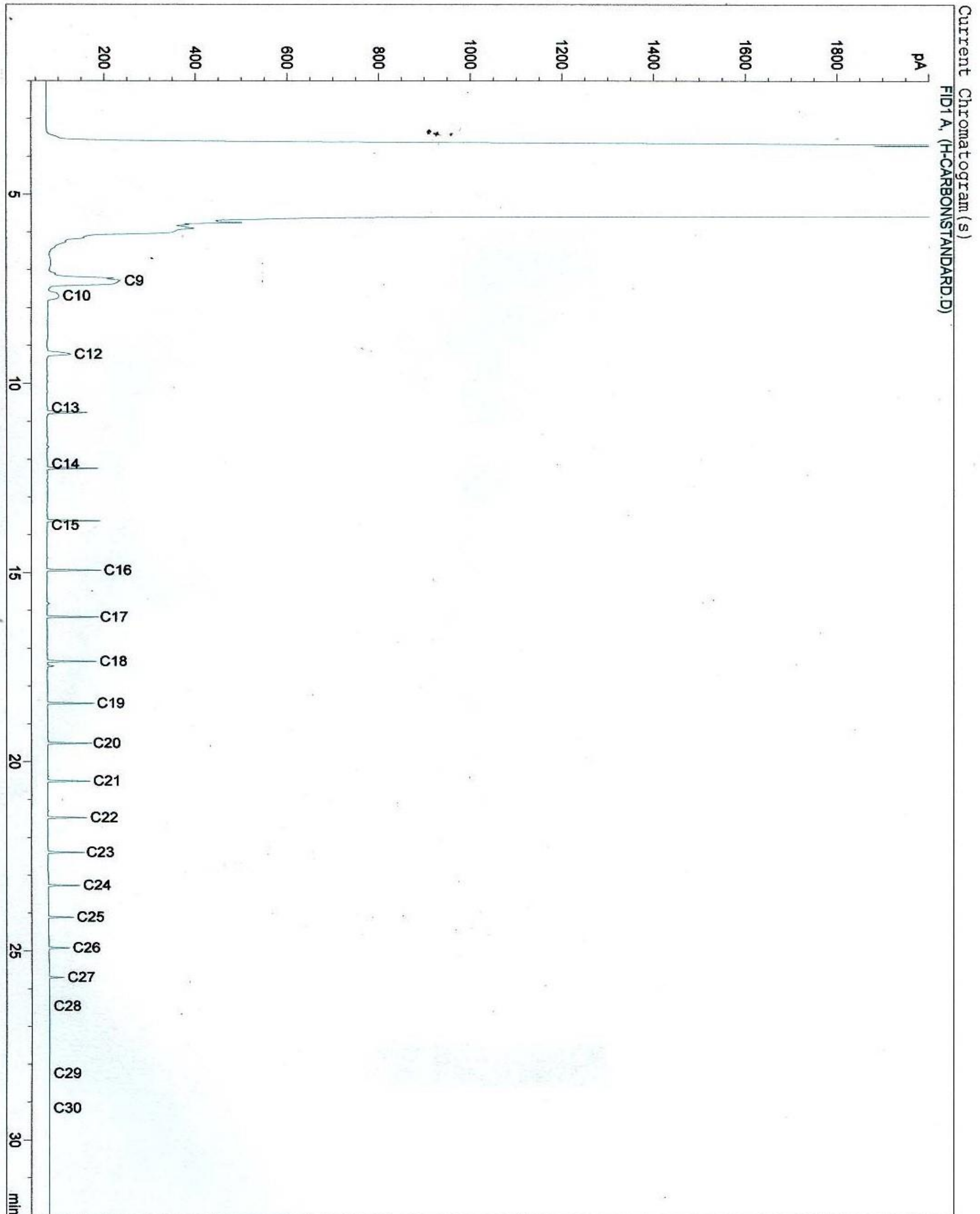

Appendix 6: Calibration curve and the correlation coefficient for oleic acid.




Appendix 7: Calibration curve and correlation coefficient for erucic acid.



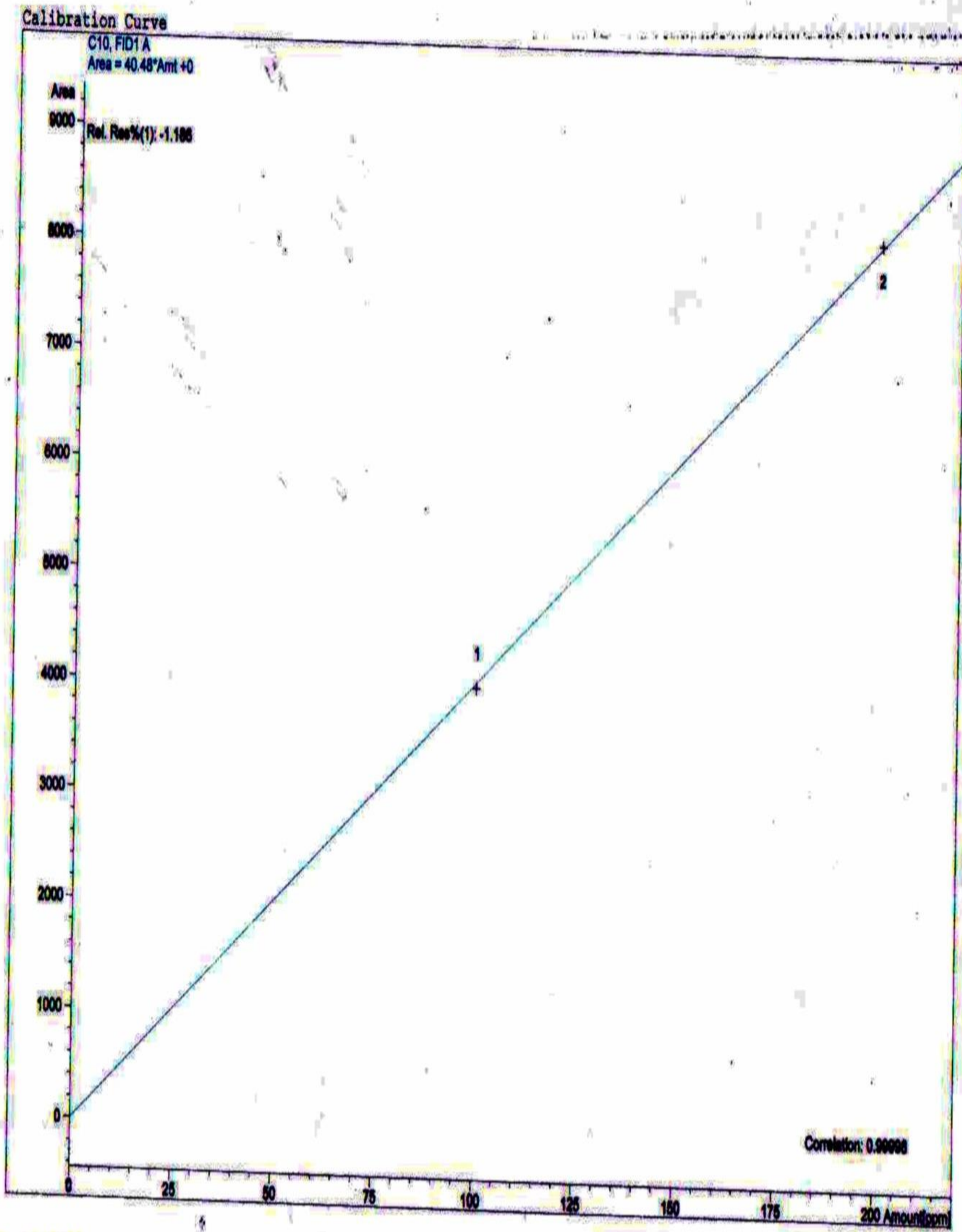

Appendix 8: Calibration curve and correlation coefficient for lignoceric acid.



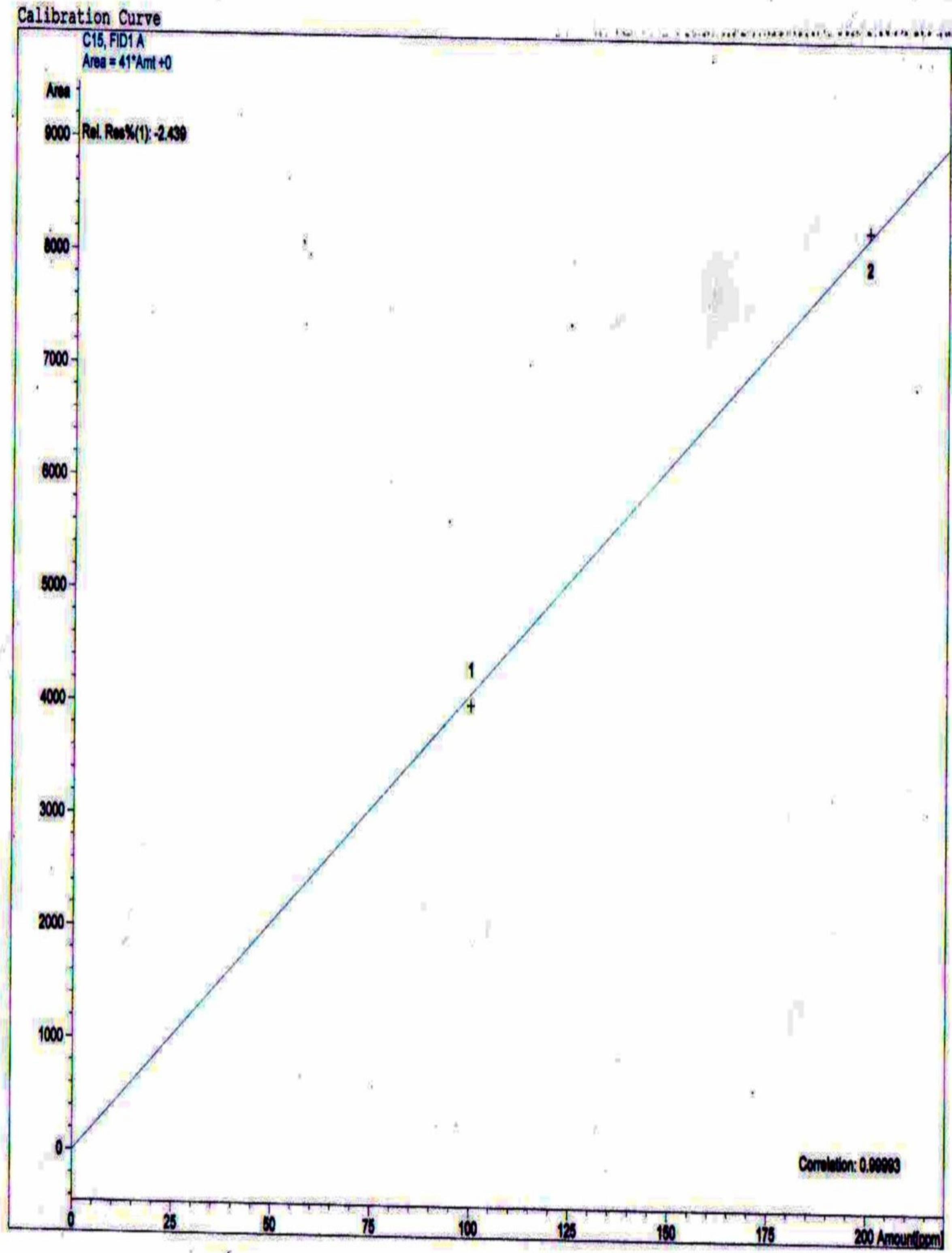

Appendix 9: Standard for Total Petroleum Hydrocarbon (TPH).

Appendix 10A: CORRELATION COEFFICIENT OF DIFFERENT HYROCARBONS AS DETERMINED BY THE GC MACHINE

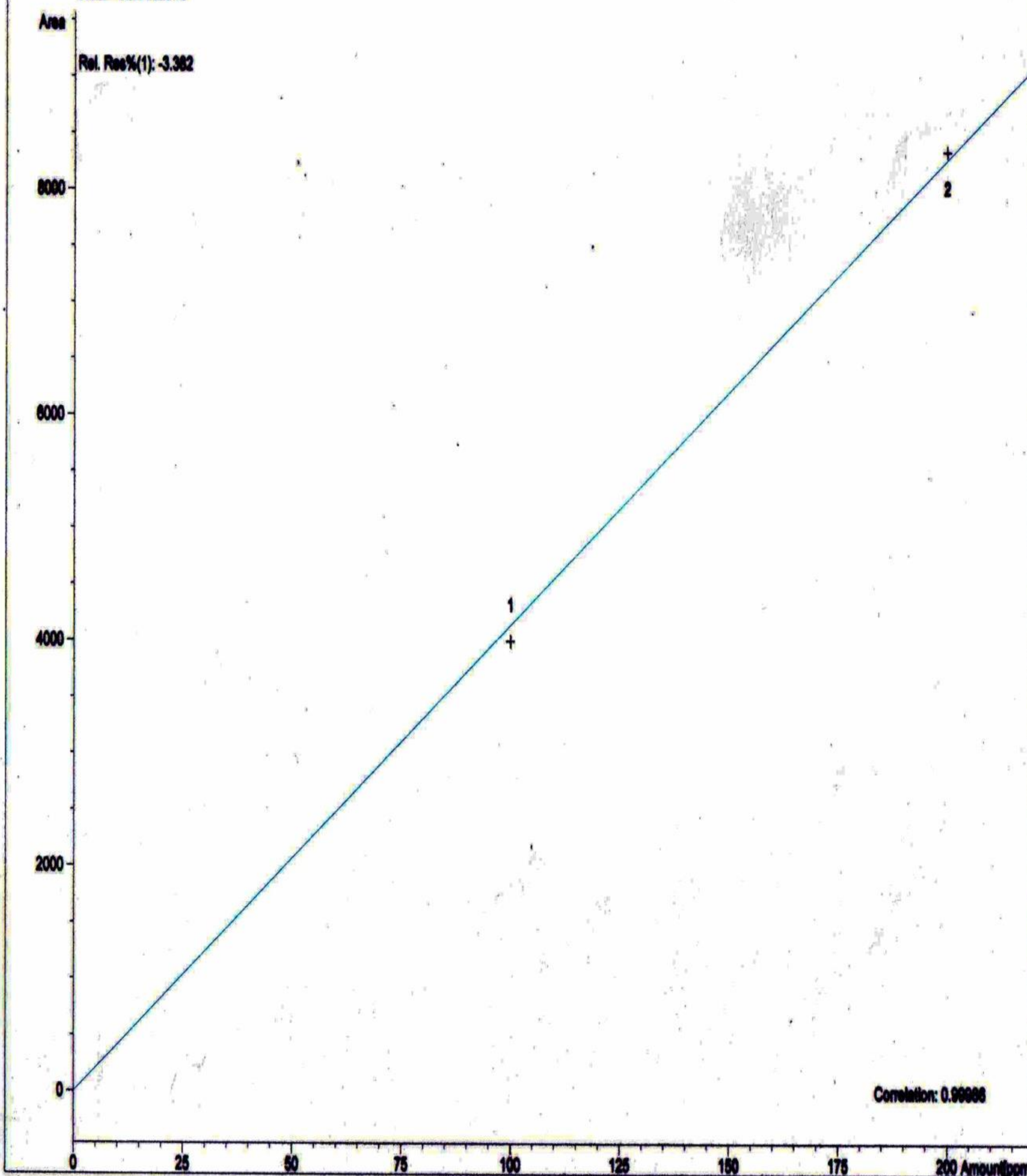
| S/N | HYDROCARBON | Correlation coefficient |
|---|---|---|
| 1. | C9 | 0.99982 |
| 2. | C14 | 0.99995 |
| 3. | C20 | 0.99972 |
| 4. | C25 | 0.99960 |
| 5. | C30 | 0.99955 |



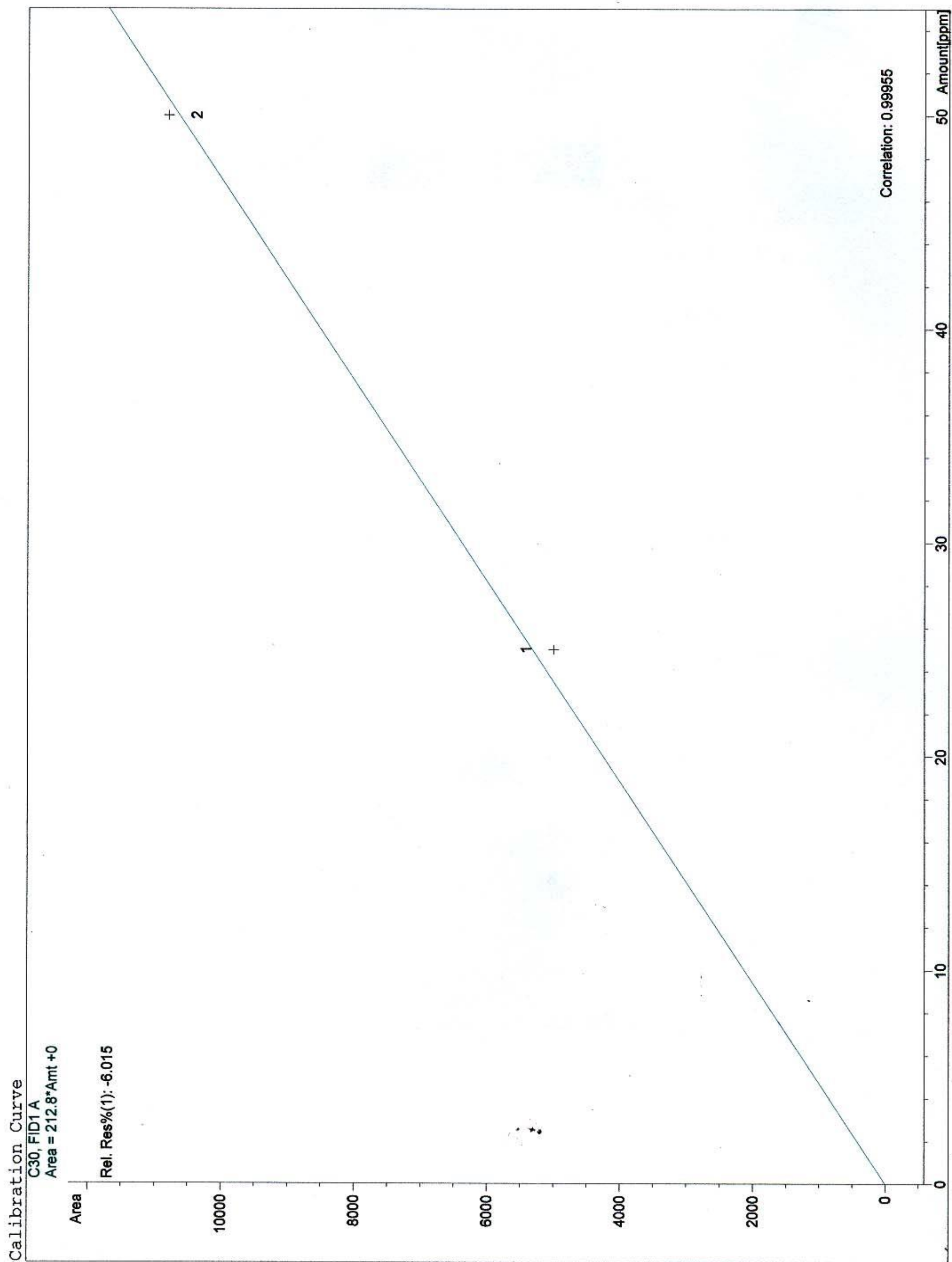

Appendix 10B: Calibration curve and correlation coefficient for C10.




Appendix 11: Calibration curve and correlation coefficient for C15.




Appendix 12: Calibration curve and correlation coefficient for C25.




Appendix 13: Callibration curve and correlation coefficient for C30.

APPENDIX 14A: MEANS SEPARATION **(USING DUNCAN'S MULTIPLE RANGE TEST)** FOR THE OPTICAL DENSITIES OF THE DIFFERENT FUNGAL SPECIES IN CO AT 530 NM

| S/N | TMT | DAY 0 | DAY 05 | DAY 10 | DAY 15 | DAY 20 | DAY 25 | DAY 30 | DAY 35 | DAY 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CO – *AO* (CONTROL 2) | $1.21^{fg}$ | $1.32^{bcd}$ | $1.30^{i}$ | $1.29^{e}$ | $0.99^{k}$ | $0.80^{mn}$ | $0.81^{klm}$ | $0.77^{ghi}$ | $0.73^{hij}$ |
| 2 | *AO* – CO (CONTROL 1 | $0.08^{l}$ | $0.25^{g}$ | $0.20^{m}$ | $1.40^{c}$ | $1.41^{b}$ | $1.38^{a}$ | $1.45^{a}$ | $1.49^{a}$ | $1.53^{a}$ |
| 3 | CO + ojt1F*AN* | $1.22^{fg}$ | $1.32^{bcd}$ | $1.37^{ef}$ | $1.36^{d}$ | $1.07^{i}$ | 1.15 | $1.16^{bcdefg}$ | $1.25^{b}$ | $1.29^{b}$ |
| 4 | CO + ojt2*AO* | $1.26^{f}$ | $1.37^{bc}$ | $1.22^{k}$ | $1.43^{ab}$ | $1.14^{f}$ | $1.22^{ef}$ | $1.20^{bcdefg}$ | $1.23^{bc}$ | $1.25^{bcde}$ |
| 5 | CO + ojt2F*AN* | $1.27^{ef}$ | $1.38^{bc}$ | $1.26^{j}$ | $1.44^{ab}$ | $1.14^{f}$ | $0.85^{m}$ | $0.88^{ijkl}$ | $0.94^{efg}$ | $1.03^{efg}$ |
| 6 | CO + ojt3*AO* | $1.25^{fg}$ | $1.36^{bc}$ | $1.37^{ef}$ | $1.43^{ab}$ | $1.06^{i}$ | $1.32^{ab}$ | $1.32^{abc}$ | $1.24^{bc}$ | $1.17^{bcdef}$ |
| 7 | CO + ojt3F*AN* | $1.26^{f}$ | $1.37^{bc}$ | $1.37^{ef}$ | $1.44^{ab}$ | $1.11^{gh}$ | $0.83^{mn}$ | $0.84^{jklm}$ | $1.01^{cdef}$ | $1.12^{bcdef}$ |
| 8 | CO + ojt4*AN* | $1.13^{h}$ | $1.24^{cd}$ | $1.36^{efg}$ | $1.44^{ab}$ | $1.13^{fg}$ | $1.29^{cd}$ | $1.34^{ab}$ | $1.26^{b}$ | $1.20^{bcdef}$ |
| 9 | CO + ont1 *AN* | $1.28^{ef}$ | $1.38^{bc}$ | $1.38^{e}$ | $1.42^{abc}$ | $1.15^{f}$ | $1.28^{cde}$ | $1.28^{abcd}$ | $1.14^{bcde}$ | $1.04^{defg}$ |
| 10 | CO + ont2*AN* | $1.24^{fg}$ | $1.35^{bc}$ | $1.36^{efg}$ | $1.41^{bc}$ | $1.14^{f}$ | $1.12^{h}$ | $1.11^{defgh}$ | $1.18^{bcd}$ | $1.26^{bcd}$ |
| 11 | CO + ont3*AO* | $1.28^{ef}$ | $1.38^{bc}$ | $1.32^{hi}$ | $1.44^{ab}$ | $1.02^{j}$ | $1.20^{fg}$ | $1.24^{abcdef}$ | $1.21^{bcd}$ | $1.13^{bcdef}$ |
| 12 | CO + sab1*AT* | $1.27^{ef}$ | $1.38^{bc}$ | $1.20^{k}$ | $1.28^{e}$ | $1.15^{f}$ | $1.06^{ij}$ | $1.03^{fghij}$ | $0.94^{efg}$ | $0.86^{gh}$ |
| 13 | CO + sab1F*AN* | $1.26^{f}$ | $1.37^{bc}$ | $1.34^{fgh}$ | $1.43^{ab}$ | $1.15^{f}$ | $1.37^{ab}$ | $1.35^{ab}$ | $1.26^{b}$ | $1.24^{bcde}$ |
| 14 | CO + sab2*AT* | $1.05^{i}$ | $1.16^{cd}$ | $1.36^{efg}$ | $1.43^{abc}$ | $1.13^{fg}$ | $1.30^{cd}$ | $1.29^{abcd}$ | $1.29^{b}$ | $1.28^{b}$ |
| 15 | CO + sab3*AO* | $1.26^{f}$ | $1.37^{bc}$ | $1.37^{ef}$ | $1.44^{ab}$ | $1.15^{f}$ | $1.00^{jkl}$ | $0.95^{hijk}$ | $1.15^{bcd}$ | $1.29^{b}$ |
| 16 | CO + sab3F*AN* | $1.03^{i}$ | $1.14^{d}$ | $1.35^{efgh}$ | $1.44^{a}$ | $1.14^{f}$ | $1.24^{def}$ | $1.19^{bcdefg}$ | $1.23^{bc}$ | $1.28^{b}$ |
| 17 | CO + sab4*AN* | $0.63^{j}$ | $0.74^{e}$ | $1.36^{efg}$ | $1.23^{f}$ | $0.83^{l}$ | $0.96^{l}$ | $0.96^{hijk}$ | $0.99^{def}$ | $1.27^{bc}$ |
| 18 | CO + sab4F*AN* | $1.27^{ef}$ | $1.38^{bc}$ | $1.36^{efg}$ | $1.44^{ab}$ | $1.15^{f}$ | $0.99^{kl}$ | $0.99^{ghijk}$ | $0.91^{fgh}$ | $0.84^{gh}$ |
| 19 | CO - *AN* | $0.13^{l}$ | $0.52^{f}$ | $0.98^{l}$ | $0.61^{k}$ | $0.41^{o}$ | $0.41^{p}$ | $0.40^{n}$ | $0.40^{j}$ | $0.33^{l}$ |
| 20 | CO + sho1 *AT* | $0.54^{k}$ | $0.89^{e}$ | $1.98^{b}$ | $1.45^{a}$ | $1.25^{d}$ | $1.24^{def}$ | $1.25^{abcde}$ | $1.22^{bc}$ | $1.01^{fg}$ |
| 21 | CO + sho3 *AO* | $2.22^{a}$ | $1.32^{bcd}$ | $2.14^{a}$ | $0.95^{i}$ | $0.79^{m}$ | $0.79^{n}$ | $0.73^{lm}$ | $0.72^{hi}$ | $0.63^{ijk}$ |
| 22 | CO + bg1*AN* | $1.29^{ef}$ | $1.27^{bcd}$ | $1.97^{b}$ | $1.23^{f}$ | $1.30^{c}$ | $1.01^{jkl}$ | $1.00^{ghij}$ | $1.01^{cdef}$ | $1.00^{fg}$ |
| 23 | CO + bg2 *AN* | $1.68^{c}$ | $0.78^{e}$ | $1.33^{fghi}$ | $1.14^{g}$ | $1.11^{h}$ | $1.04^{jk}$ | $1.33^{abc}$ | $1.24^{bc}$ | $1.22^{bcdef}$ |
| 24 | CO + ajgIv1 *AO* | $1.34^{e}$ | $0.52^{f}$ | $1.33^{fghi}$ | $0.79^{j}$ | $0.71^{n}$ | $0.68^{o}$ | $0.67^{m}$ | $0.69^{i}$ | $0.54^{jk}$ |
| 25 | CO + ajgIv2*AN* | $1.48^{d}$ | $1.50^{ab}$ | $1.78^{c}$ | $1.34^{d}$ | $1.22^{e}$ | $1.21^{f}$ | $1.20^{bcdefg}$ | $1.21^{bcd}$ | $1.14^{bcdef}$ |
| 26 | CO + oygIv1*AN* | $1.18^{gh}$ | $1.66^{a}$ | $1.37^{ef}$ | $081^{j}$ | $0.72^{n}$ | $0.64^{o}$ | $0.65^{m}$ | $0.65^{i}$ | $0.48^{kl}$ |
| 27 | CO + oygIv2*AO* | $1.42^{d}$ | $0.85^{e}$ | $2.13^{a}$ | $1.15^{g}$ | $1.15^{f}$ | $1.13^{h}$ | $1.12^{cdefgh}$ | $1.12^{bcdef}$ | $0.78^{hi}$ |

Mean values carrying different superscripts along the same column are significantly different at $P<0.0$

APPENDIX 14B: MEANS SEPARATION (USING DUNCAN'S MULTIPLE RANGE TEST) FOR THE OPTICAL DENSITIES OFTHE DIFFERENT FUNGAL SPECIES IN CRUDE OIL (CO) AT 620NM

| S/N | TMT | DAY 0 | DAY 05 | DAY 10 | DAY 15 | DAY 20 | DAY 25 | DAY 30 | DAY 35 | DAY 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CO – *AO* (CONTROL 2) | 0.47 | $0.54^{m}$ | $0.65^{l}$ | $0.55^{l}$ | $0.53^{k}$ | $0.53^{q}$ | $0.52^{n}$ | $0.53^{n}$ | $0.44^{p}$ |
| 2 | *AO* – CO (CONTROL 1) | $0.88^{b}$ | $1.18^{c}$ | $0.15^{n}$ | $1.20^{b}$ | $1.27^{bcd}$ | $1.29^{a}$ | $1.31^{a}$ | $1.34^{b}$ | $1.37^{a}$ |
| 3 | CO + ojt1*AN* | $0.90^{b}$ | $1.07^{de}$ | $1.16^{efg}$ | $1.14^{c}$ | $1.17^{f}$ | $1.01^{f}$ | $0.99^{g}$ | $1.00^{fg}$ | $1.05^{def}$ |
| 4 | CO + ojt2*AO* | $0.63^{b}$ | $0.74^{k}$ | $0.47^{m}$ | $1.16^{c}$ | $1.31^{ab}$ | $1.16^{b}$ | $1.15^{bc}$ | $1.07^{d}$ | $1.05^{def}$ |
| 5 | CO + ojt2*AN* | $0.87^{b}$ | $0.98^{fgh}$ | $1.17^{e}$ | $1.14^{c}$ | $1.28^{bc}$ | $1.00^{fg}$ | $1.03^{g}$ | $0.96^{hi}$ | $0.90^{i}$ |
| 6 | CO + ojt3*AO* | $0.99^{b}$ | $1.03^{efg}$ | $1.15^{efg}$ | $1.16^{c}$ | $1.16^{f}$ | $1.10^{c}$ | $1.08^{e}$ | $1.06^{de}$ | $1.07^{cd}$ |
| 7 | CO + ojt3*AN* | $0.62^{b}$ | $0.73^{k}$ | $1.05^{h}$ | $1.08^{d}$ | $1.25^{cd}$ | $0.95^{h}$ | $0.89^{ij}$ | $0.91^{j}$ | $0.96^{h}$ |
| 8 | CO + ojt4*AN* | $1.03^{b}$ | $1.10^{d}$ | $1.18^{e}$ | $1.16^{c}$ | $1.35^{a}$ | $1.15^{b}$ | $1.12^{cd}$ | $1.07^{d}$ | 1.07cd |
| 9 | CO + ont1*AN* | $1.00^{b}$ | $1.08^{de}$ | $1.14^{efg}$ | $1.15^{c}$ | $1.35^{a}$ | $1.15^{b}$ | $1.15^{bc}$ | $1.06^{de}$ | 1.02g |
| 10 | CO + ont2*AN* | $0.97^{b}$ | $1.04^{def}$ | $1.18^{e}$ | $1.15^{c}$ | $1.33^{a}$ | $1.12^{c}$ | $1.11^{d}$ | $1.07^{d}$ | $1.04^{efg}$ |
| 11 | CO + ont3*AO* | $1.00^{b}$ | $1.06^{de}$ | $1.16^{ef}$ | $1.04^{e}$ | $0.87^{h}$ | $1.00^{fg}$ | $0.93^{h}$ | $1.00^{fg}$ | $1.05^{def}$ |
| 12 | CO + sab1*AT* | $0.76^{b}$ | $0.87^{j}$ | $0.90^{i}$ | $1.05^{e}$ | $1.35^{a}$ | $0.75^{m}$ | $0.74^{l}$ | $1.70^{a}$ | $0.65^{lm}$ |
| 13 | CO + sab1*AN* | $0.87^{b}$ | $0.98^{fgh}$ | $1.12^{fg}$ | $1.16^{c}$ | $1.35^{a}$ | $1.15^{b}$ | $1.16^{b}$ | $1.08^{d}$ | $1.03^{fg}$ |
| 14 | CO + sab2*AT* | $0.86^{b}$ | $0.87^{j}$ | $1.15^{efg}$ | $1.15^{c}$ | $1.35^{a}$ | $1.17^{b}$ | $1.15^{bc}$ | $1.09^{d}$ | $1.05^{def}$ |
| 15 | CO + sab3*AO* | $0.87^{b}$ | $0.97^{gh}$ | $1.14^{efg}$ | $1.16^{c}$ | $1.23^{de}$ | $1.05^{d}$ | $1.15^{bc}$ | $1.09^{d}$ | $1.04^{efg}$ |
| 16 | CO + sab3*AN* | $0.73^{b}$ | $0.84^{j}$ | $1.14^{efg}$ | $1.16^{c}$ | $1.36^{a}$ | $1.10^{c}$ | $1.08^{e}$ | $1.08^{d}$ | $1.08^{c}$ |
| 17 | CO + sab4*AN* | $0.72^{b}$ | $0.83^{j}$ | $1.17^{e}$ | $1.15^{c}$ | $1.34^{a}$ | $0.92^{i}$ | $0.86^{k}$ | $0.94^{ij}$ | $1.06^{cde}$ |
| 20 | CO + sho1 *AT* | | $0.46^{m}$ | $0.92^{i}$ | $0.10^{m}$ | $0.99^{l}$ | $0.08^{r}$ | $0.08^{o}$ | $0.07^{o}$ | $0.04^{q}$ |
| 21 | CO + sho3*AO* | | $1.46^{a}$ | $1.63^{c}$ | $1.08^{d}$ | $1.01^{g}$ | $1.00^{fg}$ | $1.00^{g}$ | $0.98^{gh}$ | $0.88^{i}$ |
| 22 | CO + bg1*AN* | | $0.73^{k}$ | $1.87^{a}$ | $0.85^{h}$ | $0.85^{h}$ | $0.85^{k}$ | $0.75^{l}$ | $0.76^{l}$ | $0.66^{l}$ |
| 23 | CO + bg2*AN* | | $1.23^{c}$ | $0.78^{j}$ | $0.81^{i}$ | $0.78^{h}$ | $0.78^{l}$ | $0.75^{l}$ | $0.74^{m}$ | $0.73^{j}$ |
| 24 | CO + ajgIv1*AO* | | $0.59^{l}$ | $1.51^{d}$ | $0.75^{j}$ | $0.70^{j}$ | $0.69^{o}$ | $0.67^{m}$ | $0.68^{m}$ | $0.63^{mn}$ |
| 25 | CO + ajgIv2*AN* | | $1.39^{b}$ | $1.62^{c}$ | $1.31^{a}$ | $1.20^{ef}$ | $1.17^{b}$ | $1.15^{bc}$ | $1.15^{c}$ | $1.14^{b}$ |
| 26 | CO + oygIv1*AN* | | $0.49^{m}$ | $1.14^{efg}$ | $0.69^{k}$ | $0.65^{j}$ | $0.65^{p}$ | $0.67^{m}$ | $0.66^{m}$ | $0.62^{n}$ |
| 27 | CO + oygIv2*AO* | | $0.44^{m}$ | $1.65^{c}$ | $0.89^{g}$ | $0.89^{h}$ | $0.89^{j}$ | $0.87^{jk}$ | $0.85^{k}$ | $0.51^{o}$ |
| 28 | CO + barIv*AN* | | $0.84^{j}$ | $1.69^{b}$ | $0.91^{f}$ | $0.99^{g}$ | $0.98^{g}$ | $0.91^{hi}$ | $0.92^{j}$ | $0.74^{j}$ |

Mean values carrying different superscripts along the same column are significantly different at $P<0.05$

Appendix 15A: Means separation (using duncan's multiple range test) for the optical densities of the different fungal species in kerosine at 530nm

| S/N | TMT | DAY 0 | DAY 05 | DAY 10 | DAY 15 | DAY 20 | DAY 25 | DAY 30 | DAY 35 | DAY 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *AO* - kero (control 1) | $0.08^{ijk}$ | $0.25^{m}$ | $1.38^{a}$ | $1.40^{a}$ | $1.41^{a}$ | $1.38^{a}$ | $1.45^{a}$ | | $1.53^{a}$ |
| 2 | Kero – *AO* (control 2) | $0.03^{k}$ | $0.10^{pq}$ | $0.23^{q}$ | $0.08^{z}$ | $0.08^{r}$ | $0.08^{w}$ | $0.07^{w}$ | $0.05^{f}$ | $0.04^{tu}$ |
| 3 | Kero + ojt1F*AN* | $0.35^{f}$ | $0.55^{k}$ | $0.87^{g}$ | $0.77^{o}$ | $0.56^{k}$ | $0.77^{k}$ | $0.75^{k}$ | | $0.64^{j}$ |
| 4 | Kero + ojt2*AO* | $0.58^{c}$ | $0.78^{ef}$ | $0.78^{i}$ | $0.90^{j}$ | $0.74^{g}$ | $0.69^{m}$ | $0.67^{m}$ | | $0.58^{k}$ |
| 5 | Kero + ojt2F*AO* | $0.96^{a}$ | $1.16^{a}$ | $0.94^{b}$ | $1.32^{b}$ | $0.88^{c}$ | $0.83^{h}$ | $0.77^{i}$ | | $0.77^{h}$ |
| 6 | Kero + ojt3*AO* | $0.76^{b}$ | $0.96^{c}$ | $0.85^{h}$ | $0.79^{n}$ | $0.85^{d}$ | $0.99^{e}$ | $1.01^{c}$ | | $1.11^{c}$ |
| 7 | Kero + ojt3F*AN* | $0.63^{c}$ | $0.83^{d}$ | $0.90^{d}$ | $1.00^{d}$ | $0.83^{e}$ | $0.99^{e}$ | $0.89^{f}$ | | $0.84^{f}$ |
| 8 | Kero + ojt4*AN* | $0.59^{c}$ | $0.79^{e}$ | $0.87^{fg}$ | $0.93^{h}$ | $0.86^{d}$ | $0.81^{i}$ | $0.79^{h}$ | | $0.78^{h}$ |
| 9 | Kero + ont1*AN* | $0.57^{c}$ | $0.77^{f}$ | $0.45^{p}$ | $0.86^{l}$ | $0.43^{m}$ | $0.40^{q}$ | $0.39^{p}$ | | $0.45^{m}$ |
| 10 | Kero + ont2*AN* | $0.39^{f}$ | $0.59^{j}$ | $0.65^{k}$ | $0.86^{m}$ | $0.67^{j}$ | $0.42^{0}$ | $0.40^{o}$ | | $1.18^{b}$ |
| 11 | Kero + ont3*AN* | $0.11^{gij}$ | $0.31^{l}$ | $0.50^{n}$ | $0.47^{s}$ | $0.38^{n}$ | $0.37^{r}$ | $0.39^{p}$ | | $0.87^{e}$ |
| 12 | Kero + sab1*AT* | $0.77^{b}$ | $0.97^{c}$ | $0.92^{c}$ | $0.99^{e}$ | $0.89^{c}$ | $0.97^{f}$ | $0.94^{d}$ | | $0.96^{d}$ |
| 13 | Kero + sab1F*AN* | $0.63^{c}$ | $0.83^{d}$ | $0.86^{h}$ | $0.89^{k}$ | $0.75^{g}$ | $1.00^{d}$ | $0.87^{g}$ | | $0.74^{i}$ |
| 14 | Keor + sab2*AT* | $0.49^{d}$ | $0.69^{g}$ | $0.63^{l}$ | $0.97^{g}$ | $0.69^{i}$ | $0.80^{j}$ | $0.68^{l}$ | | $0.73^{i}$ |
| 15 | Kero + sab2F*AN* | $0.43^{d}$ | $0.63^{i}$ | $0.49^{o}$ | $0.69^{p}$ | $0.43^{m}$ | $0.41^{p}$ | $0.36^{q}$ | | $0.25^{o}$ |
| 16 | Kero + sab3*AO* | $0.94^{a}$ | $1.04^{b}$ | $0.67^{j}$ | $1.03^{c}$ | $0.72^{h}$ | $1.14^{b}$ | $1.10^{b}$ | | $0.55^{l}$ |
| 17 | Kero + sab3F*AN* | $0.63^{c}$ | $0.83^{d}$ | $0.88^{f}$ | $0.92^{i}$ | $0.80^{f}$ | $0.85^{g}$ | $0.76^{j}$ | | $0.81^{g}$ |
| 18 | Kero + sab4*AN* | $0.57^{c}$ | $0.57^{k}$ | $0.67^{j}$ | $0.64^{q}$ | $0.67^{j}$ | $0.71^{l}$ | $0.67^{m}$ | | $0.80^{g}$ |
| 19 | Kero + sab4F*AN* | $0.63^{c}$ | $0.83^{d}$ | $0.89^{e}$ | $0.98^{f}$ | $0.92^{b}$ | $1.01^{c}$ | $0.93^{e}$ | | $0.74^{i}$ |
| 20 | Kero + sho*AT* | $0.17^{g}$ | $0.15^{n}$ | $0.14^{t}$ | $0.16^{w}$ | $0.15^{p}$ | $0.13^{u}$ | $0.13^{t}$ | $0.11^{d}$ | $0.11^{r}$ |
| 21 | Kero + sho3*AO* | $0.16^{g}$ | | $0.12^{u}$ | $0.19^{v}$ | $0.15^{p}$ | $0.14^{t}$ | $0.14^{s}$ | $0.14^{b}$ | $0.14^{q}$ |
| 22 | Kero + oygIv1*AN* | $0.10^{ijk}$ | $0.10^{op}$ | $0.11^{u}$ | $0.12^{x}$ | $0.12^{q}$ | $0.11^{v}$ | $0.11^{u}$ | $0.10^{d}$ | $0.10^{r}$ |
| 23 | Kero + oygIv2*AO* | $0.13^{gi}$ | $0.23^{m}$ | $0.67^{j}$ | $0.24^{u}$ | $0.25^{o}$ | $0.24^{s}$ | $0.24^{r}$ | $0.22^{c}$ | $0.20^{p}$ |
| 24 | Kero + ajgIv1*AO* | 0.98 | $0.99^{op}$ | $1.08^{v}$ | $1.08^{y}$ | $1.07^{rs}$ | $1.16^{z}$ | $0.96^{x}$ | $0.85^{fg}$ | $0.74^{tu}$ |
| 25 | Kero + ajgIv2*AN* | $0.39^{f}$ | $0.60^{j}$ | $0.61^{m}$ | $0.61^{r}$ | $0.51^{l}$ | $0.43^{n}$ | $0.43^{n}$ | $0.42^{a}$ | $0.42^{n}$ |
| 26 | Kero + bg1*AN* | $0.09^{ijk}$ | $0.08^{q}$ | $0.21^{r}$ | $0.08^{y}$ | $0.07^{rs}$ | $0.06^{y}$ | $0.06^{x}$ | $0.04^{g}$ | $0.04^{tu}$ |
| 27 | Kero + bg2*AN* | $0.11^{gij}$ | $0.12^{o}$ | $0.08^{v}$ | $0.08^{y}$ | $0.07^{rs}$ | $0.07^{x}$ | $0.07^{x}$ | $0.06^{f}$ | $0.06^{st}$ |
| 28 | Kero + barIv1*AN* | $0.17^{g}$ | $0.17^{n}$ | $0.17^{s}$ | | | | | | |

Mean values carrying different superscripts along the same column are significantly different at $p<0.05$.

Appendix 15B: Means separation (using Duncan's multiple range test) for the optical densities of the different fungal species in kerosine at 620nm

| S/N | TMT | DAY 0 | DAY 05 | DAY 10 | DAY 15 | DAY 20 | DAY 25 | DAY 30 | DAY 35 | DAY 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *AO* – kero (control 1) | $0.88^{a}$ | $1.18^{a}$ | $1.19^{a}$ | $1.20^{a}$ | $1.27^{a}$ | $1.29^{a}$ | $1.31^{a}$ | | $1.37^{a}$ |
| 2 | kero - *AO* (control 2) | | $0.32^{o}$ | $0.22^{m}$ | $0.23^{s}$ | $0.50^{m}$ | $0.29^{o}$ | $0.27^{n}$ | $0.52^{a}$ | $0.44^{k}$ $0.51^{hi}$ |
| 3 | kero + ojt1F*AN* | $0.20^{lm}$ | $0.60^{j}$ | $0.51^{j}$ | $0.46^{o}$ | $0.45^{o}$ | $0.61^{j}$ | $0.63^{i}$ | | $0.50^{hi}$ |
| 4 | kero + ojt2*AO* | $0.27^{i}$ | $0.70^{f}$ | $0.13^{no}$ | $0.69^{h}$ | $0.66^{h}$ | $0.61^{j}$ | $0.64^{i}$ | | $0.56^{f}$ |
| 5 | kero + ojt2F*AO* | $0.58^{e}$ | $0.98^{b}$ | $0.65^{ef}$ | $0.73^{g}$ | $0.81^{c}$ | $0.74^{g}$ | $0.74^{f}$ | | $0.70^{d}$ |
| 6 | kero + ojt3*AO* | $0.77^{b}$ | $0.77^{c}$ | $0.74^{c}$ | $0.66^{j}$ | $0.69^{g}$ | $1.00^{b}$ | $0.96^{b}$ | | $0.88^{b}$ |
| 7 | kero + ojt3F*AN* | $0.77^{b}$ | $0.77^{c}$ | $0.74^{c}$ | $0.76^{f}$ | $0.76^{d}$ | $0.80^{e}$ | $0.78^{e}$ | | $0.65^{e}$ |
| 8 | kero + ojt4*AN* | | $0.69^{g}$ | $0.67^{d}$ | $0.76^{f}$ | $0.77^{d}$ | $0.75^{f}$ | $0.72^{fg}$ | | $0.74^{c}$ |
| 9 | kero + ont1*AN* | $0.66^{d}$ | $0.66^{h}$ | $0.63^{fg}$ | $0.58^{m}$ | $0.30^{r}$ | $0.50^{m}$ | $0.34^{m}$ | | $0.55^{fg}$ |
| 10 | kero + ont2*AN* | $0.66^{d}$ | $0.66^{h}$ | $0.65^{ef}$ | $0.67^{i}$ | $0.52^{l}$ | $0.54^{l}$ | $0.48^{k}$ | | $0.54^{g}$ |
| 11 | kero + ont3*AN* | $0.77^{b}$ | $0.77^{c}$ | $0.75^{c}$ | $0.41^{q}$ | $0.26^{s}$ | $0.24^{p}$ | $0.26^{n}$ | | $0.49^{i}$ |
| 12 | kero + sab1*AT* | $0.32^{h}$ | $0.72^{e}$ | $0.75^{c}$ | $0.77^{e}$ | $0.83^{b}$ | $0.80^{e}$ | $0.79^{e}$ | | $0.88^{b}$ |
| 13 | kero + sab1F*AN* | $0.69^{c}$ | $0.69^{fg}$ | $0.66^{de}$ | $0.73^{g}$ | $0.71^{f}$ | $0.90^{d}$ | $0.71^{g}$ | | $0.74^{c}$ |
| 14 | kero + sab2*AT* | $0.23^{i}$ | $0.63^{i}$ | $0.75^{c}$ | $0.61^{l}$ | $0.66^{h}$ | $0.69^{h}$ | $0.69^{h}$ | | $0.70^{d}$ |
| 15 | kero + sab2F*AN* | $0.50^{f}$ | $0.50^{m}$ | $0.49^{k}$ | $0.43^{p}$ | $0.37^{q}$ | $0.50^{m}$ | $0.43^{l}$ | | $0.31^{l}$ |
| 16 | kero + sab3*AO* | $0.66^{d}$ | $0.66^{h}$ | $0.67^{d}$ | $0.85^{b}$ | $0.60^{j}$ | $0.00^{c}$ | $0.85^{c}$ | | $0.52^{h}$ |
| 17 | kero + sab3F*AN* | $0.56^{ef}$ | $0.76^{d}$ | $0.96^{b}$ | $0.80^{c}$ | $0.75^{e}$ | $0.80^{e}$ | $0.77^{e}$ | | $0.74^{c}$ |
| 18 | kero + sab4*AN* | $0.13^{no}$ | $0.43^{n}$ | $0.61^{h}$ | $0.39^{r}$ | $0.42^{p}$ | $0.75^{f}$ | $0.64^{i}$ | | $0.73^{c}$ |
| 19 | Kero + sab4F*AN* | $0.76^{b}$ | $0.76^{d}$ | $0.59^{i}$ | $0.78^{d}$ | $0.68^{g}$ | $0.99^{c}$ | $0.82^{d}$ | | $0.70^{d}$ |
| 20 | kero + sho*AT* | $0.17^{m}$ | $0.14^{q}$ | $0.14^{n}$ | $0.13^{v}$ | $0.13^{u}$ | $0.13^{r}$ | $0.13^{op}$ | $0.12^{e}$ | $0.11^{n}$ |
| 21 | kero + sho3*AO* | $0.15^{n}$ | $0.09^{r}$ | $0.11^{op}$ | $0.16^{t}$ | $0.15^{t}$ | $0.14^{q}$ | $0.14^{o}$ | $0.14^{d}$ | $0.14^{m}$ |
| 22 | kero + oygIv1*AN* | | $0.08^{r}$ | $0.09^{q}$ | $0.11^{w}$ | $0.11^{v}$ | $0.10^{t}$ | $0.10^{q}$ | $0.10^{f}$ | $0.10^{n}$ |
| 23 | kero + oygIv2*AO* | $0.10^{o}$ | $0.08^{r}$ | $0.11^{op}$ | $0.56^{n}$ | $0.56^{k}$ | $0.55^{k}$ | $0.55^{j}$ | $0.44^{c}$ | $0.42^{k}$ |
| 24 | kero + ajgIv1*AO* | | $0.09^{r}$ | $0.09^{q}$ | $0.10^{x}$ | $0.09^{w}$ | $0.08^{u}$ | $0.08^{r}$ | $0.06^{gh}$ | $0.05^{pq}$ |
| 25 | kero + ajgIv2*AN* | $0.35^{g}$ | $0.57^{k}$ | $0.62^{gh}$ | $0.58^{m}$ | $0.48^{n}$ | $0.48^{n}$ | $0.48^{k}$ | $0.46^{b}$ | $0.46^{j}$ |
| 26 | kero + bg1*AN* | | $0.08^{r}$ | $0.08^{r}$ | $0.09^{y}$ | $0.07^{w}$ | $0.06^{v}$ | $0.06^{r}$ | $0.05^{h}$ | $0.04^{q}$ |
| 27 | kero + bg2*AN* | $0.18^{m}$ | $0.08^{r}$ | $0.08^{r}$ | $0.10^{x}$ | $0.09^{w}$ | $0.08^{u}$ | $0.07^{r}$ | $0.07^{g}$ | $0.07^{op}$ |
| 28 | kero + aggIvF | | | $0.12^{nop}$ | $0.14^{u}$ | $0.13^{u}$ | $0.12^{s}$ | $0.11^{pq}$ | $0.11^{f}$ | $0.07^{o}$ |
| 29 | kero + barIv*AN* | $0.21^{kl}$ | $0.16^{p}$ | | | | | | | |

Mean values carrying different superscripts along the same column are significantly different at $p<0.05$.

Appendix 16A: Means separation (using Duncan's multiple range test) for the optical densities of the different fungal species in diesel at 530 nm

| S/N | TMT | DAY 0 | DAY 05 | DAY 10 | DAY 15 | DAY 20 | DAY 25 | DAY 30 | DAY 35 | DAY 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | (Control 1) *A.oryzae* - diesel | 0.08$^{q}$ | 0.25$^{n}$ | 1.38$^{a}$ | 1.40$^{a}$ | 1.41$^{a}$ | 1.38$^{a}$ | 1.45$^{a}$ | | 1.53$^{a}$ |
| 2 | (Control 2) diesel - *A.oryzae* | 0.05$^{r}$ | 0.06$^{v}$ | 0.05$^{x}$ | 0.08$^{v}$ | 0.08$^{t}$ | 0.05$^{x}$ | 0.06$^{q}$ | 0.04$^{g}$ | 0.02$^{s}$ |
| 3 | diesel + ojt1F*AN* | 0.84$^{d}$ | 0.94$^{d}$ | 1.01$^{b}$ | 1.00$^{b}$ | 0.90$^{f}$ | 1.14$^{b}$ | 1.11$^{b}$ | | 0.97$^{d}$ |
| 4 | diesel + ojt2*AO* | 0.85$^{c}$ | 0.95$^{c}$ | 0.92$^{f}$ | 0.92$^{g}$ | 0.97$^{d}$ | 0.99$^{i}$ | 0.86$^{f}$ | | 0.75$^{j}$ |
| 5 | diesel + ojt3*AO* | 0.66$^{h}$ | 0.76$^{i}$ | 0.99$^{c}$ | 0.99$^{c}$ | 1.01$^{b}$ | 1.01$^{g}$ | 0.98$^{d}$ | | 0.94$^{e}$ |
| 6 | diesel + ojt3F*AN* | 0.72$^{g}$ | 0.82$^{g}$ | 0.98$^{c}$ | 0.98$^{d}$ | 0.80$^{j}$ | 1.11$^{d}$ | 1.08$^{b}$ | | 0.95$^{de}$ |
| 7 | diesel + ojt4*AN* | 0.71$^{g}$ | 0.81$^{h}$ | 0.94$^{e}$ | 0.98$^{d}$ | 0.87$^{g}$ | 1.04$^{f}$ | 0.99$^{d}$ | | 0.77$^{i}$ |
| 8 | diesel + ont1*AN* | 0.59$^{i}$ | 0.69$^{j}$ | 0.77$^{k}$ | 0.86$^{i}$ | 0.99$^{c}$ | 1.13$^{c}$ | 1.10$^{b}$ | | 0.93$^{e}$ |
| 9 | diesel + ont3*AN* | 0.74$^{f}$ | 0.84$^{f}$ | 0.81$^{i}$ | 0.94$^{e}$ | 0.95$^{e}$ | 1.10$^{e}$ | 1.04$^{c}$ | | 1.15$^{b}$ |
| 10 | diesel + sab1*AT* | 0.72$^{g}$ | 0.82$^{gh}$ | 0.88$^{g}$ | 0.93$^{f}$ | 0.98$^{c}$ | 0.91$^{l}$ | 0.86$^{f}$ | | 1.02$^{c}$ |
| 11 | diesel + sab1F*AN* | 0.51$^{k}$ | 0.61$^{l}$ | 0.70$^{m}$ | 0.81$^{k}$ | 1.01$^{b}$ | 1.00$^{h}$ | 0.91$^{e}$ | | 0.81$^{h}$ |
| 12 | diesel + sab2*AT* | 0.36$^{l}$ | 0.46$^{m}$ | 0.74$^{l}$ | 0.82$^{j}$ | 0.83$^{i}$ | 0.90$^{m}$ | 0.88$^{f}$ | | 0.86$^{g}$ |
| 13 | diesel + sab2F*AN* | 0.84$^{d}$ | 0.94$^{d}$ | 0.97$^{d}$ | 0.61$^{n}$ | 0.55$^{m}$ | 0.79$^{o}$ | 0.74$^{h}$ | | 0.54$^{l}$ |
| 14 | diesel + sab3*AO* | 0.93$^{a}$ | 1.03$^{a}$ | 0.85$^{h}$ | 0.89$^{h}$ | 0.85$^{h}$ | 0.94$^{k}$ | 0.93$^{e}$ | | 0.95$^{de}$ |
| 15 | diesel + sab3F*AN* | 0.81$^{e}$ | 0.91$^{e}$ | 0.66$^{n}$ | 0.68$^{m}$ | 0.77$^{k}$ | 0.84$^{n}$ | 0.81$^{g}$ | | 0.67$^{k}$ |
| 16 | diesel + sab4*AN* | 0.66$^{h}$ | 0.76$^{i}$ | 0.79$^{j}$ | 0.81$^{k}$ | 0.82$^{i}$ | 0.95$^{j}$ | 0.94$^{e}$ | | 0.86$^{g}$ |
| 17 | diesel + sab4F*AN* | 0.55$^{j}$ | 0.65$^{k}$ | 0.52$^{o}$ | 0.79$^{l}$ | 0.83$^{i}$ | 1.00$^{h}$ | 0.93$^{e}$ | | 0.68$^{k}$ |
| 18 | diesel + sho *AN* | 0.13$^{o}$ | 0.19$^{p}$ | 0.25$^{s}$ | 0.33$^{o}$ | 0.23$^{p}$ | 0.23$^{tu}$ | 0.22$^{lm}$ | 0.18$^{c}$ | 0.13$^{pq}$ |
| 19 | diesel + sho3*AT* | 0.15$^{mn}$ | 0.15$^{q}$ | 0.18$^{u}$ | 0.24$^{r}$ | 0.34$^{n}$ | 0.25$^{s}$ | 0.25$^{k}$ | 0.23$^{b}$ | 0.22$^{n}$ |
| 20 | diesel + bg1*AN* | 0.16$^{m}$ | 0.13$^{s}$ | 0.18$^{u}$ | 0.29$^{p}$ | 0.28$^{o}$ | 0.26$^{r}$ | 0.24$^{kl}$ | 0.15$^{d}$ | 0.15$^{op}$ |
| 21 | diesel + bg2*AN* | 0.09$^{pq}$ | 0.13$^{rs}$ | 0.21$^{t}$ | 0.27$^{q}$ | 0.17$^{s}$ | 0.17$^{w}$ | 0.14$^{op}$ | 0.12$^{ef}$ | 0.12$^{qr}$ |
| 22 | diesel + oygIv1*AN* | 0.10$^{p}$ | 0.09$^{u}$ | 0.13$^{w}$ | 0.22$^{t}$ | 0.21$^{qr}$ | 0.21$^{v}$ | 0.19$^{n}$ | 0.14$^{de}$ | 0.14$^{op}$ |
| 23 | diesel + oygIv2*AO* | 0.10$^{p}$ | 0.11$^{t}$ | 0.29$^{q}$ | 0.24$^{r}$ | 0.22$^{pq}$ | 0.22$^{u}$ | 0.12$^{p}$ | 0.11$^{f}$ | 0.10$^{r}$ |
| 24 | diesel + barIv1*AN* | 0.15$^{mn}$ | 0.14$^{r}$ | 0.21$^{t}$ | 0.24$^{r}$ | 0.23$^{p}$ | 0.23$^{t}$ | 0.19$^{mn}$ | 0.14$^{d}$ | 0.13$^{pq}$ |
| 25 | diesel + ajgIv1*AO* | 0.12$^{o}$ | 0.14$^{rs}$ | 0.16$^{v}$ | 0.18$^{u}$ | 0.17$^{s}$ | 0.17$^{w}$ | 0.15$^{o}$ | 0.13$^{de}$ | 0.10$^{r}$ |
| 26 | diesel + ajgIv*AN* | 0.90$^{b}$ | 0.97$^{b}$ | 0.92$^{f}$ | 0.68$^{m}$ | 0.68$^{l}$ | 0.68$^{p}$ | 0.58$^{i}$ | 0.36$^{a}$ | 0.36$^{m}$ |
| 27 | diesel + aggIvF*AN* | 0.14$^{n}$ | 0.22$^{o}$ | 0.28$^{r}$ | 0.23$^{s}$ | 0.20$^{r}$ | 0.26$^{r}$ | 0.20$^{mn}$ | 0.19$^{c}$ | 0.15$^{o}$ |

Mean values carrying different superscripts along the same column are significantly different at $P<0.05$.

Appendix 16B: means separation (using Duncan's multiple range test) for the optical densities of the different fungal species in diesel at 620nm

| S/N | TMT | DAY 0 | DAY 05 | DAY 10 | DAY 15 | DAY 20 | DAY 25 | DAY 30 | DAY 35 | DAY 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | (Control 1) *AO* - diesel | $0.88^{a}$ | $1.18^{a}$ | $1.19^{a}$ | $1.20^{a}$ | $1.27^{a}$ | $1.29^{a}$ | $1.31^{a}$ | | $1.37^{a}$ |
| 2 | (Control 2) diesel - *AO* | | $0.09^{rs}$ | $0.16^{q}$ | $0.08^{x}$ | $0.09^{s}$ | $0.07^{z}$ | $0.09^{t}$ | $0.06^{h}$ | $0.04^{k}$ |
| 3 | diesel + ojt1F*AN* | $0.75^{c}$ | $0.95^{de}$ | $0.80^{fg}$ | $0.85^{c}$ | $0.85^{d}$ | $0.87^{g}$ | $0.84^{e}$ | | $0.93^{b}$ |
| 4 | diesel + ojt2*AO* | $0.77^{b}$ | $0.97^{bc}$ | $0.82^{e}$ | $0.74^{i}$ | $0.92^{b}$ | $0.97^{b}$ | $0.94^{b}$ | | $0.88^{bcd}$ |
| 5 | diesel + ojt3*AO* | $0.46^{i}$ | $0.66^{k}$ | $0.80^{fg}$ | $0.86^{b}$ | $0.86^{d}$ | $0.89^{e}$ | $0.82^{ef}$ | | $0.90^{bc}$ |
| 6 | diesel + ojt3F*AN* | $0.74^{c}$ | $0.94^{e}$ | $0.79^{g}$ | $0.80^{g}$ | $0.93^{b}$ | $0.77^{m}$ | $0.73^{i}$ | | $0.90^{bc}$ |
| 7 | diesel + ojt4*AN* | $0.52^{g}$ | $0.72^{i}$ | $0.85^{d}$ | $0.85^{c}$ | $0.67^{i}$ | $0.79^{k}$ | $0.77^{h}$ | | $0.78^{bcdef}$ |
| 8 | diesel + ont1*AN* | $0.43^{j}$ | $0.63^{l}$ | $0.81^{f}$ | $0.84^{d}$ | $0.83^{ef}$ | $0.92^{c}$ | $0.87^{d}$ | | $0.83^{bcde}$ |
| 9 | diesel + ont3*AN* | $0.62^{e}$ | $0.82^{g}$ | $0.89^{b}$ | $0.79^{h}$ | $0.82^{ef}$ | $0.88^{f}$ | $0.84^{e}$ | | $0.78^{bcdef}$ |
| 10 | diesel + sab1*AT* | $0.49^{h}$ | $0.69^{j}$ | $0.83^{e}$ | $0.81^{f}$ | $0.87^{c}$ | $0.90^{d}$ | $0.91^{c}$ | | $0.92^{b}$ |
| 11 | diesel + sab1F*AN* | $0.41^{k}$ | $0.61^{m}$ | $0.73^{j}$ | $0.83^{e}$ | $0.83^{ef}$ | $0.81^{i}$ | $0.79^{gh}$ | | $0.78^{bcdef}$ |
| 12 | diesel + sab2*AT* | $0.14^{l}$ | $0.34^{n}$ | $0.68^{k}$ | $0.71^{j}$ | $0.70^{h}$ | $0.72^{o}$ | $0.70^{j}$ | | $0.78^{bcdef}$ |
| 13 | diesel + sab2F*AN* | $0.77^{b}$ | $0.97^{bc}$ | $0.87^{c}$ | $0.58^{m}$ | $0.65^{j}$ | $0.80^{j}$ | $0.64^{k}$ | | $0.67^{f}$ |
| 14 | diesel + sab3*AO* | $0.78^{b}$ | $0.98^{b}$ | $0.77^{h}$ | $0.80^{g}$ | $0.85^{d}$ | $0.82^{h}$ | $0.79^{gh}$ | | $0.77^{cdef}$ |
| 15 | diesel + sab3F*AN* | $0.71^{g}$ | $0.91^{f}$ | $0.62^{l}$ | $0.40^{p}$ | $0.58^{k}$ | $0.73^{n}$ | $0.74^{i}$ | | $0.68^{ef}$ |
| 16 | diesel + sab4*AN* | $0.55^{f}$ | $0.75^{h}$ | $0.75^{i}$ | $0.70^{k}$ | $0.82^{f}$ | $0.79^{k}$ | $0.77^{h}$ | | $0.85^{bcd}$ |
| 17 | diesel + sab4F*AN* | $0.52^{g}$ | $0.72^{i}$ | $0.40^{n}$ | $0.70^{k}$ | $0.75^{g}$ | 0.781 | $0.81^{fg}$ | | $0.73^{def}$ |
| 18 | diesel + sho *AN* | | $0.16^{p}$ | $0.13^{s}$ | $0.21^{t}$ | $0.20^{p}$ | $0.20^{u}$ | $0.19^{p}$ | $0.19^{d}$ | $0.14^{ijk}$ |
| 19 | diesel + sho3*AT* | | $0.12^{q}$ | $0.20^{p}$ | $0.19^{u}$ | $0.29^{n}$ | $0.19^{v}$ | $0.19^{p}$ | $0.18^{d}$ | $0.18^{hijk}$ |
| 20 | diesel + bg1*AN* | | $0.09^{rs}$ | $0.15^{r}$ | $0.43^{o}$ | $0.32^{m}$ | $0.31^{s}$ | $0.27^{o}$ | $0.26^{c}$ | $0.26^{hi}$ |
| 21 | diesel + bg2*AN* | | $0.09^{rs}$ | $0.13^{s}$ | $0.25^{q}$ | $0.15^{qr}$ | $0.15^{x}$ | $0.14^{qr}$ | $0.14^{ef}$ | $0.11^{jk}$ |
| 22 | diesel + oygIv1*AN* | | $0.08^{s}$ | $0.09^{t}$ | $0.28^{r}$ | $0.28^{o}$ | $0.28^{t}$ | $0.29^{n}$ | $0.25^{c}$ | $0.14^{ijk}$ |
| 23 | diesel + oygIv2*AO* | | $0.11^{q}$ | $0.62^{l}$ | $0.56^{n}$ | $0.54^{l}$ | $0.54^{q}$ | $0.44^{m}$ | $0.43^{b}$ | $0.21^{hij}$ |
| 24 | diesel + barIv1*AN* | | $0.11^{q}$ | $0.22^{o}$ | $0.31^{q}$ | $0.20^{p}$ | $0.20^{u}$ | $0.11^{s}$ | $0.10^{g}$ | $0.10^{jk}$ |
| 25 | diesel + ajgIv1*AO* | | $0.08^{s}$ | $0.21^{op}$ | $0.17^{v}$ | $0.16^{q}$ | $0.16^{w}$ | $0.16^{q}$ | $0.15^{e}$ | $0.12^{ijk}$ |
| 26 | diesel + ajgIv*AN* | | $0.96^{cd}$ | $0.83^{e}$ | $0.67^{l}$ | $0.67^{i}$ | $0.67^{p}$ | $0.58^{l}$ | $0.47^{a}$ | $0.47^{g}$ |
| 27 | diesel + aggIvF*AN* | | $0.18^{o}$ | $0.21^{op}$ | $0.16^{w}$ | $0.15^{r}$ | $0.13^{y}$ | $0.12^{rs}$ | $0.12^{fg}$ | $0.31^{h}$ |

Mean values carrying different superscripts along the same column are significantly different at $P<0.05$

Appendix 17A: means separation (using Duncan's multiple range test) for the optical densities of the different fungal species in FEO at 530 nm

| S/N | TMT | DAY 0 | DAY 05 | DAY 10 | DAY 15 | DAY 20 | DAY 25 | DAY 30 | DAY 35 | DAY 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| | FEO + ojt3*AO* | $1.28^{d}$ | $1.38^{e}$ | $1.36^{j}$ | $1.35^{jk}$ | $1.34^{j}$ | $1.35^{hi}$ | $1.32^{f}$ | | $1.28^{fg}$ |
| 2 | FEO + ont1*AN* | $1.27^{de}$ | $1.37^{ef}$ | $1.38^{hi}$ | $1.32^{l}$ | $1.33^{jk}$ | $1.33^{ijk}$ | $1.31^{f}$ | | $1.28^{f}$ |
| 3 | FEO + ojt2*AO* | $1.26^{def}$ | $1.36^{ef}$ | 1 | $1.34^{k}$ | $1.32^{k}$ | $1.34^{ij}$ | $1.32^{f}$ | | $1.30^{f}$ |
| 4 | FEO + ojt2F*AO* | $1.26^{def}$ | $1.36^{ef}$ | $1.39^{h}$ | $1.35^{jk}$ | $1.30^{l}$ | $1.35^{hi}$ | $1.33^{ef}$ | | $1.27^{fg}$ |
| 5 | FEO + ojt1F*AN* | $1.27^{de}$ | $1.37^{ef}$ | $1.37^{ij}$ | $1.36^{j}$ | $1.34^{j}$ | $1.35^{hi}$ | $1.33^{ef}$ | | $1.30^{f}$ |
| 6 | FEO + ojt4*AN* | $1.26^{def}$ | $1.37^{ef}$ | $1.37^{ij}$ | $1.00^{s}$ | $1.25^{m}$ | $1.31^{ijkl}$ | $1.28^{g}$ | | $1.20^{g}$ |
| 7 | FEO + ont1*AN* | $1.26^{def}$ | $1.36^{ef}$ | $1.38^{hi}$ | $1.36^{j}$ | $1.34^{j}$ | $1.35^{hi}$ | $1.34^{ef}$ | | $1.27^{fg}$ |
| 8 | FEO + ont3*AN* | $1.27^{de}$ | $1.37^{ef}$ | $1.37^{ij}$ | $1.34^{k}$ | $1.34^{j}$ | $1.35^{hi}$ | $1.33^{ef}$ | | $1.30^{f}$ |
| 9 | FEO + sab1*AT* | $1.15^{g}$ | $1.22^{f}$ | $1.29^{k}$ | | $0.55^{q}$ | $0.55^{p}$ | $0.51^{k}$ | | $0.48^{j}$ |
| 10 | FEO + sab1F*AN* | $1.25^{def}$ | $1.36^{ef}$ | $1.38^{hi}$ | $1.17^{q}$ | $1.33^{jk}$ | $1.34^{hij}$ | $1.31^{g}$ | | $1.27^{fg}$ |
| 11 | FEO + sab2F*AN* | $1.26^{def}$ | $1.37^{ef}$ | $1.37^{ij}$ | $1.35^{jk}$ | $1.33^{jk}$ | $1.33^{ijk}$ | | | $1.30^{f}$ |
| 12 | FEO + sab3*AO* | $1.27^{de}$ | $1.37^{ef}$ | $1.39^{h}$ | $1.22^{o}$ | $1.33^{jk}$ | $1.30^{jkl}$ | $1.26^{g}$ | | $1.22^{fg}$ |
| 13 | FEO + sab3F*AN* | $1.27^{de}$ | $1.37^{ef}$ | $1.37^{ij}$ | $1.27^{n}$ | $1.34^{j}$ | $1.29^{kl}$ | $1.21^{h}$ | | $1.28^{f}$ |
| 14 | FEO + sab4*AN* | $1.26^{def}$ | $1.36^{ef}$ | $1.37^{ij}$ | $1.28^{n}$ | $1.33^{jk}$ | $1.35^{hi}$ | $1.34^{ef}$ | $1.33^{c}$ | $1.26^{fg}$ |
| 15 | FEO + sab4F*AN* | $1.27^{de}$ | $1.37^{ef}$ | $1.37^{ij}$ | $1.28^{n}$ | $1.33^{jk}$ | $1.27^{l}$ | $1.17^{i}$ | $1.75^{ab}$ | $1.24^{fg}$ |
| 16 | FEO + sab2*AT* | $1.25^{def}$ | $1.35^{ef}$ | $1.38^{hi}$ | $1.19^{p}$ | $1.24^{m}$ | $1.30^{jkl}$ | $1.23^{h}$ | | $1.23^{fg}$ |
| 17 | *AO* – FEO (control 1) | $0.08^{k}$ | $0.25^{h}$ | $1.38^{hi}$ | $1.40^{i}$ | $1.41^{i}$ | $1.38^{gh}$ | $1.45^{c}$ | $1.94^{a}$ | $1.53^{d}$ |
| 18 | FEO – *AO* (control 2) | $0.84^{j}$ | $1.27^{ef}$ | $1.90^{b}$ | $1.76^{c}$ | $1.56^{f}$ | $1.46^{e}$ | $1.22^{h}$ | $1.21^{c}$ | $1.11^{h}$ |
| 19 | FEO + sho *AT* | $1.54^{ab}$ | $1.73^{bcd}$ | $1.80^{c}$ | $1.74^{d}$ | $1.74^{c}$ | $1.72^{a}$ | $1.62^{a}$ | $1.73^{ab}$ | $1.43^{e}$ |
| 20 | FEO + sho3*AO* | $0.91^{ij}$ | $1.85^{ab}$ | $1.94^{a}$ | $1.30^{m}$ | $1.20^{n}$ | $1.20^{m}$ | $1.26^{g}$ | $1.82^{ab}$ | $1.82^{a}$ |
| 21 | FEO + bg1*AN* | $1.61^{a}$ | $1.77^{bc}$ | $1.55^{f}$ | $1.44^{h}$ | $1.53^{g}$ | $1.50^{cd}$ | $1.41^{d}$ | $1.57^{b}$ | $1.30^{f}$ |
| 22 | FEO + bg2*AN* | $1.18^{efg}$ | $0.96^{g}$ | $1.69^{e}$ | $0.80^{t}$ | $0.83^{p}$ | $0.73^{o}$ | $0.53^{k}$ | $0.56^{c}$ | $0.56^{i}$ |
| 23 | FEO + barIv1*AN* | $1.17^{fg}$ | $1.67^{cd}$ | $1.93^{a}$ | $1.89^{a}$ | $1.92^{a}$ | $1.61^{b}$ | $1.32^{f}$ | $1.67^{b}$ | $1.61^{c}$ |
| 24 | FEO + ajgIv1*AO* | $1.39^{c}$ | $1.96^{a}$ | $1.76^{d}$ | $1.50^{g}$ | $1.51^{h}$ | $1.41^{g}$ | $1.21^{h}$ | | $1.55^{cd}$ |
| 25 | FEO + ajgIv2*AN* | $1.04^{h}$ | $1.94^{a}$ | $1.94^{a}$ | $1.66^{e}$ | $1.67^{d}$ | $1.47^{ef}$ | $1.35^{e}$ | | $1.80^{a}$ |
| 26 | FEO + oygIv1*AN* | $0.85^{j}$ | $1.93^{a}$ | $1.37^{i}$ | $1.79^{b}$ | $1.82^{b}$ | $1.72^{a}$ | $1.34^{ef}$ | $1.73^{ab}$ | $1.73^{b}$ |
| 27 | FEO + oygIv2*AO* | $1.50^{b}$ | $1.63^{d}$ | $1.90^{b}$ | $1.10^{r}$ | $1.12^{o}$ | $1.11^{n}$ | $1.01^{j}$ | $1.63^{b}$ | $1.53^{d}$ |
| 28 | FEO + aggIvF*AN* | $0.95^{i}$ | $1.40^{e}$ | $1.48^{g}$ | $1.60^{f}$ | $1.61^{e}$ | $1.54^{c}$ | $1.53^{b}$ | | $1.42^{e}$ |

Mean values carrying different superscripts along the same column are significantly different at $P<0.05$

Appendix 17B: Means separation (using Duncan's multiple range test) for the optical densities of the different fungal species in FEO at 620 nm

| S/N | TMT | DAY 0 | DAY 05 | DAY 10 | DAY 15 | DAY 20 | DAY 25 | DAY 30 | DAY 35 | DAY 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | FEO + ojt3*AO* | 1.09$^{a}$ | 1.19$^{ij}$ | 1.19$^{efg}$ | 1.15$^{mn}$ | 1.13$^{k}$ | 1.14$^{klm}$ | 1.14$^{ij}$ | | 1.06$^{jkl}$ |
| 2 | FEO + ont1*AN* | 1.08$^{abc}$ | 1.18$^{j}$ | 1.19$^{efg}$ | 1.17$^{l}$ | 1.14$^{k}$ | 1.14$^{klm}$ | 1.14$^{ij}$ | | 1.10$^{i}$ |
| 3 | FEO + ojt2*AO* | 1.06$^{c}$ | 1.18$^{ij}$ | 1.19$^{efg}$ | 1.16$^{lm}$ | 1.13$^{k}$ | 1.14$^{klm}$ | 1.15$^{ij}$ | | 1.08$^{ijk}$ |
| 4 | FEO + ojt2F*AO* | 1.09$^{a}$ | 1.19$^{ij}$ | 1.20$^{efg}$ | 1.16$^{lm}$ | 1.15$^{k}$ | 1.15$^{jkl}$ | 1.13$^{jk}$ | | 1.08$^{ijk}$ |
| 5 | FEO + ojt1F*AN* | 1.09$^{a}$ | 1.19$^{ij}$ | 1.18$^{efg}$ | 1.17$^{l}$ | 1.15$^{k}$ | 1.10$^{n}$ | 1.08$^{l}$ | | 1.11$^{i}$ |
| 6 | FEO + ojt4*AN* | 1.07$^{abc}$ | 1.18$^{jk}$ | 1.18$^{efg}$ | 0.92$^{q}$ | 1.09$^{l}$ | 1.12$^{mn}$ | 1.13$^{ijk}$ | | 1.07$^{ijk}$ |
| 7 | FEO + ont1*AN* | 1.09$^{a}$ | 1.19$^{ij}$ | 1.20$^{efg}$ | 1.17$^{l}$ | 1.14$^{k}$ | 1.16$^{ijk}$ | 1.14$^{ij}$ | | 1.07$^{ijk}$ |
| 8 | FEO + ont3*AN* | 1.09$^{a}$ | 1.19$^{ij}$ | 1.20$^{efg}$ | 1.16$^{lm}$ | 1.15$^{k}$ | 1.16$^{ijk}$ | 1.14$^{ij}$ | | 1.08$^{ijk}$ |
| 9 | FEO + sab1*AT* | 0.94$^{d}$ | 1.04$^{n}$ | 1.06$^{fgh}$ | | 0.76$^{o}$ | 0.50$^{p}$ | 0.49$^{n}$ | | 0.50$^{p}$ |
| 10 | FEO + sab1F*AN* | 1.08$^{abc}$ | 1.18$^{ij}$ | 1.19$^{efg}$ | 1.11$^{o}$ | 1.14$^{k}$ | 1.14$^{klm}$ | 1.13$^{k}$ | | 1.08$^{ijk}$ |
| 11 | FEO + sab2F*AN* | 1.06$^{bc}$ | 1.16$^{l}$ | 1.17$^{efg}$ | 1.15$^{mn}$ | 1.04$^{m}$ | 1.18$^{i}$ | | | 1.06$^{jkl}$ |
| 12 | FEO + sab3*AO* | 1.06$^{bc}$ | 1.16$^{kl}$ | 1.17$^{efg}$ | 1.16$^{mn}$ | 1.14$^{k}$ | 1.13$^{ml}$ | 1.11$^{k}$ | | 1.08$^{ijk}$ |
| 13 | FEO + sab3F*AN* | 1.08$^{abc}$ | 1.18$^{j}$ | 1.19$^{efg}$ | 1.16$^{lm}$ | 1.04$^{m}$ | 1.17$^{ij}$ | 1.15$^{ij}$ | | 1.09$^{ij}$ |
| 14 | FEO + sab4*AN* | 1.08$^{abc}$ | 1.18$^{j}$ | 1.19$^{efg}$ | 1.16$^{lm}$ | 1.13$^{k}$ | 1.12$^{mn}$ | 1.08$^{l}$ | 1.32$^{cd}$ | 1.06$^{jkl}$ |
| 15 | FEO + sab4F*AN* | 1.06$^{bc}$ | 1.16$^{kl}$ | 1.21$^{efg}$ | 1.15$^{mn}$ | 1.02$^{n}$ | 1.12$^{mn}$ | 1.11$^{k}$ | 1.39$^{c}$ | 1.09$^{ij}$ |
| 16 | FEO + sab2*AT* | 1.08$^{ab}$ | 1.18$^{ij}$ | 1.20$^{efg}$ | 1.16$^{mn}$ | 1.14$^{k}$ | 1.10$^{n}$ | 1.07$^{l}$ | | 1.05$^{klm}$ |
| 17 | *AO* – FEO (control 1) | 0.88$^{e}$ | 1.18$^{ij}$ | 1.19$^{efg}$ | 1.20$^{k}$ | 1.27$^{j}$ | 1.29$^{h}$ | 1.31$^{h}$ | 1.60$^{b}$ | 1.37$^{f}$ |
| 18 | FEO – *AO* (control 2) | 0.93$^{d}$ | 0.91$^{p}$ | 1.57$^{c}$ | 1.78$^{f}$ | 1.62$^{h}$ | 1.53$^{f}$ | 1.35$^{g}$ | 0.92$^{e}$ | 0.91$^{o}$ |
| 19 | FEO + sho*AN* | | 1.42$^{g}$ | 0.90$^{h}$ | 1.02$^{p}$ | 1.01$^{n}$ | 1.00$^{o}$ | 0.93$^{m}$ | 1.42$^{c}$ | 1.21$^{h}$ |
| 20 | FEO + sho3*AO* | | 0.96$^{o}$ | 1.98$^{b}$ | 1.71$^{i}$ | 1.51$^{i}$ | 1.43$^{g}$ | 1.43$^{f}$ | 0.98$^{e}$ | 0.98$^{n}$ |
| 21 | FEO + bg1*AN* | | 1.08$^{m}$ | 1.06$^{fgh}$ | 1.82$^{e}$ | 1.85$^{e}$ | 1.51$^{f}$ | 1.51$^{e}$ | 1.19$^{d}$ | 1.02$^{m}$ |
| 22 | FEO + bg2*AN* | | 1.49$^{f}$ | 1.05$^{fgh}$ | 1.72$^{h}$ | 1.83$^{f}$ | 1.73$^{d}$ | 1.43$^{f}$ | 1.44$^{c}$ | 1.44$^{d}$ |
| 23 | FEO + barIv1*AN* | | 2.19$^{b}$ | 2.02$^{b}$ | 2.12$^{b}$ | 2.12$^{b}$ | 2.00$^{b}$ | 2.00$^{b}$ | 2.19$^{a}$ | 2.01$^{b}$ |
| 24 | FEO + ajgIv1*AO* | | 1.89$^{d}$ | 0.99$^{gh}$ | 2.63$^{a}$ | 2.64$^{a}$ | 2.44$^{a}$ | 2.22$^{a}$ | | 1.29$^{g}$ |
| 25 | FEO + ajgIv2*AN* | | 1.60$^{e}$ | 1.36$^{de}$ | 1.91$^{d}$ | 1.92$^{d}$ | 1.72$^{d}$ | 1.52$^{e}$ | | 1.52$^{c}$ |
| 26 | FEO + oygIv1*AN* | | 2.23$^{a}$ | 1.52$^{cd}$ | 1.43$^{j}$ | 1.52$^{i}$ | 1.42$^{g}$ | 1.32$^{h}$ | 2.13$^{a}$ | 2.13$^{a}$ |
| 27 | FEO + oygIv2*AO* | | 2.05$^{c}$ | 2.21$^{a}$ | 1.95$^{c}$ | 1.95$^{c}$ | 1.93$^{c}$ | 1.73$^{c}$ | 2.05$^{a}$ | 2.02$^{b}$ |
| 28 | FEO + aggIvF*AN* | | 1.40$^{h}$ | 1.43$^{cd}$ | 1.74$^{g}$ | 1.76$^{g}$ | 1.65$^{e}$ | 1.64$^{d}$ | | 1.41$^{e}$ |

Mean values carrying different superscripts along the same column are significantly different at $P<0.05$

Appendix 18A: Means separation (using Duncan's multiple range test) for the optical densities of the different fungal species in I.O. at 530nm

| S/N | TMT | DAY 0 | DAY 05 | DAY 10 | DAY 15 | DAY 20 | DAY 25 | DAY 30 | DAY 35 | DAY 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | I.O - AO (control 2) | $1.00^{defghi}$ | $1.36^{a}$ | $1.35^{e}$ | $1.35^{bcd}$ | $1.35^{c}$ | $1.02^{m}$ | $1.04^{l}$ | | $1.16^{d}$ |
| 2 | I.O + ojt3*AO* . | $0.95^{j}$ | $1.00^{i}$ | $1.00^{t}$ | $1.10^{efg}$ | $0.88^{ijk}$ | $1.00^{m}$ | $0.99^{n}$ | | $0.79^{l}$ |
| 3 | I.O. + Ont1AN | $1.01^{cdefg}$ | $1.29^{d}$ | $1.29^{g}$ | $1.27^{bcdef}$ | $1.26^{cdef}$ | $1.10^{hij}$ | $1.12^{i}$ | | $1.04^{hi}$ |
| 4 | I.O. + Ojt1f*AN* | $1.03^{cd}$ | $1.14^{e}$ | $1.27^{hi}$ | $1.07^{g}$ | $1.11^{efg}$ | $1.12^{gh}$ | $1.15^{h}$ | | $1.15^{d}$ |
| 5 | I.O. + Ojt2fAO | $1.00^{defghi}$ | $1.00^{i}$ | $1.03^{s}$ | $1.04^{ghi}$ | $1.01^{ghij}$ | | $1.06^{k}$ | | $0.88^{k}$ |
| 6 | I.O. + Ojt3fAN | $1.01^{cdefg}$ | $1.14^{e}$ | $1.25^{j}$ | $1.35^{bcd}$ | $1.13^{efg}$ | $1.19^{e}$ | $1.21^{f}$ | | $0.72^{n}$ |
| 7 | I.O. + Ojt4AN | $0.99^{efghi}$ | $1.12^{f}$ | $1.12^{pq}$ | $1.11^{efg}$ | $1.11^{efg}$ | $1.11^{hi}$ | $1.12^{i}$ | | $1.30^{b}$ |
| 8 | I.O. + Ont1AN | $0.99^{fghi}$ | $1.07^{h}$ | $1.10^{r}$ | $1.29^{bcde}$ | $1.03^{ghi}$ | $1.07^{kl}$ | $1.14^{h}$ | | $1.14^{de}$ |
| 9 | I.O. +ONT3AN | $1.02^{cdef}$ | $1.12^{f}$ | $1.13^{op}$ | $1.22^{cdefg}$ | $1.14^{defg}$ | $1.10^{hij}$ | $1.11^{i}$ | | $1.24^{c}$ |
| 10 | I.O. +Sab1*AT* | $1.01^{defgh}$ | $1.14^{e}$ | $1.26^{ij}$ | $1.05^{gh}$ | $1.12^{efg}$ | | $1.08^{j}$ | | $1.21^{c}$ |
| 11 | I.O. + Sab1f*AN* | $1.00^{defghi}$ | $1.10^{g}$ | $1.16^{m}$ | $1.23^{cdefg}$ | $1.06^{gh}$ | $1.09^{ij}$ | $1.07^{jk}$ | | $0.94^{j}$ |
| 12 | I.O. + Sab2*AT* | $1.07^{b}$ | $1.29^{e}$ | $1.28^{gh}$ | $1.18^{defg}$ | $1.28^{cde}$ | $1.16^{f}$ | $1.14^{h}$ | | $1.09^{fg}$ |
| 13 | I.O. + Sab2fAN | $1.04^{c}$ | $1.14^{e}$ | $1.14^{mn}$ | $1.16^{defg}$ | $1.13^{efg}$ | $1.11^{hi}$ | $1.02^{m}$ | | $0.77^{lm}$ |
| 14 | I.O. + Sab3*AO* | $0.97^{hij}$ | $1.11^{fg}$ | $1.11^{qr}$ | $1.18^{defg}$ | $1.10^{fg}$ | $1.12^{gh}$ | $1.15^{h}$ | | $1.00^{i}$ |
| 15 | I.O. + Sab3f*AN* | $0.98^{ghij}$ | $1.30^{c}$ | $1.27^{hi}$ | $1.04^{ghi}$ | $1.30^{cd}$ | $0.71^{o}$ | $0.76^{p}$ | | $0.73^{mn}$ |
| 16 | I.O. + Sab4*AN* | $1.00^{defghi}$ | $1.08^{h}$ | $0.99^{t}$ | $1.09^{fg}$ | $1.06^{gh}$ | $1.09^{ijk}$ | $1.03^{lm}$ | | $1.16^{d}$ |
| 17 | I.O. + Sab4f*AN* | $1.02^{cde}$ | $1.33^{b}$ | $1.29^{g}$ | $0.88^{hij}$ | $0.99^{gij}$ | $1.05^{l}$ | $1.11^{i}$ | | $1.10^{ef}$ |
| 18 | AO – I.O. (control 1) | $0.08^{r}$ | $0.25^{j}$ | $1.38^{d}$ | $1.40^{bc}$ | $1.41^{c}$ | $1.38^{d}$ | $1.45^{d}$ | | $1.56^{a}$ |
| 19 | I.O. + aggIvf*AN*. | $0.65^{n}$ | | $1.20^{k}$ | $0.69^{klm}$ | $1.59^{b}$ | $1.09^{ijk}$ | $1.11^{i}$ | $1.00^{g}$ | $0.87^{k}$ |
| 20 | I.O. + ajgIv1AO | $1.16^{a}$ | | $1.34^{f}$ | $0.81^{jk}$ | $1.78^{a}$ | $1.14^{g}$ | $1.34^{e}$ | $1.34^{c}$ | $1.21^{c}$ |
| 21 | I.O. + ajgIv2 *AN* | $0.70^{m}$ | | $0.85^{v}$ | $1.67^{a}$ | $1.80^{a}$ | $0.65^{p}$ | | $0.55^{i}$ | $0.35^{p}$ |
| 22 | I.O. + barIv1*AN* | $0.97^{ij}$ | | | | | | | | |
| 23 | I.O. + bg1*AN* | $0.59^{op}$ | | $0.97^{u}$ | $0.59^{lmn}$ | $0.85^{jk}$ | $0.97^{n}$ | $0.78^{o}$ | $0.58^{h}$ | $0.37^{p}$ |
| 24 | I.O. + bg2*AN* . | $0.57^{p}$ | | $1.90^{a}$ | $0.49^{n}$ | $0.59^{l}$ | $1.74^{a}$ | $1.90^{a}$ | $1.05^{e}$ | $1.00^{i}$ |
| 25 | I.O. + oygIv1*AN* | $0.83^{k}$ | | $1.82^{l}$ | $0.55^{mn}$ | $0.55^{l}$ | $1.08^{jk}$ | $1.18^{g}$ | $1.08^{d}$ | $1.05^{gh}$ |
| 26 | I.O. + 0ygIv2*AO* | $0.61^{o}$ | | $1.62^{c}$ | $0.75^{jkl}$ | $0.75^{k}$ | $1.44^{c}$ | $1.82^{b}$ | $1.42^{b}$ | $1.22^{c}$ |
| 27 | I.O.+ sho*AN* | $0.75^{l}$ | | $1.72^{b}$ | $0.53^{mn}$ | $0.53^{l}$ | $1.72^{b}$ | $1.52^{c}$ | $1.44^{a}$ | $1.31^{b}$ |
| 28 | I.O. + sho3*AO* | $1.13^{a}$ | | $1.14^{no}$ | $1.43^{b}$ | $1.43^{c}$ | $1.14^{fg}$ | $1.15^{h}$ | $1.04^{f}$ | $1.02^{hi}$ |
| 29 | I.O. – AN (control 1) | $0.43^{q}$ | | $0.78^{w}$ | $0.86^{ijk}$ | $0.92^{hij}$ | $0.67^{p}$ | $0.68^{q}$ | $0.55^{i}$ | $0.44^{o}$ |

Mean values carrying different superscripts along the same column are significantly different at $P<0.05$

Appendix 18b: Means separation (using Duncan's multiple range test) for the optical densities of the different fungal species in I.O. at 620nm

| S/N | TMT | DAY 0 | DAY 05 | DAY 10 | DAY 15 | DAY 20 | DAY 25 | DAY 30 | DAY 35 | DAY 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | I.O - AO (control 2) | $0.91^{def}$ | $1.11^{c}$ | $1.11^{c}$ | $1.10^{c}$ | $1.12^{d}$ | $0.96^{e}$ | $0.98^{de}$ | | $1.07^{b}$ |
| 2 | I.O + ojt3*AO* . | $0.89^{ef}$ | $0.99^{f}$ | $0.98^{e}$ | $0.92^{ijkl}$ | $0.95^{j}$ | $1.00^{d}$ | $1.03^{c}$ | | $0.83^{g}$ |
| 3 | I.O. + Ont1AN | $0.92^{def}$ | $1.05^{de}$ | $1.06^{d}$ | $0.93^{ijk}$ | $1.03^{f}$ | $0.80^{k}$ | $0.99^{d}$ | | $0.99^{c}$ |
| 4 | I.O. + Ojt1f*AN* | $0.87^{efgh}$ | $0.99^{f}$ | $0.98^{e}$ | $0.91^{jkl}$ | $0.91^{k}$ | $0.91^{f}$ | $0.93^{gh}$ | | $1.09^{ab}$ |
| 5 | I.O. + Ojt2fAO | $0.90^{ef}$ | $0.98^{f}$ | $1.00^{e}$ | $0.94^{hij}$ | $0.80^{m}$ | $0.73^{l}$ | $0.75^{m}$ | | $0.81^{gh}$ |
| 6 | I.O. + Ojt3fAN | $0.90^{def}$ | $1.12^{c}$ | $1.12^{c}$ | $0.93^{ijk}$ | $1.11^{d}$ | $1.04^{f}$ | $1.03^{c}$ | | $0.74^{j}$ |
| 7 | I.O. + Ojt4AN | $0.88^{efg}$ | $1.03^{e}$ | $1.07^{d}$ | $0.95^{ghi}$ | $1.08^{e}$ | $0.80^{k}$ | $0.86^{j}$ | | $0.87^{f}$ |
| 8 | I.O. + Ont1AN | $0.93^{de}$ | $1.03^{e}$ | $1.04^{d}$ | $0.97^{fgh}$ | $1.02^{f}$ | $0.83^{ij}$ | $0.99^{ef}$ | | $0.99^{c}$ |
| 9 | I.O. + ONT3AN | $0.97^{cd}$ | $1.07^{d}$ | $1.07^{d}$ | $0.99^{def}$ | $1.11^{d}$ | $0.84^{i}$ | $0.96^{ef}$ | | $0.95^{d}$ |
| 10 | I.O. + Sab1*AT* | $0.88^{efg}$ | $0.97^{f}$ | $1.00^{e}$ | $0.98^{efg}$ | $0.95^{j}$ | | $0.95^{fg}$ | | $0.95^{d}$ |
| 11 | I.O. + Sab1f*AN* | $0.80^{hij}$ | $1.00^{f}$ | $1.00^{e}$ | $1.07^{c}$ | $0.99^{gh}$ | $0.99^{gh}$ | $0.91^{hi}$ | | $1.00^{c}$ |
| 12 | I.O. + Sab2*AT* | $0.93^{def}$ | $1.15^{b}$ | $1.14^{c}$ | $1.02^{d}$ | $1.13^{d}$ | $0.90^{fg}$ | $0.96^{ef}$ | | $0.98^{c}$ |
| 13 | I.O. +Sab2fAN | $0.75^{ij}$ | $0.85^{g}$ | $0.86^{g}$ | $1.01^{de}$ | $1.00^{g}$ | $0.80^{k}$ | $0.85^{j}$ | | $0.81^{gh}$ |
| 14 | I.O. +Sab3*AO* | $0.87^{efg}$ | $1.00^{f}$ | $1.11^{c}$ | $1.02^{d}$ | $0.98^{ghi}$ | $1.01^{d}$ | $0.96^{ef}$ | | $0.90^{e}$ |
| 15 | I.O. + Sab3f*AN* | $0.80^{hij}$ | $1.10^{c}$ | $1.07^{d}$ | $0.90^{klm}$ | $1.08^{e}$ | $0.67^{m}$ | $0.69^{n}$ | | $0.68^{k}$ |
| 16 | I.O. + Sab4*AN* | $0.86^{fgh}$ | $0.99^{f}$ | $0.98^{e}$ | $0.99^{def}$ | $0.96^{ij}$ | $0.84^{i}$ | $0.87^{j}$ | | $0.77^{i}$ |
| 17 | I.O. + Sab4f*AN* | $0.74^{j}$ | $0.84^{g}$ | $0.80^{h}$ | $1.01^{de}$ | $0.87^{l}$ | $0.87^{h}$ | $0.90^{i}$ | | $0.78^{hi}$ |
| 18 | AO – I.O. (control 1) | $0.88^{ef}$ | $1.18^{a}$ | $1.19^{b}$ | $1.20^{b}$ | $1.27^{c}$ | $1.29^{a}$ | $1.31^{a}$ | | $1.07^{b}$ |
| 19 | I.O. + aggIvf*AN*. | $0.81^{ghi}$ | | $1.36^{a}$ | $1.17^{b}$ | $1.27^{c}$ | $1.16^{b}$ | $1.27^{b}$ | $1.31^{a}$ | $1.11^{a}$ |
| 20 | I.O. + ajgIv1AO | $1.02^{c}$ | | $0.78^{h}$ | $0.86^{m}$ | $0.98^{hi}$ | $0.58^{p}$ | $0.78^{l}$ | $0.78^{b}$ | $0.38^{n}$ |
| 21 | I.O. + ajgIv2 *AN* | | | $0.67^{i}$ | $0.95^{fghi}$ | $1.95^{a}$ | $0.64^{n}$ | $0.57^{p}$ | $0.64^{e}$ | $0.54^{l}$ |
| 22 | I.O. + barIv1*AN* | $0.80^{hij}$ | $1.00^{f}$ | $1.00^{e}$ | $1.07^{c}$ | $0.99^{gh}$ | $0.99^{gh}$ | $0.91^{hi}$ | | $1.00^{c}$ |
| 23 | I.O. + bg1*AN* | $0.90^{ef}$ | | $0.56^{k}$ | $0.88^{lm}$ | $0.95^{j}$ | $0.56^{q}$ | $0.47^{q}$ | $0.27^{h}$ | $0.17^{r}$ |
| 24 | I.O. + bg2*AN* . | | | $0.47^{l}$ | $0.54^{p}$ | $0.74^{n}$ | $0.37^{s}$ | $0.47^{q}$ | $0.26^{h}$ | $0.21^{q}$ |
| 25 | I.O. + oygIv1*AN* | $1.79^{a}$ | | $0.77^{h}$ | $0.54^{p}$ | $0.54^{q}$ | $0.54^{qr}$ | $0.77^{lm}$ | $0.77^{c}$ | $0.67^{k}$ |
| 26 | I.O. + 0ygIv2*AO* | $1.79^{a}$ | | $0.63^{j}$ | $0.70^{n}$ | $0.70^{o}$ | $0.53^{r}$ | $0.63^{o}$ | $0.61^{f}$ | $0.31^{p}$ |
| 27 | I.O.+ sho*AN* | $0.89^{ef}$ | | $0.61^{j}$ | $0.67^{no}$ | $0.67^{p}$ | $0.61^{o}$ | $0.58^{p}$ | $0.51^{g}$ | $0.35^{o}$ |
| 28 | I.O. + sho3*AO* | | | $0.04^{m}$ | $1.34^{a}$ | $1.34^{b}$ | $0.04^{t}$ | $0.03^{r}$ | $0.02^{i}$ | $0.01^{s}$ |
| 29 | I.O. – AN (control 2) | $1.37^{b}$ | | $0.91^{f}$ | $0.66^{o}$ | $0.68^{op}$ | $0.81^{jk}$ | $0.81^{k}$ | $0.71^{d}$ | $0.51^{m}$ |

Mean values carrying different superscripts along the same column are significantly different at $P<0.05$

Appendix 19A: Means separation (using Duncan's multiple range test) for the optical densities of the different fungal species in SEO at 530 nm

| S/N | TMT | DAY 0 | DAY 05 | DAY 10 | DAY 15 | DAY 20 | DAY 25 | DAY 30 | DAY 35 | DAY 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | mss + *AO* - SEO | 0.08$^{h}$ | 0.25$^{q}$ | 1.38$^{i}$ | 1.40$^{b}$ | 1.41$^{c}$ | 1.38$^{c}$ | 1.45$^{d}$ | 1.49$^{a}$ | 1.53$^{a}$ |
| 2 | mss + SEO - *AO* | 0.87$^{f}$ | 1.16$^{n}$ | 0.17$^{q}$ | 0.29$^{o}$ | 1.16$^{h}$ | 1.00$^{k}$ | 0.99$^{m}$ | 1.01$^{d}$ | 1.03$^{f}$ |
| 3 | mss + SEO + ojt 1F*AN* | 0.99$^{ef}$ | 1.39$^{ghijk}$ | 1.01$^{o}$ | 1.36$^{fg}$ | 1.34$^{de}$ | 1.36$^{cde}$ | 1.35$^{ef}$ | 1.32$^{bc}$ | 1.30$^{b}$ |
| 4 | Mss + SEO + ojt2*AO* | 0.99$^{ef}$ | 1.39$^{ghijk}$ | 1.37$^{ij}$ | 1.39$^{c}$ | 1.37$^{cd}$ | 1.35$^{cde}$ | 1.32$^{gh}$ | 1.30$^{bc}$ | 1.28$^{bc}$ |
| 5 | Mss + SEO + ojt2*AO* | 1.01$^{ef}$ | 1.41$^{fg}$ | 1.30$^{l}$ | 1.33$^{h}$ | 1.30$^{efg}$ | 1.30$^{f}$ | 1.28$^{i}$ | 1.27$^{bc}$ | 1.28$^{bc}$ |
| 6 | Mss + SEO + ojt3*AO* | 1.01$^{ef}$ | 1.42$^{f}$ | 1.16$^{m}$ | 1.38$^{cd}$ | 1.26$^{g}$ | 1.30$^{f}$ | 1.29$^{i}$ | 1.29$^{bc}$ | 1.31$^{b}$ |
| 7 | Mss + SEO + ojt3*AN* | 1.00$^{ef}$ | 1.39$^{ghijk}$ | 1.37$^{ij}$ | 1.37$^{def}$ | 1.35$^{de}$ | 1.35$^{cde}$ | 1.35$^{e}$ | 1.32$^{bc}$ | 1.30$^{b}$ |
| 8 | Mss + SEO + ojt4*AN* | 1.01$^{ef}$ | 1.40$^{fghij}$ | 1.37$^{ij}$ | 1.33$^{h}$ | 1.34$^{de}$ | 1.25$^{g}$ | 1.21$^{j}$ | 1.25$^{c}$ | 1.30$^{b}$ |
| 9 | Mss + SEO + ont1*AN* | 1.01$^{ef}$ | 1.41$^{fgh}$ | 1.38$^{i}$ | 1.37$^{def}$ | 1.37$^{cd}$ | 1.35$^{cde}$ | 1.35$^{e}$ | 1.33$^{bc}$ | 1.30$^{b}$ |
| 10 | Mss + SEO + ont3*AN* | 1.01$^{ef}$ | 1.40$^{fghi}$ | 1.34$^{jk}$ | 1.37$^{def}$ | 1.36$^{de}$ | 1.33$^{def}$ | 1.34$^{efg}$ | 1.30$^{bc}$ | 1.28$^{bc}$ |
| 11 | Mss + SEO + sab1*AT* | 1.00$^{ef}$ | 1.39$^{hijk}$ | 1.33$^{k}$ | 1.38$^{cde}$ | 1.37$^{cd}$ | 1.35$^{cde}$ | 1.31$^{h}$ | 1.30$^{bc}$ | 1.31$^{b}$ |
| 12 | Mss + SEO + sab1F*AN* | 1.01$^{ef}$ | 1.41$^{fg}$ | 1.36$^{ijk}$ | 1.37$^{ef}$ | 1.36$^{de}$ | 1.35$^{cde}$ | 1.34$^{ef}$ | 1.31$^{bc}$ | 1.28$^{bc}$ |
| 13 | Mss + SEO + sab2*AT* | 0.98$^{ef}$ | 1.39$^{ghijk}$ | 1.38$^{i}$ | 1.39$^{c}$ | 1.37$^{cd}$ | 1.32ef | 1.35$^{e}$ | 1.34$^{bc}$ | 1.32$^{b}$ |
| 14 | Mss + SEO + sab2F*AN* | 0.98$^{ef}$ | 1.38$^{jk}$ | 1.38$^{i}$ | 1.36$^{fg}$ | 1.27$^{g}$ | 1.19$^{h}$ | 1.34$^{ef}$ | 1.22$^{c}$ | 1.21$^{cd}$ |
| 15 | Mss + SEO + sab3*AO* | 0.99$^{ef}$ | 1.37$^{k}$ | 1.36$^{ijk}$ | 1.37$^{def}$ | 1.36$^{d}$ | 1.35$^{cde}$ | 1.35$^{e}$ | 1.32$^{bc}$ | 1.29$^{b}$ |
| 16 | Mss + SEO + sab3F*AN* | 0.98$^{ef}$ | 1.38$^{jk}$ | 1.34$^{jk}$ | 1.37$^{def}$ | 1.35$^{de}$ | 1.36$^{cde}$ | 1.34$^{ef}$ | 1.32$^{bc}$ | 1.31$^{b}$ |
| 17 | Mss + SEO | 0.69$^{g}$ | 1.39$^{hijk}$ | 1.36$^{ij}$ | 1.33$^{h}$ | 1.34$^{de}$ | 1.37$^{cd}$ | 1.33$^{fg}$ | 1.31$^{bc}$ | 1.29$^{b}$ |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | + sab4*AN* | | | | | | | | | |
| 18 | Mss + SEO + sab4F*AN* | $0.99^{ef}$ | $1.38^{jki}$ | $1.35^{jk}$ | $1.37^{ef}$ | $1.33^{def}$ | $1.35^{cde}$ | $1.34^{efg}$ | $1.31^{bc}$ | $1.30^{b}$ |
| 19 | mss + SEO - *AN* | $1.52^{c}$ | $1.21^{l}$ | $1.06^{n}$ | $0.62^{n}$ | $0.42^{n}$ | $0.37^{p}$ | $0.68^{o}$ | $1.30^{h}$ | $0.24^{j}$ |
| 20 | Mss + SEO + bar Iv1*AN* | $0.69^{g}$ | $1.01^{p}$ | $0.88^{p}$ | $0.85^{k}$ | $0.55^{m}$ | $0.73^{m}$ | 0 | $1.47^{g}$ | $0.49^{h}$ |
| 21 | Mss + SEO + bg1*AN* | $1.37^{cd}$ | $1.56^{c}$ | $2.45^{a}$ | $0.80^{l}$ | $0.76^{k}$ | $0.60^{n}$ | $0.78^{n}$ | $1.50^{fg}$ | $0.45^{hi}$ |
| 22 | Mss + SEO + bg2*AN* | $1.69^{b}$ | $1.56^{c}$ | $0^{r}$ | $1.35^{g}$ | $0.70^{l}$ | $1.07^{j}$ | $1.90^{a}$ | $1.03^{d}$ | $0.96^{f}$ |
| 23 | Mss + SEO + sho1 *AT* | $1.39^{cd}$ | $1.51^{d}$ | $1.42^{h}$ | $0.79^{lm}$ | $1.29^{fg}$ | $0.51^{o}$ | $1.52^{c}$ | $0.31^{h}$ | $0.27^{j}$ |
| 24 | Mss + SEO + sho3*AO* | $0.89^{f}$ | $1.93^{a}$ | $1.56^{f}$ | $1.22^{i}$ | $0.55^{m}$ | $1.08^{j}$ | $1.15^{l}$ | $1.06^{d}$ | $0.98^{f}$ |
| 25 | Mss + SEO + oygIv*AN* | $1.35^{cd}$ | $1.13^{o}$ | $1.65^{e}$ | $0.78^{m}$ | $1.10^{i}$ | $0.76^{lm}$ | $1.18^{k}$ | $0.60^{f}$ | $0.41^{i}$ |
| 26 | Mss + SEO + oygIv2*AO* | $1.34^{d}$ | $1.46^{e}$ | $1.53^{g}$ | $0.87^{j}$ | $0.84^{j}$ | $0.80^{l}$ | $1.62^{b}$ | $0.75^{e}$ | $0.71^{g}$ |
| 27 | Mss + SEO + ajgIv1*AO* | $1.27^{d}$ | $1.55^{c}$ | $2.06^{d}$ | $1.40^{b}$ | $1.59^{a}$ | $1.55^{a}$ | $1.34^{efg}$ | $1.45^{a}$ | $1.18^{de}$ |
| 28 | Mss + SEO + ajgIv2AN | $2.07^{a}$ | $1.77^{b}$ | $2.22^{b}$ | $1.33^{h}$ | $1.11^{i}$ | $1.13^{i}$ | $0.65^{p}$ | $1.07^{d}$ | $1.03^{f}$ |
| 29 | Mss + SEO + aggIvF *AN* | $1.10^{e}$ | $1.18^{m}$ | $2.16^{c}$ | $1.59^{a}$ | $1.51^{b}$ | $1.42^{b}$ | $1.11^{m}$ | $1.38^{ab}$ | $1.11^{e}$ |

Mean values carrying different superscripts along the same column are significantly different at $P<0.05$

Appendix 19B: Means separation (using Duncan's multiple range test) for the optical densities of the different fungal species in SEO at 620 nm

| S/N | TMT | DAY 0 | DAY 05 | DAY 10 | DAY 15 | DAY 20 | DAY 25 | DAY 30 | DAY 35 | DAY 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | mss + *AO* - SEO | 0.88$^{a}$ | 1.18$^{j}$ | 1.19$^{g}$ | 1.20$^{d}$ | 1.27$^{a}$ | 1.29$^{a}$ | 1.31$^{a}$ | 1.34$^{a}$ | 1.37$^{a}$ |
| 2 | mss + SEO - *AO* | 0.79$^{f}$ | 0.99$^{p}$ | 0.16$^{o}$ | 0.16$^{k}$ | 0.98$^{g}$ | 1.14$^{bcdef}$ | 1.16$^{c}$ | 1.02$^{fg}$ | 0.91$^{fg}$ |
| 3 | mss + SEO + ojt 1F*AN* | 0.81$^{e}$ | 1.07$^{n}$ | 0.99$^{l}$ | 1.18$^{e}$ | 1.11$^{cde}$ | 1.16$^{bcd}$ | 1.15$^{cd}$ | 1.10$^{d}$ | 1.06$^{bcd}$ |
| 4 | Mss + SEO + ojt2*AO* | 0.69$^{g}$ | 0.88$^{q}$ | 1.15$^{ij}$ | 1.18$^{e}$ | 1.15$^{bcd}$ | 1.14$^{bcdef}$ | 1.07$^{g}$ | 1.11$^{cd}$ | 1.09$^{b}$ |
| 5 | Mss + SEO + ojt2*AO* | 0.70$^{g}$ | 1.02$^{o}$ | 1.07$^{k}$ | 1.17$^{e}$ | 1.15$^{b}$ | 1.15$^{bcde}$ | 1.10$^{f}$ | 1.07$^{e}$ | 1.03$^{bcd}$ |
| 6 | Mss + SEO + ojt3*AO* | 0.86$^{bc}$ | 1.14$^{kl}$ | 1.15$^{ij}$ | 1.18$^{e}$ | 1.15$^{bc}$ | 1.14$^{bcdef}$ | 1.15$^{cd}$ | 1.16$^{b}$ | 1.09$^{b}$ |
| 7 | Mss + SEO + ojt3*AN* | 0.87$^{ab}$ | 1.17$^{j}$ | 1.18$^{ghi}$ | 1.18$^{e}$ | 1.15$^{bc}$ | 1.14$^{bcdef}$ | 1.15$^{cd}$ | 1.12$^{cd}$ | 1.09$^{b}$ |
| 8 | Mss + SEO + ojt4*AN* | 0.85$^{c}$ | 1.14$^{kl}$ | 1.19$^{g}$ | 1.17$^{e}$ | 1.15$^{bc}$ | 1.14$^{bcdef}$ | 1.16$^{c}$ | 1.12$^{cd}$ | 1.10$^{b}$ |
| 9 | Mss + SEO + ont1*AN* | 0.87$^{ab}$ | 1.17$^{j}$ | 1.19$^{g}$ | 1.18$^{e}$ | 1.14$^{bcd}$ | 1.19$^{b}$ | 1.15$^{cd}$ | 1.12$^{cd}$ | 1.08$^{bc}$ |
| 10 | Mss + SEO + ont3*AN* | 0.84$^{d}$ | 1.11$^{m}$ | 1.16$^{hij}$ | 1.18$^{e}$ | 1.13$^{bcd}$ | 1.16$^{bcd}$ | 1.12$^{def}$ | 1.10$^{d}$ | 1.04$^{bcd}$ |
| 11 | Mss + SEO + sab1*AT* | 0.86$^{bc}$ | 1.16$^{jk}$ | 1.19$^{g}$ | 1.18$^{e}$ | 1.16$^{b}$ | 1.13$^{bcdef}$ | 1.16$^{c}$ | 1.13$^{c}$ | 1.09$^{b}$ |
| 12 | Mss + SEO + sab1F*AN* | 0.86$^{bc}$ | 1.13$^{l}$ | 1.18$^{gh}$ | 1.18$^{e}$ | 1.15$^{bc}$ | 1.16$^{bcd}$ | 1.14$^{cd}$ | 1.12$^{cd}$ | 1.08$^{bc}$ |
| 13 | Mss + SEO + sab2*AT* | 0.86$^{bc}$ | 1.16$^{jk}$ | 1.19$^{g}$ | 1.18$^{e}$ | 1.16$^{b}$ | 1.14$^{bcdef}$ | 1.16$^{c}$ | 1.12$^{cd}$ | 1.09$^{b}$ |
| 14 | Mss + SEO + sab2F*AN* | 0.86$^{bc}$ | 1.16$^{jk}$ | 1.19$^{g}$ | 1.18$^{e}$ | 1.09$^{e}$ | 1.18$^{bc}$ | 1.11$^{ef}$ | 1.07$^{e}$ | 1.04$^{bcd}$ |
| 15 | Mss + SEO + sab3*AO* | 0.86$^{bc}$ | 1.16$^{jk}$ | 1.18$^{gh}$ | 1.17$^{e}$ | 1.16$^{b}$ | 1.15$^{bcde}$ | 1.15$^{cd}$ | 1.12$^{cd}$ | 1.09$^{b}$ |
| 16 | Mss + SEO + sab3F*AN* | 0.85$^{c}$ | 1.13$^{l}$ | 1.17$^{ghij}$ | 1.18$^{e}$ | 1.16$^{b}$ | 1.16$^{bcd}$ | 1.15$^{cd}$ | 1.12$^{cd}$ | 1.09$^{b}$ |
| 17 | Mss + SEO + sab4*AN* | 0.86$^{bc}$ | 1.16$^{jk}$ | 1.14$^{j}$ | 1.18$^{e}$ | 1.15$^{bcd}$ | 1.15$^{bcde}$ | 1.14$^{cde}$ | 1.11$^{cd}$ | 1.09$^{b}$ |
| 18 | Mss + SEO + sab4F*AN* | 0.86$^{bc}$ | 1.16$^{jk}$ | 1.18$^{gh}$ | 1.18 | 1.13$^{bcd}$ | 1.16$^{bcd}$ | 1.14$^{cde}$ | 1.10$^{d}$ | 1.08$^{bc}$ |
| 19 | mss + SEO -*AN* | | 1.35$^{g}$ | 1.27$^{f}$ | 0.35$^{j}$ | 1.11$^{de}$ | 1.10$^{def}$ | 0.81$^{h}$ | 1.01$^{gh}$ | 1.00$^{de}$ |
| 20 | Mss + SEO + bar Iv1*AN* | | 1.22$^{i}$ | 0.78$^{n}$ | 0.96$^{g}$ | 0.78$^{h}$ | 0.66$^{j}$ | | 0.57$^{k}$ | 0.47$^{i}$ |
| 21 | Mss + SEO + bg1*AN* | | 1.54$^{e}$ | 1.72$^{c}$ | 0.98$^{g}$ | 0.99$^{fg}$ | 0.98$^{i}$ | 0.47$^{l}$ | 0.97$^{i}$ | 0.86$^{g}$ |
| 22 | Mss + SEO + bg2*AN* | | 2.50$^{a}$ | | 1.05$^{f}$ | 1.02$^{f}$ | 1.01$^{ghi}$ | 0.47$^{l}$ | 1.00$^{gh}$ | 0.87$^{g}$ |
| 23 | Mss + SEO + sho1*AT* | | 2.11$^{c}$ | 0.92$^{m}$ | 0.76$^{h}$ | 0.72$^{i}$ | 0.71$^{j}$ | 0.58$^{k}$ | 0.70$^{j}$ | 0.77$^{h}$ |
| 24 | Mss + SEO + sho3*AO* | | 2.22$^{b}$ | 2.81$^{a}$ | 1.22$^{c}$ | 1.08$^{e}$ | 1.07$^{efg}$ | 0.03$^{m}$ | 1.03$^{f}$ | 1.01$^{cde}$ |
| 25 | Mss + SEO + oygIv*AN* | | 1.48$^{f}$ | 1.61$^{e}$ | | 0.56$^{j}$ | 0.26$^{k}$ | 0.77$^{i}$ | 0.40$^{l}$ | 0.30$^{j}$ |
| 26 | Mss + SEO + oygIv2*AO* | | 1.56$^{e}$ | 1.66$^{d}$ | 1.20$^{d}$ | 1.08$^{e}$ | 1.07$^{fgh}$ | 0.63$^{j}$ | 1.00$^{gh}$ | 0.88$^{g}$ |
| 27 | Mss + SEO + ajgIv1*AO* | | 1.95$^{d}$ | 1.91$^{b}$ | 1.20$^{d}$ | 1.01$^{fg}$ | 1.00$^{hi}$ | 0.78$^{i}$ | 0.99$^{hi}$ | 0.84$^{g}$ |
| 28 | Mss + SEO + ajgIv2AN | | 2.12$^{c}$ | 1.66$^{d}$ | 1.38$^{b}$ | 1.15$^{bc}$ | 1.11$^{cdef}$ | 0.57$^{k}$ | 1.07$^{e}$ | 0.47$^{i}$ |
| 29 | Mss + SEO + aggIvF *AN* | | 1.27$^{h}$ | 1.89$^{b}$ | 2.03$^{a}$ | 1.02$^{f}$ | 1.00$^{ghi}$ | 1.27$^{b}$ | 1.00$^{gh}$ | 0.96$^{ef}$ |

Mean values carrying different superscripts along the same column are significantly different at $P<0.05$

APPENDIX 20A: TREATMENT EFFECT ON *CYNODON DACTYLON* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.1500 | 0.27 | 323.67 | 0.4855 | 1.15 | 7 | 0.3610 |
| 2 | MONTH 2 | 0.2000 | 0.33 | 313.39 | 0.6268 | 0.73 | 7 | 0.6503 |
| 3 | MONTH 4 | 0 | 0 | - | 0 | - | 7 | - |
| 4 | MONTH 6 | 0 | 0 | - | 0 | - | 7 | - |

APPENDIX 20B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *CYNODON DACTYLON* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 2 | T2 | $0.40^a$ | $0^a$ | $0^a$ | $0^a$ |
| 3 | T3 | $0^a$ | $0.20^a$ | $0^a$ | $0^a$ |
| 4 | T4 | $0.60^a$ | $0.60^a$ | $0^a$ | $0^a$ |
| 5 | T5 | $0^a$ | $0.40^a$ | $0^a$ | $0^a$ |
| 6 | T6 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 7 | T7 | $0.20^a$ | $0.40^a$ | $0^a$ | $0^a$ |
| 8 | T8 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 21A: TREATMENT EFFECT ON *CYPERUS HASPAN* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0 | 0 | - | 0 | - | 7 | - |
| 2 | MONTH 2 | 0 | 0 | - | 0 | - | 7 | - |
| 3 | MONTH 4 | 0 | 0 | - | 0 | - | 7 | - |
| 4 | MONTH 6 | 0.1750 | 0.22 | 423.78 | 0.7416 | 0.77 | 7 | 0.6150 |

APPENDIX 21B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *CYPERUS HASPAN* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 2 | T2 | $0^a$ | $0^a$ | $0^a$ | $0.40^a$ |
| 3 | T3 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 4 | T4 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 5 | T5 | $0^a$ | $0^a$ | $0^a$ | $0.80^a$ |
| 6 | T6 | $0^a$ | $0^a$ | $0^a$ | $0.20^a$ |
| 7 | T7 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 8 | T8 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 22A: TREATMENT EFFECT ON *DESMODIUM SCORPIURUS* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.9250 | 0.56 | 165.54 | 1.5312 | 3.04 | 7 | 0.0164 |
| 2 | MONTH 2 | 0.9250 | 0.36 | 236.46 | 2.1872 | 1.65 | 7 | 0.1632 |
| 3 | MONTH 4 | 0 | 0 | - | 0 | - | 7 | - |
| 4 | MONTH 6 | 0 | 0 | - | 0 | - | 7 | - |

APPENDIX 22B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *DESMODIUM SCORPIURUS* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $2.40^{ab}$ | $0.60^{b}$ | $0^{a}$ | $0^{a}$ |
| 2 | T2 | $0.80^{bc}$ | $0^{b}$ | $0^{a}$ | $0^{a}$ |
| 3 | T3 | $0.00^{c}$ | $0^{b}$ | $0^{a}$ | $0^{a}$ |
| 4 | T4 | $0.00^{c}$ | $1.20^{ab}$ | $0^{a}$ | $0^{a}$ |
| 5 | T5 | $1.20^{abc}$ | $0^{b}$ | $0^{a}$ | $0^{a}$ |
| 6 | T6 | $0.00^{c}$ | $0.80^{ab}$ | $0^{a}$ | $0^{a}$ |
| 7 | T7 | $3.00^{a}$ | $1.00^{ab}$ | $0^{a}$ | $0^{a}$ |
| 8 | T8 | $0.00^{c}$ | $3.80^{a}$ | $0^{a}$ | $0^{a}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 23A: TREATMENT EFFECT ON *EMILIA PRAETERMISSA* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0 | 0 | - | 0 | - | 7 | - |
| 2 | MONTH 2 | 0.0500 | 0.24 | 455.13 | 0.2275 | 0.83 | 7 | 0.5732 |
| 3 | MONTH 4 | 0 | 0 | - | 0 | - | 7 | - |
| 4 | MONTH 6 | 0 | 0 | - | 0 | - | 7 | - |

APPENDIX 23B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *EMILIA PRAETERMISSA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 2 | T2 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 3 | T3 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 4 | T4 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 5 | T5 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 6 | T6 | $0^a$ | $0.20^a$ | $0^a$ | $0^a$ |
| 7 | T7 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 8 | T8 | $0^a$ | $0.20^a$ | $0^a$ | $0^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 24A: TREATMENT EFFECT ON *EUPHORBIA HIRTA* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.0500 | 0.37 | 414.04 | 0.2070 | 1.00 | 7 | 0.4520 |
| 2 | MONTH 2 | 0.1500 | 0.45 | 387.30 | 0.5809 | 2.67 | 7 | 0.0302 |
| 3 | MONTH 4 | 0.1500 | 0.29 | 352.99 | 0.5295 | 0.97 | 7 | 0.4730 |
| 4 | MONTH 6 | 0.1750 | 0.29 | 460.70 | 0.8062 | 0.92 | 7 | 0.5078 |

APPENDIX 24B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *EUPHORBIA HIRTA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^a$ | $0^b$ | $0^a$ | $0^a$ |
| 2 | T2 | $0,20^a$ | $0^b$ | $0.40^a$ | $0.20^a$ |
| 3 | T3 | $0^a$ | $0^b$ | $0^a$ | $0^a$ |
| 4 | T4 | $0^a$ | $1.20^a$ | $0^a$ | $0^a$ |
| 5 | T5 | $0^a$ | $0^b$ | $0^a$ | $0^a$ |
| 6 | T6 | $0.20^a$ | $0^b$ | $0^a$ | $0^a$ |
| 7 | T7 | $0^a$ | $0^b$ | $0.20^a$ | $0.20^a$ |
| 8 | T8 | $0^a$ | $0^b$ | $0.60^a$ | $1.00^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 25A: TREATMENT EFFECT ON *GOMPHRENA CELOSOIDES* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0 | 0 | - | 0 | - | 7 | - |
| 2 | MONTH 2 | 1.3500 | 0.55 | 201.1139 | 2.7150 | 1.53 | 7 | 0.1971 |
| 3 | MONTH 4 | 0.0500 | 0.28 | 632.45 | 0.3162 | 1.0 | 7 | 0.4520 |
| 4 | MONTH 6 | 0.1250 | 0.24 | 463.53 | 0.5794 | 0.84 | 7 | 0.5636 |

APPENDIX 25B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *GOMPHRENA CELOSOIDES* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^a$ | $0.40^{ab}$ | $0^a$ | $0^a$ |
| 2 | T2 | $0^a$ | $1.60^{ab}$ | $0^a$ | $0^a$ |
| 3 | T3 | $0^a$ | $2.60^{ab}$ | $0^a$ | $0.40^a$ |
| 4 | T4 | $0^a$ | $0^b$ | $0^a$ | $0^a$ |
| 5 | T5 | $0^a$ | $0^b$ | $0.40^a$ | $0.60^a$ |
| 6 | T6 | $0^a$ | $1.80^{ab}$ | $0^a$ | $0^a$ |
| 7 | T7 | $0^a$ | $4.20^a$ | $0^a$ | $0^a$ |
| 8 | T8 | $0^a$ | $0.20^b$ | $0^a$ | $0^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 26A: TREATMENT EFFECT ON *HETEROTIS ROTUNDIFOLIA* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 1.0250 | 0.59 | 117.98 | 1.2093 | 5.10 | 7 | 0.0008 |
| 2 | MONTH 2 | 2.3000 | 0.54 | 139.62 | 3.2113 | 2.96 | 7 | 0.0186 |
| 3 | MONTH 4 | 0.1500 | 0.19 | 342.72 | 0.5141 | 0.59 | 7 | 0.7548 |
| 4 | MONTH 6 | 0.5000 | 0.17 | 262.41 | 1.3120 | 0.56 | 7 | 0.7782 |

APPENDIX 26B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *HETEROTIS ROTUNDIFOLIA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^{d}$ | $0^{c}$ | $0.40^{a}$ | $1.20^{a}$ |
| 2 | T2 | $1.80^{abc}$ | $0.60^{bc}$ | $0.20^{a}$ | $0.60^{a}$ |
| 3 | T3 | $3.20^{a}$ | $0^{c}$ | $0^{a}$ | $0.20^{a}$ |
| 4 | T4 | $2.20^{ab}$ | $2.00^{abc}$ | $0.20^{a}$ | $0.40^{a}$ |
| 5 | T5 | $0^{d}$ | $6.40^{a}$ | $0^{a}$ | $0.60^{a}$ |
| 6 | T6 | $0.60^{bc}$ | $5.00^{ab}$ | $0.40^{a}$ | $1.00^{a}$ |
| 7 | T7 | $0.40^{cd}$ | $0.60^{bc}$ | $0^{a}$ | $0^{a}$ |
| 8 | T8 | $0^{d}$ | $3.80^{abc}$ | $0^{a}$ | $0^{a}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 27A: TREATMENT EFFECT ON *IPOMEA INVOLUCRATA* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.2250 | 0.36 | 200.53 | 0.4512 | 1.53 | 7 | 0.1992 |
| 2 | MONTH 2 | 0.4500 | 0.38 | 180.88 | 0.8139 | 1.44 | 7 | 0.2274 |
| 3 | MONTH 4 | 0.9000 | 0.29 | 92.61 | 0.8334 | 1.40 | 7 | 0.2450 |
| 4 | MONTH 6 | 0.9750 | 0.28 | 54.65 | 0.5328 | 1.09 | 7 | 0.3935 |

APPENDIX 27B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *IPOMEA INVOLUCRATA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0.20^{a}$ | $0.80^{a}$ | $1.00^{a}$ | $1.00^{a}$ |
| 2 | T2 | $0^{a}$ | $0.80^{a}$ | $0.40^{a}$ | $0.60^{a}$ |
| 3 | T3 | $0^{a}$ | $0.20^{a}$ | $0.60^{a}$ | $0.80^{a}$ |
| 4 | T4 | $0.60^{a}$ | $0^{a}$ | $1.20^{a}$ | $1.20^{a}$ |
| 5 | T5 | $0.20^{a}$ | $0^{a}$ | $0.20^{a}$ | $1.00^{a}$ |
| 6 | T6 | $0^{a}$ | $0.40^{a}$ | $1.20^{a}$ | $1.40^{a}$ |
| 7 | T7 | $0.20^{a}$ | $1.20^{a}$ | $1.40^{a}$ | $1.00^{a}$ |
| 8 | T8 | $0^{a}$ | $0.20^{a}$ | $1.20^{a}$ | $0.80^{a}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 28A: TREATMENT EFFECT ON *LANTENA CAMARA* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.6000 | 0.43 | 124.60 | 0.7476 | 2.45 | 7 | 0.0428 |
| 2 | MONTH 2 | 1.7000 | 0.61 | 71.5701 | 1.2167 | 3.94 | 7 | 0.0041 |
| 3 | MONTH 4 | 11.7250 | 0.47 | 52.26 | 6.1274 | 1.79 | 7 | 0.1290 |
| 4 | MONTH 6 | 4.9750 | 0.43 | 58.69 | 2.9198 | 1.31 | 7 | 0.2822 |

APPENDIX 28B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *LANTENA CAMARA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^{b}$ | $3.40^{a}$ | $7.80^{b}$ | $3.40^{a}$ |
| 2 | T2 | $0.40^{ab}$ | $2.80^{ab}$ | $19.00^{a}$ | $4.20^{a}$ |
| 3 | T3 | $1.00^{ab}$ | $2.20^{abc}$ | $11.20^{ab}$ | $5.80^{a}$ |
| 4 | T4 | $1.00^{ab}$ | $1.80^{bcd}$ | $13.40^{ab}$ | $5.20^{a}$ |
| 5 | T5 | $0.20^{b}$ | $0.20^{d}$ | $9.00^{b}$ | $6.20^{a}$ |
| 6 | T6 | $0.80^{ab}$ | $0.60^{cd}$ | $13.40^{ab}$ | $6.60^{a}$ |
| 7 | T7 | $1.40^{a}$ | $1.40^{bcd}$ | $11.80^{ab}$ | $6.00^{a}$ |
| 8 | T8 | $0^{b}$ | $1.20^{bcd}$ | $8.20^{b}$ | $2.40^{a}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 29A: TREATMENT EFFECT ON *LAPORTEA AESTUANS* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0 | 0 | - | 0 | - | 7 | - |
| 2 | MONTH 2 | 0 | 0 | - | 0 | - | 7 | - |
| 3 | MONTH 4 | 0 | 0 | - | 0 | - | 7 | - |
| 4 | MONTH 6 | 0.0500 | 0.45 | 387.30 | 0.1936 | 2.67 | 7 | 0.0302 |

APPENDIX 29B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *LAPORTEA AESTUANS* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 2 | T2 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 3 | T3 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 4 | T4 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 5 | T5 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 6 | T6 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 7 | T7 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 8 | T8 | $0^a$ | $0^a$ | $0^a$ | $0.40^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 30A: TREATMENT EFFECT ON *MALVASTRUM COROMANDELIANUM* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.2250 | 0.33 | 247.03 | 0.5558 | 1.38 | 7 | 0.2541 |
| 2 | MONTH 2 | 0.8000 | 0.63 | 147.86 | 1.1749 | 5.96 | 7 | 0.0003 |
| 3 | MONTH 4 | 0 | 0 | - | 0 | - | 7 | - |
| 4 | MONTH 6 | 0 | 0 | - | 0 | - | 7 | - |

APPENDIX 30B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *MALVASTRUM COROMANDELIANUM* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^a$ | $3.80^a$ | $0^a$ | $0^a$ |
| 2 | T2 | $0.40^a$ | $0.20^b$ | $0^a$ | $0^a$ |
| 3 | T3 | $0^a$ | $0.80^b$ | $0^a$ | $0^a$ |
| 4 | T4 | $0.80^a$ | $1.20^b$ | $0^a$ | $0^a$ |
| 5 | T5 | $0.40^a$ | $0.20^b$ | $0^a$ | $0^a$ |
| 6 | T6 | $0^a$ | $0^b$ | $0^a$ | $0^a$ |
| 7 | T7 | $0.20^a$ | $0^b$ | $0^a$ | $0^a$ |
| 8 | T8 | $0^a$ | $0.20^b$ | $0^a$ | $0^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 31A: TREATMENT EFFECT ON *MARISCUS ALTERNIFOLIUS* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.225 | 0.30 | 271.52 | 0.6109 | 1.60 | 7 | 0.1771 |
| 2 | MONTH 2 | 0.2500 | 0.28 | 451.03 | 1.1276 | 0.89 | 7 | 0.5291 |
| 3 | MONTH 4 | 0.1000 | 0.21 | 398.66 | 0.3986 | 0.72 | 7 | 0.6567 |
| 4 | MONTH 6 | 0.0750 | 0.25 | 478.09 | 0.3586 | 0.86 | 7 | 0.5483 |

APPENDIX 31B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *MARISCUS ALTERNIFOLIUS* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^{b}$ | $0^{a}$ | $0.40^{a}$ | $0.20^{a}$ |
| 2 | T2 | $1.00^{a}$ | $0.20^{a}$ | $0.20^{a}$ | $0^{a}$ |
| 3 | T3 | $0.20^{ab}$ | $1.40^{a}$ | $0^{a}$ | $0^{a}$ |
| 4 | T4 | $0.20^{ab}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| 5 | T5 | $0^{b}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| 6 | T6 | $0^{b}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| 7 | T7 | $0.40^{ab}$ | $0.20^{a}$ | $0.20^{a}$ | $0.40^{a}$ |
| 8 | T8 | $0^{b}$ | $0.20^{a}$ | $0^{a}$ | $0^{a}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 32A: TREATMENT EFFECT ON *MITRACARPUS VILLOSUS* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 2.1750 | 0.56 | 147.24 | 3.2024 | 3.97 | 7 | 0.0039 |
| 2 | MONTH 2 | 5.5000 | 0.45 | 112.36 | 6.1800 | 1.55 | 7 | 0.1926 |
| 3 | MONTH 4 | 0 | 0 | - | 0 | - | 7 | - |
| 4 | MONTH 6 | 0.0500 | 0.28 | 632.45 | 0.3162 | 100 | 7 | 0.4520 |

APPENDIX 32B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *MITRACARPUS VILLOSUS* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $8.60^{a}$ | $3.20^{a}$ | $0^{a}$ | $0^{a}$ |
| 2 | T2 | $1.80^{b}$ | $7.60^{a}$ | $0^{a}$ | $0.40^{a}$ |
| 3 | T3 | $0.40^{b}$ | $2.80^{a}$ | $0^{a}$ | $0^{a}$ |
| 4 | T4 | $1.40^{b}$ | $4.80^{a}$ | $0^{a}$ | $0^{a}$ |
| 5 | T5 | $3.60^{b}$ | $2.20^{a}$ | $0^{a}$ | $0^{a}$ |
| 6 | T6 | $0^{b}$ | $2.80^{a}$ | $0^{a}$ | $0^{a}$ |
| 7 | T7 | $1.60^{b}$ | $9.60^{a}$ | $0^{a}$ | $0^{a}$ |
| 8 | T8 | $0^{b}$ | $11.00^{a}$ | $0^{a}$ | $0^{a}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 33A: TREATMENT EFFECT ON *OLDENLANDIA CORYMBOSA* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.3750 | 0.35 | 212.32 | 0.7962 | 1.21 | 7 | 0.3293 |
| 2 | MONTH 2 | 0.7000 | 0.49 | 134.31 | 0.9402 | 3.56 | 7 | 0.0074 |
| 3 | MONTH 4 | 0.5000 | 0.26 | 251.14 | 1.2557 | 0.87 | 7 | 0.5420 |
| 4 | MONTH 6 | 1.8500 | 0.47 | 150.06 | 2.7761 | 3.46 | 7 | 0.0085 |

APPENDIX 33B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *OLDENLANDIA CORYMBOSA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^{a}$ | $2.40^{a}$ | $0.80^{a}$ | $0.80^{b}$ |
| 2 | T2 | $0.80^{a}$ | $1.20^{ab}$ | $1.00^{a}$ | $5.60^{a}$ |
| 3 | T3 | $0.60^{a}$ | $0.80^{b}$ | $0^{a}$ | $0.80^{b}$ |
| 4 | T4 | $1.00^{a}$ | $0.40^{b}$ | $0^{a}$ | $0^{b}$ |
| 5 | T5 | $0.40^{a}$ | $0^{b}$ | $0.40^{a}$ | $1.60^{b}$ |
| 6 | T6 | $0^{a}$ | $0.40^{b}$ | $0^{a}$ | $0^{b}$ |
| 7 | T7 | $0.20^{a}$ | $0.40^{b}$ | $1.40^{a}$ | $5.40^{a}$ |
| 8 | T8 | $0^{a}$ | $0^{b}$ | $0.40^{a}$ | $0.60^{b}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 34A: TREATMENT EFFECT ON *OPLISMENUS BURMANNII* AT THE DIFFERENT TIMES.

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0 | 0 | - | 0 | - | 7 | - |
| 2 | MONTH 2 | 0.2000 | 0.31 | 547.72 | 1.0954 | 1.00 | 7 | 0.4520 |
| 3 | MONTH 4 | 0 | 0 | - | 0 | - | 7 | - |
| 4 | MONTH 6 | 0 | 0 | - | 0 | - | 7 | - |

APPENDIX 34B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *OPLISMENUS BURMANNII* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^a$ | $1.40^a$ | $0^a$ | $0^a$ |
| 2 | T2 | $0^a$ | $0.20^a$ | $0^a$ | $0^a$ |
| 3 | T3 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 4 | T4 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 5 | T5 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 6 | T6 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 7 | T7 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 8 | T8 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 35A: TREATMENT EFFECT ON *PANICUM MAXIMUM* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.6750 | 0.50 | 120.26 | 0.8117 | 3.29 | 7 | 0.0111 |
| 2 | MONTH 2 | 1.4000 | 0.59 | 81.94 | 1.1472 | 4.39 | 7 | 0.0022 |
| 3 | MONTH 4 | 0.5500 | 0.56 | 123.89 | 0.6814 | 4.15 | 7 | 0.0030 |
| 4 | MONTH 6 | 0.5500 | 0.36 | 165.14 | 0.91 | 1.37 | 7 | 0.2573 |

APPENDIX 35B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *PANICUM MAXIMUM* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^{b}$ | $0.80^{bcd}$ | $1.00^{ab}$ | $0.60^{a}$ |
| 2 | T2 | $0.60^{b}$ | $2.40^{ab}$ | $0.20^{bc}$ | $0.80^{a}$ |
| 3 | T3 | $2.00^{a}$ | $2.80^{a}$ | $0^{c}$ | $0^{a}$ |
| 4 | T4 | $0.80^{b}$ | $1.80^{abc}$ | $1.60^{a}$ | $1.00^{a}$ |
| 5 | T5 | $0.20^{b}$ | $2.40^{ab}$ | $0^{c}$ | $0^{a}$ |
| 6 | T6 | $1.00^{ab}$ | $0.60^{cd}$ | $1.20^{a}$ | $1.00^{a}$ |
| 7 | T7 | $0.80^{b}$ | $0.40^{cd}$ | $0.20^{bc}$ | $0^{a}$ |
| 8 | T8 | $0^{b}$ | $0^{d}$ | $0.20^{bc}$ | $1.00^{a}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 36A: TREATMENT EFFECT ON *PASPALUM SCROBICULATUM* AT THE DIFFERENT TIMES.

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0 | 0 | - | 0 | - | 7 | - |
| 2 | MONTH 2 | 2.0200 | 0.70 | 104.72 | 2.1205 | 7.53 | 7 | <0.0001 |
| 3 | MONTH 4 | 0.3500 | 0.22 | 304.72 | 1.0665 | 0.74 | 7 | 0.6396 |
| 4 | MONTH 6 | 0.8250 | 0.37 | 166.37 | 1.3726 | 0.71 | 7 | 0.6632 |

APPENDIX 36B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *PASPALUM SCROBICULATUM* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^{a}$ | $8.20^{a}$ | $0.60^{a}$ | $0.80^{a}$ |
| 2 | T2 | $0^{a}$ | $2.20^{b}$ | $0.40^{a}$ | $0.80^{a}$ |
| 3 | T3 | $0^{a}$ | $2.00^{b}$ | $0^{a}$ | $0.60^{a}$ |
| 4 | T4 | $0^{a}$ | $1.20^{b}$ | $0^{a}$ | $0.60^{a}$ |
| 5 | T5 | $0^{a}$ | $0.60^{b}$ | $0^{a}$ | $1.20^{a}$ |
| 6 | T6 | $0^{a}$ | $0^{b}$ | $0^{a}$ | $0.80^{a}$ |
| 7 | T7 | $0^{a}$ | $1.40^{b}$ | $0.80^{a}$ | $1.80^{a}$ |
| 8 | T8 | $0^{a}$ | $0.60^{b}$ | $1.00^{a}$ | $0^{a}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 37A: TREATMENT EFFECT ON *PHYLANTHUS AMARUS* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0 | 0 | - | 0 | - | 7 | - |
| 2 | MONTH 2 | 0 | 0 | - | 0 | - | 7 | - |
| 3 | MONTH 4 | 0.4250 | 0.37 | 282.28 | 1.1997 | 2.04 | 7 | 0.0847 |
| 4 | MONTH 6 | 0.0250 | 0.28 | 632.45 | 0.1581 | 1.00 | 7 | 0.4520 |

APPENDIX 37B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *PHYLANTHUS AMARUS* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^{a}$ | $0^{a}$ | $0.80^{ab}$ | $0^{a}$ |
| 2 | T2 | $0^{a}$ | $0^{a}$ | $0^{b}$ | $0.20^{a}$ |
| 3 | T3 | $0^{a}$ | $0^{a}$ | $0^{b}$ | $0^{a}$ |
| 4 | T4 | $0^{a}$ | $0^{a}$ | $0.20^{b}$ | $0^{a}$ |
| 5 | T5 | $0^{a}$ | $0^{a}$ | $0^{b}$ | $0^{a}$ |
| 6 | T6 | $0^{a}$ | $0^{a}$ | $0^{b}$ | $0^{a}$ |
| 7 | T7 | $0^{a}$ | $0^{a}$ | $2.20^{a}$ | $0^{a}$ |
| 8 | T8 | $0^{a}$ | $0^{a}$ | $0.20^{b}$ | $0^{a}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 38A: TREATMENT EFFECT ON *SCHWENCKIA AMERICANA* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.0500 | 0.28 | 632.46 | 0.3162 | 1.00 | 7 | 0.4520 |
| 2 | MONTH 2 | 0.1500 | 0.45 | 387.30 | 0.5809 | 2.67 | 7 | 0.0302 |
| 3 | MONTH 4 | 0.0250 | 0.28 | 632.46 | 0.1581 | 1.00 | 7 | 0.4520 |
| 4 | MONTH 6 | 0 | 0 | - | 0 | - | 7 | - |

APPENDIX 38B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *SCHWENCKIA AMERICANA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^{a}$ | $1.20^{a}$ | $0^{a}$ | $0^{a}$ |
| 2 | T2 | $0.40^{a}$ | $0^{b}$ | $0^{a}$ | $0^{a}$ |
| 3 | T3 | $0^{a}$ | $0^{b}$ | $0^{a}$ | $0^{a}$ |
| 4 | T4 | $0^{a}$ | $0^{b}$ | $0^{a}$ | $0^{a}$ |
| 5 | T5 | $0^{a}$ | $0^{b}$ | $0.20^{a}$ | $0^{a}$ |
| 6 | T6 | $0^{a}$ | $0^{b}$ | $0^{a}$ | $0^{a}$ |
| 7 | T7 | $0^{a}$ | $0^{b}$ | $0^{a}$ | $0^{a}$ |
| 8 | T8 | $0^{a}$ | $0^{b}$ | $0^{a}$ | $0^{a}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 39A: TREATMENT EFFECT ON *SENNA OBTUSIFOLIA* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.4250 | 0.31 | 394.73 | 1.6776 | 1.35 | 7 | 0.2652 |
| 2 | MONTH 2 | 0.3500 | 0.30 | 385.79 | 1.3503 | 0.84 | 7 | 0.5651 |
| 3 | MONTH 4 | 0 | 0 | - | 0 | - | 7 | - |
| 4 | MONTH 6 | 0 | 0 | - | 0 | - | 7 | - |

APPENDIX 39B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *SENNA OBTUSIFOLIA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 2 | T2 | $2.40^a$ | $0^a$ | $0^a$ | $0^a$ |
| 3 | T3 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 4 | T4 | $1.00^a$ | $0.60^a$ | $0^a$ | $0^a$ |
| 5 | T5 | $0^a$ | $0.20^a$ | $0^a$ | $0^a$ |
| 6 | T6 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 7 | T7 | $0^a$ | $1.60^a$ | $0^a$ | $0^a$ |
| 8 | T8 | $0^a$ | $0.40^a$ | $0^a$ | $0^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 40A: TREATMENT EFFECT ON *SETARIA BARBATA* AT THE DIFFERENT TIMES.

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 6.0750 | 0.67 | 76.91 | 4.6723 | 7.87 | 7 | <0.0001 |
| 2 | MONTH 2 | 3.5750 | 0.89 | 55.49 | 1.9839 | 32.46 | 7 | <0.0001 |
| 3 | MONTH 4 | 1.3500 | 0.39 | 286.80 | 3.8718 | 1.77 | 7 | 0.1338 |
| 4 | MONTH 6 | 2.6750 | 0.32 | 264.23 | 7.0680 | 1.40 | 7 | 0.2444 |

APPENDIX 40B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *SETARIA BARBATA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $18.00^{a}$ | $0.20^{d}$ | $2.20^{ab}$ | $6.00^{a}$ |
| 2 | T2 | $3.40^{bcd}$ | $0.80^{d}$ | $0^{b}$ | $0.60^{a}$ |
| 3 | T3 | $1.80^{cd}$ | $0^{d}$ | $0^{b}$ | $0^{a}$ |
| 4 | T4 | $1.40^{cd}$ | $0.40^{d}$ | $0^{b}$ | $0^{a}$ |
| 5 | T5 | $7.20^{bc}$ | $0.40^{d}$ | $0^{b}$ | $0.40^{a}$ |
| 6 | T6 | $9.40^{b}$ | $4.60^{c}$ | $0.20^{b}$ | $0.40^{a}$ |
| 7 | T7 | $7.40^{bc}$ | $8.60^{b}$ | $1.80^{ab}$ | $3.80^{a}$ |
| 8 | T8 | $0^{a}$ | $13.60^{a}$ | $6.60^{a}$ | $10.20^{a}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 41A: TREATMENT EFFECT ON *SIDA ACUTA* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.3750 | 0.28 | 632.46 | 2.3717 | 1.0 | 7 | 0.4520 |
| 2 | MONTH 2 | 0.3250 | 0.36 | 428.49 | 1.3926 | 1.0 | 7 | 0.4520 |
| 3 | MONTH 4 | 0.3500 | 0.40 | 442.63 | 1.5492 | 2.04 | 7 | 0.0848 |
| 4 | MONTH 6 | 0.2750 | 0.46 | 285.01 | 0.7838 | 1.20 | 7 | 0.3333 |

APPENDIX 41B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *SIDA ACUTA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^a$ | $0^a$ | $0^b$ | $0^a$ |
| 2 | T2 | $3.00^a$ | $1.00^a$ | $0^b$ | $0.80^a$ |
| 3 | T3 | $0^a$ | $0^a$ | $0^b$ | $0^a$ |
| 4 | T4 | $0^a$ | $0^a$ | $2.80^a$ | $0.80^a$ |
| 5 | T5 | $0^a$ | $1.60^a$ | $0^b$ | $0.60^a$ |
| 6 | T6 | $0^a$ | $0^a$ | $0^b$ | $0^a$ |
| 7 | T7 | $0^a$ | $0^a$ | $0^b$ | $0^a$ |
| 8 | T8 | $0^a$ | $0^a$ | $0^b$ | $0^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 42A: TREATMENT EFFECT ON *SOLENOSTEMON MONOSTACHYUS* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0 | 0 | - | 0 | - | 7 | - |
| 2 | MONTH 2 | 0 | 0 | - | 0 | - | 7 | - |
| 3 | MONTH 4 | 0.1500 | 0.32 | 430.02 | 0.6450 | 1.06 | 7 | 0.4114 |
| 4 | MONTH 6 | 0.3500 | 0.27 | 552.76 | 1.9346 | 0.94 | 7 | 0.4903 |

APPENDIX 42B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *SOLENOSTEMON MONOSTACHYUS* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^a$ | $0^a$ | $0^a$ | $2.40^a$ |
| 2 | T2 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 3 | T3 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 4 | T4 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 5 | T5 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 6 | T6 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 7 | T7 | $0^a$ | $0^a$ | $0.80^a$ | $0.40^a$ |
| 8 | T8 | $0^a$ | $0^a$ | $0.40^a$ | $0^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 43A: TREATMENT EFFECT ON *SPIGELIA ANTHELMIA* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.1000 | 0.28 | 632.46 | 0.6324 | 1.0 | 7 | 0.4250 |
| 2 | MONTH 2 | 0 | 0 | - | 0 | 0 | 7 | 0 |
| 3 | MONTH 4 | 0.0500 | 0.24 | 455.13 | 0.2276 | 0.83 | 7 | 0.5732 |
| 4 | MONTH 6 | 0.0750 | 0.28 | 356.45 | 0.2673 | 0.75 | 7 | 0.6326 |

APPENDIX 43B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *SPIGELIA ANTHELMIA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $0^a$ | $0^a$ | $0^a$ | $0.20^a$ |
| 2 | T2 | $0.80^a$ | $0^a$ | $0^a$ | $0^a$ |
| 3 | T3 | $0^a$ | $0^a$ | $0^a$ | $0.20^a$ |
| 4 | T4 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 5 | T5 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 6 | T6 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 7 | T7 | $0^a$ | $0^a$ | $0.20^a$ | $0^a$ |
| 8 | T8 | $0^a$ | $0^a$ | $0.20^a$ | $0.20^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 44A: TREATMENT EFFECT ON *SYNEDRELLA NODIFLORA* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 4.3000 | 0.57 | 92.63 | 3.9830 | 4.56 | 7 | 0.0017 |
| 2 | MONTH 2 | 10.700 | 0.56 | 87.09 | 9.3188 | 3.33 | 7 | 0.0105 |
| 3 | MONTH 4 | 0 | 0 | - | 0 | - | 7 | - |
| 4 | MONTH 6 | 2.1250 | 0.48 | 113.58 | 2.4135 | 3.16 | 7 | 0.0137 |

APPENDIX 44B: MEAN SEPARATION FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *SYNEDRELLA NODIFLORA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | Month 2 | Month 4 | Month 6 |
|---|---|---|---|---|---|
| 1 | T1 | $4.60^{bc}$ | $5.40^{bc}$ | $0^{a}$ | $2.00^{abcd}$ |
| 2 | T2 | $8.00^{ab}$ | $18.60^{ab}$ | $0^{a}$ | $3.80^{ab}$ |
| 3 | T3 | $2.60^{bc}$ | $24.00^{a}$ | $0^{a}$ | $0.20^{cd}$ |
| 4 | T4 | $11.60^{a}$ | $10.40^{bc}$ | $0^{a}$ | $0.40^{bcd}$ |
| 5 | T5 | $3.40^{bc}$ | $2.60^{c}$ | $0^{a}$ | $0^{d}$ |
| 6 | T6 | $1.00^{c}$ | $7.60^{bc}$ | $0^{a}$ | $1.80^{abcd}$ |
| 7 | T7 | $3.20^{bc}$ | $13.60^{abc}$ | $0^{a}$ | $5.20^{a}$ |
| 8 | T8 | $0^{d}$ | $3.40^{c}$ | $0^{a}$ | $3.60^{abc}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 45A: TREATMENT EFFECT ON *TALINUM TRIANGULARE* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.5500 | 0.47 | 208.86 | 1.1487 | 2.41 | 7 | 0.0457 |
| 2 | MONTH 2 | 1.7250 | 0.57 | 223.72 | 3.8591 | 1.12 | 7 | 0.3807 |
| 3 | MONTH 4 | 0 | 0 | - | 0 | - | 7 | - |
| 4 | MONTH 6 | 0.1250 | 0.30 | 408.48 | 0.5106 | 0.86 | 7 | 0.5469 |

APPENDIX 45B: MEANS SEPARATION FOR THE EFFECT OF TREATMENTS ON *TALINUM TRIANGULARE*

| s/n | Treatment | Baseline Mean | 2ND Month | 4TH Month | 6TH Month |
|---|---|---|---|---|---|
| 1 | T1 | $2.40^a$ | $0^a$ | $0^a$ | $0.60^a$ |
| 2 | T2 | $0.60^b$ | $1.60^a$ | $0^a$ | $0^a$ |
| 3 | T3 | $0.60^b$ | $2.00^a$ | $0^a$ | $0^a$ |
| 4 | T4 | $0^b$ | $0^a$ | $0^a$ | $0^a$ |
| 5 | T5 | $0.60^b$ | $0^a$ | $0^a$ | $0^a$ |
| 6 | T6 | $0^a$ | $1.60^a$ | $0^a$ | $0^a$ |
| 7 | T7 | $0.20^b$ | $3.60^a$ | $0^a$ | $0.20^a$ |
| 8 | T8 | $0^a$ | $5.00^a$ | $0^a$ | $0.20^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

APPENDIX 46A: TREATMENT EFFECT ON *TRIDAX PROCUMBENS* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.0750 | 0.46 | 308.61 | 0.2314 | 1.00 | 7 | 0.4520 |
| 2 | MONTH 2 | 0.2000 | 0.27 | 561.80 | 1.1236 | 0.95 | 7 | 0.4850 |
| 3 | MONTH 4 | 1.0000 | 0.33 | 407.43 | 4.0743 | 0.92 | 7 | 0.5043 |
| 4 | MONTH 6 | 0.4250 | 0.36 | 422.55 | 1.7958 | 1.00 | 7 | 0.4520 |

APPENDIX 46B: MEANS SEPARATION FOR THE EFFECT OF TREATMENTS ON *TRIDAX PROCUMBENS*

| s/n | Treatment | Baseline Mean | 2ND Month | 4TH Month | 6TH Month |
|---|---|---|---|---|---|
| 1 | T1 | $0.20^a$ | $1.40^a$ | $2.00^a$ | $1.40^a$ |
| 2 | T2 | $0^a$ | $0.20^a$ | $5.00^a$ | $2.00^a$ |
| 3 | T3 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 4 | T4 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 5 | T5 | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| 6 | T6 | $0.20^a$ | $0^a$ | $0.20^a$ | $0^a$ |
| 7 | T7 | $0.20^a$ | $0^a$ | $0.60^a$ | $0^a$ |
| 8 | T8 | $0^a$ | $0^a$ | $0.20^a$ | $0^a$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 47A: TREATMENT EFFECT ON *AXONOPUS COMPRESSUS* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 0.6000 | 0.34 | 129.29 | 0.7757 | 1.52 | 7 | 0.2015 |
| 2 | MONTH 2 | 0.4750 | 0.35 | 222.41 | 1.0564 | 1.07 | 7 | 0.4068 |
| 3 | MONTH 4 | 0.6000 | 0.44 | 208.10 | 1.2486 | 2.35 | 7 | 0.0511 |
| 4 | MONTH 6 | 0.7000 | 0.23 | 177.86 | 1.2450 | 0.15 | 7 | 0.9930 |

Appendix 47B: MEAN SEPARATION **(USING DUNCAN'S MULTIPLE RANGE TEST**) FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *AXONOPUS COMPRESSUS* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean values | $2^{nd}$ Month mean values | $4^{th}$ Month mean values | $6^{th}$ Month mean values |
|---|---|---|---|---|---|
| 1 | **T1** | $0.20^{ab}$ | $0.40^{bC}$ | $0^{b}$ | $0.60^{b}$ |
| 2 | **T2** | $1.00^{ab}$ | $1.60^{a}$ | $0.40^{b}$ | $0.00^{b}$ |
| 3 | **T3** | $0.60^{ab}$ | $1.80^{a}$ | $0^{b}$ | $0.60^{b}$ |
| 4 | **T4** | $0.40^{ab}$ | $0.40^{ab}$ | $0.80^{b}$ | $1.40^{a}$ |
| 5 | **T5** | $1.10^{ab}$ | $0.90^{b}$ | $0.20^{b}$ | $1.00^{a}$ |
| 6 | **T6** | $0.40^{ab}$ | $0^{C}$ | $0.60^{b}$ | $0.40^{b}$ |
| 7 | **T7** | $1.00^{ab}$ | $0.60^{b}$ | $0.20^{b}$ | $0.20^{b}$ |
| 8 | **T8** | $0.00^{b}$ | $0.20^{bC}$ | $1.70^{a}$ | $1.20^{a}$ |

Mean values carrying different superscripts along the same column are significantly different at $P < 0.05$

Appendix 48A: TREATMENT EFFECT ON *COMMELINA ERECTA* AT THE DIFFERENT TIMES

| S/N | TIME | WEED MEAN | R-SQUARE | COV | ROOT MSE | F-VALUE | DF | P-Value |
|---|---|---|---|---|---|---|---|---|
| 1 | BASELINE | 1.3500 | 0.41 | 127.03 | 1.7150 | 2.23 | 7 | 0.0620 |
| 2 | MONTH 2 | 1.3000 | 0.34 | 160.57 | 2.0874 | 1.05 | 7 | 0.4207 |
| 3 | MONTH 4 | 1.3000 | 0.38 | 95.66 | 1.2436 | 0.67 | 7 | 0.6993 |
| 4 | MONTH 6 | 0.6750 | 0.33 | 150.12 | 8.375 | 1.17 | 7 | 0.3535 |

Appendix 48B: MEAN SEPARATION (**USING DUNCAN'S MULTIPLE RANGE TEST**) FOR THE EFFECT OF THE DIFFERENT TREATMENTS ON *COMMELINA ERECTA* AT THE DIFFERENT TIMES

| s/n | Treatment | Baseline Mean | 2nd Month mean values | 4th Month mean values | 6th Month mean values |
|---|---|---|---|---|---|
| 1 | T1 | $3.60^{a}$ | $0.60^{c}$ | $2.00^{a}$ | $0.60^{ab}$ |
| 2 | T2 | $1.80^{ab}$ | $0.20^{c}$ | $0.60^{a}$ | $0.00^{b}$ |
| 3 | T3 | $1.80^{ab}$ | $2.60^{a}$ | $1.60^{a}$ | $0.90^{a}$ |
| 4 | T4 | $0.40^{b}$ | $1.00^{c}$ | $1.60^{a}$ | $1.40^{a}$ |
| 5 | T5 | $1.60^{ab}$ | $1.80^{b}$ | $1.60^{a}$ | $1.00^{a}$ |
| 6 | T6 | $1.60^{ab}$ | $1.60^{b}$ | $1.20^{a}$ | $0.40^{ab}$ |
| 7 | T7 | $1.20^{ab}$ | $2.80^{a}$ | $1.40^{a}$ | $0.20^{b}$ |
| 8 | T8 | $0.00^{b}$ | $2.60^{a}$ | $1.20^{a}$ | $1.20^{a}$ |

Along each column, mean values carrying different superscripts are significantly different at $p < 0.05$.

Appendix 49: Meteorological data covering the entire duration of sampling and field mycoremediation work

| NIGERAN METEOROLOGICAL AGENCY, LAGOS | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | |
| | | | | | | | T MIN | | | | | | |
| **STN** | **YEAR** | **JAN** | **FEB** | **MAR** | **APR** | **MAY** | **JUN** | **JUL** | **AUG** | **SEP** | **OCT** | **NOV** | **DEC** |
| Lagos | 2007 | 23.8 | 25.1 | 24.7 | 24.7 | 24.2 | 23.3 | 23.7 | 22.9 | 23.5 | 23.5 | 24.4 | 23.7 |
| Lagos | 2008 | 24 | 27.4 | 27.9 | 25.5 | 26.3 | 25.4 | 24.9 | 23.3 | 23.9 | 24.4 | 25.6 | 24.5 |
| Lagos | 2009 | 24.9 | 21 | 26.8 | 26 | 25.8 | 25.1 | 24.2 | 24.6 | 24.8 | 24.4 | 25.3 | 25 |
| Lagos | 2010 | 25.6 | 25.1 | 26.7 | 26.1 | 25.2 | 24.6 | 24.1 | 24 | 24.4 | 25.2 | 26.3 | 25.7 |
| | | | | | | | | | | | | | |
| | | | | | | | T MAX | | | | | | |
| **STN** | **YEAR** | **JAN** | **FEB** | **MAR** | **APR** | **MAY** | **JUN** | **JUL** | **AUG** | **SEP** | **OCT** | **NOV** | **DEC** |
| Lagos | 2007 | 31.1 | 31.8 | 31.9 | 32.2 | 31.1 | 27.2 | 28.8 | 27.4 | 27.9 | 29 | 30.8 | 31.6 |
| Lagos | 2008 | 31.1 | 32.9 | 32.9 | 31.4 | 31.5 | 30.6 | 28.4 | 27.9 | 28.4 | 29.7 | 31.3 | 31 |
| Lagos | 2009 | 30.5 | 31.3 | 31.5 | 31.2 | 30.7 | 30.2 | 28.7 | 28.5 | 28.7 | 29.4 | 30.3 | 31.1 |
| Lagos | 2010 | 31.1 | 31.7 | 32.3 | 31.8 | 31.5 | 29.7 | 29.3 | 28.2 | 29 | 29.9 | 31.3 | 31.4 |
| | | | | | | | | | | | | | |
| | | | | | **RELATIVE HUMIDITY @ 09 HOURS (%)** | | | | | | | | |
| **STN** | **YEAR** | **JAN** | **FEB** | **MAR** | **APR** | **MAY** | **JUN** | **JUL** | **AUG** | **SEP** | **OCT** | **NOV** | **DEC** |
| Lagos | 2007 | 61 | 80 | 77 | 75 | 80 | 83 | 81 | 85 | 86 | 75 | 73 | 70 |
| Lagos | 2008 | 68 | 74 | 74 | 75 | 76 | 79 | 83 | 81 | 83 | 79 | 77 | 76 |
| Lagos | 2009 | 83 | 78 | 75 | 76 | 76 | 79 | 85 | 80 | 79 | 82 | 78 | 78 |

| Lagos | 2010 | 81 | 62 | 73 | 74 | 76 | 83 | 80 | 82 | 85 | 79 | 78 | 78 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | |
| | | | | | **RELATIVE HUMIDITY @ 15 HOURS (%)** | | | | | | | | |
| **STN** | **YEAR** | **JAN** | **FEB** | **MAR** | **APR** | **MAY** | **JUN** | **JUL** | **AUG** | **SEP** | **OCT** | **NOV** | **DEC** |
| Lagos | 2007 | 45 | 69 | 68 | 71 | 73 | 79 | 73 | 76 | 73 | 70 | 65 | 62 |
| Lagos | 2008 | 71 | 75 | 75 | 76 | 79 | 80 | 82 | 79 | 78 | 80 | 72 | 75 |
| Lagos | 2009 | 75 | 76 | 74 | 74 | 78 | 98 | 83 | 86 | 83 | 81 | 78 | 66 |
| Lagos | 2010 | 68 | 52 | 68 | 71 | 74 | 79 | 76 | 79 | 81 | 76 | 75 | 71 |
| | | | | | | | | | | | | | |
| | | | | | | | **RAINFALL (mm)** | | | | | | |
| **STN** | **YEAR** | **JAN** | **FEB** | **MAR** | **APR** | **MAY** | **JUN** | **JUL** | **AUG** | **SEP** | **OCT** | **NOV** | **DEC** |
| Lagos | 2007 | 0 | TR | TR | 16.4 | 146.9 | 487.4 | 104.5 | 53.2 | 129.9 | 98.2 | 7.1 | 0 |
| Lagos | 2008 | Trace | 0 | 39.2 | 36 | 40 | 194.2 | 126.3 | 78.6 | 214.6 | 86 | 65.5 | 58.8 |
| Lagos | 2009 | 5.3 | 88 | 43.1 | 172.9 | 274.7 | 434.7 | 267 | 19.7 | 126.6 | 246 | 8 | 22.1 |
| Lagos | 2010 | 70.8 | 14.3 | 36.9 | 160.9 | 215.4 | 679.3 | 270.2 | 216.1 | 229.1 | 116.3 | 73.8 | 0 |