MSI Journal of Multidisciplinary Research (MSIJMR)

GC-MS Profiling and Phytotoxicity Activity of Essential oil Extract of Elaeis Guineensis Jacq. seeds.

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The authors declare that no funding was received for this work.

OPEN ACCESS

Received: 20-August-2025
Accepted: 01-September-2025
Published: 23-September-2025

This article is published in the **MSI Journal of Multidisciplinary Research (MSIJMR) ISSN 3049-0669 (Online)**

The journal is managed and published by MSI Publishers.

Volume: 2, Issue: 9 (September-2025)

ABSTRACT: This study investigates the phytotoxic effects of Elaeis guineensis essential oil of the seeds, extracted via Hot Water Floatation (HWF) approach with a yield of 20.7%, on plant growth. Bioassays demonstrated a concentration dependent reduction in both shoot and root lengths. At the lowest concentration (Treatment 1), shoot and root lengths were reduced by 21% and 29%, respectively, while higher concentrations in Treatments 2 and 3 led to reductions of up to 47%, with root growth consistently more affected. This heightened sensitivity in roots is likely due to their role as the initial site of uptake for phytotoxic compounds, resulting in oxidative stress, hormonal imbalance, and disrupted nutrient absorption. GC-MS analysis identified 14 compounds, including medium and long-chain fatty acids such as n-decanoic (14.88%), dodecanoic (9.49%) tetradecanoic (7.61%), and n-hexadecanoic acids (5.42%), comprising approximately 39% of the total oil. These fatty acids are known to compromise cell membrane integrity and impede physiological functions essential for germination and growth.

The findings suggest that the essential oil of E. guineensis possesses significant allelopathic potential, with its fatty acid content being a primary contributor to its bioactivity. This highlights the oil's potential application as a natural bio-herbicide

Keywords: *Phytotoxicity, antigermination, essential oil, allelochemicals, GC-MS, Elaeis guineensis.*

Introduction

Plant essential oils (EOs) are volatile, natural occurring complex compounds characterized by a strong odour and are formed by plants as secondary metabolites. They usually occur as a mixture of different compounds of lower molecular weights [1]. Numerous studies have demonstrated the diverse biological activities of essential oils, including their antibacterial [2], antifungal [3], anti-inflammatory, antioxidants [4] properties.

Past and present research have delved into essential oil as alternatives in replacing several of the Active Pharmaceutical Ingredients (API) which are considered resistant to their respective microbes. The nature of these oils is such that they are friendly and pose less harmful effects when used as therapeutics. The increasing demand for products that posed fewer health risks have driven the search for natural and organic ingredients in processed foods, beverages, personal care items, and cosmetics [5]. This trend has propelled the industrial essential oils market, which was valued at over USD 7.51 billion globally in 2018 and is anticipated to grow by 9% by 2026 [6].

Essential oils are intricate blends of volatile compounds typically found in low concentrations across various plant parts, including flowers, buds, bark, herbs, wood, fruits, roots, seeds, leaves, and branches [7]. These compounds consist of relatively low molecular weight organic molecules containing carbon, hydrogen, oxygen, and occasionally nitrogen and sulfur; chlorine and bromine also may be found less frequently, particularly in seaweed volatiles [8]. In general, these components are <500 Da in molecular weight and contain only 1–3 oxygen atoms [9]. Essential oils (EOs) consist of a wide variety of molecules, including both terpenoid and non-terpenoid compounds, with their composition influenced by factors such as the plant

species and extraction methods employed. The non-terpenoid components primarily include short- chain alcohols, phenylpropanoids, acids, ketones, esters, and aldehydes, which are typically produced through the metabolic transformation or degradation of phospholipids and fatty acids [10-12].

Essential oils can be extracted from plants parts such as, leaves, stems, flowers and seeds using several methods. Methods employed in the extraction of EO include the following Steam distillation, Cold pressing, Solvent extraction, Maceration and Supercritical fluid distillation (CO2 extraction). The choice of method is dependent on the plant part and study purpose. In recent, studies showed that when essential oil is extracted and not conserved well, it decomposes and loses its effectiveness in biological studies [13].

In recent years, extensive research has focused on finding suitable alternatives to chlorinated and phosphate-based herbicides, due to growing concerns over their negative impacts on both the environment and human health. These effects extend to ecosystems and aquatic environments [14]. Despite these concerns, the use of such herbicides remains widespread, driven by the global need to increase food production for a growing population. Therefore, it is crucial to develop safer, more sustainable alternatives that consider environmental consequences. Nature, as the world's largest chemical laboratory, offers a vast source of potential solutions that researchers can explore and that includes plant essential oils (EOs) are volatile [15].

Elaeis guineensis Jacq., commonly known as the African oil palm, is native to the equatorial tropical rainforests of Africa, particularly along the Gulf of Guinea. It belongs to the Arecaceae (palm family) and thrives in tropical climates characterized by high rainfall and temperatures. The seeds are composed of three layers: the exocarp (outer skin), the mesocarp (fibrous pulp rich in palm oil), and the endocarp (a hard shell that encloses the kernel or endosperm, which is the source of palm kernel oil). Due to its dual oil sources, E. guineensis has significant economic and industrial value, particularly in the food, cosmetic, and biofuel industries [16]. Some studies have reported the presences of the following chemicals presents in the oil, terpenoids, fatty acids, flavonoids, saponin, glycosides and steroids [17].

Several studies have reported the medicinal usage of this plant including the oil from the seeds to cure several ailments. In Ghana, the oil is extracted from the seeds through various means. In commercial-scale production, a small production plant is locally constructed to extract the oil. The researchers observed that the residual oil decarded usually affect the surrounding grasses given an indication of the presence of allelochemicals in the oil. However, there is paucity of data on the use of Elaeis guineesis oil for phytotoxicity studies. It is against this backdrop that this study seeks to investigate the essential oils of Elaeis guineensis seeds and explored it as possible source of allelochemicals through standard antigermination assay, and profile the oil using GC-MS to identify the types of compounds present in the oil.

Materials and Methods Materials

Elaeis guineensis seeds were collected in June 2023 at Cape Coast in the Central region of Ghana. Specimen identification was done by the curator of the UCC Botanical Garden. The nuts were stored in sealed plastic bags. They were stored at 4ºC for later use.

Reagents and chemicals used in this study were purchased from Merck, Ghana and all organic solvents were redistilled and dried according to standard procedures before being used. Petri-dis and Whatman No 41 filter papers were purchased from the market. Seeds were purchased from seeds store in Ghana.

General Experimental Procedure Sample Preparation

The seeds were identified by the assistant curator of the botanical garden of UCC Mr. Vicent Appoley. Samples were taken and crushed using an electric blender into coarse particles. Sample of the crushed particles were taken for extraction while the remaining crushed nuts were sealed in plastic bags and stored at 4 ºC. This was to prevent bacterial infestation.

Extraction Procedure

The Hot Water Flotation (HWF) method is a traditional technique for extracting edible oil, commonly practiced in rural communities across many developing African countries including Ghana. Its main advantage over other small-scale methods, such

as expellers or ghanis, lies in its simplicity. The tools involved such as a pestle and mortar, boiling and kneading pans, seed roasters, and calabashes are inexpensive and easily accessible [18]. However, some researchers note that the method often results in low oil yields and can be both labour-intensive and time consuming. The method may be applied to most oilseeds with varying degrees of oil yield. 200 g of the crushed seeds was placed in a round bottom flask and water was added. The mixture was then boiled for 2 hours at ~80 ºC with more water added periodically to compensate for evaporation. Figure 1 illustrates the extraction procedure. After 2 hours, the content was removed, and cold water was added. The mixture was allowed to stand at room temperature for 30 minutes, resulting in the formation of two immiscible layers; an oil layer and a water layer. The oil layer was carefully decanted and dried completely by passing it over $MgSO_4$. This yielded 20.7% of the essential oil extracted.

Figure 1. Extraction of oil from Elaeis guineensis seeds using the Hot Water Floatation (HWF) approach.

Phytotoxicity assay

Phytotoxic bioassays on extracts are typically performed in Petri dishes™ by placing a filter paper impregnated with the extract inside the dish. The paper is then moistened with distilled water. A control is set up using the same procedure, but with a non-impregnated filter paper. Seeds are added to the dishes, which are then sealed [18]. This method is widely recognized as the standard approach for studying phytotoxicity.

Treatments

Three treatments (TR) were prepared with increasing concentrations of essential oil:

Treatment 1 (TR1): 100 µL of the essential oil was dissolved in 5 mL of ethyl acetate. The solution was poured into a Petri dish lined with a double layer of Whatman No. 41 filter paper.

Treatment 2 (TR2): 200 µL of the essential oil was dissolved in 5 mL of ethyl acetate. The resulting solution was poured into a Petri dish lined with a double layer of Whatman No. 41

filter paper. Treatment 3 (TR3): 400 µL of the essential oil was dissolved in 5 mL of ethyl acetate. This solution was poured into a Petri dish lined with a double layer of Whatman No. 41 filter paper. A control experiment was also set up alongside the treatments. In the control, 5 mL of 100% ethyl acetate was poured into a Petri dish lined with a double layer of Whatman No. 41 filter paper. All treatments and the control were placed inside a fumehood for 24 hours to allow complete evaporation of the ethyl acetate, leaving the filter papers impregnated with the essential oil extract.

Seeds of Lactuca sativa were placed in each Petri dish, and 5 mL of water was added. The dishes were sealed with parafilm to create a conducive environment for germination. The picture of the first trial experiment is shown in supplementary document page 4.

GC-MS- Experiment of E. guineensis Oil

G-C-MS was performed on the oil prepared as follows; 100 µL of the oil was taken and dissolved in 1 mL of ethyle acetate. The solution was filtered and sample submitted for analysis. The samples were submitted on Shimadzu GC-MS-QP2010 Gas Chromatograph Mass Spectrometer Ultra system equipped with a DB-SMS column (28 m in length × 0.25 mm in diameter × 0.25 µm film thickness). Spectroscopic detection by GC–MS involved an electron ionization system that utilized high-energy electrons. Pure hydrogen gas was the used carrier gas with a flow rate of 1 mL/min. The initial temperature was held at 50°C for 2 minutes, followed by an increase from 50 to 290°C with an increasing rate of 20°C/min and a

holding time 15 min. Two microliter (2 µL) of the prepared 100 µL/mL in ethyl acetate was injected. The MS was operated in EI mode at 70 eV. Peaks from the compounds in the oil were identified based on computer matching of the mass spectra with the National Institute of Standards and Technology (NIST) library.

Results and Discussions

The extracted oil from the seeds yielded 20.7%. This oil was stored in the fridge at 4 ºC for later usage. This was to prevent degradation and fungal infestation.

The results of the phytotoxicity activity of the essential oil were demonstrated by using Lactuca sativa seeds in a tree replicate experiment. Figure 5 below shows the germinated seed incubated inside Petri DisHh™ after 7 days. The germinated seeds were observed to have a physical difference between the control and that of the experimental. The root and shoot lengths were measured and compared with the control as an indication of phytotoxic effect of the essential oil. Table 1 shows the average mean values of both the root and shoot lengths, while Figure 2 shows the bar chart of the root and shoot lengths compared with the control, Figure 3 shows some of the germinated seeds in the Petri Dish after 7 days at laboratory temperature.

Table 1. A table showing the mean values of the shoot and root lengths of the germinated seeds after 7 days of incubation in Petri Dish™

Parameters	Control	TR1	TR2	TR3
Shoot length	**19**	**15**	**14**	**10**
Root length	**38**	**27**	**25**	**21**

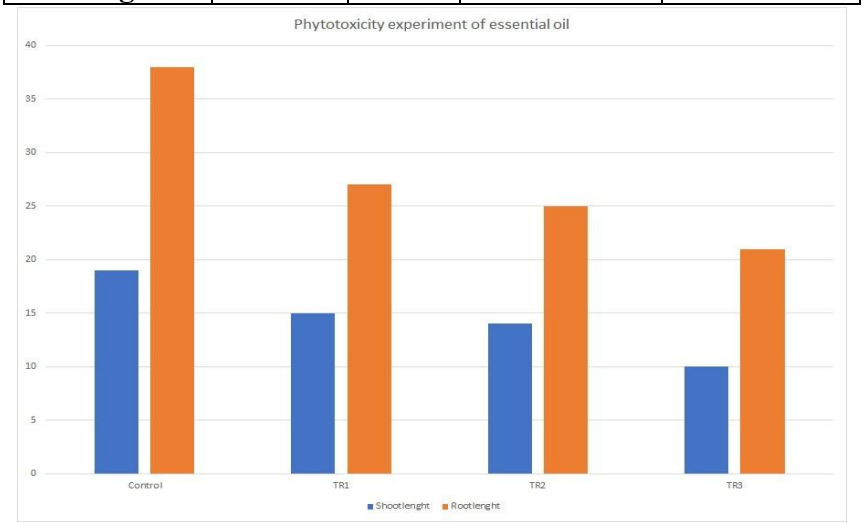

Figure 2. A bar chart showing the root and shoot lengths of germinated Lactuca sativa seed after 7 days

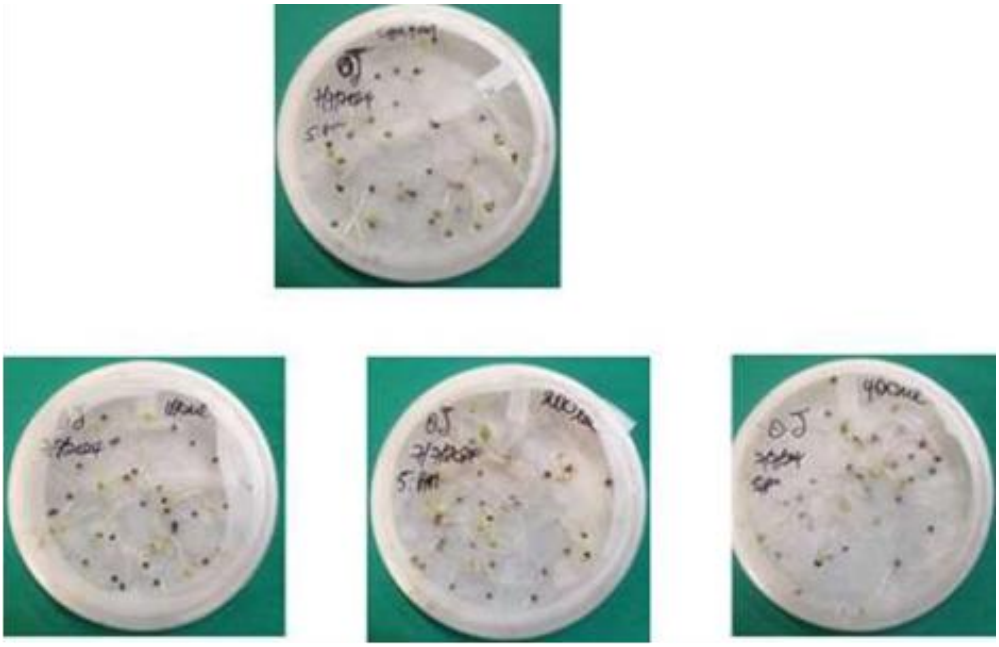

Figure 3. Phytotoxicity experiment. The setup shows germination of the seed after 7 days of incubation at laboratory temperature.

The determination of the percentage reduction of the root and shoot lengths is given by the relation below:

$$Percentage\ Reduction\ of\ Lengh\ = [\frac{Nc - Ne}{Nc}]X\ 100$$

Where, *Nc* = Length of the shoot/root in control experiment

Ne = Length of the shoot/root in experimental set-up

From the bar chart in figure 4, the data showed 21% and 29% of shoot and root length reductions respectively compared with the control in Treatment 1(TR1). This reduction shows a minimal concentration of phytotoxic principle present in the essential oil. The concentration in TR1 could be low and hence exhibited a minimal effect. In Treatment 2 (TR2), the shoot length reduction compared with the control was 26% while the root length had a reduction of 34%. Comparing these results to TR1 it is clear a minimal increase in the essential oil concentration affected both the root and shoot lengths. This could be attributed to a minimal increased in the phytotoxic principle in the oil. In Treatment 3 (TR3), the greatest effect was seen, the root and shoot length were observed to have been reduced by 45% and 47 % respectively. This again could be attributed to the increased in phytotoxic principle's

concentration in the TR3. A thorough analysis revealed that the root length is greatly affected compared to the shoot length. This could be due to the fact that roots are the first point of contact to plant nutrients and hence would take up the phytotoxic principle. When this happened, it results into factors such oxidative stress, hormonal imbalance, disruption of cell biological process and hindering of nutrients uptake [19-21].

GC-MS Analysis and Discussion

Analysis of the GC-MS data identified 14 distinct compounds, which were categorized into fatty acids, fatty acid esters, and sterols. Consistent with our findings, Cruz-Estrada et al. (2019) reported that medium-chain fatty acids led to a 38–52% reduction in root length and a 50–60% reduction in leaf length in Lolium perenne.[22]. Among the components detected in our analysis were n-decanoic acid (1), dodecanoic acid (2), tetradecanoic acid (3), and n- hexadecanoic acid (4). These fatty acids were present in varying proportions within the essential oil, accounting for 14.88%, 9.49%, 7.61%, and 5.42%, respectively. Table 2 below shows the compounds identified with their characteristics. In a similar study, Magdalena et al. (2022) analyzed various herbal extracts for their allelopathic potential using FT-Raman spectroscopy and identified fatty acids and flavonoids as the primary agents responsible for inhibiting germination and growth in white mustard and oilseed rape. Among the extracts tested, sunflower extract exhibited the highest level of permeability and caused the most significant disruption to the plants' physiological and biochemical functions [23]. Sunflower is considered to be one of the most important oil plants having 22–55% oil content [24]. The presence of much oil in this plant could explain the behaviour in terms of cell permeability and inhibition of biological process.

The phytotoxic effects observed in Elaeis guineensis oil may be largely attributed to its significant fatty acid content, which makes up approximately 39% of the total extract. Fatty acids are known to play a critical role in allelopathic interactions due to their ability to disrupt cellular structures, particularly the integrity of cell membranes. When absorbed by seeds or seedlings, these compounds can compromise membrane permeability, leading to ion leakage, enzyme inhibition, and reduced nutrient

transport. Such disruptions eventually hinder key physiological processes involved in germination and growth. The high concentration of fatty acids in the oil suggests a strong potential for bioactivity, aligning with previous findings that associate medium and long-chain fatty acids with growth inhibition in various plant specie.

Table 2. List of compounds identified from the GC-MS analysis of the essential oil.

S/N	Retention time (t_R)	RI Index	Name of Compound	M/z
1	11.2	1372	n-Decanoic acid	172.27
2	13.9	1570	Dodecanoic acid	200.8
3	16.1	1769	Tetradecanoic acid	228.38
4	18.8	1968	n-Hexadecanoic acid	256.43
5	23.0	2175	Oleic Acid	282.47
6	23.5	2167	Octadecanoic acid	284.48
7	35.8	4336	Dodecanoic acid, 1,2,3-propanetriyl ester	639.02
8	36.2	2085	Glycerin 1-monolaurate	274.40
9	42.6	2450	4-Nitrophenyl laurate	321.42
10	43.5	2596	Cholest-5-en-3-ol	386.66
11	43.9	2739	Stigmasta-5,22-dien-3-ol	412.70
12	44.9	2545	Cholest-5-ene, 3-methoxy-	400.69
13	54.1	2689	9-Octadecenoic acid (Z)-2,3-dihydroxypropyl ester	356.55
14	55.3	2383	Oleic acid, butyl ester	324.55

Conclusion

The study demonstrated a clear dose-dependent phytotoxic effect of Elaeis guineensis essential oil on plant growth, with increasing concentrations in Treatments 1 to 3 resulting in progressively greater reductions in both shoot and root lengths. Notably, root length was more severely affected, likely due to its direct exposure to the phytotoxic components, leading to physiological stress and impaired nutrient uptake. GC-MS analysis revealed a significant presence of medium and long-chain

fatty acids such as n-decanoic acid and n-hexadecanoic acid which are known to disrupt cell membranes and inhibit key biological processes. The findings align with previous studies highlighting the allelopathic potential of fatty acids in plant extracts. Overall, the high fatty acid content (39%) in E. guineensis oil strongly supports its role in the observed phytotoxicity and suggests potential for further investigation into its use as a natural bio-herbicide

Authors' Contribution

Conceptualization, Mahama Alhassan, Emmanuel Oppong; Methodology, Jacinta Ochire, Churchlife Agava Agbetum and Jacob Amenano; Writing-Review and Editing, Ibrahim Chikowe, Mahama Alhassan, Emmanuel Kyame Oppong.

Acknowledgement

Special thanks to the laboratory technicians of the University of Education, Winneba for providing all the needed support during this study and Mr. Charles Kwaku Torgbo for the help he provided in laboratory.

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