CHEMICAL COMPOSITION OF *OCIMUM KILIMANDSCHARICUM* AND *OCIMUM AMERICANUM* AND THEIR BIOACTIVITIES AGAINST *SITOPHILUS ZEAMAIS*

BY

MATHU R. DAVIS (B.Ed. Science)

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DECLARATION

This is my original work and has not been presented for award of degree in any other university.

Mathu R. Davis

Sign…………………………. Date………………………..

This thesis has been submitted for examination and approval through my supervisors.

Prof. Caroline Langat Thoruwa

Department of Chemistry

Signature.. Date..

Prof. Ahmed Hassanali

Department of Chemistry

Signature... Date..

Dr. Wilber Lwande

Behavioural and Chemical Ecology Department

International Centre for Insect Physiology and Ecology

Signature………………………………. Date……………………………

DEDICATION

To my parents Eliud and Mary Mathu who I love very much. My brother Amos Mathu and my fiancée Jane Muthoni for the great support they gave me. God bless you all.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BC	Before Christ
CAB	Commonwealth Agricultural Bureaux
DEET	N, N-diethyl-m-toluamide
DCM	Dichloromethane
FID	Flame ionization detector
GC-MS	Gas Chromatography/Mass Spectrometry
GC	Gas Chromatography
HP	Hewlett Packard
ICIPE	International Centre of Insect Physiology and Ecology
LC	Least Concentration
MDG	Millennium Development Goal
MS	Mass spectrometer/spectrometry
NIR	Near-Infrared
NIST	National Institute of Standards and Technology
SE	Standard error
SNK	Student-Newman-Keuls

ABSTRACT

The maize weevil, *Sitophilus zeamais* (Motchulsky), the lesser grain borer, *Rhyzopertha dominica* (Fabricius), and the Angoumois grain moth, *Sitotroga cerealella* (Oliver), are serious pests of stored grain in Kenya. The control of these insect pests relies heavily on the use of synthetic insecticides by better off farmers; but there is an increasing cost of application and erratic supply in developing countries. In addition, small-scale farmers cannot afford these commercial products and there increasing cases of resistance development by the pests. In contrast with synthetic pesticides, the traditional uses of plants-derived pesticides are usually simple, eco-friendly, cost–effective and accessible to communities with minimal external input. The major challenge is lack of sufficient research on the efficacy of these products, epigenetic (chemotypic) differences associated with a given plant species growing in different environments. Also there is lack of effective downstream promotion of promising local plants and their products. In this study, essential oils of *Ocimum kilimandscharicum* growing in Limuru, Ngong, Kasarani, Kakamega and Trans-Nzoia regions and *Ocimum americanum* from Machakos region were screened for their repellence and fumigant toxicity against maize weevils. The aerial parts of the plants were hydrodistilled using Clevenger apparatus. Analyses of the essential oils collected, and identification of their constituents was performed with Gas Chromatograph (GC) with FID detector and GC-linked Spectrometer (MS). Comparison of mass spectra of individual constituents and with NIST data led to the identification of prominent constituents. From the analyses, 9 constituents were identified in both *O. kilimandscharicum* essential oils and 9 from *O. americanum.* Camphor was the major constituent in *O. kilimandscharicum* growing in all areas. 1,8-Cineole, which was a minor component in *O. kilimandscharicum,* was the major component in *O. americanum*. Subtractive blend mortality bioassays showed that camphor is responsible for 82% activity of *O. kilimandscharicum* oils, while 1,8-cineole contributed 18% of the activity, with the other compounds contributing less than 5%. Probit analysis showed that essential oils of plants collected from Limuru and Ngong regions were more toxic against the maize weevils. Plants from Kakamega region had the lowest activity both in fumigation and damage assays. Essential oils of *O. kilimandscharicum* from Trans-Nzoia and Ngong regions had the highest repellence at 93% against the maize weevils. *O. kilimandscharicum* essential oil was more repellent to maize weevil than *O. americanum*. The germination of the maize seeds was not affected when the seeds were treated with the essential oils of both *O. americanum* and *O. kilimandscharicum.* Maize grains treated with higher dosage of *O. kilimandscharicum* had the highest germination rate at 90%. The ground powdered materials provided less protection against *S. zeamais*. Essential oils could be used by small scales farmers as maize protectants against maize weevils. This study lays ground for using essential oils by small scales farmers to control post-harvest pests.

CHAPTER 1

INTRODUCTION

1.1 Background information

For a long period of time human beings have stockpiled foodstuff in times of plenty for use during leaner periods or for sale at a later date. Equally, the stored-products are infested by insects leading to food shortage. It is thought that between 5,000 and 10,000 BC, human society commenced settled agriculture and began to produce grains, fibres and skin. A vast new resource then become available which attracted a selected band of insects that feed on dry materials of animals and plant origin (Rees, 2004). Among this is the maize weevil *Sitophilus zeamais* which is one of the most serious pests of farm-stored grain and basket or bag stored grain in stores under tropical and sub-tropical condition. If left unchecked, infection of *S. zeamais* can result in devastating damage of stored corn. An annual post-harvest loss by *Sitophilus species* could meet the minimum annual food required of at least 48 million people (FAO, 2011). The weevil causes damages to stored maize grains by boring the grain and eating the inner part which reduces maize weight and quality in terms of consumption and germination.

The loss of food grains caused by weevils has been a challenge to farmers. The most common, *S. zeamais* and the resent coming of *Prostephanus trancatus,* the larger grain borer has given farmers a lot of problems. The huge post harvest losses and deterioration caused by these pests is a major obstacle to food security in developing countries (Asawalam and Hassanali, 2006). Other species of insect commonly found in stored corns and sorghum are *Rhyzopertha dominica* (Fabricius), *Tribolium castaneum* (Herbst), *S. granaries*, *S. oryzae*, all of the family, Curculionidae (Peng, 1998). The main pest of corn in granaries in tropics is the maize weevils (Kouninki *et al.,* 2007).

Insects also play an important role as facilitators of the aflatoxin-producing fungus, *Aspergillus flavus* link and related fungi, in both pre-harvest and post-harvest corns (Nault, 1997). In particular, maize weevils facilitate the growth of *A. flavus*, aflatoxin production in corn by increasing surface area susceptible to fungal infection and increasing moisture content as a result of weevil metabolic activity. The food insecurity is a global crisis and FAO has warned of increase in food prices (Stacey *et al.*, 2007). In Eastern and Southern Africa alone, food losses are valued at $1.6 billion per year, or about 13.5 percent of the total value of grain production (FAO, 2011). Worldwide, FAO estimates that 842 million people were undernourished in 1999-2001. The losses caused by maize weevils has great impact to the economy, resulting to low prices and lack of access to market for poor quality grain, or nutritional, arising from poor quality or contaminated food (FAO, 2011). Several methods of pest control have been used in various fields. This includes application of insecticides to structural surface and grains, temperature and moisture control, proper sanitation and integrated pest management and control activities. (Dowdy and McGaughey, 1998).

Thus, there is an urgent need to come up with subtle insect control agents which will help to substantially reduce the negative impact of post-harvest insects, but are less toxic or non-toxic to man and non-target animals, non-polluting to environment and readily degradable.

The plant-derived pesticides have low mammalian toxicity and can effectively prevent and/or suppress insects pest especially in storage (Golob and Hedges, 1982). Extracts from a variety of plants extracts have potent but subtle insect pest-control modes of action. Insect pest-control properties have being found to affect the biology of target insects in different modes such as ovicides, repellents, anti-feedants, fumigants and contact toxicants and insecticides (Watanabe *et al.*, 1993).

Other plant-derived pesticides is the plant powder. Many plant powders have been found to be very effective in the control of *S. zeamais* attacking maize grains in the storage (Adedire and Ajayi, 1996; Asawalam and Hassanali, 2006; Asawalam *et al.*, 2006; Kabeh and Jalingo, 2007; Mulungu *et al.,* 2007; Parugrug and Roxas, 2008; Ukeh *et al.,* 2009; Danjumma *et al.,* 2009). However, studies on the effects of these powders on the insect pest attacking guinea corn grains have not been given much attention. Plant powders are cheap, easily biodegradable and readily available and will not contaminate food products in acting as protectants in small-scale storage systems (Ukeh *et al.*, 2009).

Herbal extract that are not harmful to the environment have shown to be effective natural preservatives (Sen, 2001). In addition, plant-based products are renewable in nature and cheaper. Also some plants have more than one chemical as an active principle responsible for their biological properties. These may be either for one particular chance of developing quick resistance to different chemicals (Saxena *et al.,* 1989). Plants-derived pesticides can be transferred into practical application in natural crop protection, which can help the small-scale farmers (Duke, 1990).

Repellents have been used in control of insect. One of the well known synthetic commercial repellents is N, N-diethyl-m-toluamide (DEET). Traditionally, use has been made of plants such as *Ocimum, spp* (Labiatae) (Chogo and Crank, 1981), *Artemisia* (Asteraceae) and *Calmus* species. These plants have been smouldered to produce smoke carrying chemical compounds that repel insects in the maize granaries. Essential oil from plants is being used by man to control maize weevils.

1.2 Hypotheses

- **i.** Essential oils from *O. kilimandscharicum* and *O. americanum* are toxic and repellent to the maize weevil.
- **ii.** The composition of essential oil of each plant species growing in different locations varies and this may affect its performance.

1.3 General objective

To identify the constituents of essential oils of *O. kilimandscharicum* and *O. americanum* that contributes to their toxicity and repellence against *S. zeamais* and chemotypic (epigenetic) variations of these in plants growing in different ecologies that may impact their application.

1.3.1 Specific objectives

- **i.** To undertake GC-MS (gas chromatography-linked mass spectrometry) analyses of essential oils and identify the major constituents.
- **ii.** To undertake subtraction and blend bioassays to identify the constituents responsible for repellence and toxicity of oils against maize weevils.
- **iii.** To determine repellent and toxicity of the essential oils through fumigation and repellence assays.
- **iv.** To compare the activities of both *O. americanum* and *O. kilimandscharicum* through fumigant toxicity, repellence, and damage assessment to a certain the best species.
- **v.** To assess the implications of compositional differences between the chemotypes of the two plant species through damage assay in downstream technology transfer initiatives.
- **vi.** To determine the germination (viability) of maize grains treated with essential oils of *O. kilimandscharicum* and *O. americanum.*

vii. To determine the bioactivity of powdered plant materials of the two plants *O. kilimandscharicum* and *O. americanum* against the maize weevils.

CHAPTER 2

LITERATURE REVIEW

2.1 Weevils

Weevils belong to the family Curculiniodea. Most weevils have elongated heads which appear as pronounced snout (rostrum). Weevils are the most common pest destroying food grains in the tropics. The main insects causing destruction and losses to cereals include *S. oryzae, S. zeamais, T. castanium, S. granaries* and larger grain borer *P. truncates* (Dustan and Magazini; 1981 Peng, 1998). Of major concern is the maize weevil.

2.1.1 Maize weevil

Maize weevil is a cosmopolitan pest of stored product (Longstaff, 1981). Adult *S. zeamais* excavates a shallow pit in the seed and lays a single egg inside, sealing it with a waxy plug. The eggs hatch in approximately 3 days, depending on the humidity and moisture content of the grain. The larvae, which are approximately 4 mm, white and legless, begin to feed within a single grain while developing, which takes approximately 18 to 23 days, pupating inside it and finally begin the transformation into the adult weevil form, much like a butterfly. Adult leaves through an emergence hole. Other secondary pests feed through the same hole (Longstaff, 1981). Like other *Sitophilus* species, favourable condition such as 27 ^{0}C and 70% relative humidity completes the life cycle in about 35 days. The adult *S. zeamais* takes 5-8 months before they die (Walker and Farrell, 2003).

2.1.2 General biology *of S. zeamais*

The maize weevils is the most important insect pest of stored maize in tropical and sub-tropical countries. The adults are small and brown to brown snout weevils about 3-6 mm long. They have a life span of several months to one year. Female select a spot on the grain surface then chew small hole in grain kernels and use their ovipositors to insert one egg per hole. Each hole is then plugged with a gluey secretion usually known as egg plug (Ukeh *et al.*, 2007). Eggs are laid throughout most of the adult life, but the majority of the eggs are laid within the first 6 weeks and more than 150 eggs may be laid per female. Eggs are laid at a temperature of 15^{o}C and 35 ^{0}C and at grain moisture content over 10% but not above 32%.

The incubation period of the egg is between 2-6 days at 25 ^{0}C. Larvae are cannibalistic and larger ones may prey upon less developed individual should they meet (Rees, 2004). There are four larval instances all of which bore through the cavity hollowed out in the seed and develop within the grain. Pupation takes place within the grain and the newly developed adult may spend several days within the cavity before chewing it and leaves a large characteristic emergence hole called exit-hole. The total development period ranges from about 35 days under optimal condition to over 110 days in unfavorable conditions.

The actual duration of the life-cycle also depend upon the type and quality of grain that is infected (Haines, 1991). An index of environmental suitability indicated that between 25-30 ^{o}C and 65-75% temperature and relative humidity respectively are the optimal environmental conditions for growth of maize weevils population on stored maize (Throne and Cline 1994; Bekele *et al.*, 1995). Mating of S. *zeamais* does not occur before the adult are three days old (Walgenbach *et al.*, 1987) but the insects continuous to feed on grain throughout its life span.

2.2 Bioactivity of plants extracts against *S. zeamais*

Insects damage in stored food grains may amount to 10-40% in countries where modern storage technologies have not been introduced (Shaaya *et al.*, 1997). Among the stored products is *S.*

zeamais an important primary pest of stored maize in the tropics (Wakefield *et al.*, 2005). It is an internal feeder and causes considerable loss to stored maize affecting the quality and quantity of the grain. More frequently, fumigation with appropriate chemical substance such as methyl bromide and aluminum phosphide are the most economical and convenient tools for managing the maize weevils because of their case of penetration into the commodity while leaving minimal residues (Bond, 1984). But safety and environmental impact concerns about the continuous application of these chemical substances has prompted the research for more environmental sound and novel methods for the control of storage pests.

Globally, the management of stored products pests using plants products has been the subject of much research (Isman, 2006), and there has been a growing interest in research concerning the possible us of plants extracts as alternative of synthetic insecticides in stored product protection (Obeng-ofori and Reichmuth, 1997; Shaaya *et al.*, 2007; Sahaf *et al.*, 2007). A large number of plants species used traditionally as medicines have been reported to posses bioactives against several insects species (Ivbijaro and Agbal, 1986; Singh and Upadhyay, 1993; Bekele *et al.*, 1995). Natural repellant produced by edible plants represents a vital approach for ecochemical control of stored- products insects pest (Adler *et al.*, 2000; Rozman *et al.*, 2007). Plants essential oil may act as fumigants, contact insecticide, repellant, deterrent and antifeedant to storage insect species (Hassanali *et al.*, 1997).

2.3 Economic damage caused by of *S. zeamais*

After harvest, growers generally store the product for various purposes such as providing food throughout the year, future planting and sale at a later date when products prices might have increased to make a profit (Demisjie *et al.,* 2008). However, during this post-harvest period, stored crop products are usually liable to infestation and depreciation by various stored-product insects pest. Appert (1987) reported total post-harvest crop losses of 40% in the hot, humid regions and more than 10% in the dry region of the world. Other estimates of the crop losses have been given as 10-20% world wide and 25-40% in tropical region (Hill and Walter, 1990; Larry, 2000). Maize weevil causes damage on stored maize grain by boring the grain and eating the inner part which reduces maize weight and quality in terms of consumption and germination (Adda *et al.,* 2000). Damage caused by *S. zeamais* on stored cereal can be extremely high. It is reported that up to 18.3% weight loss occurred due to *S. zeamais* infestation when single maize kernel were exposed to ovipositing adults and kept at 27^{o}C and 70% relative humidity for only 37 days (Adams, 1976). *S. zeamais* have continued to persist and pose major problems to food security in Africa (Markham *et al.,* 1994). These problems have increased in traditional crop varieties and thus have been replaced by improved, high-yielding varieties with shorter growth cycles but which are generally more susceptible to insect damage.

2.4 Sources of infestation

In Kenya, fields infestation of standing crops and store to store infestation appears to be most prevalent. This is common in the temperature climate where the majority of infestation commence after maize is harvested. For example, if the store has not been cleaned properly, cryptic weevils hidden in the storage structure could be left behind from the previous stock ready to infest the new intake commodity (Cox and Collins, 2002). Also grain residues in commercial grain elevators, boot pit and tunnel have been reported to contain large number of storage pests including *S. zeamais* and empty bins are serving as sources of infestation for new grains in the United States (Pomeranz, 1978). In elevators, the source of insects that infest new grain could be previously infested grain present when the new crop is received, spills, trucks and trailers used to transport grains (Dowdy and McGaughey, 1998).

It is also very likely that stored products insects like most phytophagous insects, use chemical released or general pest species require finding their host plants in a patchy environment and plants release hundreds of volatile organic compounds and many of these from grains have been identified as short-chain alcohols, aldehydes, fatty acids, ketones, ester, terpenes and hetecyclic compounds (Maarse, 1991). For example wheat germ contains about 15% lipid and 60% triglyceride (Pomeranz, 1978), and unsaturated triglyceride has been reported to elicit aggregation responses from the granary weevils, *S. granaries* (Nawrot and Czaplicks, 1982). *S. granaries* is a sibling species of *S. zeamais* that is prevalent in temperature regions. The use of carob pod in combination with wheat kernels has been reported to enhance trap catch of *S. zeamais* and its siblings species *S. oryzae* around traditiohby 8nal African granaries (Likhayo and Hodges, 2000). The main volatile compounds of maize grains, namely hexanoic acid, nonanoic acids, nonanal, decanal, 2-phenylethanol and vanillin have been reported to be the main attachment for *S. zeamais* and *S. oryzae* (Pike *et al.*, 1994; Hodges *et al.*, 2000).

Infestation of stored maize by *S. zeamais* can be visualized as a process of invasion, colonization and population changes driven by its response to grain degradation, intra and inter-specific interaction and the arrivals and of new colonizers (Arbogast and Throne, 1997). The behavior of the weevils on stored grains is affected by the inter-play of different chemical, physical and biotic factors. Factors such as store temperature, relative humidity, light intensity, grain size and variety pests density and the presence of other insects including parasitoids and predators, as well as micro-organisms such as fungi will greatly affect the behavior of the maize weevils for successful utilization of the environment (Cox and Collins, 2002).

2.5 Existing control strategies

2.5.1 Manipulation of storage conditions

Physical control methods such as heat or cold can be manipulated in a stored-product environment to eliminate pest infestation or slow down their populations. Low temperatures are commonly used to manage stored-product insect because between 1 ^{o}C and 5 ^{o}C, depending on acclimation and the species, most stored-product pests are unable to move and reproduce. Insects are killed at temperatures lower than 0 ^{o}C as the lower the temperature, the faster the insects will succumb to cold injury (Beckett *et al.,* 2007). Aeration of the grain bulks with ambient air is one of the methods extensively used after harvest to cool the grain. The air is passed through the grain at relatively low volume, thereby preventing grain quality by slowing down population growth, minimizing moisture evaporation and limiting the build-up of hot spot (Darby, 1998). Aerating the grain bulk with chilled or refrigerated air has also been reported to be very effective in the prevention of pest build-up in storage (Fields *et al.*, 1992; Burks *et al.*, 2000).

The use of elevated temperatures in stored product protection has the advantage of giving complete disinfection, being rapid and pests are not likely to develop resistance to it. Various methods of applying heat have been developed that disinfect grain on-farm and in stores, as well as in processing facilities. Heat disinfection in structures involves raising the temperature of the facility to 50-60 ^{o}C and maintaining these temperatures for 24-36 h (Dowdy and Fields, 2002; Wright *et al.*, 2002; Dosland *et al.*, 2006). Heat treatment of the structures can be performed using gas, electric or stream heaters. Depending on the size and nature of the facility, long periods of heating may be necessary for adequate penetration of wall voids and equipment to kill insects harboring in them (Beckett *el al.*, 2007).

2.5.2 Sampling and trapping

The presence of *S. zeamais* in stored maize is not easily noticeable except when infestation has become very high, but this disadvantage can be overcome though a mixture of sampling, inspection and trapping to detect low level infestations. Grains are sampled at intake, during transportation for the presence of insects mainly for assessment of grade and quality. In the developed world, hand probes which have been replaced with pneumatic sample probe can be inserted into the back of the truck using a mechanical arm. Maize moving through a handling system can be sampled using a diverter system where a small percentage of the grain is continuously diverted into a sampling system. To check for insect, the sample seeds are passed through a mechanical system attended by a specialist. Probes can be developed to detect noise made by insects when infesting bulk grain, and there are electronic sensors which detect chemical odours released by stored-product insects. Hidden infestations are detected by radio photography using soft X-ray and near-infrared (NIR) spectroscopy (Rees, 2004; Toews *et al.*, 2006; Neethirajan *et al.*, 2007). Use of traps has also been employed to monitor and detect insect population and distribution in stored products (Dendy *et al.*, 1991).

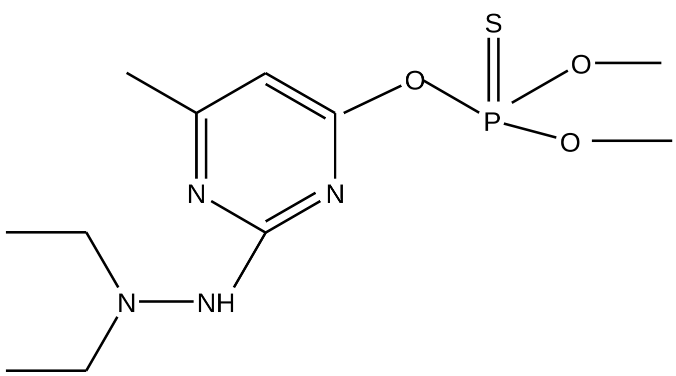
2.5.3 Synthetic fumigants

The pre-harvesting grain management strategies are quite involving and most farmers fail to adhere to it. The practices like timely harvesting, proper drying and cleaning of maize before storage are some of the precautions farmers are yet to follow. Stores are expected to be swept and disinfected using pest control chemicals following the recommended residual spray fumigation or by contact insecticides. This is the process of holding the stored commodity with phosphine (PH_3) in order to kill any infesting organism (Walker and Farrell, 2003).

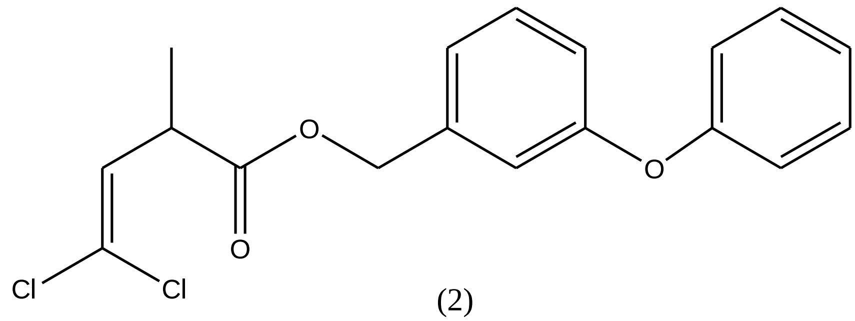
Contact insecticides may also be used to support fumigation. The fumigant gases were in common usage: methyl bromide and phosphine. Methyl bromide is a very effective fumigant, although more dangerous to use than phosphine. It penetrates the insect body through the respiratory system, causing death (Walker and Farrell, 2003). Methyl bromide acts rapidly, controlling insects in less than 48 h in space fumigations and it has a broad spectrum of activity, controlling not only insects but also mites, nematodes and plant-pathogenic microbes. Phosphine from metal phosphines such as aluminum phosphine is formulated as solid tablets, pellets powder in sachets or used in phosphine generators to control insect in all development stages; also mites and rodents. The combination of 80-100 ppm phosphine with heat at 30-36 ^{0}C and carbon dioxide at 3-7% concentrations, exhibited 100% insect control in 24-36h (Fields and White, 2002). Fumigation may reach all part of the storage and stored commodity, and usually be effective in all stage of the pest species while leaving minimal residues. Methyl bromide has been declared an ozone-depleting substance and was banned completely in 2005 in western countries. However, methyl bromide is still used in the fumigation of granaries for the protection of stored product commodities in developing countries. It is expected that developing counties will follow suit by drastically reducing their consumption of methyl bromide and completely phase out its application by 2015 (Fields and White, 2002).

2.5.4 Contact insecticides

Several contact insecticides such as Pirimiphos-methyl (1), Permethrin (2), Malathion (3) and Chlorpyrifos (4) have been widely used as grain protectants against stored-product pest (Snelson, 1987). Pirimiphos-methyl (1), a powder, is in combination with permethrin (2) (trade name Actellic Super) or with deltamethrin (trade name Sofagrain). Pirimiphos-methyl (1) is a fast-acting broad-spectrum insecticide with both contact and vapour action (Walker and Farrell, 2003). Others are; Chlorpyrifos (3), which has an advantage over the insecticide products as it is effective against a wide range of insect pests and Malathion (4) (Chai, 2008).



(1)



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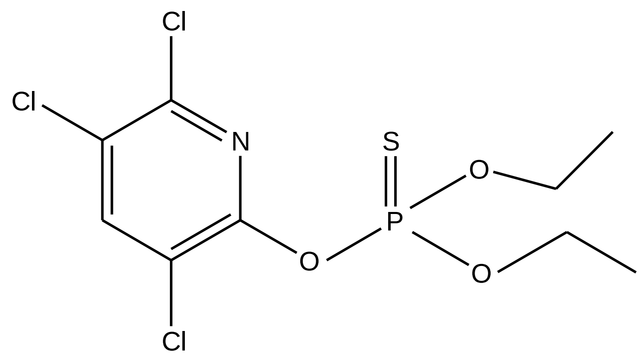

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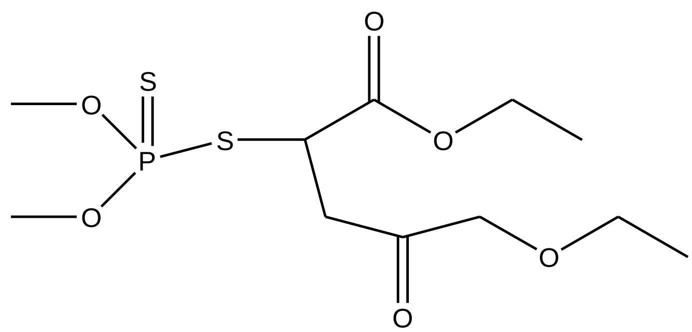


(4)

Continuous application of these chemicals has led to the development of insect resistance throughout the world (Subramanyam and Hagstrum, 2000). These insecticides were favored for stored grain protection because of their relatively low mammalian toxicity and suitable rates of degradation, but are currently not considered safe to be sold on the market based on toxicological re-evaluation (Fields and White, 2002; Beckett *et al.*, 2007).

Contact insecticides contain substances which can penetrate through the insect cuticle entering tissues and poisoning leading to death (Chai, 2008). The problem associated with this chemical insecticides includes environmental pollution, high skill required for useful application, coupled with development of resistant strain has led to the reintroduction of the bio-insecticides. Attempts have been even made to use natural compounds as template in the synthesis of the pesticides. Structural manipulation could lead to improvement of activity, toxicological properties, altered environmental effects, or discovery of an active compound that can be economically synthesized (Duke, 1990). Such attempt has been used in the synthesis of pyrethroid insecticides. Pyrethroids are man-made pesticides similar to the natural pyrethrins, which are produced by chrysanthemum flowers (pyrethrum). Pyrethriods insecticides are safe and biodegradable. The pyrethrum, being one of the most important plants used as safe insecticide, has in fact given hope to explore on more plants. Pyrethrins are insecticides derived from extract of Chrysanthemum flowers. The plant extract pyrethrins are used to control various insect pests (Duke, 1990).

2.6 Essential oils

Essential oils are often found in plants, in the sub-cuticular space of glandular hairs, in cell organelles in idioblast, in excretory cavities and canals or exceptionally in heart woods (Kilgore, 1967). They are odourous plants components that can be separated from the plants materials by steam distillation. Some oil bearing plants are attractive to certain animal and plants where as other are repellents (McGraw-Hill, 1987). Essential oils have a wide and varied application. They are used in the manufacture of perfumes, cosmetics and toilet soaps (Sheath and

Reinnescius, 1986), as flavoring material in candy, chewing gum, ice cream and for flavoring alcoholic properties and are valuable in medicine and dentistry (Chhabra *et al*, 1990).

2.6.1 Composition of essential oil

Each type of essential oil is generally a mixture of variety of chemical compounds ranging from two to three major constituents and to over a hundred for rare constituents (Chhabra *et al.*, 1990). Chemical analysis has shown that essential oil are chiefly liquid and more or less volatile components of many classes of organic substances which includes acrylic, cyclic, aromatic and heterocyclic compounds (Pinder, 1960), which may be broadly classified as:

(a) Nitrogen and sulphur containing compounds such as alkyl isothiocyanate which is found in mustard oil (*Brassica nigrah*) (Guenther, 1975).
(b) Terpene hydrocarbons, alcohols, aldehydes, ketones and lactones (Arcander, 1960)
(c) Aromatic compounds such as eugenol, the main constituents of clove oil (Simonsen, 1953).
(d) Miscellaneous compounds including unbranched long-chain compounds of the terpenes, monoterpenes and sesquiterpenes are the most abundant components of essential oil (Pinder, 1960).

2.7 Plants under investigation

2.7.1 *Ocimum kilimandscharicum*

O. kilimandscharicum (Labiatae) is one of the native plants in East Africa and was introduced in India and some parts of Turkey. It is an evergreen aromatic perennial under shrub belonging to the lamiceae family. It thrives as a natural rounded woody shrub that can reach 2m high in warm temperature regions of the tropics but can be propagated both by seeds and vegetative. The plant has pubscent quadrangular banchlets with simple leaves that are opposite and long narrow at the base and deeply serrated (Warrier *et al.,* 1994). The leaves contain aromatic oil which is the essence of the plant. The oil is removed by either distillation or solvent extraction methods. The oil constitutes liquid oil and white solid crystal .The pure crystal possesses a characteristic odour and taste of natural camphor. The seeds are black and very small, oval shaped and about 1mm in the middle and 2mm long. Once the rubs are established it can be harvested three times per annum for more than three years. The plant is not grazed or browsed by animals but has a rooting system and perennial habits which prevent soil erosion where it’s grown.

Plate 2.7.1: Succulent stems and leaves of *O. kilimandscharicum* plant

2.7.2 *Ocimum americanum*

Commonly known as hoary basil. It’s an erect, branched aromatic herb reaching a height of 30-100cm tall, with opposite light green, silky leaves. The flowers are small, white in colour and arranged in a terminal spike. It is distinguished from the others species by its fruiting calyx which has a dense cluster of hairs on the inside. Its cultivated in Africa and tropical Asia while in Kenya, it is found in dry areas, and mostly in north eastern and parts of West Pokot region. It’s often found growing on roadsides, in fields, in teak forest and open waste place. It prefers sunny, wind-sheltered spots. It occurs at elevations between sea level and 500m or even up to 2000m.

Recently, there has been much research into the health benefits conferred by the essential oils found in it. Scientific studies have established that compounds in the oil have potent antioxidant, antiviral and antimicrobial properties. It is traditionally used for supplementary treatment of stress, asthma and diabetes in India. A 1989 study of the essential oil showed antifungal and insect-repelling properties. A similar study also confirmed that extract from the plant are very toxic to mosquitoes and maize weevils (Weaver *et al.*, 1991).

Plate 2.7.2: Succulent stems and leaves of *O. americanum* plant

CHAPTER 3

MATERIALS AND METHODS

3.1 General procedures

3.1.1 Solvents for assay

Both solvents (acetone and hexane) used were GC-grade analytical reagent obtained from Aldrich chemical co. Inc.

3.1.2 Glassware preparation

All glassware used in the study were soaked in concentrated nitric acid for 24 hours, rinsed with distilled water and dried in an oven at 50 ^{0}C for 12 hours.

3.2 Experimental procedures

3.2.1 Collection and Preparation of plants materials

Based on their ethno botanical information, the plants of *O. kilimandscharicum* were collected from five different regions in Kenya (Kakamega, Trans-Nzoia, Ngong, Kasarani and Limuru), while *O. americanum* was collected in Machakos. It was identified by a botanist, Mr. Karimi Lucas of Kenyatta University and specimens deposited in the microbial sciences herbarium, at Kenyatta University (Table 2.1).

Table 2.1: Plant specimens

S/No	Specimen	Name of plant	Region	Parts used
1	MD 01/2011	*O. kilimandscharicum*	Kasarani	Succulent stem and leaves
2	MD 02/2011	*O. kilimandscharicum*	Limuru	Succulent stem and leaves
3	MD 03/2011	*O. kilimandscharicum*	Ngong	Succulent stem and leaves
4	MD 04/2011	*O. kilimandscharicum*	Kakamega	Succulent stem and leaves
5	MD 05/2011	*O. kilimandscharicum*	Trans-Nzoia	Succulent stem and leaves
6	MD06/2011	*O. americamum*	Machakos	Succulent stem and leaves

The dried plants were crushed to fine powder using a laboratory mill. The powders were weighed and stored in air-tight containers at room temperature. For the extraction of the essential oil, fresh plant materials (consisting of succulent stem and leaves) were collected and hydrodistilled for 8 hours (Jembere *et al.*, 1995).

3.2.2 Extraction of the essential oils

The essential oils from the plants samples were extracted by steam distillation using modified Clevenger apparatus. Various quantities (700-1300g) of each of the plant material were put into a 5 litre round-bottom flask and 1500ml of water added. The flask was then be fitted with the Clevenger apparatus and a double pocket condenser. The plant materials were steam-distilled for

8 hours. Each essential oil was collected on water layer in the Clevenger apparatus. It was separated, dried with anhydrous sodium sulphate and the solvent removed using rotary evaporator and stored in amber-coloured vials at 0 ^{o}C in fridge for bio-assay.

3.3 Maize grains for bioassay

Untreated maize grains, were purchased locally from Kiminini in Trans-Nzoia County, Kenya and disinfected in an oven at 40 ^{o}C for 4 hours and kept in open jars before use. Temperature above 40 ^{o}C has been shown to reduce moisture content of the seeds and interfere with the normal reproduction of the storage insect fed on such seed (Asawalam *et al.,* 2006).

3.4 Rearing of weevils

S. zeamais were obtained from International Centre for Insect Physiology and Ecology (ICIPE) laboratory and reared in a glass jars at room temperature in whole maize grains maintained at temperatures 27±2 ^{o}C, 65-70% relative humidity and 12 hours: 12 hours light: dark regime. After three weeks of oviposition, the parent adults *S. zeamais* were removed by sieving the grains (mesh size 2.0 mm). The maize weevils were transferred to another jar for further rearing. The fully grown adults were used for bioassays (Adjoudji *et al.,* 2007).

3.5 Analyses of the essential oils

3.5.1 Gas Chromatography (GC)

Gas chromatographic separation was performed on a 6890N Gas chromatography (GC) (Agilent Technologies) equipped with split-Splitless injector (230 ^{0}C and flame ionization detector (FID), 53mm i.d., 2.65 μm film thickness). The oven temperature programme comprised of initial temperature of 30 ^{o}C for 0.5 min, rose to 150 ^{o}C at 5 ^{o}C/min, held at 150 ^{o}C (0.1min) and further to 250 ^{o}C at 10 ^{0}C/min and finally held at 250 ^{o}C for 45 min. Results was obtained with an enhance integrator (HP Chemstation).

3.5.2 Gas Chromatography-Mass spectrometry (GC-MS) analyses of essential oils

GC-MS analyses was performed using a fused silica capillary column (50m x 0.32mm i.d., film thickness 0.52 µm, DB-1, J and W Scientific) attached to a cool on column injector, which was directly coupled to HP 5972 MSD. Ionization was by electron impact (70 eV, source temperature 250 ^{o}C). Helium was the carrier gas. The oven temperature was maintained at 30 ^{o}C for 5 min, and programmed at 5 ^{o}C/min to 250 ^{0}C. The calculation of retention indexes was made through co-injection with an n-alkenes series (Van Den Dool and Kratz, 1963). Tentative identification of the oil constituents was based on the retention indexes (Adams, 1995) and by comparison of mass spectra with databases (NIST, 2005).

3.6 Toxicity of individual constituents and blends of the essential oils in Petri dish assays

In a minimum dose of 1.0 mg per cm^2 of the filter paper, *O. kilimandscharicum* essential oil caused 100% mortality of *S. zeamais*. Each major compound from the essential oil (camphor, 1,8-cineole, limonene, caryophyllene, linalool, 4-terpineol, comphene) in the amount present at that dose of the oil (1.0 mg per cm^2), was tested against the maize weevils. The number of the dead weevils were counted after 24 hours. The blends of the constituents compounds of the oil were also done to certain the effects of combined compounds.

3.7 Comparative fumigant toxicity tests of the essential oils

These were conducted in a pyrex glass petri dishes, 10 cm diameter (Weaver *et al.,* 1991). A sample of each essential oil was weighed, dissolved in 1 ml of acetone and delivered to a Whatman No 1 filter paper (9 cm diameter). Various doses of the essential oil (0.125-2.5 mg cm^{-2}) for *O. kilimandscharicum* and essential oil (3.0-5.0 mg cm^{-2}) for *O. americanum* were used to identify the minimum doses which gave 100% mortality against the maize weevils. Each dose of the essential oil was mixed with one ml of acetone solution and then sprayed into the filter paper. It was then left for 20 minutes for acetone to evaporate. 10 pairs of maize weevils of mixed sex were introduced into the petro dishes and kept for 24 h in the laboratory maintained at 26-27 ^{0}C and 60-65% relative humidity.

The number of insects were counted after 24 hours. Insects were considered dead if they were immobile and did not react to the three probing with a blunt dissecting probe. The experiments were repeated for different concentration of essential oils form the different regions.

3.8 Repellence test using Y-shaped olfactometer

The method developed at the International Centre of Insects Physiology and Ecology (ICIPE) Nairobi, Kenya was used in this experiment (Hassanali *at el.,* 1990). A Y shaped glass olfactometer was used for bioassay repellancy of the essential oils on maize weevil, *S. zeamais*. 20 weevils ere introduced at the base of the Y-tube and left to move freely towards the Y-junction. Airflow was maintained and the excess test material removed by an aspirator. Different quantities of the essential oil (0.5-2 micro-litres) in acetone was applied on the test filter paper discs (1.8 cm diameter) while the control contained only acetone. A 60 watts bulb was placed 15 cm from the entrance. This was due to the fact that the maize weevils were photo tactic in nature.

The maize weevils migrated on either the control or the treated part of the olfactometer. Five replicate per concentration of the test sample were run and scoring done where the number moved to either the control or treated. The scoring was done 20 minutes after the introduction of the weevils.

3.9 Toxicity and damage assessment assay for weevils

Based on Awonyinka *et al.* (2006) method, which is a modification of Scott and Mackibben (1978) method, essential oils were applied to the grains at the rate of 0.96, 4.8, 24 and 96 mg/20g of grain dissolved in 1 ml of acetone for *O. kilimandischaricum* and *O. americanum*. 20 g of disinfested grains were weighed and treated by spraying with the different concentration of the test sample. The treated grains were allowed to dry for about 30 minutes then transferred into glass vials (50ml). Five blank controls were run periodically consisting of acetone treated grain. Five replicates of each concentration were run. 10 weevils were introduced in each vial. The assay was incubated at 32 ^{0}C and relative humidity of 70% at a photoperiod of 12:12 hours. After 30 days damage assessment was carried out on treated and untreated grains. 50 grains were taken from each jar and the number undamaged and damaged (grains with characteristic holes) grains were counted and weighed. Percentage weight loss was calculated, using FAO (1985) method (Bekele, 1994).

3.10 Effect of essential oil on the germination potential of the maize seeds

Germination of the maize grains treated with hexane and essential oils (Limuru) was determined in petri dishes (9-cm diameter) lined with moist filter papers. Ten grains were randomly selected from every replicate of each treatment and placed in the petri dish for 96 hours. The petri dishes were watered for 48 hours to maintain maximum humidity. Germination was determined by observing the emergence of the radical. Each of the experiment was replicated five times (Law-Ogbomo and Egharevba, 2006).

3.11 Effect of plant powdered materials on mortality of *S. zeamais*

Exactly one gram and two grams of each plant material were weighed and thoroughly mixed with 20 g of maize grains in a glass jar so as to obtain 5% and 10% doses, respectively. Twenty adult *S. zeamais* were introduced into each of the glass jars. Five replicates of each dose level were prepared for the two powdered plants material. The number of dead and alive weevils was recorded daily for a period of two weeks. Adults *S. zeamais* were considered dead where no response was observed after probing with a soft brush (Arannilewa *et al.*, 2006). Untreated maize was used as control.

3.12 Data analyses

The percentage repellence values were computed as:-

$$Percentage\ Repellence = \frac{Nc - Nt}{Nc + Nt} \times 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqn\ 1$$

Where Nc=Number in the control

Nt=Number in the treated

The percentage repellence data were analyzed using analysis of variance (ANOVA) after arcsine transforming them. The results were presented in various forms of tables and graphs.

The damage analysis through weight loss was determined using the method adopted from FAO, (1985) where,

$$\%\ Weight\ loss = \frac{UNd - DNu}{U(Nd + Nu)} \times 100\% \ldots\ldots\ldots\ldots\ldots..Eqn\ 2$$

Where U= weight of undamaged grains,

Nu= number of undamaged grains,

D= weight of damaged grains,

Nd= number of damaged grains.

The experiments were set up in a randomized design, replicated five times for the laboratory tests. The data obtained in different levels and treatments were analyzed using analysis of variance (ANOVA) R-statistical package and significant different means ($p<0.05$) were separated using Student-Newman-Keuls (SNK) test. The results were presented in various forms of tables and graphs.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characterization of the constituents of plant essential oil

Based on the GC profiles of the oil samples, the plants constituents present were identified by GC-MS and co-injections with the standards. The identification of the constituents was based on NIST library of mass spectra data against the standards.

4.1.1 Essential oil composition of *O. kilimandscharicum* plant

From the GC profiles (Appendix 1.e to 1.d) and (Figure 4.1) of essential oils of *O. kilimandscharicum*, total of 9 major compounds were identified.

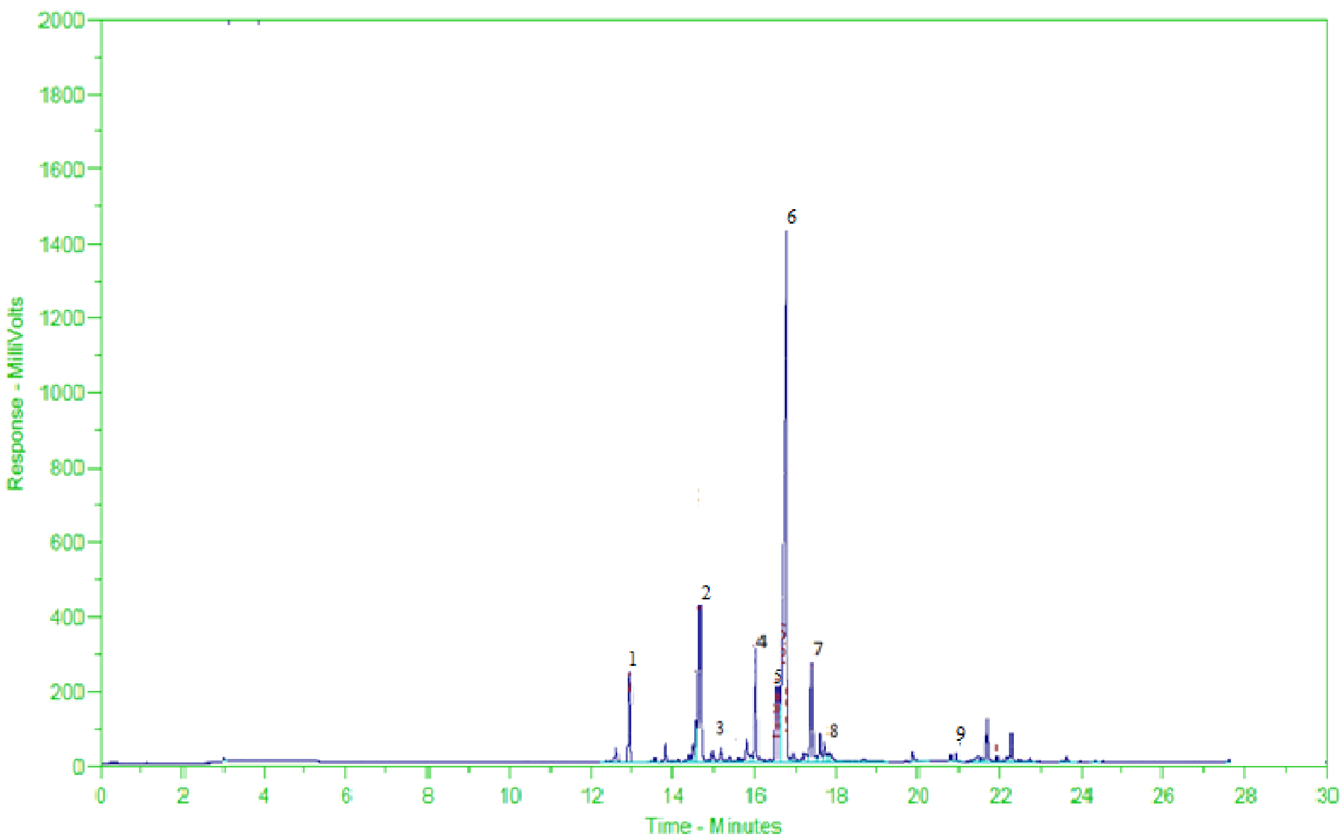
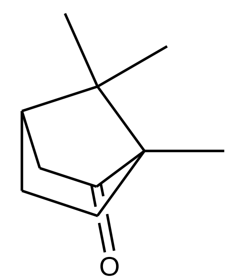


Figure 4.1: Representative GC profile for *O. kilimandscharicum* essential oil from Trans-Nzoia

Table 4.1: List of identified compounds from *O. kilimandscharicum* essential oil from Trans-Nzoia

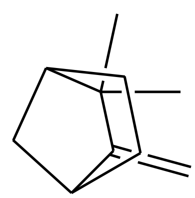
	Compound	Retention time	% peak Area
1	Camphene	13.02	13.57
2	Limonene	14.73	3.435
3	1,8-Cineole	14.77	0.609
4	Ocimene	15.02	0.905
5	Camphor	16.79	45.51
6	Linalool	16.93	4.926
7	Myrtenol	17.54	4.525
8	4-Terpineol	17.26	4.625
9	Caryophyllene	20.73	0.238

All the essential oils from the five regions had the 9 major compounds but in different percentage compositions: namely camphor (5), camphene (6), limonene (8), linalool (7), caryophyllene (10), 4-terpineol (12), 1,8-cineole (13) and ocimene (11) (Table 4.1).



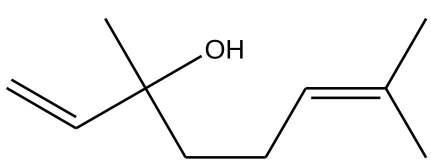
(5)

Camphor



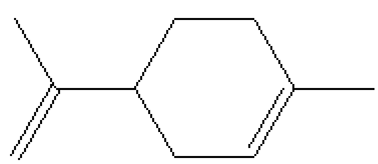
(6)

Camphene



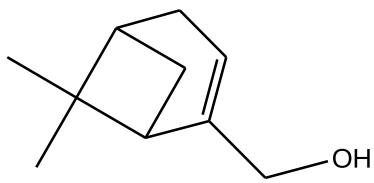
(7)

Linalool



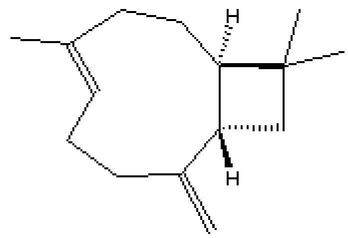
(8)

Limonene



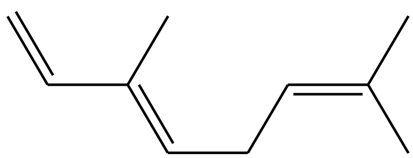
(9)

Myrtenol



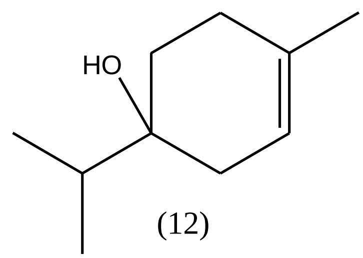
(10)

Caryophyllene



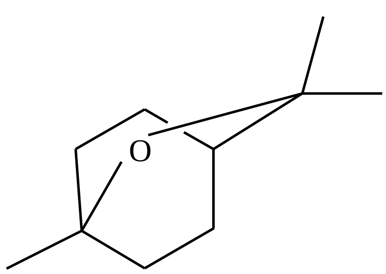
(11)

Ocimine



(12)

4-Terpineol



(13)

1,8-Cineole

4.1.2 Essential oil compositions of *O. kilimandscharicum* from the Kasarani

Comparison of mass spectra of individual constituents and with NIST data through GC-MS led to the identification of 9 major compounds (Table 3). The compound with the highest percentage peak area was camphor (5) with 36.1 %, while the least compound from Kasarani region was 1,8-cineole (13) with below 1%. These compounds were compared with the other 9 major compounds from the five other regions (Table 5).

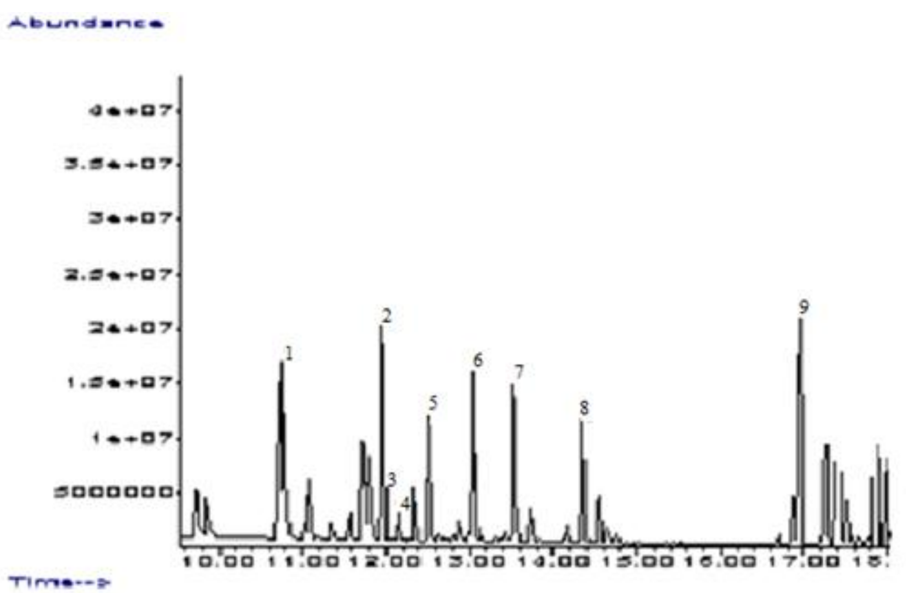


Figure 4:2: GC-MS profile for *O. kilimandscharicum* essential oil from Kasarani

Table 4.2: Compounds identified from essential oil of *O. kilimandscuricum* from Kasarani

	Compound	Retention time	% peak Area
1	Camphene	10.42	5.65
2	Limonene	11.82	21.2
3	1,8-Cineole	11.88	0.80
4	Ocimene	12.13	1.27
5	Camphor	13.90	36.1
6	Linalool	13.12	2.00
7	Myrtenol	14.66	2.43
8	4-Terpineol	14.37	4.82
9	Caryophyllene	17.84	2.48

GC and GC-MS analyses showed that the essential oils of *O. kilimandscharicum* in all the regions contained different proportions of terpenes, monoterpenes, oxygenated monoterpenes, sesguiterpenes hydrocarbons and alcohols. Among the monoterpenes compounds are ocimene (11), camphene (6), and limonene (8). Oxygenated monoterpenes compounds were camphor (5), linalool (7) and 1,8-cineole (13) while sesquiterpenes contained compound like caryophyllene (10). Terpenes, monoterpenes and sesquiterpenes are the most abundant components of essential oils (Pinder, 1960).

Terpenes are the most widespread and important secondary plant compounds and can exert toxic, deterrent, antifeedant and repellent effect on insect herbivores. They are dominant compounds of many natural volatile blends and are responsible for many of the characteristic smell of plant oils, resins, fruits and flowers. Terpenoids chemistry may vary among plants due to factors which may include environmental and genetic influences (Langenheim, 1994; Powell and Raffa, 1997; Wang and Lincoln, 2004). This is evident in this research where the percentage composition of all major compounds varies.

4.1.3 Essential oil composition of *O. americanum* from Machakos

From the GC profiles of essential oils (figure 3), constituents present were identified by GC-MS and co-injection with the standards. 9 major compounds among them α-pinene (15), camphene (6), 1,8-cineole (13), linalool (7), camphor (5), myrcene (14), 4-terpineol (12), β-pinene (16), caryophyllene (10) (Table 4).

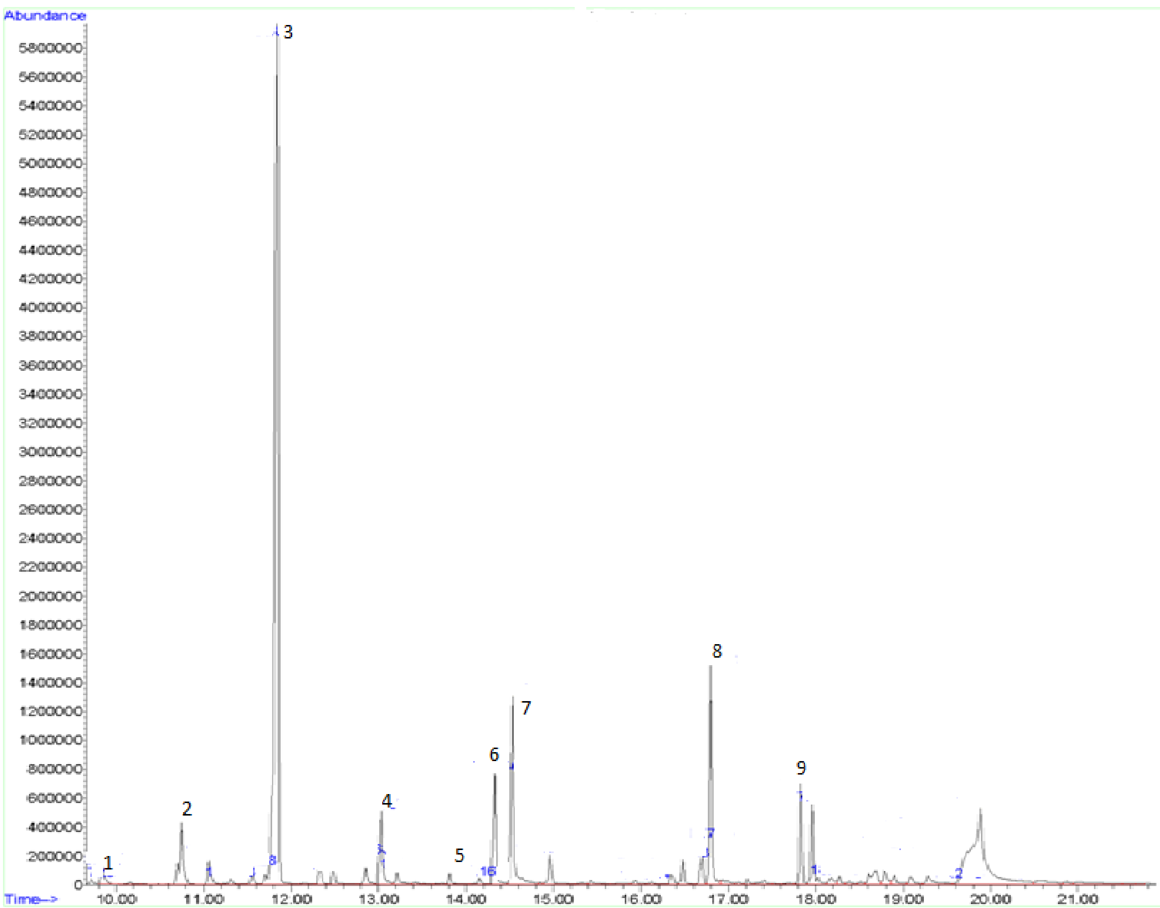


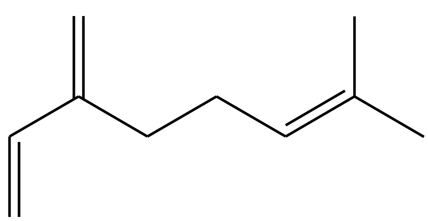
Figure 4.3: GC-MS profile for *O. americanum* essential oil from Machakos

1,8-cineole (13) was the major compound in *O. americanum* essential oil with 38.39%. This was followed by linalool (7) which had 22.25%. The level of camphene (6) was the lowest at 0.1% (Table 4.3). Camphor (5) which was more abundant in *O. kilimandscharicum* was quite low in

O. americanum with below 1%. Like in *O. kilimandscharicum*, the constituents of *O. americanum* are a mixture of monoterpenes and sesquiterpenes. The activities of both plants are mostly attributed to the presence of their major compounds although the combined effect of all the constituents plays a big part in its activity (Asawalam and Hassanali, 2006).

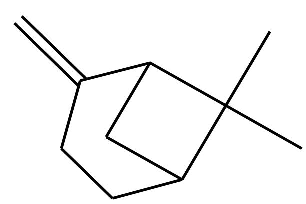
Table 4.3: List of compounds identified from *O. americanum* essential oil

	Compound	Retention time	% Peak Area
1	Pinene (alpha)	9.85	0.94
2	Camphene	10.75	0.10
3	1,8 Cineole	11.84	38.39
4	Linalool	13.03	22.25
5	Camphor	13.82	0.36
6	Myrcene	14.33	0.91
7	4-Terpineol	14.54	3.87
8	Pinene (beta)	16.80	0.82
9	Caryophyllene	17.83	2.54



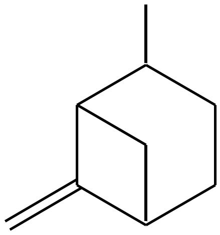
(14)

Myrcene



(15)

α-Pinene



(16)

β-Pinene

4.1.4 Percentage composition of major compounds of *O. kilimandscharicum* growing in different regions

Essential oils of *O. kilimandscharicum* from the five regions had all the nine major compounds but in different percentage proportions namely camphor (5), camphene (6), limonene (8), linalool (7), caryophyllene (10), 4-terpineol (12), 1,8-cineole (13) and ocimene (11) (Table 4.4).

Table 4.4: Percentage composition of major compounds of *O. kilimandscharicum* growing in different regions

	Camphor	Camphene	Limonene	Linalool	Myrtenol	Caryophylene	4-Terpineol	1,8-Cineole	Ocimene
Trans-Nzoia	45.51%	13.58%	3.44%	4.93%	4.52%	0.23%	4.52%	0.60%	0.90%
Limuru	40.09%	23.51%	1.73%	0.41%	1.25%	0.97%	2.14%	1.73%	0.84%
Ngong	37.92%	28.73%	0.47%	0.14%	1.08%	1.02%	0.62%	1.51%	0.39%
Kasarani	36.16%	5.65%	21.21%	2.00%	2.43%	2.48%	4.82%	0.80%	1.27%
Kakamega	32.78%	24.15%	20.86%	0.84%	0.29%	0.18%	1.71%	0.15%	0.18%

The percentage composition of camphor (5) in all the regions was quite high, more than 30% of all the compounds present in all the individual regions with Trans-Nzoia having the highest composition at 45.51%. Plants from Kakamega had the lowest composition of camphor at

32.78%. Comphene (6) was high in all the regions except Kasarani with below 10%. Kakamega had the highest composition of comphene (6) at 24.15% while Kasarani had the highest composition of limonene (8) at 21.21%. 1,8-cineole (13) was less than 1% in all the regions except Limuru and Ngong. The percentage composition of the major compounds from Limuru and Ngong regions essential oils did not differ much in their chemical composition. This could be attributed to the same climatical conditions in the two regions with both the regions having a constant temperature throughout the year ranging between 10-28 ^{o}C. The two regions also share the same deep reddish brown clay soil with soil pH ranging from 5 to 6.5.

Regions with high altitude favoured higher percentage compostion of camphor (5). Limuru and Ngong regions have high altitudes with Limuru having 2500m above the sea level and Ngong is 2460m above the sea level. Trans-Nzoia which boarders Cherengani hills and Mt. Elgon has a high altitude of 1900m above the sea level. The other two regions have low altitudes with Kakamega having the lowest at 1500m above the sea level while Kasarani had 1700m above the sea level.

Generally, the composition of essential oils of the same plants differs geographically. Terpenoids chemistry varies among plants due to factors which may include environmental and genetic influences (Langenheim, 1994; Powell and Raffa, 1997; Wang and Lincoln, 2004). Chemical analysis has shown that essential oil are chiefly liquid and more or less volatile components of many classes of organic substances which includes acrylic, cyclic, aromatic and heterocyclic compounds and the ecosystem affects the genetic control of a plant (Pinder, 1960).

4.2 Effect of individual constituents and blends of the essential oil on mortality of *S. zeamais* in Petri dish assay

In relative amount present in the minimum lethal dose of essential oil from Kasarani, none of the seven individual components (camphor, 1,8-cineole, limonene, caryophyllene, linalool, 4-terpineol, camphene) that were tested had significant toxic effect against *S. zeamais*. However, the mixture of these compounds gave 100% mortality (Table 4.5).

Table 4.5: Effect of individual constituents and blends of the essential oil on mortality of *S. zeamais* in Petri dish assay

	Compunds	%Mortality Mean±SE
1	Essential oil (Kasarani)	100.0 ± 0.0^{a}
2	Camphor	0.0 ± 0.0^{e}
3	1,8-Cineole	0.0 ± 0.0^{e}
4	Limonene	0.0 ± 0.0^{e}
5	Caryophyllene	0.0 ± 0.0^{e}
6	Linalool	0.0 ± 0.0^{e}
7	4-Terpineol	0.0 ± 0.0^{e}
8	Camphene	0.0 ± 0.0^{e}
9	2 to 8	100.0 ± 0.0^{a}
10	3 to 8 minus 2	18.0 ± 3.7^{d}
11	2 to 8 minus 3	82.0 ± 5.8^{b}
12	2 to 8 minus 4	94.0 ± 4.0^{a}
13	2 to 8 minus 5	98.0 ± 2.0^{a}
14	2 to 8 minus 6	96.0 ± 2.4^{a}
15	2 to 8 minus 7	94.0 ± 2.4^{a}
16	2 to 7 minus 8	96.0 ± 2.4^{a}
17	2 plus 3	42.0 ± 3.7^{c}
18	2 plus 4	12.0 ± 3.7^{d}
19	2 plus 3 plus 4	48.0 ± 3.7^{c}
20	Hexane	0.0 ± 0.0^{e}
	p-value	<0.001

Mean mortality (±SE) followed by different small letter(s) within the same column are significantly different and ($p<0.05$, $\alpha=0.05$, SNK).

When one compound from the total combined was removed, there was a reduction in percentage mortality with the highest drop observed when camphor was absent, giving 18% mortality. When

1,8-cineole was absent from the total mixture, there was 82% mortality of the maize weevils. When limonene was removed, the mortality was 94%, same applies to 4-terpineol. Subtraction of individual compounds of caryophyllene, camphene and linalool from the mixture resulted to more than 95% mortality. Thus, subtractive assays provide additional insight into the relative contributions of these components to the mortality of the full blend. Absence of camphor in the blend resulted in the largest drop in the toxic action of the resulting blend, identifying it as the most important component of the active blend followed by 1,8-cineole as the second most active compound.

From the results, camphor was responsible for 82% within the blend while 1,8-cineole contributed 18% activity in relative amount present in the oil. A blend between the two compounds resulted to 42% relative to the proportion of the essential oil from Kasarani. With the addition of limonene combined with both camphor and 1,8-cineole, there was an increase in the activity by 6% to 48%.

From these results, addition of each individual major compound resulted to an increase in the activity of the essential oil on the maize weevils. This shows that more active compounds gain synergism between themselves resulting to increase in mortality. In the present study, the major component in *O. kilimandscharicum* is camphor while the major component in *O. americanum* is 1,8-cineole.

These results stress the importance of evaluating plants components in blends to elucidate their full potency in a given bioactivity. A number of previous studies on the effect of essential oil on post-harvest and other pest focused on the identification of active components rather than mixtures (Weaver *et al.,* 1991; Seck *et al.,* 1993). Bekele and Hassanali (2001), found that in relative amount present in the minimum lethal dose of essential oil from Kakamega, camphor in the blend containing limonene, 4-terpeneol, 1,8-cineole, camphere and caryophyllene had the highest drop with 22%. This value is close to what is found in the present study.

From the present study, the toxic effect of the essential oil *O. kilimandscharicum* is attributed to higher percentage of camphor, while the toxicity of *O. americanum* can be attributed to higher percentage of 1,8-cineole. Obeng-Ofori *et al*. (1997) found 1,8-cineole to be highly repellent and toxic to *S. granaries, S. zeamais, T. castaneum,* and *P. truncates.*

The implication of these results in practice is that blend as a whole rather than specific components could constitute a control agent with sufficient broad spectrum of bioactivity to be deployed in stored-product pest control.

4.3 Toxic effect of essential oils in petri dish assays

From the results (Table 4.6), essential oil of *O. americanum* and *O. kilimandscharicum* from different regions are both toxic to the maize weevils but with varying degrees of activity. Essential oil of *O. kilimandscharicum* from Limuru had the lowest LC_{50} at a dose of 0.30 $mgcm^{-2}$ and 0.63 $mgcm^{-2}$ dose at LC_{95}. This was closely followed by Ngong with 0.37 $mgcm^{-2}$ at LC_{50} and 0.64 $mgcm^{-2}$ at LC_{95}.

Table 4.6: Dose-response effects of essential oils of *O. kilimandscharicum* growing in different regions and *O. americanum* from Machakos on mortality of maize weevil

	LC_{50}	LC_{95}
Kakamega	0.77 ± 0.049^{b}	1.59 ± 0.104^{b}
Limuru	0.30 ± 0.023^{e}	0.63 ± 0.055^{e}
Kasarani	0.43 ± 0.025^{c}	0.74 ± 0.059^{d}
Trans-Nzoia	0.42 ± 0.027^{c}	0.80 ± 0.064^{c}
Ngong	0.37 ± 0.021^{d}	0.64 ± 0.044^{e}
Machakos	3.55 ± 0.065^{a}	4.73 ± 0.139^{a}

Mean mortality (±SE) followed by different small letter(s) within the same column are significantly different and ($p<0.05$, $\alpha=0.05$, SNK)

O. kilimandscharicum from Limuru had high concentrations of the major compounds that were responsible for the toxicity of the essential oil, with 40.09% camphor, 1.73% 1,8-cineole and 23.51% camphene (Table 4.4). Ngong region had the second highest level of activity. This can also be attributed to the high level of the major compounds with 37.92% camphor, 1.51%, 1,8-cineole and 28.73% camphene. However there was no significant difference between the levels of activities of the oils from the two regions at LC_{95}. This means essential oils from Limuru and Ngong regions provided the best activity against the maize weevil compared to the plants of the same species in the different regions.

Kakamega had the lowest level of activity with 0.77 $mgcm^{-2}$ at LC_{50} and 1.59 $mgcm^{-2}$ at LC_{95}. This is could be attributed to low levels of the two major compounds camphor and 1,8- cineole. Trans-Nzioa region had the highest level of camphor at 45.51% but with low level of 1,8-cineole at 0.60%. This shows that more active compounds gain synergism between themselves resulting to increase in mortality and toxicity effect. *O. americanum* from Machakos region had high values of both the LC_{50} and LC_{95}. This could be attributed to low level of camphor in that region.

The LC_{50} result found from Kakamega is similar to Bekele and Hassanali (2001), who found that *O. kilimandscharicum* from that region, had an activity of 0.74mg per cm^2 against 0.77mg per cm^2 from this study.

The toxicity, fumigant and repellent effect of some of these main constituents of essential oil have been demonstrated by other researchers. Five monoterpenoids namely terpinen-4-ol, 1,8-cineole, linalool, limonene and camphor have been reported to elicit direct toxicity and fumigant activity against 3-day old eggs, third instar larvae and pupae of *T. cofusum* (Stamopoulus *et al.*, 2007).

4.4 The repellent effect of essential oil

4.4.1 The repellent effect of the essential oils of *O. kilimandscharicum* from the five regions

O. kilimandscharicum essential oil was found to be repellent against *S. zeamais*, with repellence level varying with percentage of active compounds and also the dosage. At 0.5 µl/disc dose, Limuru and Kakamega had the lowest repellency level. Increase in dosage to 1.0 µl/disc in all the regions resulted to an increase in repellence, with the highest repellence at 1.0 µl/disc being Ngong at 88%. When the dose was increased by 50% to 1.5 µl/disc, Trans-Nzoia region had the highest repellence at 93%, with the other regions registering an increase in repellence (Table 4.7).

Table 4.7: Repellent effects of different doses of *O. kilimandscharicum* essential oils from different regions using a Y-shaped olfactometer

	Dose			
Site	0.5µl/disc	1.0 µl/disc	1.5 µl/disc	p-value
Limuru	72.00±4.40[b]	78.20±3.50[ab]	88.40±3.50[a]	0.043
Kakamega	72.40±2.16[b]	80.40±4.07[ab]	86.40±4.07[a]	0.036
Kasarani	76.00±3.77[b]	80.80±4.08[ab]	86.20±4.08[a]	0.028
Ngong	78.80±4.39[ab]	88.40±4.11[a]	91.20±4.11[a]	0.024
Trans-Nzoia	74.00±3.78[b]	86.60±2.62[a]	93.60±2.62[a]	0.007
p-value	0.702	0.290	0.578	

Mean values followed by the same small letter(s) within the same row are not significantly different

From the results, there is no significance difference in repellence for dose of the same concentration within the region. The high repellence rate in both Ngong and Trans-Nzoia regions could be attributed to the high levels of camphor (5) and 1,8-cineole (13) (Table 4.4). Trans-Nzoia region has the highest composition of camphor (5) at 45.51% while Ngong region has the second highest composition of 1,8-cineole (13) at 1.5%. The high presence of linalool (highest at 4.98%) in Trans-Nzoia region also played part in repellence. Plants that constitutively produce high levels of linalool proved to be a major compound of the essential oils conferring repellent activities (Ryan and Byne, 1988).

The effect of different plant materials on the maize weevil may depend on several factors such as chemical composition of the crude oil, part of the plant extracted, geographical location of the plant, variation in insect behavior and species susceptibility (Casida, 1990). Some of these factors may have contributed to the variation in repellence within the same dose response.

4.4.2 Repellent effect of essential oils of *O. americanum* from the Machakos region

The essential oil of *O. americunum* was repellent to *S. zeamais* for all the doses ranging from 0.5-1.5 µl/disc (Table 4.8).

Table 4.8: Repellent effect of essential oils of *O. americanum* using a Y-shaped olfactometer from the Machakos region

Dose	0.5 µl/disc	1.0 µl/disc	1.5 µl/disc	p-value
Repellence	42.80±4.76[b]	61.80±2.31[a]	65.00±4.16[a]	0.003

At a minimal dosage of 0.5 µl/disc, *O. americanum* had 42.80% repellence. Increases in dosage response by 50% resulted to an increase in repellence. The highest dosage of 1.5 µl/disc gave the highest repellence at 65%. Analysis of variance indicate significance difference ($p<0.05$) between the dose response against the *S. zeamais*.

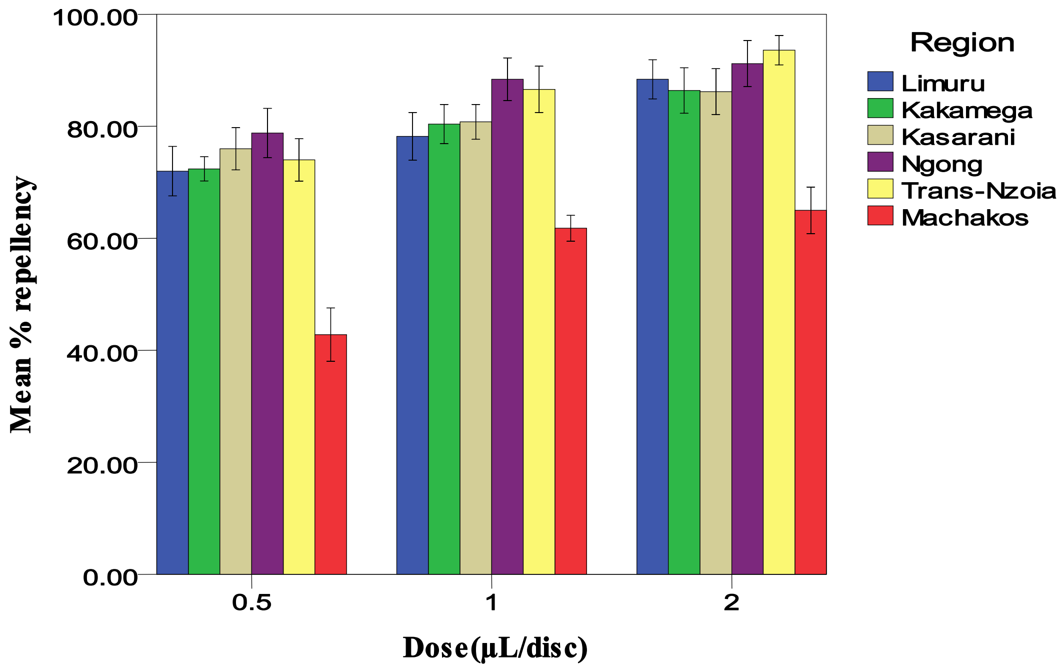
4.4.3 Mean repellence of *O. americanum* and *O. kilimandscharicum* plants from different region against maize weevils *S. zeamais*

The bar graph in (Figure 4.4), indicate an increase in the mean repellence with dose response for both *O. americanum* and *O. kilimandscharicum* plants. However, at equal concentration the mean percentage repellence of *O. americanum* is lower than that of *O. kilimandscharicum.*

This could be attributed to the high concentration of camphor (5) which is has more than 30% composition in all *O. kilimandscharicum* plants essential oil against 0.3% in *O. americanum.* The activity of essential oil mainly depend upon the major components they posses such as linalool

(7), 1,8-cineole (13) and camphor (5) (Asawalam and Hassanali, 2006). These results show that terpenoids blends of *O. kilimandscharicum* are highly repellent to maize weevils than *O. americanum*.

Figure 4.4: A graph of Mean repellence of *O. americanum* and *O. kilimandscharicum* plant from different regions against maize weevils *S. zeamais*



1,8-cineole at appropriate doses was found to be intrinsically toxic and repellent to post-harvest pests (Obeng-Ofori *et al.*, 1997). In a study with different blends, camphor was found to be a major active compound responsible for the highest activity of the essential oil (Bekele and Hassanali, 2001). This has also been demonstrated in the present study. Earlier studies by Jembele *et al.*, (1995) on repellence of different test materials of *O. kilimandscharicum* indicated that 0.3 g /250 g of grains showed 79% repellence on *S. zeamais*. This value, together with values from this research shows that *O. kilimandscharicum* is highly repellent against *S. zeamais*.

4.5 Damage assessment infested maize grains treated with essential oils

4.5.1 Assessment of the weight loss to the maize grains treated with essential oils of *O. kilimandscharicum*

Maize grains treated with essential oils of *O. kilimandscharicum* from the different regions were monitored after every two days for a period of 30 days to ascertain the level of damage to the grains caused by the *S. zeamais*, using the international method of weight and count FAO, 1985.

Table 4.9: Mean weight loss of the maize grain treated with essential oils of *O. kilimandscharicum*

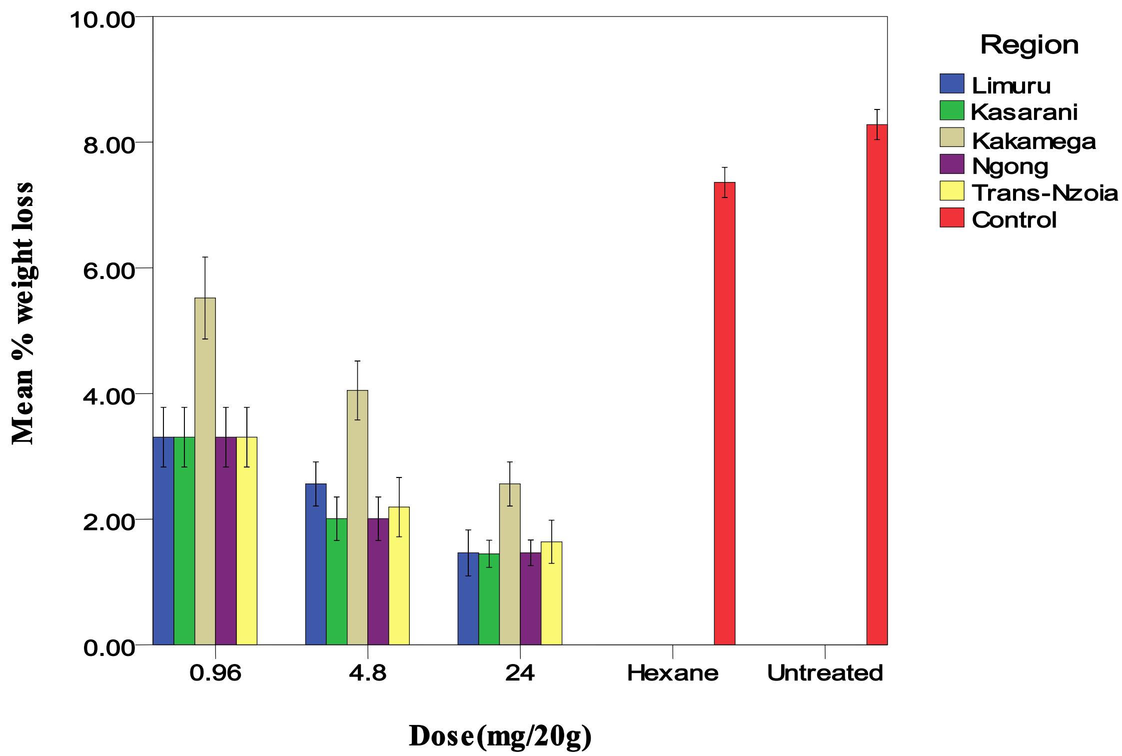
	Dose (mg/20g)		
	0.96 mg	4.8 mg	24 mg
Limuru	3.29 ± 0.49^{Bb}	2.56 ± 0.35^{Bb}	1.46 ± 0.37^{b}
Kasarani	3.30 ± 0.38^{Bb}	2.01 ± 0.36^{Bb}	1.45 ± 0.22^{c}
Kakamega	5.52 ± 0.65^{Ab}	4.05 ± 0.47^{Abc}	2.56 ± 0.35^{c}
Ngong	3.33 ± 0.48^{Bb}	2.01 ± 0.35^{Bbc}	1.46 ± 0.21^{c}
Trans-Nzoia	3.31 ± 0.27^{Bb}	2.19 ± 0.47^{Bb}	1.64 ± 0.34^{b}
p-value	0.021	0.009	0.078
Untreated control = 8.28 ± 0.65^{a}		Hexane treated = 7.36 ± 0.65^{a}	

Mean values followed by the same small letter(s) within the same row are not significantly different from one another while mean values within the same column followed by same capital letters are not significantly different (SNK, α=0.05). One way ANOVA showed a highly significant variation of the % weight loss between various doses (P<0.001)

After 30 days, the maize grain treated with 0.96 mg of the essential oil in all the regions showed significant weight loss. The essential oil from Kakamega had the highest weight loss at 0.96 mg dose of 5.52 %. There was no significant difference in weight loss in the other regions at 0.96 mg dose. When the dose was increased to 4.8 mg in 20g of the maize seeds, there was a decrease in the weight loss in all the regions (Table 4.9).

A further increase in dose to 24 mg of *O. kilimandscharicum* essential oils led to a more decrease in the weight loss across all the regions. At this dose, Kakamega region had the highest weight loss of all the regions followed by Trans-Nzoia. This could have been attributed to the low levels of camphor and 1,8-cineole in the essential oil from the region (Table 4.4).

Figure 4.5: Mean weight loss of the maize grain treated with essential oils of *O. kilimandscharicum* in the five regions



From these results, there is a high significant variation in the percentage weight loss between various doses ($p<0.001$). The grains treated with essential oils from the different regions had less level of damage compared to both the untreated grains and hexane treated control ($\alpha=0.05$) (Figure 4.5). There was no significant difference in the untreated control and hexane treated control.

The maize weevil causes damage on stored maize grain by boring the grain and eating the inner part which reduces maize weight and quality in terms of consumption and germination (Adda *et al.*, 2000). Damage caused by *S. zeamais* as stored cereal can be extremely high. It is reported that up to 18.3% weight loss occurred due to *S. zeamais* infestation when single maize kernel were exposed to ovipositing adults and kept at 27 ^{o}C and 70% relative humidity for only 37 days (Adams, 1976; Adams *et al*,. 1995). The behavior of the weevils on stored grains is affected by the inter-play of different chemical, physical and biotic factors. Factors such as store temperature, relative humidity, light intensity, grain size and variety pest's density and the presence of other insects including parasitoids and predators, as well as micro-organisms such as fungi will greatly affect the behavior of the maize weevils for successful utilization of the environment (Cox and Collins, 2002).

4.5.2 Assessment of the weight loss to the maize grains treated with essential oils of *O. americanum*

Maize treated with *O. americanum* was monitored for a period of 30 days to a certain the level of damage through weight loss by the *S. zeamais*, using the international method of weight and count FAO, 1985. After 30 days, the least concentration of 0.96 mg of *O. americanum* essential oil in 20 g of the maize seeds had the highest mean weight loss at 6.99%. The weight loss dropped slightly to 6.91% with a dose of 4.8 mg. There was no significant difference in weight loss between the four doses of hexane treated, untreated control, 0.96 mg and 4.8 mg (Table 4.10).

But when the concentration was increased to 24 mg, the weight loss reduced tremendously to 3.3% from the previous 6.91%. At a concentration of 96 mg, there was a further decrease in weight loss to 2.2%.

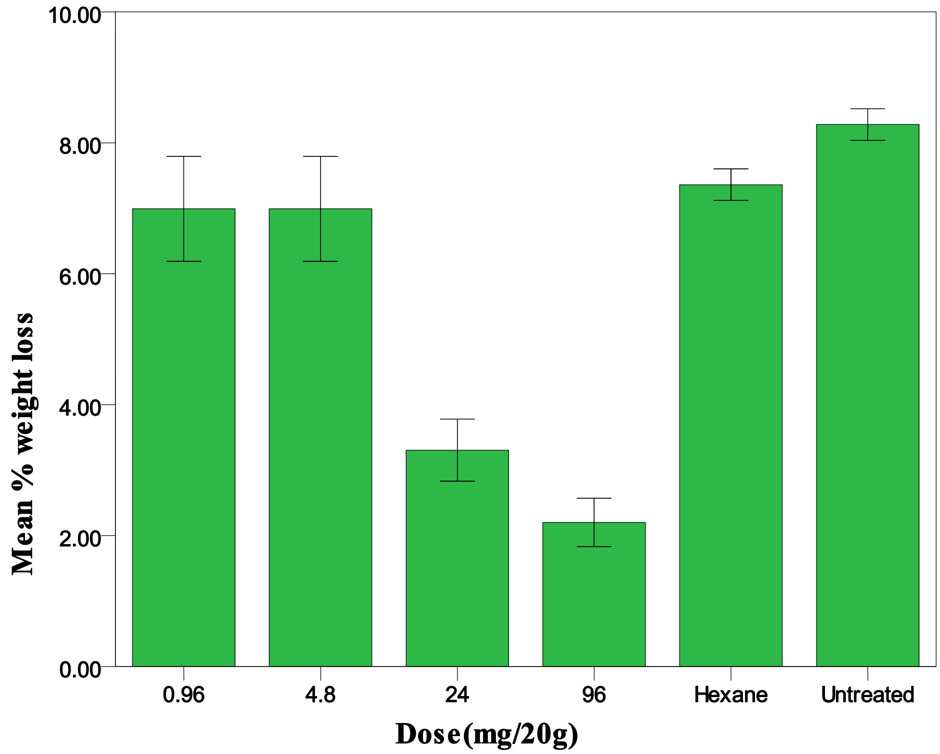
Table 4.10: Mean weight loss of the maize grain treated with essential oils of *O. americanum*

Treatment	**Mean ±SE**
Untreated	8.28 ±0.68[a]
Hexane	7.36 ±0.68[a]
0.96 mg/20g	6.99±0.80[a]
4.8 mg/20g	6.91±0.51[a]
24 mg/20g	3.30±0.48[b]
96 mg/20g	2.20±0.80[b]
p-value	<0.001

Mean values followed by the same small letter(s) within the same column are not significantly different from one another (SNK, $\alpha = 0.05$).

Weight loss caused by *S. zeamais* was significantly higher in the control compared with the grains treated with the essential oils of *O. americanum* with high dosage. The essential oil enhances feeding activity of the maize weevils with no noticeable feeding on grains treated with the highest dose (Figure 4.6).

Figure 4.6: Mean weight loss of the maize grain treated with essential oils of *O. americanum* from Machakos



Results from this study demonstrate practically that the toxic and repellent effects of *O. americanum* could be used to prevent *S. zeamais* to stored maize by masking the odours from grains in order to make the weevil unable to detect the presence of the food and oviposition site. The use of locally available plants materials for stored products protection is a common practice and has more potential in substance and traditional farm storage conditions, in developing and under-developing countries (Weaver and Subramanyam, 2002; Nikpay, 2007). This study also shows that essential oil from *O. kilimandscharicum* plant is better than *O. americanum* in the protection of maize against the maize weevils over a period 30 days.

4.6 Effect of *O. kilimandscharicum* (Kasarani) *and O. americanum* (Machakos) essential oils on the germination of maize grains.

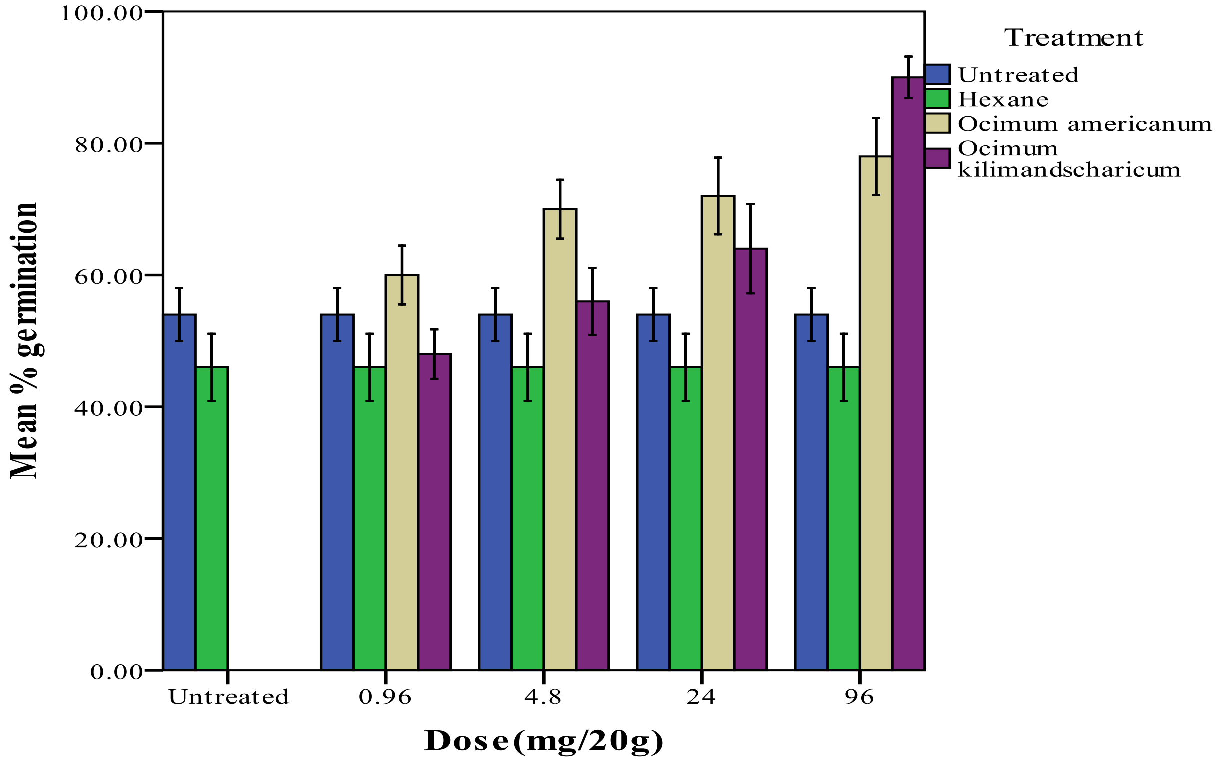
Treatment of the maize grains with essential oils of *O. kilimandscharicum* and *O. americanum* did not affect the viability of the grains. Maize seeds treated with the least concentration of 0.96mg in both plants showed no significant difference with both untreated and hexane treated control (Table 4.11).

Table 4.11: Mean percentage germination for maize grain treated with *O. kilimandscharicum* (Kasarani) and *O. americanum* (Machakos) essential oils

Treatment	0.96 mg/20g Mean±SE	4.8 mg/20g Mean±SE	24 mg/20g Mean±SE	96 mg/20g Mean±SE
Untreated control	54.00±4.00	$54.00±4.00^{a}$	$54.00±4.00^{a}$	$54.00±4.00^{a}$
Hexane (control)	46.00±5.10	$46.00±5.10^{a}$	$46.00±5.10^{a}$	$46.00±5.10^{a}$
Ocimum kilimandscharicum	48.00±3.74	$56.00±5.10^{ab}$	$64.00±6.78^{bc}$	$90.00±3.16^{b}$
Ocimum americanum	60.00±4.47	$70.00±4.47^{b}$	$72.00±5.83^{c}$	$78.00±5.83^{b}$
p-value	0.140	0.018	0.022	<0.001

The germination rate of both *O. kilimandscharicum* and *O. americanum* increased with an increase in concentration (Figure 4.7). The maize grains with the highest germination rate were when treated with the highest dose of 96mg *O. kilimandscharicum* oil, with a germination rate of 90%. With the same dose, maize seeds treated with *O. americanum* essential oil had a germination rate of 78%. The high rate of germination from the two plants at higher concentrations could have been attributed to their effectiveness in killing the maize weevils hence no mojar damages occured on the maize grains. From the present study, *O. kilimandscharicum* oil contains high concentration of camphor which gain synergism with other compounds resulting to an increase in mortality and toxicity effect.

Figure 4.7: Mean percentage germination for maize seed grain treated with *O. kilimandscharicum* and *O. americanum* essential oils



The germination of the untreated seeds was poor since the grains were largely affected by *S. zeamais*. *S. zeamais* causes damage on stored maize grains by boring the grain and eating the inner part which reduces maize weight and quality in terms of consumption and germination (Adda *et al.*, 2000).

The germination of maize seeds were not much affected with the maize grains treated with *O. kilimandscharicum* and *O. americanum* of high concentration (Figure 4.7). This shows that it can be used to treat seeds for germination.

4.7 Mortality effect of dried and grounded powder of *O. kilimundscharicum* on maize weevil

Results in this study indicate that the effect of dried and grounded powder of *O. kilimandscharicum* as contact insectides on maize weevils is not very effective. 1 g of *O. kilimandscharicum* in 20 g of the maize seeds caused 6% mortality after 48h while 2 g caused 10% mortality after the two days (Table 4.12).

Table 4.12: Mortality effect of dried and grounded powder of *O. kilimundscharicum* on maize weevil

Days	1 g/20g %Mortality(Mean±SE)	2 g/20g %Mortality(Mean±SE)	control	p-value
2	6.0±4.0	10.0±4.5	0.0±0.0	0.118
4	$4.0±2.4^{b}$	$16.0±5.8^{a}$	$0.0±0.0^{b}$	0.006
6	$14.0±3.7^{a}$	$18.0±5.1^{a}$	$0.0±0.0^{b}$	0.005
8	10.0±4.5	10.0±4.5	0.0±0.0	0.082
14	$12.0±2.0^{a}$	$14.0±2.4^{a}$	$0.0±0.0^{b}$	<0.001
p-value	0.075	0.716	-	

% (mean±SE) Mortality followed by the different small letter(s) within the same row are significantly different and ($p<0.05$, $\alpha=0.05$, SNK)

There was a progressive increase in mortality with increase in the exposure of the *S. zeamais* in the grounded mixture for both concentrations up to day 6. The highest mortality rate was experinced after 6 days for both concentrations with the highest rate being 18% with 2 g concentration and 14% when 1g concentration was used. After 6 days, the rate of mortality reduced in both concentrations. The activity of the essential oils decreases with time because they are highly volatile. Oils with high contents of hydrocarbons monoterperpenes compounds lose activity quicker than those containing oxygenated monoterpenes compound (Owolabi *et al.*, 2009). From the results, it is evident that the efficancy of grounded powder as protectant against the maize weevil was dose dependent with higher dose providing better protection with higher mortality on the *S. zeamais*. Research finding from several countries confirm that some plants powders, essential oils or their constituents not only repel insects but also have contact and fumigant insecticidal action against specific stored-product pests (Isman, 2000; Rajendran and Sriianjini, 2008).

4.7 Mortality effect of dried and grounded powder of *O. americanum* on maize weevil

From the results, the effect of grounded powder on the maize weevils was dose-dependent with the higher dose of 2 g *O. americanum* providing greater protection than the corresponding 1g of *O. americanum* dose. At a dosage of 1 g of *O. americanum,* there was a 2% mortality of *S. zeamais* while the corresponding 2 g dose had killed 10% of the maize weevils after 2 days (Table 4.13). There was a progressive increase in mortality with increase in the period of exposure for both doses.

Table 4.13: Mortality effect of dried and grounded powder of *O. americanum* on maize weevil

Days	1 g/20g % Mortality(Mean±SE)	2 g/20g %Mortality(Mean±SE)	control	p-value
2	2.00±2.00^{ab}	10.00±4.47^{b}	0.00±0.00^{a}	0.044
4	4.00±2.45	10.00±4.47	0.00±0.00	0.064
6	8.00±2.00^{b}	8.00±2.45^{b}	0.00±0.00^{a}	0.012
8	10.00±4.47	11.00±4.47	0.00±0.00	0.082
14	6.00±2.45^{b}	14.00±2.45^{c}	0.00±0.00^{a}	<0.001
p-value	0.194	0.297		

Mean values followed by the same small letter(s) within the same row are not significantly different from one another (SNK, α=0.05)

Although the mode of action of these plants powders is not clearly understood, it was observed that the repellent and pungent odours from these plants caused the insects to climb to the wall of the containers soon after introduction thereby limiting adequate feeding. Also the physical abrasion of the insect cuticle with the resultant loss of body haemolymph or partial blockage of the spiracles (Ogunwolu *et al.,* 1998; Oparaeka and Kuhiep, 2006) may have contributed to mortalities in suffocation and death. The observation that higher dosage of *O. kilimandscharicum* and *O. americanum* powders caused higher mortality could be due to either repellent or feeding deterrence effects on the weevils or a combination of the two. From the two results on the effect of dried and grounded powder on maize weevil, *O. kilimandscharicum* essential oil provided better protection against the maize weevil than *O. americanum* at equal dosage.

Bekele *et al.,* (1995) also reported that the repellent effect of dried ground leaves (25 g/250 g of maize seeds) of *O. kilimandscharicum* against *S. zeamais, R. dominica and S. cerealella* resulting in lower weight loss and number of damaged maize seeds compared with untreated grains. In Tanzania, 10% (w/w) leaf powders of eucalyptus (*Eucalptus macrorhyncha*), pawpaw (*Carica papaya)*, neem (*Azadirachta indica)* and lantana, (*Lantana camara)* were toxic to maize weevils and significantly reduced grain damage and weight loss (Mulungu *et al.*, 2007). Under small farmer scale condition as in the case of this study, powder treatment may protect stored grains for some time (Bekele, 2002).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATION

5.1 CONCLUSIONS

Several researches have been done in different countries to determine the efficacy of botanical plants for the protection of stored products against pests. This research was conducted to determine the composition and activities of essential oils of *O. kilimandscharicum* and *O. americanum* from different geographical regions for the protection of stored grains against maize weevils. The overall goal is to develop alternatives to the highly toxic and persistent pesticides being used today. The two plants from the different regions were *O. kilimandscharicum* from (Ngong, Kasarani, Kakamega, Limuru and Trans-Nzoia) and *O. americanum* from Machakos, all in Kenya. The study revealed several important facts:

(i) The composition of essential oils in a plant species growing in different location varies and this therefore affects its performance. The essential oils of *O kilimandscharicum* from Limuru and Tran-Nzoia regions had the highest mortality rate against the maize weevils over a period of 24 hours, while that from Kakamega region showed the least activity at both LC_{50} and LC_{95}. This could be attributed to low levels of the major compounds responsible for toxicity and mortality.

(ii) The level of maize damage depends largely on the dose applied and partly on the region as revealed in the damage assessment and fumigation assay. From the five regions, essential oils of *O. kilimandscharicum* from Kakamega region had the highest mean percentage weight loss after a period of 30 days.

(iii) Essential oils of *O. kilimandscharicum* from different regions and *O. americanum* from Machakos showed significant repellent activities against *S. zeamais*. This demonstrated potency in repellence against *S. zeamais* and could be one of the basis of their use in traditional methods of grain protection. There was no significant difference in repellence between the *O. kilimandscharicum* plants with the same dose from different regions but the maize weevils were more strongly repelled by *O. kilimandscharicum* than O. *americanum* plant.

(iv) Ground powdered materials provided less protection against the maize weevils compared to the essential oils. The essential oils of both *O. americanum* and *O. kilimandscharicum* caused high mortality to the *S. zeamais* than the ground powdered plant materials. This therefore demonstrates the potential of the plants for the use against the *S. zeamais*.

(v) Subtractive bioassays showed the relative roles of different constituents and the reasons underlying differential activities of the essential oils from the different regions. The activity of essential oils from the two plants *O. americanum* and *O. kilimandscharicum* differed significantly, with that of *O. kilimandscharicum* proving to be a better protectant against the maize weevil than that of *O. americanum*. Many of the major compounds found in *O. kilimandscharicum* are found in *O. americanum* with camphor being the major compound in *O. kilimandscharicum* and 1, 8-cineole being the major compound in *O. americanum*.

(vi) The subtractive bioassays with different blends provided an additional insight on an optimum combination for protection against the maize weevil. This was best demonstrated by combining both camphor and 1, 8-cineole which proved to be effective against the maize weevils.

(vii) The seed viability of the maize treated with essential oils of *O. kilimandscharicum* and *O. americanum* were determined. It was noted that the germination (viability) of the maize seeds was not affected with the maize seed treated with the essential oils of both *O. americanum* and *O. kilimandscharicum*. These therefore can be used to treat seeds grains for planting.

5.2 RECOMMENDATIONS FROM THE STUDY

(i) Materials from plants could be used to control pests and thus substitute other expensive synthetic pesticides.

(ii) Farmers can improve their traditional methods of maize protection by using the oil extracts rather than the ground plants powder.

(iii) Plant essential oils could be used by farmers as maize protectant against maize weevils. This could best be enhanced by the development of a simple hydro-distillation device for use by farmers.

(iv) Through further research, appropriate combinations from different plants need to be identified for improved protection against post-harvest pests.

5.3 RECOMMENDATION FOR FURTHER STUDY

From the research, the following recommendations for further investigations are suggested:

(i) Further studies on the activities of the pure compounds and different blends are recommended to identify the compounds responsible for repellence of *S. zeamais*.

(ii) A research study should be carried out to determine the effect of essential oils on the aflatoxin producing fungus, *Aspergillus flavus*.

(iii) More work needs to be carried in elucidating the chemical composition of the oils under different seasons of the year.

REFERENCES

Adams, J.M. (1976). Weight loss caused by development of *Sitophilus zeamais* (Motsch) in maize. *Journal of Stored Product Research*, **32**: 105-113.

Adams, R.P. (1995). Identification of essential oil components by Gas Chromatography/mass spectroscopy. Allured, Illinois.

Adda, C., Borgemeister, C., Biliwa, A., Meikle, W.G., Markham, R.H. and Poehling, H.M. (2000). Intergrated pest management in post-harvest maize: a case study from the Republic of Togo (West Africa). *Agriculture*, *Ecosystem and Environment*, **93**: 305-321.

Adedire, C.O. and Ajayi, T.S. (1996). Assessment of the insecticidal properties of some plants extracts as grain protestant against maize weevils *Sitophilus zeamais* (Mot). *Nigerian Journal of Entomology*, **13**: 93-101.

Adjoudji, O., Ngassoum, M.B, Essia-Ngang, J.J., Ngamo, L.S.T and Ndjuenkeu, R. (2007). Insecticidal activity of spicy plant oils against *Sitophilus zeamais* in stored maize in Cameroon. *Agricultural Journal*, **2**: 192-197.

Adler, C., Ojimelukwe, P. and Leon, T.A. (2000). Utilization of phytochemicals against stored product insects. *Intergrated Protection of Stored Products, IOBC Bulleting*, **23**: 169-175.

Appert, J. (1987). The storage of food: Gain and seeds Macmillan publishers Ltd, London, pg. 146.

Arannilewa, S.T., Ekrakene, T. and Akinne, J.O. (2006). Laboratory evaluation of four medicinal plants as protectants against the maize weevil, *Sitophilus zeamais*, (Motsch).

Arbogast, R.T. and Throne, J.E. (1997). Insect infection of farm-stored maize in south California: Towards characterization of habitat. *Journal of Stored Products Research*, **33**: 187-198.

Arcander, S. (1960). Perfume and Flavour Material of Natural Origin Det. Hoffenabergke Establishment, Denmark, pg. 300-302.

Asawalam, E.F., and Hassanali A. (2006). Constituents of the essential oil of *vernonia amygdalina* as maize weevil protectants. Tropical and subtropical Agrosystems, **6**:95:102.

Asawalam, E.F., Emosairue, S.O. and Hassanali A. (2006). Essential oil of *Ocimum grattissimum(*Labiatae) as *Sitophilus zeamais* (Coleoptera: Curculionidae) protectant. *African Journal of Biotechnology*, **20**: 3771-3776.

Awoyinka O.L., Oyewole, B., Amos O., Onasoga. (2006). Comparative pesticidal activity of dichromethane extracts of *Piper nigrum* against *Sitophilus zeamais* and *Callosobruchus maculates*. *African Journal Biotechnology*, **24**:2446-2449.

Beckett, S.J. Fields, P.G. and Subramanyam Bh. (2007). Disinfestation of stored products and associated structures using heat. Heat treatment of postharvest pest control strategy and practice, pg. 112-237.

Bekele, A.J. (1994). Effect and some use of some Ocimum plants species and their essential oil on some storage insects pests. PhD thesis (University of Nairobi), pg. 200.

Bekele, and Hassanali, A., (2001). Blend effect in the toxicity of essential oil constituents of *Ocimum kilimandscharicum* and *Ocimum kenyense* (Labiatae) on two post harvest insects pests. Phytochemisty, **57**: 385-391.

Bekele, A.J., Obeng-Ofori, D., Hassanali, A. and Nyamasyo, G.H.N. (1995). Products derived from the leaves of *Ocimum kilimandscharicum* as post-harvest grain protestants against the infestation of three major stored products insect pests. *Bulletin of Entomological Research*, **85**: 361-367.

Bekele, J. (2002). Evaluation of toxicity potential of *Milletia ferruginea* (Hochest) Baker against *Sitophilus zeamais* (Motsch). *International journal of pest Management*, **48**(1): 29-32.

Bond, E.J. (1984). Manual of fumigation for insect control FAO, Plant production and protection paper 54.

Burks, C.S., Johnson,J.A., Maier, D.E. and Heaps, J.W. (2000). Temperature. In: Subramanyam, Bh. and Hagstrum, D.W. (eds), *Alternatives to Pesticides in Stored-products IPM*. Boston, Massachusetts, Kluwer Academic Publishres, pg. 73-104.

Casida, J.H. (1990). Pesticide mode of action, evidence for implication of a finite number of biochemical targets. *In*: Casida, J.E. (*ed*), *Pesticides and alternatives .Innovative Chemical and Biological Approaches to Pest Control*, Amsterdam, Elsevier. pg. 11-22.

Chai, M.K., (2008). Development and validation of a solid phase micro extraction method for simultaneous determination of pesticide residues in fruits and vegetables by gas chromatography Ph.D. university of Malaya Kuala Lumpur, pg. 339.

Chhabra, S.C., Mahunnah, R.L.A. and Mshiu, E.N. (1990). J. *Ethnopharmacology*, **28**: 255-283.

Chogo, J.B. and Crank, G. (1981). Chemical composition and biological activity of the Tanzanian Plant *Ocimum suave*. J. *Natural. Products*. **44**: 309.

Cox, P.D. and Collins, L.E. (2002). Factor affecting behavior of beetle pests in stored grains, with particular reference to the development of lures. *Journal of Stored Product Research*, **38**: 95-115.

Danjumma, B.J.,Majeed, S.B.,Yabaya, M.A., Dike M.C., Bamaiya, L. 2009. Effects of some plants powders in the control of *Sitophilus zeamais*. *Journal of Science Research*, **4**: 313-316.

Darby, J. (1998). Putting grain aeration in order with generalized categories. In: Banks H.J., Wright, E.J. and Damcevski, K.A. (eds), *Stored grain in Australia*. Proceedings of the Australian Postharvest Technical Conference , Canberra, 26-29 may 1998. CSIRO, Canberra, pg. 203-208.

Demisjie, G., Teshome, A., Abakemal, D. and Tadesse, A. (2008). Cooking oils and “Triplex” in the control of *Sitophilus zeamais* in farm stored maize. *Journal of Stored Product Research*, **44**: 173-178.

Dendy, J., Dobie, P., Smith, J.A. and Urono, J.L. (1991). Trials to assess the effectives of new synthetic pheromone mixtures for trapping *Prostephanus truncates* (Horn) (Coleoptera: Bostrichidae) in maize stores. *Journal of Stored Products Research*, **27**: 69-74.

Dosland, O., Subramanyam, Bh., Sheppard, K. and Mahroof, R. (2006). Temperature modification for insect control. In: Heaps, J. (ed), *Insect Management for food storage and processing*, 2nd edition. Minnesota, American Association for Clinical Chemistry, St Paul, pg. 89-103.

Dowdy, A.K. and Mc Gaughey, W.H. (1998). Stored-product insect activity outside of grain masses in commercial grain elevators in the mid western United states. *Journal of Stored Products Research,* **38:** 11-12.

Dowdy, A.K. and Fields, A.K. (2002). Heat combined with diatomaceous earth to control the confused flour beetle (Coleoptera: Tenebrionidae) in a flourmill. *Journal of Stored Products Resaerch,* **38**: 11-22.

Duke, S.O. (1990). Natural pesticides from plants pg. 511-517. In: J. Janicks and J.E. Simon (eds), Advances in new crops. Timber Press, Portland.

Dunstan, W.R. and Magazini, I.A. (1981). Outbreak and new records. The Larger grain borer on stored products. FAO *Plant Protection Bulletins*, **10**: 588-590.

Fields, P.G. and White, N.D.G. (2002). Alternative to methyl bromide treatments for stored-product and quarantine insects. *Annual Review of Entomology*, **47**:331-359.

Fields, P.G.,Van Loon, I., Dolinski, N., Harris, L. and Burkholder, W.E. (1992). The distribution of Rhyzopertha dominica in Western Canada. *The Canadian Entomologist*, **125**: 317-328.

Food and Agricultural Organization of the United Nations (FAO) (2011). Prevantion of post-harvest food losses. Training series No 10(122), Food and Agricultural organization of united Nations. Roma, Italy, pg. 120.

Food and Agricultural organization of the United Nations (FAO) (1985). FAOSTAT.database (http//apps.fao.org/faostat).

Golob and Hedges, R. (1982). Study of an outbreak of *Prostephanus truncates* (Horn) in Tanzania. *Tropical product institute*, London, England. **16**: 23

Guenther, E. (1975). Essential oils. Vol **1I**. Krieger, New York, pg. 734.

Haines, C.P. (1991). Insects and Arachnids of tropical stored products: Their biology and identification (A training manual). Natural Resources Institute, Chatham, UK, pg. 246.

Hassanali, A., Lwande, W., Ole Sitayo, N., Moreka, L., Chapya, A. and Nokoe, S.K. (1997). Leaves of *Ocimum suave* and flower buds of *Eugenia aromatic* as maize weevil (*Sitophilus zeamais*, Motschulsky) repellents. *Discovery and Innovovation,* **2**: 91-95.

Hill, D.S. and Walter, J.M. (1990). Pest and diseases of Tropical crops. Field Handbook, Longhman scientists and technical, UK, pg. 432.

Hodges, R.J., Hall, D.R., Mbugua, J.N. and Likhayo, P.W. (2000). Grain injury models for *Prostephanus truncates* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) in rural maize stores in West Africa. *Journal of Economic Entomology*, **93**: 1338-1346.

Isman, M.B. (2006). Botanical Insecticides, Deterrents, and Repellents in Modern Agriculture and an increasingly required World. *Annual Review of Entomology,* **51**: 45-66.

Isman, M.B. (2000). Plants essential oils for pest and diseases management. *Crop Protection*, **19**: 603-608.

Ivbijaro, M.F. and Agbaje, M. (1986). Insecticidal activities of *Piper guineense* and *Capsicum species* on the cowpea bruchid, *Callosobruchus maculates* F. *Insect Science and its Application*, **7**: 353-357.

Jembere, B.D., Obeng-Ofori, A., Hassanali and Nyamasyo G.N.N., (1995). Products derived from the leaves of *Ocimum kilimandscharicum* (Labiatae) as post-harvest grain protectants against the infestation of three major stored product insect pests. *Bulletin of Entomological Research*, **85**: 361-626.

Kabeh, J. D. and Jalingo, M. G. D. S. S. (2007). Pesticidal effect of bitter leaf plant *Vernonia amygladina* (Compositae) leaves and pirimiphos-methyl on larvae of *Callosobruchus maculatus* (Coleoptera: Bruchidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae). *International Journal of Agriculture and Biology*, **9**: 452 – 454.

Kilgore, W.W. (1967). Pest control. Hall, Belfast, pg. 269-270.

Kouninki, H., Hance, T., Noudjou, F.A., Lognay, G., Malaisse, F., Ngassoum, M.B., Mapongmetsem, P.M., Ngamo, L.S.T. and Haubruge, E. (2007). Toxicity of some terpenoids of essential oils of Xylopic from Cameroon against *Sitophilus zeamais* Motshuksky. *Journal of Applied Entomology,* **131**: 269-274.

Langenhein, J.H. (1994). Higher plant terpenoids: A phytocentric overview of their ecological roles. *Journal of Chemical Ecology,* **20**: 1223-1280.

Larry, P.P. (2000). Entomology and pest management, fourth edition, Printice-Hall, India, pg. 203-212.

Law-Ogbomo, K.E. (2007). Reduction of post-harvest loss caused by *Callosobruchus maculates* (F.) in three varieties of Cowpea treated with palms. *Journal of Entomology,* **4**: 194-201.

Likhayo, P.W. and Hodges, R.J. (2000). Field monitoring *Sitophilus zeamais* and *Sitophilus oryae* (Coleoptera: Curculionidae) using refuge traps baited with synthetic pheromone and cracked wheat. *Journal of Stored Products Research*, **36**: 341-353.

Longstaff, B.C. (1981). The effect of aeration upon insect population growth. In Champ, B.R. and Highley, E (eds). Proceedings of the Australian development assistance course on the preservation of stored cereals CSIRO, Canberia, pg. 459-465.

Maarse, H. (1991). *Volatile compounds in food and beverages*. Marcel Dekker Inc., New York.

Markham, R.H., Bosque-Perez, N., Bosqemeister, C. and Meikle, W.G. (1994). Developing pest management strategies for the maize weevil, *Sitophilus zeamais* and the larger grain borer, *Prostephanus truncatus* , in the humid and sub-humid tropics. (FAO), *Plant Protection Bulleting,* **42**: 97-116.

McGraw-Hill (1987), Encyclopadia of science and Technology. McGraw-Hill Book Co. 6 pg. 443.

Mulungu, L.S., Lupenza, G., Reuben, S.O.W.M. and Misangu, R.N. (2007). Evolution of botanical products as stored grain protectants against maize weevils, (*Sitophilus zeamais*) on maize. *Journal of Entomology*, **4**: 258-262.

Nault, L.R. (2007). Arthropods transmission of plants viruses, *New synthesis Entomology* **90**: 521-541.

Nawrot, J. and Czaplicki, E. (1982). Behaviour of granary weevil beetle towards some substances extracted from natural products. *Zeszty Problrmowe Postepow Nauk Rolnicych*, **202**: 183-191.

Neethirajan, S., Karunakaran, C., Jayas, D.S. and White, N.D.G. (2007). Detection techniques for stored-product insects in grain. *Food Control*, **18**: 157-162.

NIST. (2005) NIST/EPA/NIH mass spectral library version 2.0 Office of the standard Reference Data Base, National Institute of Standards and Technology, Gaithersburg, Maryland.

Nikpay, A. (2007). Insecticidal efficacy of tree vegetable oils as post-harvest grain protectants of stored wheat against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). *Insect Science*, **14**: 145-150.

Obeng-Ofori, D. and Reichmuth, C . (1997). Biological activity of eugenol, a major compoment of *Ocimum suave* (Wild) against four species of stored product coleopteran. *International Journal of Pest Management,* **43**: 89-94.

Obeng-Ofori, D., Reichmuth, C., Bekele, J. and Hassanali, A. (1997). Biological activity of 1,8-cineole, a major component of essential oil of the *Ocimum kenyense* (Ayobangira) against stored product beetles. *Journal of Applied Entomology,* **121**: 237-243.

Ogunwolu, E.O., Igoli, J.O. and Longe, N.N. (2008). Reduction in reproductive fitness of *Callosobruchus maculates* F. exposed to *Zanthoxylum zanthoxyloides* (Lam.) Watern. *Journal of Herbs, Spices and Medical Plants*, **6**: 19-27.

Oparaeke, A.M. and Kuhiep, G.C. (2006). Toxicity of powders from indigenous plants against *Sitophilus zeamais* Motsch on stored grains. *Journal of Entomology,* **3**: 216-221.

Owolabi, M.S., Oladimeji M.O., Lajide, L, Singh G., Marimuthu P. and Isidorov V.A (2009). Bioactivity of three plants derived essential oils against the maize weevils *Sitophilus zeamais* (Motschusky) and cowpea weevils *Callosobruchus maculates* (fabticus). *Electronic journal of envornmental chemistry,* **8**: 828-835.

Parugrug, A.M. and Roxas, C. (2008). Insecticidal action of five plants against maize weevils, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). *King Mongkut's institute of Technology Ladkrabang Science and Technology Journal*, **8**(9): 24-38.

Peng, W.K. (1998). Insects of domestic corns and sorghum stored in steel silos in Taiwan. *Plant protection Bulletin*, **40**: 309-304.

Pike, V., Smith, J.L., White, R.D. and Hall, D.R. (1994). Studies of responses of stored-products pests, *Protephanus truncates* (Horn) and *Sitophilus zeamais* (Motsch.), to food volatiles. In: Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R. (eds), *Proceeding of 6th International Working Conference on Stored-Product Protection*, Canberra, Australia. Wallingford, UK, CAB Internatial, pg. 566-569.

Pinder, A.R. (1960). Chemistry of Terpenes. Chapman and Hall, Belfast, pg. 3-4.

Pomeranz, T. (1978). Cereals 78: Better nutrition for the worlds millions. *In proceeding of the sixth international Cereal and Bread Congress*. American Association of Cereal Chemists Inc., USA, pg. 272.

Powell, J.S. and Raffa, K.F. (1997). Sources of variation in concentration and composition of folier monoterpenes in Tamarack (*Larix laricina*) seedlings: Roles of nutrient availability, time of season, and plant architecture. *Journal of Chemical Ecology*, **25**: 1771-1797.

Rajendran, S. and Sriranjini, V. (2008). Plant products as fumigants for stored-product insect control. *Journal of Stored Products Research*, **44**: 1-30.

Rees, D. (2004). Insects of stored product CSIRO Publishing, Collingwood, Austradia, pg. 181.

Roark, R.C. (1947). Some promising insecticidal plants. *Economic Botany*, **1:** 437-439.

Ryan, M.F. and Byne, O. (1988). Plants-insect coevolution and inhibition of acetylcholinesterase. *Journal of Chemical Ecology*, **14**: 1965-1975.

Rozman, V., Kalinovic, I. and korunic, Z. (2007). Toxicity of naturally occurring compounds of Limiaceae and Lauraceae to three stored products insects. *Journal of Stored Product Research*, **43**: 349-355.

Sahaf, B.Z., Moharramipour, S. and Meshkatalsadat, M.H. (2007). Chemical constituents and fumigants toxicity of essential oil from *Carum copticum* against two stored products beetles.

Saxana, B.P., Sharma, P.R., Thappa, R.K. and Tikku, K. (1989). Temperature-induced sterilization for control of three stored grain bettles. *Journal of Stored Product Research,* **28**: 67-70.

Scott, W.P. and Mackibben G.H. (1978). Toxicity of black pepper extract to boll weevils. *Journal of Economics Entomology*, **71**: 343-344.

Seck, D., Lognay, G., Haubruge, E., Wathelet, J.P., Marlier, M., Gaspar, C. and Sererin M. (1993). Biological activity of the shrub *Boscia senegalensis* on stored grain insects. *Journal of Chemical Ecology*, **19**: 377-390.

Sen, B. (2001). Determination of effects of some plants phenols on wood protection. PhD thesis, University of Karaelmas institute of Science. Zonguldak, Turkey, pg. 295.

Shaaya, E., Kostjukovsky, M., Eilberg, J. and Sukprakarn, C. (1997). Plant oils as fumigants and contact insecticides for the control of stored-product insects. *Journal of Stored Products Research*, **33**: 7-15.

Sheath, H.B. and Reinescius , G. (1986). Flavour Chemisty and Technology. AVL Pudl. Co., Pakistan, Pg. 207.

Simonsen, J.L. (1953). The Terpenes, Vol **1**. Cambrige University press, London pg. 10.

Singh, G. and Upadhyay, R.K. (1993). Essential oils as potent source of natural pesticides. *Journal of Scientific and Industrial Research*, **52**: 676-683.

Snelson, J.T. (1987). Grains protectants. Monograph no. 3. *Australian Centre for internation Aricultural Research*. Canberra, Auastralia, pg. 224.

Stacey, R. Shahla, S. Kathryn, Q. and Bright, M. (2007). Food security assessment. *Enomimic Research Service,* pg. 1-26.

Stamopoulos, D.C., Damos, P. and Karagianidou, G. (2007). Bioactivity of five monoterpenoid vapours to *Tribolium confusum* (duVal) (Coleoptera: Tenebrionidae). *Journal of Stored Products Research,* **43**: 571-577.

Subramanyam, Bh and Hagstrum, D.W. (2000). Botanicals. *In*: Subramanyam, Bh and Hagstrum, D.W. (eds), *Alternatives to Pesticides in Stored Product IPM*. Boston, Kluwer Academic Publishers, pg. 303-320.

Toews, M.D., Pearson, T.C. and Campbell, J.F. (2006). Imaging and automated detection of *Sitophilus oryzae* (Coleoptera: Curculioniddae) pupae in hard red winter wheat. *Journal of Economic Entomology,* **99**: 583-592.

Ukeh, D.A., M.A. Birkett, J.A., Pickett, A.S. Bowman, and A.J. Luntz. (2009). Repellent activity of alligator pepper, *Aframomum melegueta*, and ginger, *Zingiber officinale* against maize weevils, *Sitophilus zeamais*. Phytochemistry, **70**: 751-758.

Van Den Dool, H. and Kratz, P.D.J.A. (1963). Generalisation of the retention index system including linear temperature programmed gas-liquid partition chromatography, **11**: 463-47.

Walgenbach, C.A., Phillips, J.K., Faustini, D.L. and Burkholder, W.E. (1987). Male produced aggregation pheromone of the maize weevil *Sitophilus zeamais* and interspecific attraction between three Sitophilus species. *Journal of Chemical Ecology*, **9**: 831-841.

Walker, D.J. and Farrell, G. (2003). *Food Storage Manual*. Chatham, UK: Natural Recourses Institute/Rome: World Food Programme. Pg. 121-175.

Wakefield, M.E., Bryning, G.P. and Chambers, J. (2005). Progress towords a lure to attrack three stored product weevils, *Sitophilus zeamais* Motschulsky, *S. oryzae* (L.) and *S. granaries* (L.) (Curculionidae). *Journal of Stored Products Research,* **41**: 145-161.

Wang, M. and Lincoln, D. (2004). Effect of light intensity and artificial wounding on monoterpenes production in *Myrica cefera* from two different ecological habitants. *Canadian Journal of Botany*, **82**: 1501-1508.

Warrier, P.K., V.P.K. Nambiar and C. Mankutty. (1994). Indian Medicinal Plants. Orient Longman; Chennai, India, pg. 341-345.

Watanabe, K., Shono, Y., Kakimizu, A., Okada, A.M. and Mishumura, H. (1993). New mosquito repellent for *Eucalptus camaldelensis*. *Journal of Agriculture and Food chemistry*, **41**: 2164-2166.

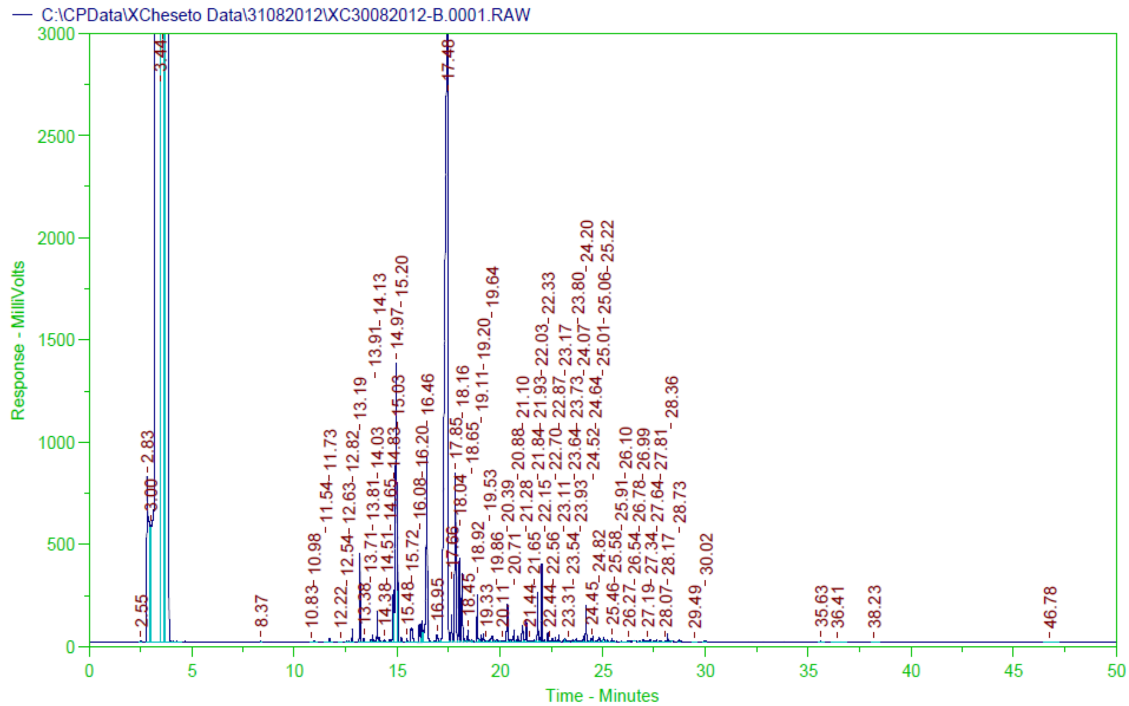
Weaver, D.K., Dunkel, F.V., Ntezupubanza, L., Jackson, L.L. and Stock, D.T. (1991). The efficacy of Linalool, a major component of freshly-milled *Ocimum americumum* (Lamiaceae), for protection against stored product Coleoptera. *Journal on stored Product Research*, **27**: 213-220.

Weaver, D.K. and Subramanyam, Bh.(2002). Botanicals. *In*: Subramanyam, Bh and Hagstrum, D.W. (eds.), *Alternatives to Pesticides in Stored-Product IPM*, Boston, Kluwer Academic Publishers, pg. 303-320.

Wright, E.j., Sinclair, E.A. and Annis, P.C. (2002). Laboratory determination of the requirement for control of *Trogoderma variabile* (Coleoptera: Dermestidae) by heat. *Journal of Stored Products Research*, **38**: 147-155.

Appendix 1.a GC trace for essential oil from Kakamega

Chrom Perfect Chromatogram Report GC-Kakamega



Sample Name = **DCM CLEAN OUT**

Instrument = 5890-S2+GC-2
Heading 1 =
Heading 2 =

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Method Description = 40 min run for Volats 35 to 280 Degree | Method Date = 4/17/2012 8:12:59 PM
Calibration File Name = | Calibration Version = 0
Internal/External = [No data] | Calibration Date = [No data]

Run Time = 50 | Data Sampling Rate = 10
Amount Injected = 1 | Dilution Factor = 1
Sample Weight = 1 | Int Std Amount = 0

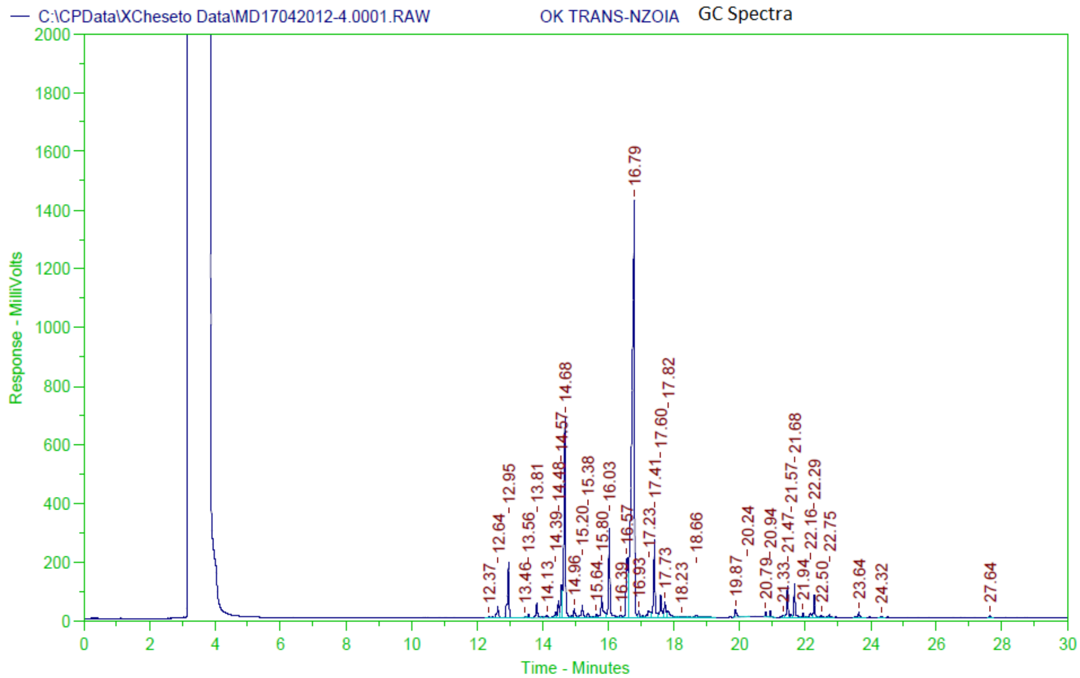
Peak Width = 0.05 | Peak Threshold = 5

Operator = XC | Manual Integration = not manually integrated

Appendix 1.b

GC trace for essential oil from Trans-Nzoia

Chrom Perfect Chromatogram Report



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Method Description = 40 min run for Volats 35 to 280 Degree
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Method Version = 2
Method Date = 4/17/2012 8:12:59 PM
Calibration Version = 0
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Run Time = 50
Amount Injected = 1
Sample Weight = 1

Data Sampling Rate = 10
Dilution Factor = 1
Int Std Amount = 0

Peak Width = 0.05

Peak Threshold = 5

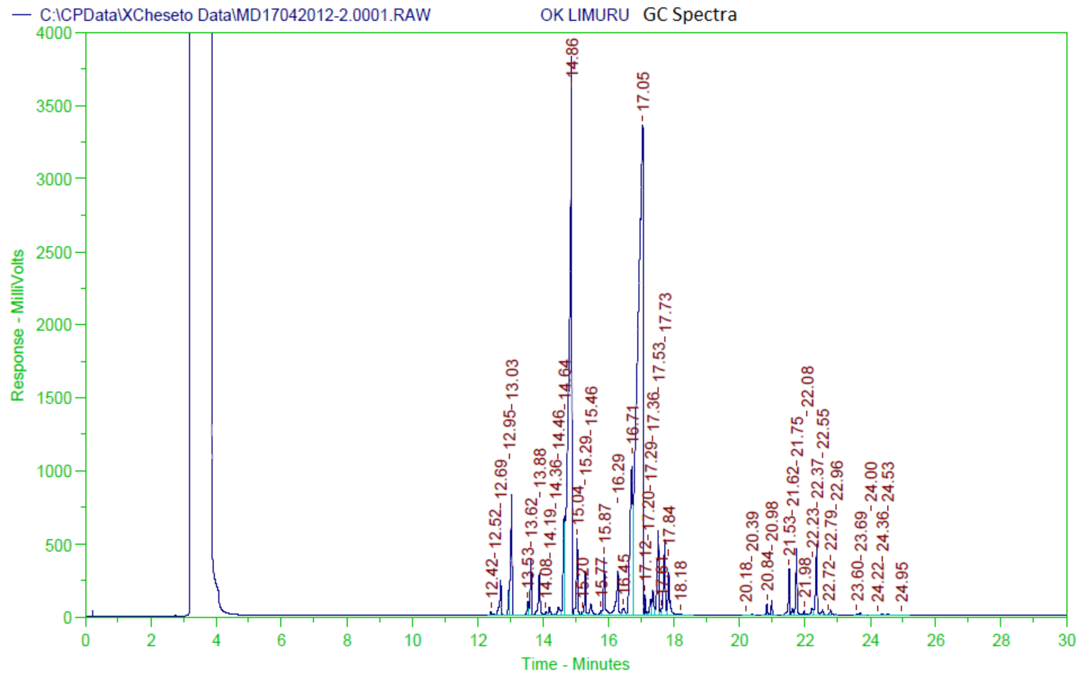
Operator = XC

Manual Integration = manually integrated

Appendix 1.c

GC trace for essential oil from Limuru

Chrom Perfect Chromatogram Report



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Method Description = 40 min run for Volats 35 to 280 Degree
Calibration File Name =
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Method Date = 4/17/2012 8:12:59 PM
Calibration Version = 0
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Run Time = 50
Amount Injected = 1
Sample Weight = 1

Data Sampling Rate = 10
Dilution Factor = 1
Int Std Amount = 0

Peak Width = 0.05

Peak Threshold = 5

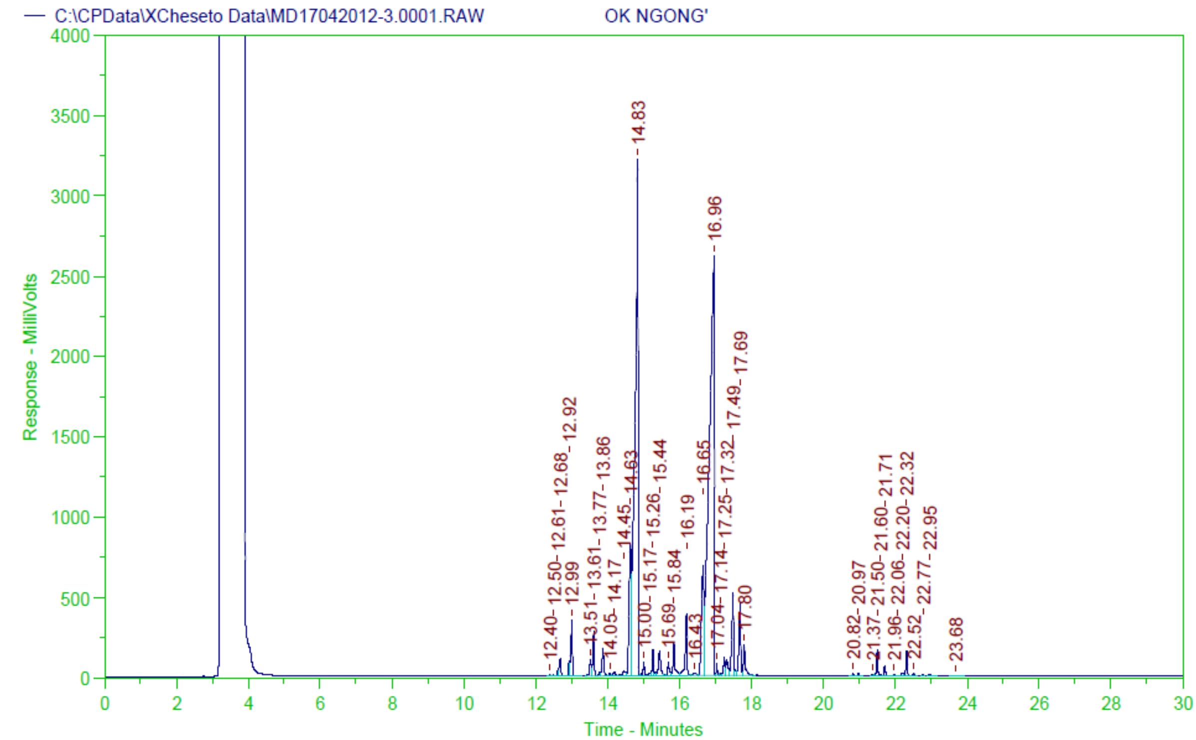
Operator = XC

Manual Integration = manually integrated

Appendix 1.d

GC trace for essential oil from Ngong

Chrom Perfect Chromatogram Report



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Calibration File Name =
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Calibration Date = [No data]

Run Time = 50
Data Sampling Rate = 10
Amount Injected = 1
Dilution Factor = 1
Sample Weight = 1
Int Std Amount = 0

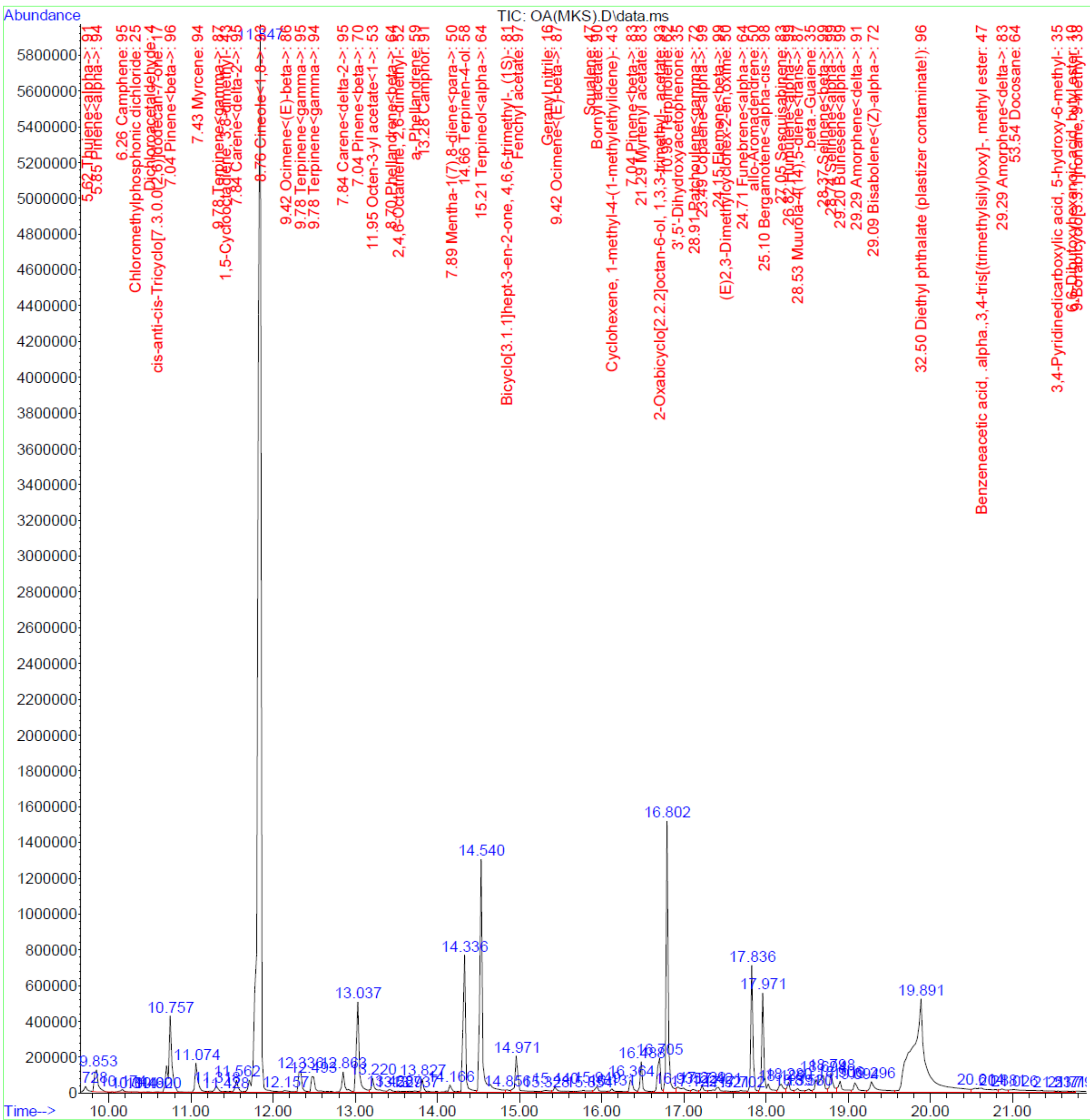
Peak Width = 0.05
Peak Threshold = 5

Operator = XC
Manual Integration = manually integrated

Appendix 1.e

GC trace for essential oil from Machakos

File :C:\msdchem\1\DATA\bw\20112012\OA(MKS).D
Operator :
Acquired : 22 Nov 2012 6:43 using AcqMethod DCM VOLATILES 35-280 XTD 40MINUTES .M
Instrument : ICIPE MSD
Sample Name: OA (MKS)
Misc Info : OIL
Vial Number: 25



The MS spectra for the major compounds identified in *Ocimum kilimandscharicum* and *Ocimum americanum* essential oils

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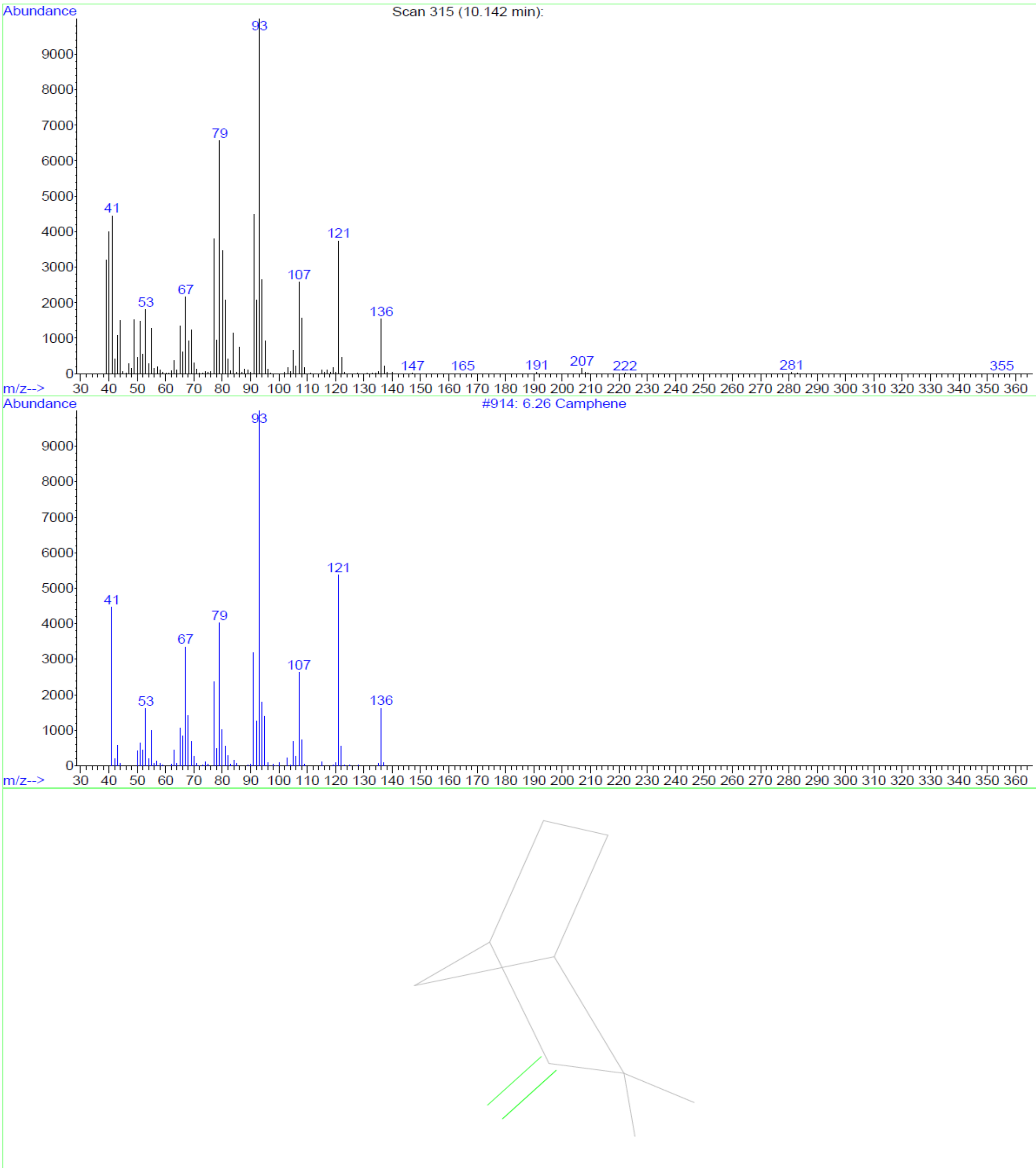

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ID               : 13.28 Camphor
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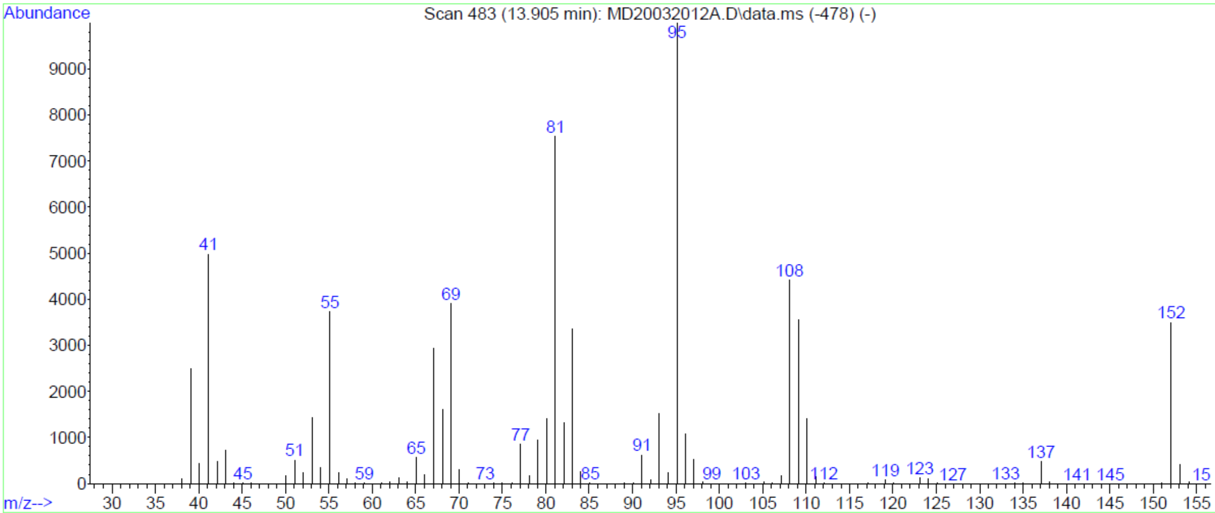

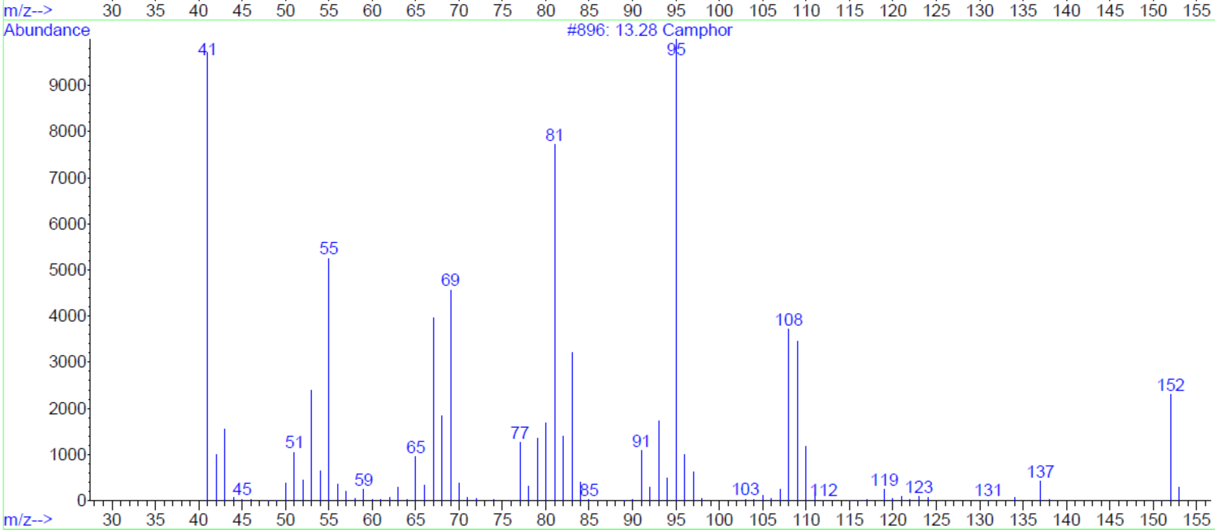

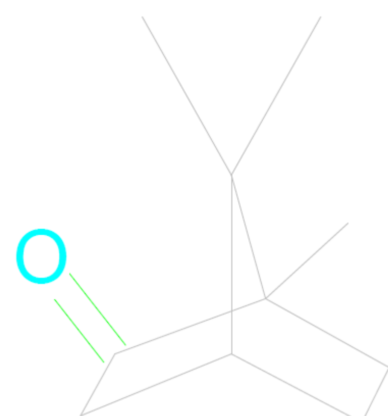

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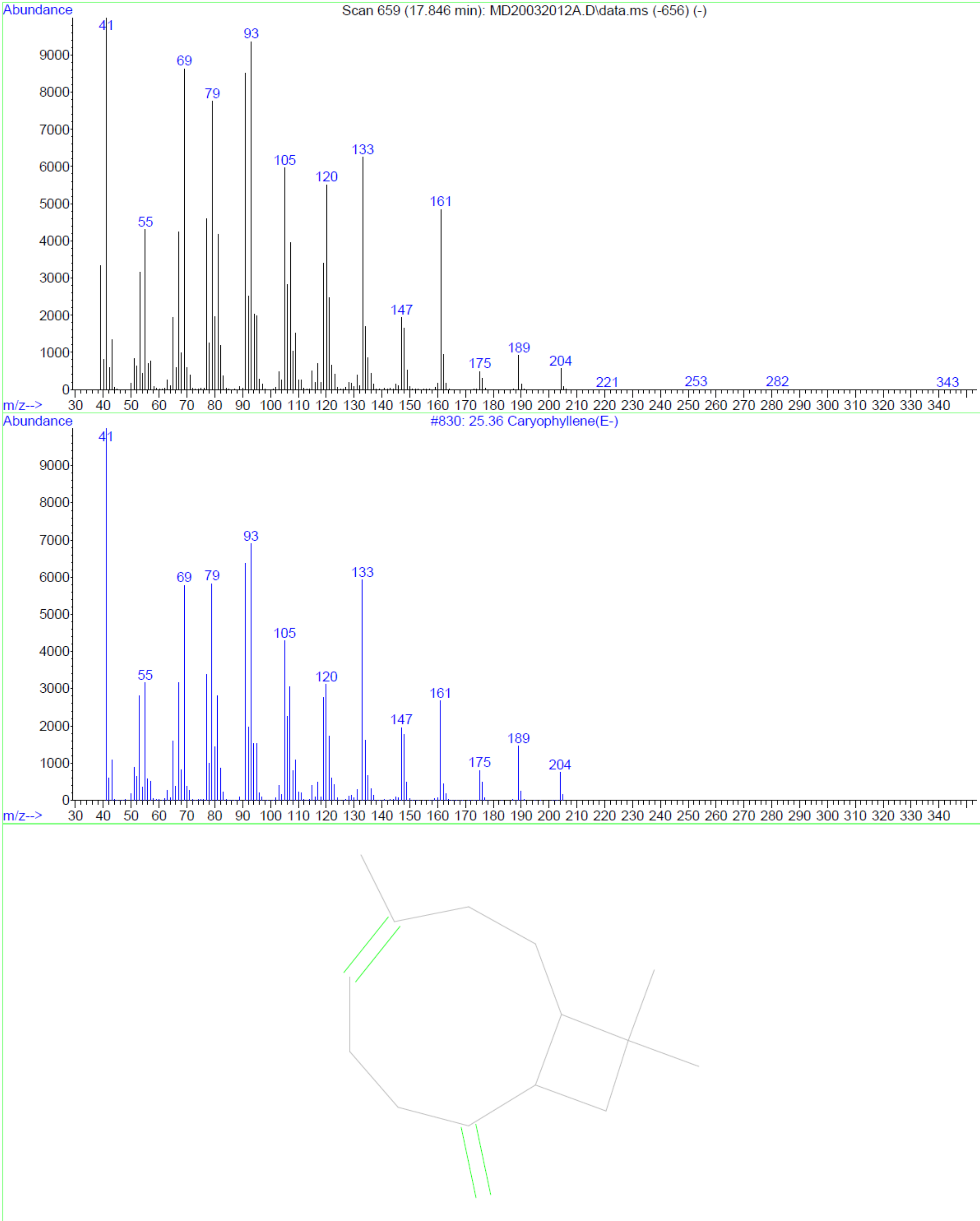

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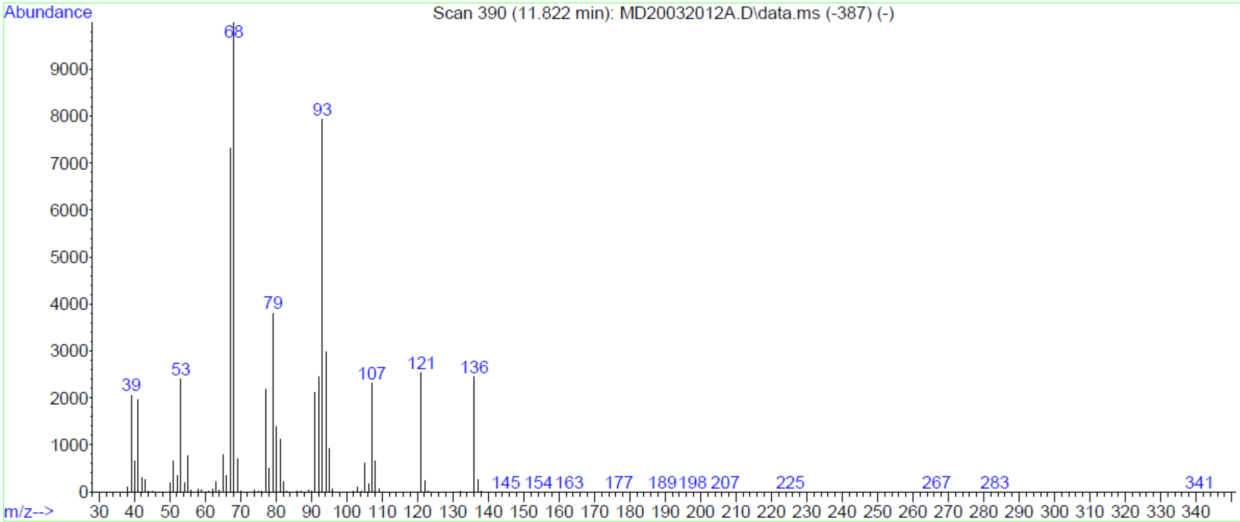

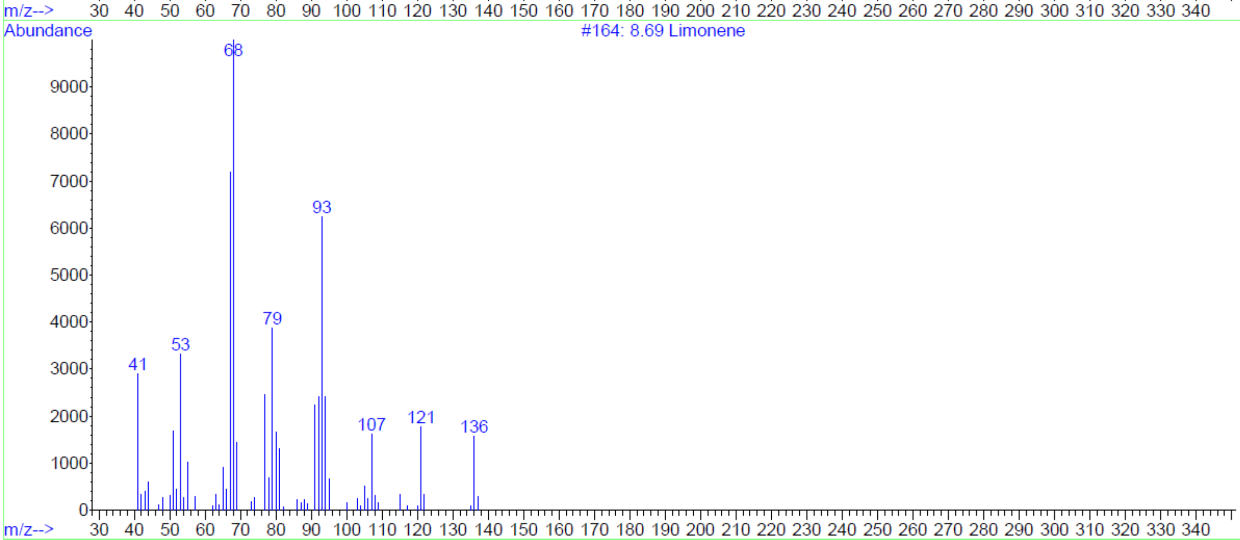

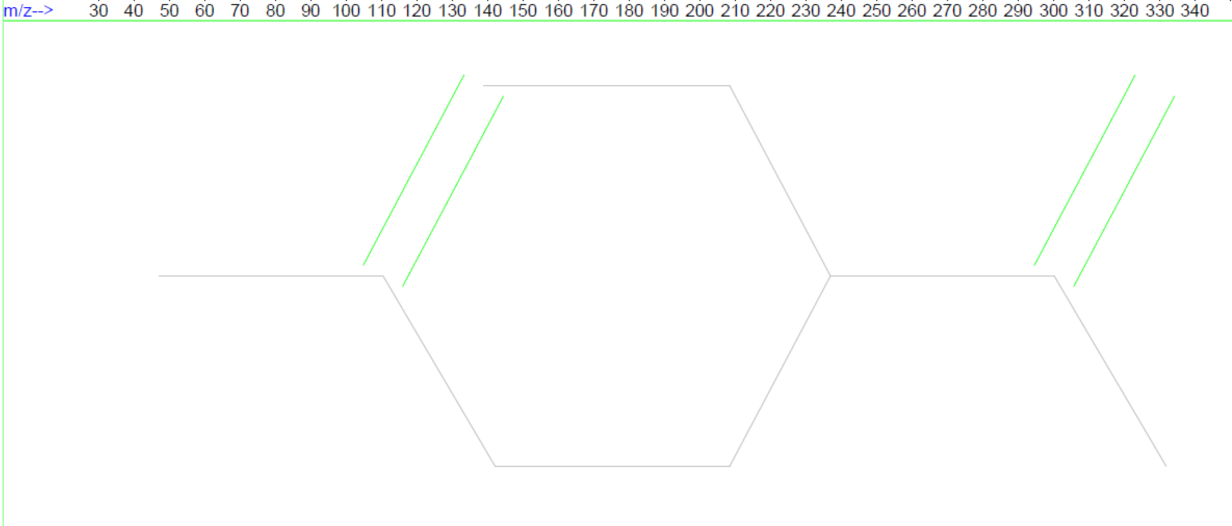

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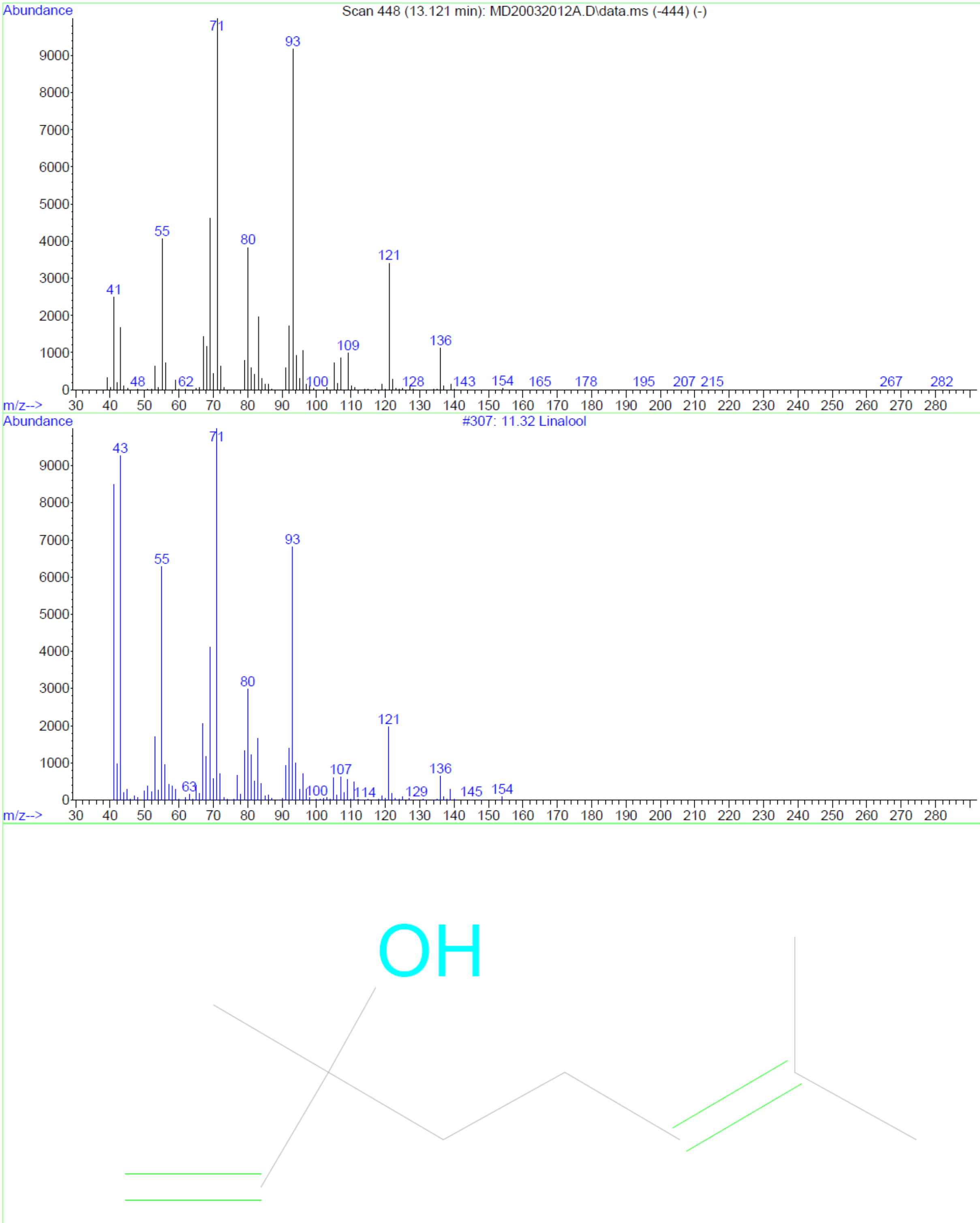

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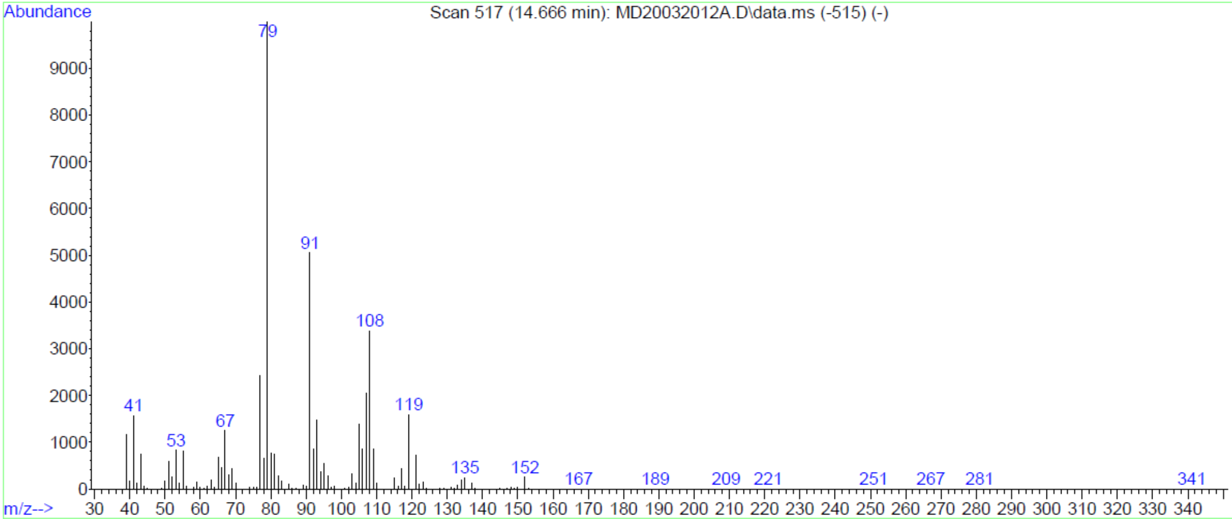

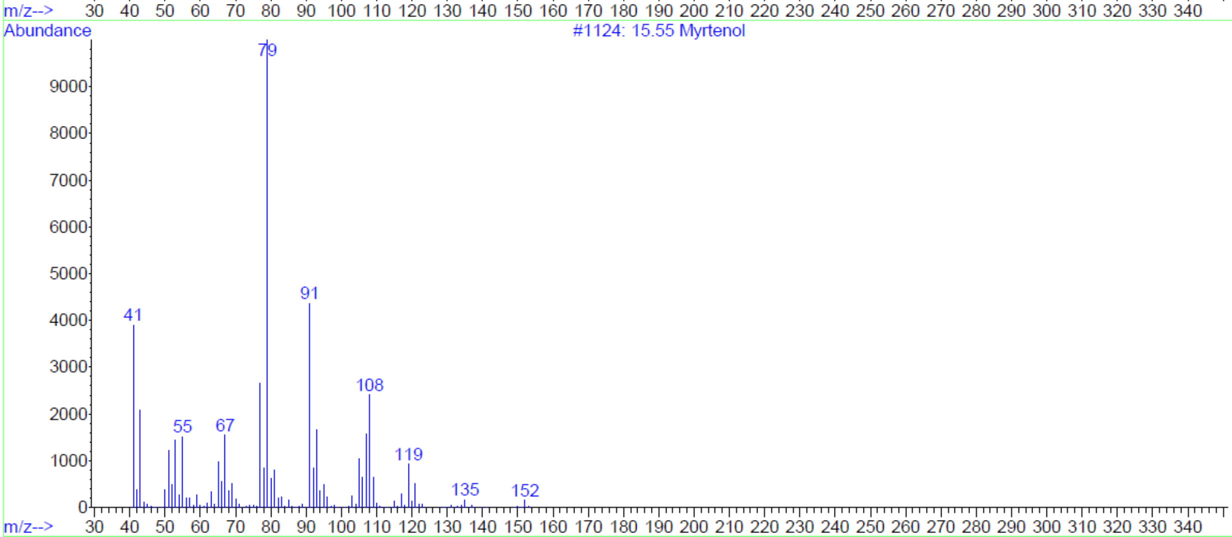

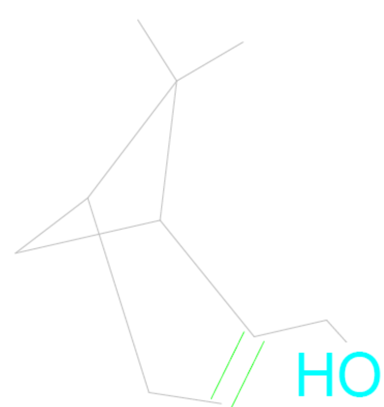

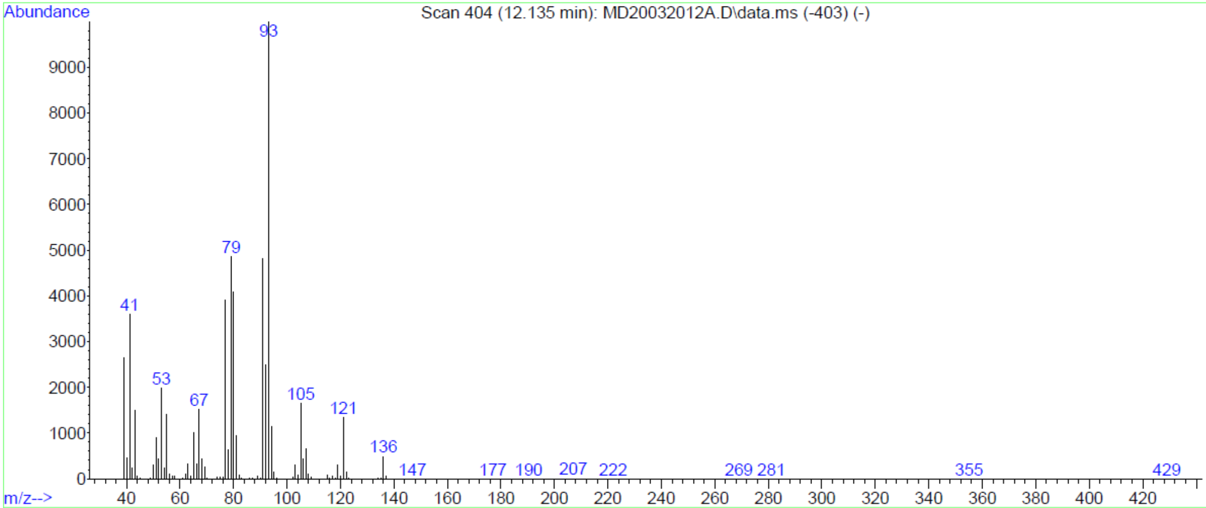
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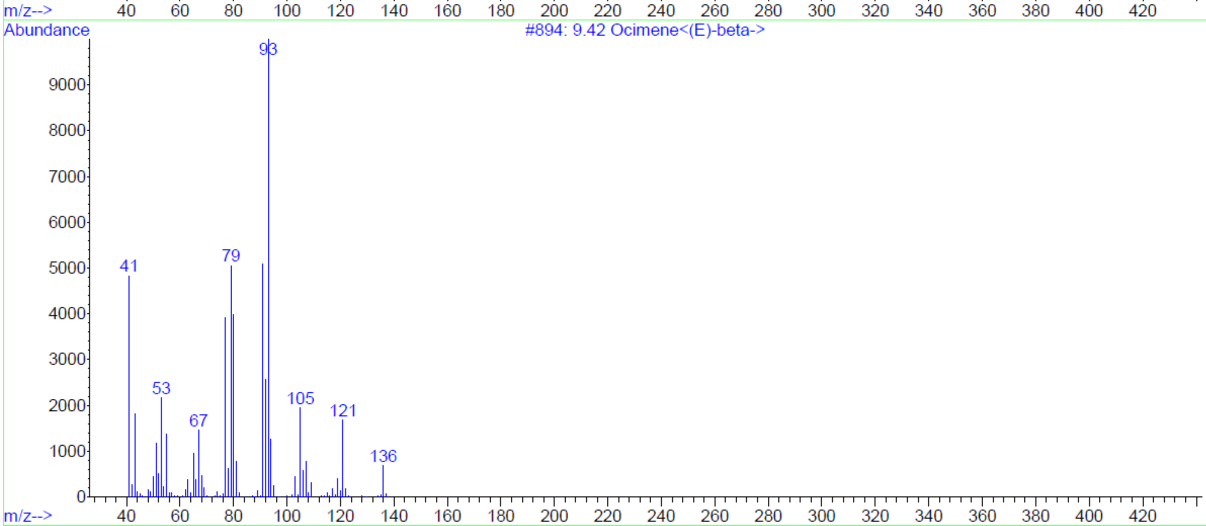
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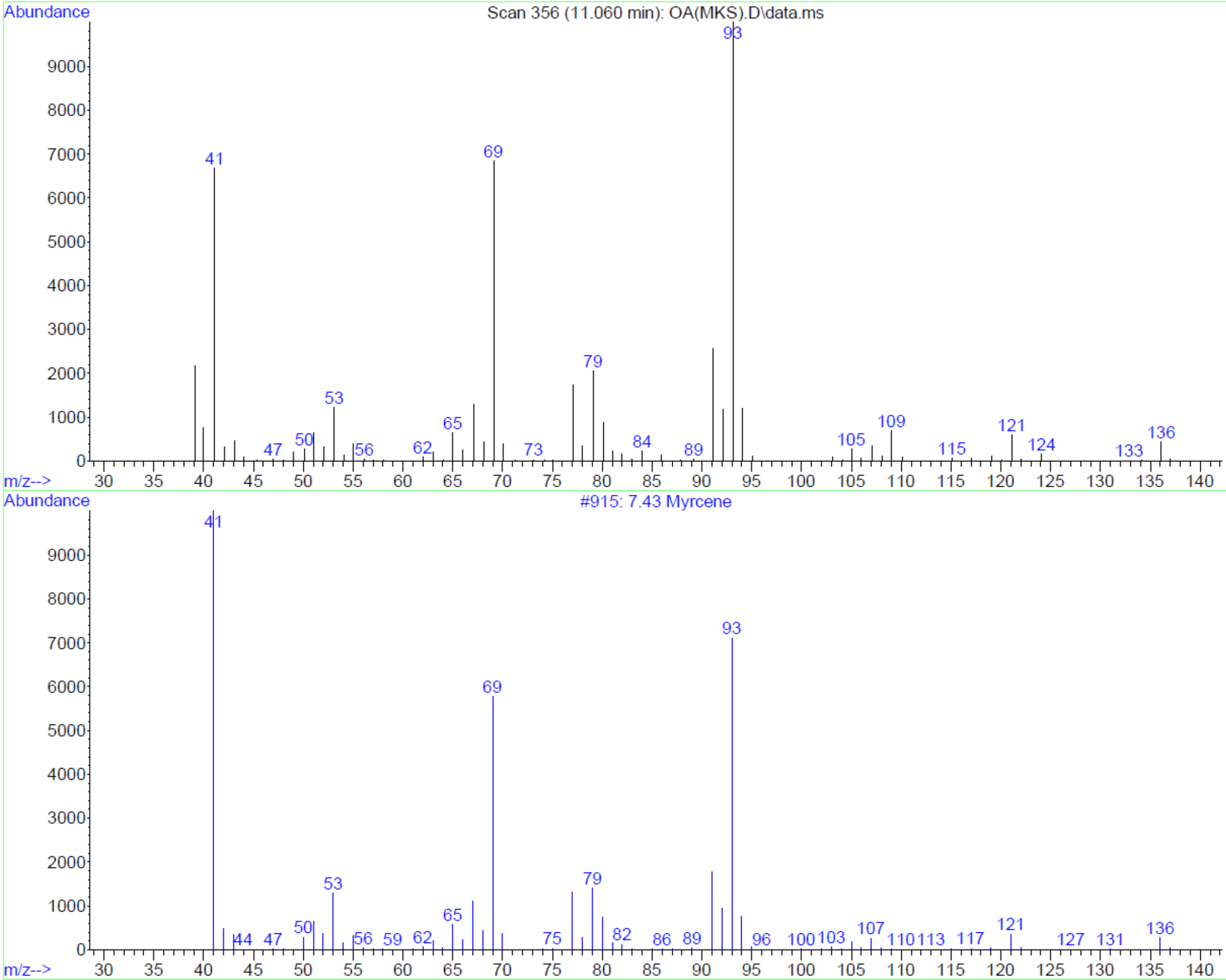

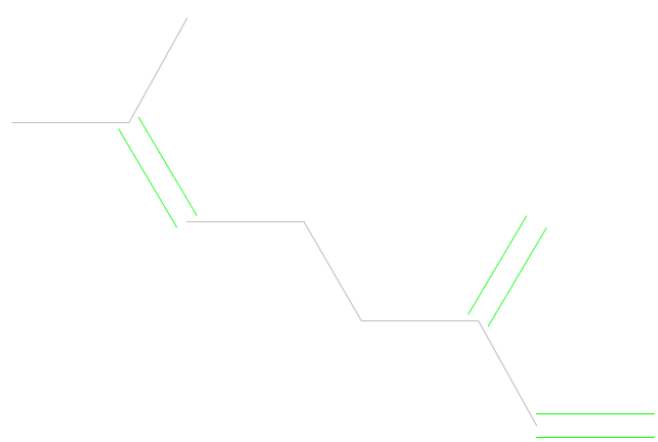

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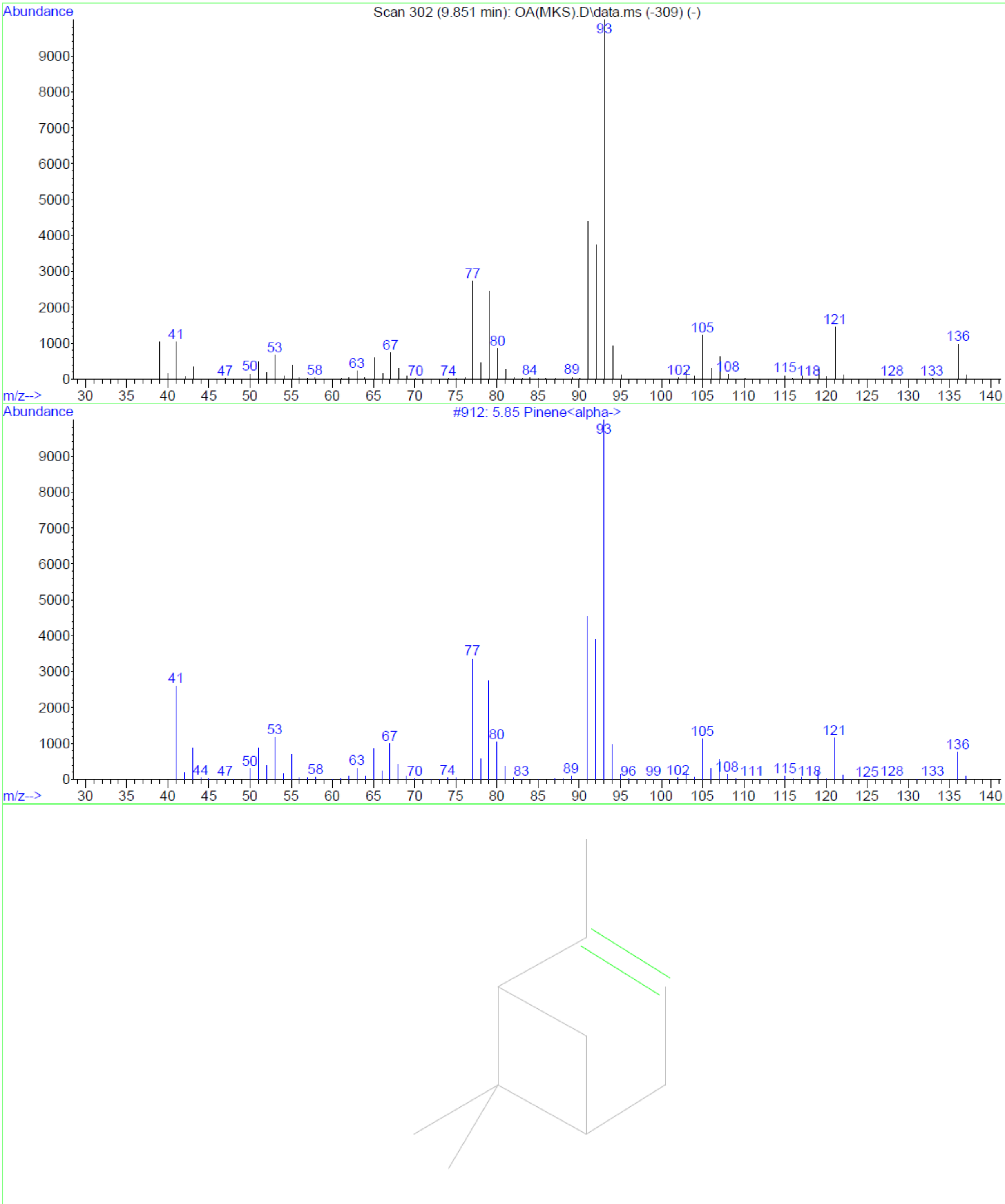

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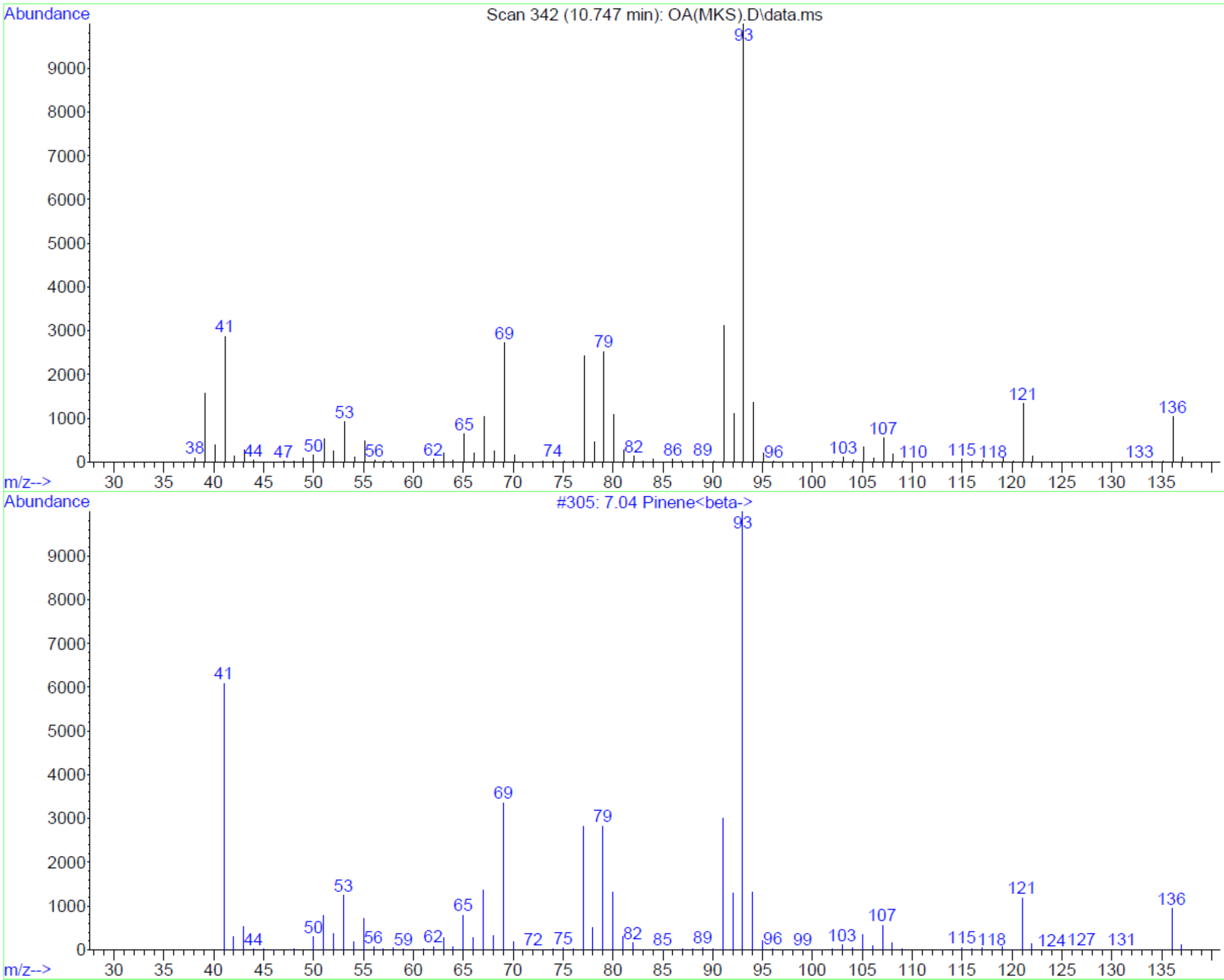

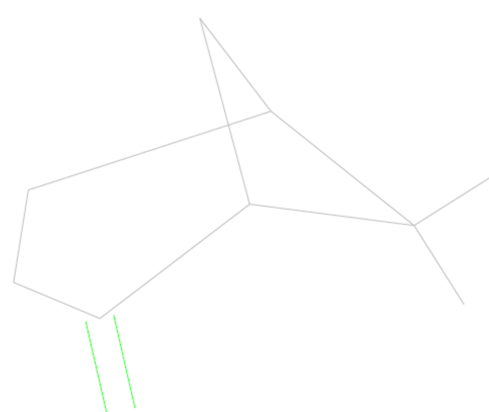

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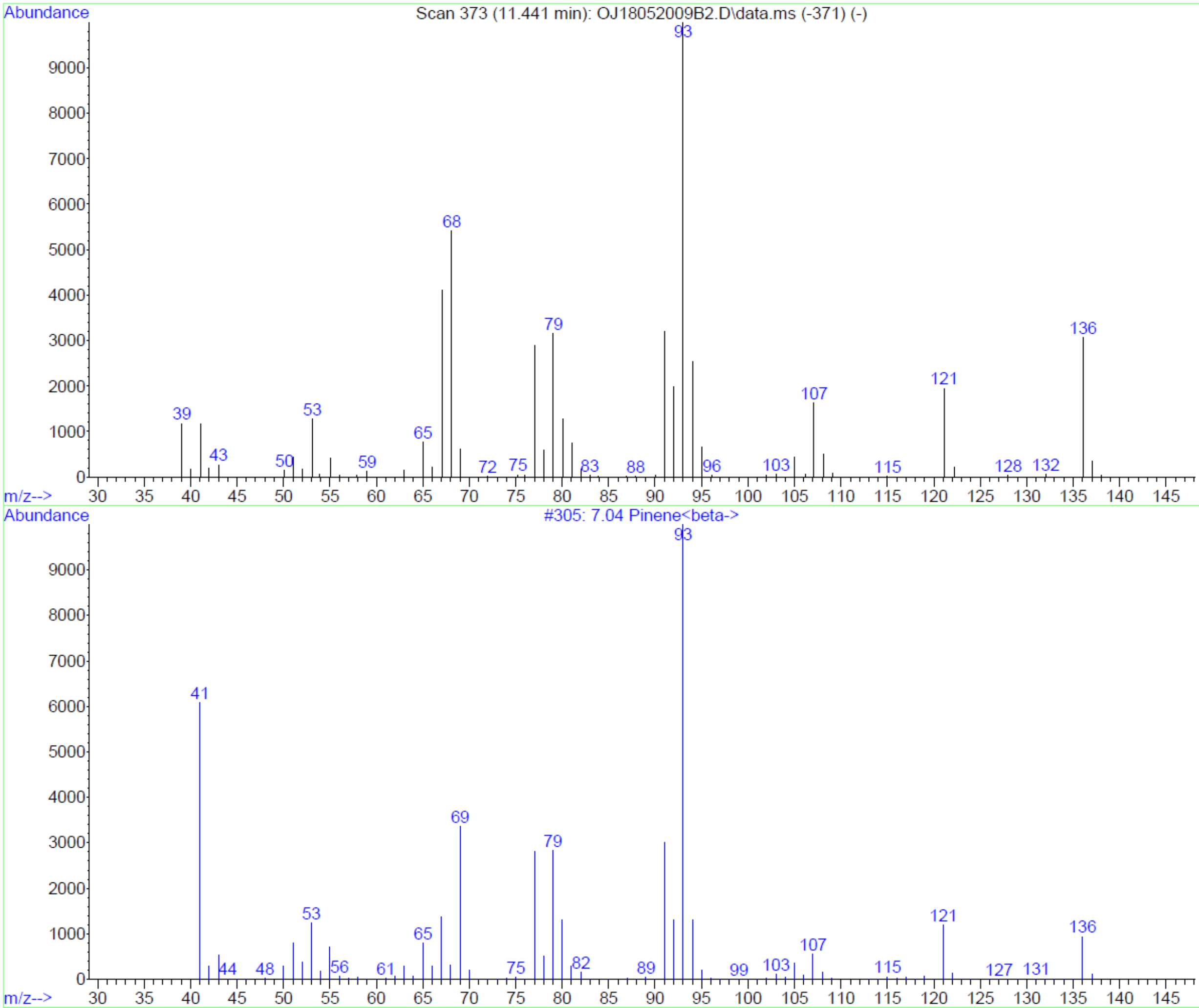

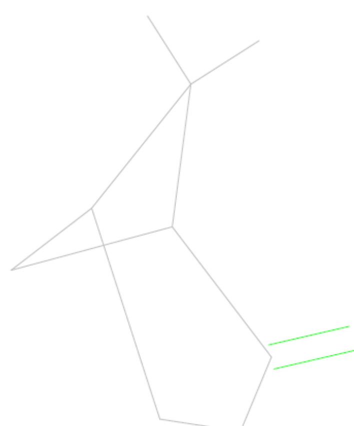

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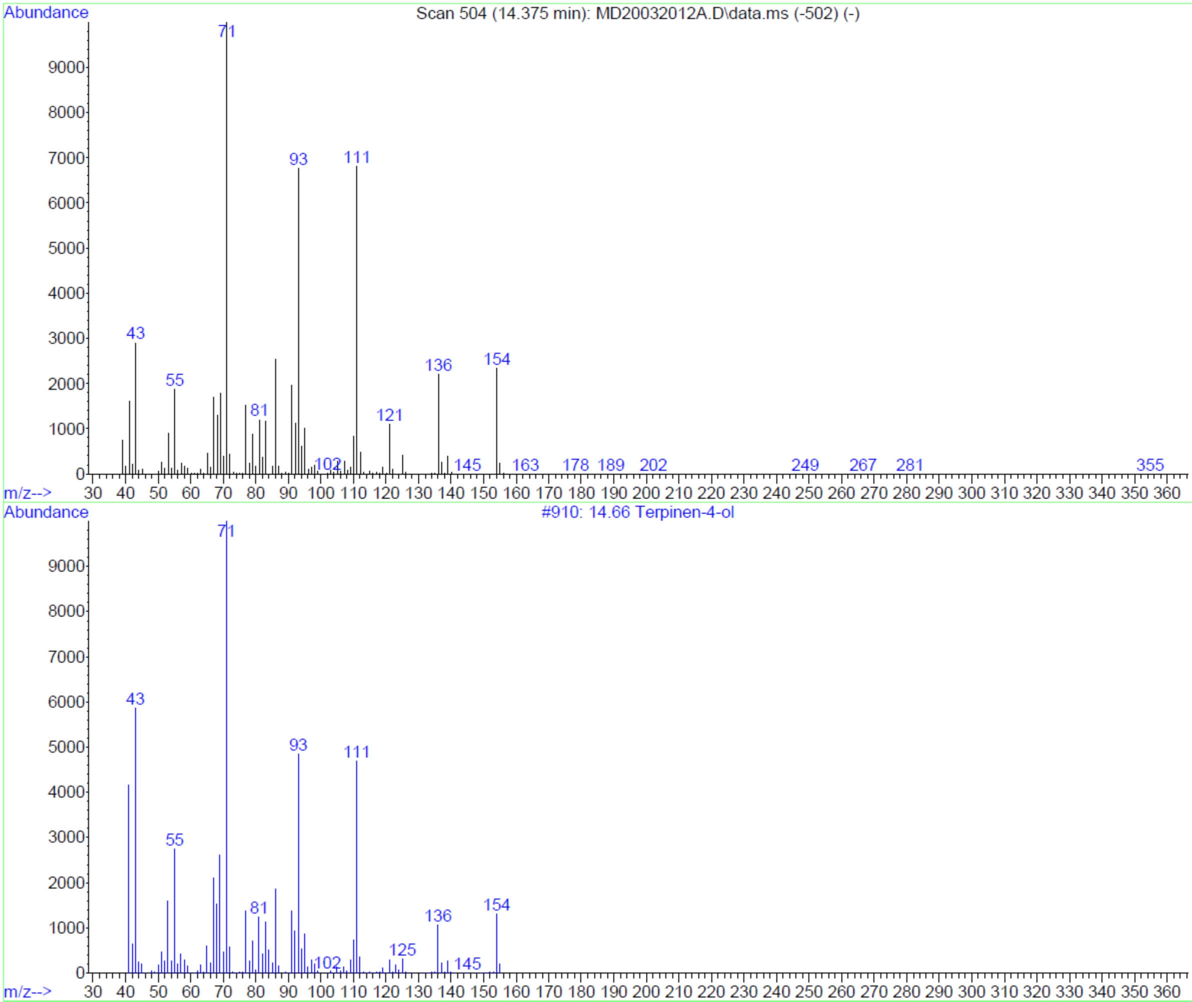

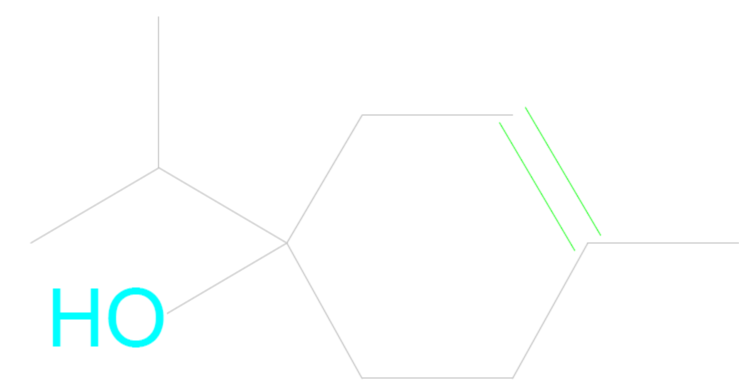

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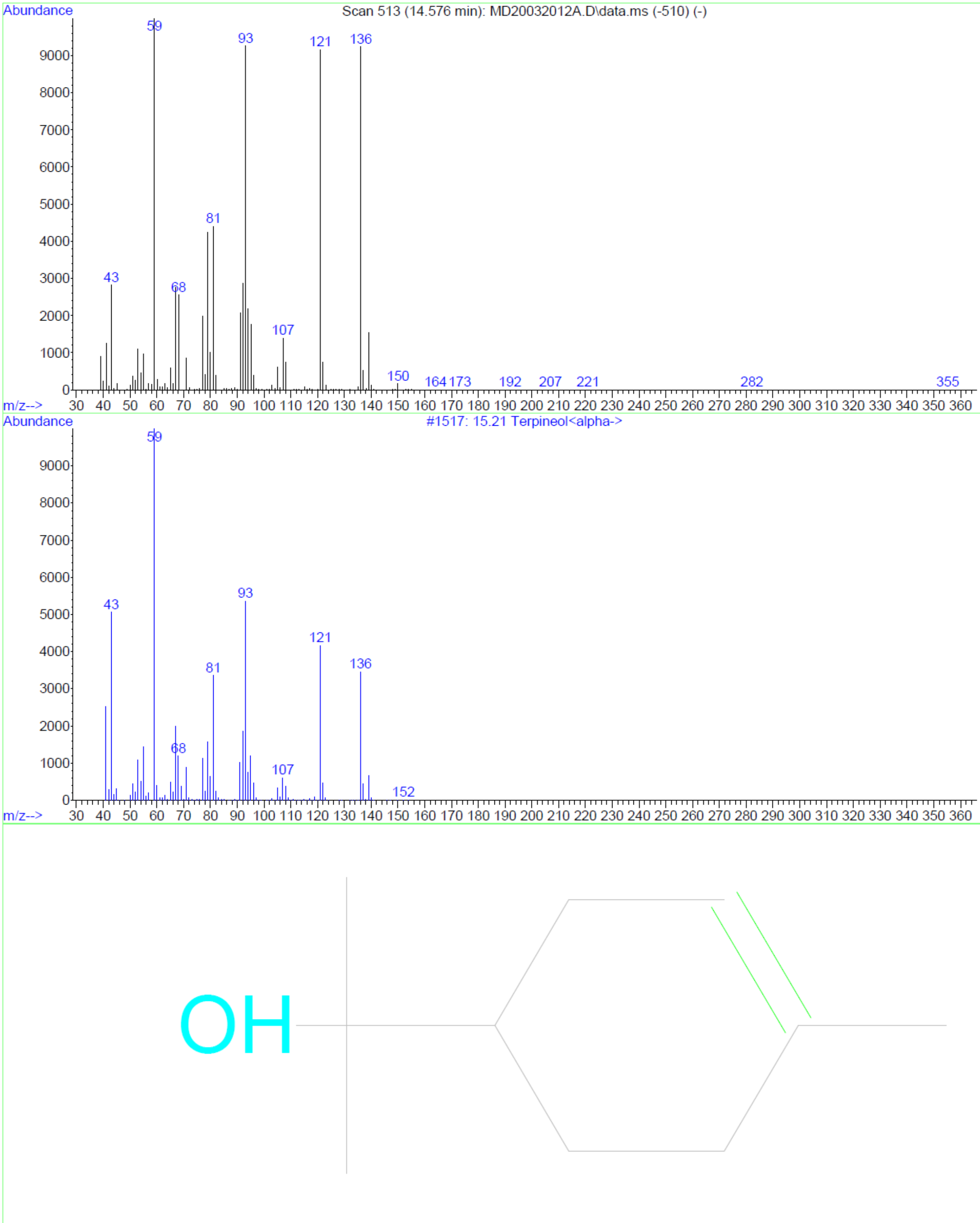

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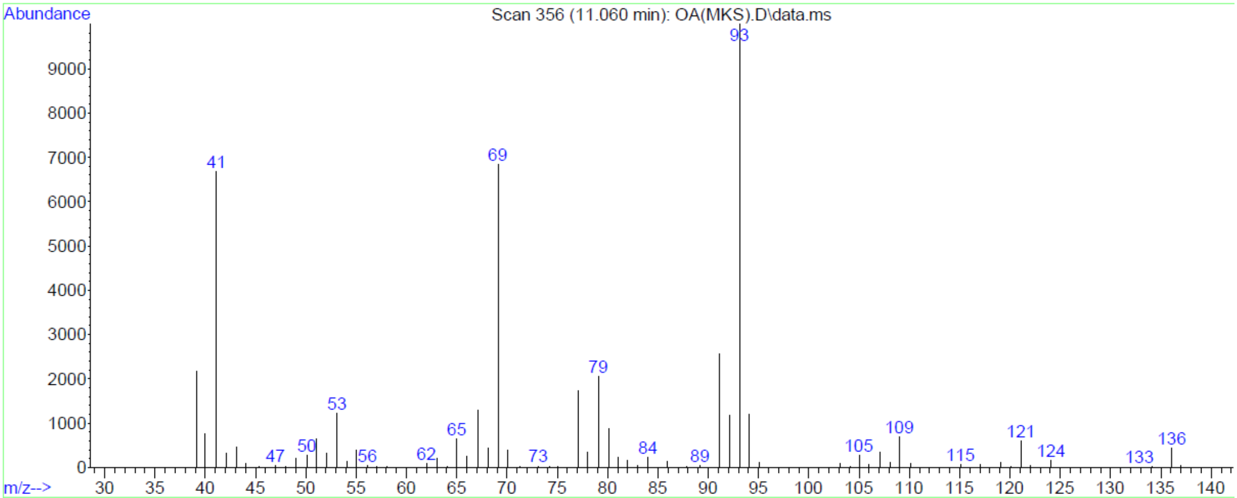

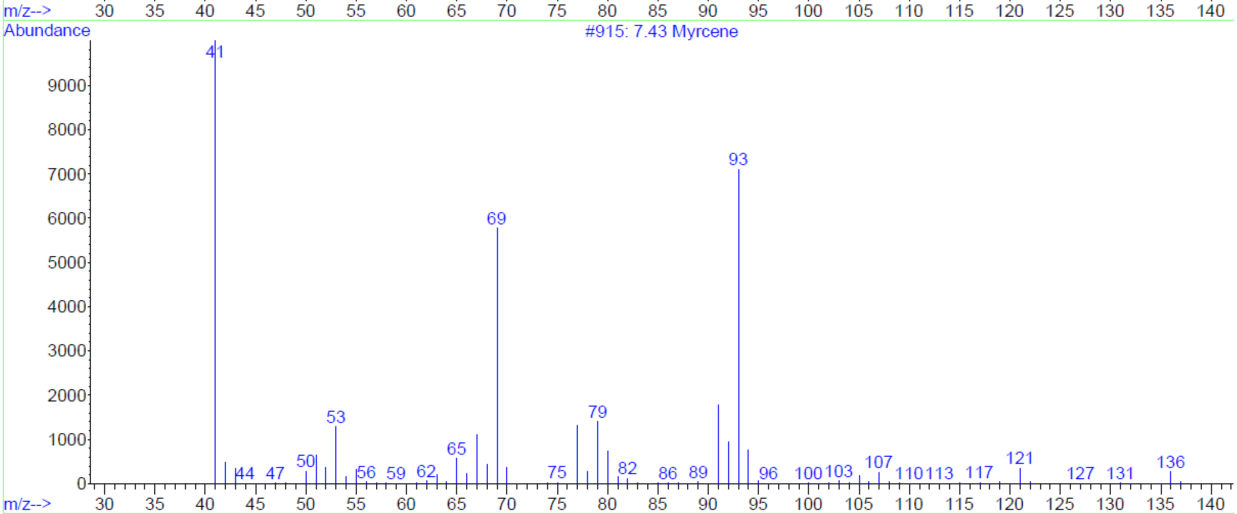

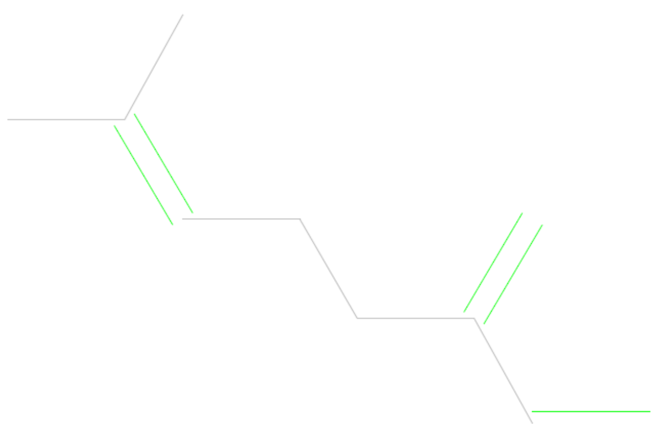

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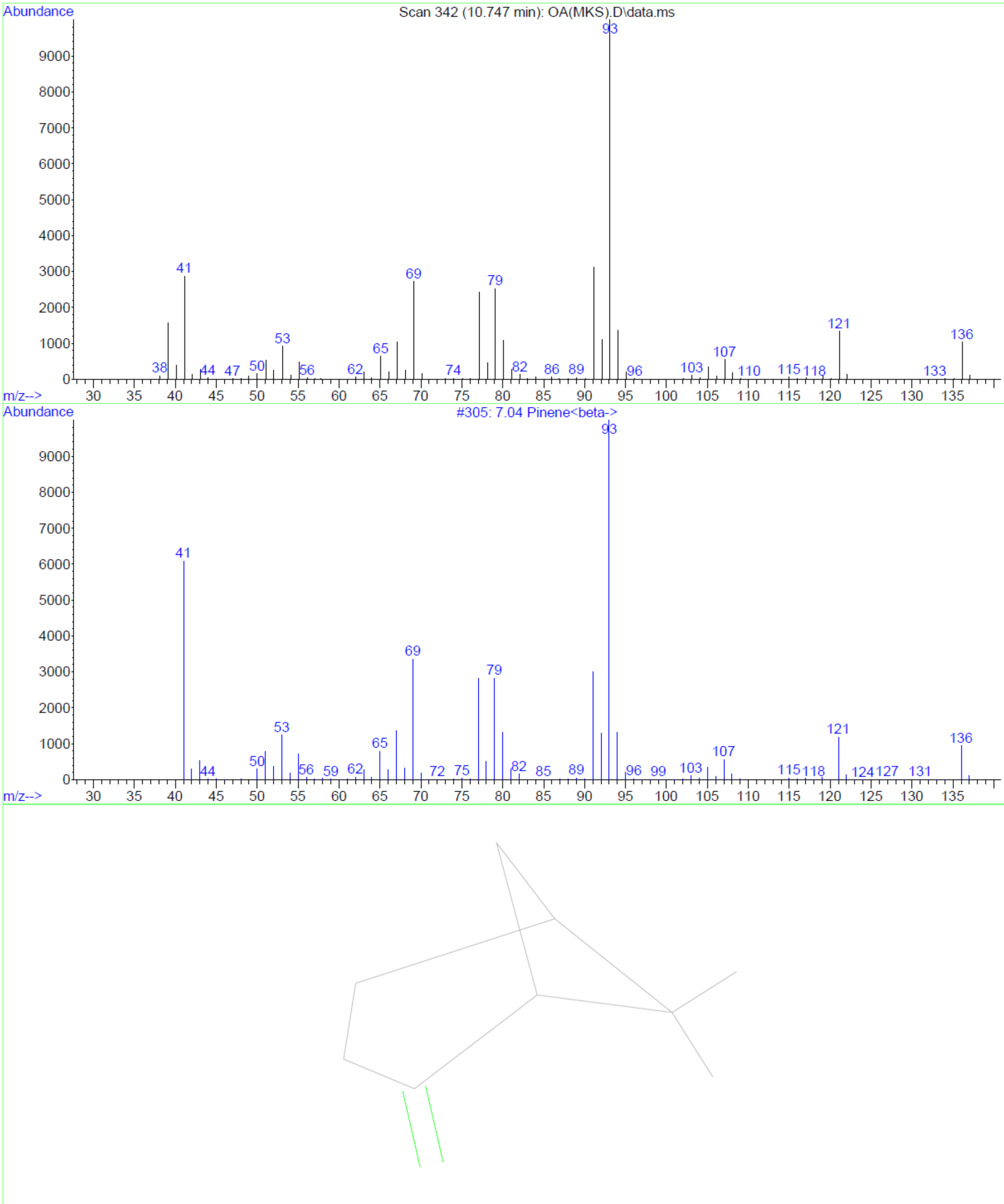

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