Deanship of Graduate Studies

Al-Quds University

Evaluation of Local *Micromeria fruticosa* and Its Essential Oil Effects on Weed Germination

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Evaluation of Local *Micromeria fruticosa* and Its Essential Oil Effects on Weed Germination

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III

Dedication

To my dad's spirit

To my mom, who have always given me power to keep on

To my sisters and brothers, Azeezah, Hussein, Sondos, Tuqa, Shahd, and Shehab Aldein for their support, help, encouragement and patience

To my best friend and cousin Aya

To all my supportive friends during this journey

Asma'

Declaration

Declaration

I certify that the thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not be submitted for a higher degree to any other university or institution.

Signed:.....

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Date: 15/1/2020

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All thanks are express to my mom, sisters, and brothers for their help, support, and encouragement. This work would not have been completed without their unwavering love and support given to me at all times.

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For you all…

Thank you from the deepest of my heart

Asma'

Abstract

Micromeria fruticosa is an evergreen shrub aromatic plant and endemic to the eastern Mediterranean region including Palestine. There was no information found about the yield of the *M. fruticosa* aerial parts and its essential oil (EO). In order to evaluate that, 586 plants were grown at Al-arroub station of the National Agriculture Research Center "NARC" in Hebron. Thereafter, plants were harvested eight times within two years. Then after each harvest, the EO was extracted by steam-distillation from the fresh material. The GC-MS analysis was performed on interval hourly samples took during the EO extraction for the August/2018 and October/2018 harvests. Effect of seasonal and extraction time on the EO profile were evaluated. Also, the effect of the EO on weed germination was determined on soil samples infested by common weed species with three different concentrations (0.05, 0.1. and 0.2%).

The results shown the average aerial parts production of *M. fruticosa* for every plant as fresh weight for the seasons of 2017 and 2018, were 0.20 and 0.22 Kg, respectively. The yearly yield of aerial parts for 2017 and 2018 was 680 and 748 Kg/dunam, respectively. Oil density was 0.9459 ± 0.00184 and percentage of average oil production as fresh matter basis was 0.82 and 0.80% for the season 2017 and 2018, respectively. Moreover, the results appeared that the late summer months (August-October) show the highest percentages of the EO extracted from *M.fruticosa*. A total of 191 compounds were identified in all samples with abundant of the monoterpene pulegone in all with varied proportions. The GC-MC analysis found that the EO got from August and October/2018 harvests reveal high percentages of pulegone (66.22 and 63.75%, respectively), which is the compound that causes inhibition of germination for weeds' seeds, with 2 hours of extraction.

The EO severely affected on the number of germinated weeds. It also observed that the inhibition rate was markedly increased with the increasing of the EO concentration, where the mean of the number of germinated weeds for the concentrations of 0.05, 0.1 and 0.2% was decreased by 24, 27 and 63% respectively compared to the control group $P \leq 0.001$.

The present study results showed that the EO of *M. fruticosa* could be used in biocontrol practices, especially in organic farms, as an anti-germination agent.

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List of abbreviations

Symbol	Abbreviation
M. fruticosa	*Micromeria fruticosa*
EOs	Essential Oils
B.C	Before Christ
MAPs	Medicinal and Aromatic Plants
NARC	National Agricultural Research Center
NAHA	National Association for Holistic Aromatherapy
BHT	Butylated hydroxytoluene
GC-MS	Gas Chromatography/Mass Spectrometry
FW	Fresh Material
DW	Dry Material
DM	Dry Matter
LABS	Linear Alkyl Benzene Sulfonate
SLS	Sodium Lauryl Sulfate
NaOH	Sodium hydroxide
Ppm	part per million

1. CHAPTER ONE:

GENERAL INTRODUCTION

1.1. Introduction

For centuries, essential oils (EOs) and plant extracts have been extensively used by most of the ancient civilizations in folk medicine, aromatherapy, flavors or preservatives in food and beverage industries, perfumes, cosmetics, and even as biological agents (King, 2018; Nieto, 2017; Tongnuanchan & Benjakul, 2014). EOs, which also known as volatile oils or ethereal oils, are plants secondary metabolites consist of a complex mixture of compounds (Dagli, Dagli, Mahmoud, & Baroudi, 2015; Sharma, Haider, Andola, & Purohit, 2011). They can be obtained from different parts of aromatic plants (roots, stems, flower, seeds, buds, fruits, leaves, bark, etc.) (Nieto, 2017).

Globally, weeds considered the most critical and threatening obstacle for agriculture yields, compared to the other pests (insects and pathogens) (Tetteh, Norman, & Amoatey, 2011). Weeds consider as the leading economic obstacles in crop production, where they cause yield losses reached about 45% yearly, affecting on the quality of the final product, reduce the biodiversity of agriculture systems, some of them has allelopathic properties on the origin host plant, and other encourage the growth of other pests (Sanganyado, 2015; Tetteh et al., 2011).

In order to control weed germination, many methods have been improved; the most popular one is chemical herbicides because of their fast action and is less labor-intensive than other weed control methods. Nevertheless, the use of herbicides during production led to several long-term harmful effects on the environment, crops, soil, water, food contamination, human health, and even on products taste (Issa, Sawalha, Sultan, & Yaghmour, 2017; Lubeck, 2018). The consequences on human health representing in herbicides residue in crops and food contaminations, as it is known these chemicals kill or control the weeds, but on the other hand it has a harmful impacts on other non-targets organisms including human even it causes diseases for them such as; cancer, asthma, some reproductive system disorders, cancers, congenital disorders, fertility problems, lung diseases, and hepatotoxicity (Enibtawi, 2017; Keikotlhaile & Spanoghe, 2011).

Through recent years, some feature stories and newspaper reports interested about the effects of commonly used pesticides and herbicides in Palestine on environment and human health, reported that some globally banned pesticides, which proven its connected with many diseases including cancers and its accumulation in the final crops, are still using in

Palestinian territories, because of this it is hard for the Palestinians crops to export especially to the European Union. One of the most dangerous chemicals still using by Palestinians farmers is glyphosate, it is the common active ingredient in most synthetic herbicides, which also are internationally banned where researches approved its consequences on human health by causing cancerous tumors (Abu shanab, Sawalha, Al-khalili, & Dagher, 2016; Altaweel, 2016; En'erat, 2007; Harb, Shoaibi, & Qadous, 2016).

The limited agriculture information for most of the Palestinian farmers about the correct practices of using pesticides and herbicides with mostly no help from the agricultural extension workers is another serious problem, causing many harmful effects on the final products because of the misusing of pesticides thereby affecting on the consumers' health. Also, there is no routine examination and supervising for the pesticide's accumulation in the crops by the Ministry of Health (Abu shanab et al., 2016; Altaweel, 2016).

However, since the early of the previous century, the world is moving toward organic farming, which depending on agriculture practices have no harm to the environment and human health. Wherefore, find a natural alternative to the chemical pesticides is the best solution for this new agriculture system (Harb et al., 2016). So many scientific works conducted on using economic options like EOs in order to use it as a natural herbicide and pesticide.

Inhibition of the germination and growth of some plants including weeds by inhibitory substances releases by other plants in their vicinity resulted from chemical interactions between them, which can cause enhancement or inhibition of growth, has been known as allelopathy (Dudai et al., 1993). There have been some studies examining the potentiality of EOs for managing weeds and work as a bio-herbicide (Atak, Mavi, & Uremis, 2016; Ramezani, Saharkhiz, Ramezani, & Mohammad Hossein, 2008; Tworkoski, 2002). Many other kinds of research confirmed their effectivity against pests; insects, microbial, and bacterial pathogen (Sharma et al., 2011).

Most of the ancient civilizations have arisen on the east coast of the Mediterranean Sea have shown an interested in plants folk therapy over the years. Also, aromatic plants have been used in traditional remedies for treat abdominal pains, diarrhea, eye infections, heart illness, hypertension, exhaustion, stress, colds and open injuries (Al-Hamwi et al., 2011; Salameh, 2018; Telci & Ceylan, 2007). On the other hand, the oils and the solutions extract

from these plants introduce in medicines industry; where the active ingredients can work as anti-microbial, anti-oxidant, anti-cancer, anti-rheumatic, anesthetic, sedatives, and antiseptic (Abu-Gharbieh & Ahmed, 2012; Nieto, 2017; Tongnuanchan & Benjakul, 2014).

In searching for economic plants to be used as an a natural herbicide, *M. fruticosa* which is an endemic evergreen shrub grown mainly in wild regions in eastern Mediterranean countries including Palestine (Ali-shtayeh et al., 2008; Dudai, Larkov, Ravid, Putievsky, & Lewinsohn, 2001). It is known as Qurnya, Ishbit esh-shai, Duqat 'Adas, Zie'ttman, and Zaa'tar balat in Arabic, these common names are using in the Levant region, where the English name is Thyme-leaved savory (Abu-rabia, 2012; Abu-Reidah, Arráez-Román, Al-Nuri, Warad, & Segura Carretero, 2018). It has been used in traditional therapy for many years, where it shows medicinal value against heart diseases, open wounds, cold, abdominal pain, headache, hypertension (Putievsky et al., 1995; Salameh, Shraim, & Jaradat, 2018). Also, it shows biological activities, where it works as anti-microbial, anti-fungal, anti-bacterial, and anti-oxidants (Telci & Ceylan, 2007).

For the Palestinian community, *M. fruticosa* which is usually grown in the wild habitat in the mountains between clefts and chalky rocks, considered as one of the aromatic plants that could have economic importance (Abu-Reidah et al., 2018). For Palestinians people, it is generally consumed as herbal tea, food flavor agents, helps to calm the nerves, and for traditional medicinal purposes in colds, relieve abdominal, stomach and bowel pains. Furthermore, the boiled leaves are using to cure wounds, eye and skin infections, cough, heart, and respiratory system disorders (Abu-rabia, 2012; Ali-shtayeh et al., 2008; Salameh, 2018).

It has been found that the effective material in the plant is the EO which extracted from leaves and flowers (Kırımer, Ozek, Baser, & Harmandar, 1993; Kırımer, Tümen, Ozek, & Baser, 1993; Telci & Ceylan, 2007). The concentration and composition of the oil are varied due to some factors such as; climate, environmental factors, subspecies, genetic factors, seasons, vegetative stage, cultivation conditions and stress on the plant (Al-Hamwi et al., 2011; Mehalaine & Chenchouni, 2018; Telci & Ceylan, 2007).

Many worthy types of research interests in the EO of *M. fruticosa* and studied it in many aspects. As a result of recent studies, it has been shown that the extract and the EO of *M. fruticosa* has biological activities such as; analgesic and gastroprotective activities, anti-

lipase, anti-amylase, anti-oxidant, anti-tumor, anti-inflammatory, and anti-microbial (Abu-Gharbieh & Ahmed, 2012; Abu-Gharbieh, Ahmed, & Ahmed Khan, 2013; Al-Hamwi et al., 2015; Salameh, 2018). Other studied focused on the chemical composition and the concentrations of main constituents of the EO and how it influenced by some factors including the extracted parts, environmental and genetic factors, season, subspecies, climate, and location (Al-Hamwi et al., 2011; Putievsky et al., 1995; Salameh et al., 2018; Telci & Ceylan, 2007) to choose the oil with best chemical profile suitable for the concerned purpose.

Also, there have been few studies examining the possibility of the EO of *M. fruticosa* for inhibited germination and work as bio-herbicide mostly conducted in Israel. These studies refer the inhibition activities to the dominated compound in the oil, Pulegone (Dudai, Ben-Ami, Chaimovich, & Chaimovitsh, 2004; Dudai et al., 2001; Dudai et al., 1993; Dudai, Poljakoff-Mayber, Mayer, Putievsky, & Lerner, 1999).

It is known that Palestine generally and Hebron in particular, suffering from water crisis and varied rainfall amount from winter to another (Harb et al., 2016). So, it should keep in mind all of these obstacles when decided to start cultivation a wild species in order to use its EO as a natural product, as *M. fruticosa* in this case. Fortunately, with the potentiality to resist drought climate and no need for costly horticulture practices and agriculture caring products (pesticides and fertilizers) *M. fruticosa* can cultivate and grow up.

For our knowledge, there is no published research discussed the yield of the plant, the yield of the EO and the variation in the EO chemical profile, regarding the season of harvest or the time of the extraction, and the possibility of using this plant as a commercial crop in Palestine. Moreover, there is no published research discussed the inhibition potentiality of *M. fruticosa* EO against the common weed species with different concentrations and in different seasons and using it as a natural anti-germination agent in Palestine.

In the present study, we report on the possibility to use *M. fruticosa* as a commercial crop, assess for its yearly aerial parts and EO yield, seasonal and extraction duration affecting on the oil concentration and the chemical composition of it. Finally, it examines the activity of the EO as a germination inhibitor of some common weed species (plumed cockscomb, squirting cucumber, wild oat, cheeseweed mallow, and poaceae) in Palestine, and their possible use as a natural anti-germination agent.

1.2. Objectives

The major purposes of this study are investigating the potentiality of using the EO of *M. fruticosa* growing in Palestine as natural germination inhibitor (bio-herbicide) with effective cost. However, in order to achieve our objectives, several methods were subjected to this study. The minor goals of this study were:

- To evaluate the yield of *M. fruticosa* aerial parts.
- To evaluate the EO production from the fresh matter.
- To determine the time of harvest and the elongation of steam distillation extraction with the highest percentage of the EO and pulegone by comparing the chemical profile using the GC-MS.
- To examine the effect of the EO of *M. fruticosa* with different concentrations on weed seeds germination.

2. CHAPTER TWO:

LITERATURE REVIEW

2.1. Essential oils

2.1.1. What are the EOs?

Essential oils are highly volatile compounds obtained mainly from aromatic plants, defined as having a strongly odorous component (Tongnuanchan & Benjakul, 2014). EOs can be found only in 10% of the plant kingdom (Jilani & Dicko, 2012). However, the amount of EOs existed in aromatic plants is about 0.01% to 10%, most of them ranging between 1 - 2% (Sharma et al., 2011).

Essential oils are classified as secondary metabolites in aromatic plants (Al-Hamwi et al., 2015; Sutili, Gatlin, Heinzmann, & Baldisserotto, 2017) and characterized as hydrophobic solvents, so they are soluble in lipids/organic solvents, but poorly or non-soluble in water because most of them have lower density than water (Bakkali, Averbeck, Averbeck, & Idaomar, 2008).

The chemical compounds found in EOs fall into two major groups, the first are terpenes derived compounds which consist of hydrocarbon terpenes (monoterpenes and sesquiterpenes) and terpenoids, where the other group contains the oxygenated compounds, including alcohols, ethers, phenols, aldehydes, ketones, acids, and esters (Dagli et al., 2015; Nieto, 2017; Sutili et al., 2017; Tongnuanchan & Benjakul, 2014).

EOs can found in oil glands in many parts of plants such as roots, stems, flower, seeds, buds, fruits, leaves, and bark (Kar, Gupta, & Gupta, 2018; Nieto, 2017). In order to obtain these etheric organic compounds, many methods have been developed (Surburg & Panten, 2006). The most common and effective methods are hydro and steam distillation especially for commercial production (Tongnuanchan & Benjakul, 2014).

There are around 3000 EOs are known and discovered (Nieto, 2017), but only about 300 compounds are commercially available and used by fragrance, pharmaceutical, and food flavor industrials (Kar et al., 2018; Thosar, Basak, Bahadure, & Rajurkar, 2013; Wei & Shibamoto, 2010).

2.1.2. Importance of the EOs for the Plants:

Mainly, plants produce EOs for two reasons: defense and communication. In nature, they play the primary role in the pollination process by attracting pollinators by their aroma (Kar et al., 2018). On the other hand, EOs work as allelopathic agents in some cases, where plants release chemicals to deter other competing vegetation in its zone from growing (Dudai et al., 1999).

On the protection side, EOs have significant effects on plants where they work as defense compounds against herbivores and other harmful insects by reducing their appetite (Blowman, Magalhães, Lemos, Cabral, & Pires, 2018; Sharma et al., 2011). These essence aromatic compounds are working as a chemical defense for the plant, helping it to prevent infections by pathogens caused by fungi, bacteria or viruses, and this can be considered the real function of EOs (Sachin, Bhalerao, Patil, & Desai, 2016; Sharma et al., 2011).

2.1.3. Uses and benefits of the EOs:

Humankind has been known EOs by ancient Egyptians, where they utilized them before 4000 B.C in traditional medicine and perfumes. (Jilani & Dicko, 2012). Then, other ancient cultures such as; Chinese, Indian, Romanian, Greek, and Mesopotamia used the EOs for their therapeutic values in order to improve the health and mood for the patient (King, 2018).

There are many uses and benefits for EOs. Generally, they are used in traditional medicine, in the treatment of inflammation, abdominal pains, eyes and skin infections and bronchitis (Nieto, 2017; Telci & Ceylan, 2007). Furthermore, they are used in agricultural industries, dental products, the tobacco industry, pharmaceuticals, cosmetics, perfumes and finally in food and beverage industries as natural additives or preservations (Kar et al., 2018; Sachin et al., 2016).

However, in recent years science becomes more interested in studying the biological activities of these EOs. Including using them as an anti-oxidant, anti-cancer, anti-viral and anti-microbial which includs anti-bacterial and anti-fungal (Cai & gu, 2016; Loizzo et al., 2008; Nieto, 2017; Sachin et al., 2016; Silva & Junior, 2010; Tongnuanchan & Benjakul, 2014).

Some studies reported that EOs extracted from different species of aromatic plants have a natural anti-oxidant activity such as; tea tree (*Melaleuca alternifolia*), Guggul (*Commiphora wightii*), oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), clove (*Eugenia caryophyllata*) leaf and basil (*Ocimum basilicum*) (Caldefie-Chezet et al., 2004; Ljiljana, Jelena, Dragan, & Dušica, 2016; Siddiqui, Thomas, & Prasad, 2013; Wei & Shibamoto, 2010).

In the last few years, some published researches proved that EOs extract from several MAPs acts as an anti-cancer agent versus liver, colon, brain, leukemia, oral, breast, lung, and prostate cancerous cells (Blowman et al., 2018; Nieto, 2017; Yousefzadeh et al., 2014).

2.1.3.1. EOs in aromatherapy:

According to National Association for Holistic Aromatherapy (NAHA), aromatherapy or essential oil therapy, defined as "the art and science of utilizing naturally extracted aromatic essences from plants to balance, harmonize and promote the health of body, mind, and spirit. It seeks to unify physiological, psychological and spiritual processes to enhance an individual's innate healing process" (NAHA, 2016).

However, the term "Aromatherapy" emerges in 1937 by the French chemist and perfumer Rene- Maurice Gattefosse, who was named the father of aromatherapy, in his book "Gattefosse's Aromatherapy" (Sachin et al., 2016). Aromatherapy gained broader attention in the United States during the 1980s, and day by day the popularity and applying fragrant oils for therapy purposes is rising and expanding (King, 2018).

Two main theories explain the way that EOs act among human bodies. First theory talking about that the EOs absorbed out of the skin into body cells and tissues then working on as anti-inflammatory, antiseptic, antioxidant, antimicrobe and so on, while the other opinion supposes that the smell nerve motivates by EOs and send signs to the specific part of the brain, which in its turn release chemicals leading to relaxations and quietness (Jilani & Dicko, 2012).

2.2. Herbicides

2.2.1. Weeds and its harmful effects on the productivity of plants:

Generally, weeds defined as any undesired wild plant which emerges around cultivated plants in filed or garden so prevent it from growing correctly (Lubeck, 2018).

Globally, weeds regarded as the most threatening and dangerous obstacle for agriculture yields, compared to the other pests (insects and pathogens) (Tetteh et al., 2011; Tworkoski, 2002). Weeds consider as the main economic issue in the crop production, where they cause yield losses reached about 45% annually, affecting on the quality of the final product, reduce the biodiversity of agriculture systems, some of them has allelopathic properties on the origin host plant, and other encourage the growth of other pests (Sanganyado, 2015; Tetteh et al., 2011).

Over the years, many methods have been developed to fight weeds seed germination (Lubeck, 2018; Telkar et al., 2015). Conventional herbicides, which utilize agrochemicals are the most common and commercial way (Telkar et al., 2015). However, nowadays, the world moving towards the green agriculture, so depending on natural biological agents to control weeds is the most effective, sustainable and eco-friendly method (Cai & gu, 2016; Telkar et al., 2015).

2.2.2. Consequences of the synthetic (chemicals) pesticides and herbicides:

Chemical herbicides, the popular way to fight weed growth, depends on using strong toxically chemicals to stop weeds spread (Telkar et al., 2015). However, there are many defects in this way; the most critical one is its harmful effects on human health and the environment (Lubeck, 2018). Contaminated crops, soil, and water by artificial pesticides and herbicides residues consider another serious problem (Mossa, 2016; Sharma et al., 2011).

In compared to the animal's tissue, plants considering more delicate and prone to biotic and abiotic infections, so using pesticides are necessary in this case to manage diseases. However, these chemical pesticides listed as the major causes diminished productivity and quality for the product (Dhaliwal & Sharma, 2016).

Since the 70s of last century, many studies discussed the correlation between the agrochemical pesticides and various health effects and diseases (Biswas, Rahman, Kobir, Ferdous, & Banu, 2014). Some studies proved that many commercial types of herbicides including Glyphosate, one of the most synthetic weed killer using all over the world, cause dangerous diseases such as; several types of cancers, some respiratory issues, hypertension, diabetes, Parkinson's and Alzheimer's diseases, and even reproductive problems (Biswas et al., 2014; Nicolopoulou-Stamati, Maipas, Kotampasi, Stamatis, & Hens, 2016; Owens, Feldman, & John, 2010).

In Palestine, more than half of agriculture lands are depending on synthetic pesticides to manage pests and growth of weeds (Issa et al., 2017). The annual rate for use pesticides in the West Bank and Gaza Strip have estimated about 502.7 tones, consist in 123 types, and 160 types of pesticides with 893.3 tone, respectively (PCBS, 2010). More than 11% form each internationally banned due to health reasons (PCBS, 2010). These banned pesticides cause damage to the soil, water, environment, and animal and human health (Issa et al., 2017).

2.2.3. Natural alternatives to chemical pesticides and herbicides:

It is well known the adverse side effect of chemical pesticides on human health, environment and even on products taste. Wherefore, find a natural alternative is the best solution. So many scientific works conducted on using economic options like EOs in order to use them as herbicides and pesticides.

Bio-herbicides (bio-pesticides) or green- herbicides refer to a safe, eco-friendly weeds control method depends on using natural or living organisms; such as pathogens, natural products, and EOs extract from plants (Cai & gu, 2016; Sharma et al., 2011; Telkar et al., 2015).

Bio-herbicides mostly use in organically sustainable horticulture as an alternative to the dangerous chemical herbicides (Ramezani et al., 2008). Despite the cure effects of EOs, it has toxic effects on several harmful pests so it can be used as biopesticides (Mossa, 2016; Sachin et al., 2016).

In the last two decades, many studies confirmed that using EOs as an insecticide and herbicide considered a new effective method to fight and control pests and weeds (Mossa, 2016; Sachin et al., 2016). Due to the lipophilic nature of EOs, so it is easy to pass into the insect's body and then leading to biochemical dysfunction and thereby to death (Mossa, 2016).

2.2.3.1. EOs as bio-herbicides, bio-pesticides, and germination inhibitor:

Allelopathy is a phenomenon where some plants release natural chemical compounds, which can work as an anti-germination or anti-growth for other plants growing in their nearby (Dudai et al., 1993; Ramezani et al., 2008). Throughout the years, its proved that some EOs have allelopathic properties (Dudai et al., 1993).

There have been numerous studies examining the potential of EOs for controlling weeds and work as a natural herbicide (Tworkoski, 2002). On the other hand, some other pieces of researches showed their effectivity against pests; insects, microbial and bacterial pathogens (Sharma et al., 2011).

Tworkoski (2002) authenticated that the EO extracted from cinnamon, with eugenol as the active and dominated ingredient with percentage up to 84%, had a high level of herbicidal activities against some weed types, which are johnsongrass, Common Lambsquarters, and Common Ragweed. Moreover, the other examined EOs which took from clove, red thyme, and summer savory had herbicidal properties, but with less efficiency.

The EOs obtained from four plants (*Eucalyptus nicholii, Rosmarinus officinalis* L., *Chamaecyparis lowsoniana, and Thuja occidentalis*) were examined and applied as a natural herbicide against three different species of weeds affecting badly on vegetable productivity in Iran. The paper discussed the inhibition properties for the four EOs with various concentrations on germination percentage of the examined weed's species. It is founded that the EOs extracted from *Eucalyptus nicholii* with concertation of 300ppm possess the most efficient allelopathic properties (Ramezani et al., 2008).

However, EOs that can be used as herbicide should be economical. Also, plants used should be available and has good economic production. Regarding the literature, Palestine has a wide variety of plants can be used as an herbicide; one of those plants is the *M. fruticosa*.

2.3. *Micromeria fruticosa* and its EO

2.3.1. *Micromeria fruticosa*:

Micromeria fruticosa (L.) Druce (Lamiaceae) is a perennial dwarf shrub grows and endemic in the eastern Mediterranean region including Palestine (Telci & Ceylan, 2007). Like most of the aromatic plants, *M. fruticosa* widely has been used in traditional herbal therapy (Abu-Gharbieh et al., 2013).

In folk medicine, *M. fruticosa* has been used since ancient time. This evergreen aromatic plant helps in wounds heal, headache relief, reduce abdominal pain, treat heart diseases, reduce eyes and skin infections and relieve diarrhea (Dudai et al., 2001; Salameh et al., 2018; Telci & Ceylan, 2007).

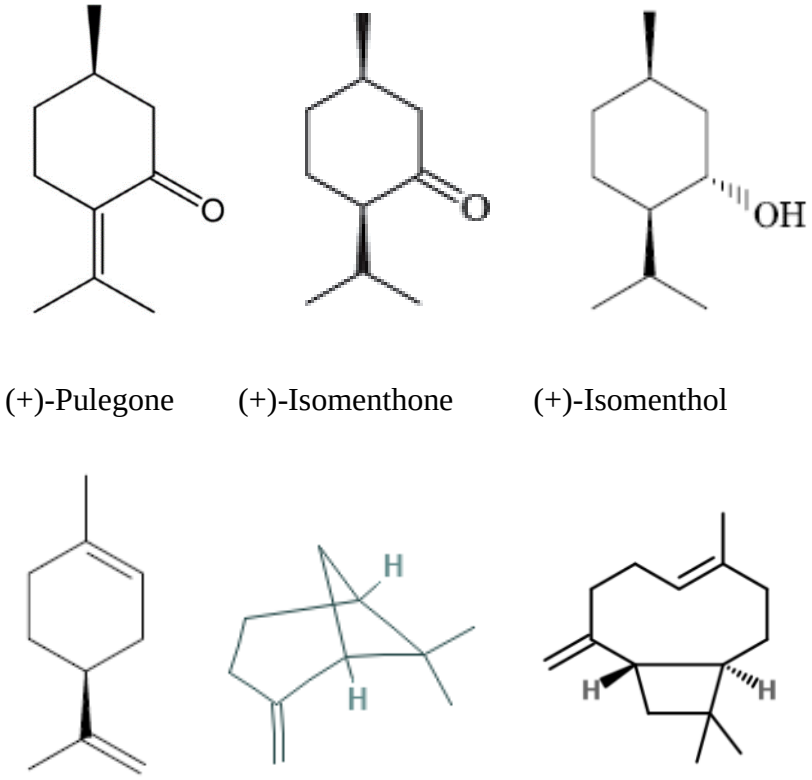
For Palestinian society, *M. fruticosa* is using in herbal tea and add to the food as a fragrance and flavor agent. *M. fruticosa* considered as one of the most vital MAPs in the Palestinian traditional remedies and culture, many people are boiling its leaves and flowers with water to make a syrup used as a treatment for abdominal, stomach, and bowels pains. Moreover, the boiled leaves are using to cure wounds, eye and skin infections, cough, heart, and respiratory system disorders (Ali-shtayeh et al., 2008; Salameh, 2018).

2.3.2. The EO of *M. fruticosa* and the pulegone:

There are more than 20 chemical compounds exist in the *M. fruticosa* EO, pulegone considers as the main and most abundant one with a percentage of up to 80% in some cases (Dudai et al., 2001; Dudai et al., 1993). These compounds present in different organs (flowers, stalk, and leaves, …etc.) with different percentages depending on the organ and the season of harvest (Dudai et al., 1993).

The main chemical compounds exist in the EO of *M. fruticosa* mostly belong to the terpenes group. Some of them are monoterpenes such as; pulegone, isomenthol, isomenthone, D-limonene, menthol, alpha-pinene, beta-pinene, piperitone, piperitenone oxide. Where others are having sesquiterpenes structure like beta-caryophyllene and germacrene D (Dudai et al.,

2001). Figure (2.1), presents the chemical structure for the main violate compounds in the EO of *M. fruticosa*.



(+)-Pulegone (+)-Isomenthone (+)-Isomenthol

D-Limonene beta-Pinene beta-Caryophyllene

Figure 2.1: Chemical structure of the main components in the EO of *M. fruticosa* (Dudai et al., 2001; Salameh, 2018)

Pulegone is a monoterpene ketone in which chemical structures is classified as a potent vigorous inhibitor for the seed germination (allelopathic agent), so it is suggested to be used as a natural herbicide (Dudai et al., 2004; Dudai et al., 1993).

Pulegone is a chemical compound exists in the EOs of some aromatic plants including *M. fruticosa*. It has an oily nature with no color. On the room temperature, it is in the liquid state. Its boiling point is high reaches 224°C. Molar mass is 153.23 g/mol, and its density is 0.9346 g/ml. Also, it is entering in some foods or drinks flavors and dental products (NTP, 2011).

2.3.3. Biological activities for the *M. fruticosa* EO and extracts:

A study conducted by the Faculty of Pharmacy; Beirut Arab University was testing the biological activities (anti-oxidant and anti-microbial) of the ethanolic extract of *M. fruticosa*. The results showed that the ethanolic extract of *M. fruticosa* has an anti-oxidant property mostly due to the presence of phenolic acids and flavonoids. It has been proved that it is more powerful than the commercial anti-oxidant BHT (Al-Hamwi et al., 2015).

At the same study, an anti-microbial activity is also was discover in the *M. fruticosa* ethanolic extract. The study examined the extract against three types of bacteria and two kinds of fungal. The results showed it could work as anti-microbial on a positive gram bacteria *Staphylococcus aureas* and against widespread fungal pathogen *Candida albicans* (Al-Hamwi et al., 2015).

The EO obtained from *Micromeria fruticose*, which collected from Nablus, showed anti-tumor properties against two types of cancer cells (Human Colon Tumor cells (HCT) and Mammary Carcinoma F7 (MCF7)) mostly attributed to the oxygenated constituents in the oil. Where the aqueous extract exhibited anti-tumor properties, analgesic activities, anti-inflammatory properties, and gastroprotective activities (Abu-Gharbieh & Ahmed, 2012; Abu-Gharbieh et al., 2013). In other research, it is proved that the pulegone itself can cause cancerogenic for some tissues in mice and rates like liver, renal, and urinary bladder. However, that regraded to synthetic pulegone or when use it purely not as EO have many compounds (NTP, 2011).

In Palestine, a new study on the EO of *M. fruticosa* collected from three different regions in the West Bank had been done in 2018. It recommended to use the EO of *M. fruticosa* in food preservation, treat some chronic diseases like diabetes, the cure for open wounds and skin infections. The paper proved the potentiality of the EOs obtained from *M. fruticosa* to work as anti-lipase, anti-amylase, anti-oxidant, and anti-microbial agents (Salameh, 2018; Salameh et al., 2018).

2.3.4. Variation in chemical profile of the EO of *M. fruticosa* according to the season of harvest:

Generally, the chemical composition for the EOs varied according to many factors such as; climatic, annual, seasonal, geographic, and genetic factors (Mehalaine & Chenchouni, 2018). The effect of seasonal variation on the chemical composition of the EO extract from *M. fruticosa* has been studied and discussed before (Al-Hamwi et al., 2011; Dudai et al., 2001; Putievsky et al., 1995).

Under controlled environmental conditions, this experiment has occurred to observe the impact of seasonal variation on the chemical composition of the EO of *M. fruticosa*. GC-MS was used to identify the chemical profile for the monthly EO samples. Results reported that the direct primary reason behind the seasonal composition variation is the leaf maturation. Pulegone, which is the main component in the EO of *M. fruticosa*, showed dramatically variation in its percentage throughout the months. The highest percentage registered during summer months maximumly in June, where its percentage up to 80% (Dudai et al., 2001).

Another published from Lebanon about the same issue was done in 2011. It compared the chemical profile of the EOs extracted from two samples of *M. fruticosa* in two different months. One took in July (full flowering stage), where the second harvested in October. The EO extracted from the two samples analyzed by GC-MS to recognize the chemical composition. Results exhibit apparent differences in the chemical composition for the samples. The percentage for some compounds like pulegone, D-Limonene, Menthone, and menthol declined from the July sample to the October sample. On the other hand, other compounds showed an increase in their percentage. Two compounds (Neomenthol and Sabinene) appeared only in the full flowering stage sample (Al-Hamwi et al., 2011).

2.3.5. Using *M. fruticosa* essential oils especially pulegone as germination inhibitors:

Dudai et al. (1993) deduced that the inhibition activities in the EO extracted from *M. fruticosa* mostly related to pulegone. Moreover, they proved that there is a relationship between the time that plant prone to the pulegone and the effect of blocking of germination

and growth for the exhibited plant. Finally, they recommended the EO of *M. fruticosa* in general and pulegone, in particular, can be used as a bio-herbicide.

In 1999, another research worked on 32 aromatic plants studied the possibility of their EOs as germination inhibitors. The chemicals in the EO of *Micromeria fruticose*, especially pulegone with a percentage reached up to 59.7% from the total oil, listed as the one the most effective EOs can use and recommend as natural herbicides (Dudai et al., 1999).

In another study, it has been founded that some of monoterpenes compounds in EOs which existing in aromatic plants such as; carvone, pulegone, artemisia ketone, carveol, linalool, alpha-terpineol, gamma-terpinene, para-cymene, and delta-3-carene and their metabolites can work as germination inhibitors (Dudai et al., 2004).

3. CHAPTER THREE:

MATRIALS AND METHODS

3.1. Growing of *M. fruticosa*

In February 2017, 586 plants of *M. fruticosa* were planted in 80 cm between rows and 40 cm within the row (Figure 3.1). The plants were obtained from the local market and then planted in 200 m^2 plot at NARC, Al-arroub station in Hebron. The location is 890 m above sea level and the annual temperatures varied from -2 to 40 °C. The plants were irrigated one time weekly from June to October using drip irrigation, no fertilizers or pesticides were used. Also, mechanically removed of weeds every month from May to October. The aerial parts of the plants were harvested eight times from May 2017 to June 2019. After each harvest, the fresh material was weighted and a 100 gm of a pool of sample was taken to determine the dry matter content of the aerial parts, and then directly sent to Florastina factory, which is located in Beitummar-Hebron-West Bank, in order to extract the EO.

Figure 3.1: The cultivated *M. fruticosa* plants used in the experiments.

3.2. The EO Extraction

The fresh aerial parts were subjected to three or four hours of steam distillation Clevenger-type apparatus (Figure 3.2) as soon as the plant were collected in order to extract the EO; the steam temperature was 85°C under zero pressure, and the steam condensation was 12 L/hour at 20-25°C.

During the extraction time, one sample of 5 ml was taken every hour and stored in dark glass bottles for GC-MS analyze. Samples were sent to Pyrenessences laboratory (address: - 2, chemin de la plaine - 11340 Belcaire, France) for chemical profile determination.

Figure 3.2: Actual steam distillation setup used (Clevenger-type apparatus).

3.3. Calculations

3.3.1. Plants production:

During two years, period, May/2017 - June/2019, the plants were harvested eight times approximately every four months and then the yield was divided on the numbers of plants cultivated to estimate the average production for each plant.

3.3.2. Dry matter estimation:

After each harvest the pool of fresh collected samples was weighted and then dried at 105°C for 24 to determine the water content.

Dry matter content in the samples were determined using the following equation:

$$\% \text{ DW} = = \frac{\text{DW}}{\text{FW}} * 100\%$$

Where; DW: Dry Weight, FW: Fresh Weight.

3.3.3. Evaluation for the EO percentage:

The data of the plant's production was evaluated during 24 months at Al-Alroub station, approximately one harvest every 4 months. Aerial parts of the plants were sent to be weighted and extracted by Florastina factory for essential oils.

The percentage of the EO as a fresh matter basis is evaluated using the following equation:

$$\%\text{EO} = \frac{\text{Weight of the EO}}{\text{DW of the harvest}} * 100\%$$

Where; DW: Dry Weight, EO: Essential Oil.

The weight of the EO was determined by calculation the density of the EO by weighting 1 ml of the EO (45 times), and then calculate the total weight of the produced EO.

3.4. Chemical analysis

3.4.1. Samples analysis (GC-MS Analysis and Identification of Components):

EOs samples were stored in dark glass bottles and then sent to the Pyrenessences laboratory, France, for GC-MS analysis.

The EO components were identified by using a 6890 Agilent gas chromatographs equipped with an VF WAX (polar) fused silica capillary column with 60 m (length) x 0.25 mm (diameter), and 0.25 µm (film thickness). Helium was used as the carrier gas with 23 psi.

The oven temperature was held at 60°C for 5 min and then increased from 60°C to 250°C at a rate of 2°C/min. Samples consisting of 1µl of 10% solution in hexane were injected. Quantitative data were obtained electronically from FID area percent data. GC-MS analyses were performed using Agilent system model 6890 with 5973 mass selective detector equipped with an VF WAX (polar) fused silica capillary column (60 m x 0.25 mm, film thickness 0.25 µm). For GC/MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium was used as the carrier gas with 30 psi. Identification of the components was done by a combination of retention times (Pyrenessences database) and mass spectra library NKS 75 000 records. Percentages are calculated from GC/FID peaks areas without using corrections factors.

3.5. Experimental design

3.5.1. Soil samples:

A pool of samples was taken from an infested soil with unwanted weed seeds (plumed cockscomb, squirting cucumber, wild oat, cheeseweed mallow, and poaceae) in order to conduct two trials. Samples were mixed well together until homogenized, then filtered from impurities. soil was distributed in 160 pots of 340 cm^3 for each, by factorial design in 8 groups, each one contains 20 pots, as shown in Figures (3.3 and 3.4).

The first trial was conducted at the beginning of February, and the other one started in the middle of April. In addition to the control, the three treatments were distributed in 5 pots in each group (Figure 3.5).

Figure 3.3: Pool of soils samples distribution in 8 groups (First step of the experiment).

Figure 3.4: Distribution of the treatments where every pot is an experimental unit (Second step of the experiment).

Control	0.2%	0.05%	0.2%	0.1%
0.1%	0.05%	0.1%	Control	0.05%
0.2%	Control	0.2%	0.05%	Control
0.05%	0.1%	Control	0.1%	0.2%

Figure 3.5: Distribution of the treatments in each group, every pot is considered as experimental unit within plot.

3.5.2. Treatments preparation:

The EO used in this experiment was from the harvest of August/2018. The Plants used, extraction method, conditions of extraction, and the chemical analysis aforementioned in Experiment 1.

The volumes of the EO were 2, 4, and 8 ml in order to prepare the 0.05, 0.1, 0.2% concentrations. Since the EO has low water solubility, we added some surfactants to the mixture. The treatments were prepared by dissolving the EO with 2 ml of surfactants (LABS, SLS, and NaOH) in 4 litter of water, as follow:

1. 0.05% (dissolve 2ml of the EO of *M. fruticosa* in 4L of water with 2 ml of surfactants).
2. 0.1% (dissolve 4ml of the EO of *M. fruticosa* in 4L of water with 2 ml of surfactants).
3. 0.2% (dissolve 8ml of the EO of *M. fruticosa* in 4L of water with 2 ml of surfactants).
4. Control group (4L of water with 2 ml of surfactants).

For each group of treatment every pot was irrigated by 100 ml of its solution and the number of weeds were taken every 4 days for 24 days. Soil was kept wet until the water drained from the bottom of the pots during the experimental period. Figure (3.6) shows the experiment groups contain germinated weeds at the last day of record.

Figure 3.6: The experiment groups at the end of experimental period

3.6. Statistical analysis

Treatments were distributed randomly in 4 concentrations (0.0, 0.05, 0.1, and 0.2%) in factorial design as shown in Figures (3.3, 3.4 and 3.5). The effect of the concentrations on weed germination were studied in this experiment. The effect of concentrations during the time was also studied.

One group from the eight groups in each trial was deleted because it ruined by ants' attack, as the experiment is conducted in an open field. So, it was isolated and excluded from the experiment.

The effect of the EO concentration on weeds germination during the time were analyzed using the SAS statistical package (SAS 9.4, 2013). Repeated measures using the PROC MIXED procedure and following the model.

$$Y = \mu + T_i + D_j + TRT_k + T*TRT_{ik} + TRT*D_{kj} + T*TRT*D_{ikj} + \varepsilon_{l(ijk)}$$

Where T_i (d.f.=1) represents the numbers of trials conducted in the experiment, D_j (d.f.=5) represents the day of recording, TRT_k (d.f.=3) represent the different concentrations of the used EO, $T*TRT_{ik}$ (d.f.=3) the interaction between trials and concentration, $TRT*D_{kj}$ (d.f.=15) the interaction between concentration and days of recording, $T*TRT*D_{ikj}$ (d.f.=20) the interaction between trials, concentration and the days of recording, and ε_{ikj} (d.f.=9) represent the experimental error. The interaction $T*D_{ij}$ was used as repeated measurements and differences between mean values were tested using the Tukey-Kramer test.

4. CHAPTER FOUR:

RESULTS AND DISCUSSION

4.1. Results

4.1.1. Aerial parts and EO yield:

Table (4.1) presents the vegetative/biomass yield of *M. fruticosa* and its EO within seasons under the conditions mentioned in the previous chapter. Plants yield and EOs proportion as dry matter basis are varied among seasons. For the seasons of 2017 and 2018, the average aerial parts production was 0.2 and 0.22 Kg respectively for every plant as fresh weigh. The yearly yield of aerial parts for 2017 and 2018 was 680 and 748 Kg/dunam, respectively. Oil density was 0.9459 ± 0.00184 and percentage of average EO production as fresh matter basis was 0.82% and 0.80% for the season 2017 and 2018, respectively.

Table 4.1:The seasonal vegetative/biomass yield of aerial parts and EO for *M. fruticosa* within two years.

Harvesting date	Total FW of aerial parts (Kg)	Avg FW per plant (Kg)	Aerial parts yield (Kg/dunam)	%DW	DW (Kg)	EO (ml)	% EOs as DW basis (w/w) %	% EOs as FW basis (v/w) %	% EOs as FW basis (w/w) %	EO yield (L/ dunam)
May 2017	6.658	0.011	37.4	_	_	45	_	0.68	0.64	261.1
August 2017	32.418	0.055	187.0	_	_	340	_	1.05	0.99	1.973
October 2017	42.500	0.073	248.0	_	_	427	_	1.00	0.95	2.477
December 2017	30.500	0.052	176.8	_	_	160	_	0.52	0.50	0.928
Total	112.080	0.200	680.0	_	_	972	_	0.87	0.82	5.639
April 2018	70.000	0.119	404.6	_	_	495	_	0.71	0.67	2.872
August 2018	30.400	0.052	176.8	30.39	9.24	309	3.16	1.02	0.96	1.793

October 2018	26.500	0.045	153.0	31.36	8.31	273	3.11	1.03	0.97	1.584
Total	126.900	0.220	748.0	_	_	1077	_	0.85	0.80	6.249
June 2019	50.300	0.086	292.4	27.84	14.01	471	3.18	0.94	0.89	2.733

Avg = Average, FW = Fresh Weight; DW = Dry Weight, _ = no data avalible, dunam = 1000m^2, Kg = Kilogram, L = litter, ml = milliliter

Regarding to the EO recovery during the extraction time, results were: For the three hours of extraction 70, 24 and 6% for the first, second and third hour. While for the four hours of extraction results were 70, 23, 5 and 2%, respectively.

4.1.2. GC-MS Results:

The chemical analysis appeared that there are 191 compounds found with different percentages in the EOs samples. The total various compounds found in the nine samples were isolated and compared (Appendix 1). However, there are just 33 characterized compounds ranging from 92% to 98.5% in the nine samples (Table 4.2), classified into dominated oxygenated constituents (71.6 - 81.9%) mainly monoterpenoid represented in ketones and non-oxygenated components, with a relative percentage ranging between (11.2 - 18.2%), dominated by terpene hydrocarbons among which beta-caryophyllene was the major compound (4 – 6.7%) as appeared in Table (4.3).

The most abundant component in all of nine samples was pulegone with a proportion range of (52.32 - 70.28%). Results show decreasing in the percentage of pulegone by 3.7% from the August sample to the October sample. Figures (4.1 and 4.2) represent the GC-MS peaks for the EO of *M. fruticosa* for August and October total samples, respectively. The chromatographic profile for other samples which were taking during the extraction process mentioned in Appendix 2.

The proportion of beta-pinene, limonene, beta-caryophyllene, pulegone, alpha-humulene (alpha-caryophyllene), piperitenone, piperitenone oxide, caryophyllene oxide, and others dropped from the August samples to the October samples. Reciprocally, the proportion of menthol increased by 63.3% from the August sample to the October sample, and others are

increased in low rates such as; cis-beta-ocimene, 3,8-p-menthadiene, 3-octanol, 1-octen-3-ol, isomenthone, neomenthol, neoisomenthol, germacrene D, piperitone, bicyclogermacrene, and delta-cadinene.

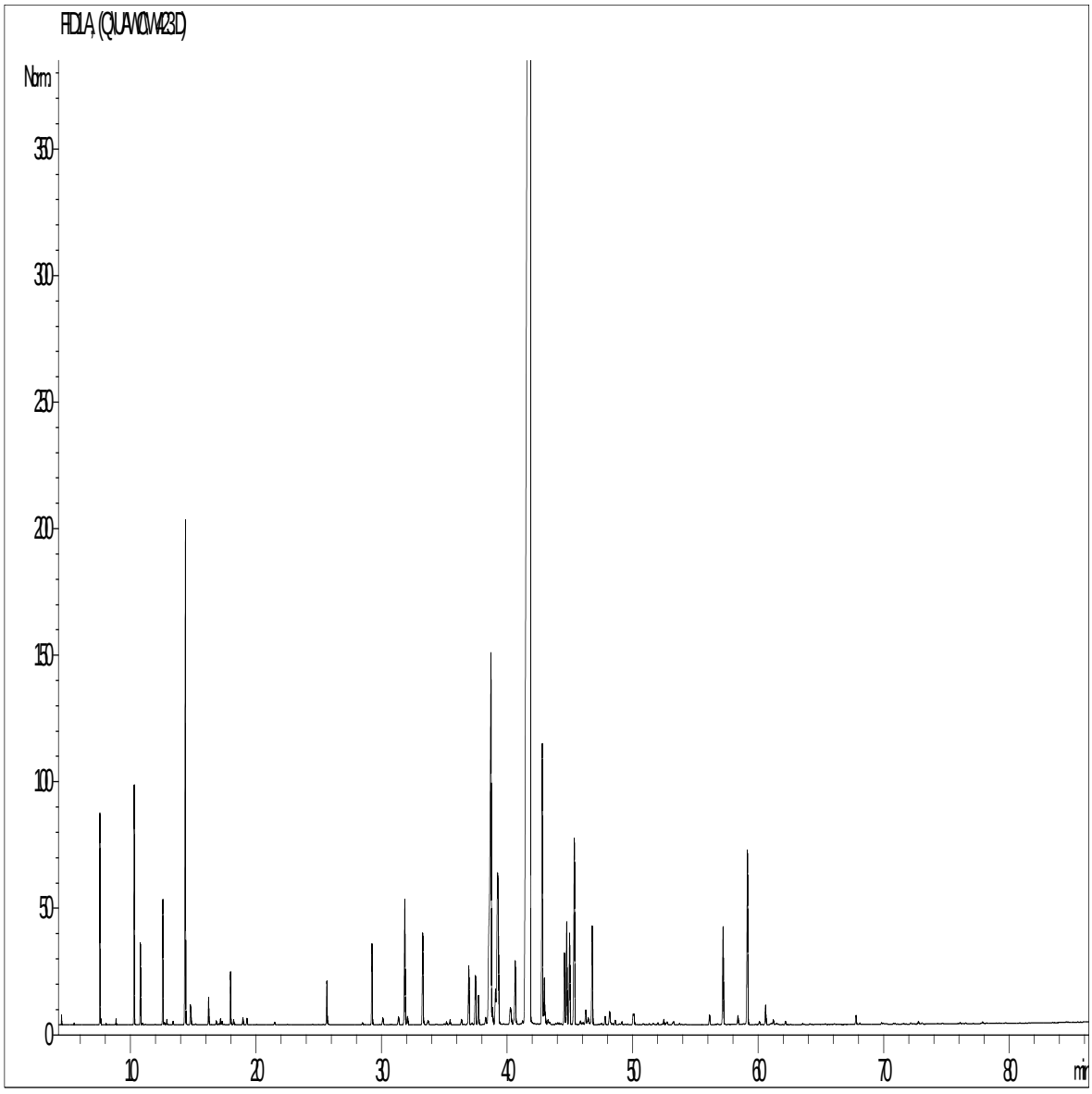


Figure 4.1: Chromatographic profile for the EO of *M. fruticosa* sample (Total sample for Aug/2018 harvest).

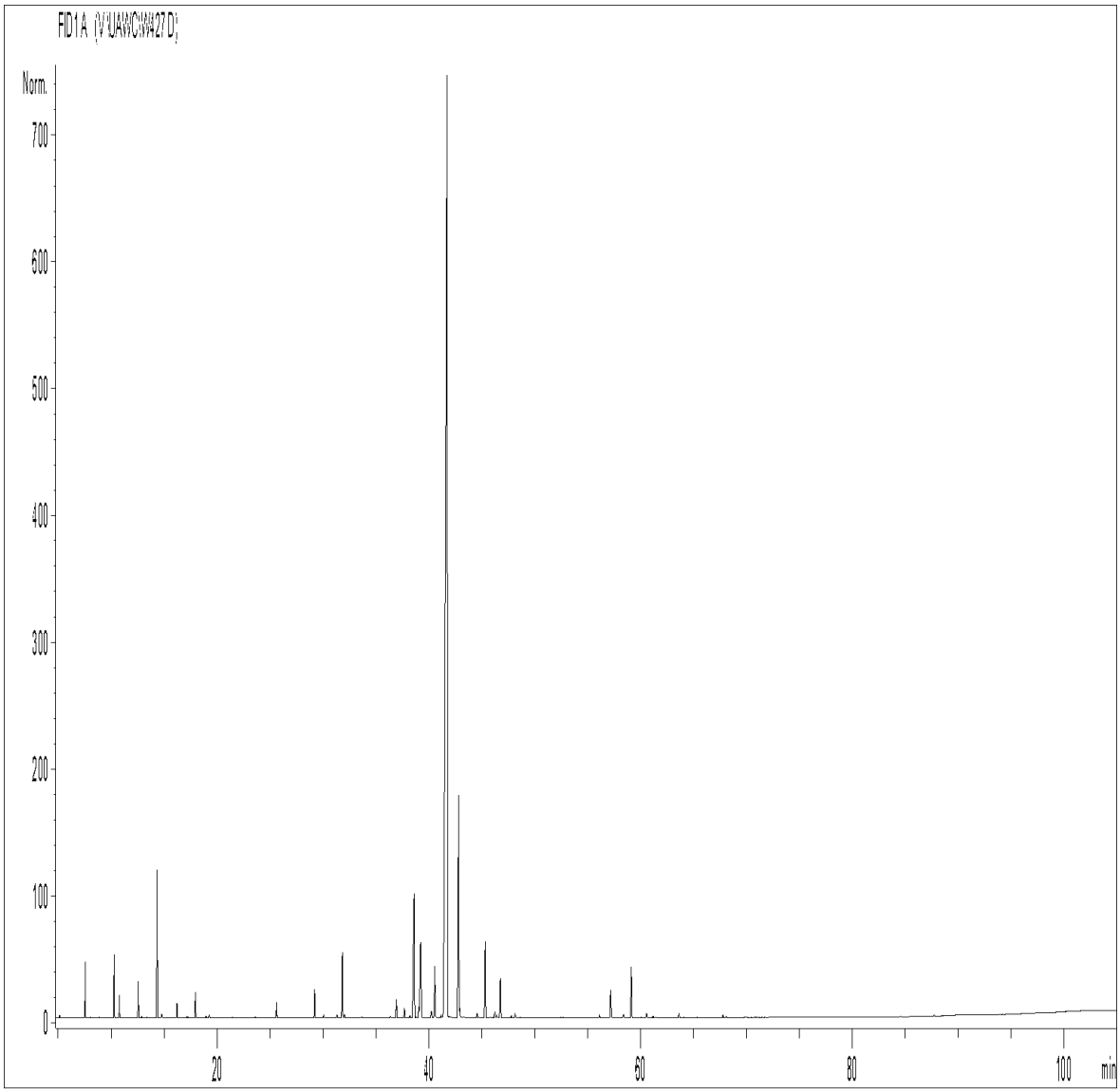


Figure 4.2: Chromatographic profile for the EO of *M. fruticosa* sample (Total sample for Oct/2018 harvest).

However, some compounds detected only in the samples of August which are; alpha-phellandrene,alpha-p-dimethylstyrene, camphor, beta-pinocamphone, alpha-pinocamphone, linalool, bornyl acetate, myrtenyl acetate, verbenone , alpha-curcumene , menthadienol isomer , myrtenol, campholenol, calamenene, geraniol, e-methyleugenol, epoxy-6,7-humulene, eugenol, and carvacrol. On the other hand, others figured only in October samples which are; 2-pentanone, 4-hydroxy-4-methyl-, gurjunene isomer, citronellyl acetate, piperitone oxide, ledene, nerolidol, pentadecanone, trimethyl-, fokienol, eudesma-7-en-4-ol.

Furthermore, as it observed in the supplementary table 1 (Appendix 1) that there are many compounds appeared in in the samples which took during the extraction process and not appeared in the total samples analysis such as; isovaleraldehyde, thuyadiene, pinadiene, menthatriene isomer, alpha-gurjunene, linalyl acetate, pinocarvone, epsilon-cadinene, camphene hydrate, alpha-trans-bergamotene, isopulegol isomer, hotrienol, 2-undecanone, umbellulone, 1-nonen-3-ol, menthadienol isomer, estragole, cis-verbenol, e-tagetenone (trans-ocimenone), beta-curcumene, sabinol isomer, nerol, isocarvacrol, alpha-bisabolol, and beta-asarone in August samples. Where furan, menthatriene isomer, ylangene, beta-cubebene, beta-elemene, alpha-bulnesene, cadina-1,4-diene, ledol, gleenol, eudesmol isomer, and delta-cadinol in October samples.

Table 4.2: The main chemical compounds of the EO of *M. fruticosa*.

			August					October			
Number	RT (min)	Compound	H1	H2	H3	H4	Tot	H1	H2	H3	Tot
1	41.8	PULEGONE	52.32	68.31	70.28	67.78	66.22	62.51	66.05	63.36	63.75
2	38.7	beta-CARYOPHYLLENE	6.67	4.48	5.45	5.4	4.56	3.93	4.57	5.47	4.03
3	14.4	LIMONENE	3.64	3.5	2.13	1.45	3.39	3.94	1.73	0.69	2.98
4	39.2	COMPONENT Mw=154	2.39	2.6	1.95	1.96	2.46	3.55	2.82	1.58	3.18
5	42.8	MENTHOL	1.94	2.46	2.62	2.53	2.42	6.19	6.99	7.51	6.6
6	45.3	GERMACRENE D	1.1	2	2.57	2.15	1.78	2.16	2.24	3.6	2.2
7	59.1	PIPERITENONE OXIDE	0.94	1.82	1.83	1.6	1.58	1.38	1.61	1.6	1.47
8	10.3	beta-PINENE	1.73	1.28	0.61	0.32	1.27	1.48	0.46	0.16	1.05
9	31.8	ISOMENTHONE	1.01	1.08	0.97	0.93	1.06	1.88	1.71	1.13	1.77

10	39.1	NEOMENTHOL	0.29	0.31	0.25	1.49	0.97	1.5	1.28	2.25	1.67
11	7.6	alpha-PINENE	1.5	0.84	0.39	0.16	0.9	1.09	0.27	0.1	0.75

RT = Retention Time. H1 = after one hour. H2 = after two hours. H3 = after three hours. H4 = after four hours. Tot = Total sample.

Table 4.3: Relative percentages for the main volatile constituents identified from the EO of *M. fruticosa*.

Constituents	Relative Percentage								
	H1	H2	H3	H4	Tot	H1	H2	H3	Tot
Oxygenated constituents									
Alcohols	9.37	5.36	4.79	7.33	7.01	11.90	11.04	12.11	11.23
Ketones	61.84	73.56	74.37	73.50	71.96	68.06	70.99	68.30	68.53
Epoxide	0.17	0.12	0.17	0.24	0.18	_	0.09	0.24	0.13
Ether	0.24	0.11	0.04	0.04	0.12	0.1	0.04	_	0.08
Total oxygenated constituents	71.61	78.89	79.16	80.90	79.01	78.67	81.89	80.48	79.70
Non-oxygenated constituents (hydrocarbons)									
Monoterpene	8.78	7.35	4.46	2.78	7.22	8.88	3.89	2.01	6.65
Sesquiterpene	9.37	7.91	9.97	8.45	7.79	7.45	8.55	11.89	7.75
Total non-oxygenated constituents	18.15	15.26	14.43	11.23	15.01	16.33	12.44	13.90	14.40
Total main constituents	92.15	96.75	95.54	94.09	96.48	98.55	97.15	95.96	97.28

H1 = after one hour. H2 = after two hours. H3 = after three hours. H4 = after four hours. Tot = Total sample. _ = no compound detected.

4.1.3. The Effect of *M. fruticosa* EO on weeds germination:

Results (Table 4.4) and (Figure 4.3) show dropping in the number of weeds in all pots treated with solutions that contain EO. However, high significations with the control group P<0.001 were noted after the fourth day of record for all the solutions contains EO.

While, differences between and within days were disappeared from the sixteenth day of record for the concentrations of 0.05% and 0.1%. It was noted that the concentration with 0.2% had high significations with all groups of treatments within and between days P< 0.001. Also, for all treatments there were no differences from the sixteenth day of record.

Results indicated a significant decrease in the number of weeds for the concentrations 0.05, 0.1 and 0.2% by 40, 73 and 98% in the first day of record, and this decrease was also significant in the last day of record by 24, 27 and 63%, respectively.

Table 4.4: The Least square mean of the effect of *M. fruticosa* EO with different concentrations on the number of germinated weeds during 24 days for each trail.

Treatment	Recording days (D)						D*TRT	Trials	
	D4	D8	D12	D16	D20	D24	s.e.m	T1	T2
Control	$_{F}1.5^{ab}$	$_{E}4.7^{a}$	$_{D}6.1^{a}$	$_{CAB}7.525^{a}$	$_{AB}7.9^{a}$	$_{B}7.527^{a}$	0.277	$_{A}8.7^{a}$	$_{B}3.1^{ab}$
0.05%	$_{F}0.9^{bc}$	$_{E}2.7^{b}$	$_{D}4.4^{b}$	$_{CAB}5.63^{bc}$	$_{AB}5.69^{bc}$	$_{B}5.67^{bc}$	0.274	$_{A}5.9^{b}$	$_{B}2.5^{bc}$
0.1%	$_{F}0.4^{cd}$	$_{E}1.8^{c}$	$_{D}3.6^{c}$	$_{CBA}5.1^{c}$	$_{BA}5.3^{c}$	$_{A}5.5^{c}$	0.275	$_{A}5.1^{c}$	$_{B}2.1^{c}$
0.2%	$_{FE}0.03^{d}$	$_{ED}0.5^{d}$	$_{D}1.2^{d}$	$_{CBA}2.3^{d}$	$_{BA}2.6^{d}$	$_{A}2.8^{d}$	0.275	$_{A}2.3^{d}$	$_{B}0.8^{d}$

D: the recording day; TRT: Treatment; T: Trail; s.e.m: standard error of the mean; 0.05%, 0.1% and 0.2% indicate the concentrations of *M. fruticosa* EO in the treatments.

$^{a, b, c,}$ and ^{d} Different lowercase superscript letters within columns, besides letters indicate values have no significantly different at (P<0.05).

$_{A, B, C, D, E,}$ and $_{F}$ Different uppercase subscript letters within rows, besides letters indicate values have no significantly different at (P<0.05).

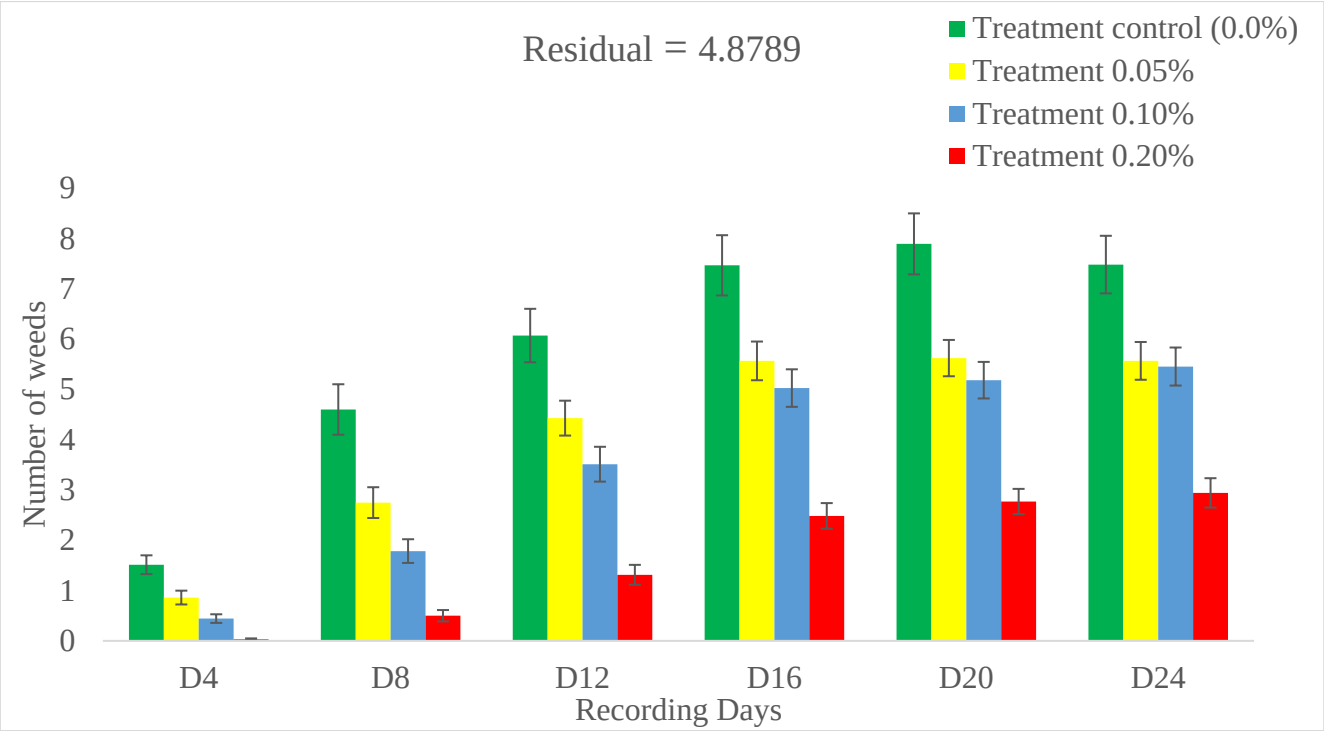


Figure 4.3: The effect of the EO of *M. fruticosa* with different concentrations on the number of germinated weeds during 24 days for each trail.

4.1.4. Effect of season on weeds germination:

Table 4.5 and Table 4.6 illustrate the least square mean of the number of weeds treated with the same concentrations but in two different seasons; first trail (Table 4.5 and Figure 4.5) was done in February/2019, where the second trail (Table 4.6 and Figure 4.6) was started in the second half of April and ended at the beginning of May/2019.

It's clear that there were huge differences in the numbers of weeds germination between the two trails in all aspects. Numbers of weeds were more in trial 1 than in trial 2 in all the days of record $P<0.001$. Also, in both trials differences between the days were disappeared from the sixteenth day of record as shown in Figure (4.4).

Effects of concentrations were the same significations within and between the days of record in both trials (Figures 4.5, 4.6 and Tables 4.5, 4.6).

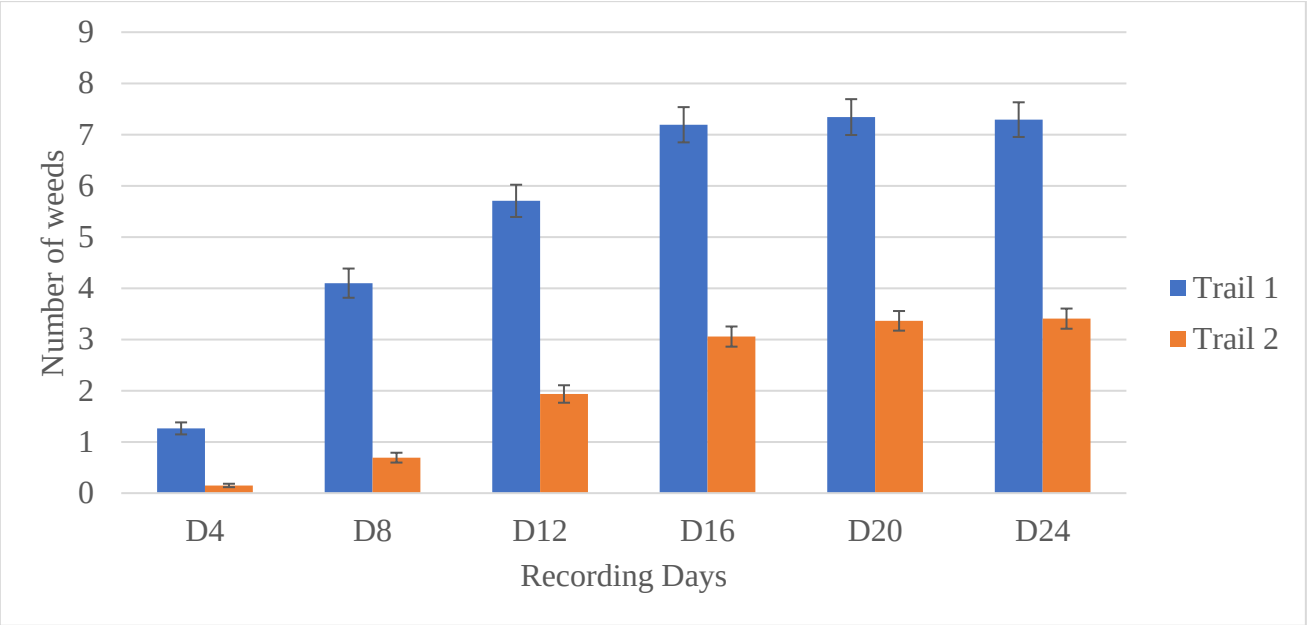


Figure 4.4: The effect of the EO of *M. fruticosa* applied in two different seasons on the number of germinated weeds during 24 days.

(Trails: Trail 1: the winter trial, Trail 2: the spring trail)

Table 4.5: The Least square mean of the effect of *M. fruticosa* EO on the number of germinated weeds during 24 days (Winter Trail, Feb/2019).

Treatment	Recording days (D)						D*TRT
	D4	D8	D12	D16	D20	D24	s.e.m
Control	$_{F}2.6^{ab}$	$_{E}7.7^{a}$	$_{D}9.1^{a}$	$_{BAC}10.9^{a}$	$_{AC}11.5^{a}$	$_{C}10.5^{a}$	0.388
0.05%	$_{F}1.6^{bc}$	$_{E}4.6^{b}$	$_{D}6.2^{bc}$	$_{ACB}7.7^{bc}$	$_{CB}7.5^{bc}$	$_{B}7.6^{bc}$	0.378
0.1%	$_{F}0.9^{cd}$	$_{E}3.2^{c}$	$_{D}5.6^{c}$	$_{CBA}6.87^{c}$	$_{BA}6.89^{c}$	$_{A}7.2^{c}$	0.382
0.2%	$_{FE}0.06^{d}$	$_{E}0.9^{d}$	$_{D}2.1^{d}$	$_{CBA}3.4^{d}$	$_{BA}3.5^{d}$	$_{A}4^{d}$	0.382

D: the recording day; TRT: Treatment; T: Trail; s.e.m: standard error of the mean; 0.05%, 0.1% and 0.2% indicate the concentrations of *M. fruticosa* EO in the treatments.

$^{a, b, c,}$ and ^{d} Different lowercase superscript letters within columns, besides letters indicate values have no significantly different at ($P<0.05$).

$_{A, B, C, D, E,}$ and $_{F}$ Different uppercase subscript letters within rows, besides letters indicate values have no significantly different at ($P<0.05$).

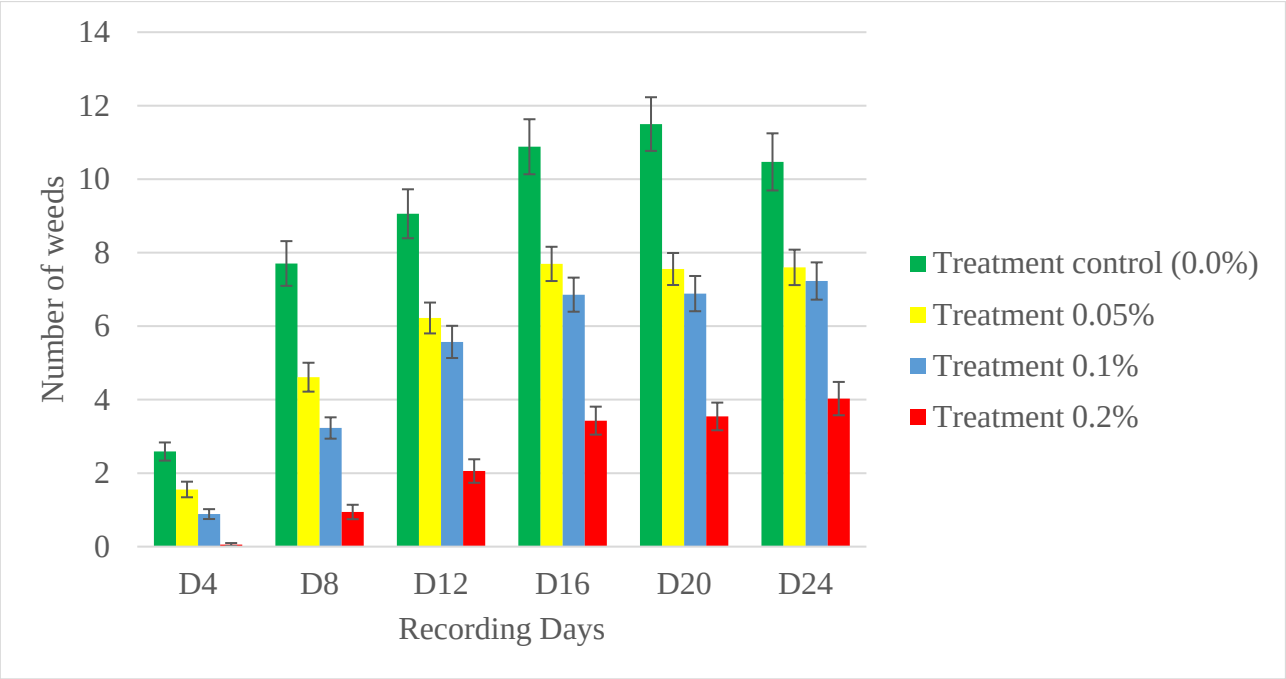


Figure 4.5: The effect of the EO of *M. fruticosa* with different concentrations on the number of germinated weeds during 24 days (Winter Trail, Feb/2019).

Table 4.6: The Least square mean of the effect of *M. fruticosa* EO on the number of germinated weeds during 24 days (Spring Trail, end of Apr/2019).

Treatment	Recording days (D)						D*TRT
	D4	D8	D12	D16	D20	D24	s.e.m
Control	$_{F}0.4^{abcd}$	$_{E}1.6^{ab}$	$_{DC}3.2^{ab}$	$_{CBA}4.2^{abc}$	$_{BA}4.4^{abc}$	$_{A}4.6^{acb}$	0.382
0.05%	$_{FE}0.2^{bcd}$	$_{E}0.9^{bcd}$	$_{DC}2.7^{b}$	$_{CAB}3.6^{bc}$	$_{AB}3.8^{bc}$	$_{B}3.7^{cb}$	0.382
0.1%	$_{FE}0^{cd}$	$_{E}0.3c^{d}$	$_{D}1.6^{c}$	$_{CBA}3.4^{c}$	$_{BA}3.7^{c}$	$_{A}3.8^{b}$	0.382
0.2%	$_{EFD}0^{d}$	$_{FD}0^{d}$	$_{DC}0.3^{d}$	$_{CAB}1.1^{d}$	$_{AB}1.6^{d}$	$_{B}1.5^{d}$	0.382

D: the recording day; TRT: Treatment; T: Trail; s.e.m: standard error of the mean; 0.05%, 0.1% and 0.2% indicate the concentrations of *M. fruticosa* EO in the treatments.

$^{a, b, c,}$ and ^{d} Different lowercase superscript letters within columns, besides letters indicate values have no significantly different at ($P<0.05$).

$_{A, B, C, D, E,}$ and $_{F}$ Different uppercase subscript letters within rows, besides letters indicate values have no significantly different at ($P<0.05$).

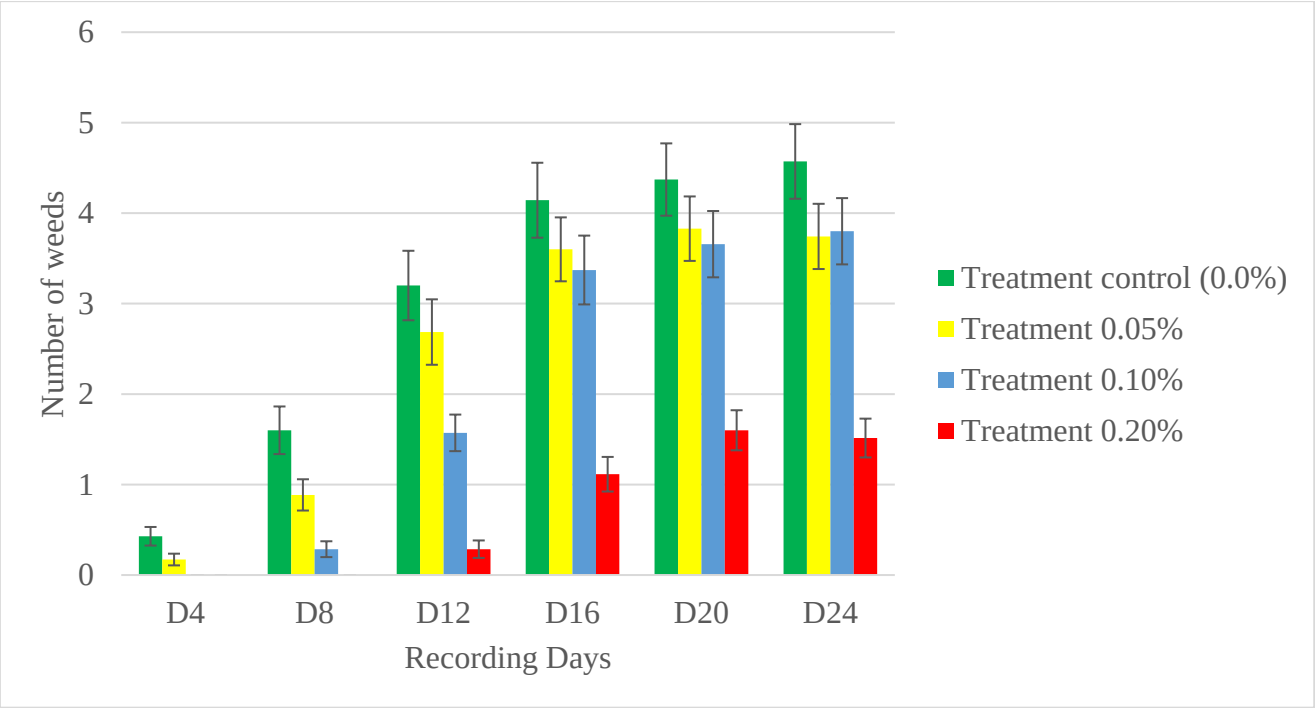


Figure 4.6: The effect of the EO of *M. fruticosa* with different concentrations on the number of germinated weeds during 24 days (Spring Trail, end of Apr/2019).

4.2. Discussion

4.2.1. Aerial parts and the EO yield:

There were no available data describing the production for both vegetative/biomass and EO of the *M. fruticosa* in Palestine. However, the production of the plant in increasing with the growing stage in the second-year was increased about 12% than the first-year with no difference in the percentage of the EO percentage as a fresh weight basis.

The variation in percentages of the EO and chemical profile in each harvest may back into the time of harvest, the growth stage, climate, and stress on the plants. However, all the EO percentages were obtained in this survey were much higher than the result of a recent study worked by Salameh et al. (2018), which reported that the mean rate of the EO extracted from samples collected in Hebron was 0.70 ± 0.17% calculated on moisture-free basis, the huge differences could be due to the extraction method.

In another work the EO obtained from *M. fruticosa* was collected before the flowering stage in March from Nablus in Palestine also got a yield lower than what we get in this research (2.2% v/w calculated on a dried weight basis) (Abu-Gharbieh & Ahmed, 2012).

Comparing to the outcomes obtained from Turkey, the percentages we got are higher than what Telci and Ceylan (2007) got, where they reported that the EO content in a sample collected at the flowering stage was 2.4%. The closest percentage to ours was recorded in Lebanon by Al-Hamwi et al. (2011), where the percentage of EO obtained from a sample was collected in July (full flowering stage) reaches 2.8% which was calculated on a dried weight basis.

The difference in the percentages of EOs obtained maybe refers to the species, extraction method, and the harvesting and extraction protocol. The plants were extracted fresh for three hours by steam distillation Clevenger-type apparatus and in the morning in order to avoid the evaporation of the oils. Also, the condenser tube used was 36 meters of length with a condensation temperature of 20 °C. However, in the other studies, the hydro-distillation method was applied on a dry material for 2-3 hours (Abu-Gharbieh & Ahmed, 2012; Al-Hamwi et al., 2011; Telci & Ceylan, 2007). An ultrasonic microwave method was used in another study and also after drying the material (Salameh et al., 2018). So, these above-mentioned different conditions could be the reasons behind the variances in oil percentages beside the other uncontrolled effects like climate, genetic, environmental, and geographical factors. The amount of extracted plants also could be a reason of observed varied between this study and previous researches, where all of previous used small amount (<500 gm) of fresh material comparing to the amount this study used.

It is observed that the highest percentages of extracted EO were at the pre-flowering stage or the early flowering stage, as the plant close to the flowering stage as the percentage of the EO is dropped. Because of most of the EO storage and accumulation in the leaves, and when the percentage of flower tissue increases the percentage of leaves tissues decreases (Dudai et al., 2001; Putievsky et al., 1995).

The extraction process was taken 3-4 hours, but it would be more cost-effective with good pulegone percentages if it just two hours, where its notice that there is no variation in the pulegone percentage between the second and third hour (Table 4.2). Moreover, it just produced only 6% or less of EO in the last hour.

4.2.2. GC-MS analysis:

The GC-MS test analyzed under the conditions mentioned earlier (in the previous chapter) led to the identification of 191 compounds, listed in supplementary Table 1, but most of them appeared in negligible traces amounts (<0.05%). Where the research was focusing on the proportion of pulegone as the component causes inhibition for seed germination (Dudai et al., 1993). It is pronounced that the variation in the pulegone percentage during extraction is not high except in the first hour of August samples, where it was 52.32% then dramatically up to 68.31% at the second hour of analysis. Regards to the seasonal variation, the variation in pulegone percentages between the total samples in August and October is less than 3%.

A previous study was conducted in Palestine on the EO of *M. fruticosa* collected from Hebron in April, shows that the pulegone percentage is 74.43% (Salameh et al., 2018), which higher than both percentages we got in August and in October. The proportion of pulegone in EO obtained from a sample collected pre-flowering stage (March) from Nablus, Palestine was 58.5% lower than this study results in August and October (Abu-Gharbieh & Ahmed, 2012) (Table 4.7).

There are similarities in increasing pulegone content from August to October between the present study and those described by (Al-Hamwi et al., 2011) which conducted in Lebanon. However, the percentages they got in both months were lower than what this study found. Also, for that growing in Turkey, the pulegone proportion is much lower than what this study got (Kırımer, Ozek, et al., 1993; Telci & Ceylan, 2007) (Table 4.7).

This observed variation in the percentages of the EO and the chemical profile, thereby the proportion of compounds including pulegone in the EO of *M. fruticosa,* affected by numerous of factors including geographic, elevation over the sea, climatic conditions (average of rainfall and temperature), season, and the stress on the plant (Abu-Gharbieh & Ahmed, 2012; Salameh et al., 2018). Even few researches connect between the maturity of leaves and the amount of EO can extract from them (Dudai et al., 2001; Putievsky et al., 1995).

From this study, we can conclude that the elongation of the extraction process can also affect the chemical composition of the EO and on the concentrations of these constituents. These factors should be studied well and used as a tool to choose the perfect conditions to extract

the EO with the highest pulegone percentage for use as an anti-germination agent with a suitable cost for farmers.

Table 4.7: Pulegone percentage in the EO of *M. fruticosa* from different origin collected in different times.

Origin	Sample harvesting time	State of extracted sample	Sample amount (gm)	Extraction method	% Pulegone	References
Hebron, Palestine	April, 2017	Dry	100	Ultrasonic Microwave	74.43%	(Salameh et al., 2018)
Nablus, Palestine	Before the flowering stage (March, 2010)	Dry	300	Hydro-distillation	58.5%	(Abu-Gharbieh & Ahmed, 2012)
Lebanon	Full flowering stage (July,2010)	Dry	-	Hydro-distillation	30.41%	(Al-Hamwi et al., 2011)
Lebanon	October, 2010	Dry	-	Hydro-distillation	13.35%	(Al-Hamwi et al., 2011)
Turkey	at the flowering stage in 2004	Dry	20	Hydro-distillation	16.65%	(Telci & Ceylan, 2007)
Turkey	-	Dry	-	Hydro-distillation	33.4%	(Kırımer, Ozek, et al., 1993)

= no data available

Although the steam distillation considered as the most widely ideal process for a large scale EO production, there is an obvious drawback of this process may be the induction of chemical compound changes during the extraction due to convert on between isomers or

enantiomers for the same compound or converted from a compound to another by some reactions such as; oxidation, reduction, hydrogenation, dehydrogenation, dehydration and others affected by some factors during the collection for the final oil including changing in temperature, expose to the air and mixing all obtained oils together at the end. So, some compounds detected by GC-MC in the hourly samples, but it did not find out in the total samples.

4.2.3. Effects of the EO on weed germination:

Most studies on allelopathy activities of the *M. fruticosa* EO have described the phenomenon but did not consider applications for weed control in agriculture. In the present study examined the primary steps towards a possible practical application for this EO as seed germination control against some common weed species in Palestine affecting severely on cultivated plants and causing high yield losses yearly.

In the present study, the decrease in the number of weeds was relatively increased with increasing the concentration of the *M. fruticosa* EO and the oil exhibited a high phytotoxic effect. The high phytotoxicity of *M. fruticosa* mostly attributed to pulegone as the main bioactive component that negatively affected the germination of weed seeds, with a percentage up to 66.22% of the EO according to the GC-MC analysis results aforementioned in the previous chapter (Table 4.2).

Pulegone is a ketonic monoterpene compound, which is the abundant compound in the EO of *M. fruticosa*, and that matches with previous studies (Abu-Gharbieh & Ahmed, 2012; Al-Hamwi et al., 2011; Dudai et al., 2001; Salameh et al., 2018; Telci & Ceylan, 2007). Pulegone shows germination inhibition properties, and this is consistent with what has been found in the previous study conducted by Dudai et al. (2004), who reported that the monoterpenes such as pulegone act as potent seed germination inhibitor against wheat seeds.

Others have shown that the monoterpene is not enough for the compound to have potent inhibition activities when they compared pulegone inhibition properties to menthone which have the same structure but have no carbon-carbon double bond. Menthone have only one-third of the activity of pulegone (Dudai et al., 1993), this result is on agree with this study finding, where the percentage of pulegone and menthone in the used oil was 66.22 and 1.06%, respectively. The results obtained in this study also revealed that pulegone had an

adverse impact on the germination of some weed species in varying proportions according to the applied concentration.

The effectivity of pulegone in the EO is varied from previous studies (Dudai et al., 2004; Dudai et al., 1993; Dudai et al., 1999) probably because part of prevention or inhibitor material for the germination of seeds in the extracted plant may lose their inhibitory power during extraction methods, or it may be related to different solubility of the active constituents in the different solutions. Moreover, the genetic, seasonal and climatic factors could be the reason that affected the effectivity of pulegone on the EO.

All studied discussed the possibility of using *M. fruticosa* as a germination inhibitor conducted under controlled conditions in the laboratory. Dudai et al. (1999) reported that the EO of *M. fruticosa* is one of three best EOs among 32 have inhibitory activities against some species of weed and wheat seeds were sowed in Petri dishes, they refer this potency to the dominated compound, pulegone, with percentage reaches 59.7%. It also revealed good results when they applied the EO in clay soil against Amaranth (weed species); the experiment was performed in pots in a greenhouse (Dudai et al., 1999).

In another study, also conducted in the lab using Petri dishes and applied the EO, with pulegone percentage up to 70%, in the gas phase because of its low solubility in water, the results showed completely prevented wheat seed germination, and highly recommended the EO of *M. fruticosa* as a candidate bio-herbicide (Dudai et al., 1993).

On the last day of record, it is observed that the average number of counted weeds was less than the previous record, that may be back into died of some weeds where the experiment conducted in an open field, thereby, it is exposed to the environmental effects which include insect attack and climatic effects (wind, rain, …etc).

4.2.4. Effects of season on weeds germination:

Differences between the two trials in the number of weeds could be because of leaching of the solutions or due to the high humidity of the soil in the winter. thus, give more opportunities for the seeds to be germinated.

This issue wasn't discussed in any previous work, so when applying the EO of *M. fruticosa* as an herbicidal agent it has to keep in mind the season and the weather conditions

if the cultivation in open area as in our case. Furthermore, it is recommended to increase the concentration of the EO in winter months so it will give better inhibition results.

4.2.5. Effects of EO concentration on the weed germination:

There are no significant differences for all treatments between days from the day sixteenth of record till the last day, and that may back into owing limiting numbers of seeds in the pots.

The allopathic effects increased with the EO concentration increase in the solutions this trend was similar to that reported elsewhere (Dudai et al., 1993).

5. CHAPTER FIVE:

CONCLUSION AND RECOMINDATIONS

5.1. Conclusion

The results of this study presented the possibility of cultivating and growing *M. fruticosa* as a commercial plant in Palestine to obtain the EO and applied it as a natural germination inhibitor. *Micromeria fruticosa* has a very good yield of the EO if it compared with other aromatic plants. More studies are needed to determine the environmental effect of the EO in order to use it as a natural herbicide.

The GC-MC analysis found that the summer months generally are the best time to harvest *M. fruticosa* in order to obtain the highest percentage of pulegone with 2 hours of steam distillation subject on fresh material.

Results show that the EO of *M. fruticosa*, as an endemic plant in Palestine, exhibit good results in working as germination inhibitor against some common weeds (plumed cockscomb, squirting cucumber, wild oat, cheeseweed mallow, and poaceae) in Palestine, more studies are needed on its effects on human health and environment.

This study suggested that the use of the EO of *M. fruticosa* at 0.2% concentration could be applied for inhibiting the germination of some common weed species seeds (plumed cockscomb, squirting cucumber, wild oat, cheeseweed mallow, and poaceae) that spread in Palestine, with a percentage up to 63%. As the EO concentration dropped, the effectivity of it to work as germination inhibitor decline too. The results also suggested that the EO could be used for biological control of weeds as pre-emergence, especially in organic farming.

5.2. Recommendations

In refer to this work, the following recommendations would be outlined:

- More analyses for the chemical profile of the EO in other seasons are needed, especially early of summer, in order to choose the composition with the pulegone maximum percentage to determine the harvesting time.
- Pot experiments and field studies on specific weed species which affected economical plants in Palestine like a vegetable, such as a broomrape which parasite the tomato plants causing severe loss in its production.
- Evaluate the effects of the EO on plant production and the quality of the final crop.
- The maximum concentration of the EO can apply as herbicides without causing pulegone toxic side effects.

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Appendices

Appendix 1: Supplementary Tables

Supplementary Table 1: The chemical composition of the EO of *M. fruticosa*

			August					October			
Number	RT (min)	Compound	H1	H2	H3	H4	Total	H1	H2	H3	Total
1	41.8	PULEGONE	52.32	68.31	70.28	67.78	66.22	62.51	66.05	63.36	63.75
2	38.7	beta-CARYOPHYLLENE	6.67	4.48	5.45	5.4	4.56	3.93	4.57	5.47	4.03
3	14.4	LIMONENE	3.64	3.5	2.13	1.45	3.39	3.94	1.73	0.69	2.98
4	39.2	COMPONENT Mw=154	2.39	2.6	1.95	1.96	2.46	3.55	2.82	1.58	3.18
5	42.8	MENTHOL	1.94	2.46	2.62	2.53	2.42	6.19	6.99	7.51	6.6
6	45.3	GERMACRENE D	1.1	2	2.57	2.15	1.78	2.16	2.24	3.6	2.2
7	59.1	PIPERITENONE OXIDE	0.94	1.82	1.83	1.6	1.58	1.38	1.61	1.6	1.47
8	10.3	beta-PINENE	1.73	1.28	0.61	0.32	1.27	1.48	0.46	0.16	1.05

9	31.8	ISOMENTHONE	1.01	1.08	0.97	0.93	1.06	1.88	1.71	1.13	1.77
10	39.1	NEOMENTHOL	0.29	0.31	0.25	1.49	0.97	1.5	1.28	2.25	1.67
11	7.6	alpha-PINENE	1.5	0.84	0.39	0.16	0.9	1.09	0.27	0.1	0.75
12	46.8	BICYCLOGERMACRENE	0.56	0.97	1.34	0.01	0.89	1.05	1.29	2.06	1.12
13	57.2	PIPERITENONE	0.49	0.96	0.3	1.16	0.89	0.64	0.95	1.42	0.8
14	44.8	BORNEOL	3.36	0.42	0.25	1.13	0.88			0.01	
15	45	VERBENONE	3.41	0.37	0.17	0.84	0.81	_	_	_	_
16	33.3	CAMPHOR	3.3	0.41	0.18	0.49	0.79	_	_	_	_
17	12.6	beta-MYRCENE	0.93	0.75	0.41	0.27	0.7	0.95	0.33	0.12	0.64
18	40.6	NEOISOMENTHOL	0.52	0.59	0.6	0.63	0.61	1.39	1.5	1.47	1.44
19	29.2	1-OCTEN-3-OL	0.63	0.63	0.38	0.37	0.59	0.8	0.51	0.15	0.66
20	44.6	alpha-TERPINEOL	1.88	0.33	0.25	0.72	0.57	0.1	0.12	0.16	0.11

21	36.9	Cis-ISOPULEGONE	0.45	0.53	0.49	0.47	0.51	0.51	0.5	0.38	0.5
22	37.5	BORNYL ACETATE	1.82	0.22	0.02	0.03	0.49	_	_	_	_
23	10.8	SABINENE	0.63	0.43	0.17	0.11	0.43	0.55	0.14	0.03	0.38
24	43	alpha-HUMULENE	0.93	0.33	0.4	0.66	0.43	0.21	0.26	0.41	0.26
25	35.4	LINALOOL	0.14	0.03	0.02	0.05	0.4	_	_	_	_
26	17.9	3.8-p-MENTHADIENE	0.14	0.36	0.62	0.38	0.36	0.44	0.76	0.83	0.55
27	25.6	3-OCTANOL	0.33	0.33	0.21	0.2	0.31	0.43	0.27	0.09	0.36
28	39.1	TERPINENE-4-OL	_	_	_	0.29	0.31	0.31	0.31	0.23	0.31
29	37.7	Trans-ISOPULEGONE	0.15	0.26	0.4	0.37	0.26	0.2	0.36	0.43	0.27
30	40.2	ISOPULEGOL ISOMER	0.27	0.26	0.21	0.21	0.26	1.39	0.27	0.17	0.27
31	60.6	CARYOPHYLLENE OXIDE	0.17	0.12	0.17	0.24	0.18	_	0.09	0.24	0.13
32	16.2	Cis-beta-OCIMENE	0.2	0.19	0.13	0.09	0.17	0.43	0.2	0.08	0.3

33	38.8	SESQUITERPENE	_	_	_	0.19	0.16	0.03	0.04	0.05	0.04
34	46.3	KETONIC COMPOUND Mw=154	0.32	0.04	0.09	0.13	0.15	0.21	0.17	0.03	0.01
35	48.2	delta-CADINENE	0.12	0.13	0.21	0.23	0.13	0.1	0.19	0.35	0.14
36	14.8	1.8-CINEOLE	0.24	0.11	0.04	0.04	0.12	0.1	0.04	_	0.08
37	32	ELEMENE ISOMER	0.08	0.09	0.04	0.09	0.09	0.1	0.11	0.12	0.1
38	43.4	SESQUITERPENE	0.02	0.01	_	0.08	0.09	0.01	0.01	0.02	0.02
39	46.4	PIPERITONE	0.22	0.09	0.03	0.12	0.09	0.06	0.04	0.19	0.2
40	50	MYRTENOL	0.35	0.04	0.02	0.12	0.09	_	_	_	_
41	50.1	CAMPHOLENOL	0.34	0.04	0.02	0.13	0.09	_	_	_	_
42	31.3	MENTHOFURAN	0.04	0.08	0.14	0.12	0.08	0.07	0.12	0.25	0.1
43	56.1	AROMATIC COMPOUND	0.04	0.07	0.04	0.05	0.08	0.04	0.04	0.04	0.07
44	58.4	Z-JASMONE	0.06	0.08	0.12	0.11	0.08	0.07	0.11	0.19	0.09

45	67.8	SPATHULENOL	0.02	0.04	0.07	0.07	0.08	0.03	0.04	0.14	0.08
46	14.8	beta-PHELLANDRENE	0.16	0.06	0.04	0.04	0.07	0.06	0.03	0.01	0.04
47	38.6	ISOPULEGOL	_	0.72	0.57	0.1	0.07	0.06	0.09	0.15	0.08
48	47.8	Trans-ISOPIPERITENOL	0.08	_	0.05	0.05	0.07	0.08	0.06	0.04	0.06
49	30.1	MENTHONE	0.06	0.06	0.06	0.06	0.06	0.08	0.08	0.07	0.08
50	18.9	TERPINOLENE	0.08	0.05	0.04	0.04	0.05	0.06	0.04	0.02	0.04
51	33.7	alpha-PINOCAMPHONE	0.04	0.05	_	_	0.05	_	_	_	_
52	36.9	NEOISOPULEGOL	0.02	0.05	0.05	0.05	0.05	0.05	0.05	0.03	0.04
53	52.7	Trans-CARVEOL	_	0.01	0.03	0.06	0.05	0.01	0.02	0.02	0.02
54	4.5	ACETONE	0.02	0.02	0.01	0.03	0.04	0.02	0.02	0.02	_
55	17.1	Trans-beta-OCIMENE	0.05	0.04	0.03	0.02	0.04	0.05	0.03	0.01	0.04
56	19.3	TERPINOLENE ISOMER	0.02	0.05	0.08	0.05	0.04	0.06	0.11	0.11	0.07

57	43.4	E-beta-FARNESENE	0.05	0.04	0.07	0.06	0.04	0.02	0.04	0.05	0.03
58	45.9	EREMOPHILENE	0.07	0.04	_	0.04	0.04	0.02	0.01	0.02	0.02
59	48.6	CITRONELLOL	0.08	0.03	0.03	0.05	0.04	0.02	0.02	0.04	0.02
60	61.2	2-ALLYL-4-PHENOL	0.01	0.04	0.09	0.09	0.04	0.02	0.04	0.17	0.04
61	8.8	CAMPHENE	0.06	0.02	0.01	0.01	0.03	0.02	_	_	0.02
62	12.9	psi-LIMONENE	0.04	0.03	0.02	0.01	0.03	0.04	0.02	_	0.03
63	16.8	gamma-TERPINENE	0.06	0.02	0.02	0.02	0.03	0.02	0.02	0.01	0.01
64	18.2	p-CYMENE	0.07	0.02	0.01	0.02	0.03	_	_	_	0.01
65	35.2	beta-PINOCAMPHONE	0.1	_	_	0.01	0.03	_	_	_	_
66	40.7	SESQUITERPENE	_	_	0.04	0.03	0.03	0.06	0.02	_	_
67	48.9	alpha-CURCUMENE	0.04	0.02	0.01	0.02	0.03	_	_	_	_
68	52.7	ISOPIPERITENONE	_	_	0.02	0.03	0.03	0.01	0.02	0.02	0.01

69	53.3	GERANIOL	0.06	0.02	_	0.04	0.03	_	_	_	_
70	60.1	ISOCARYOPHYLLENE OXIDE	0.02	0.02	0.01	0.03	0.03	0.07	_	0.02	0.02
71	62.2	E-METHYLEUGENOL	0.12	0.01	_	0.04	0.03	_	_	_	_
72	87.8	AROMATIC COMPOUND	_	0.02	0.09	0.09	0.03	0.02	0.06	0.24	0.05
73	7.6	alpha-THUYENE	0.04	0.02	0.01	_	0.02	0.03	_	_	0.02
74	13.4	alpha-TERPINENE	0.06	0.02	0.01	0.02	0.02	0.01	0.01	_	_
75	17.3	3-OCTANONE	0.03	0.02	0.01	_	0.02	0.03	0.01	_	0.02
76	21.5	CYCLOHEXANONE.3-METHYL	_	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02
77	28.5	alpha-p-DIMETHYLSTYRENE	0.03	0.01	0.01	0.01	0.02	_	_	_	_
78	36.6	Trans-p-MENTH-2-EN-1-OL	0.04	_	_	_	0.02	0.02	0.02	0.03	0.02
79	41.9	ALLO-AROMADENDRENE	_	_	_	0.02	0.02	_	_	0.03	0.04
80	43.9	MYRTENYL ACETATE	0.04	_	_	0.03	0.02	_	_	_	_

81	46.1	VALENCENE	0.05	0.02	0.03	0.04	0.02	_	_	0.01	_
82	48.2	gamma-CADINENE	0.02		0.03	0.03	0.02	_	0.01	0.04	_
83	51.3	beta-DAMASCENONE	_	_	0.02	0.02	0.02	_	_	0.02	_
84	52	SESQUITERPENE Mw=202	0.02	0.02	_	_	0.02	_	_	_	_
85	53.2	p-CYMENE-8-OL	0.08	0.01	0.03	0.02	0.02	_	0.01	0.02	_
86	61.5	AROMATIC COMPOUND	_	_	0.05	0.05	0.02	_	0.02	0.13	0.02
87	72.8	beta-NOOTKATOL	_	0.02	0.04	0.04	0.02	_	0.01	0.05	0.02
88	77.9	SESQUITERPENIC EPOXIDE	0.06	_	_	0.03	0.02	_	_	0.02	_
89	12.7	alpha-PHELLANDRENE	0.06	_	_	0.01	0.01	_	_	_	_
90	33.7	alpha-COPAENE	_	_	0.02	0.06	0.01	0.04	0.05	0.06	0.04
91	34.3	beta-BOURBONENE	0.02	0.01	0.04	_	0.01	0.01	0.01	0.01	0.01
92	44	TERPENIC ESTER	0.07	_		0.03	0.01	_	_	_	_

93	44.3	gamma-MUUROLENE	0.03	_	0.01	0.02	0.01	_	0.01	0.05	_
94	47.2	Trans-PIPERITOL	0.02	0.08	0.01	0.01	0.01	_	0.01	_	_
95	48.4	SESQUITERPENE	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.01
96	49.6	MENTHADIENOL ISOMER	0.02	_	_	0.01	0.01	_	_	_	_
97	52.3	AROMATIC COMPOUND	0.02	_	_	_	0.01	_	_	_	_
98	52.8	CALAMENENE	0.11	0.1	_	0.02	0.01	_	_	_	_
99	53.9	E-GERANYLACETONE	0.02	_	_	0.02	0.01	_	_	0.01	_
100	61.1	MYRTO LACTONE A	_	_	0.04	0.04	0.01	_	0.01	0.08	0.01
101	63.5	Epoxy-6.7-HUMULENE	0.02	_	_	0.01	0.01	_	_	_	_
102	69.8	EUGENOL	0.05	0.01	0.03	0.03	0.01	_	_	_	_
103	70.1	SESQUITERPENOL	0.01	0.01	0.01	0.02	0.01	_	_	_	_
104	72.2	CARVACROL	0.06	0.01	_	0.02	0.01	_	_	_	_

105	73	alpha-CADINOL	_	0.01	0.03	0.03	0.01	_	0.01	0.07	0.01
106	5.1	ALIPHATIC ESTER	_	_	_	_	_	_	_	_	0.03
107	5.5	ISOVALERALDEHYDE	0.01	_	_	_	_	_	_	_	_
108	8.1	FURAN. 2.5-DIETHYLTETRAHYDRO-	_	_	_	_	_	_	0.01	0.02	_
109	10.9	THUYADIENE + PINADIENE	0.03	_	_	_	_	_	_	_	_
110	17	MENTHATRIENE ISOMER	_	_	0.01	_	_	_	_	0.01	_
111	23.6	2-PENTANONE. 4-HYDROXY-4-METHYL-	_	_	_	_	_	_	_	_	0.03
112	25.7	KETONIC COMPOUND	0.01	_	_	_	_	_	_	_	_
113	25.8	MENTHATRIENE ISOMER	_	_	_	_	_	0.01	_	_	_
114	33.3	YLANGENE	_	_	_	_	_	_	0.02	_	_
115	34.4	KETONIC COMPOUND Mw=138	_	_	_	_	_	_	_	0.01	_
116	35.1	alpha-GURJUNENE	_	_	0.01	_	_	_	_	_	_

117	35.2	beta1-CUBEBENE	_	_	_	0.02	_	0.01	_	0.01	_
118	36.1	LINALYL ACETATE	0.01	_	_	_	_	_	_	_	_
119	36.4	PINOCARVONE	0.07	_	_	_	_	_	_	_	_
120	37.2	FENCHOL + SESQUITERPENE	0.03	_	_	_	_	_	_	_	_
121	37.5	epsilon-CADINENE	_	_	0.15	_	_	_	_	_	_
122	37.5	beta-CUBEBENE	_	_	_	_	_	_	_	0.01	_
123	38.1	beta-ELEMENE	_	_	_	_	_	_	0.01	0.01	_
124	38.2	CAMPHENE HYDRATE	0.04	0.07	_	_	_	_	_	_	_
125	38.5	alpha-trans-BERGAMOTENE	_	_	0.09	_	_	_	_	_	_
126	38.8	ISOPULEGOL ISOMER	0.54	_	_	_	_	_	_	_	_
127	39.4	HOTRIENOL	0.05	_	_	_	_	_	_	_	_
128	39.6	2-UNDECANONE	0.03	_	_	_	_	_	_	_	_

129	39.7	SESQUITERPENE	_	_	_	0.01	_	_	_	_	_
130	41.2	UMBELLULONE	0.04	_	_	_	_	_	_	_	_
131	41.2	GURJUNENE ISOMER	_	_	_	_	_	_	0.06	0.06	0.06
132	41.3	1-NONEN-3-OL	_	0.03	_	_	_	_	_	_	_
133	41.8	SESQUITERPENE	0.03	_	_	_	_	_	_	_	_
134	41.9	MENTHADIENOL ISOMER	0.05	_	_	_	_	_	_	_	_
135	42.5	ESTRAGOLE	0.01	_	_	_	_	_	_	_	_
136	43	CITRONELLYL ACETATE	_	_	_	_	_	0.02	0.03	0.02	0.03
137	43	Cis-VERBENOL	0.19	0.04	_	_	_	_	_	_	_
138	44.2	E-TAGETENONE	_	_	0.02	_	_	_	_	_	_
139	44.8	PIPERITONE OXIDE	_	_	_	_	_	0.03	0.03	0.02	0.03
140	44.8	LEDENE	_	_	_	_	_	_	_		
141	45.5	ALIPHATIC ESTER	0.01	_	_	_	_	_	_	_	_

142	46	alpha-BULNESENE	_	_	_	_	_	_	0.01	_	_
143	46.4	ALIPHATIC ALCOHOL		_	_	_	_	_	_	_	_
144	46.5	SESQUITERPENE	_	_	_	_	_	_	_	0.04	0.06
145	46.5	alpha-MUUROLENE	_	_	0.02	1.17	_	0.02	0.02	0.02	0.02
146	46.7	alpha-ZINGIBERENE	0.05	0.02	_		_		_	_	_
147	47.4	beta-CURCUMENE	0.02	_	_	_	_	_	_	_	_
148	49.1	beta-SESQUIPHELLANDRENE	0.08	_	_	0.04	_	_	_	_	_
149	49.6	CADINA-1.4-DIENE	_	_	_	_	_	_	_	0.01	_
150	50.8	alpha-AMORPHENE	_	_	0.02	_	_	_	_	0.02	_
151	50.9	SABINOL ISOMER	0.04	_	_	0.01	_	_	_	_	_
152	51.3	KETONIC COMPOUND	_	_	_	_	_	_	0.02	0.02	0.01
153	53.7	TERPENIC ESTER	0.03	_	_	_	_	_	_	_	_
154	54.2	ALIPHATIC ALCOHOL	0.02	_	_	_	_	_	_	_	_

155	55	AROMATIC COMPOUND	_	_	_	_	_	_	_	0.01	_
156	56.8	AROMATIC COMPOUND	_	_	0.01	_	_	_	_	_	_
157	61	PHENYLETHYLIC ESTER	0.03	_	_	_	_	_	_	_	_
158	61.6	SESQUITERPENIC EPOXIDE	0.01	0.01	_	_	_	_	_	0.01	_
159	62.6	AROMATIC COMPOUND	_	_	0.02	0.02	_	_	_	0.03	_
160	63	LEDOL	_	_	_	_	_	_	_	0.01	_
161	63.6	NEROLIDOL	_	_	_	_	_	0.1	0.1	0.3	0.12
162	64.1	GERMACRENE D-4-OL	0.02	0.01	_	_	_	0.01	_	_	_
163	64.5	GLEENOL	_	_	_	_	_	_	_	0.02	_
164	64.6	BENZOIC COMPOUND	0.01	_	_	_	_	_	_	_	_
165	65.4	GLOBULOL	_	_	0.02	0.02	_	_	_	0.05	_
166	65.8	VIRIDIFLOROL	_	_	0.01	0.01	_	_	_	0.04	_

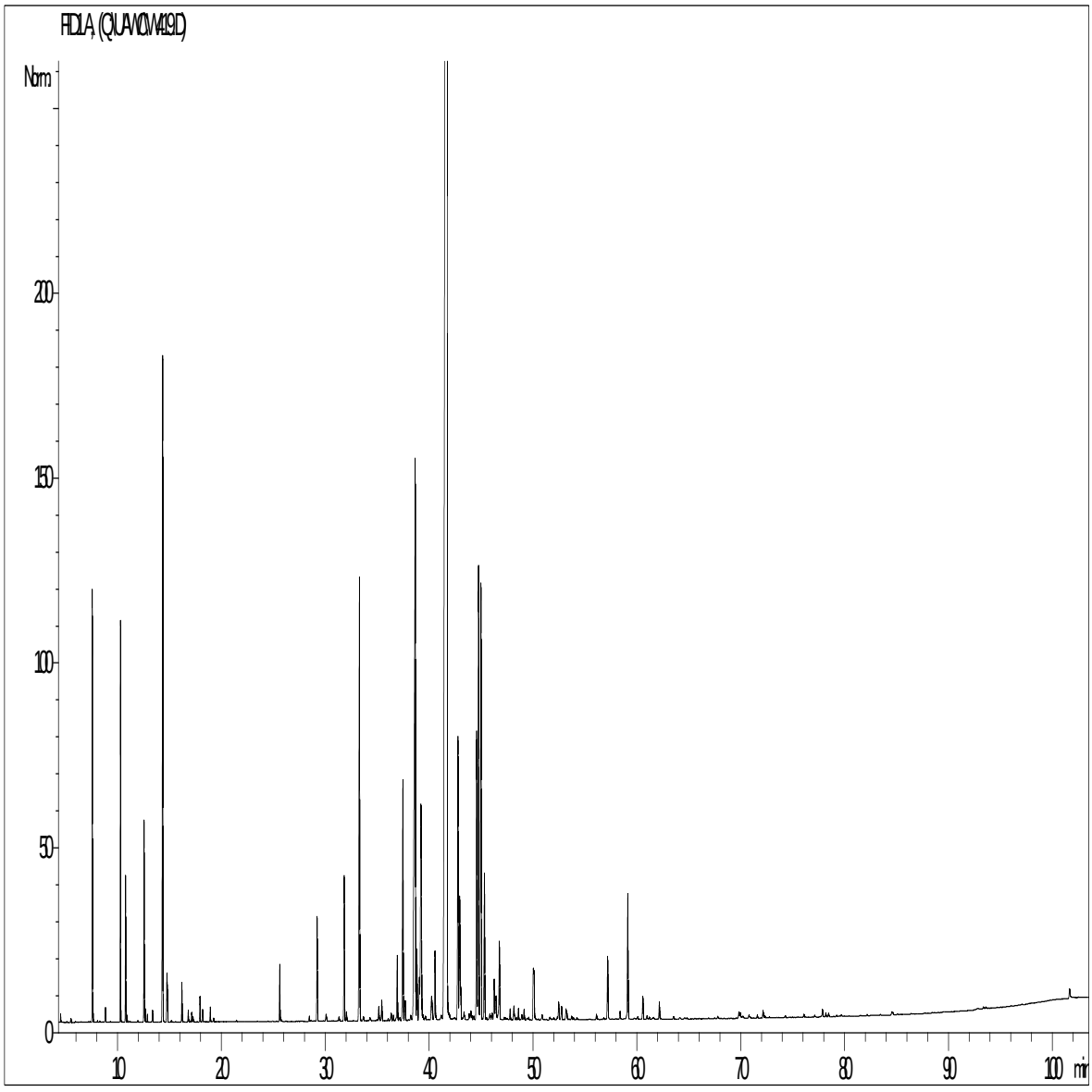
167	66.9	EUDESMOL ISOMER	_	_	_	_	_	_	_	0.01	_
168	68.1	PENTADECANONE. TRIMETHYL-	_	_	_	_	_	0.02	0.04	0.11	0.03
169	70	ISOTHYMOL	0.05	_	_	0.02	_	_	_	0.02	_
170	70	FOKIENOL	_	_	_	_	_	0.03	0.02	0.07	0.03
171	70.1	T-CADINOL	_	_	0.01	0.01	_	_	_	0.01	_
172	70.2	DITERPENE Mw=272	0.02	_	_	_	_	_	_	_	_
173	70.8	THYMOL	0.03	_	_	0.01	_	_	_	0.01	_
174	70.8	DITERPENE Mw=272	0.01	_	_	_	_	_	_	_	_
175	70.9	alpha-MUUROLOL	_	_	0.01	0.02	_	_	_	0.03	_
176	71.5	delta-CADINOL	_	_	_	_	_	_	_	0.02	_
177	71.6	ISOCARVACROL	0.02	_	_	_	_	_	_	_	_
178	72.3	alpha-BISABOLOL	0.01	_	_	_	_	_	_	_	_

179	74	EUDESMA-7-EN-4-OL	_	_	_	_	_	0.01	0.01	0.04	0.02
180	74.3	SESQUITERPENOL	0.01	_	_	_	_	_	0.01	0.01	_
181	76.1	DITERPENE Mw=272	0.02	_	_	_	_	_	_	_	_
182	77.1	CARYOPHYLLA-3.7-DIEN-6-OL	0.02	0.01	_	0.02	_	_	_	0.01	_
183	78.2	beta-ASARON	0.03	_	_	_	_	_	_	_	_
184	78.5	SANTALOL ISOMER	0.03	_	_	_	_	_	0.01	_	_
185	84.5	13-HEXYLOXACYCLOTRIDEC-10-EN-2-ONE Mw=280	0.04	_	_	_	_	_	0.02	_	_
186	91.3	AROMATIC COMPOUND	_	_	_	0.02	_	_	0.02	0.03	0.01
187	93.6	MYRISTIC ACID	0.02	_	_	_	_	_	_	_	_
188	101.7	PALMITIC ACID	0.09	_	_	0.04	_	_	0.04	0.04	0.05
189	45.9	COMPOUND Mw=152	_	_	0.03	_	_	_	_	_	_
190	51.6	NEROL	0.02	_	_	_	_	_	_	_	_

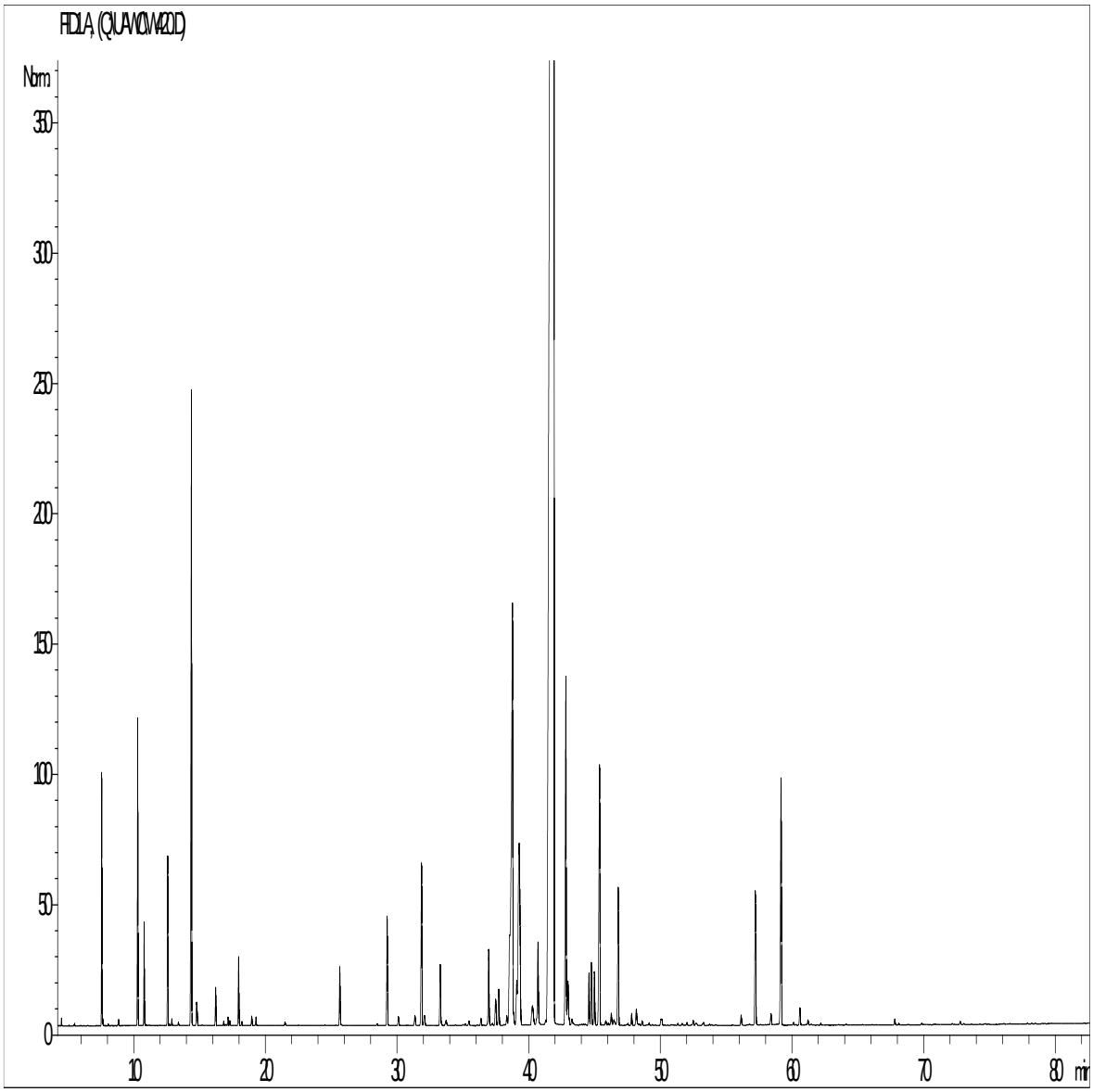
191	52.5	ALIPHATIC ALCOHOL Mw=150	0.13	0.03	_	_	_	_	_	_	_

RT = Retention Time. H1 = after one hour. H2 = after two hours. H3 = after three hours. H4 = after four hours. Tot = Total sample. _ = no compound detected.

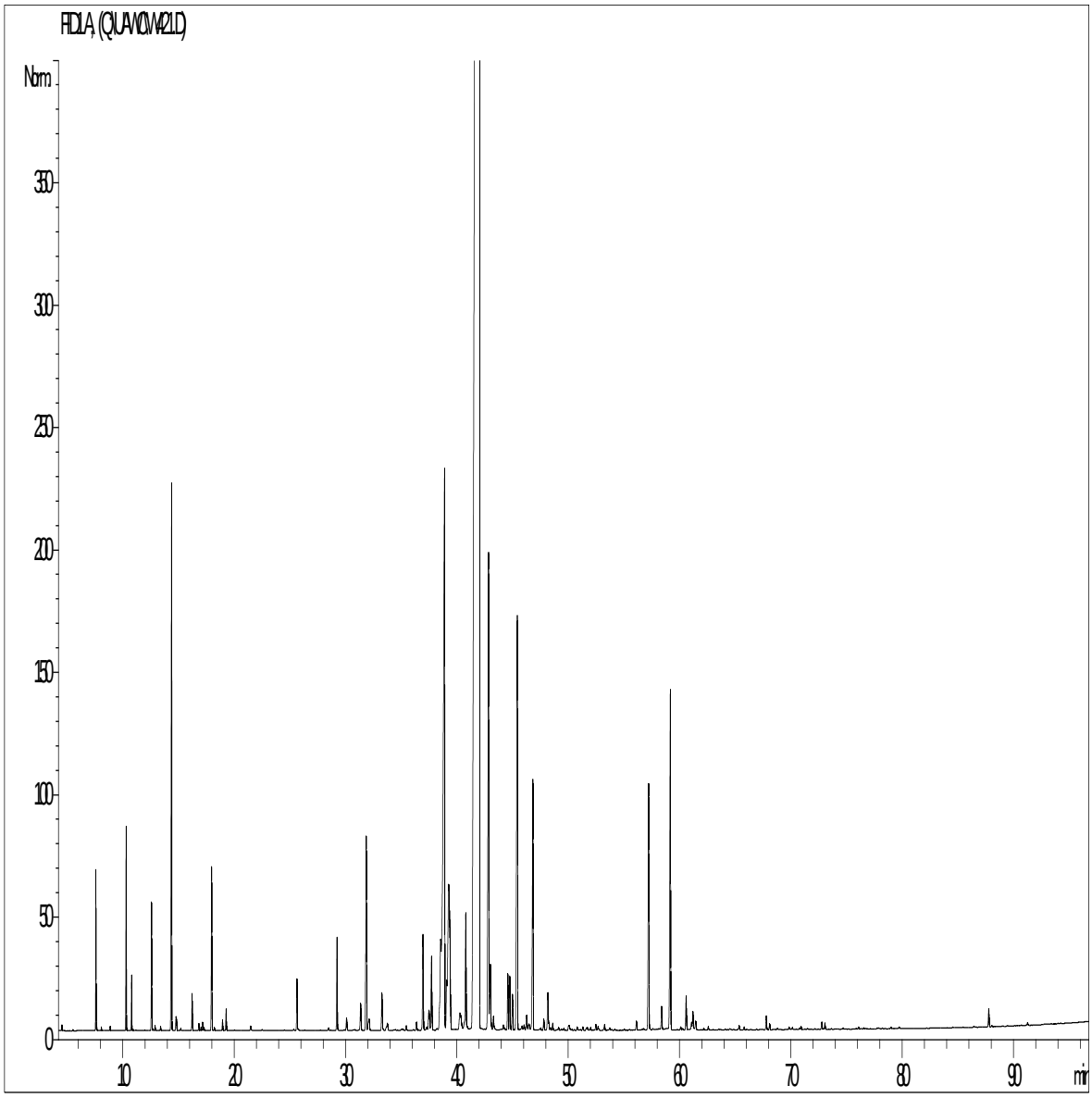
Appendix 2: Chromatographic profiles for the GC-MS results.



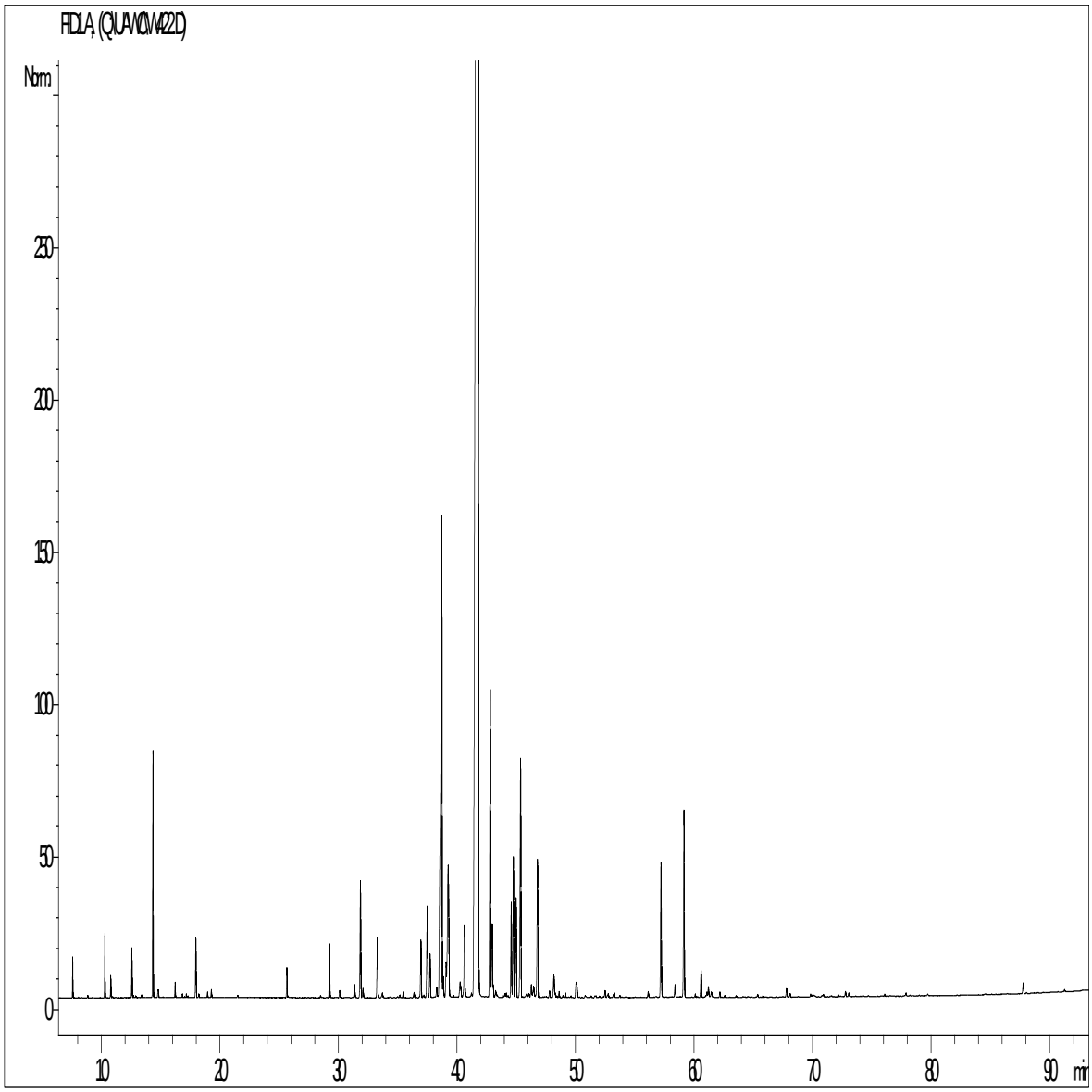
Chromatographic profile for the EO of *M. fruticosa* sample (after 1-hour extraction for Aug/2018 harvest).



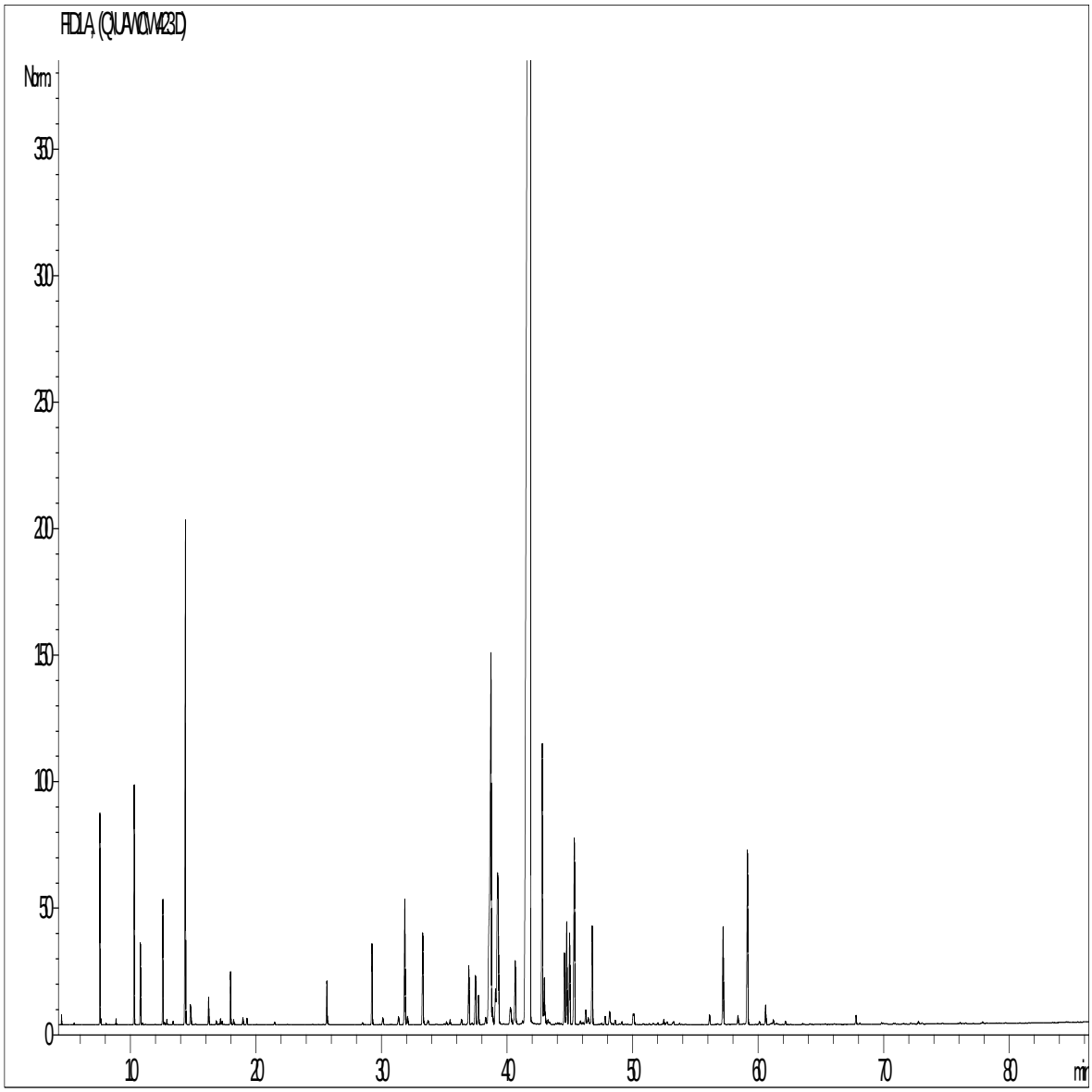
Chromatographic profile for the EO of *M. fruticosa* sample (after 2-hour extraction for Aug/2018 harvest).



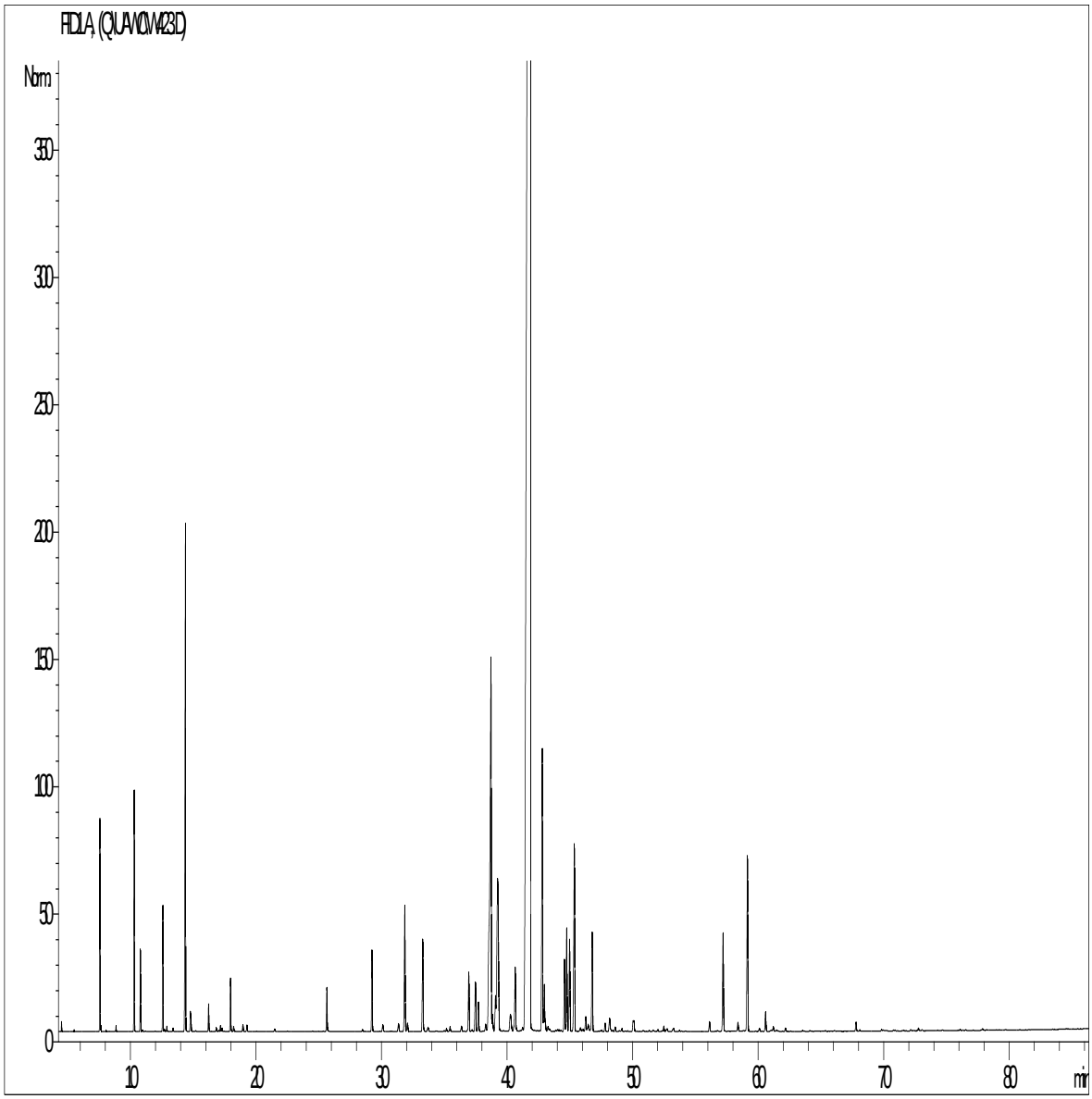
Chromatographic profile for the EO of *M. fruticosa* sample (after 3-hour extraction for Aug/2018 harvest).



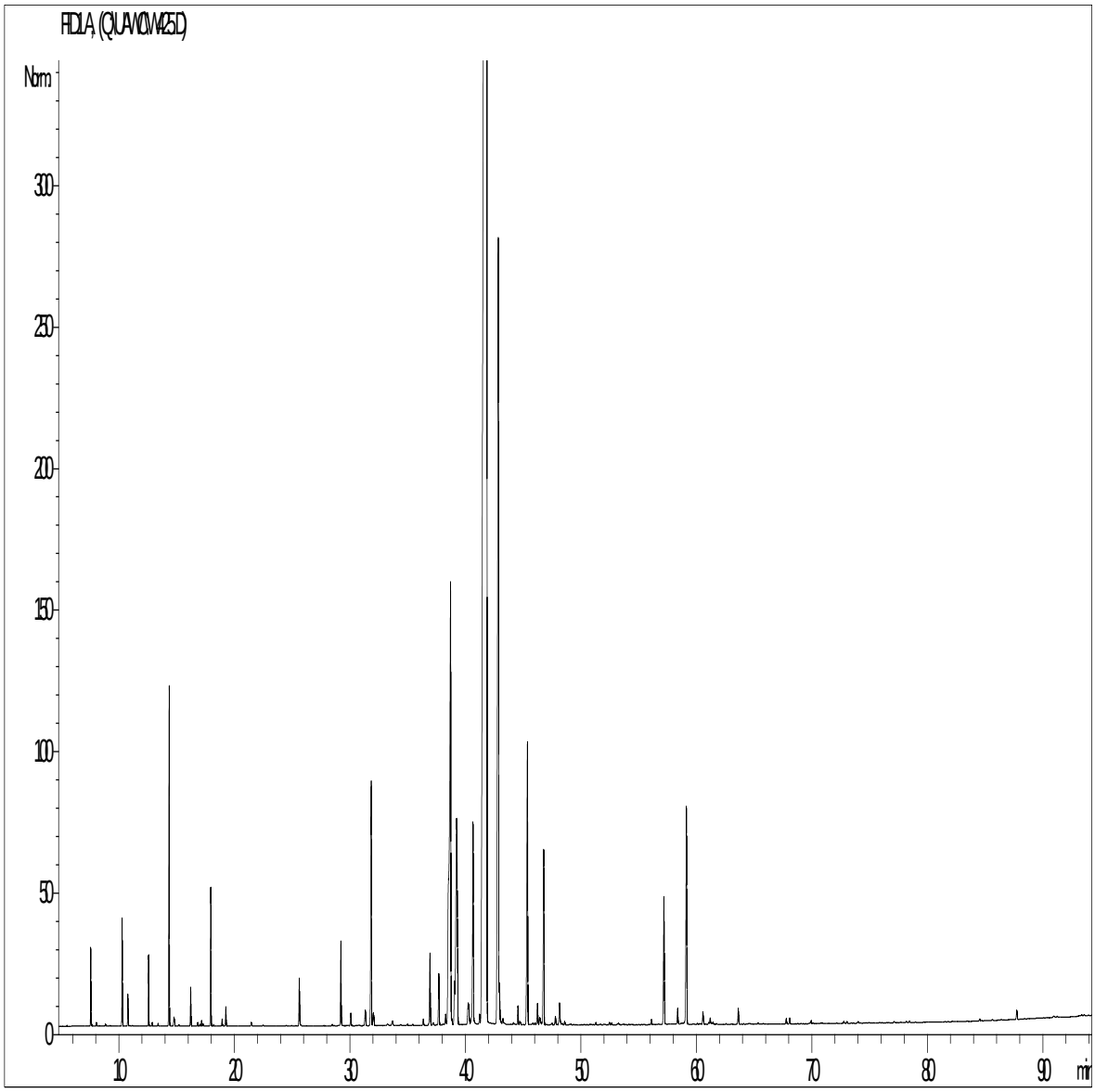
Chromatographic profile for the EO of *M. fruticosa* sample (after 4-hour extraction for Aug/2018 harvest).



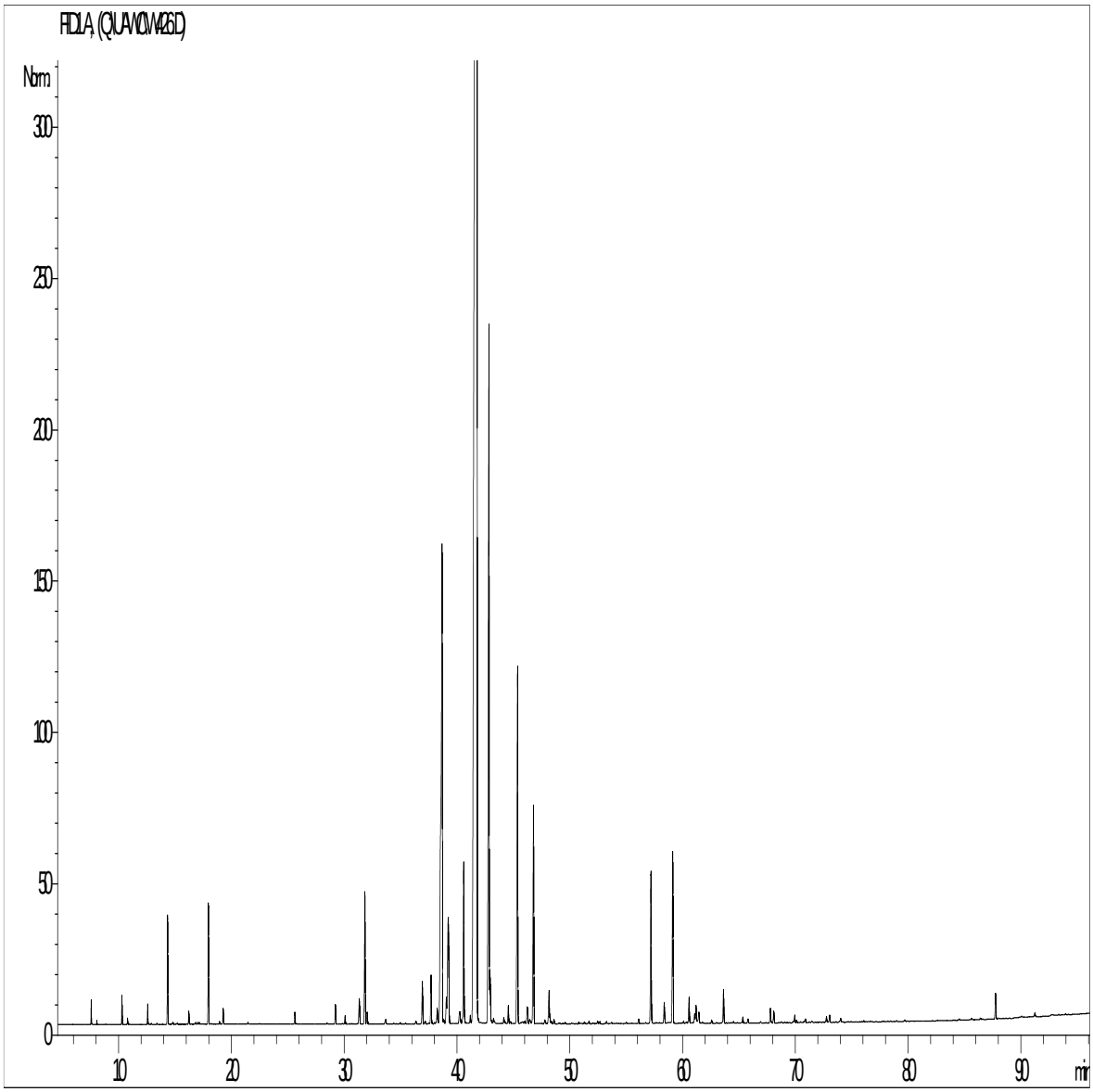
Chromatographic profile for the EO of *M. fruticosa* sample (Total sample for Aug/2018 harvest).



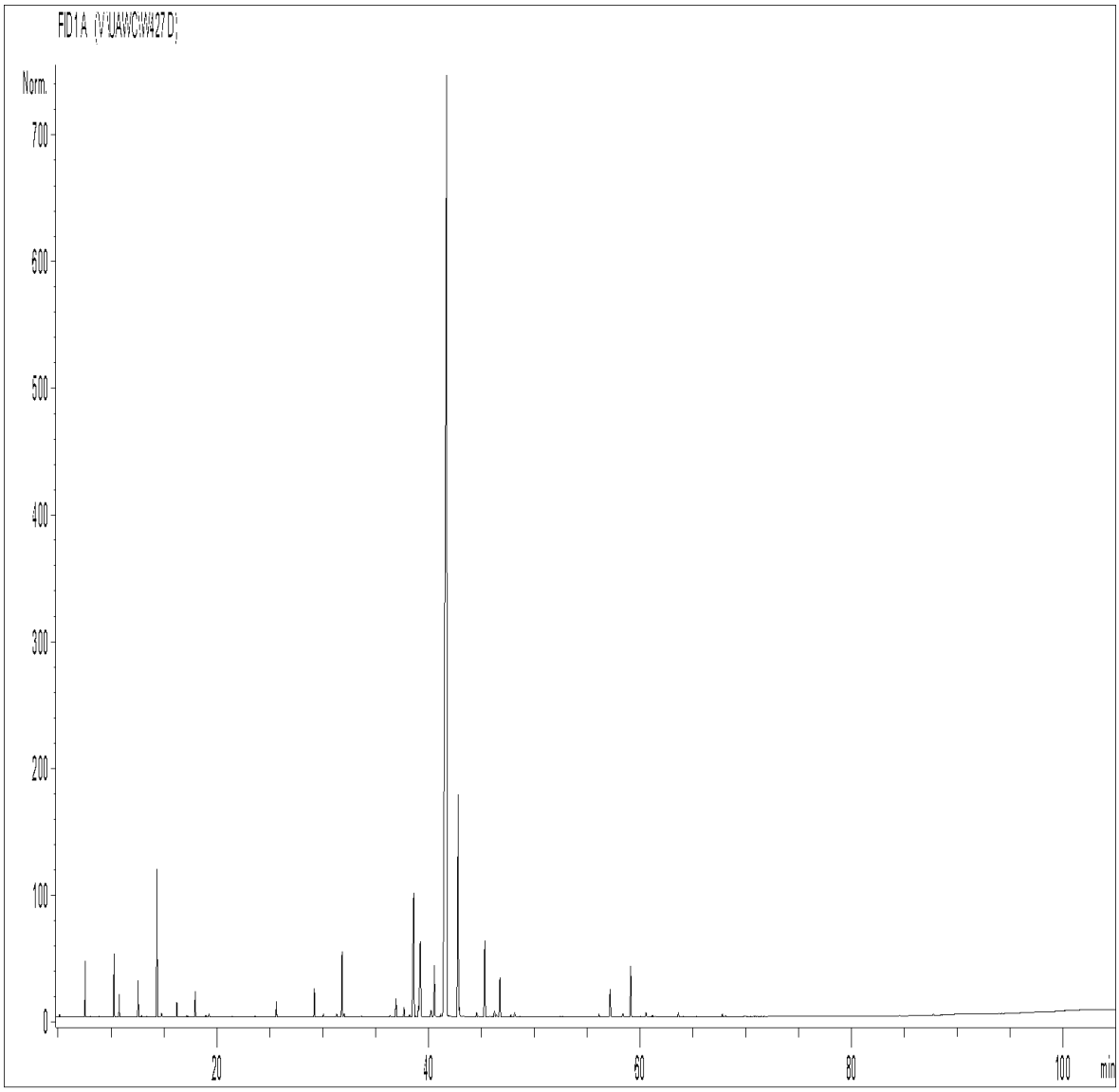
Chromatographic profile for the EO of *M. fruticosa* sample (after 1-hour extraction for Oct/2018 harvest).



Chromatographic profile for the EO of *M. fruticosa* sample (after 2-hour extraction for Oct/2018 harvest).



Chromatographic profile for the EO of *M. fruticosa* sample (after 3-hour extraction for Oct/2018 harvest).



Chromatographic profile for the EO of *M. fruticosa* sample (Total sample for Oct/2018 harvest).

Abstract in Arabic

تقييم إنتاجية القرنية المحلية ودراسة أثر استخدام زيتها العطري على تثبيط نمو الأعشاب

إعداد : اسماء محمد مشارقه.

بإشراف: الدكتورعبد المحسن العلامي.

الملخص:

تعتبرالقرنية واحدة من النباتات العطرية دائمة الخضرة، والتي تنمو بشكل عام في دول حوض البحر المتوسط وفي فلسطين بشكل خاص. نظراً لعدم توفر دراسات سابقة ومعلومات كافية حول إنتاجية هذه النبتة في فلسطين وإمكانية استخدام زيتها كمثبط إنبات للأعشاب الضارة تم عمل هذه الدراسة.

لتحقيق أهداف الدراسة تم حساب الإنتاجية للأجزاء الخضرية من النباتات (الأوراق والأزهار) ثمان مرات خلال سنتين ل 568 شتلة زرعت في ممحطة العروب التابعة للمركز الوطني للبحوث الزراعية – الخليل. بعد ذلك، تم استخلاص الزيت العطري للنباتات مباشرة بعد كل عملية حصاد عن طريق التقطير بالبخار. خلال عملية الاستخلاص لحصاد شهري اغسطس وأكتوبرمن عام 2018 تم اخذ عينات من الزيت الناتج بعد كل ساعة وعينة بعد انتهاء العملية ليتم تحليلها على جهاز الكروماتوجرافيا الغازية ومطياف الكتلة GC-MS لمعرفة المركبات وتراكيزها في كل عينة لاختيارالوقت والمدة الأنسب للاستخلاص بحيث تكون نسبة البوليجون أعلى ما يمكن لاستخدام الزيت كمانع انبات لبذور الاعشاب. ثم تم اختبار الزيت الذي تم استخلاصه من حصاد شهر اغسطس/2018 كمثبط للانبات على بذور العديد من الأعشاب الضارة الشائعة في فلسطين(عرف الديك، الشوفان البري، نجليات، قثاء الحمار، والخبيزة). تم عمل التجربة في اوعية وضعت في بيئة مفتوحة ومعرضة للتغيرات المناخية والبيئية بحيث تكون قريبة من الظروف الطبيعية التي تزرع فيها معظم النباتات والمحاصيل في فلسطين. استخدمت ثلاثة تراكيز مختلفة من الزيت (0.05، 0.1 و0.2%) لاختيار التركيز الأفضل في العمل كمثبط للإنبات من بينها.

بالنسبة للنتائج فقد تبين أن معدل انتاج النبتة السنوي في كل من عامي 2017 و 2018 كان 0.2 و 0.22 كغم، على التوالي. أما بالنسبة للزيت المستخرج فقد تم حساب كثافته وقد كانت 0.9459 ± 0.00184، أما نسبة معدل إنتاج الزيت العطري المستخرج من النبات طازجا (بدون تجفيف) فقد كان 0.82 و 0.8% لعامي 2017 و2018، على التوالي، وأعلى نسبة من الزيت تكون في نهاية الأشهر الصيفية (أغسطس-أكتوبر) كما أظهرت النتائج. بالنسبة للتحليل الكيميائي لعينات الزيت التسعة التي تم أخذها خلال عملية الاستخلاص لحصاد شهري اغسطس واكتوبر من عام 2018 والتي تم تحليلها عن طريق جهاز الكروماتوجرافيا الغازي ومطياف الكتلة GC-MS أظهرت وجود 191 مركب كيمائي بنسب متفاوتة. كان البوليجون هو المركب الرئيسي والأكثر وفرة بشكل واضح في جميع العينات ولكن بنسب متفاوتة. تبين أيضاً من خلال التحليل الكيميائي أن نسبة البوليجون كانت عالية في عينتي شهري آب وتشرين الثاني لعام 2018، حيث يعد البوليجون المركب الأساسي المسؤول عن تثبيط الإنبات للبذور، أما بالنسبة لأفضل مدة زمنية في استخلاص الزيت فقد تبين ساعتين هي المدة الأفضل اقتصادياً وكيميائياً. وكذلك تبين أن الزيت المستخرج من القرنية له أثر كبير على عدد الأعشاب التي تظهروتنمو حول المحاصيل الزراعية. وقد وجد أيضاً أنه بزيادة تركيز الزيت فإن نسبة التأثير تزيد بشكل ملحوظ. حيث أن معدل عدد الأعشاب النابتة قد قل بنسبة 24، 27 و 63% بالمقارنة بالعينة المحايدة لكل من الثلاث تراكيز التي استخدمت 0.05، 0.1 و0.2%، على التوالي.

وفي النهاية فقد بينت الدراسة الحالية أن الزيت العطري للقرنية يمكن استخدامه كمثبط لإنبات وكمبيد عشبي طبيعي بذور الاعشاب و بديل للمبيدات الكيماوية الضارة، وبالأخص في الزراعة العضوية.