CHEMICAL COMPOSITION AND ACTIVITY OF VOLATILE COMPOUNDS AND ESSENTIAL OILS IN TOMATO ACCESSIONS AGAINST THE TOBACCO SPIDER MITE, *TETRANYCHUS EVANSI* BAKER AND PRITCHARD.

LUCY KANANU MURUNGI
(Bsc. Hons)

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF MASTER OF SCIENCE DEGREE IN HORTICULTURE TO THE DEPARTMENT OF HORTICULTURE OF JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY (J.K.U.A.T).

2005

DECLARATION

This thesis is my original work and has not been presented in any other University for examination.

CANDIDATE

Lucy Kananu Murungi

Sign ..... Date.....

SUPERVISORS

This thesis has been submitted for examination with our approval as university and institutional Supervisors.

Prof. Stephen G. Agong,

DVC, Administration Planning Development (APD),

Jomo Kenyatta University of Agriculture and Technology (JKUAT),

P.O. Box 62000,

Nairobi.

Sign: ..... Date.....

Dr. Peter W. Masinde,

Lecturer, Department of Horticulture,

Jomo Kenyatta University of Agriculture and Technology (JKUAT),

P.O. Box 62000,

Nairobi.

Sign: ..... Date.....

Dr. Markus Knapp,

Coordinator, Red Spider Mite Project (RSM),

International Center of Insect Physiology and Ecology (ICIPE),

P.O. Box 30772,

Nairobi.

Sign: ..... Date.....

Prof. Ahmed Hassanali,

Head, Behavioral and Chemical Ecology Department (BCED),

International Center of Insect Physiology and Ecology (ICIPE),

P.O. Box 30772,

Nairobi.

Sign: ..... Date.....

DEDICATION

This thesis is dedicated with deepest love and affection to my husband George and my parents, Mr. & Mrs. Murungi, for their love, generosity, wisdom and strength that have always inspired me to be the best I can.

ACKNOWLEDGEMENT

I wish to register my sincere appreciation to my ICIPE Supervisors Dr. Markus Knapp and Prof. Ahmed Hassanali for their generous support of this research work and for numerous valuable comments throughout the research study. Permission to enroll for the study was granted by JKUAT through the BPS to whom I am also grateful. My gratitude also goes to my University Supervisors: Prof. Stephen Agong, DVC APD and Dr. Masinde for their guidance in planning of the research work, providing advice and suggestions that contributed greatly to the completion of this study. I am indebted to Dr. Ngamau, the Chairman of Horticulture Department for his initial letter that secured my placement at ICIPE and all staff members of JKUAT Horticulture department for their moral support. I am also indebted to Dr. Hans Herren, who was then Director General ICIPE, for granting me the opportunity to carry out my study at ICIPE and Prof. Onesmo Ole Moiyoi, Director Research and Partnerships for approving my stipend under Dissertation Research Internship Programme (DRIP) through the Capacity Building Department at ICIPE. I wish also to thank the technical staff of the Red Spider Mite Project; Carlos Kyallo who assisted a lot in taking care of the tomatoes in the green house, also Bernard Muia and Miriam Kungu for their assistance in handling mites. I also thank Edward Nyandat, Wanyama and other staff and students of Behavioral and Chemical Ecology Department (BCED) for their advice and practical support in laboratory experiments. The course work and research funds were provided by the Red Spider Mite Project based at ICIPE funded by German Federal Ministry for Economic Cooperation and Development (BMZ). Above all, I thank the Almighty God for his abundant grace He showered upon me throughout my course of study.

LIST OF ABBREVIATIONS

°C	Degrees centigrade
APD	Administration Planning and Development
AVRDC	Asian Vegetable Research and Development Center
BCED	Behavioral and Chemical Ecology Department
CABI	Centre for Agriculture and Biosciences International
CO_2	Carbon dioxide
DVC	Deputy Vice Chancellor
FAO	Food Agriculture Organization
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometer
GTZ	German Agency for Technical Co-operation
ICIPE	International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
JKUAT	Jomo Kenyatta University of Agriculture and Technology
L: D	Light: Dark
PI	Plant Introduction
PROC GLM	Procedure for Generalized Linear Model
RH	Relative Humidity
SAS	Statistical Analysis System
SNK	Student-Newman-Keuls
χ^2	Chi-square

ABSTRACT

Development of tomato varieties resistant to *Tetranychus evansi* Baker and Pritchard requires a thorough knowledge of the key factors contributing to the resistance. The overall objective of this study was to establish the chemical composition and activity of leaf emitted volatile phytochemicals and compounds in essential oils against the tobacco spider mite *T. evansi* in cultivated tomato (*Lycopersicon esculentum* Mill.) and wild *Lycopersicon* species in order to provide information for the development of resistant tomato varieties.

Seven tomato accessions were used in order to establish the volatile compounds and essential oils present. Volatile compounds were obtained by trapping with adsorbents (Silica C-18) suspended from the top of glass chambers enclosing potted plants. Chemical composition and quantification of volatile compounds was studied in the laboratory using gas chromatography-mass spectrometer (GC-MS). The response of the adult female mites was studied in the laboratory under room temperature conditions. Six tomato accessions were evaluated by either testing the response of mites to plant odors in a Y-tube olfactometer or plant acceptance using leaf disks. The accessions were compared to Money Maker, a *T. evansi* susceptible variety grown in Kenya that served as control. Essential oils were obtained by steam distillation using Dean-Stark apparatus and the chemical composition was established by GC-MS. Essential oil distilled from the various accessions was diluted to 0.01 in order to establish the response of mites under glass slide bioassays. Mite response was compared for treated cotton balls with essential oil of the test accession and untreated ones (without any essential oil), which served as a control.

The analyses of volatile phytochemicals revealed 2-tridecanone as the most abundant methyl ketone in accession 51 (70.7%) while it occurred at lower concentrations in accessions 13, 162, 428 and 460 ranging between 0.1-1.5%. 2-Undecanone was also identified in accessions 13, 51 and 428 ranging between 0.01-0.2% 3-methy-2-butanone was also established in the volatile compounds in various accessions. Sesquiterpenes that were established included β-phellandrene in accessions 13 (0.03%) and 428 (0.02%).

α-Terpinene occurred in accessions 1 (0.05%) and 13 (0.01%) while α-pinene and δ^3-carene occurred in accessions 13 (0.01%) and 1 (0.2%) respectively. The number of mites that responded positively to olfactometer stimuli in whole potted tomato plants and leaf disk bioassay was significantly lower than in the control (Money Maker) in four accessions (51, 428, 460 and 13).

Several methyl ketones and sesquiterpenes were established in the essential oils at varying levels. 2-Tridecanone occurred in accessions 51, 162, 182, 428 and 460 and ranged between 1.1-49.0%, while 2-undecanone occurred in accessions 51, 182, 428 and 460 and it ranged between 0.3-13.0%. Other methyl ketones such as 2-Pentadecanone, 2-dodecanone, 2-decanone, β-demascenone, β-ionone and 3-Methyl-2-butanone occurred in different accessions at varying proportions.

β-Caryophyllene, β-phellandrene, δ-elemene, β-elemene, α-humulene and δ-cadinene were found to be the most widespread sesquiterpenes in all the accessions used in our experiment with β-caryophyllene occurring in accessions 1, 13, 51, 162, 182, 428 and 460 and ranging between 0.8-20% while β-phellandrene occurred in accessions 1, 13, 162, 428 and 460 and ranging between 0.3-4%. δ-Elemene occurred in accessions 1, 13, 162, 182, 428 and 460 and it ranged between 0.1-7.0% while β-elemene occurred in accessions 13, 51, 162, 182 and 428 and it ranged between 0.2-2.5%.

α-Humulene occurred in accessions 1, 13, 51, 162, 182, 428 and 460 and it ranged between 0.1-16.0%, while δ-cadinene occurred in accessions 13, 51, 162, 182, 428 and 460 and it ranged between 0.03-1.5%. Other terpenes that were found only in trace amounts in a few accessions are β-bisabolene, terpinolene, α–pinene, β-pinene, α-terpinene, camphene, δ^3-carene, azulene and ρ-cymene. Limonene occurred in accession 428 at 13.0 %. The response of the mites to different concentrations of essential oils was not significantly different in all accessions in the olfactometer bioassay. Mite response to essential oils (0.01 concentration) was significantly lower than the control (untreated cotton balls) in accessions 13, 51, 162, 182, 428 and 460 while accession 1 (Money Maker) did not show any significant differences from the control on glass slide bioassay.

Conclusively, this study reveals some chemical compounds in volatiles and essential oils in foliage of tomato accessions, which can be used in development of resistant tomato varieties.

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CHAPTER ONE

1.0: GENERAL INTRODUCTION

The tomato, *Lycopersicon esculentum* Mill., belongs to the vast family of Solanaceae, which also includes potatoes, capsicums, brinjals and black nightshade. Tomato is part of the everyday diet of most people as an important source of lycopene (the pigment that gives the fruit its red colour), vitamin C, niacin and thiamin. The fruit is used raw or cooked, made into soup, sauce, juice, ketchup, paste, puree or powder, canned and used unripe in chutneys (Hill and Waller, 1994). An antioxidant property of lycopene has raised interest in tomato as a vegetable with anticancer properties (Di Mascio *et al.*, 1989). Also tomato fruits and leaves are used as medicine for treatment of earache and urinary tract diseases (FAO, 1993). Tomato has an annual world production of about 108.499 million metric tons, with 12.428 million metric tons being produced in Africa (FAO, 2002).

Tomato was ranked the second most important vegetable in Kenya with a production of 260,000 metric tons in 1999 surpassed only by brassicas with 351,000 metric tons. However, with an average of about 15 metric tons per hectare (Anonymous, 2000), tomato yields are still far below the potential of the crop. Average yields as low as 7 t/ha have been reported from Tanzania (Swai, 1995) and 10 t/ha from Uganda (Mwaule, 1995) while yields as high as 100 t/ha have been reported from commercial farms in Zimbabwe (Varela, 1995). Tomato is also a major cash crop and a source of livelihood for many farmers in the high rainfall districts of Central, Western, Eastern, Nyanza and Rift Valley provinces in Kenya. Tomato production in the coastal region is mainly practiced in drier areas, by use of irrigation.

Production problems in Kenya include pests and diseases, high cost of inputs, poor seed quality and adverse weather conditions. Also, marketing of tomato is uncoordinated and disorganized leading to exploitation of farmers by middlemen. Poor production planning leading to over-supply in some months hence low prices also occur.

The major diseases of tomato include; bacterial wilt caused by *Ralstonia solanacearum* Smith, fusarium wilt caused by *Fusarium oxysporium* Snyder and Hansen, early blight caused by *Alternaria solani* Ell. and Martin (Sor), late blight caused by *Phytopthora infestans* Mont. de Bary, septoria leaf spot caused by *Septoria lycopersici* Spegazzini, bacterial canker caused by *Clavibacter michiganense* Smith (MOARD, 2000). The tomato is also attacked by a number of serious arthropod pests (Lange and Bronson, 1981). Among the major arthropod pests that attack tomato in Kenya are peach aphids (*Myzus persicae* Sulzer), African bollworm (*Helicoverpa armigera* Hübner), tobacco white flies (*Bemisia tabaci* Gennadius), leaf miners (*Liriomyza trifolii* Burgess), onion thrips (*Thrips tabaci* Lindeman), and russet mites (*Aculops lycopersici* Massee) (Varela *et al.*, 2003).

The tobacco spider mite *Tetranychus evansi* Baker and Pritchard is also an important pest of tomato in Kenya and other countries in eastern and southern Africa (Knapp *et al.*, 2003; Saunyama and Knapp, 2003). Because of their great reproductive capacity, the mites are able to destroy plants within a short period of time if environmental conditions are favorable to their development as is the case in hot and dry weather. To date, farmers in Africa rely on frequent application of highly toxic acaricides to control *T. evansi*, which results in both contamination of the farmers produce and the environment. However, these acaricides applications are often ineffective (Sibanda *et al.*, 2000; Saunyama and Knapp, 2003).

This has resulted in speeding up of mite resistance to chemicals faster than most pests due to their short life cycle as observed with *Tetranychus urticae* Koch (Yoon *et al.*, 2001; James and Price, 2002) and *T. cinnabarinus* Boisduval (Guo *et al.*, 1998). Resistance to many arthropod pests has been reported in the wild tomato species; *Lycopersicon hirsutum* var. *glabratum* C. H. Mull, *L. hirsutum* var. *hirsutum* Humb and Bonpl, *L. peruvianum* Mill and *L. pennellii* Correll D'Arcy (França *et al.*, 1984; Gentile *et al.*, 1969; Rick, 1973), but the species do not have immediate commercial value. Plants contain a rich diversity of phytochemicals that have been implicated as important mediators of insect-plant associations (Rosenthal and Janzen, 1979).

Volatile metabolites of *Lycopersicon* species are of interest because of their roles in host defense against arthropod herbivores (Buttery *et al.*, 1990, 1993; Carter *et al.*, 1989a; Lin *et al.*, 1987). Glandular trichomes that accumulate large quantities of terpenes and other essential oils have been found to be associated with insect resistance in a number of species in the genus *Lycopersicon* (Fery and Kennedy, 1987; Lin *et al.*, 1987). Breeding programmes aimed at introgressing arthropod resistance from wild *Lycopersicon* species into the cultivated tomato often face methodological difficulties of keeping uniform conditions for infestations necessary for resistance selection (Stevens and Rick, 1986).

1.1.0: Justification and Objectives

1.1.1: Justification

The main management strategy for the control of spider mites employs frequent application of acaricides. This approach is not sustainable due to various reasons: build up of acaricide resistance, environmental safety and health risks for farmers and consumers, non-availability or prohibitive cost of acaricides, and inadequate spraying equipment. Thus, the increasing concerns about the overuse of pesticides have led to farmers seeking more environmentally benign methods for controlling arthropod pests. Plant resistance and biological control are among the non-chemical strategies for mite control. Resistant varieties are environmentally friendly and in general a cost-effective method of reducing pest damage; rendering them a most desirable tool for resource-poor smallholder agriculture. The resistance comes with the seed and needs little extra cost to make it work (Ampofo, 1995). Resistance to tetranychid mites seems to be a rather common but largely unexploited trait (Ponti, 1982).

Pest resistance levels of current tomato cultivars are not sufficiently high to permit a significant reduction in the amount of pesticides used in the tomato crop. Several tomato varieties and wild *Lycopersicon* accessions with resistance to different tetranychid species (mostly *T. urticae*) have been identified (Farrar and Kennedy, 1991). Only one study dealt with *T. evansi* (Silva et al., 1992) identifying an accession of *L. hirsutum* var. *glabratum* as the most resistant genotype.

Development of cultivars with increased levels of arthropod resistance would be an important component for integrated pest management programs aimed at reducing pesticide applications, allowing for diminished environmental degradation.

1.1.2: Objectives

Overall objective

To evaluate the role of volatile compounds and essential oils obtained from foliage of various tomato accessions against the tobacco spider mite, *T. evansi,* in order to provide information for the development of resistant tomato cultivars.

Specific objectives

- To identify and quantify the major volatile compounds found in the leaves of various tomato accessions.
- To obtain and identify major compounds in essential oils from leaves of various tomato accessions.
- To establish the response of female tobacco spider mites to volatile compounds and compounds in essential oils obtained from foliage of different tomato accessions.

CHAPTER TWO

2.0: REVIEW OF LITERATURE

2.1: Tomato and allied species

Tomato, *L. esculentum* is an annual diploid species (2n = 24) (Rick 1971). It is a self-fertilizing plant whose origin is not well established but thought to be Mexico (Esquinas-Alcazar, 1981). The tomato plants have either determinate or indeterminate growth. Their phenology comprises a vegetative period of about 5-7 weeks after planting and a reproductive period of 5-8 weeks, starting 6 weeks after planting. This period is critical for the yield formation. The yields range from 5-60 tons per hectare depending on the variety and the season. Most cultivated varieties are susceptible to various mite groups, mainly Eriophyidae, Tarsonemidae and Tetranychidae (Meyer, 1996). Several wild species exist within the genus *Lycopersicon* including *L. peruvianum, L. hirsutum, L. cheesmanii* Riley and *L. pimpinellifolium* Mill (Williams *et al.*, 1980).

2.2: Spider mite taxonomy

Spider mites of importance in crop protection belong to the class Arachnida, order Acari, suborder Prostigmata, superfamily Tetranychoidae, family Tetranychidae with the genus *Tetranychus* accounting for most of the family members. More than 79 species distributed worldwide are included in this genus and 11 species are known as vegetable feeders in South Africa (Meyer, 1996; Bolland *et al.*, 1998; CAB International, 2000).

2.3: Origin and distribution of tobacco spider mite, *T. evansi*

T. evansi is probably of South American origin and was first recorded in Africa on tobacco in Zimbabwe in 1979 (Blair, 1983). Since its introduction into Zimbabwe, the mite has slowly been moving northwards. Nowadays it is one of the major constraints in tomato production in Mozambique, Malawi, Namibia, Zimbabwe and Zambia (Knapp *et al*, 2003). *T. evansi* was first recorded as *T. marianae* McGregor from northeastern Brazil (Silva, 1954) and Mauritius (Moutia, 1958), and was later redescribed as *T. evansi* (Baker and Pritchard, 1960; Moraes *et al.*, 1987) from the material collected in Mauritius.

T. evansi has also been reported from Reunion, Seychelles and Rodriguez (Gutierrez, 1974; Gutierrez and Etienne, 1986), Congo (Bonato, 1999), Morocco (El Jaouani, 1988), Tunisia (Bolland *et al.*, 1998), Virgin Islands (Moraes *et al.*, 1987), U.S.A (Schuster, 1959; Moraes *et al.*, 1987). Recently, it was also found in Spain (Ferragut and Escudero, 1999) and Portugal (Bolland and Vala, 2000). In March 2001, *T. evansi* was found in a laboratory culture at the International Center of Insect Physiology and Ecology (ICIPE) originally from a mite collection from Mwea Irrigation Scheme in Central Kenya (Knapp *et al.*, 2003). *T. evansi* can develop within temperature ranges of 10-36 °C which explains the high adaptability of this phytophagous mite to various environmental conditions and its wide distribution (Bonato, 1999).

2.4: Host range of *T. evansi*

T. evansi attacks a wide range of wild and cultivated plants but solanaceous plants are the preferred hosts, which include tomato, potato, eggplant, nightshade, *Physalis* sp. and tobacco (Moutia 1958; Silva, 1954). *T. evansi* was reported on tomato plants in California (Oatman *et al.*, 1967), the Lower Rio Grande Valley of Texas (Schuster, 1959), Brazil (Silva, 1954), and Mauritius (Moutia, 1958), on wild species of *Solanum* and *Physalis* in Brazil (Silva, 1954), on eggplants in the Lower Rio Grande Valley of Texas (Schuster, 1959) and Mauritius (Moutia, 1958), and on potato, peanut and *Solanum nigrum* L. in Mauritius (Moutia, 1958).

2.5: Damage and crop loss by *T. evansi*

Mites generally pierce single plant cells and suck out the cell content. The first symptoms of injury are chlorotic stipples on the leaves; larger areas subsequently turn yellow and leaves become convex (plate 1). Fine webbing (plate 2) is clearly visible when infestation is severe (Meyer, 1996). The result of this damage to leaf tissue is reduced chlorophyll content and reduced photosynthesis, CO_2 assimilation and transpiration. Disturbance of metabolic processes results in decreased growth, flowering and cropping (Mathews and Tunstall, 1994). Crop yields are diminished as essential plant processes are affected.

Yields in tomatoes may be reduced by a mite-induced physiological shock, by reduced size and numbers of fruit, and sun scalded fruit arising from loss of leaves (McKinney, 1992). *T. evansi* is regarded as one of the key tomato pests in Zimbabwe (Sibanda *et al.*, 2000) and yield losses of up to 90% have been recorded (Knapp, pers. comm.).

Plate 1: chlorotic stipples on tomato plants caused by mite infestation (Source: ICIPE)

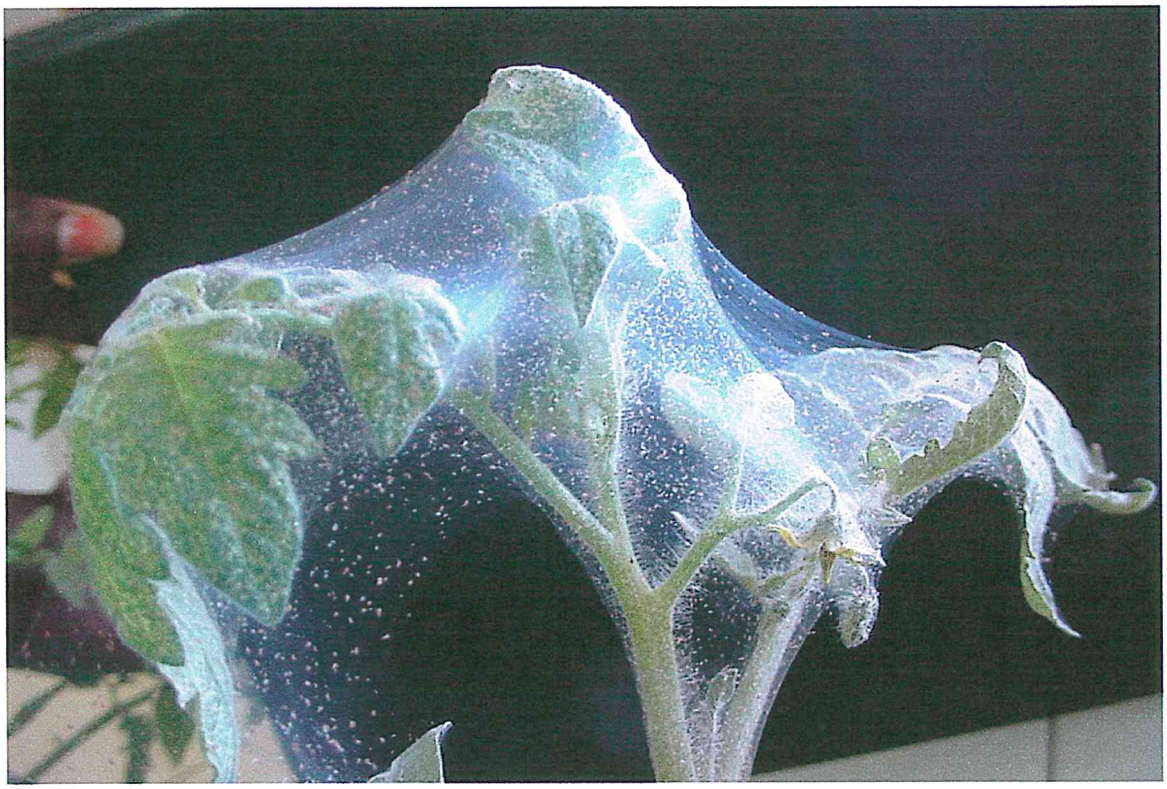

Plate 2: Webbing due to heavy mite infestation (Source: ICIPE)

2.6: The life cycle of *T. evansi*

The life cycle of tetranychid mites is a typical epimorphosis, the stages being egg, larva, protonymph, deutonymph and adult (plate 3). The three immature stages are each followed by a quiescent stage; protochrysalis, deutochrysalis and teliochrysalis, respectively (Meyer, 1996). Oviposition begins a day after emergence from the deutonymph and females reach their maximum egg-laying capacity on the fourth day. At this time they may oviposit up to 10-15 eggs per day, after which the rate of oviposition decreases (Qureshi *et al.*, 1969). The eggs are whitish and are laid singly on the underside of the leaf. Eggs may be covered with webs (plate 2), the presumed purpose of which is to regulate humidity and to protect them from mite predators (Gerson, 1985). They hatch after 4 to 7 days at a temperature of 25-30 ^{0}C. The larva is six legged, pinkish and slightly larger than the egg; it lasts 3-5 days. There are two reddish nymphal stages characterized by four pairs of legs. The total nymph period lasts 6-10 days.

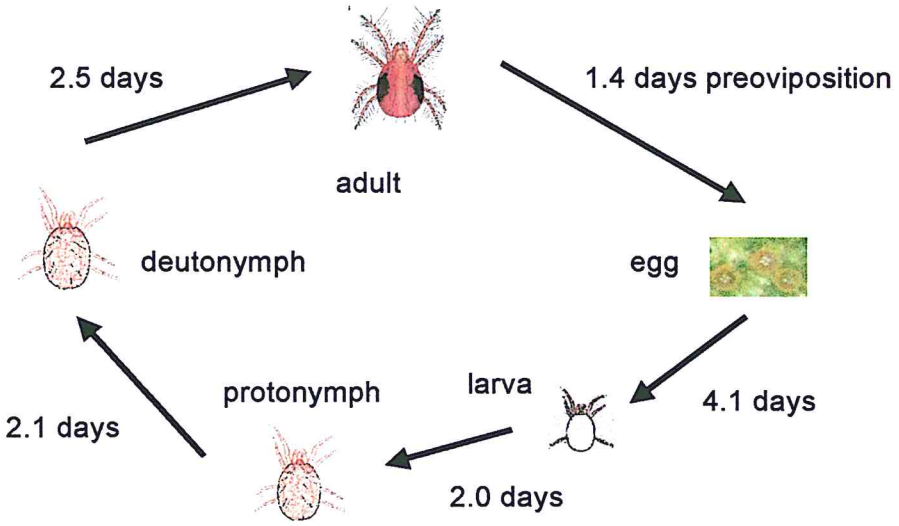

Plate 3: The life cycle of *T. evansi*. (Data source: Bonato, 1999).

Adult females are oval, range red with an indistinct dark blotch on each side of the body and 0.5 mm long. Males are smaller and straw to orange colored (Meyer, 1996). There are no conspicuous changes in morphology after individuals have entered the first nymphal stage. Reproduction by *T. evansi* involves arrhenotokous parthenogenesis with unmated females producing only male progeny and mated females producing both male and female offsprings. Although the potential sex ratio of tetranychid mites is under genetic control (Mitchell, 1972), the actual sex ratio of progeny produced by individual females is also dependent upon the amount of sperms a female receives during mating, host quality, population density and temperature (Wrensch, 1985).

Males can be distinguished from females by their more slender bodies that are pointed at the rear, and by their proportionately longer legs and are usually smaller than females. There is however, a marked difference between males and females in the rate of development. The early maturing males locate and remain near the female teliochrysalis until the females emerge. Copulation takes place almost immediately after emergence of the young females. This explains why, in a normal bisexual population, the females are nearly always mated (Meyer, 1996).

2.7: Natural enemies of spider mites

Many arthropods are predacious on spider mites (Helle and Sabelis, 1985). The greatest attention has been focused on phytoseiid predators (predatory mites) because a number of them possess attributes that enable them to control spider mite populations effectively at low densities, and have an extraordinary ability to recover from starvation, and to reproduce after finding a new prey patch (Sabelis, 1985). Some species of phytoseiids consume other species of mites and pollen when spider mites are scarce or absent (Overmeer, 1985). For instance, *Phytoseiulus persimilis* Athias-Henriot has been introduced into many countries for the control of spider mites in greenhouse crops (Oomen, 1988).

The predator is known to exert remarkable control of *T. urticae* on bean plants in green houses (Chant, 1961). However, attempts to control tobacco spider mites with *P. persimilis* in Zambia and Zimbabwe failed (Jensen and Mingochi, 1988; Sibanda, 1995). Moraes and McMurtry (1985, 1986) investigating the suitability of *T. evansi* as prey for *P. persimilis,* and seven other phytoseiid mites found that none of the phytoseiids were effective predators of *T. evansi*. Their oviposition and survivorship were very low on this prey.

Further investigations showed that *T. evansi* is physiologically unsuitable as prey for *P. persimilis.* Two important predators *Stethorus vinsoni* Kapur (Coccinellidae) and *Feltiella* sp. (Cecidomyiidae) were reported from Mauritius (Moutia, 1958). *Oligota fageli* Williams (Staphylinidae) is a predator that occurs in Kenya, South Africa and Yemen (Botha *et al.*, 1994; Chazeau, 1985; Knapp, 1997).

It is usually found when red spider mite numbers are already high and is not able to control mite outbreaks under field conditions. Its rearing is difficult due to its delicate larvae and pupation in the soil. In Brazil, the mites do not affect tomato production severely, probably because natural enemies keep the mite population under control. Due to the fact that the mite is an introduced pest to Southern Africa, it is likely that the natural enemies are not present. However, a few predators have been found associated with *T. evansi* in Africa but they are not effective in controlling the spider mites (Sarr *et al.*, 2002).

2.8: Control of spider mites

2.8.1: Cultural control

Several cultural techniques that can reduce the mite populations have been employed and they are aimed at disrupting the life cycle and hence reducing the mite population (Tindall, 1983). These techniques include crop rotation, proper field sanitation, uprooting and burning of old crops. Since hot and dry environmental conditions are favorable to the development of mite populations, reducing the planting distance and applying overhead irrigation reverses these conditions and thus depresses the mite populations. Tulisalo (1974) developed a program of water sprays in form of mist to inhibit mite infestations. He reported that in high relative humidity, the females had a shorter life span and laid eggs at a slower rate.

However, on tomatoes the use of mist sprays should be avoided to prevent the development of *Botrytis* leaf infections (Meyer, 1996). Attempts have been made to reduce mite build-up by varying the fertilizer regime applied to the crops. Large quantities of nitrogen or a deficiency of potassium increases the amount of soluble nitrogen in the plant so that sharp increases in the rate of population growth by mites follow such fertilizer regimes (Markkula and Tiittanen, 1969).

2.8.2: Chemical control

Chemical acaricide applications are the most common strategies used for control of spider mites (Cranham and Helle, 1985). However, the response of spider mites to pesticides can range from processes such as the evolution of pesticide resistance via metabolic detoxifying mechanisms (Rizzieri *et al.*, 1988; Fergusson-Kolmes *et al.*, 1991) to behavioral responses such as dispersal behavioral changes (Fisher and Wrench, 1986) and influences upon oviposition site selection (McPherson *et al.*, 1989).

Current control practices of *T. evansi* in Africa involve frequent applications of highly toxic acaricides with long pre-harvest intervals, which result in pesticide contamination of the farmer's produce and the environment. In addition, poor application techniques used by African farmers limit the success of chemical control. Chemical control of spider mites requires regular distribution of the spray solution on all plant parts including the lower surfaces of the leaves. Pruning and staking of tomatoes makes it easier to cover the plants with acaricides and therefore improves spider mite control (Saunyama and Knapp, 2003).

2.8.3: Biological control

Biological control of tetranychid mites has utilized predators from the families Bdellidae, Anystidae, Coccinellidae, Cecidomyiidae, Stigmaeidae, Staphylinidae, Phytoseiidae and Cheyletidae; but predaceous mites of the family Phytoseiidae are the most important ones. There are only two reports of phytoseiid mites associated with *T. evansi* and there are no indications that they can significantly reduce its population (Moraes and McMurtry, 1983). Moraes and McMurtry (1985, 1986) reported that *T. evansi* was not a suitable prey for *P. persimilis. T. evansi* is a newly introduced pest in Africa and there are no known indigenous predators that feed on it, except in Malawi and Zimbabwe where a Staphylinid beetle, *Oligota* sp., was found preying on it (Knapp pers. comm.).

Three groups of fungi belonging to the orders of Entomophthorales, Deuteromycetes and Ascomycetes are known to cause epizootics in mite populations (Van der Geest *et al.*, 2000); but the most frequently encountered are the Entomophthorales.

The genus *Neozygites* is the commonest among the fungi that attack mites in the order of Entomophthorales. *Neozygites floridana* Weiser and Muma has subsequently been observed on several species of spider mites on various agricultural crops. It was reported on *Tetranychus tumidus* Banks on cotton (Saba, 1971), on *T. evansi* on tomato in Brazil (Humber *et al.*, 1981), on *T. ludeni* Zacher on bean in India (Rameseshiah, 1971), on *Oligonychus hondoensis* Ehara on cedar in Japan (Nemoto and Aoki, 1975), on *T. urticae* on field corn in North Carolina, USA (Brandenburg and Kennedy, 1982), and on the cassava green mite (CGM), *Mononychellus tanajoa* Bondar, in Venezuela (Agudella-Silva, 1986), Brazil (Delalibera *et al.*, 1992), and Kenya (Bartkowski *et al.*, 1988).

2.8.4: Host plant resistance

Host plant resistance (HPR) is defined as the relative amount of heritable qualities possessed by a plant, which influence the ultimate degree of pest damage (Painter, 1951). Painter recognized three types of host plant resistance: (1) antibiosis, in which the pest is killed or is not able to complete its life cycle; (2) non-preference (called antixenosis; Kogan and Ortman, 1978) in which the pest is either repelled or not attracted to, or inhibited from feeding on the plant; and (3) tolerance, in which the plant supports as many pests as other plants but shows less yield reduction.

HPR to insect pests has been used as the principal method in providing the level of control needed as well as an adjunct to other methods such as biological control and cultural methods (Smith, 1989). HPR as a single pest management factor has the following advantages; (i) resistance is specific towards a single pest or a couple of related pests with no direct effects on non-target insects; (ii) it is cumulative, i.e. the desired effect on the pest population is compounded in successive generations; (iii) it is a constant attribute of the plant with the exception of an occasional upsurge of biotypes; (iv) it is of low cost to the farmers (only seed) which is perhaps a major factor effect in developing countries; (v) it has no detrimental effects to the environment; (vi) it is compatible with other control measures such as biocontrol used to keep a pest below the economic threshold (Bergman and Tingey, 1979).

HPR has also a number of disadvantages; (i) resistance may not be expressed in every environment in which the variety is grown; (ii) high levels of resistance may lead to development of new insect biotypes; (iii) there is a genetic limitation of absence of preadaptive resistance genes; (iv) the time required to incorporate resistance in commercially accepted cultivars is long and therefore may not solve sudden or very localized pest problems; (v) the problem of conflicting resistance traits whereby certain plant characteristics may act as resistance factors for some insect pests but induce susceptibility to others (Smith, 1989).

2.8.5: Resistance of tomatoes to mites

Plant resistance to arthropod herbivores is often mediated by phytochemicals that negatively affect the feeding, growth, or reproduction of the attacking pest (Karban and Baldwin, 1997; Walling, 2000). Natural resistance of tomato to many herbivores is attributed to both constitutive and inducible defensive phytochemicals (Farrar and Kennedy, 1992). Induced resistance can be defined as a change in plant resistance resulting from herbivore infestation, which depends on the density of the attacking herbivores (Karban and Baldwin, 1997; Underwood, 2000). This is in contrast to constitutive plant resistance, which is the constant level of resistance a plant has regardless of herbivore attack.

Trichomes both glandular and non-glandular are prominent features of the foliage and stems of *Lycopersicon* spp. Four types of glandular trichomes occur most commonly (Luckwill, 1943). Of these, type IV and type VI trichomes have been associated with high levels of arthropod resistance. Type IV trichomes have a short, multicellular stalk on a monocellular base and produce a droplet of exudates at their tip. High densities of type IV glandular trichomes and the presence of high levels of toxic acyl sugars in their exudates play a major role in the resistance of *L. pennellii* (Goffreda *et al.*, 1989) to a number of arthropods.

These include aphids, whiteflies, tomato fruitworm (*Helicoverpa zea* Boddie), beet armyworm (*Spodoptera exigua* Huebner), the agromyzid leafminer (*Liriomyza trifolii* Burgress) and the South American tomato pinworm (*Tuta absoluta* (Meyrick) (Blauth *et al.*, 1998; França *et al.*, 1989; Gentile and Stoner, 1968; Goffreda and Mutschler, 1989; Goffreda *et al.*, 1990; Hartman and St. Clair, 1999; Hawthorne *et al.*, 1992; Heinz and Zalom, 1995; Leidl *et al.*, 1995; Nombela *et al,* 2000; Ponti *et al.*, 1983). The presence of type IV glandular trichomes in *L. pennellii* 'LA-716' that secrete acyl sugars is controlled by at most two unlinked genes in crosses of *L. pennellii* with *L. esculentum* (Lenke and Mutschler, 1984). While acyl sugars are encountered mainly in *L. pennellii* and other wild species, their transfer to the cultivated tomato, *L. esculentum*, would contribute to increased levels of pest resistance in the commercial species.

High densities of type VI trichomes which have a four-celled glandular head on a short multicellular stalk, and a monocellular base (Luckwill, 1943), have been implicated in resistance of *L. hirsutum* f. *typicum* to two-spotted spider mite (*T. urticae*) (Carter and Snyder, 1985, 1986; Weston *et al.,* 1989). They have also been implicated in resistance of several *Lycopersicon* species to a number of other arthropod pests e.g. in entrapment of aphids and other small arthropods (Duffey, 1986; McKinney, 1938).

The trichome tips contain several phenolics [primarily rutin (80%-90%)] but also chlorogenic acid and conjugates of caffeic acid and polyphenol oxidase and peroxidase. The glandular tips of type VI trichomes of *L. hirsutum* f. *typicum* also contain several sesquiterpenes, including zingiberene γ-elemene, δ-elemene, α-curcumene, and α-humulene, which are acutely toxic to *S. exigua*. Zingiberene, the predominant sesquiterpene in the tips of type VI trichomes of at least some accessions, is also toxic to Colorado potato beetle (*Leptinotarsa decemlineata* Say) larvae and has been implicated in resistance to that important pest (Carter *et al.*, 1989b). Patterson *et al.* (1975) showed that resistance in tomatoes to *T. urticae* was due to avoidance and toxicity caused by sesquiterpenoids. The 13-carbon methyl ketone, 2-tridecanone, is one of the most thoroughly studied arthropod resistance factors in tomato (Williams *et al.*, 1980; Kennedy and Dimock, 1983).

2-Tridecanone is acutely toxic to a number of phytophagous species when assayed in contact bioassays (Dimock and Kennedy, 1983; Kennedy and Sorenson, 1985; Lin *et al.*, 1987; Williams *et al.*, 1980). Studies on the contact toxicity of 2-tridecanone in the two-spotted spider mite (*T. urticae*) have shown strong acaricidal properties of this compound (Chatzivasileiadis and Sabelis 1997, 1998).

Another constituent of the tips of the wild tomato type VI trichomes was identified as the 11-carbon methyl ketone, 2-undecanone (Farrar and Kennedy, 1987). One accession of *L. hirsutum* f. *glabratum*, PI 134417, has been investigated extensively and possesses multiple defenses against a number of phytophagous arthropods due the presence 2-tridecanone and 2-undecanone, in the tips of the type VI glandular trichomes, which abound on the foliage and stems (Farrar and Kennedy, 1991; Williams *et al.*, 1980).

These ketones comprise 90% of the tip contents of type VI trichomes of PI 134417, but trace amounts are present in the type VI trichomes of *L. esculentum* (Dimock and Kennedy, 1983; Lin *et al.*, 1987). Carter and Snyder (1985) found a positive correlation of both the mite mortality and repellence with the density of type IV and type VI trichomes in *L. esculentum* X *L. hirsutum* F_2 hybrids, and Weston *et al.* (1989) found a negative correlation of tomato susceptibility to mites with 2-tridecanone levels in the tips of type VI trichomes. Chatzivasileiadis *et al.* (1999) established that 2-tridecanone and 2-undecanone are toxic to *T. urticae* with the LC_{50} being similar with the formulated acaricide amitraz, although 2-tridecanone was more toxic than 2-undecanone.

The sesquiterpene content of tomato leaf oil varies considerably among species, with caryophyllene and humulene being widespread and reported from *L. esculentum*, *L. hirsutum*, *L. pimpinellifolium*, *L. peruvianum*, *L. cheesmanii*, *L. chilense*, and *L. chmielewski* (Lundgren *et al.*, 1985). Environmental and developmental factors that promote photosynthesis or availability of photosynthate concomitantly increase terpene accumulation (Croteau and Johnson, 1984; Gershenzon and Croteau, 1990). Monoterpene concentrations are also generally higher in young leaves and peak at or before flowering (Croteau *et al.*, 1981; Loomis and Croteau, 1973).

CHAPTER THREE

3.0: MATERIALS AND METHODS

3.1: Biological material and experimental conditions

3.1.1: Tomato accessions

Tomato accessions tested comprised of commercial varieties available in Kenya and material from tomato germplasm collections at ICIPE, AVRDC and JKUAT (Table 1). The seedlings were raised in a screen house under ambient conditions at ICIPE. Seeds were pre-germinated in a soil enriched with compost in plastic seedling trays. Seedlings were transplanted after six weeks into pots filled with a mixture of soil, compost and sand (3:2:1 on volume basis) and placed on benches in a completely randomized design. The plants were watered weekly and no fertilizers or pesticides were applied. Plants that were one to two months after transplanting were used in the experiments.

Table 1: Tomato (*Lycopersicon sp*) accessions, their names, species and source.

Accession No	Accession name	Species	Source
1 (control)	Money Maker	*L. esculentum*	Simlaw Seeds
13	Marglobe	*L. esculentum*	Simlaw Seeds
51	PI 134417	*L. hirsutum*	ICIPE
162	JKUAT 22/202183	*L. esculentum*	JKUAT
182	JKUAT 19	*L. esculentum*	JKUAT
428	LA 2185	*L. peruvianum*	AVRDC
460	LO 3279	*L. esculentum*	AVRDC

3.1.2: Spider mite culture

A stock culture of *T. evansi* was established in a rearing room at ICIPE. The mite culture was maintained at a room temperature of 25 °C and 50-70% relative humidity (RH) under a photoperiod of light: dark (L: D) 12:12 hours on Money Maker plants.

3.2: Trapping of volatile compounds

Whatman filter paper and wire gauze were folded to make packets measuring 2.5 cm x 4.0 cm. 100 mg of adsorbent material (Silica C-18) was introduced into the sachets and sealed by clipping with pins. Each adsorbent packet was cleaned separately in a sohxlet using dichloromethane (DCM) for 72 hours. The packets were handled using a pair of forceps, placed in aluminium foil and dried in an oven at 30 °C for 3-4 days. The adsorbents were then 'activated' in an inert atmosphere of purified nitrogen in a gas chromatography oven for 72 hours. 'Activated" adsorbents were handled with a pair of forceps, placed in clean aluminium foil and then in an airtight container to avoid contamination as they were being carried to the green house. The volatiles from the plants were obtained by trapping using a glass chamber 39 x 39 x 52 cm, whereby the clean sachets containing adsorbent material (Silica C-18) were introduced by suspending them from the top of the chamber (Plate 4).

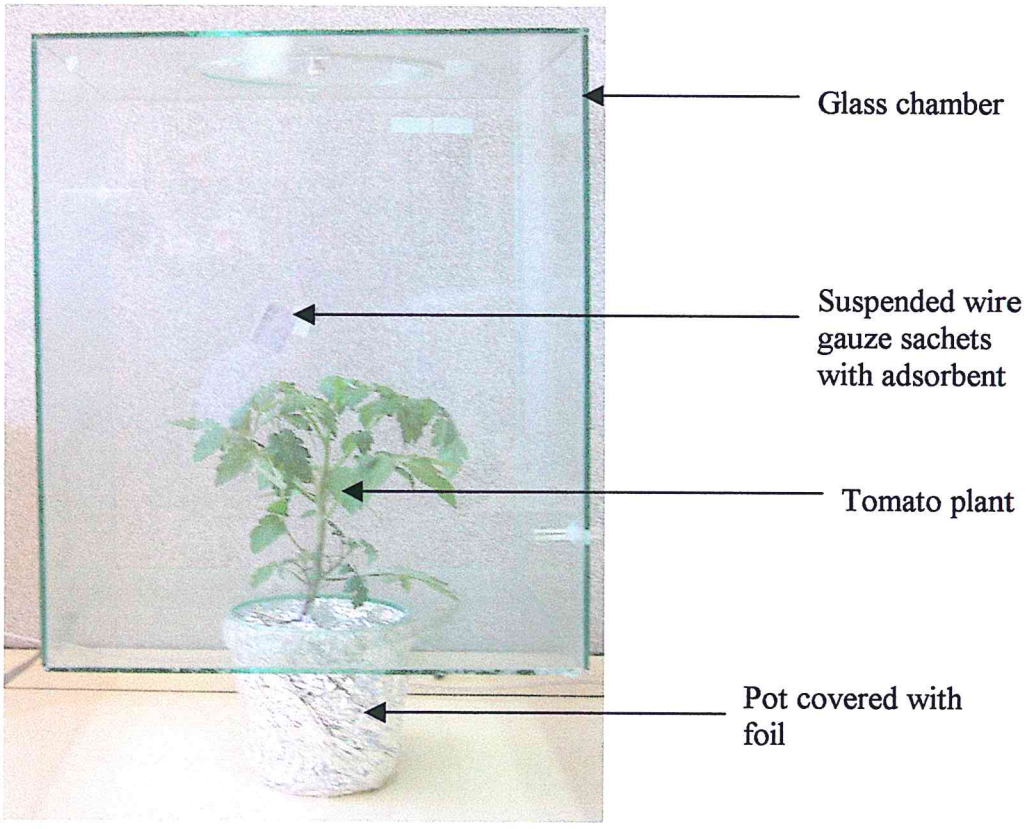

Plate 4: Set-up for trapping volatile compounds in the green house

The volatiles were trapped for 24 hours under green house conditions. The pots and soil were covered with aluminium foil to prevent contamination of the adsorbent material. After trapping, the sachets were handled with forceps, wrapped in aluminium foil, placed in an airtight container and stored under -21°C. Volatile compounds were then eluted with 2 ml of dichloromethane, by emptying the adsorbent material into clean glass wool placed in a Pasteur pipette, which was then concentrated to 0.5 ml using inert nitrogen for Gas Chromatography (GC) or Gas Chromatography-Mass Spectrometer (GC-MS) analysis.

3.3: Harvesting and steam distillation of plant material for essential oils

Approximately 50 g of fresh leaves from each accession was randomly harvested, wrapped in aluminium foil and stored at -21°C. The foliage of each accession was then steam distilled in a water recycling apparatus (Dean-Stark) (Plate 5) for 8 hours. The essential oils collected on the hexane layer were separated and dried with anhydrous sodium sulphate and stored in dark colored vials.

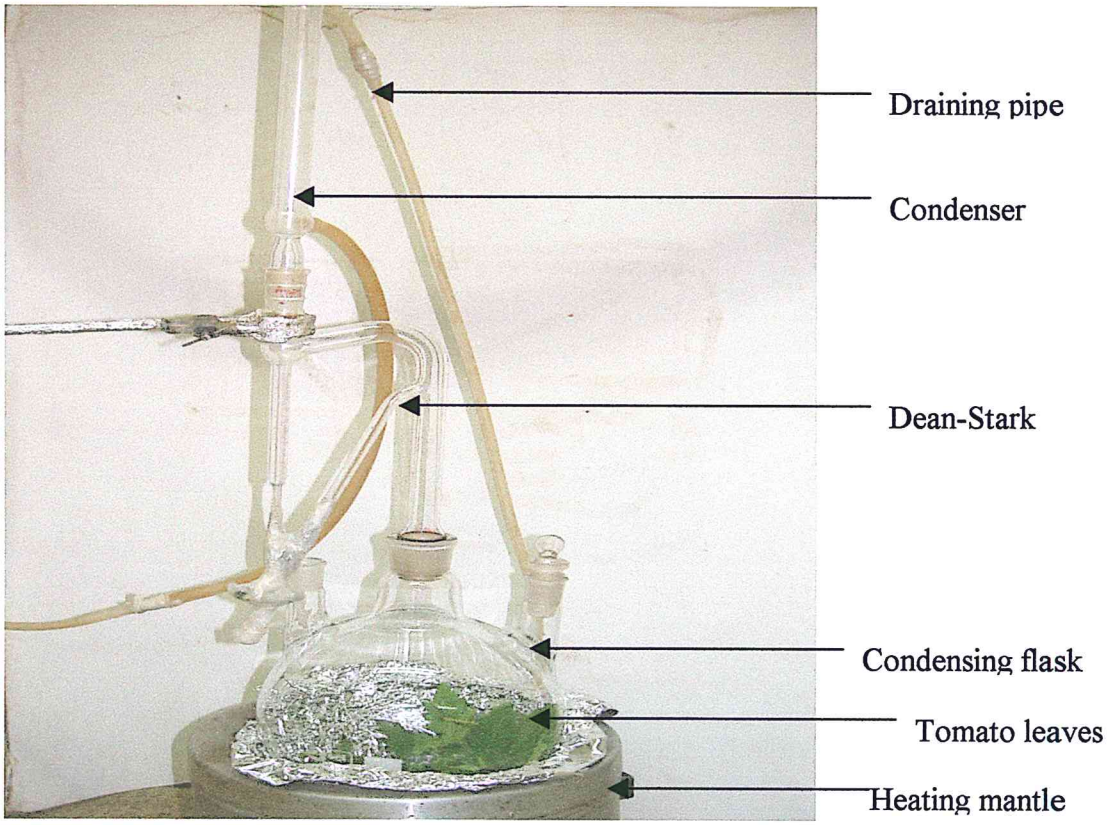

Plate 5: Steam distillation apparatus set-up in the laboratory

3.4: Purification and characterization of volatile compounds and essential oils

Characterization and identification of the chemical composition of the volatile compounds and essential oils was done by gas chromatography (GC), gas chromatography-mass spectrometer (GC-MS) respectively. 2 µl of the sample of each accession was injected into the capillary column of the gas chromatography (GC) or gas chromatography-mass spectrometer (GC-MS). The temperature program started at 50 °C for 5 min on the lag phase and rose to 280 °C at the rate of 5 °C/min for 30 min.

GC separation was done in a capillary gas chromatograph, Hewlett Packard (HP) model series II equipped with a splitless capillary injection system, a flame ionization detector (FID) coupled to an integrator (HP 3393 A series II). The separation was done on a cross-linked methyl silicon capillary column 50 m x 0.2 mm (internal diameter) x 0.33 mm truck (film thickness). White spot nitrogen was used as a carrier gas at a flow rate of 0.7 ml per minute. GC-MS analysis was carried out on a HP 8060 series II spectrometer. The spectrometer was operated in the electron (EI) mode at 70 eV and an emission current of 200 microamperes. The temperature was held at 180 °C and the multiplier voltage was 300 V. GC-MS database was used to identify the molecular structures and names of identified compounds.

3.5: Response of *T. evansi* to plant odors in a Y-tube olfactometer

A glass Y-tube olfactometer (4 cm inner diameter, 12 cm length of trunk, 17 cm length of the arms and 5 cm length of small glass tubes placed inside) modified from Sabelis and Van de Baan (1983) was used to assess the response of spider mite females to odors from different treatment categories. A wire was placed at the entrance of the trunk to aid the mites to walk to either arms of the olfactometer (Figure 1). Airflow at the entry was set at 2 l/min and at the exit 4.5 l/min. Each olfactometer arm was connected by a Teflon® tube (0.5 cm inner diameter) to a glass chamber/ glass tube that were used for holding the source of test odors. Two pressure pumps (Air Cadet vacuum/pressure station, Cole Palmer Instrument Co., USA) were used to pump air into and out of the system while two flow meters (Cole Palmer Instrument Co., USA) regulated the airflow.

Additional Teflon® tubes conveyed air from the inlet pressure pump through an activated charcoal filter where it was purified, then through one flow meter and into the separate treatment jars. The second flow meter was connected between the stem of the olfactometer and the second pump, which exhausted air out of the system. White Styrofoam boards (30 cm high) screened the observation arena.

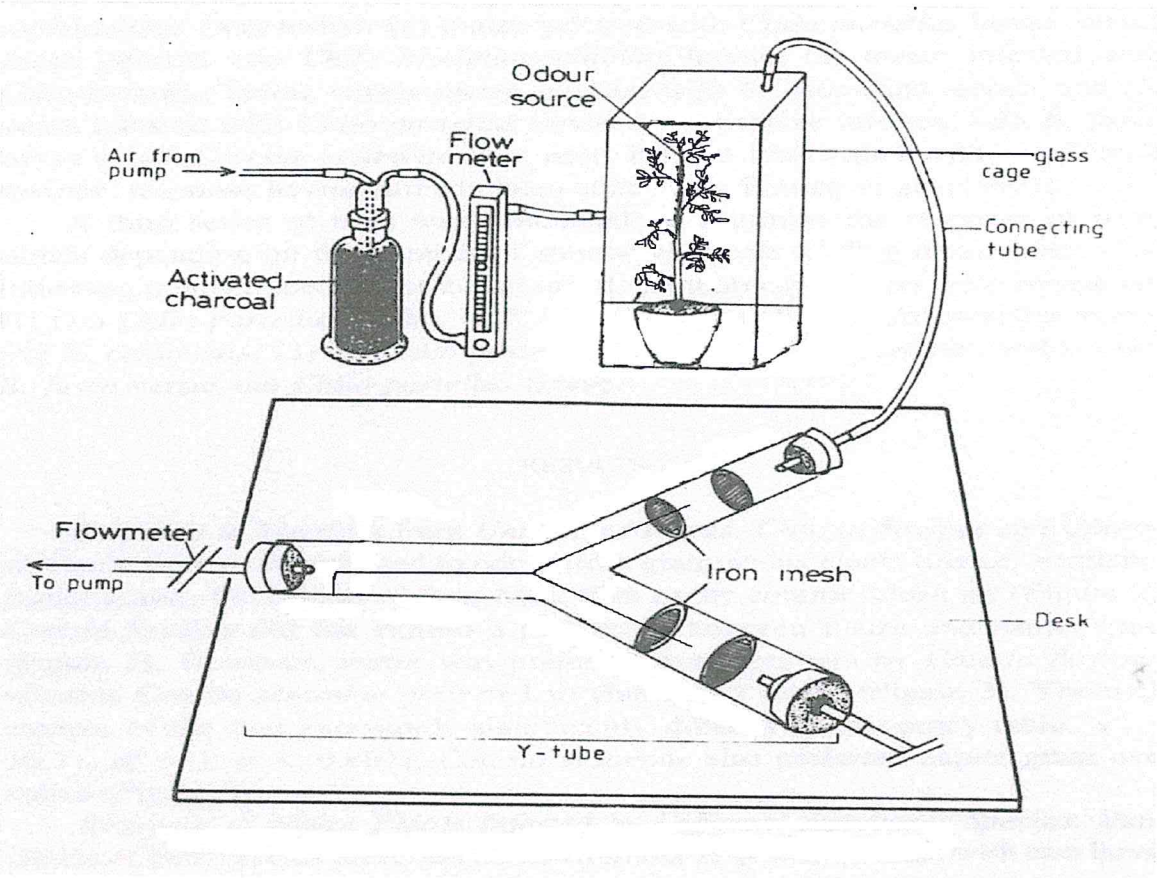

Figure 1: A representation of the Y-tube olfactometer set-up in the laboratory (modified after Ngi-Song *et al.*, 1996).

A potted plant of the test accession was maintained in a glass chamber connected to one arm of the olfactometer and another chamber with a Money Maker plant connected to the other arm of the olfactometer (control). At the start of each bioassay, a female was placed on the wire at the entrance of the trunk on which the mites walked on to either end of the olfactometer arms (Figure 1).

A positive (+) response to the odor stimulus was recorded when the females that oriented themselves towards the odour source from the test accession reached the far end of the arm within 10 min from the start of the experiment.

Those mites that followed the control air stream in the opposite arm were recorded as showing a negative response (-). Mites that walked up and down without reaching one of the arms within 10 min were recorded as showing no response (0). Each bioassay was carried out on four different days with 10 mites per each bioassay, which means a total of 40 mites per bioassay. Before the start of the bioassays, the olfactometer was tested with blank cages to ascertain that mites displayed an equal response for the two arms of the olfactometer. Adult female mites were randomly collected and used in the bioassay. The experiment was carried out under room temperature conditions.

3.6: Glass slide bioassays

A glass slide was placed in a petri dish with moist cotton wool and used to access the response of spider mites to different treatments placed on either end of the marked glass slide section as described by van de Boom *et al.*, 2003. The treatments were connected via a glass bridge (length: 5 cm, width: 2.5 cm, thickness: 1 mm) (Plate 6 and 7). At the beginning of each experiment, 10 spider mites were placed at the center of the glass bridge and were allowed to make a choice between the two treatments within a period of 10 min. Spider mites that made no choice were excluded from the statistical analysis.

3.6.1: Plant acceptance (leaf disk bioassay)

Fresh leaf disks of 25 mm diameter were made using a cork borer and cut in halves. To investigate whether the spider mites' response was affected by different accessions, half leaf disk of the test accession was placed ventral side up on one side of the petri dish on the glass slide section and a leaf section of the Money Maker was placed on the other side as a control (as described by van de Boom *et al.*, 2003). The leaf sections were connected via a glass bridge (length: 5 cm, width: 2.5 cm, thickness: 1 mm) (Plate 6).

At the beginning of each experiment, 10 spider mites were placed at the center of the glass bridge and were allowed to make a choice between the two accessions within a period of 10 min. The experiment was replicated six times using fresh leaf disks for each accession. Spider mites that made no choice were excluded from the statistical analysis.

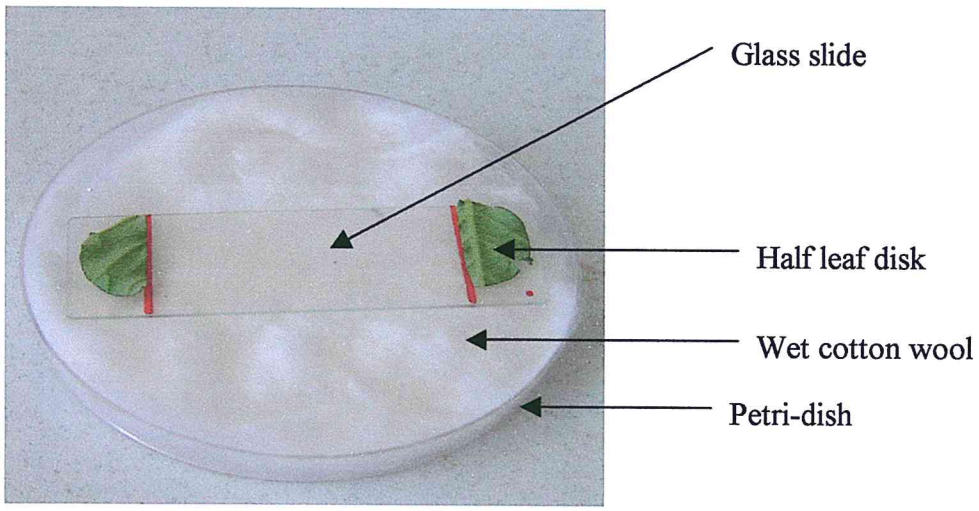

Plate 6: Leaf- disk bioassay set up in the laboratory

3.6.2: Preparation of essential oils for bioassays

The hexane that was used to collect the oil was removed by concentrating each sample to dryness using nitrogen gas. Vials containing crude extract of essential oil matter were weighed and the weight of the crude extract determined (Table 3). 1 ml of dichloromethane (DCM) was added to each vial containing the crude extract in order to make serial dilutions for use in the olfactometer bioassays. 0.1 ml of the solution was drawn from the sample using a syringe and put in a clean and dry vial. It was then topped to 10 ml using DCM to make 0.01 concentrations.

Table 2: Weights (g) of crude extracts with essential oils

Accession	Weight of crude extract (g)
1	0.05
428	0.03
460	0.04
51	0.03
162	0.03
182	0.04
13	0.04

3.6.3: Response of *T. evansi* to essential oils concentration

The essential oils at 0.01 concentrations were used for the bioassay. Small balls of cotton wool were connected via a glass bridge (length: 5 cm, width: 1.5 cm, thickness: 1 mm), which was placed in a Petri dish with moist cotton wool (Plate 7). They were placed on a 1 x 2.5 cm section at the end of the glass slide (modified from van de Boom *et al.*, 2003). 10 µl of essential oil of the test accession was put on one ball of cotton wool placed on one side of the glass slide and untreated cotton ball placed on the other side as a control. At the beginning of each experiment, 10 spider mites were placed at the center of the glass bridge and were allowed to make a choice between the test accession and the empty cotton ball within a period of 10 min. The experiment was replicated six times for each accession.

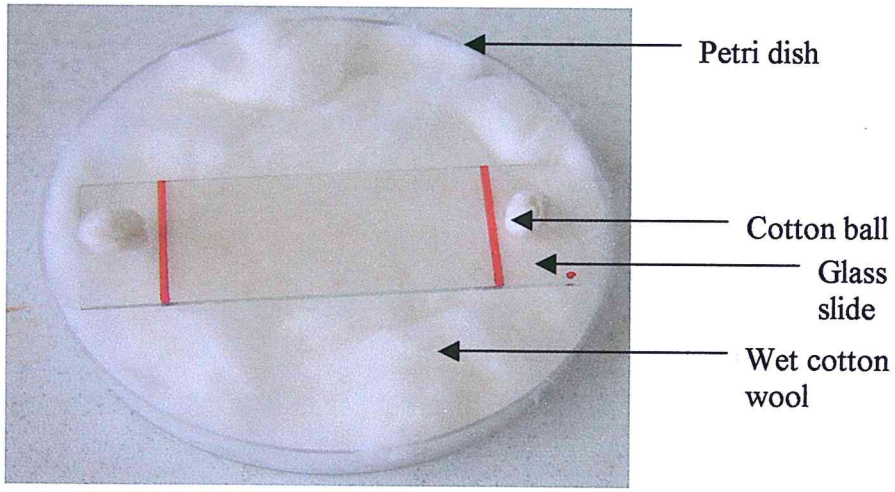

Plate 7: Set-up for essential oils bioassay in the laboratory

3.7: Statistics

To analyze the chromatographic data, the relative abundance of a compound was computed as the area percentage (i.e. the area of the peak as a percentage of the total area of all the selected peaks) for each identified peak.

To test whether the mites were responsive to plant odors of Money Maker or the test accession, the total number of mites arriving at each side of the treatment was estimated and subjected to $\chi 2$ test (n= 197 and 237 spider mites for the olfactometer bioassay and leaf disk bioassay respectively). Means and standard errors were calculated using SAS Proc GLM (SAS Institute, 1990).

To test whether the mites were attracted to the odors of the untreated cotton balls or essential oil of the test accession on the glass slide bioassay, the total number of mites arriving at each side of the treatment was determined and subjected to $\chi 2$ test. Thus n= 188 spider mites for the glass slide bioassays. Means and standard errors were calculated using SAS proc GLM (SAS Institute, 1990).

CHAPTER FOUR

4.0: RESULTS AND DISCUSSION

4.1: RESULTS

4.1.1: Volatile compounds in tomato accessions

2-Tridecanone was the most abundant methyl ketone in accession 51 (70.7%) while it occurred at lower concentrations in accessions 13, 162, 428 and 460 ranging between 0.1-1.4%. Accessions 1 (Money Maker) and 182 did not reveal any presence of 2-tridecanone. 2-Undecanone was also identified in accessions 13, 51 and 428 ranging between 0.01-0.2% while it was absent in accessions 1, 162, 182 and 460. The level of 3-methy-2-butanone in accessions 1, 13, 51, 162, 428 and 460 ranged between 0.2-3.3% while it was absent in accession 182. Sesquiterpenes that were established included β-phellandrene in accessions 13 (0.03%) and 428 (0.02%). α-Terpinene occurred in accessions 1 (0.05%) and 13 (0.01%) while α-pinene and δ^3-carene occurred in accessions 13 (0.01%) and 1 (0.2%) respectively (Table 3).

Table 3: Chemical composition and relative abundance (%) of volatile compounds

Compound	**1**	**13**	**51**	**162**	**182**	**428**	**460**
2-Tridecanone	-	0.1	70.7	1.4	-	0.9	0.7
2-Undecanone	-	0.01	0.2	-	-	0.04	-
3-Methyl-2-butanone	0.9	0.4	3.3	2.1	-	0.2	0.9
β-Phellandrene	-	0.03	-	-	-	0.02	-
α-Terpinene	0.05	0.01	-	-	-	-	-
α -Pinene	-	0.01	-	-	-	-	-
δ^3-Carene	0.2	-	-	-	-	-	-

The identification of compounds was based on integration of peaks in the GC-MS total ion chromatogram (Appendix 1 & 2).

4.1.2: Chemical composition of essential oils

Several methyl ketones and sesquiterpenes were established in the essential oils at varying levels. 2-Tridecanone occurred in accessions 51, 162, 182, 428 and 460 and ranged between 1.1-49.0%, while 2-undecanone occurred in accessions 51, 182, 428 and 460 and it ranged between 0.3-12.3%. 2-Pentadecanone occurred in accessions 1, 13, 51, 162, 428 and 460 and it ranged between 1.4-3.3% while 2-dodecanone occurred in accessions 51, 182 and 428 and it ranged between 0.3-0.6%. 2-decanone occurred only in accession 51 and 428 at trace amounts of 0.04-0.06% respectively, while β-demascenone and β-ionone occurred in accessions 1 at 0.02% and 1.7% respectively. 3-Methyl-2-butanone occurred in all accessions and ranged between 0.01-0.6% (Table 4).

β-Caryophyllene, β-phellandrene, δ-elemene, β-elemene, α-humulene and δ-cadinene were found to be the most widespread sesquiterpenes in all the accessions used in our experiment with β-caryophyllene occurring in accessions 1, 13, 51, 162, 182, 428 and 460 and it ranged between 0.8-19.8% while β-phellandrene was found in accessions 1, 13, 162, 428 and 460 and it ranged between 0.3-3.1%. δ-Elemene occurred in accessions 1, 13, 162, 182, 428 and 460 and it ranged between 0.1-6.9% while β-elemene occurred in accessions 13, 51, 162, 182 and 428 and it ranged between 0.1-2.2%. α-Humulene occurred in accessions 1, 13, 51, 162, 182, 428 and 460 and it ranged between 0.1-15.4%, while δ-cadinene occurred in accessions 1, 13, 51, 182, 428 and 460 and it ranged between 0.03-1.3% (Table 4).

Other terpenes that were found only in trace amounts in a few accessions are β-bisabolene, which occurred in accessions 51, 182 and 460 at 0.01% respectively while terpinolene occurred in accessions 1 (0.1%), 162 (0.6%) and 460 (0.4%). α–pinene occurred in accessions 162 (0.02%) and 428 (4.9%), while β-pinene occurred in accessions 1(0.06%) and 428 (7.4%). α-Terpinene occurred in accessions 13, 162, 428 and 460 at 0.01-0.1%. Camphene occurred in accession 460 (0.02%) and 428 (0.1%) while δ^3-carene occurred in accession 460 (0.01%). Azulene and ρ-cymene occurred in accession 162 (0.03%) and 460 (0.1%) respectively, while limonene occurred in accession 428 at 13.0 % (Table 4).

Table 4: Chemical composition and relative abundance (%) of compounds in essential oils

Compound	1	13	51	162	182	428	460
2-Undecanone	-	-	12.3	-	7.1	2.4	0.3
2-Dodecanone	-	-	0.6	-	0.4	0.3	-
2-Tridecanone	-	-	48.5	1.1	27.1	39.6	12.6
2-Pentadecanone	3.3	1.6	2.4	2.5	-	1.9	1.4
2-Decanone	-	-	0.04	-	-	0.06	-
β-Demascenone	0.02	-	-	-	-	-	-
β-Ionone	1.7	-	-	-	-	-	-
3-Methyl-2-butanone	0.6	0.04	0.01	0.03	0.02	0.04	0.03
β-Phellandrene	0.3	2.0	-	2.4	-	2.1	3.1
β-Caryophyllene	2.1	0.8	2.9	19.8	5.4	2.7	7.8
δ –Elemene	0.4	6.9	-	5.4	0.1	2.8	0.7
β-Elemene	-	2.2	0.1	1.2	0.2	1.0	-
α-Humulene	0.6	15.4	0.8	4.9	1.4	3.0	0.1
δ-Cadinene	0.1	1.3	0.1	-	0.1	0.7	0.03
β-Bisabolene	-	-	0.01	-	0.01	-	0.01
Terpinolene	0.1	-	-	0.6	-	-	0.4
α –Terpinene	-	0.01	-	0.04	-	0.1	0.01
α –Pinene	-	-	-	0.02	-	4.9	-
β –Pinene	0.06	-	-	-	-	7.4	-
Camphene	-	-	-	-	-	0.1	0.02
δ^3-Carene	-		-	-	-	-	0.01
Azulene	-	-	-	0.03	-	-	-
ρ-Cymene	-	-	-	-	-	-	0.1
Limonene	-	-	-	-	-	13.0	-

The identification of compounds was based on integration of peaks in the GC-MS total ion chromatogram (Appendix 1 & 2).

4.1.3: Response to plant odors in olfactometer tests

The six *Lycopersicon* accessions that were compared to Money Maker (accession 1), a variety of *L. esculentum* susceptible to *T. evansi,* revealed varying differences in the olfactometer bioassay. Accessions 13, 51, 428 and 460 showed significant differences to Money Maker whereas accessions 162 and 182 did not show significant differences (Figure 2).

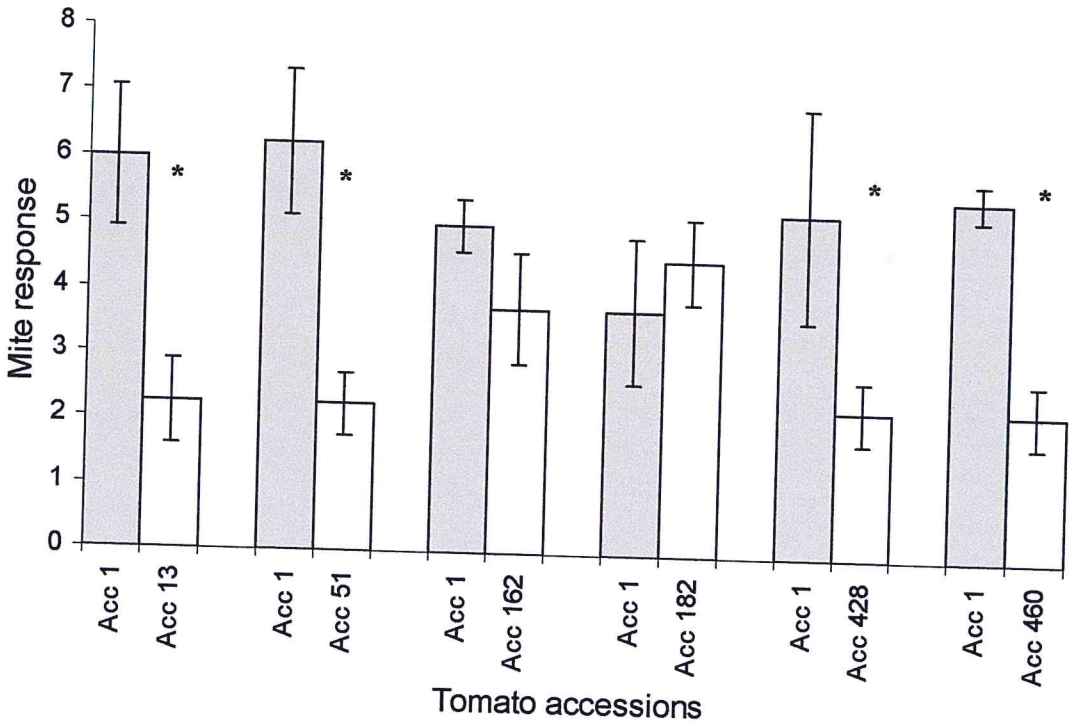

Figure 2: *T. evansi* response (Mean ± S.E) to whole plant odors in a Y-tube olfactometer (n=30-35). Vertical bars indicate standard errors. Asterisks (*) indicate significant differences between Money Maker (acc 1) and the test accessions (Chi-square test ($\chi 2$), P=0.05). (n=varies with pair wise comparisons since mites that made no choice were excluded from the analysis). Acc stands for accession.

4.1.4: Plant acceptance (leaf disk bioassay)

The six *Lycopersicon* accessions that were compared to Money Maker (accession 1) revealed varying differences in the leaf disk bioassay. Accessions 13, 51, 428 and 460 showed significant differences to Money Maker whereas accessions 162 and 182 did not show significant differences (Figure 3).

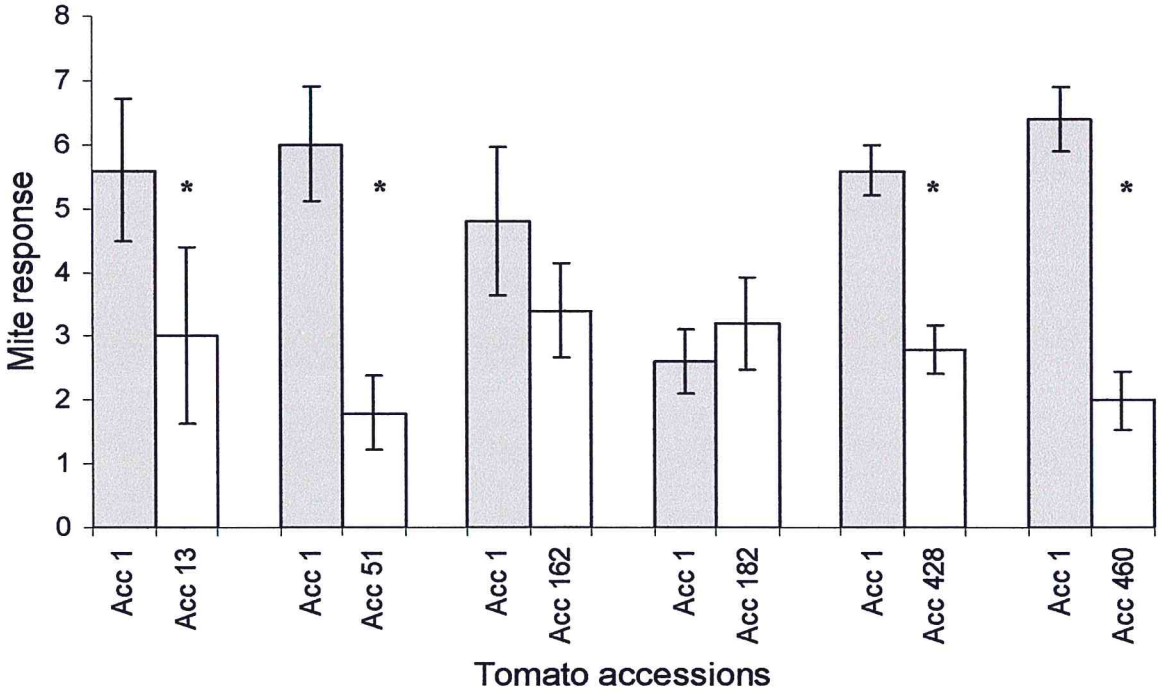

Figure 3: *T. evansi* response (Mean ± S.E) to leaf-disk emitted volatile compounds (n=29-44). Vertical bars indicate standard errors. Asterisks (*) indicate the accessions, which were significantly different from Money Maker (Chi-square test ($\chi 2$), P=0.05). (n=varies with pair wise comparisons since mites that made no choice were excluded from the analysis). Acc stands for accession.

4.1.5: Response of *T. evansi* to essential oils concentration

Spider mite preference to 10µl of essential oils at a concentration of 0.01 reveals that accessions 51, 162, 428 and 460 are significantly different from empty cotton balls when pair wise comparisons are made whereas accessions 1, 13 and 182 were not significantly different (Figure 4).

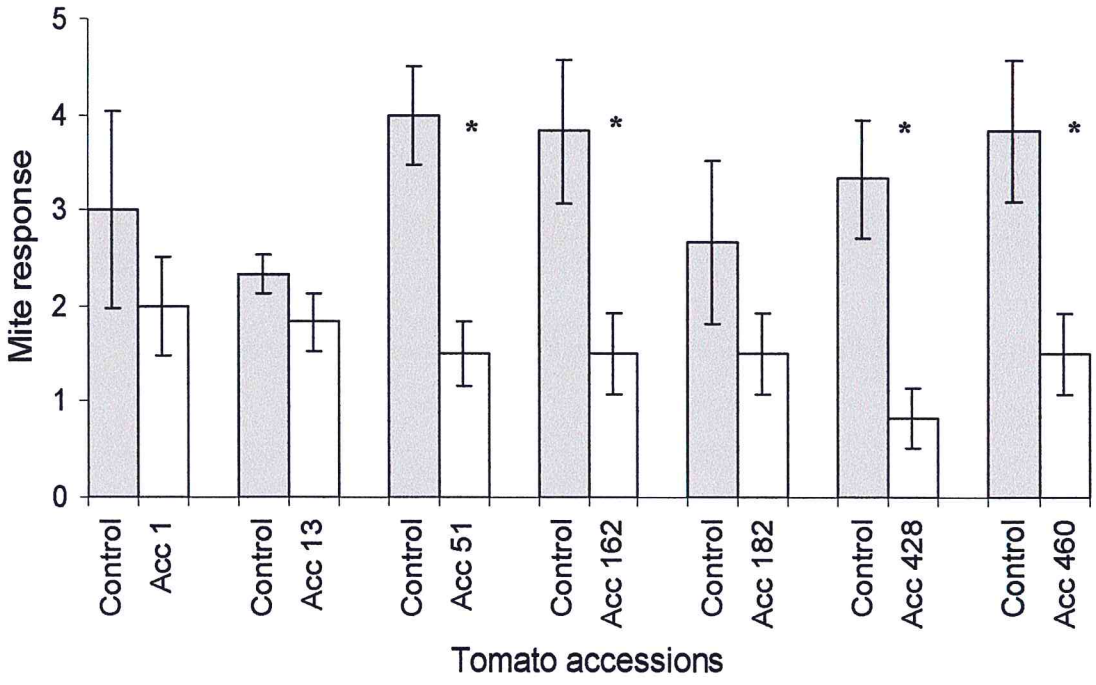

Figure 4: *T. evansi* response (Mean ± S.E) to essential oil compounds (n=25-33). Vertical bars indicate standard errors. Asterisks (*) indicate accessions that were significantly different from untreated cotton balls (without essential oil) (Chi-square test ($\chi 2$), P=0.05). (n=varies with pair wise comparisons since mites that made no choice were excluded from the analysis). Acc stands for accession.

4.2: DISCUSSION

This study has provided important clues regarding the chemical composition of volatiles and essential oils in leaves of the wild and cultivated *Lycopersicon* accessions, which may form a basis for the development of spider mite resistant cultivars. Several methyl ketones were present in both wild and cultivated tomato accessions. Accession 51 in the study that was found to contain the highest amount of 2-tridecanone is of *L. hirsutum* f. *glabratum* 'PI 134417' that has been found to have strong acaricidal properties to *T. urticae* (Chatzivasileiadis and Sabelis, 1997). The olfactometer and leaf disk bioassay results concur with Silva *et al.,* 1992 who identified an accession of *L. hirsutum* f. *glabratum* as the most resistant genotype to *T. evansi*. Nevertheless, accession 428, also a wild tomato accession, was unattractive to spider mites both in the olfactometer and leaf disk bioassays though it contained low amounts of 2-tridecanone.

Though 2-tridecanone occurred in lower amounts in accessions 13 and 460 as compared to accession 162, the two former accessions seem to be unattractive to spider mites in both olfactometer and leaf disk bioassays whereas the latter accession was not significantly different from Money Maker (accession 1) which is highly susceptible to spider mite damage. Also the presence of 2-tridecanone in essential oils of accessions 51, 162, 428 and 460 could have contributed to the significant differences in response of spider mites when they were compared to empty cotton balls using glass slides in the experiment. 2-Tridecanone is a 13-carbon methyl ketone (Williams *et al.*, 1980; Kennedy and Dimock, 1983), which has been thoroughly studied, in arthropod resistance factors in tomato.

2-Undecanone an important 11-carbon methyl ketone (Farrar and Kennedy, 1987) was also established in accessions 13, 51 and 428 although it was less abundant than 2-tridecanone (Kennedy *et al.*, 1991; Lin *et al.*, 1987). 2-Undecanone is known to synergize the activity of 2-tridecanone (Lin *et al.*, 1987; Farrar and Kennedy, 1987) and contribute to spider mite repellence in *Lycopersicon* sp. (Carter and Snyder, 1985) although synergism was not established in the experiments.

2-Undecanone was absent in accessions, 460 and 162 which further contradicts the chemistry behind the repellence of accession 460 to spider mites in the olfactometer and leaf disk bioassays in this experiment. The presence of 2-decanone, 2-pentadecanone and 2-dodecanone in essential oils may have significant effects on spider mite repellence in tomato accessions that contained them through their synergistic effects on the activity of 2-tridecanone, though this was not established in this study. Lack of significant effects on spider mites in accession 162 in the glass slide bioassays using essential oils is not understood since it contains good amounts of the important ketones.

Accessions 1 (Money Maker) and 182 did not reveal any presence of 2-tridecanone and 2-undecanone in the volatile studies and this may be the reason they were preferred by the spider mites in both leaf disk and olfactometer bioassays. Accession 1 (Money Maker) was also found to contain trace amounts of β-demascenone and β-ionone in essential oil analysis. These compounds are norisoprenoids, which are volatile C9-C13 fragments from the degradation of C40 carotenoids, which have been shown to have significant aroma impact in tomatoes (Weeks, 1986). The presence of these aroma components in foliage of Money Maker plants may be the reason why this accession is very susceptible to spider mite attack due to the level of attraction as depicted in the glass slide bioassays. Further research is required to elucidate this question. The presence of 3-methyl-2-butanone in nearly all the accessions may be speculated to have some synergistic effects on 2-tridecanone and other ketones, but no previous work related to this was established.

The sesquiterpene content of tomato leaf oil varied considerably amongst accessions with β-caryophyllene, β-phellandrene, δ-elemene, β-elemene, α-humulene and δ-cadinene being widespread amongst the wild and cultivated accessions. Lundgren *et al.* (1985) established that caryophyllene and humulene are the most widespread sesquiterpenes in *Lycopersicon* species as was the case with essential oils where β-caryophyllene and α-humulene occurred in all the accessions.

Leaves of cultivated tomato have large glandular trichomes (type VI) (Luckwill, 1943) and their major volatiles are terpenoids such as β-phellandrene and δ^3-carene (Buttery *et al.*, 1987; Dicke *at al.*, 1998) as found in the experiments. They occurred in very low proportions to cause any significant effects as depicted in the bioassays especially in Money Maker. Accession 182, which lacked some of these major compounds in the volatiles, was a rather preferred accession as established in the olfactometer and leaf disk bioassays. α-Pinene, β-pinene and camphene were established in accessions 1 (Money Maker), 162 and 460 respectively although in minor proportions. The occurrence of these sesquiterpenes in these *L. esculentum* accessions could be a possible contributor to spider mite preference especially in the case of accession 1 which is known to be very susceptible to spider mites.

These compounds have been previously reported to play a role in eliciting behavioral responses in some adult phytophagous insects. α-Pinene in combination with camphene is known to be attractive to the leafhopper, *Amrasca devastans* Distant (Saxena and Saxena, 1974). α-Pinene and β-pinene have been known to stimulate oviposition of the Eastern spruce budworm, *Choristoneura fumiferana* Clemens (Städler, 1974) and could possibly contribute to the preference of mites to Money maker as was established in olfactometer and leaf disk bioassays, since it was found to contain some amount of α-pinene.

Large quantities of terpenes and other essential oils accumulate in glandular trichomes that have been found to be associated with insect resistance in a number of species in the genus *Lycopersicon* (Fery and Kennedy, 1987; Lin *et al.*, 1987; Snyder and Carter, 1984). Limonene, which was established in accession 428, may have played a role in enhancing repellence to spider mites in the olfactory perception and glass slide bioassays for essential oils. Al Rouz and Thibout (1988) reported that limonene repels the leek moth while Dormont *et al.* (1997) reported that limonene and β-phellandrene in combination with other terpenes prevent insect attack of pine cones.

Previous studies carried on the oviposition of *T. evansi* per day for 10 days on these accessions showed that 460 and 13 had a mean egg laying capacity of 0.3 and 1.5 respectively as compared to 1, 162 and 182 which had 40.3, 50.2 and 45.6 respectively while accessions 51 and 428 showed a mean egg laying capacity of 0.8 and 0 respectively (ICIPE, unpublished data).

The resistance mechanisms involved in deterring oviposition in accessions 13, 51, 428 and 460 are those associated with repellence of trichome exudates (Cantelo *et al.*, 1974) as has been identified in our experiments. Mites die quickly when they receive a lethal dose of 2-tridecanone through either cuticular contact with the trichome exudates or vapor action or both (Kennedy and Yamamoto, 1979; Williams *et al.*, 1980). This could probably be the reason why the oviposition was low in these accessions in past trials. Carter and Snyder (1985) also established that the density of type VI glandular trichomes contribute to variation in spider mite responses which probably resulted to differences in oviposition capacity in this study.

However, care has to be taken since varying environmental conditions such as photoperiod, light intensity and plant nutrient status may alter the resistance mechanisms of the plants. For instance, Good and Snyder (1988) demonstrated that interspecific hybrids of *L. esculentum* x *L. hirsutum* grown in winter and summer expressed differential levels of mite resistance, trichome numbers and sesquiterpenes content. Snyder and Hyatt (1984) found that artificial changes in photoperiods also changed the density of different types of trichomes. Trichome numbers change with canopy height in *L. esculentum* leading to different densities of the tomato rust mite *Aculops lycopersici* (Massee) (Leite *et al.*, 1999). Barbour *et al.* (1991) also detected that high fertilization levels can reduce the density of trichomes and the level of sesquiterpenes important for the resistance of *L. hirsutum* to several insect and mite species. More research is required to elucidate the question on effect of environmental conditions.

CHAPTER FIVE

5.0: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

The present study reveals that some of the compounds present in whole intact plants are similar to those that are present in essential oils. 2-tridecanone and 2-undecanone are the major volatile phytochemicals that were established in the wild *Lycopersicon* species although the relative abundance was lower for 2-tridecanone in essential oil of accession 51 than it was in volatile compounds obtained from intact plants. Also, essential oils revealed a number of methyl ketones and sesquiterpenes than volatile compounds from intact plants. These differences can be attributed to various factors affecting the release of the volatiles and the time course of their emission. The technique used in collecting the volatiles should provide important information without disturbing the plant. Time is very important in all plant studies, because most chemicals show a time dependent release profile (Loughrin *et al.*, 1990). The technique that was used to trap volatiles in this study was taking a long time i.e. 24 hours and thus unable to provide precise information on the time course of release of compounds emitted over short periods of time.

Furthermore, the handling of plant material before and during the collection of the compounds is crucial and can affect the release of these compounds. Techniques requiring maceration or chopping of the plant material, followed by distillation allow enzymatic and chemical reactions to occur that cause changes in the volatile composition of the sample (Blight, 1990) as was done in our experiments. Although such procedures allow for the isolation of large amounts of specific compounds, they do not provide information on what was emitted by a living plant. For example, Tollsten and Bergström (1988) showed that intact plants of a number of *Brassica* species released a different blend of volatile chemicals to macerated plant material.

In addition, mechanical injury of plant tissue induces changes in the chemistry of the intact parts of the plant (Paré and Tumlison, 1997). The use of glass chambers in our experiment may have protected that plant from any physical damage, but the effect of inclosing the plant may not be established which could have resulted in chemical changes within the plant.

Indirect selection for high allelochemical content in cultivated tomato would be an efficient technique for pest resistance (Juvik *et al*, 1982). The accession *L. hirsutum* f. *glabratum* PI 134417 is a particular promising source of resistance because it can be easily intercrossed with *L. esculentum* (Taylor, 1986). Selection for high 2-tridecanone concentration in tomato leaflets has been effected in segregating populations of the cross between *L. esculentum* x *L. hirsutum*. f. *glabratum* 'PI 134417' (Barbosa and Maluf, 1996) and was shown to effectively increase the levels of resistance to the South American tomato pinworm *T. absoluta* (Maluf *et al.*, 1997).

Breeding programmes aimed at introgressing arthropod resistance from wild *Lycopersicon* species into the cultivated tomato face methodological difficulties to keep uniform conditions of infestation necessary to select for resistance (Stevens and Rick, 1986). Direct selection for arthropod resistance is also expensive and slow. Therefore they should be efficiently studied and should be based on an easy to measure trait as suggested by Juvik *et al.* (1982), especially if it imparts resistance to multiple pests.

Tomato accessions could be grown in different environmental conditions in future, so as to follow closely the variations of the chemical composition based on day length, temperature, light intensity and all interactions amongst these variables. In addition, sampling of the leaves for steam distillation to obtain essential oils could be done according to leaf sites on the plant i.e. either top, middle or bottom for each of the tomato accessions to check the chemical variation within the plant. Also mite responses could be studied at certain dosages of the major compounds on main developmental stages of the spider mites i.e. larvae and adult.

Nevertheless, there could be a possibility to develop a technique of isolation of volatile chemicals released by individual leaves of each accession when they are intact. In conclusion, this experiment reveals that both wild and some cultivated *Lycopersicon* accessions contain volatile compounds and constituents of these compounds in essential oils, which may form a basis for the development of resistant tomato varieties.

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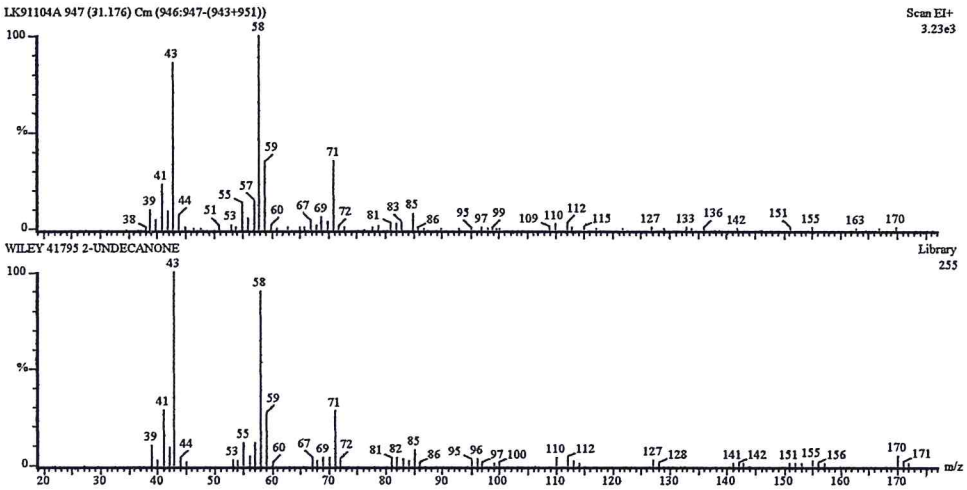
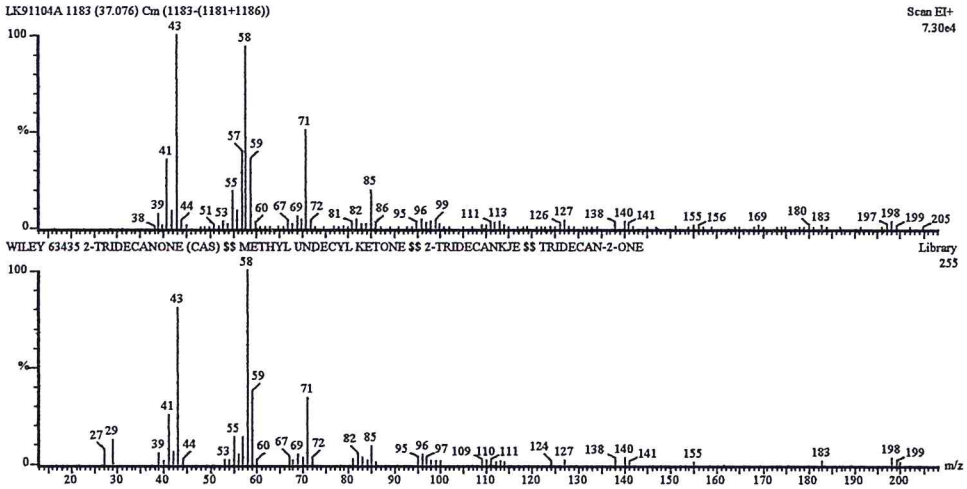
APPENDIX

Appendix 1: GC-MS spectrums of some major identified compounds



1 (a) 2-undecanone



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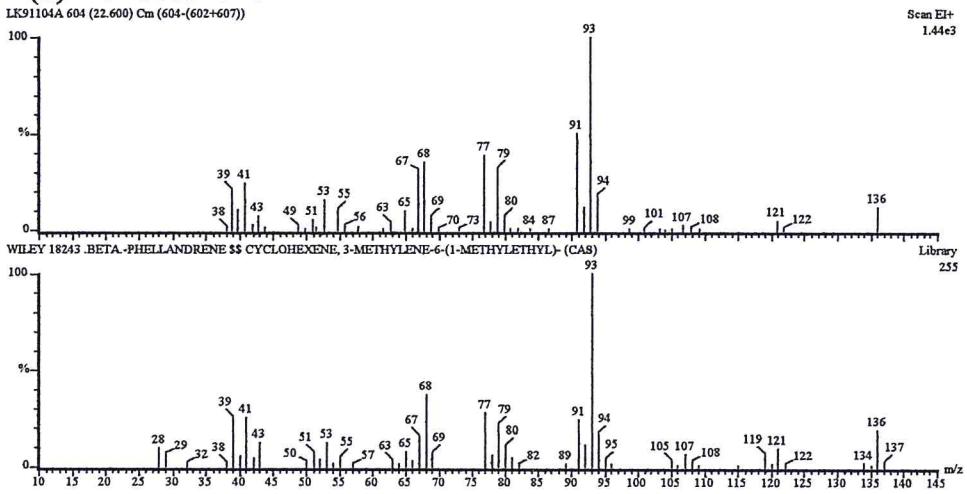
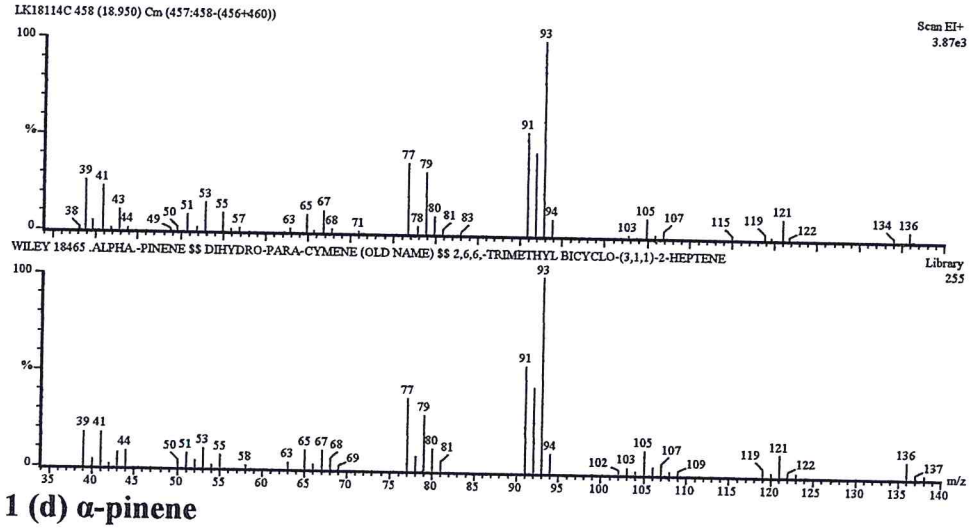
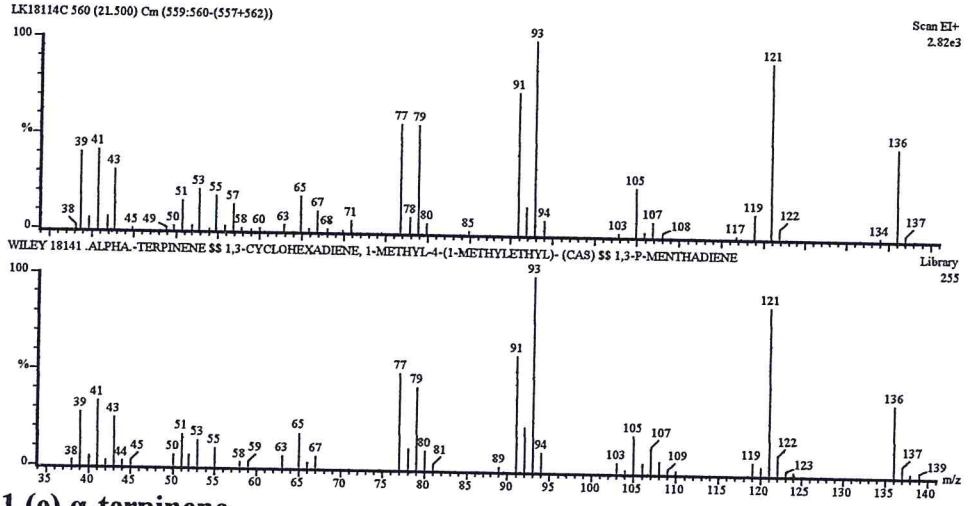


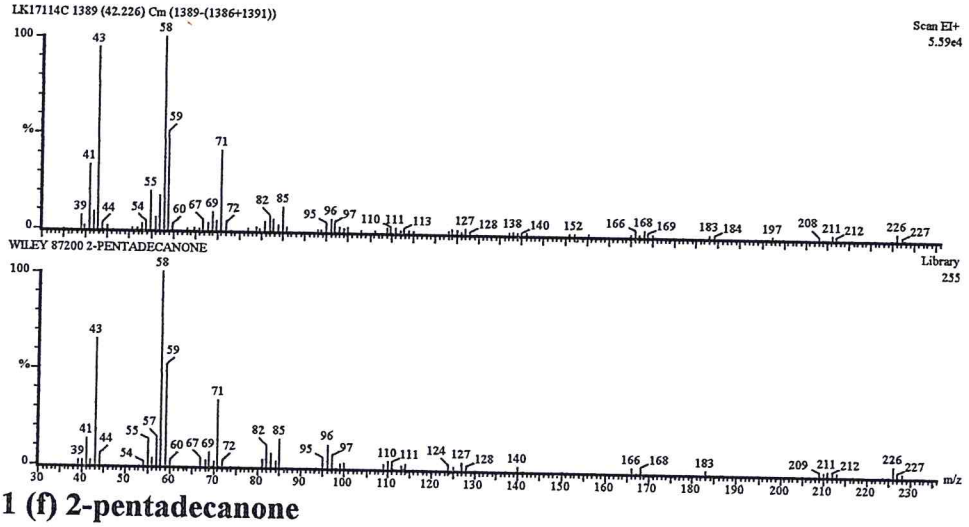
1 (c) β-phellandrene



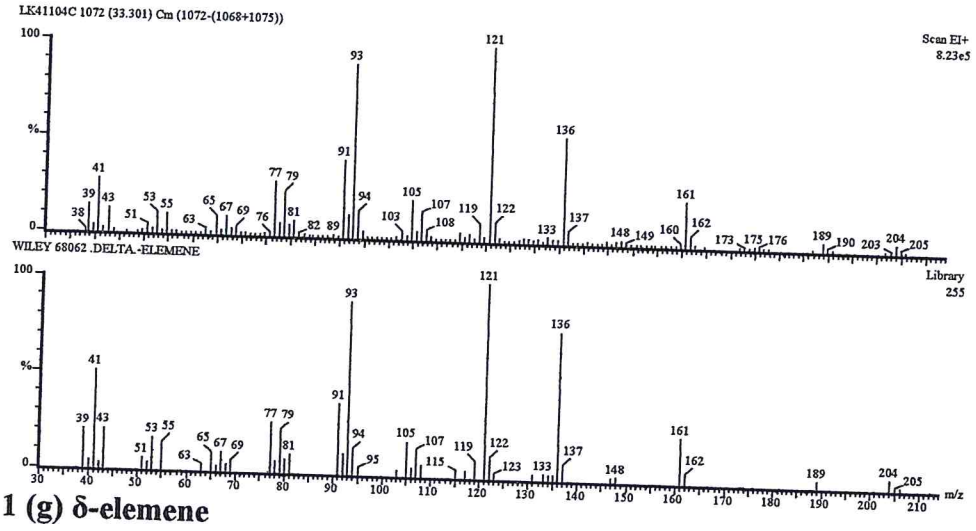
1 (d) α-pinene



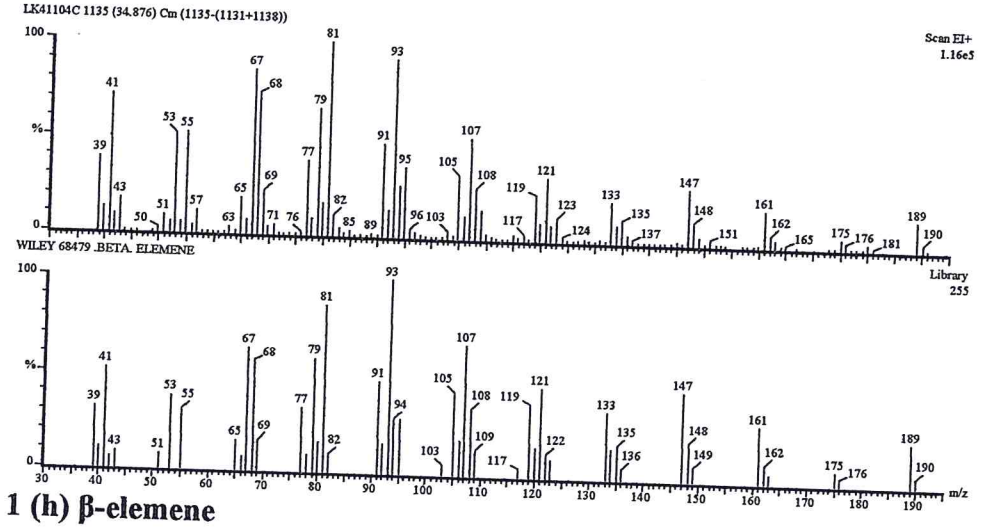
1 (e) α-terpinene



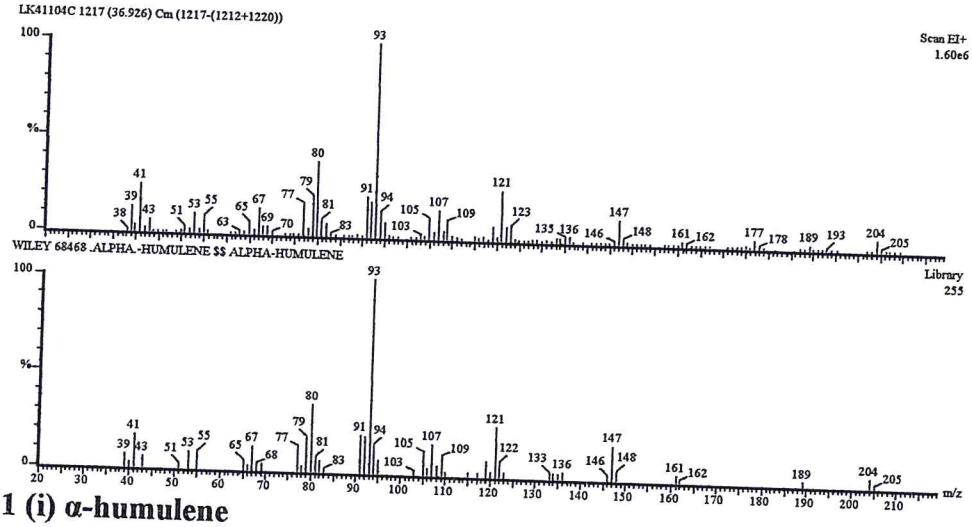
1 (f) 2-pentadecanone



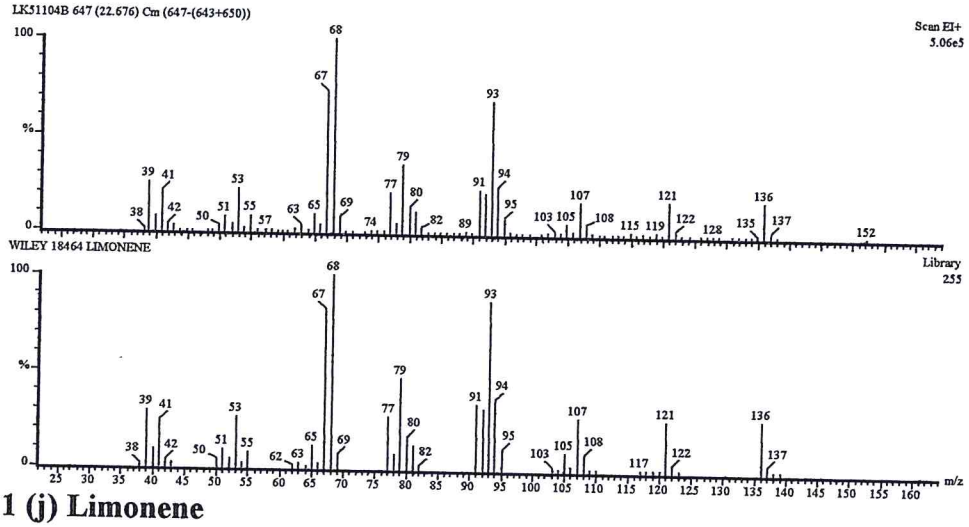
1 (g) δ-elemene



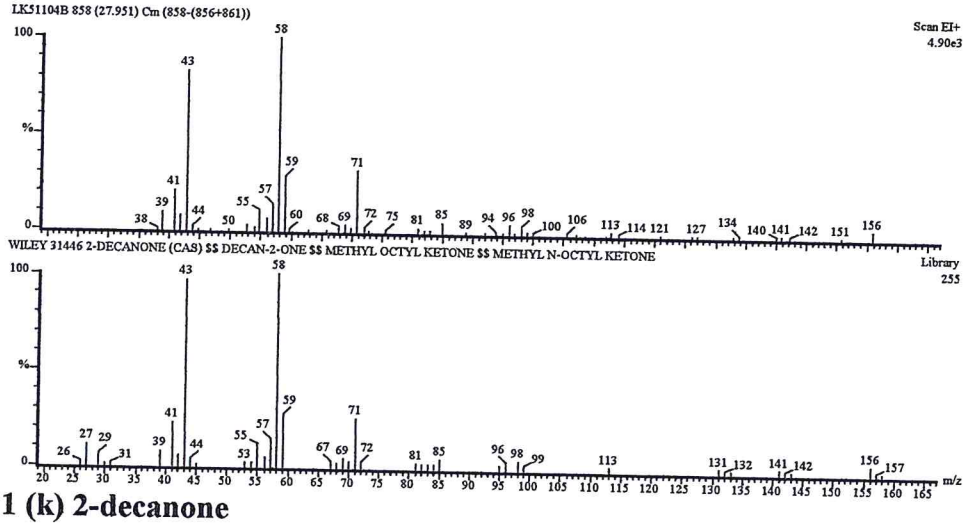
1 (h) β-elemene



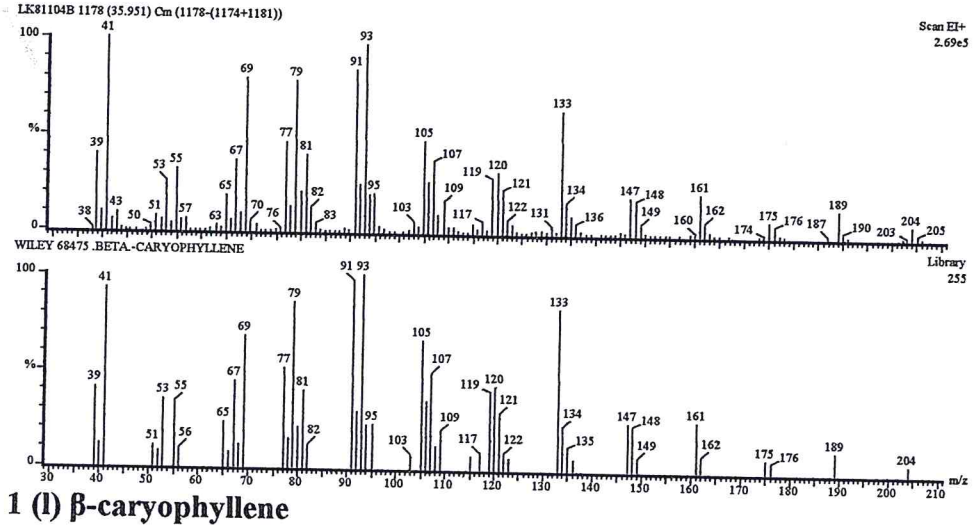
1 (i) α-humulene



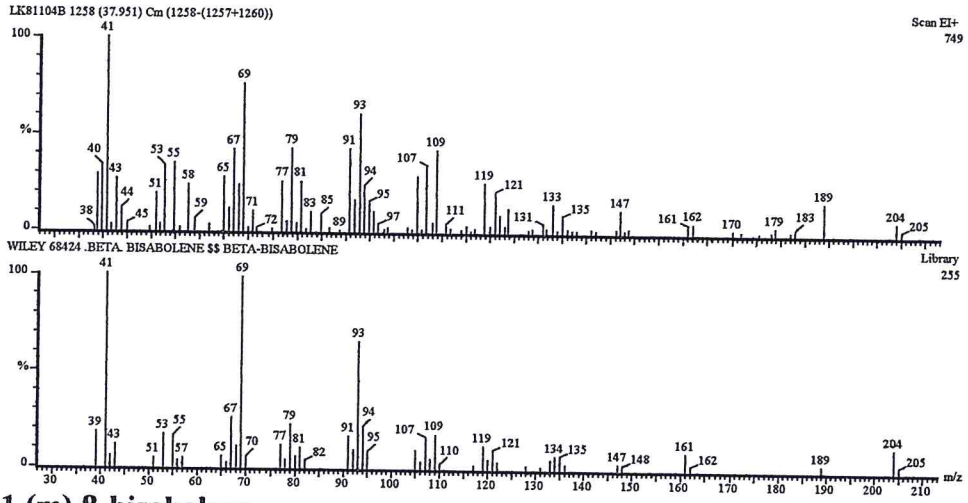
1 (j) Limonene



1 (k) 2-decanone



1 (l) β-caryophyllene



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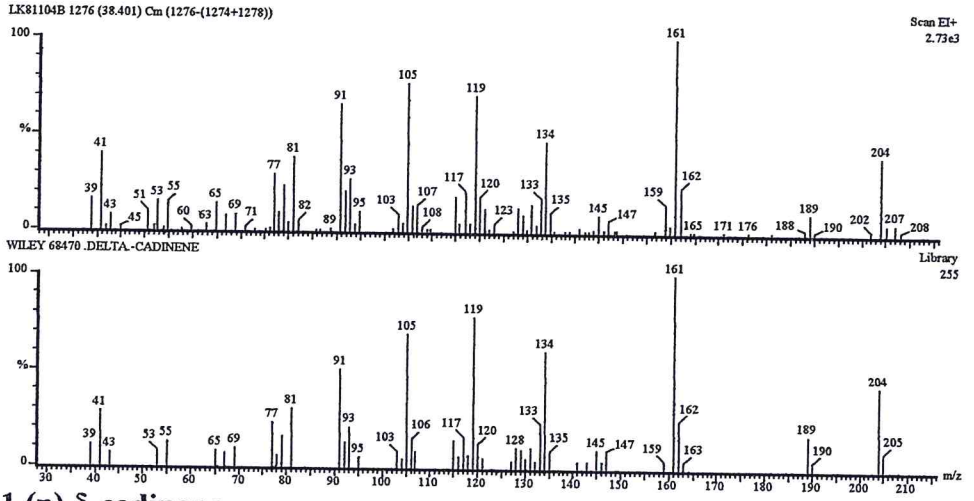
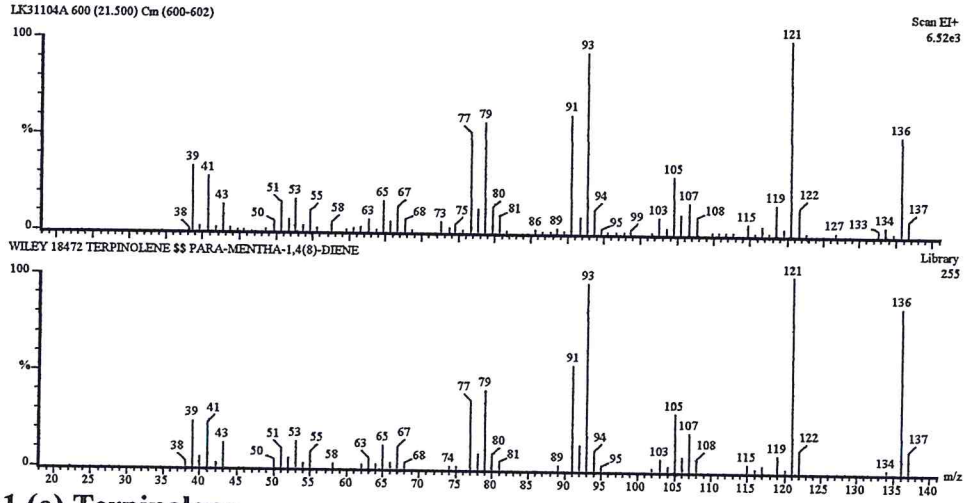
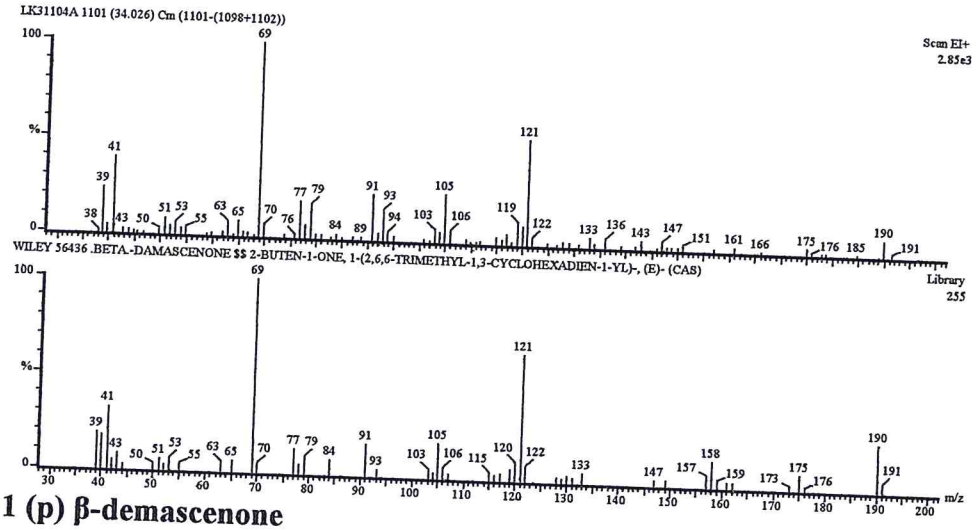


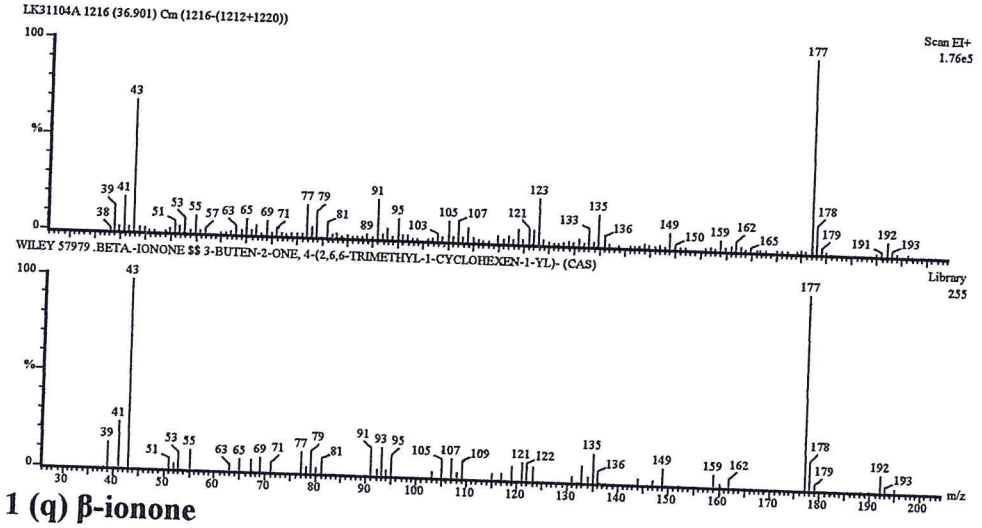
1 (n) δ-cadinene



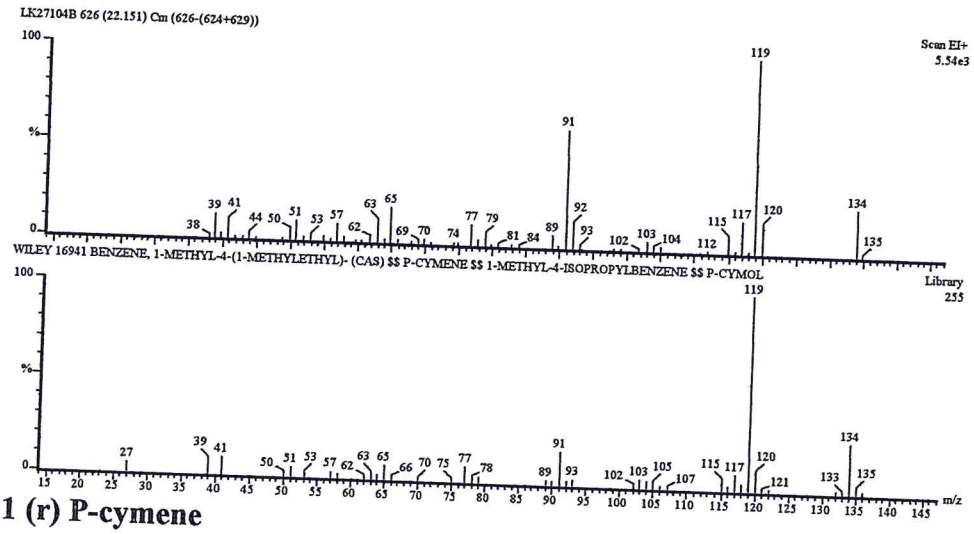
1 (o) Terpinolene



1 (p) β-demascenone



1 (q) β-ionone



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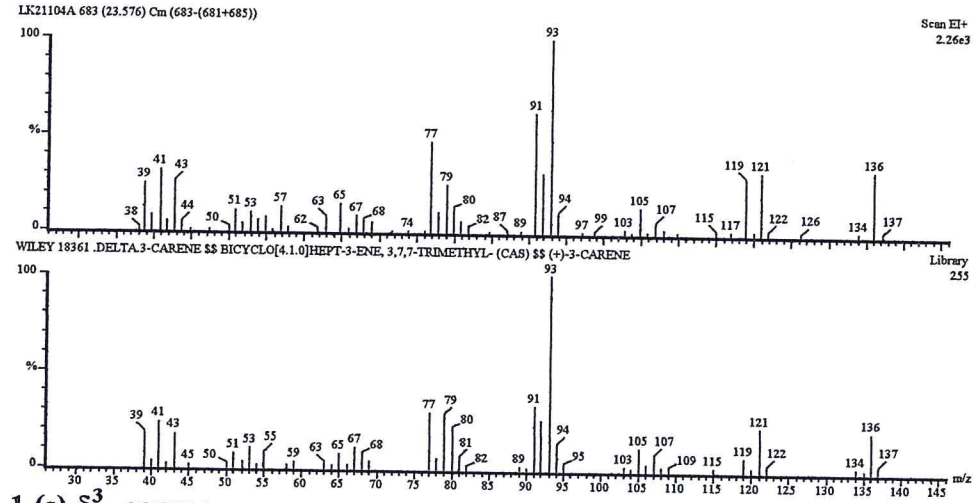
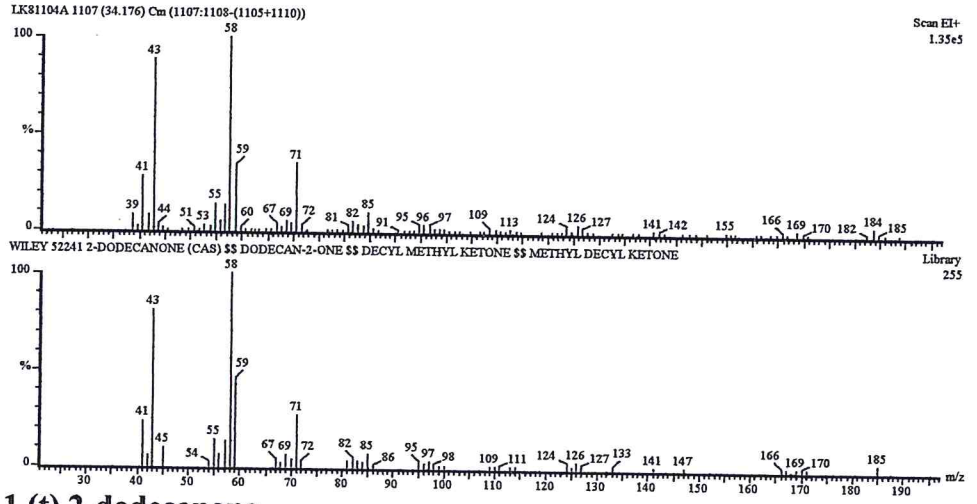


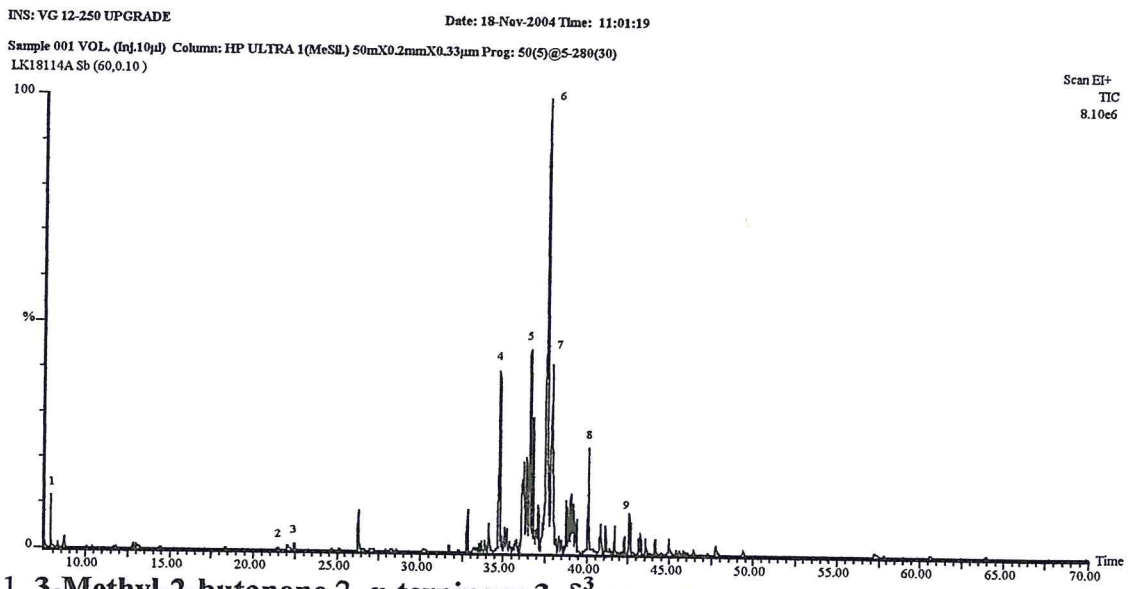
1 (s) δ^3- carene



1 (t) 2-dodecanone

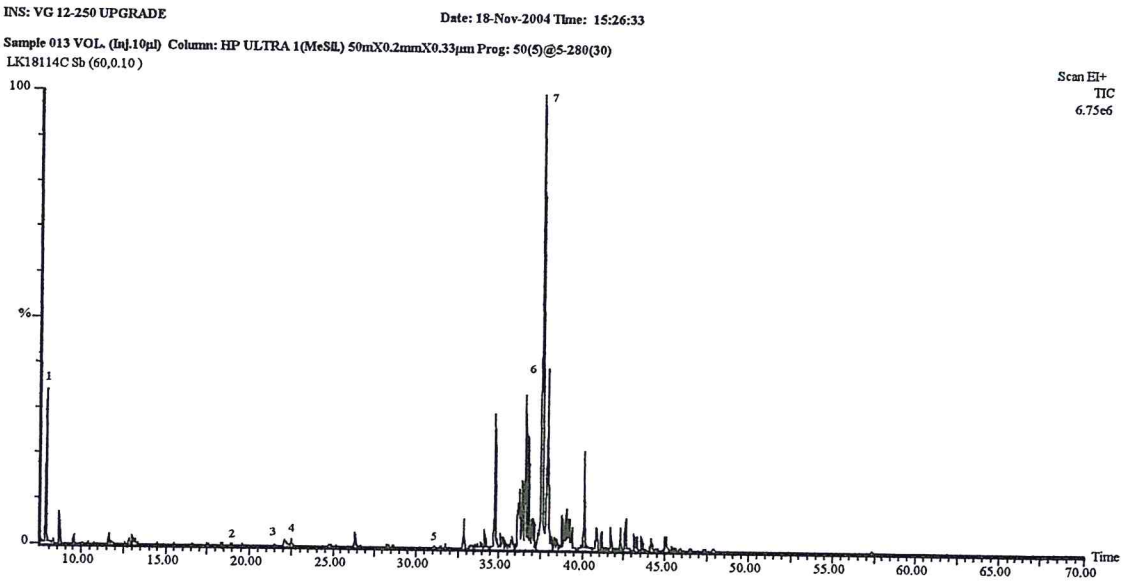
Appendix 2: GC-MS spectrum of chromatogram profiles of volatile compounds

2 (a) Accession 1



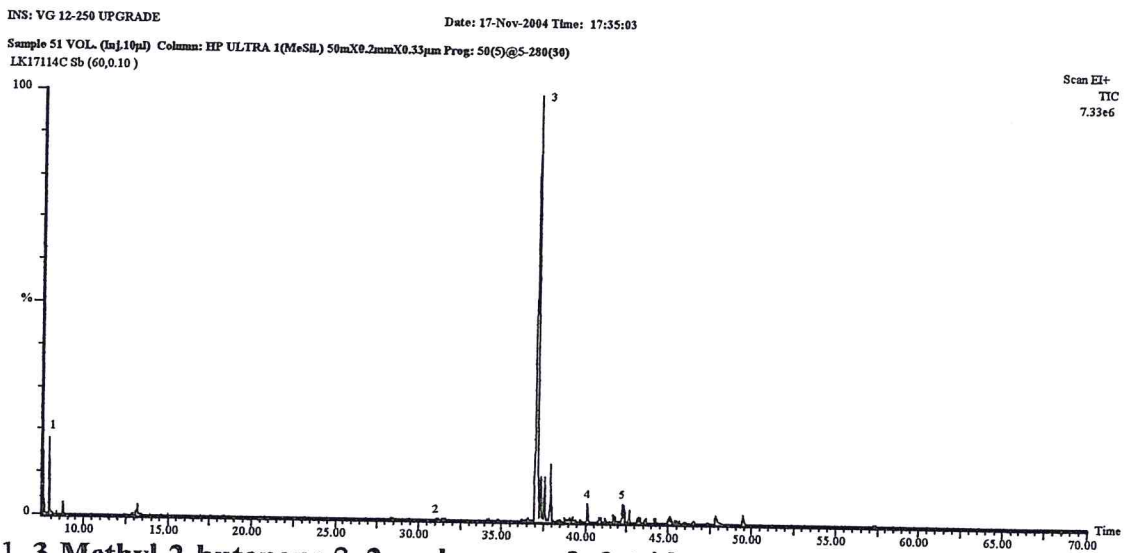
1. **3-Methyl-2-butanone** 2. **α-terpinene** 3. **δ^3-carene**

2 (b) Accession 13



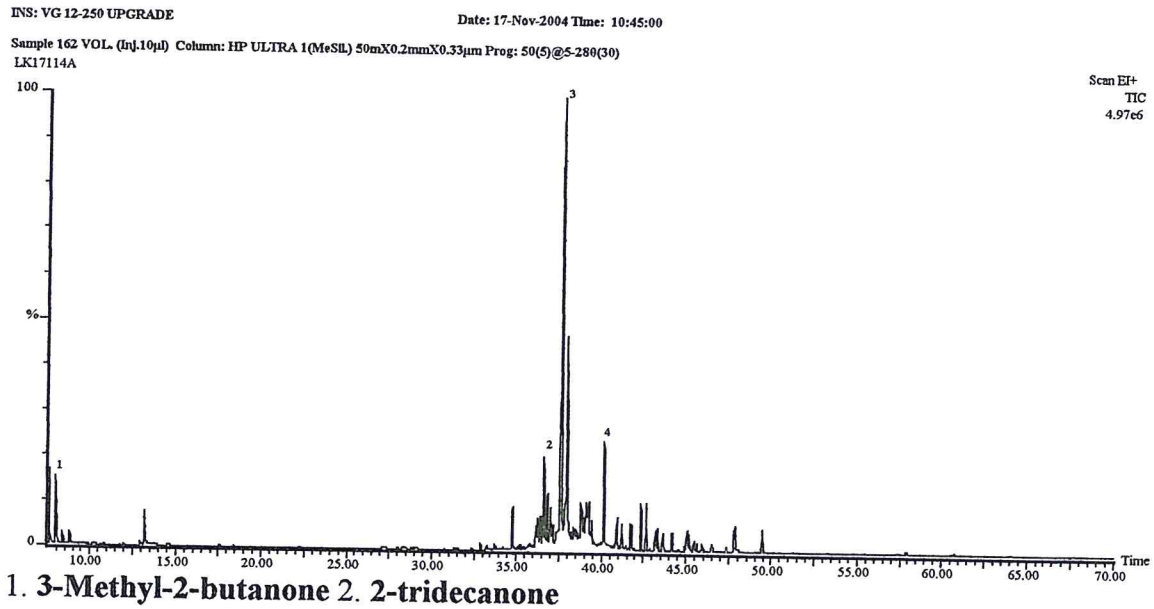
1. **3-Methyl-2-butanone** 2. **α-pinene** 3. **α-terpinene** 4. **β-phellandrene** 5. **2-undecanone** 6. **2-tridecanone**

2 (c) Accession 51



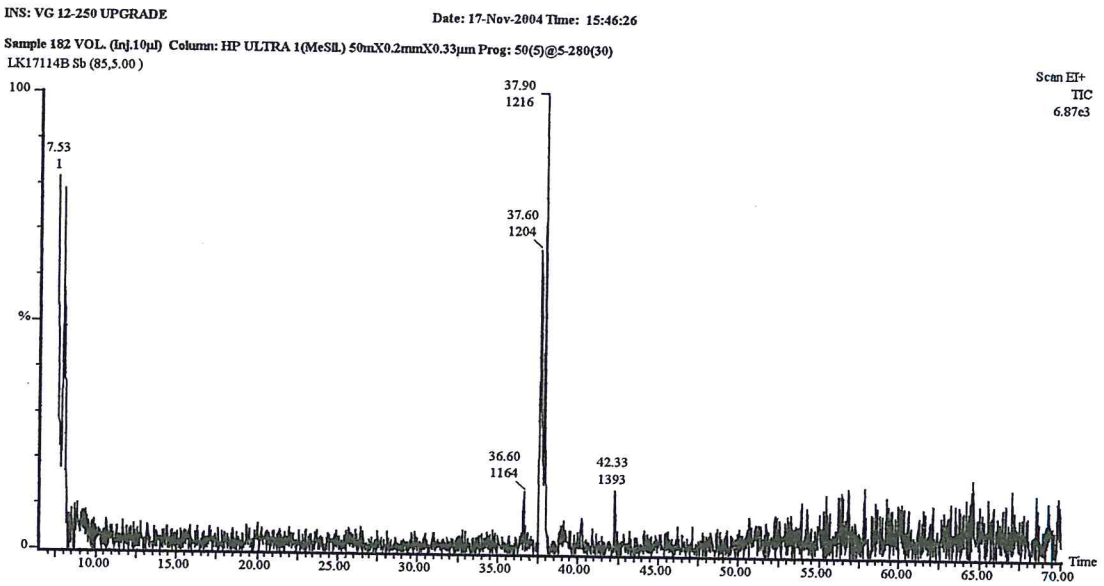
1. **3-Methyl-2-butanone** 2. **2-undecanone** 3. **2-tridecanone** 5. **2-pentadecanone**

2 (d) Accession 162

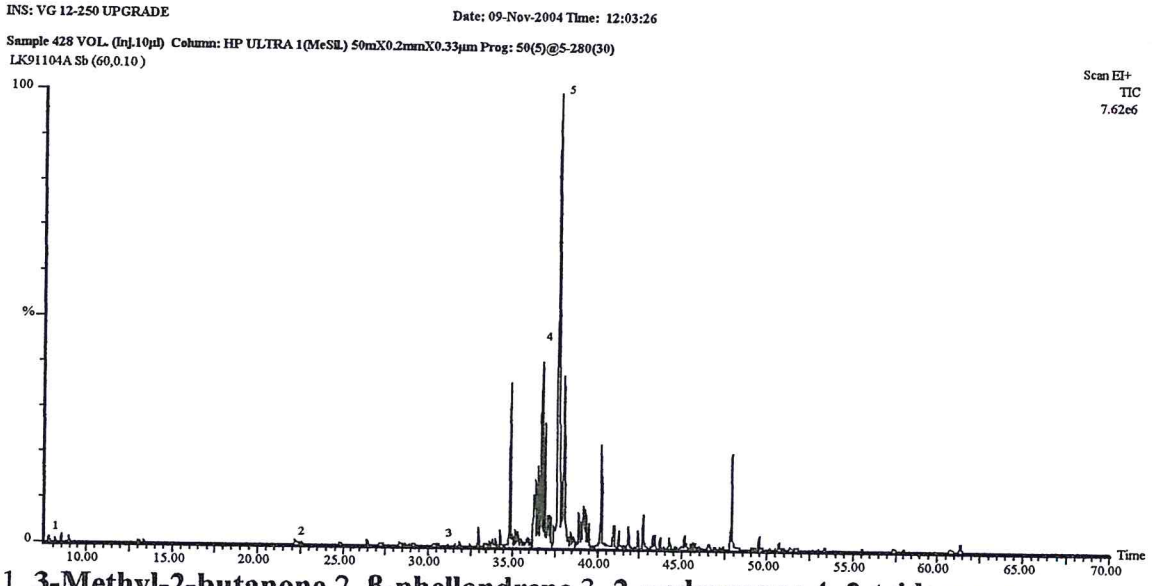


1. **3-Methyl-2-butanone** 2. **2-tridecanone**

2 (e) Accession 182

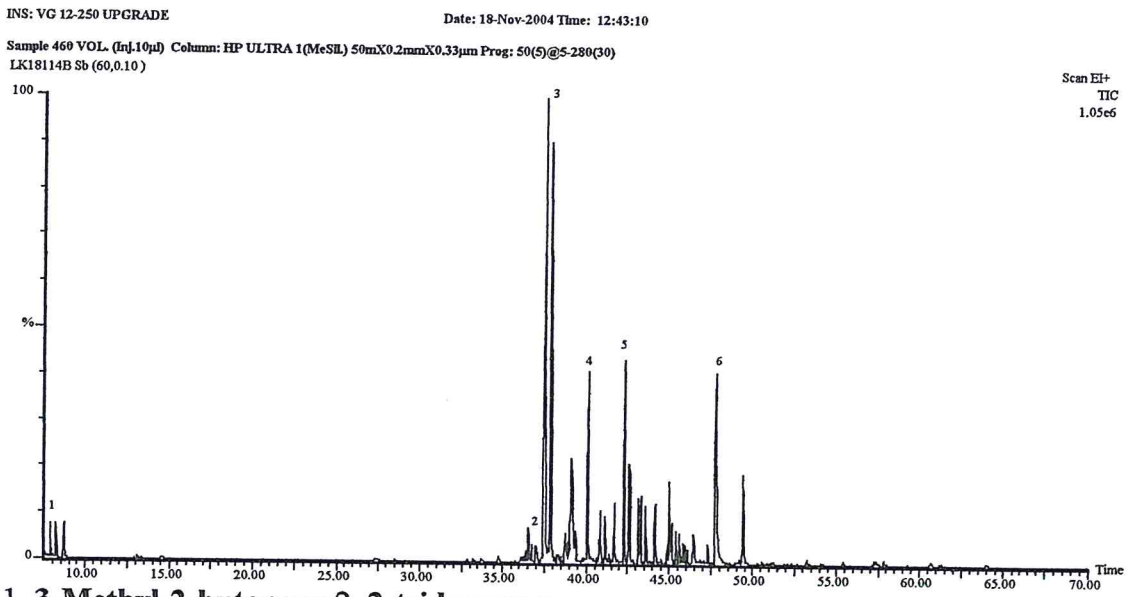


2 (f) Accession 428



1. **3-Methyl-2-butanone** 2. **β-phellandrene** 3. **2-undecanone** 4. **2-tridecanone**

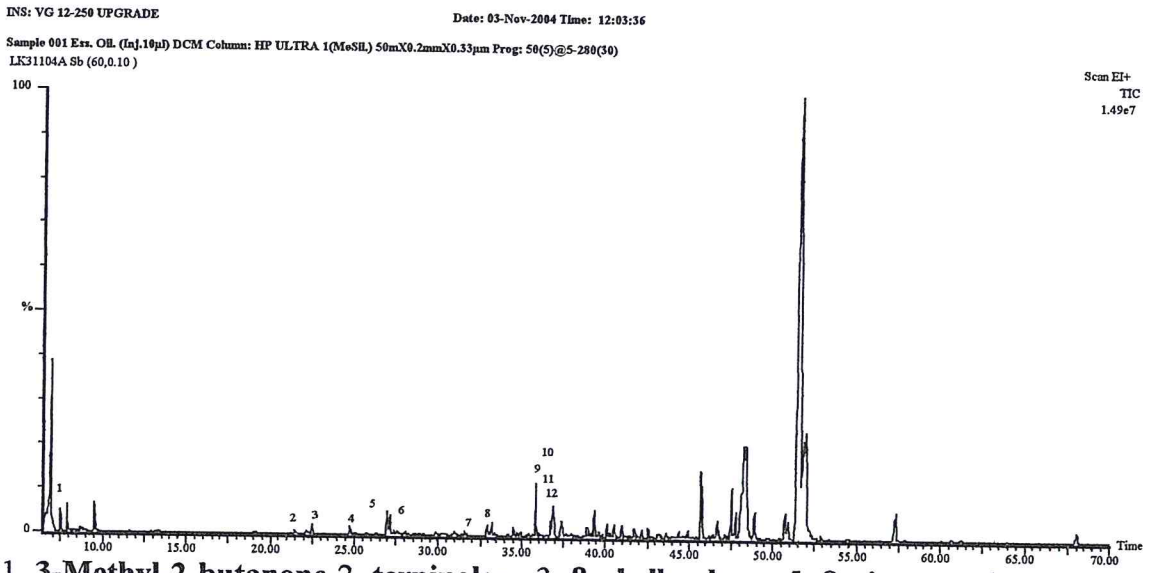
2 (g) Accession 460



1. **3-Methyl-2-butanone** 2. **2-tridecanone**

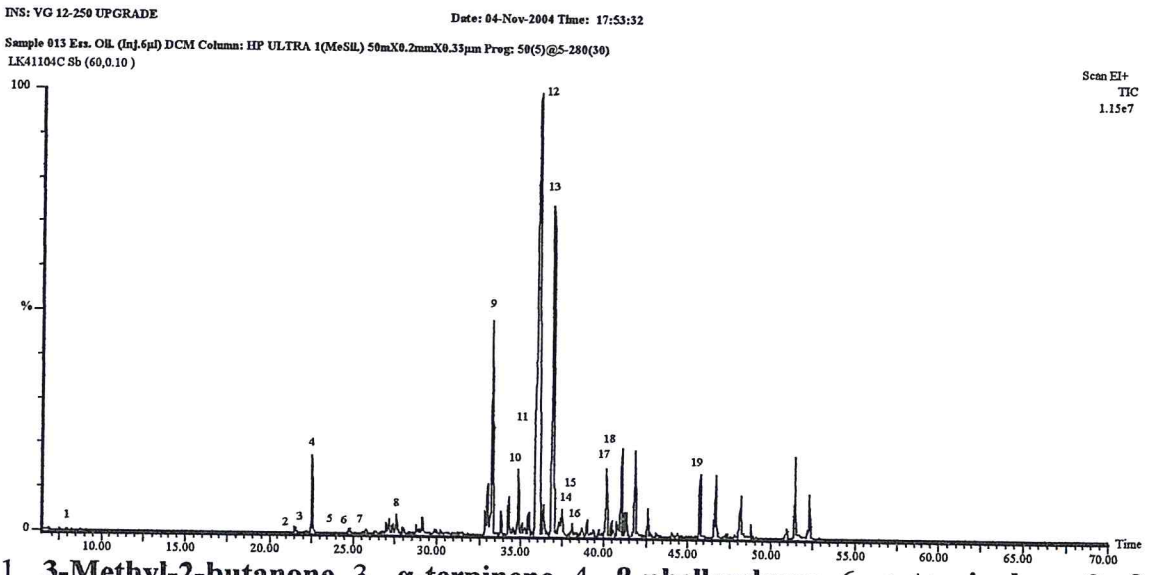
Appendix 3: GC-MS spectrum of chromatogram profiles of essential oils

3 (a) Accession 1



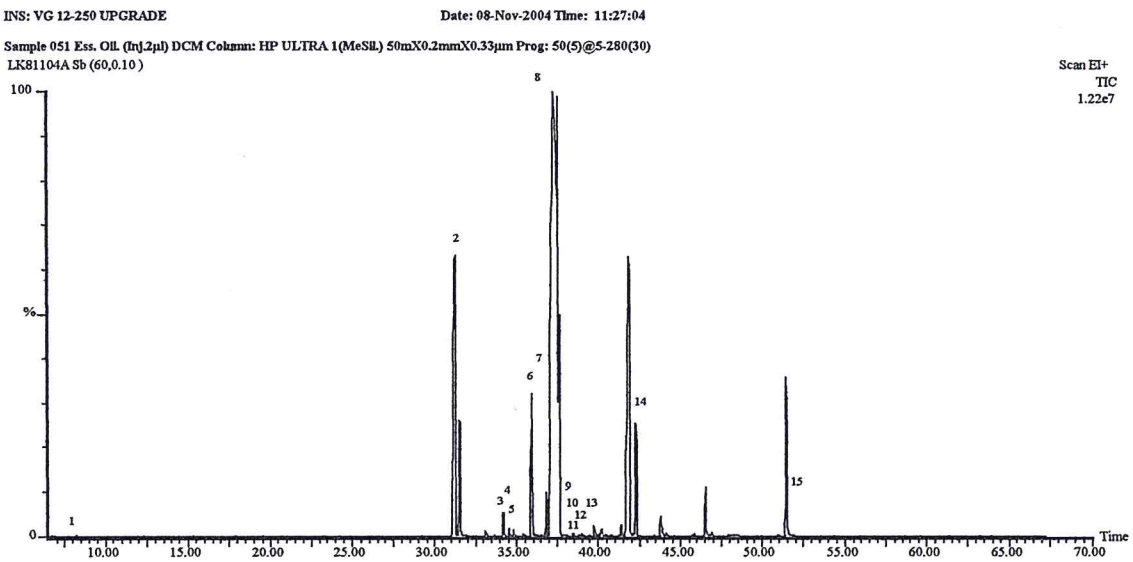
1. **3-Methyl-2-butanone** 2. **terpinolene** 3. **β-phellandrene** 5. **β-pinene** 8. **δ-elemene** 9. **β-caryophyllene** 11. **α-humulene** 12. **β-ionone**

3 (b) Accession 13



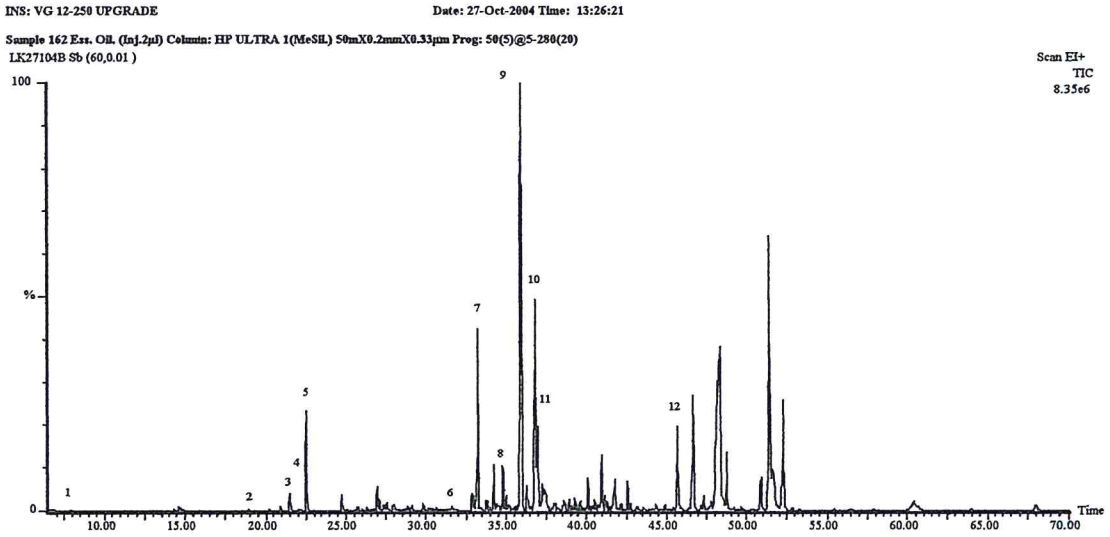
1. **3-Methyl-2-butanone** 3. **α-terpinene** 4. **β-phellandrene** 6. **α-terpinolene** 9. **δ-elemene** 10. **β-elemene** 11. **β-caryophyllene** 13. **α-humulene** 14. **δ-cadinene** 19. **2-pentadecanone**

3 (c) Accession 51



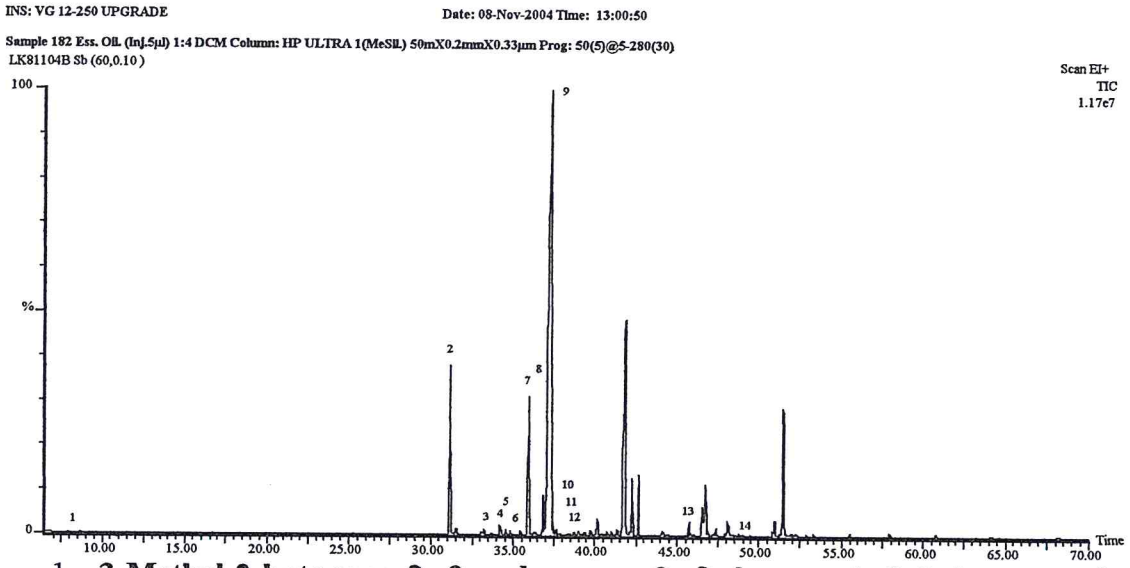
1. **3-Methyl-2-butanone** 2. **2-undecanone** 3. **2-dodecanone** 5. **β-elemene** 6. **β-caryophyllene** 7. **α-humulene** 8. **2-tridecanone** 10. **β-bisabolene** 11. **δ-cadinene** 12. **2-decanone** 14. **2-pentadecanone** 15. **2-nonadecanone**

3 (d) Accession 162



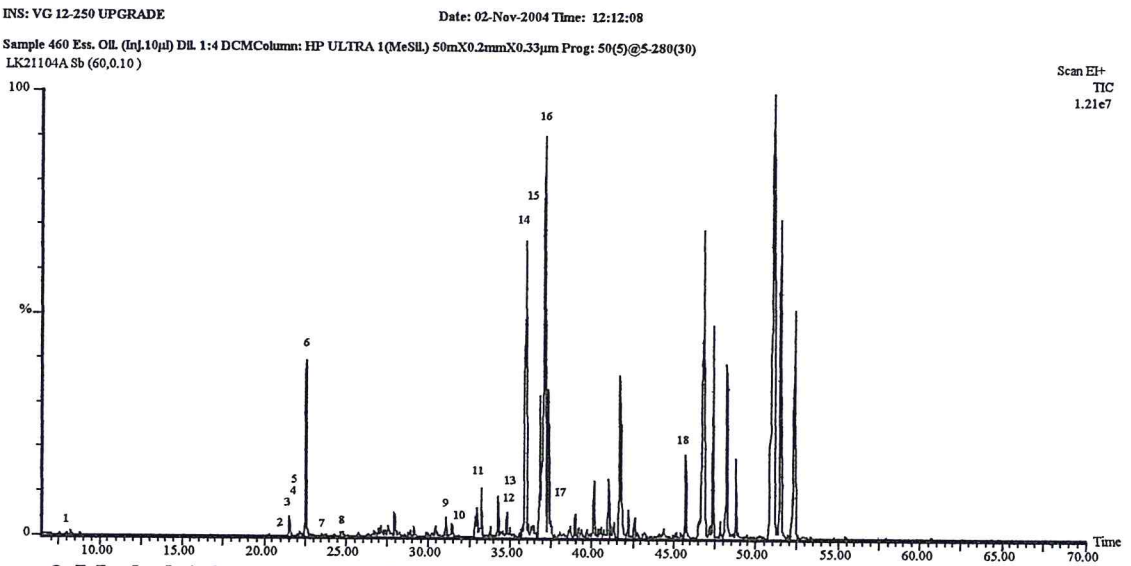
1. **3-Methyl-2-butanone** 2. **α-pinene** 3. **terpinolene** 4. **α -terpinene** 5. **β- phellandrene** 6. **2-undecanone** 7. **δ-elemene** 8. **β-elemene** 9. **β-caryophyllene** 10. **α-humulene** 11. **2-tridecanone** 12. **2-pentadecanone**

3 (e) Accession 182



1. **3-Methyl-2-butanone** 2. **2-undecanone** 3. **δ-elemene** 4. **2-dodecanone** 5. **β-elemene** 7. **β-caryophyllene** 8. **α-humulene** 9. **2-tridecanone** 11. **β-bisabolene** 12. **δ-cadinene** 13. **2-pentadecanone**

3 (f) Accession 460



3-Methyl-2-butanone 2. β-bisabolene 3. terpinolene 4. α-terpinene 5. ρ-cymene 6. β-phellandrene 7. δ^3-carene 8. α-humulene 9. 2-undecanone 11. δ-elemene 12. Camphene 14. β-caryophyllene 16. 2-tridecanone 17. δ-cadinene 18. 2-pentadecanone.