ANALYSIS OF VOLATILE PHYTOCHEMICALS FROM WILD NON-HOST PLANT, *Melinis minutiflora* P. BEAUV., AND WILD HOST PLANT *Pennisetum purpureum* (K.) SCHUMACH. OF *Chilo partellus* (SWINHOE)

BY

KIMANI SOLOMON MBUTHIA

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DECLARATION

I hereby declare that this is my own original work, and that it has not been presented for degree in any other University.

Signed .....

Kimani Solomon Mbuthia

Kenyatta University

This thesis has been submitted for examination with our approval as supervisors.

Signed.....

Prof. Sumesh Chander Chhabra

Department of Chemistry

Kenyatta University

Nairobi.

Signed.....

Dr. Wilber Lwande

Department of Chemical Ecology

International Center of Insect Physiology and Ecology

Nairobi.

DEDICATION

WITH LOVE TO DAD AND MUM.

YOUR LOVE, CARE AND PRAYERS HAVE BEEN THE FOUNTAIN OF MY INSPIRATION.

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LIST OF ABBREVIATIONS

ADH	Alcohol dehydrogenase
ATP	Adenosine triphosphate
CAB	Commonwealth Agricultural Bureaux
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
GC	Gas Chromatography
GC-EAG	Gas Chromatography-Electroantenography
GC-MS	Gas Chromatography-Mass Spectrometry
HP	Hewlett Packard
ICIPE	International Centre of Insect Physiology and Ecology
ICRISAT	International Crop Research Institute for Semi-Arid Tropics
IPP	Isopentyl Pyrophosphate
ITC	Isothiocyanate
Lpx	Lipoxygenase
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced
NBS	National Bureau of Standards
NIST	National Institute of Standards and Technology

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ABSTRACT

Semiochemicals that could be involved in host and non-host plant selection by *Chilo partellus* (Swinhoe) were identified from volatiles released by wild host plant, *Pennisetum purpureum* (K.) Schumach. and wild non-host plant, *Melinis minutiflora* P. Beauv. Volatiles were trapped using Porapak Q adsorbent for 24 hrs and analyzed using GC, GC-MS, and GC co-injection with authentic samples.

Compounds confirmed to be present in the headspace of *P. purpureum* were toluene, p-xylene, α-pinene, ß-pinene, (Z)-3-hexenyl acetate, (+)-2-carene, limonene, 1,8-cineole, nonanal, 4,8-dimethylnona-1,3,7-triene, decanal, ß-caryophyllene and α-humulene. On the other hand, headspace volatiles of *M. minutiflora* contained hexanal, α-pinene, sabinene, myrcene, (Z)-3-hexenyl acetate, limonene, 1,8-cineole, (E)-ß-ocimene, terpinolene, 4,8-dimethylnona-1,3,7-triene, methyl salicylate, α-cubebene, α-ylangene, ß-cubebene, ß-caryophyllene and α-humulene. The latter results compared significantly with those of the field trapping using reverse-phase adsorbent. However, the headspace volatiles of *M. minutiflora* varied significantly from its steam distilled oil which contained toluene, hexanal, 3-hexenol, 2-heptanone, heptanal, (E)-2-octenal, nonanal, methyl salicylate, decanal, ß-caryophyllene and α-humulene. A GC comparison of the airborne volatiles from *M. minutiflora* recovered from activated charcoal, Porapak Q and reverse phase

traps showed no discernible qualitative difference in the components present.

Electrophysiologically active compounds located by GC-EAG were identified as: sabinene, 2-pentylfuran, (E)-ß-ocimene, terpinolene, 4,8-dimethylnona-1,3,7-triene, ß-cubebene and ß-caryophyllene. These compounds have been found to elicit behaviour in some important phytophagous insects. Hence, subtractive behavioural assays could be carried out to determine the attractive and repulsive substances for oviposition and feeding behaviour. This can lead to an alternative method of controlling and managing this insect pest.

CHAPTER 1

INTRODUCTION

1.1 General Considerations

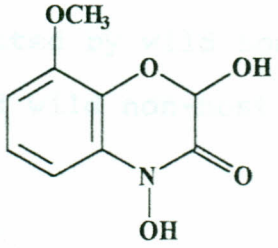
Cereal crops, particularly maize, sorghum and millet, serve as major staple food for millions of people in different parts of Africa [1-3]. Sorghum and millet are traditional staple cereals in most eastern and southern African countries. They used to be the only cereals grown until the introduction of maize about 75 years ago [4].

Although agricultural technology has brought about a significant and rapid increase in productivity of various cereal crops in most parts of Africa, the impact of such technology has been limited by the vulnerability of these crops to insect pests among several other constraints such as climatic factors, plant diseases and weeds. In the tropics several species of insect pests are known to damage these crops [5-7], of which lepidopteran stem borers are ubiquitous and a major pest. Fifteen species in two families, Pyralidae and Noctuidae have been found to attack maize and sorghum (Appendix 1). *Chilo partellus* (Swinhoe), *Busseola fusca* (Fuller), *Sesamia calamistis* (Hampson) and *Eldana saccharina* (Walker) are of significant importance. Losses due to stem borers as high as 80-100% in sorghum and 23-100% in maize have been reported [8,9].

Most cereal stem borers of maize and sorghum are generally polyphagous and have several graminaceous wild hosts in addition to more than one cultivated crop [8,10]. Several non-cultivated wild host plants of these stem borers have been recorded and documented by various workers (Appendix 2). During off season when there are no cultivated crops in the field, the stem borers remain present on wild host plants, or hibernate in crop residue and subsequently infest the cultivated host after planting [11,12]. These wild habitats, often harbour food sources for these pest insect species and may encourage their outbreak in the neighbouring agroecosystem [7]. On the contrary, the adjacent wild habitats can be used to suppress insect outbreak by keeping the pest on the so-called wild trap plants. Intercropping host and non-host plants may also support lower insect pest levels than the corresponding monocultures [13,14]. Hence a more complete understanding of the role of such wild hosts and non-hosts in determining outbreak of stem borers can generate suitable management strategies.

Control against this pest has mainly been by the use of insecticides. However, the social and environmental repercussions like resistance or tolerance to insecticides, increased cost, health hazards and ecological imbalances have encouraged scientists to search for alternative methods of crop protection [15, 16]. The use of resistant cultivars is one such approach [17]. Developing a new strategy to protect crops against insect pests by the use of non-polluting and specific

method requires a basic knowledge of insect-plant relationships. For example 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) found in young maize leaves, has been recognised as the major feeding deterrent in both field and laboratory studies to *Ostrinia nobilalis* (Hubner). DIMBOA acts on growth and development of corn borer larvae by non-competitive inhibition of digestive protease [18, 19].



2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one **(DIMBOA)**

It is now well documented that different behavioural responses shown by an insect, which lead to the selection of its host plant (as opposed to non-host plant), are influenced in part by volatile phytochemicals. It is, therefore important to investigate the plant volatiles that mediate plant-pest interaction for a better understanding of the underlying principles. Based on such knowledge:

(a) An alternative method of controlling and managing stem borers can be developed, if volatile compounds from host and non-host plants and their role in stem borer plant interaction is understood.

(b) Synthetic samples of host plant attractants could be

used in developing traps for the stem borers, while synthetic non-host plant repellants could be used in repelling stem borer pests from cultivated crops.

(c) Breeding of cultivated crop cultivars that are resistant to a particular stem borer will be possible.

The present research sought to study *Chilo partellus* (Swinhoe) behaviour (oviposition and feeding) related volatile phytochemicals emitted by wild host plant, *Pennisetum purpureum* (K.) Schumach., and wild non-host plant, *Melinis minutiflora* P. Beauv.

1.2 Objectives of the Study

(1) To collect behaviour-mediating volatile phytochemicals from wild host plant, *P. purpureum* and wild non-host plant, *M. minutiflora* of *C. partellus*.

(2) Identify the composition of the volatile blend using GC, GC-MS, and confirm the identity of the constituents by GC co-injection with authentic samples.

(3) To provide chemical data, which can be used later for behavioural studies of *C. partellus*. Such data may be useful in understanding (and the eventual management) of the attractive or repulsive substances for oviposition and feeding behaviour of this insect pest.

1.3 The Spotted Stem Borer, *Chilo partellus* (Swinhoe)

1.3.1 Introduction

The spotted stem borer, *Chilo partellus* (Swinhoe), of the family Pyralidae is one of the most important pest in tropical Africa and Indian sub-continent [20]. Essentially it is a pest in lowland areas and seldom found above an altitude of 1500 m [2]. *C. partellus*, which is native to Asia, is suspected to have been introduced in Africa from Indian sub-continent. It was first reported in Africa in the early 1930's in Malawi [21], and later reported in Uganda [5], and Tanzania [12]. By 1977, it had spread to Ethiopia, Kenya, Malawi, Mozambique, Somalia, South Africa, Sudan, Tanzania and Uganda [22].

1.3.2 Bioecology

Adults are nocturnal and live 2-3 days, during which each female lay 200-600 scale-like eggs in batches of 10-80 overlapping eggs on the underside of leaves of the host plant, mostly near the midrib, with a preference to young (3-4 weeks after germination) rather than older plants [23,24]. Larvae hatch after 4-5 days. The damage caused by the larvae feeding in young plants involves the attack of the growing point and cause "dead hearts", which is characterized by dead central leaf resulting in 100% crop loss. In older plants the larvae bore into the main stem, which is subsequently hollowed out over a

considerable length. Stem tunnelling reduces plant vitality, the grain filling process and promotes lodging of plants as they mature. Plants thus affected have poor growth, reduced yield and are more susceptible to wind damage and secondary infections. Larval development is completed in 2-4 weeks and larvae pupate in the damaged stem. The pupal period lasts for 5-12 days, and during the growing season the life-cycle is completed in 25-50 days [23]. The life cycle may be continuous in areas where suitable conditions for host plant growth are permanently present. Usually however, the cycle is interrupted by cool or dry season during which plant growth is impossible.

1.3.3 Host and Non-host Plants

The major cultivated host plants of *C. partellus* are maize, sorghum, millet, sugarcane, rice and wheat [1, 23], while the non-cultivated host plants include wild grasses *Pennisetum purpureum* (K.) Schmach., *Penicum maximum* Jacq., *Hyparrhenia refu* (Nees) Stepf., *Vossia cuspidate* (Roxb.) W. Greff and *Sorghum verticulliflorum* Steud. [23, 25]. However, previous work have recorded that cowpea (*Vigna ungiculata* (L.) Walp.) cassava (*Manihot esculenta* Crantz.) and simsim (*Sesamum indicum* L.) are cultivated non-host plants [26, 27], while *Melinis minutiflora* P. Beauv. is a wild non-host plant of *C. partellus*.

Pennisetum purpureum **(K.) Schmach.**

P. purpereum (family Gramineae), a non-cultivated host plant of *C. partellus*, is commonly known as Napier or elephant grass. It is a robust perennial with culms 2 to 4 metres tall. This grass is native of Africa but is extensively grown in Central America for forage [28].

Melinis minutiflora **P. Beauv.**

M. minutiflora (family Gramineae), commonly known as molasses grass, is one of the few grasses on which *C. partellus* have not been found. It is also known to be an anti-tick pasture plant in South America and Australia [29]. In Africa the brown ear tick is the vector of the pathogen which causes East Coast fever in animals. Identification of volatiles from this plant may constitute new avenues of pest control, by screening the volatiles for bioactivity against pest species. *M. minutiflora* is a strong scented perennial which is native to tropical America and Africa.

1.3.4 Control of *Chilo partellus*

Various control measures have been used in attempts to reduce the losses due to *C. partellus* including chemical control, cultural practices, biological control and use of host plant resistance.

Pesticides for control of *C. partellus* include the use of

endosulfan, trichlorfon, synthetic pyrethroids, dichlorodiphenyltrichoroethane (DDT) and carbaryl. However, in general, chemical control is considered unsatisfactory because of the cryptic nocturnal habits of the adult moth and the protection afforded by the stem of the host crop to the developing stages [9].

Cultural practices have been attempted with different limited levels of success. These include the destruction of crop residue after harvest by farmers [1], destruction of wild host plants in the proximity of the field [5] and intercropping of host and non-host plants. Aspects of the ovipositional responses in *C. partellus* have been studied and results have showed that ovipositional behaviour of this pest in sorghum-cowpea and maize-cassava intercropping system supported low egg mass per five plants than the corresponding monocultures [1, 14, 30] (Appendix 3).

In biological control, the role of local parasitoids and predators in suppressing *C. partellus* population in East Africa has been examined and some species of natural enemies have been identified, including several of larval and pupal parasitoids. The most common parasitoid being *Cotesia sesamiae* and *Cotesia flavipes* [31]. Plants may defend themselves against herbivores by enhancing the effectiveness of the natural enemies of herbivores (indirect defence). For example carnivores are known to discriminate between plants with and without herbivores on the basis of volatiles. Recent chemical analysis of volatiles from

infested plants have shown that plant start emitting volatile infochemicals that attract the natural enemies upon herbivore damage [32]. Plants also produce toxins, growth inhibitors, digestibility reducers, repellents, and other secondary products to directly defend themselves against herbivores.

Plant resistance to attack by *C. partellus* may also reduce the intensity of infestation by the pest [33]. A series of sorghum cultivars produced by various national and international breeding programs have been evaluated for resistance, and varieties have been identified with different levels of preference for oviposition, feeding and establishment by *C. partellus* [34]. In these studies however, assessment of levels of resistance has been based on stem borer performance on the plant without consideration of the underlying molecular characteristics mediating pest plant interaction [35].

1.4 Host Plant Selection

The term "host plant specificity" refers to the range of plant species on which a given insect is known to occur in nature. "Host plant selection" is the behavioural sequence by which an insect distinguishes between host and non-host plant. Host selection is a function of the female moth as she will deposit eggs within its host range. In the presence of several utilizable hosts, *C. partellus* will deposit eggs almost exclusively on maize or sorghum in preference to the wild

grasses. Oviposition behaviour is of utmost interest to plant protection specialists because it determines the level of damage.

Both biophysical and biochemical factors are involved in guiding the ovipositing female to the potential host plants. The insect's orientation behaviour is followed by recognition behaviour in which the plant is either accepted or rejected [36].

1.4.1 Biophysical Factors

Physical factors involved in host plant orientation and recognition include both visual and tactile stimuli. Colours and shapes can be perceived by insects especially when in locomotion. Ovipositing citrus butterflies, *Papilo demoleus* L. for example, respond to greenish or yellowish green colour [37,38].

Tactile factors come into play after the orientation phase of the host selection, and are frequently involved in the recognition phase that immediately precede oviposition or feeding. The physical characteristics of the surface on which the female deposits eggs are of great importance. The choice of oviposition site is influenced by the nature of leaf surface. That is, the amount of pubescence, crevices and cavities. The smooth areas of the plant were noted to be the preferred sites for oviposition, by *C. partellus* with up to 92% preference for 3-4 weeks old maize plant [39].

1.4.2 Biochemical Factors

Physical features are rarely unique to a single plant species. However, chemical cues may show a higher degree of specificity, and hence play a major role in mediating the behavioural steps leading to oviposition. Chemical communication system involve two types of allelochemical substances emitted by the plant and detected by the moth: (1) orientation to a plant and landing may depend on the presence of volatile compounds emanating from the plant, for example, phenylacetaldehyde by *Ostrinia nubilalis*; and (2) other compounds acting on contact, for example, soluble sugars which affect the amount of time the insect remains on the plant and stimulate or inhibit oviposition by an unknown mechanism [40].

Volatile compounds emanating from plants are thought to play a major role in the orientation of insects to their hosts and in avoidance of unsuitable plants. Yet only a few examples of specific chemicals involved in attracting or repelling gravid females to oviposition sites are known. These chemicals activate the olfactory organs of an insect that is searching for host plant, from a distance, and show a directed movement towards the odour source. At close range the insect may obtain additional information especially with respect to differences between individual plants. For example, some individual plants in a cabbage field are more attractive to oviposition by *Pieris brassicae* (L.), because they contain higher than average amounts

of volatile allyl nitriles, which attract the cabbage butterfly [36].

If an insect depends on volatile cues to guide it to host plant for oviposition, non-host odours could interfere with orientation. Intercropping experiments in the field and in greenhouse generally support this idea, and individual chemical constituents have been used to inhibit oviposition in the laboratory [41].

After alighting on a plant, a gravid insect depends on a combination of physical and chemical stimuli at the surface to assess the acceptability of that plant for oviposition. The total process of host selection, therefore depend on the combined input from different chemical as well as the physical stimuli.

1.5 Plant Volatile Phytochemicals

1.5.1 Composition of Plants Volatile Blends

Two alternative hypotheses can be considered in regard to the composition of plant volatile blends [42]:

(1) Plant odours are highly specific and composed of compounds not found in unrelated plant species, or

(2) Plant odour specificity is achieved by particular ratio between constituent chemical compounds, which are generally distributed among the plant species.

1.5.2 General Odour Components

General odour components are formed via biosynthetic pathways generally present in plants. Examples are the production of leaf alcohols, aldehydes, and derivatives from unsaturated fatty acids [43], fermentation products like ethanol and ethyl acetate, and terpenes derived from the polymerization of isopentyl pyrophosphate [44]. Some of the identified general odour components and their distribution in the headspace of various plants are listed in Tables 1 and 2, respectively.

Table 1: Some of the Reported General Odour Components

(1) Leaf alcohols and aldehydes

Aliphatic alcohols: 1-pentanol, 1-hexanol, 1-nonanol, *cis*-2-penten-1-ol, *cis*-2-hexen-1-ol, *trans*-2-hexen-1-ol, *cis*-3-hexen-1-ol, 1,5-dimethyl-4-hexen-1-ol, 2,6-nonadien-1-ol.

Aromatic alcohols: m-cresol, phenol, Isoeugenol, salfrol, eugenol, methyl salicylate.

Aliphatic aldehydes: hexanal, heptanal, nonanal, *trans*-2-hexanal, hept-2-enal, nona-2,6-dienal.

(2) Isoprenoids

Monoterpenes

Hydrocarbons: α-pinene, ß-pinene, ocimene, limonene, sabinene, terpinolene, camphene, α-terpinene, myrcene, 3-carene, 2-carene, thujene.

Alcohols: (-)-borneol, Isoborneol, (-)-carueol, fenchyl alcohol, terpene-4-ol, geraniol, linalool, menthol.

Aldehydes: (-)-myrtenal, perillaldehyde, citronellal.

Ketones: carvenone, menthone, thujone.

Sesquiterpenes

Hydocarcarbons: caryophyllene, humulene, germecrene, farnesene, coapene, cubebene, bisabolene, bergamotene.

Alcohols: cedrol, phytol, fernesol.

Table 2: General Oduor Components Identified from Various Plants

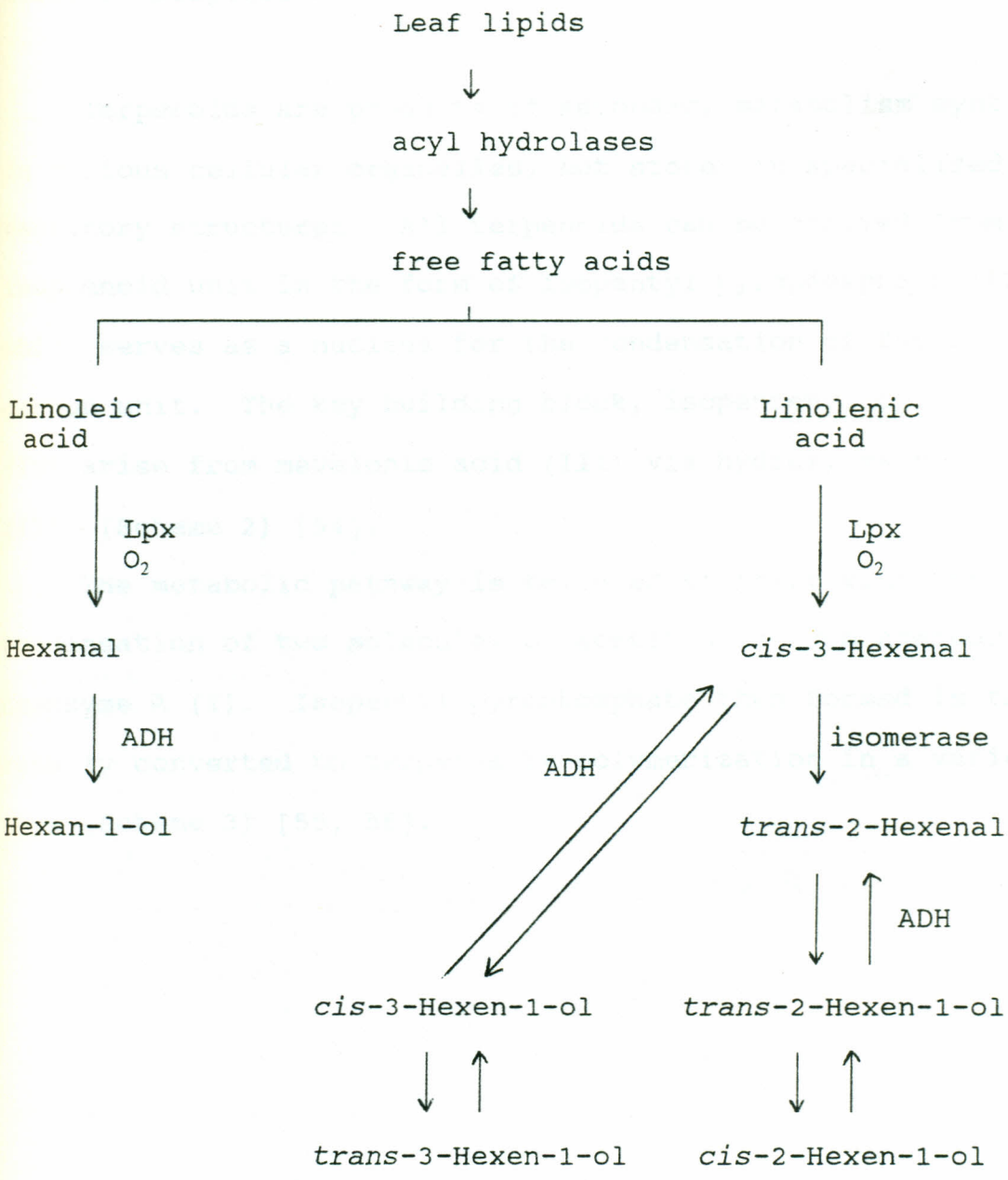
Plants	Method of Analysis	Compounds identified	Ref
Sorghum bicolor (L.) Moench. (cultivated host plant of *C. partellus*).	Trapped using Tenax TA, and volatiles desorbed directly into GC-MS	Toluene, hexanal, (Z)-3-hexen-1-ol, m-xylene, o-xylene, decanal and nonanal.	45
Maize, *Zea mays* L., (cultivated host plant of *C. partellus*)	Trapped using activated charcoal, extracted with DCM concentrated and injected into GC-MS	Myrcene, 2-heptanone, 4,8-dimethylnona-1,3,7-triene, (Z)-3-hexenyl acetate, (Z)-2-hexenal, cyclosativene, α-copaene and cedrene.	46
Cowpea, *Vigna unguiculata* (L.) Walp. (cultivated non-host plant of *C. partellus*)	Modification of Ref. 46 above	Hexanal, 1-nonene, α-pinene, ß-pinene, (Z)-3-hexenyl acetate, hexyl acetate, limonene, (E)-ß-ocimene, nonanal, cedrene and 1,8-cineole.	47
Cabbage, *Brassica oleracca* L.	Trapped using Porapak Q, extracted with DCM concentrated and injected into GC-MS	α-Pinene, ß-pinene, myrcene, 1,8-cineole, n-hexyl acetate, *cis*-3-hexenyl acetate and dimethyl trisulphide*	48

* Specific Odour Component.

1.5.2.1 Green Leaf Volatiles

Green leaf volatiles (GLVs), are aliphatic six-carbon primary alcohols, aldehydes and acetates. They are metabolites of the oxidative degradation of fatty acids and are often produced when enzymes are liberated due to plant tissue damage, for example, 2-hexenal, 1-hexanol, 2-hexen-1-ol and 3-hexen-1-ol. Lipolitic acyl hydrolases liberate free fatty acids from endogenous membrane lipids. The polyunsaturated fatty acids, linoleic and linolenic acids are oxidized by the action of lipoxygenase to hexanal, and *cis*-3-hexenal, respectively (Scheme 1) [43].

Alcohol dehydrogenase converts hexanal to 1-hexanol, whereas *cis*-3-hexenal easily isomerises to *trans*-2-hexenal and is converted to *cis*-3-hexen-1-ol. *Trans*-2-hexen-1-ol is formed from *trans*-2-hexenal. *Trans*-3-hexen-1-ol and *cis*-2-hexen-1-ol possibly originate from isomerisation during processing and storage of plants products. This biosynthetic pathway is operative in several plant species, for example, potatoes, tomatoes, tea leaves, peas, apples and other fruits, legumes like soybeans, and some cereal grains, and is regarded as well distributed in many other plant species [49-51].

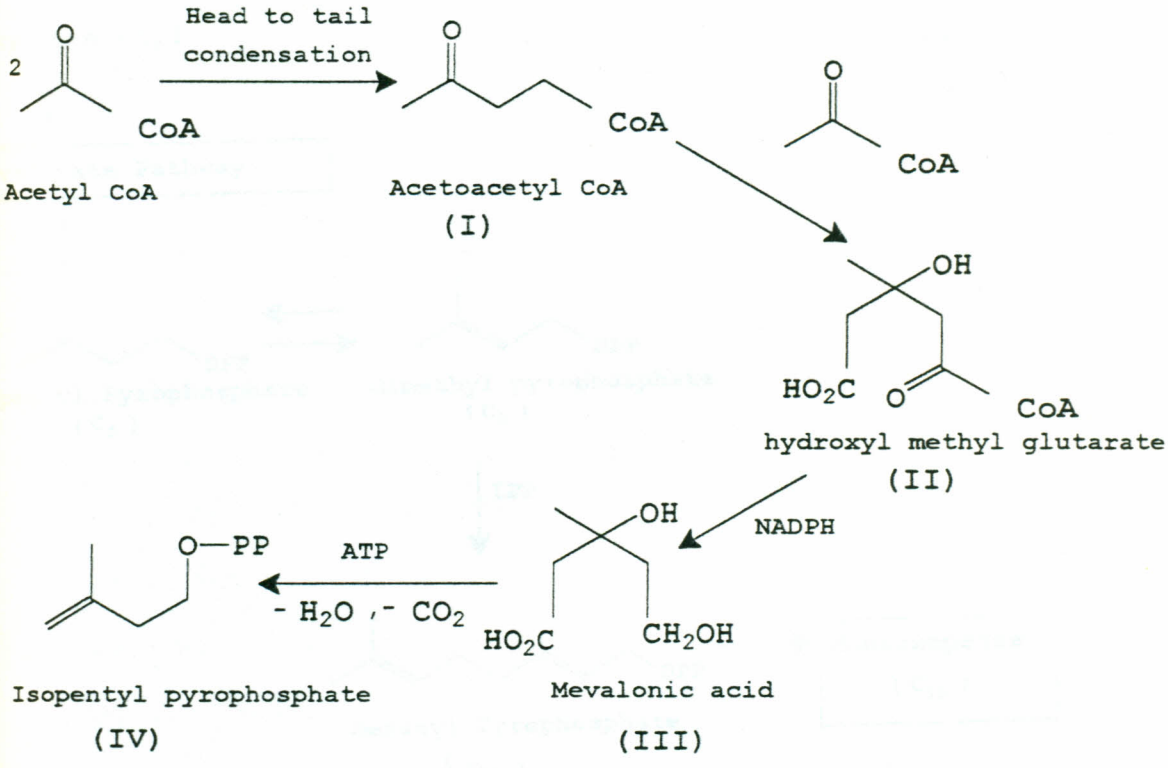


Scheme 1: Biosynthesis of Leaf Aldehydes and Alcohols Constituting a GLV Complex [50,52,53].

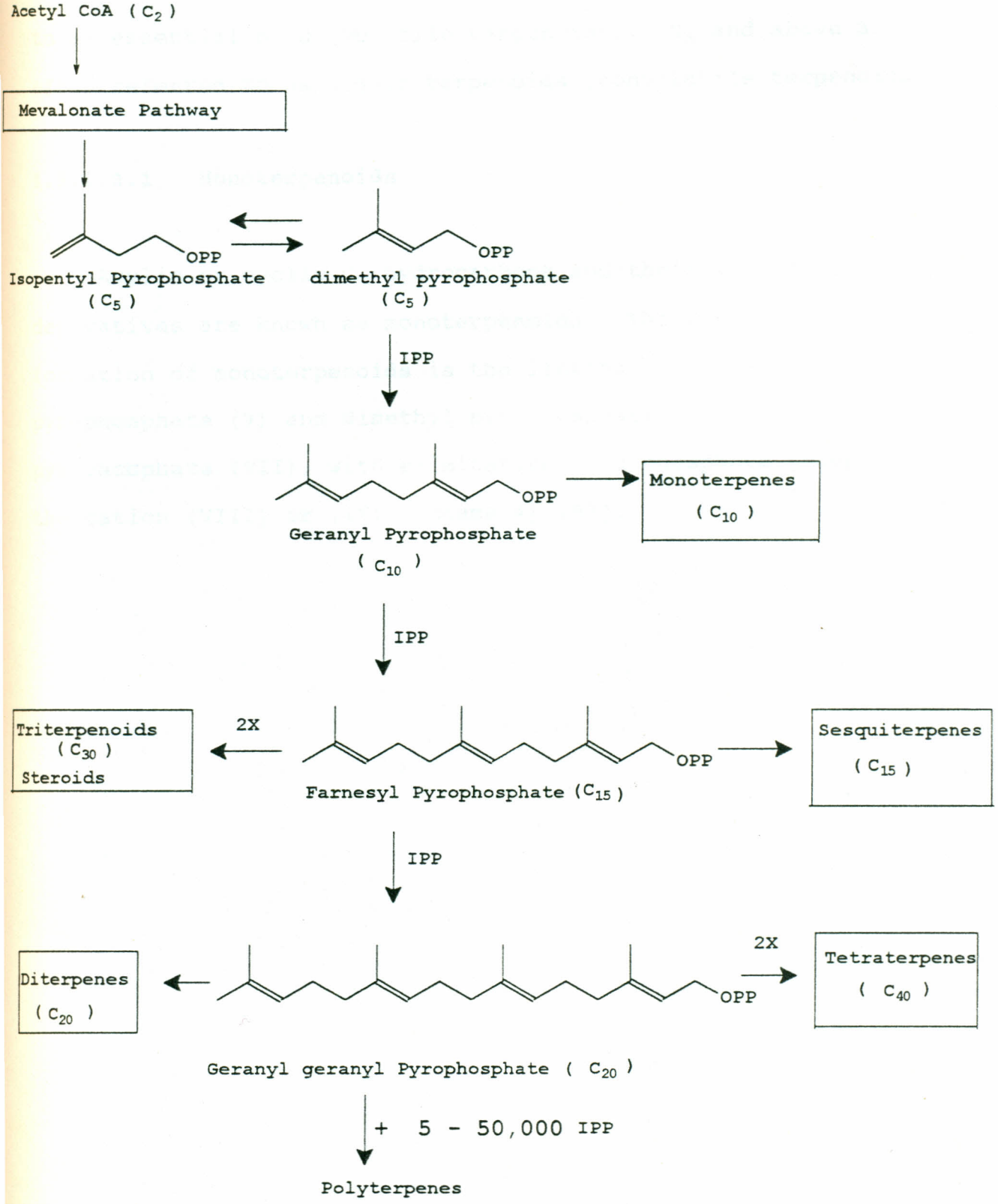
1.5.2.2 Terpenoids

Terpenoids are products of secondary metabolism synthesized in various cellular organelles, but stored in specialized secretory structures. All terpenoids can be derived from an isoprenoid unit in the form of isopentyl pyrophosphate (IPP) which serves as a nucleus for the condensation of further 5-carbon unit. The key building block, isopentenyl pyrophosphate (IV) arise from mevalonic acid (III) via hydroxy methyl glutarate (II), (Scheme 2) [54].

The metabolic pathway is believed to start with the condensation of two molecules of acetic acid from acetoacetyl coenzyme A (I). Isopentyl pyrophosphate thus formed is then readily converted to terpenes by polymerization in a variety of ways (Scheme 3) [55, 56].



Scheme 2: Biosynthesis of Isopentenyl Pyrophosphate from Acetyl CoA

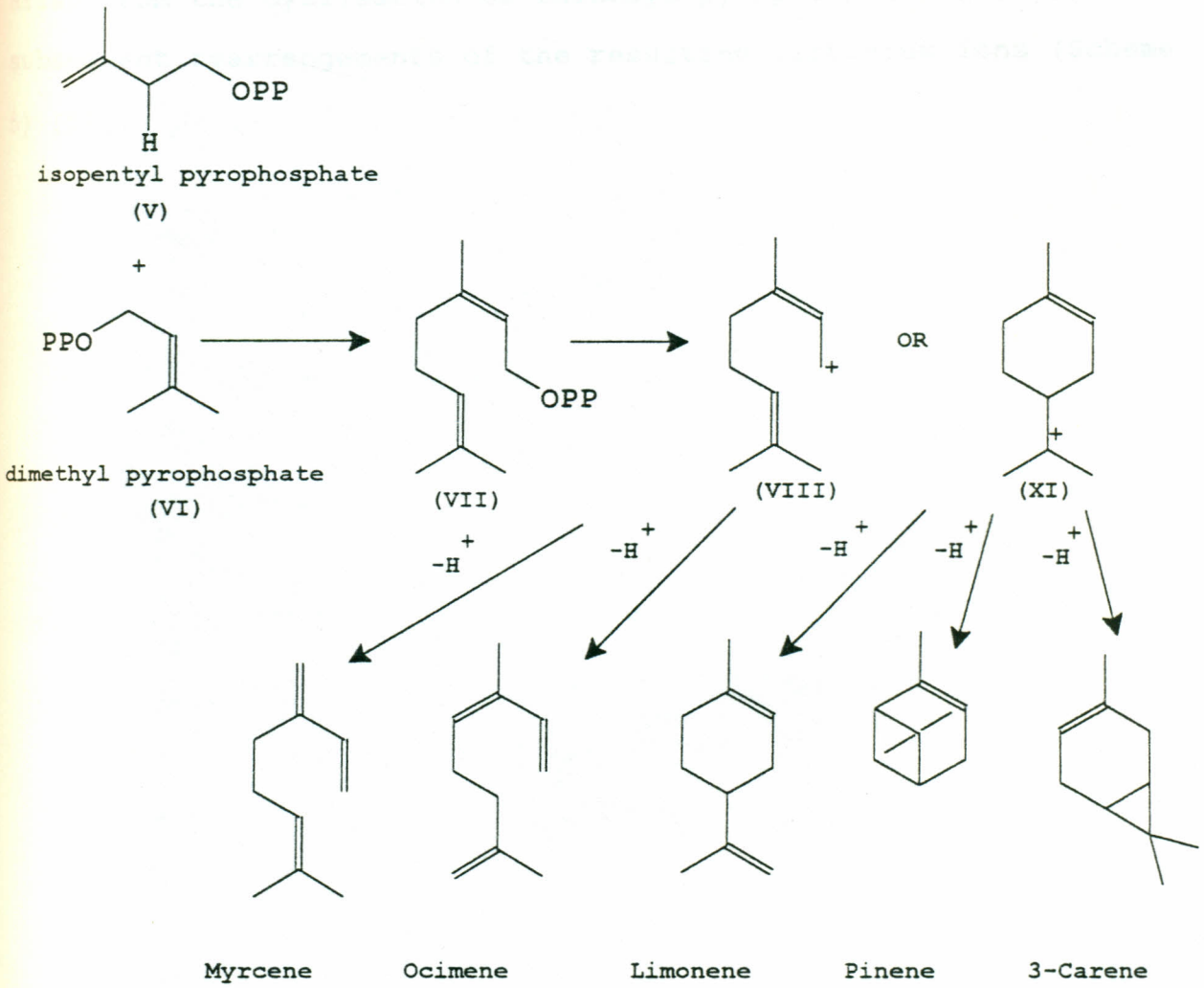


Scheme 3: Biogenesis of Terpenoids

The C_{10} and C_{15} compounds are often referred to as lower terpenoids when they occur together, they are commonly referred to as essential oils (volatile terpenoids). C_{20} and above are often referred to as higher terpenoids (nonvolatile terpenoids).

1.5.2.2.1 Monoterpenoids

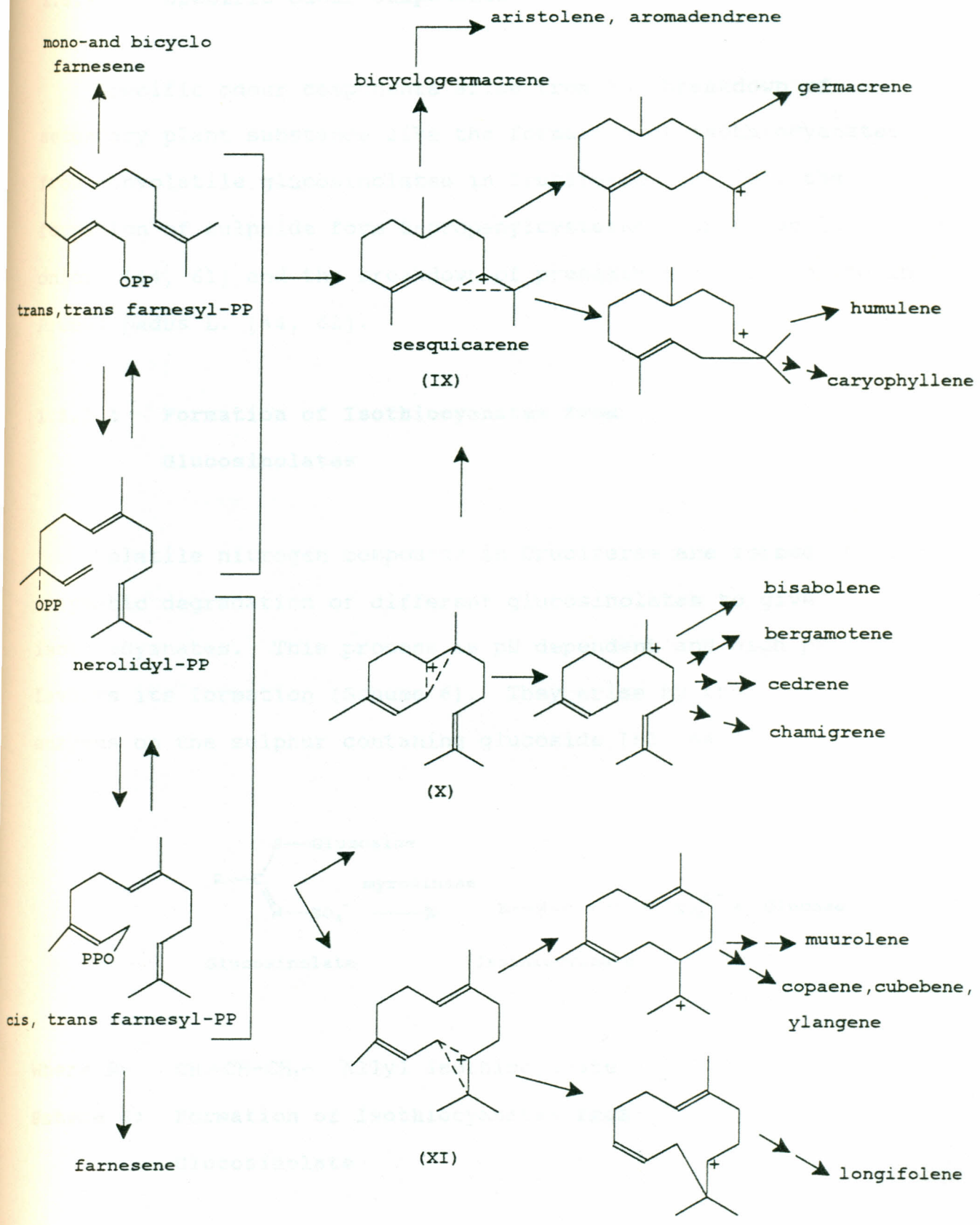
Acylic or cyclic C_{10} hydrocarbons and their oxygenated derivatives are known as monoterpenoids. The first stage in the formation of monoterpenoids is the linking of isopentyl pyrophosphate (V) and dimethyl pyrophosphate (VI) to give geranyl pyrophosphate (VII), with elimination of a phosphate group via the cation (VIII) or (IX) (Scheme 4) [57].



Scheme 4: Possible Ways for the Formation of Monoterpenoids from Geranyl Pyrophosphate

1.5.2.2.2 Sesquiterpenoids

These are C_{15} hydrocarbons or their oxygenated analogs. They arise from the cyclisation of farnesyl pyrophosphate and the subsequent rearrangements of the resulting carbonium ions (Scheme 5) [58].



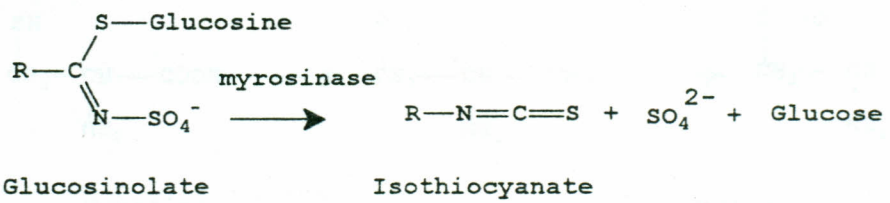
Scheme 5: Biogenesis of Sesquiterpenoids

1.5.3 Specific Odour Components

Specific odour components arise from the breakdown of secondary plant substance like the formation of isothiocyanates from nonvolatile glucosinolates in Cruciferae [59, 60], the formation of sulphide form S-propenylcysteine sulphoxide in onions [44, 61] and the breakdown of prunasin to benzaldehyde in *Prunus padus* L. [44, 62].

1.5.3.1 Formation of Isothiocyanates from Glucosinolates

Volatile nitrogen compounds in Cruciferae are formed through enzymatic degradation of different glucosinolates to give isothiocyanates. This process is pH dependent and high pH favours its formation (Scheme 6). They arise by the action of enzymes on the sulphur contaning glucoside [63, 64].



Where R= CH_2=CH-CH_2- Allyl isothiocyanate

Scheme 6: Formation of Isothiocyanates from Glucosinolate

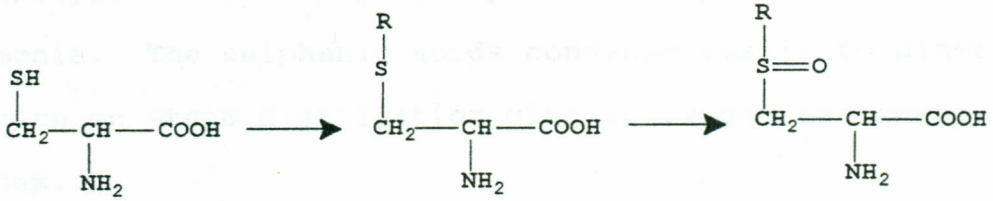
The enzyme myrosinase, which is active in the cleavage of glucosinolates, is localized in special cells. Destruction of tissue structure liberate the enzyme and glucosides are cleaved, hence the smell of cruciferae [65].

Some examples of Isothiocyanates (ITC):

Isopropyl-ITC	But-3-enyl-ITC
Butyl-ITC	Pent-4-enyl-ITC
Sec-Butyl-ITC	Benzyl-ITC
Allyl-ITC	

1.5.3.2 Formation of Sulphides from S-Propenylcysteine

In the formation of these substance cysteine is first alkylated, and the alkylcysteines thus formed are oxidized to alliins (Scheme 7).

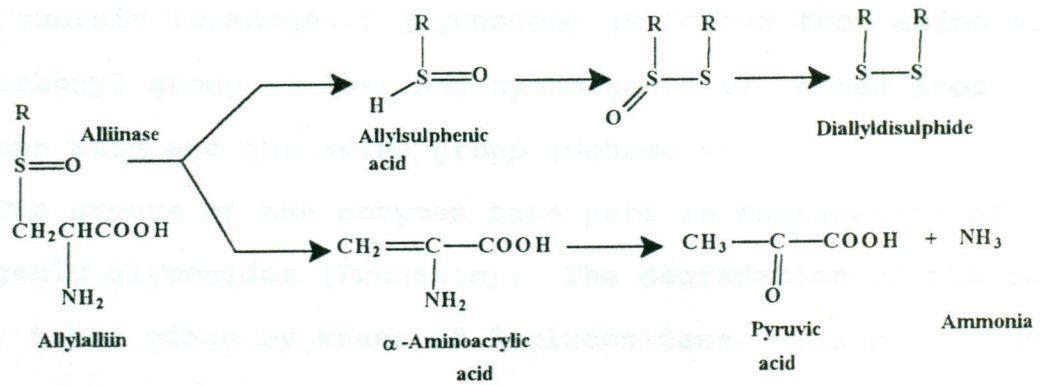


Cysteine Alkylcysteine

Scheme 7: Formation of Alliin

Alliins are degraded to an alkyl sulphenic acid and

α-aminoacrylic acid by the action of enzyme alliinase, which is formed only in the genera *Allium* and *Nothoscordum* (Scheme 8).



Where R = CH_3-CH=CH-

Scheme 8: Formation of Sulphides

Allylsulphenic acid is formed from allylalliin and propenylsulphenic acid is formed from propenylalliin by this reaction. Propenylsulphenic acid is probably the tear-producing substance which is liberated on crushing the cells of onion, *Allium capa* L. [44].

α-Aminoacrylic acid decomposes spontaneously into pyruvic acid and ammonia. The sulphenic acids condense easily to dimeric compounds which on steam distillation give up oxygen and change to disulphides.

Examples of sulphides:

Dimethyl disulphide

Methyl pentyl sulphide

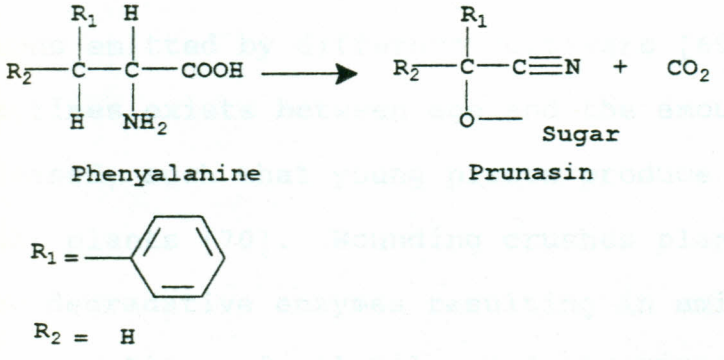
Dimethyl trisulphide

Ethyl methyl trisulphide

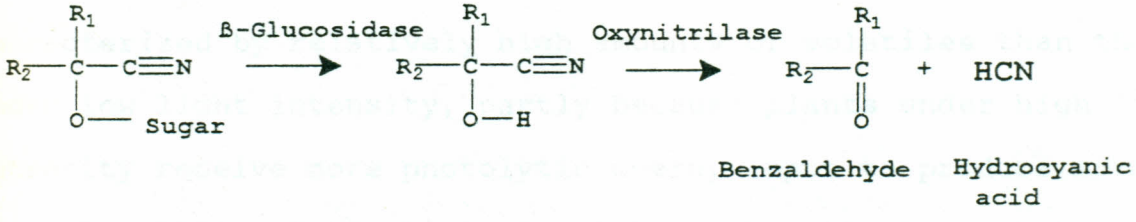
1.5.3.3 Breakdown of Prunasin to Benzaldehyde

Prunasin (cyanogenic glycoside) is formed from amino acids. The carboxyl group is lost and cyano group is formed from α-carbon atom and the amino group (Scheme 9).

Two groups of the enzymes take part in degradation of cyanogenic glycosides (Prunasin). The degradation of the sugar moisty takes place by means of ß-glucosidase. The cyanohydric are degraded to aldehydes and ketones and hydrocyanic acid by means of oxynitrilase (Scheme 10) [44].



Scheme 9: Amino acids as Precursors of Prunasin



Scheme 10: Breakdown of Prunasin to Benzaldehyde

1.5.4. Factors Affecting Volatiles Released by Plants

Composition of the volatile blend emitted by plant varies within plant species depending upon the taxonomic group to which the plant belongs [66-68], plant cultivar [69], plant age [70], plant wounding [71], light intensity [72], season and geographical location [73].

Plant species differ greatly in chemical composition and it is no surprise that there are large qualitative and quantitative differences in the composition of the plant volatile (Table 2). Data about the effect of the plant cultivar on the composition of volatile has shown a marked difference in composition of the blends emitted by different cultivars [69]. Inverse correlation sometimes exists between age and the amount of volatiles released, such that young plants produce more volatiles than older plants [70]. Wounding crushes plant cells and releases some degradative enzymes resulting in emission of higher concentrations of volatiles and sometimes change in the composition of the components. Mechanically damaged maize, for example, will in addition produce anisole, (Z)-3-hexenyl-1-ol and (E)-ß-farnesene (Table 2). Light also affects the composition of volatile blend. Plants under high light intensity are characterized by relatively high amounts of volatiles than those under low light intensity, partly because plants under high light intensity receive more photolytic energy input to produce more volatiles [74]. Production of volatiles by intact plant follow a distinct seasonal trend [75].

1.5.5 Plant Volatiles as Olfactory Cues

Volatile phytochemicals are major early cues to insect, and thus especially affect the orientation and oviposition of adult female [76, 77] as shown in Table 3.

Table 3: Plant Odour Components that Elicit Behavioural Responses in some Adult Phytophagous Insects

Species	Class [a]	Compounds	Class [b]	Ref
Lepitinotarsa decemlineata (Say)	A	*trans*-2-Hexenal, *cis*-3-Hexyl acetate, *cis*-3-Hexen-1-ol, *trans* 2-Hexen-1-ol	G	78 79
Popillia japonica Newm.	T	Phenethyl propanoate, Eugenol, Geraniol	G	80
Delia antiqua (Meign.)	AO	Propanethiol, Dipropyl disulfide,	S	81 82 83
	O	Dipropyl trisulfide, Methyl propyl Trisulfide, Propenyl Propyl disulfide		
	T	2-Phenylethanol, Pentanoic acid	G	84
Delia brassicae (Bouche)	AOT	Allylisothiocynate	S	85 86
	A	Hexyl acetate	G	87
Psila rosae (F.)	OT	*trans*-Methylisoeugenol, *trans* Arasone	S	88 89
	T	Hexanal, *trans*-2-Hexenal	G	88

[a] Compounds are attractive (A), stimulate oviposition (O) or increase trap catches (T).

[b] Chemicals are classified according to their distribution in plant species, as general (G) or specific (S) odour components.

1.6 Methods of Isolation of Plant Volatiles

Traditional techniques such as solvent extraction, steam distillation and distillation under reduced pressure lead to destruction of plant cells. This may result in release of enzymes that are normally present in the intact plant cells and may give rise to other compounds which are not present in volatiles of intact plants [90, 91]. Enzyme action also degrades some of the plant volatile compounds. However, extracting air from around plants (Headspace analysis) is the best indicator of chemicals usually available in the plants' surroundings and most important in host-plant location by insects [92, 93].

1.6.1 Headspace Analysis

Headspace collection is being used increasingly to collect volatile substances from the air around plants. The advantages of this system are that it requires little plant material, it avoids artifacts from impure solvents and it provides an estimate of the concentration of the volatile chemicals that the insects normally experience during host-plant location in the field. Its most serious disadvantage, however, is that many of these chemicals are present in such small quantities that the usual analytical techniques are often operating close to their limits.

Headspace is carried out using either a static or a dynamic system. In the static system, chemicals released by the plants

are allowed to reach an equilibrium level in a closed container before a large gas tight syringe is used to sample the air. In the dynamic system an air stream is used to flush the volatile chemicals from around plants through a trap containing adsorbent such as Porpak Q (styrene ethyl vinyl benzene-divinyl benzene copolymer, EVB-DVB) [48], activated charcoal [46], Tenax-GC (2,6-diphenyl paraphenylene oxide polymer) [45], Supelpak-2 (Amberlite XAD-2 resin) [94], glass wool [95], and C_{18}-Bonded Silica [96], or using cryogenic traps [97] Table 4.

Table 4: Examples of Cryogenic Trap Coolants

Temperature of traps	Composition of Cooling mixture	Ref.
-21°C	Crushed Ice /NaCl (3:1)	98
-39°C	Crushed Ice /$CaCl_2$ (1.2:2)	98
-55°C	Crushed ice /$CaCl_2$ (1.4:2)	98
-63°C	Liquid N_2 /$CHCl_3$ (1:1)	98
-78°C	Solid CO_2 /acetone	98
-95°C	Liquid N_2 /acetone	99
-120°C	Liquid N_2 /ethanol solution	98
-186°C	Liquid Ar	100
-196°C	Liquid N_2	98

Desorption of volatile components from the traps, is achieved by extraction using suitable solvents or heating (in case of heat stable adsorbents such as Tenax TA and Tenax GC)

[45, 47]. However, the thermal desorption may lead to decomposition of some organic compounds and therefore solvent extraction has considerable advantage. Among the desireable qualities of an adsorbent are its trapping efficiency, thermal stability, lack of conterminating artifacts and the efficiency with which it releases the trapped materials.

In cryogenic traps, there is generally a significant amount of water concurrently collected with the volatile organic compounds [101]. Since all water vapour is trapped together with all the volatile compounds, the advantage of this method can be exploited only if these compounds can be further separated from water. Desiccants (like, potassium carbonate) and porous polymers (like, Porapak Q) have been used for excluding water during the concentration of the volatile trace compounds in air. Concentration on porous polymer is possible because of low retention volume of water relative to the volatile compounds of interest.

1.7 Identification of Plant Volatiles

In order to identify the constituents of volatile plant chemicals a combination of various analytical techniques are needed. The most important being gas chromatography (GC), mass spectrometry (MS) and combined gas chromatography-mass spectrometry (GC-MS), and/or nuclear magnetic resonance (NMR).

1.7.1 Gas Liquid Chromatography (GLC)

Gas liquid chromatography is the technique of choice for the separation and analysis of thermally stable and volatile organic compounds. Separation is accomplished by partitioning the components of a chemical mixture between a moving (mobile) gas phase and stationary liquid phase held on a solid support. Since the partition coefficients are different, the individual components are carried along the column at different rates and emerge from the opposite end of the column in distinct "zones". A detector such as, Flame Ionization Detector (FID), Thermal conductivity Detector (TCD), Electron-Capture Detector (ECD) is present at the exit of the separation column of the GC, from which the electronic signal is fed into a recorder which draws out a set of peaks corresponding to the quantitative percentage of each component present in the same mixture. The retention time of the test chemical is compared with that of a known compound [102].

1.7.1.1 Retention Time (Rt)

There are three fundamentals concerning retention times obtained on a given instrument with a given column operating under specific conditions.

(i) If the retention time of component A, $(Rt)_A$ is equal

to retention time of unknown component $(Rt)_{uk}$, this does not confirm that the unknown component is A. This prevents GC from being an exceptional qualitative method of analysis. However, if the retention times are the same in several different columns, then the two compounds may be the same.

(ii) If $(Rt)_A$ does not equal $(Rt)_{uk}$, then indeed with absolute certainty we can say that the unknown is not component A.

(iii) If we have no discernable peak at $(Rt)_A$, this component A is not present in the sample within the limits of detection.

The systematic method for expressing retention data uses the Kovats retention indices (RI) [102]. These indices indicate where components will appear on a chromatogram with respect to straight chain alkanes injected with the sample. The index of an unknown compound X is described as:

$$I_x = 100 \left[n \frac{\log R_x - \log_z}{\log R_{z+n} - \log R_z} + Z \right]$$

where,

R_x = Rt for unknown x

R_z = Rt for normal alkane having z carbons

R_{z+n} = Rt for normal alkanes having z+n carbons.

n = the difference in the number of carbon atoms for the normal alkanes.

Retention Indices (RI) for a normal paraffin is 100 times the number of carbon atoms in the compound regardless of the column used for chromatographic conditions. During this work, it was not possible to use the Kovats indices to identify the peaks, however, uncorrected retention times were used to make tentative identification of the components in the samples. This was dependent on the availability of the authentic samples.

1.7.2 Mass Spectrometry (MS)

In mass spectrometry a sample is introduced into a highly evacuated chamber, of pressure between 10^{-6}-10^{-7} mm Hg ≡ 10^{-4} NM^{-2}, maintained by the use of the diffusion pumps. Molecules are then bombarded with a beam of electrons with sufficient energy to effect its ionization (70ev). This may lead to ionization of the sample molecule resulting in the formation of the molecular ion.

$$M + e^- \longrightarrow M^+ + 2e$$

The electron removed from the sample may originate from:

(1) a lone pair of electrons on a heteroatom such a oxygen, sulfur or nitrogen.

(2) a pair of electron in the π orbital of a multiple bond, or

(3) a pair of electrons in the σ orbitals of a single bond.

Energy in excess of that required to ionize a molecule may be dissipated by fragmentation of the molecular ion, into smaller

ions (fragment ions, or daughter ions) [103].

$$M^+ \text{ ----------> } M_1 + M_2^+ \quad \text{or} \quad M^+ \text{ ----------> } M_1^+ + M_2$$

The molecular ions, the fragment ions and the fragment radical ions are separated by deflection in a variable magnetic field according to their mass and charge, and generate a current (the ion current) at the collector in proportion to their relative abundance. A mass spectrum is a plot of relative abundance against the ratio mass/charge (m/e value). The preference of fragment ions in a spectrum provides valuable information concerning the structure of molecule.

Basically compounds are identified by mass spectrum by comparing the spectrum of the unknown with those in reference collection (NIST or NBS libraries) to find one with ions of similar m/e value and relative abundance.

1.7.3 Gas Chromatography-Mass Spectrometry (GC-MS)

The combination of the two techniques was developed as a powerful method for analysis and characterization of small amounts of compounds present in complex mixtures [102].

Gas chromatography is used for separation of compounds according to their distribution between a mobile gas phase and the stationary phase, so that the eluted compounds may have their mass spectra recorded. Mass spectra obtained rapidly on

compounds emerging from a gas chromatographic column afford informative correlation of mass spectrometric and chromatographic data. Full use is made of the separating power of GC together with the structural information derivable from MS. This gives exceptional power of discrimination between closely related structures.

CHAPTER 2

EXPERIMENTAL

2.1 General Experimental Procedures

2.1.1 Reagents and Reference Compounds

All solvents (acetone, dichloromethane and hexane) were obtained from Merck Chemical Co. (Germany), reference compounds were obtained from Aldrich (England), Fluka (Switzerland) and Sigma Chemical Co. (England) while adsorbents were from Chrompack (Netherlands) and J.T. Baker (U.S.A).

2.1.2 Cleaning of Adsorbents

Adsorbents were cleaned by soxhlet extraction for 48 hrs in a thimble, held in place at the upper end by glass wool plug, using dichloromethane (CH_2Cl_2, DCM). It was then removed from the soxhlet, wrapped with aluminium foil and allowed to dry for 12 hrs at room temperature.

The adsorbent was packed into glass columns (traps) and washed again with DCM for another 48 hrs.

To ensure that the adsorbent was clean, one of the packed column was extracted with 4 ml of pure DCM into a clean vial and the resulting solution concentrated under nitrogen (N_2) stream at 0°C to approximately 10 μl. 4 μl of the solution was injected

into gas chromatograph (GC) to confirm that the traps were clean.

2.1.3 Distillation of solvents

Impure solvents were double distilled, 5 ml of the distillate was then concentrated to 10 μl. To confirm its purity, 4 μl was injected into the GC.

2.1.4 Cleaning of Glassware

All reusable glassware, that is, collection chamber, glass tubing, columns and sample vials were washed with hot water, rinsed with cold water, acetone and finally with distilled water. The use of soap as a cleaning agent was avoided as much as possible to minimize soap artifacts. Then the glassware was dried at 110°C for 1 hr in a clean oven.

2.2 Plant Materials

Melinis minutiflora P. Beauv. and *Pennisetum purpureum* (K.) Schumach., were collected from the farm yard at International Centre of Insect Physiology and Ecology (ICIPE) Nairobi, and identified at Kenya National Museum under reference numbers KNU/H62/95 and KNU/H8/96, respectively by the Botanist in Charge, Mr. G. Mwachala.

These plants were propagated into the allocated plot (for

field trapping), while others were planted in pots, and allowed to grow under the field conditions. Plants were watered every morning to ensure that they did not dry. During plant development, potted plants were regularly checked for root exposure or other accidental damage. Damaged plants were rejected as damage may affect the physiology and subsequently emission of unusual volatiles by plants [104, 105]. Approximately 3-4 weeks old plants were used in these experiments.

2.2.1 Plant Treatments

For *Melinis minutiflora*, two potted plant (about 10 stems) were carefully removed from the pots and the soil washed off with water held in a trough. The plants were then immediately transferred into a clean beaker filled with water.

For *Pennisetum purpureum*, approximately 10 stems of the plant were obtained from the field. This was done by cutting the plants near their stems, just above the roots, with a pair of scissors and the cut ends immersed immediately into a beaker of water [106, 45]. Although cutting the plant like this may lead to formation of some metabolic artifacts, the damage was less severe as compared to steam and reduced-pressure distillation, where the whole plant is macerated and subjected to high temperature.

In both cases this was done to exclude soil volatiles.

2.3 Insects (*Chilo partellus*)

Insects were obtained from ICIPE mass-reared colonies. Larvae were reared until pupation on artificial diet based on roscoco bean and sorghum leaf powder [107]. They were kept under natural illumination and held in a rearing room with temperatures between 25-28°C and relative humidity of 65-70%. Pupae were collected on moist cotton wool and kept in a perspex cage under a reversed photo period. On the day of emergence they were allowed to mate, then the gravid fed females of *C. partellus* were used in electrophysiology test.

2.4 Collection and Analysis of Volatiles

2.4.1 Headspace Collection of Volatiles

2.4.1.1 Laboratory Collection

Apparatus for collecting volatiles from plants. A glass chamber (30 cm X 30 cm X 30 cm) **(a)**, which consisted of an air tight collection chamber (45 cm tall) was devised. The collection chamber was divided into two parts; a lower part that consisted of a 3 litre beaker **(b)** in which 1 litre beaker **(c)** containing plant was placed, and the upper part which consisted of a 5 litre beaker **(d)**, used to enclose plant. The lower part was isolated from the upper part by suspending the 5 litre beaker over four aluminium strips **(e)** which were hanging from 3 litre

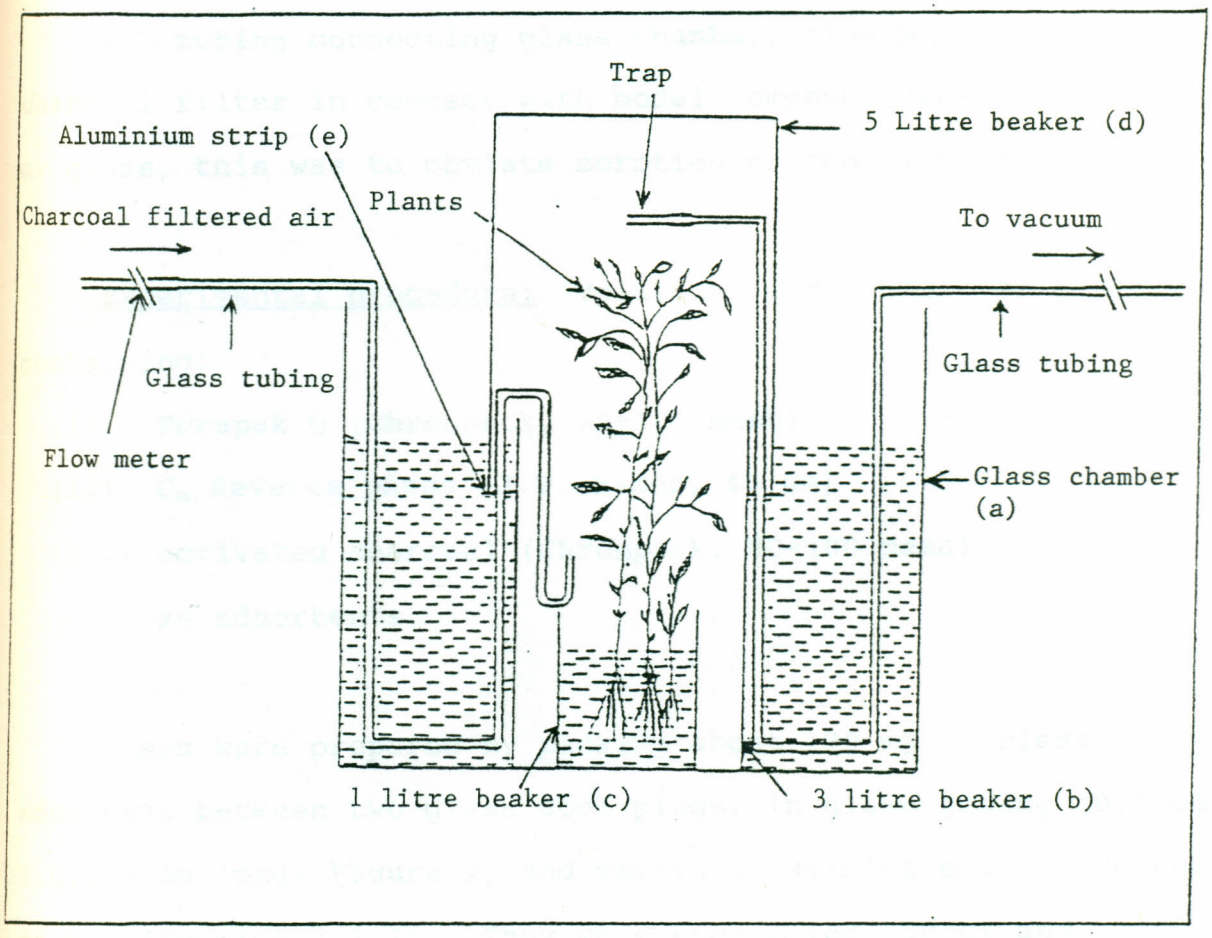


Figure 1: Setup of Glass Chamber for Collection of Airborne Plant Volatiles by Air Entrainment

beaker **(b)**.

The collection chamber was made air tight by addition of distilled water into the glass chamber **(a)** to about 2 cm below the mouth of 3 litre beaker **(b)**, and the system held from floating by means of wet sand contained in 4 glass bottles (Figure 1).

All tubing connecting glass chamber, flow meters, and charcoal filter in contact with model compounds was either teflon or glass, this was to obviate sorption or contamination.

Experimental procedure: Volatiles were collected in traps containing:

(i) Porapak Q (Chrompack, 80-100 mesh)

(ii) C_{18} Reverse phase (J.T. Baker, 40 μm)

(iii) Activated charcoal (Chrompack, 80-100 mesh)

as adsorbents.

Traps were prepared by packing about 120 mg of clean adsorbent between two glass wool plugs, in glass tubing (0.5 cm I.D., 5 cm long) Figure 2, and washed by soxhlet extraction for 48 hrs (Section 2.1.2). They were then dried (50°C) and conditioned in readiness for trapping.

(i) Porapak Q trap was conditioned by heating it under N_2 stream at 110°C for 30 min using the GC oven.

(ii) Activated charcoal trap was conditioned by heating it under N_2 stream at 250°C for 30 min.

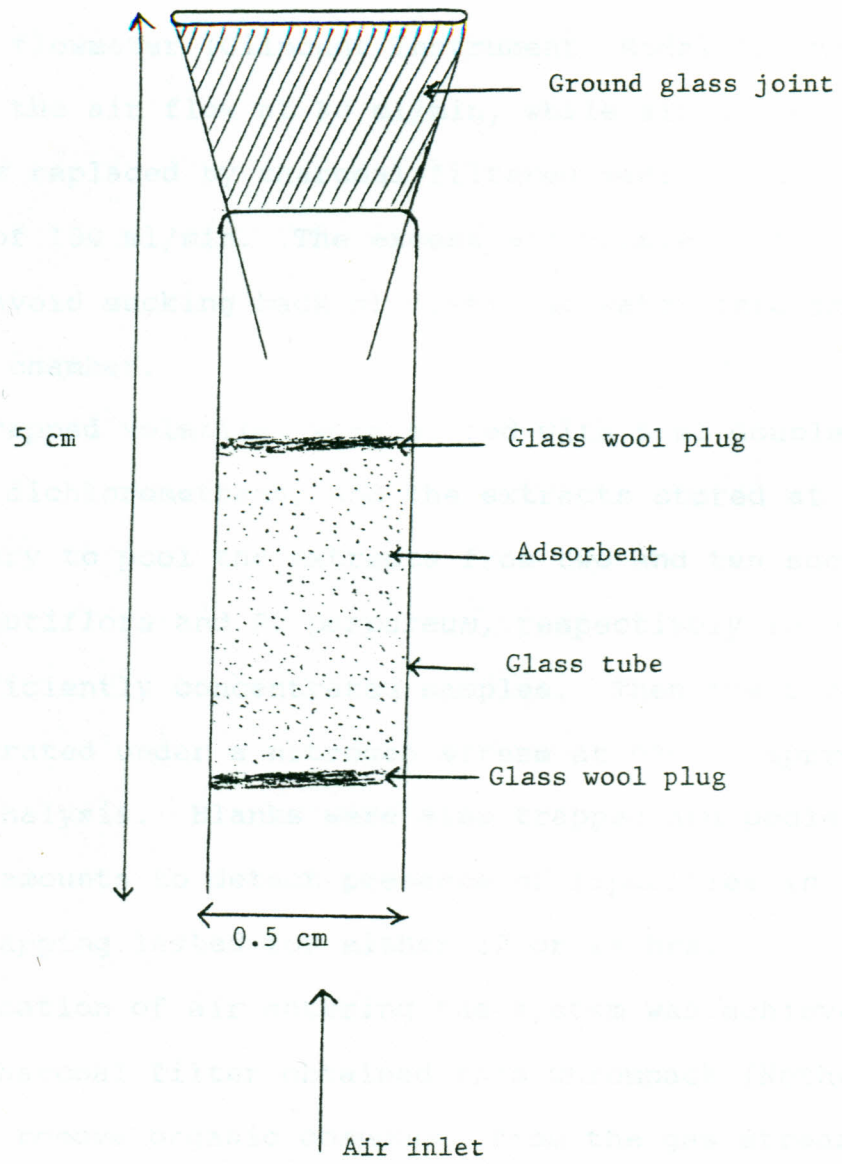


Figure 2: A Trap Packed with Adsorbent

(iii) Reverse phase trap did not require conditioning.

Charcoal filtered medical air (East African Oxygen) was drawn over the plants in a glass chamber and subsequently through a push-pull odour collecting system (Figure 1). Air was sucked through the trap using a vacuum pump (GAST, Model DOA-P136-BN), and an air flowmeter (Gilmount Instrument, Model 150 mm) controlled the air flow at 80 ml/min, while air inside collection chamber was replaced by charcoal filtered medical air at an air flow rate of 100 ml/min. The excess air bubbled out of the system to avoid sucking back of distilled water into the collection chamber.

The trapped volatiles were eluted with 5 ml double distilled HPLC grade dichloromethane, and the extracts stored at -20°C. It was necessary to pool the extracts from two and ten such samples from *M. minutiflora* and *P. purpureum*, respectively in order to obtain sufficiently concentrated samples. Then the pooled sample was concentrated under a nitrogen stream at 0°C to approximately 50 µl for analysis. Blanks were also trapped and pooled to equivalent amounts to detect presence of impurities in the system. Trapping lasted for either 12 or 24 hrs.

Purification of air entering the system was achieved using activated charcoal filter obtained from Chrompack (Netherlands), this was to remove organic compounds from the gas stream.

All experiments were carried out at room temperatures, 25-28°C.

2.4.1.2 Field Collection

Suitable plants were selected from the plot (ICIPE, Nairobi) for field trapping, and the base of the selected plant was covered with a sheet of aluminium foil to minimize contamination of the adsorbent with soil volatiles.

1.5 g of clean reverse phase was put into each of the five 4 ml wheaton 225532 glass sample bottle (about 1.5 cm I.D., 2.0 cm long), tied at the neck with stainless steel wires, and then suspended above the test plant with clean glass rods, in one such set-up.

The suspended adsorbent together with the test plant were partially enclosed by a glass chamber, held by four stainless steel wires 2 cm above the ground and the setup was left for 12 hrs overnight (Figure 3).

The adsorbent was transfered from the glass containers into a clean funnel plugged with a small piece of clean glass wool. It was then extracted with 5 ml HPLC grade DCM, and the extract concentrated under N_2 at 0°C to approximately 50 μl, for analysis.

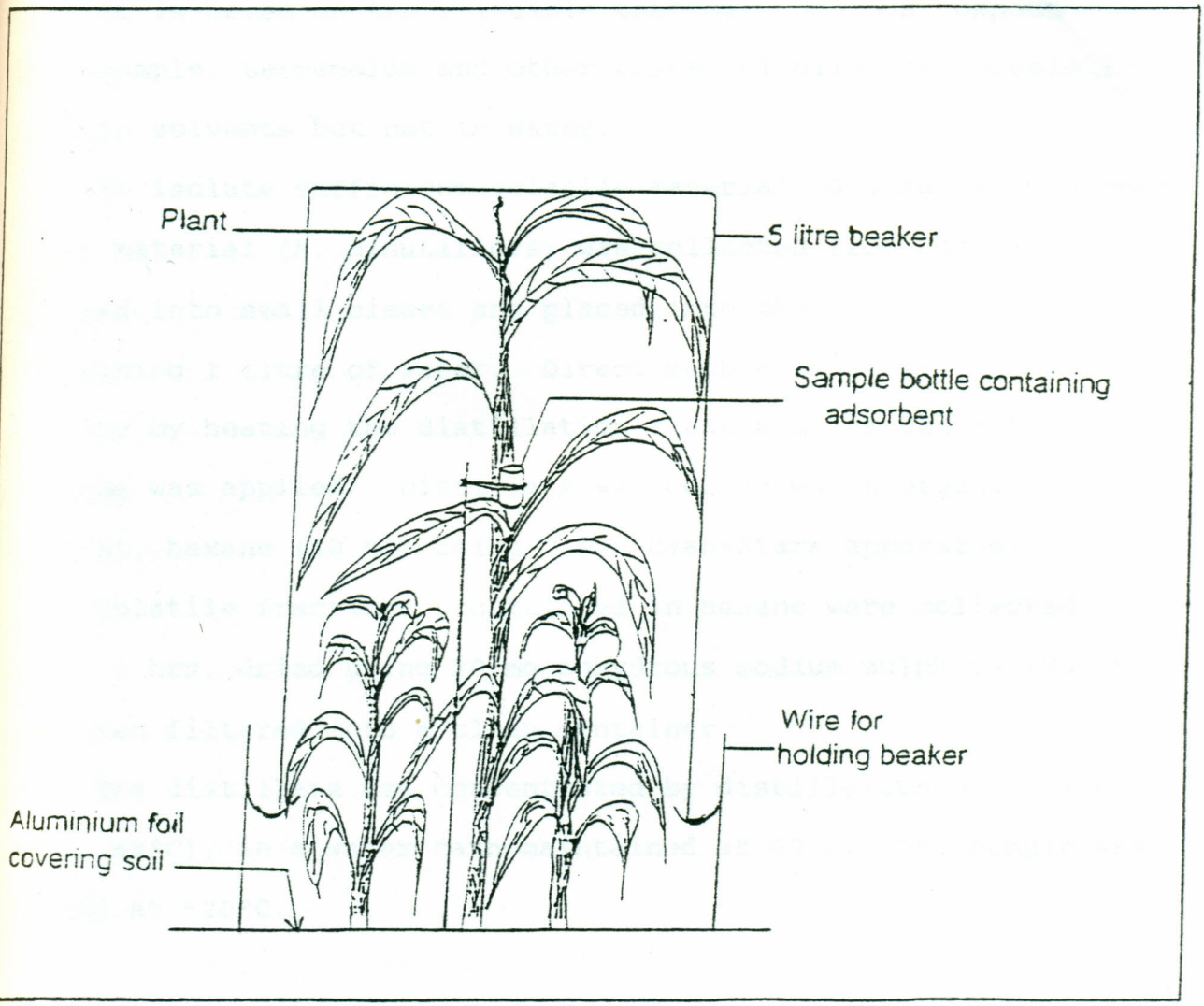


Figure 3: Setup for Collection of Airborne Plant Volatiles in the Field.

2.4.2 Collection of *M. minutiflora* Volatiles by Steam Distillation

It is based on the principle that many odorous compounds, for example, terpenoids and other essential oils are soluble in organic solvents but not in water.

To isolate sufficient volatile material, 0.5 kg of the fresh plant material (*M. minutiflora*) was collected from the farm chopped into small pieces and placed into the distillation flask containing 1 litre of water. Direct method of generating steam in situ by heating the distillation flask and its contents to boiling was applied. Distillate was collected in organic solvent, hexane (10 ml) using clean Dean-Stark apparatus.

Volatile fractions accumulated in hexane were collected after 6 hrs, dried using 20 mg anhydrous sodium sulphate (Na_2SO_4) and then filtered into a clean container.

The distillate was concentrated by distillation of hexane (b.p. 69°C), in a water bath maintained at 69°C. The sample was stored at -20°C.

2.5 Analyses of Volatiles

Aliquots of volatiles collected were analyzed by gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) and GC co-injection with authentic samples.

2.5.1 Gas Chromatography (GC) Analyses

GC analyses of the collected volatiles was performed on a Hewlett Packard (HP) 5890 series II gas chromatograph equipped with a splitless injector and a Hewlett Packard 3396 series II integrator, methylsilicone capillary column (HP, 50 m X 0.2 mm I.D. X 0.33 μm film thickness) and a flame ionization detector (FID). The oven temperature was programmed from 50°C isothermal for 5 min, increased at 5°C/min to 280°C and held at 280°C for 10 min. Detector and injector temperature were maintained at 250°C each. The carrier gas used was nitrogen with a flow rate of 0.90 ml/min. The percentage of the compounds recovered was calculated on the bases of the peak areas.

2.5.1.1 Separation of 1,8 Cineol and Limonene

1,8-Cineol (M.W. 136, b.p. 156-157°C) and Limonene (M.W. 154, b.p. 156-157°C) were co-eluting in a fused methylsilicone column. Their separation was achieved using a Supelcowax column (60 m X 0.25 mm I.D. X 0.32 μm film thickness). GC oven temperature program was 60°C isothermal for 10 min, increased at 5°C/min to 220°C, and held at 220°C for 10 min.

2.5.2 Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

GC-MS analyses of the collected volatiles was carried out using a VG analytical 70-250 magnetic sector and a VG masslab 12-250 quadrople mass spectrometer. For the VG Analytical 70-250, a methylsilicone capillary column (50 m X 0.2 mm I.D. X 0.33 μm) fitted in a HP 5890 GC was directly coupled to MS and integrated data system. Ionization was achieved by electron impact at 70 ev, 230°C. The initial GC oven temperature was maintained at 30°C for 5 min and then programmed at 5°C/min to 180°C. This was done at Rothemsted Research Centre, Harpenden, United Kingdom.

For the VG masslab 12-250, chromatographic separations were achieved using a fused HP methylsilicone column (50 m X 0.2 mm I.D. X 0.33 μm film thickness) fitted into a HP 5890 GC and directly coupled to the MS and data system. Ionization was achieved by electron impact 70 ev, 210°C. The GC oven temperature was programmed from 50°C (5 min) to 280°C (10 min) at the rate of 5°C/min. All injections were made in the splitless mode.

The tentative identification of the unknown compounds was done by comparison of its mass spectra to the NIST library installed in the computer. The identity of the compounds was confirmed by GC comparison of the sample retention time with that of the authentic and peak enhancement by co-injection.

2.5.3 Co-injection and Peak Enhancement

After the MS library search it was necessary to confirm the identity of the candidate compounds. This was done by injecting a known volume V_1 of the sample into the GC; the peak area of the candidate compound was noted (A_1). Then, its corresponding authentic sample was diluted from a stock solution prepared in the ratio 1 μl : 5 ml DCM, such that volume V_2 injected into the GC had approximately the same peak area as the candidate compound ($A_2 = A_1$). This was done on condition that the two had the same retention time.

Volume (V_1) of the test sample and volume (V_2) of the authentic sample were drawn from their respective vials in that order using a 10 μl syringe and co-injected into the GC. If the peak area of the candidate compound was enhanced by almost twice its original peak area, then, its identity was confirmed.

The GC oven temperature program and column were the same as those used in the GC analyses, section 2.5.1.

2.6 Electrophysiology

2.6.1 Antennal preparation

Electroantennograms (EAGS) were recorded on antennae of one-day old, mated, and fed naive female of *C. partellus*. The antenna was cut off from the head capsule at the level of the

scape. The cut part of the antenna was inserted into the recording electrode (a glass micropipette containing a saline solution) and the tip was inserted into the indifferent electrode. Both electrodes were appropriately connected to the universal AC/DC UNDS amplifier (Syntech., The Netherlands). The test sample was injected into the GC for Gas chromatography-Electroantennography (GC-EAG).

2.6.2 Gas Chromatography-Electroantennography (GC-EAG)

2.6.2.1 GC-EAG Analyses of *P. purpureum* Volatiles

Coupled GC-EAG analyses were performed on a HP fused methylsilicone column (50 m X 0.2 mm I.D. X 0.33 μm film thickness) fitted into a HP 5890 series II GC, whose GC oven temperature program was, 50°C (10 min) then increased at a rate of 5°C/min to 280°C (10 min), with nitrogen as the carrier gas. The column effluent was slit in a 1:1 ratio into two 50 cm long deactivated fused columns connected to the FID and the delivery tube placed in such way that the effluent passed over the antenna. The FID and EAG signals were monitored synchronously using a GC-EAG interface card installed in PC (Harvard Professional Computer, American Megatrend Inc.).

2.6.2.2 GC-EAG Analyses of *M. minutiflora* Volatiles

Separation of the samples was achieved on an AI 93 gas chromatograph equipped with a cold on-column injector and an FID. A methylsilicone column (50 mm X 0.2 mm I.D. X 0.33 μm film thickness) was used, with hydrogen as the carrier gas. The GC oven temperature was programmed from 40°C (1 min.) and then increased at rate of 10°C/min to 250°C. This work was done at Rothamsted Research Centre, United Kingdom.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Analyses and Identification of Volatiles

Volatiles from *M. minutiflora* and *P. purpureum* were analyzed using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), and then they were screened for activity using gas chromatography-electroantennography (GC-EAG). Constituents of the volatiles were identified by direct comparison of their mass spectra, to the NIST mass spectra data base, using computer library search and confirmed by GC co-injection with authentic samples.

This procedure could be stated in three parts:

(i) "MS" Mass spectra analysis

(ii) "RT" Retention time comparison

(iii) "GC-co" Peak enhancement by co-injection

From which a compound was considered identified. Due to inaccessibility of some standards, some of the major GC peaks could not be further confirmed by GC co-injection.

3.1.1 Chemical Composition of Headspace Volatiles Emitted by *M. minutiflora*

Most of the preliminary work was done using *M. minutiflora*.

Volatiles were trapped at various time (0.5, 1, 2, 4, 6, 12, 24 and 36 hrs) to establish the optimal period, for 12 hrs (day time) and 12 hrs (night time) using Porapak Q, and for 24 hrs using Porapak Q, C_{18} reverse phase and activated charcoal as adsorbents.

3.1.1.1 Analyses of *M. minutiflora* Volatiles Collected at Various Time

Gas chromatographic analyses revealed that 24 hrs collection was optimal (Table 5; Figure 4), with a consistent occurrence of 25 compounds. Out of which 16 (comprising of 64% of the sample) were positively identified. 2-pentylfuran, α-bergamotene, (E)-ß-farnesene, α-muurolene, germacrene, eremophilene, ß-chamigrene and 4,8,12-trimethyltrideca-1,3,7,11-tetraene were partially or tentatively characterized as possible candidates due to lack of reference standards, while decanal was in trace amounts (< 0.1%). The confirmed compounds were found to belong to three classes: (a) isoprenoids; which included monoterpenes, α-pinene, sabinene, myrcene, limonene, 1,8-cineole, (E)-ß-ocimene, terpinolene and 4,8-dimethylnona-1,3,7-triene, and sesquiterpenes, α-cubebene, α-ylangene, ß-cubebene, ß-caryophyllene and α-humulene, (b) fatty acid derivatives; hexanal and (Z)-3-hexenyl acetate and (c) phenolics; methyl salicylate.

Table 5: Chemicals Extracted in Headspace Volatiles of *M. minutiflora* Using Porapak Q for 24 hrs

Peak No.	Compound	Retention Time	Relative %[1]	Method of Identification
1.	Hexanal	12.38	0.96	MS, RT, GC-co
2	α-Pinene	19.38	0.70	MS, RT, GC-co
3	Sabinene	20.23	0.56	MS, RT, GC-co
4	2-Pentylfuran	20.63	0.63	MS
5	Myrcene	20.73	0.79	MS, RT, GC-co
6	(Z)-3-Hexenyl acetate	20.88	1.13	MS, RT, GC-co
7	Limonene and 1,8-cineole[2]	22.38	2.35	MS, RT, GC-co
8	(E)-ß-Ocimene	22.88	8.13	MS, RT, GC-co
9	Terpinolene	24.53	2.24	MS, RT, GC-co
10	4,8-Dimethylnona-1,3,7-triene	25.48	28.12	MS, RT, GC-co
11	Methyl salicylate	27.78	1.85	MS, RT, GC-co
12	Decanal	27.98	t	MS
13	α-Cubebene	33.50	0.45	MS, RT, GC-co
14	α-Ylangene	34.40	0.45	MS, RT, GC-co
15	ß-Cubebene	34.70	2.94	MS, RT, GC-co
16	ß-Caryophyllene	35.85	24.19	MS, RT, GC-co
17	α-Bergamotene	36.10	1.20	MS
18	(E)-ß-Farnesene	36.50	0.26	MS
19	α-Humulene	36.73	8.84	MS, RT, GC-co
20	α-Muurolene	37.08	1.64	MS
21	Germacrene	37.40	5.04	MS
22	Eremophilene	37.58	3.23	MS
23	ß-Chamigrene	37.80	1.99	MS
24	4,8,12-Trimethyltrideca 1,3,7,11 tetraene	39.33	2.30	MS

[1] based on the integration of peaks in the GC

[2] the two compounds were co-eluting

t trace amount < 0.1%

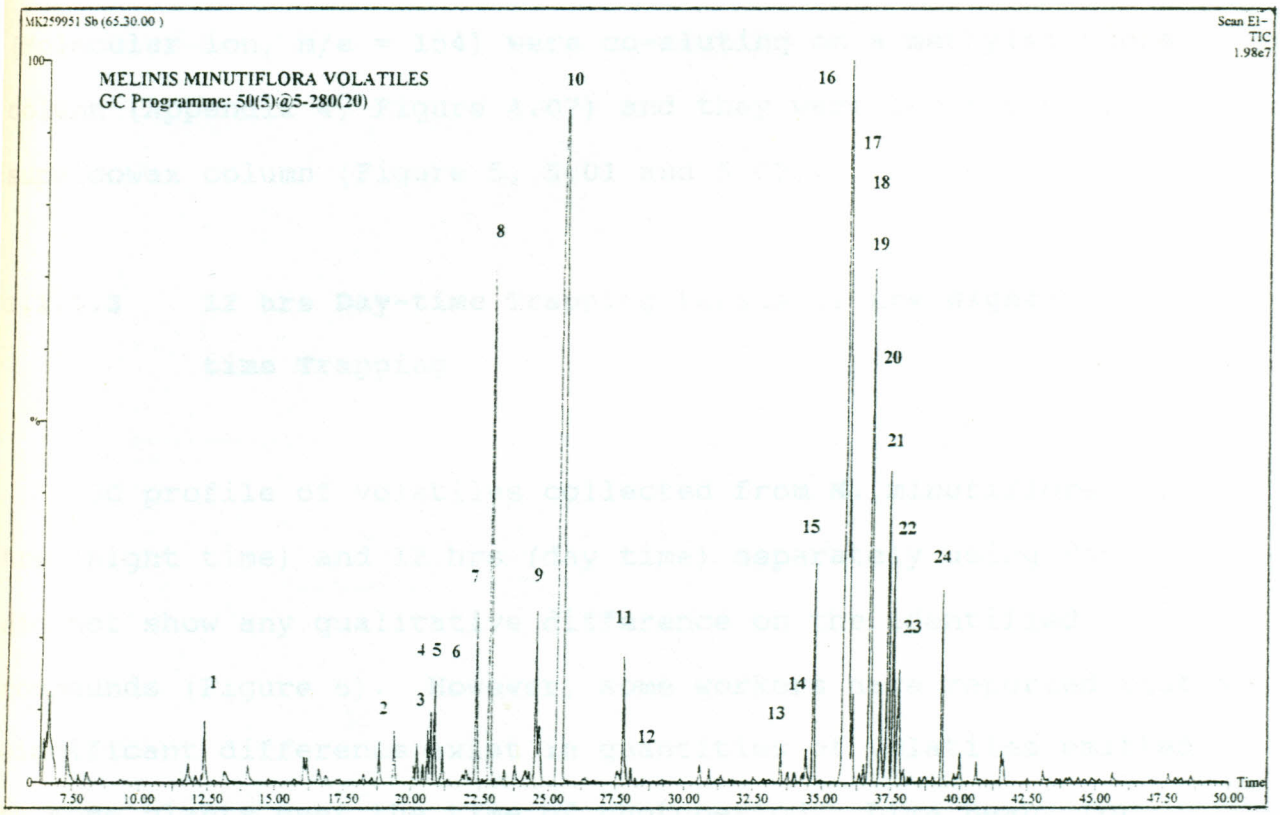


Figure 4: A Chromatogram of *M. minutiflora* Volatiles Trapped for 24 hrs using Porapak Q .

Their spectra and structures are given in Appendix 4; Figures 4.01 to 4.24. None of these compounds were found in the profiles from the blank.

3.1.1.2 Limonene and 1,8-Cineole

Limonene (Molecular ion, m/e= 136) and 1,8-cineole (Molecular ion, m/e = 154) were co-eluting on a methylsilicone column (Appendix 4, Figure 4.07) and they were separated by a supelcowax column (Figure 5, 5.01 and 5.02).

3.1.1.3 12 hrs Day-time Trapping Versus 12 hrs Night-time Trapping

GC profile of volatiles collected from *M. minutiflora*, 12 hrs (night time) and 12 hrs (day time) separately using Porapak Q did not show any qualitative difference on the identified compounds (Figure 6). However, some workers have reported that a significant difference exist in quantities of volatiles emitted by some plants over the time of photoperiod. Lima Bean, for example, showed relatively high amounts of volatile components in the headspace of leaves under high light intensity than under low light intensity [32]. Plants under high light intensity receive more photolytic energy to produce more volatiles.

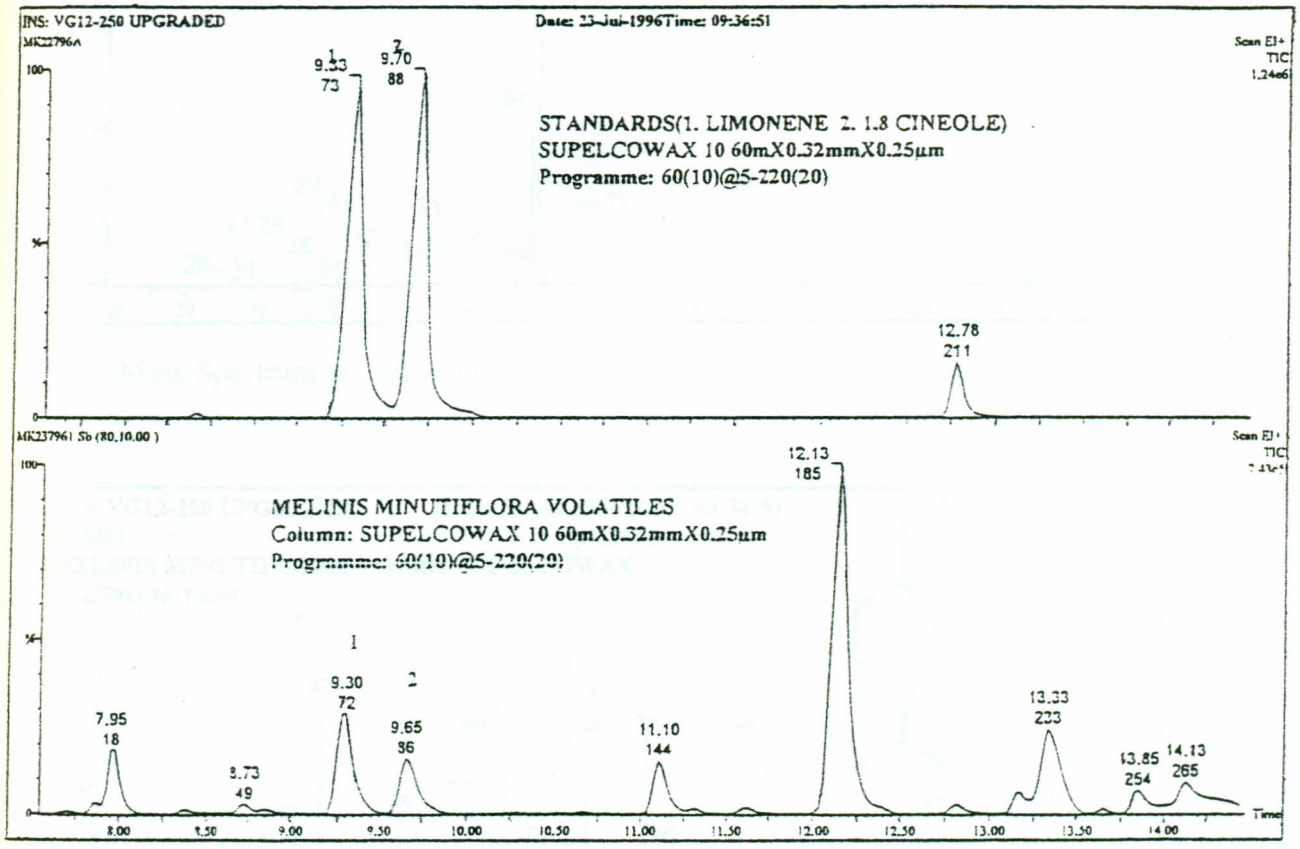
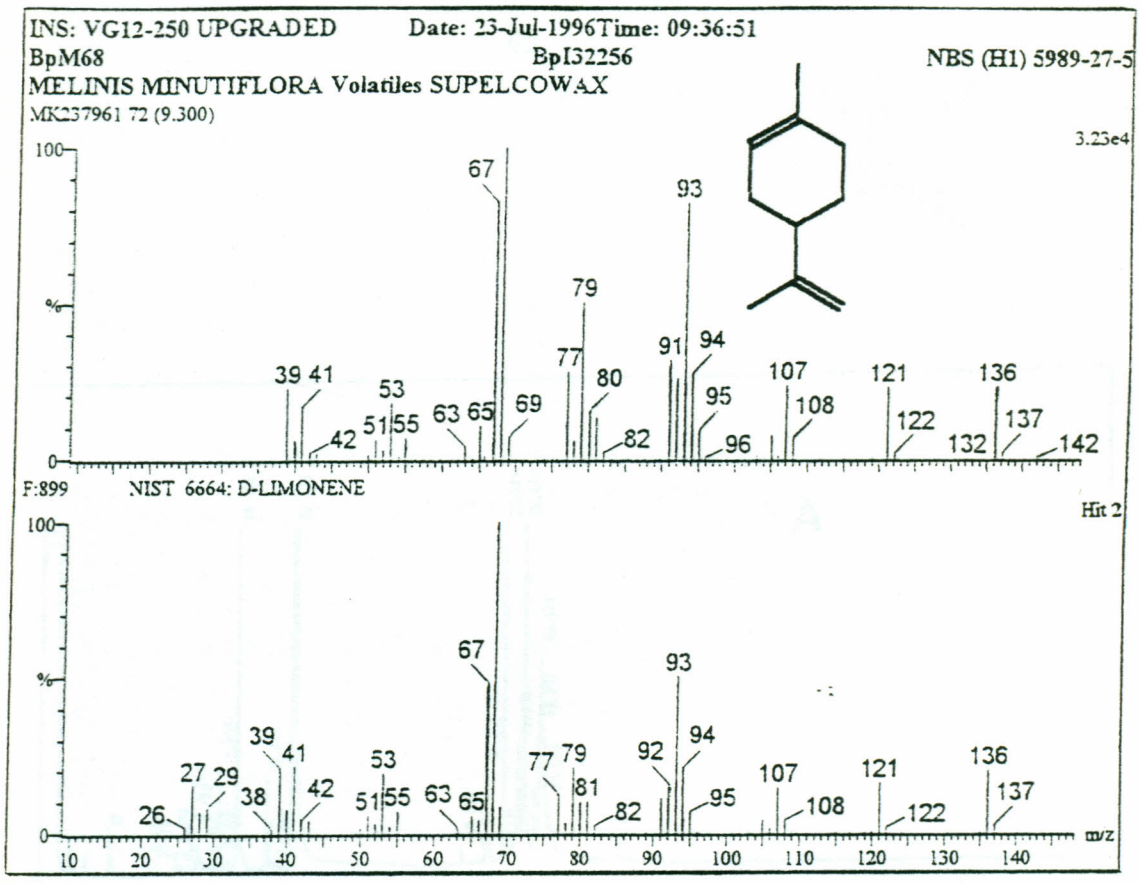
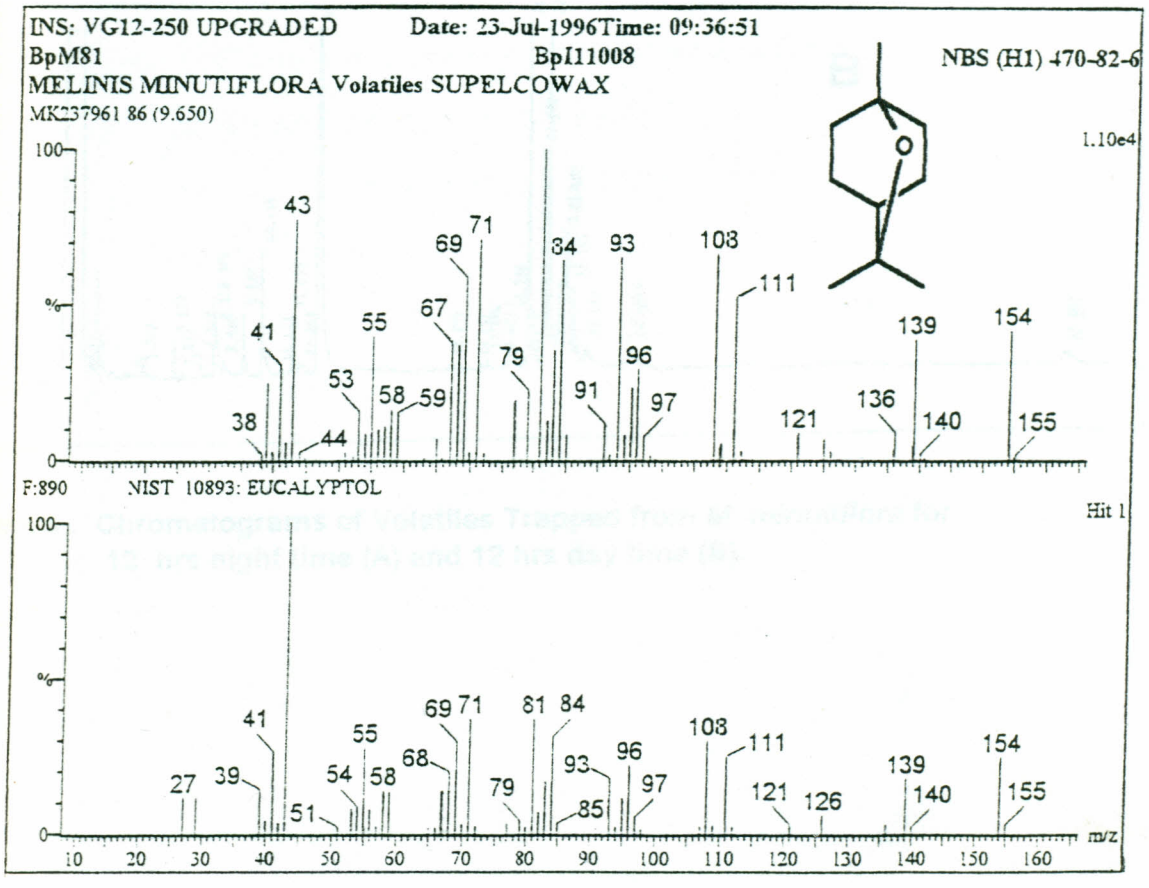


Figure 5: Chromatograms Showing Separation of Limonene (1) and 1,8- Cineole (2) on a Supelcowax Column.



5.01 Mass Spectrum of Limonene



5.02 Mass Spectrum of 1,8-Cineole

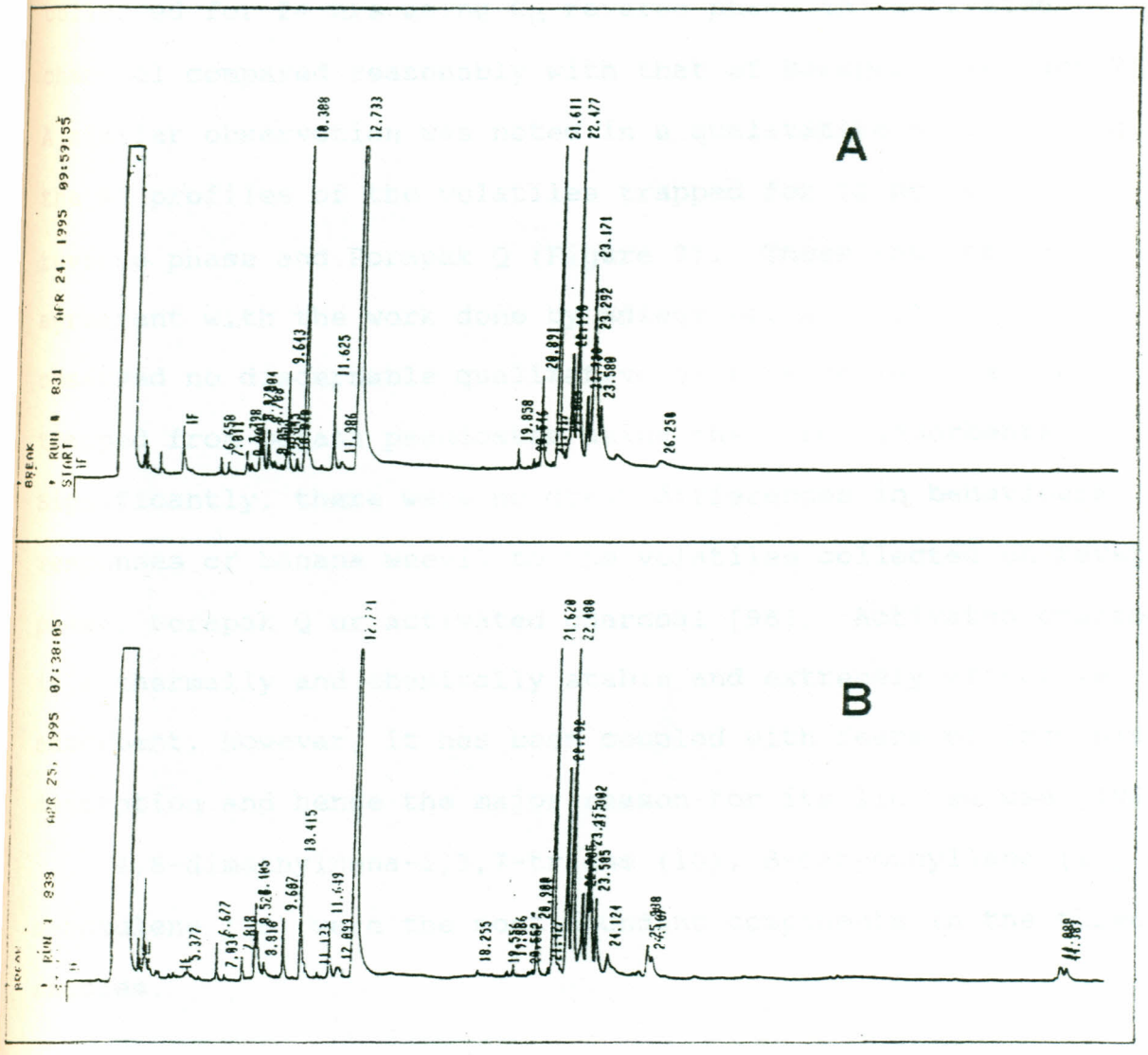


Figure 6 : Chromatograms of Volatiles Trapped from *M. minutiflora* for 12 hrs night time (A) and 12 hrs day time (B).

3.1.1.4 Analysis of *M. minutiflora* Volatiles Trapped Using Various Adsorbents

A qualitative comparison of gas chromatogram of volatiles collected for 24 hrs using C_{18} reverse phase and activated charcoal compared reasonably with that of Porapak Q (Figure 7). A similar observation was noted in a qualitative comparison of the GC profiles of the volatiles trapped for 12 hrs using C_{18} reverse phase and Porapak Q (Figure 8). These results were in agreement with the work done by Ndiege et. al. (1994), who observed no discernable qualitative difference in volatiles trapped from banana pseudostem using the three adsorbents. Significantly, there were no great differences in behavioural responses of banana weevil to the volatiles collected on reverse phase, Porapak Q or activated charcoal [96]. Activated charcoal is a thermally and chemically stable and extremely effective adsorbent, however, it has been coupled with fears of incomplete desorption and hence the major reason for its limited use [108].

4,8-dimethylnona-1,3,7-triene (10), ß-caryophyllene (16) and α-humulene (19) were the most abundant components in the three samples.

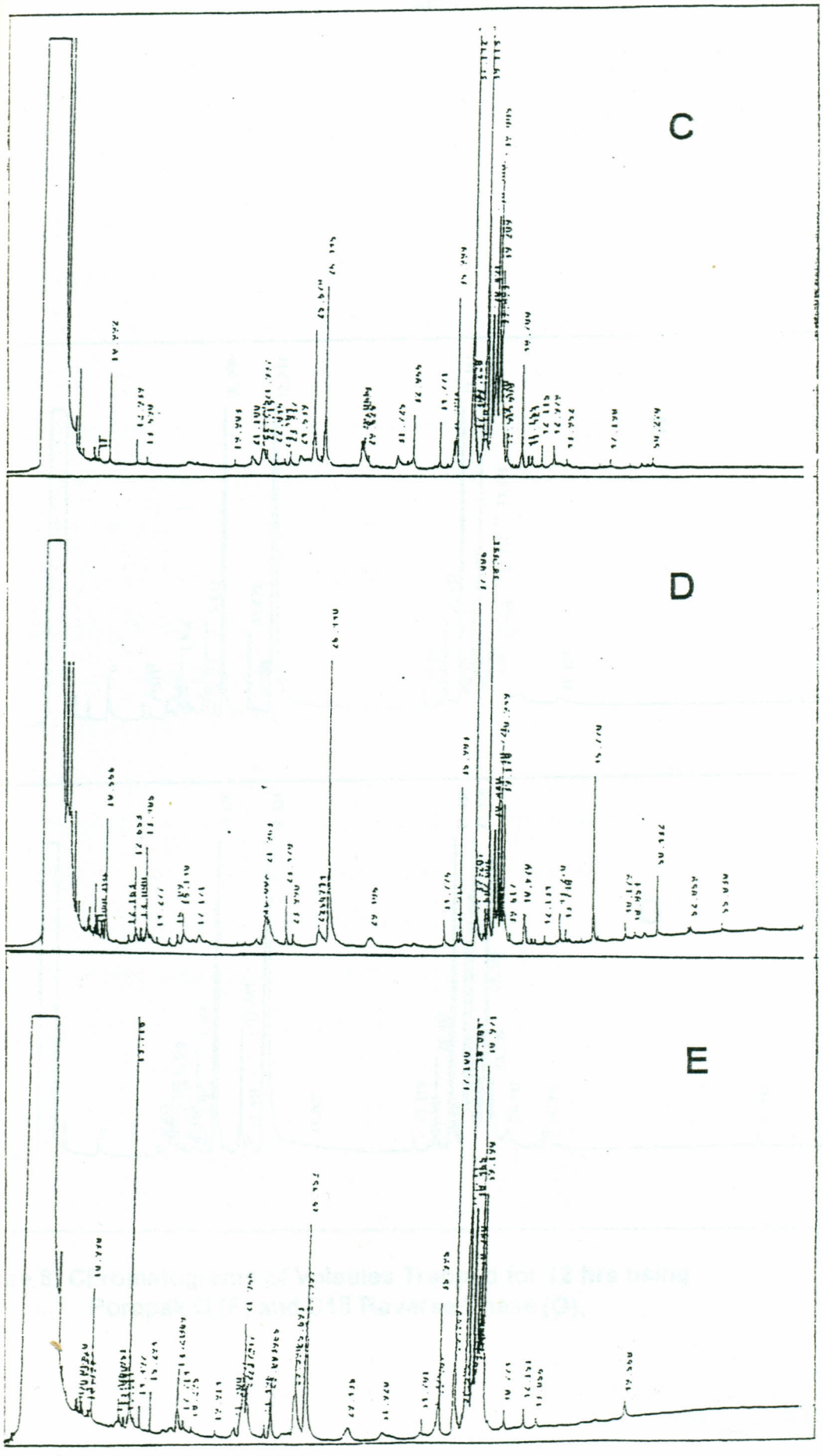


Figure 7: Chromatograms of Volatiles Trapped from *M. minutiflora* using Porapak Q (C), Activated Charcoal (D) and C18 Reverse Phase (E)

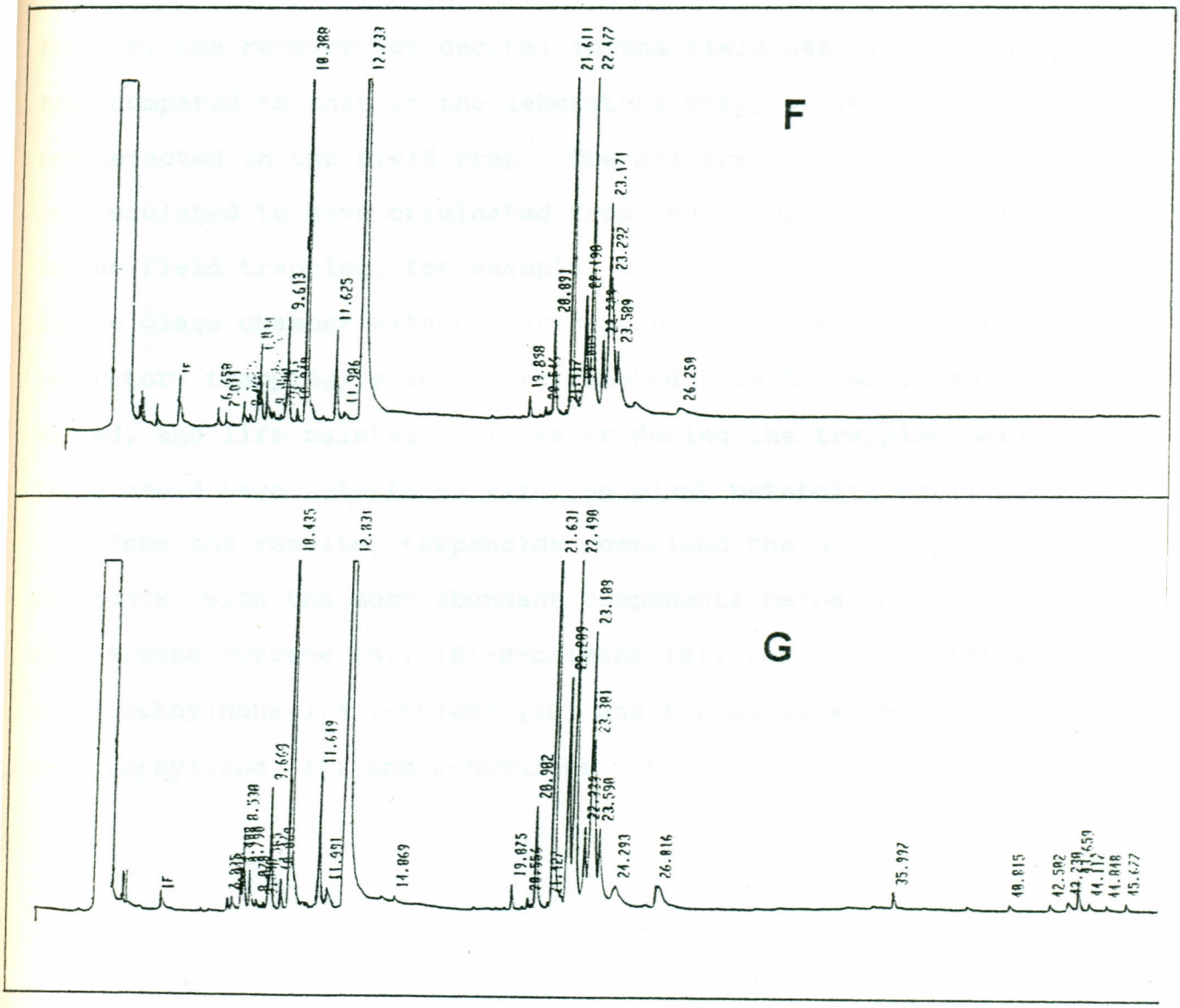


Figure 8: Chromatograms of Volatiles Trapped for 12 hrs using Porapak Q (F) and C18 Reverse Phase (G).

3.2 Chemical Composition of Field Trapped Volatiles Emitted by *M. minutiflora*

GC profiles of the volatiles recovered from the field using C_{18} reverse phase (Table 6, Figure 9), compared qualitatively with that recovered in the laboratory using Porapak Q adsorbent. However, the recovery of decanal in the field was significantly high compared to that in the laboratory trap, while α-pinene was not detected in the field trap. The differences in results could be speculated to have originated from the trapping techniques. In the field trapping, for example, the test plant was enclosed in the glass chamber without further interference, while in the laboratory trapping, plants were removed from the soil, roots washed, and life maintained in water during the trapping period. These could have interfered with the plant metabolic activity.

From the results, terpenoids comprised the majority of compounds, with the most abundant components being the monoterpene myrcene (5), (E)-ß-ocimene (8), terpinolene (9) and 4,8-dimethylnona-1,3,7-triene (10) and the sequiterpene ß-caryophyllene (16) and α-humulene (19).

Table 6: Headspace Field Collected Volatile Blend of *M. minutiflora*

Peak No.	Compound	Retention Time	Relative[a] %
1.	Hexanal	12.33	2.77
2	α-Pinene	-	-
3	Sabinene	20.20	1.27
4	2-Pentylfuran	20.68	2.35
5	Myrcene	20.85	12.85
6	(Z)-3-Hexenyl acetate	21.13	3.00
7	Limonene and 1,8-cineole	22.33	1.56
8	(E)-ß-Ocimene	22.83	6.46
9	Terpinolene	24.50	6.21
10	4,8-Dimethylnona-1,3,7-triene	25.35	26.11
11	Methyl salicylate	27.75	0.48
12	Decanal	28.05	3.02
13	α-Cubebene	33.48	0.33
14	α-Ylangene	34.40	0.84
15	ß-Cubebene	34.65	1.27
16	ß-Caryophyllene	35.73	8.34
17	α-Bergamotene	36.05	0.65
18	(E)-ß-Farnesene	36.45	0.43
19	α-Humulene	36.68	11.60
20	α-Muurolene	37.05	3.17
21	Germacrene	37.33	1.53
22	Eremophilene	37.53	3.21
23	ß-Chamigrene	37.75	2.15
24	4,8,12-Trimethyltrideca-1,3,7,11- tetraene	39.30	0.38

[a] based on the integration of peaks in the GC.

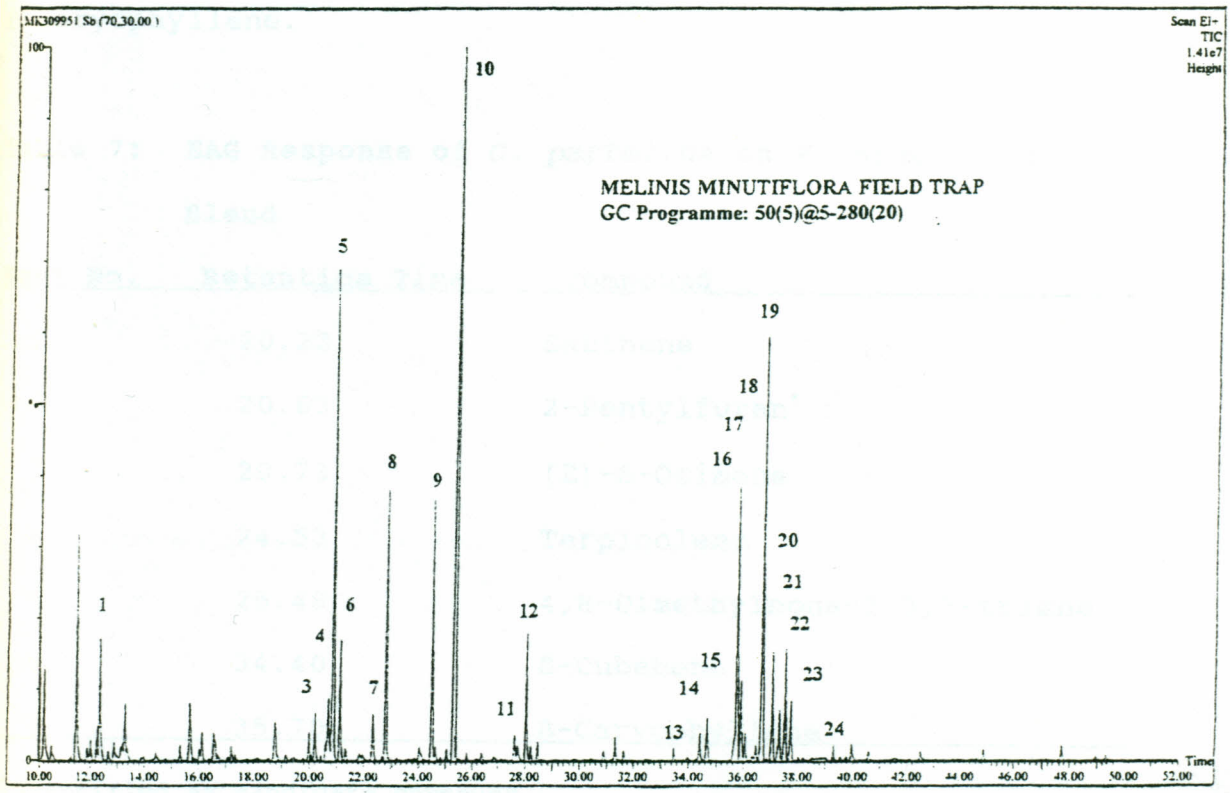


Figure 9 : A Chromatogram of Volatiles Trapped from the Headspace of *M. minutiflora* in the Field.

3.3 GC-EAG Analysis of *M. minutiflora*

Seven compounds were identified as electrophysiologically active, when volatiles emitted by *M. minutiflora* were screened for activity on *C. partellus*. Both strong and weak responses were recorded (Table 7, Figure 10). The identified EAG active peaks included sabinene, 2-pentylfuran, (E)-ß-ocimene, terpinolene, 4,8-dimethylnona-1,3,7-triene, ß-cubebene and ß-caryophyllene.

Table 7: EAG Response of *C. partellus* on *M. minutiflora* Volatile Blend

Peak No.	Retention Time	Compound
1	20.23	Sabinene
2	20.63	2-Pentylfuran*
3	20.73	(E)-ß-Ocimene
4	24.53	Terpinolene
5	25.48	4,8-Dimethylnona-1,3,7-triene
6	34.40	ß-Cubebene
7	35.70	ß-Caryophyllene

* Identified as candidate compound

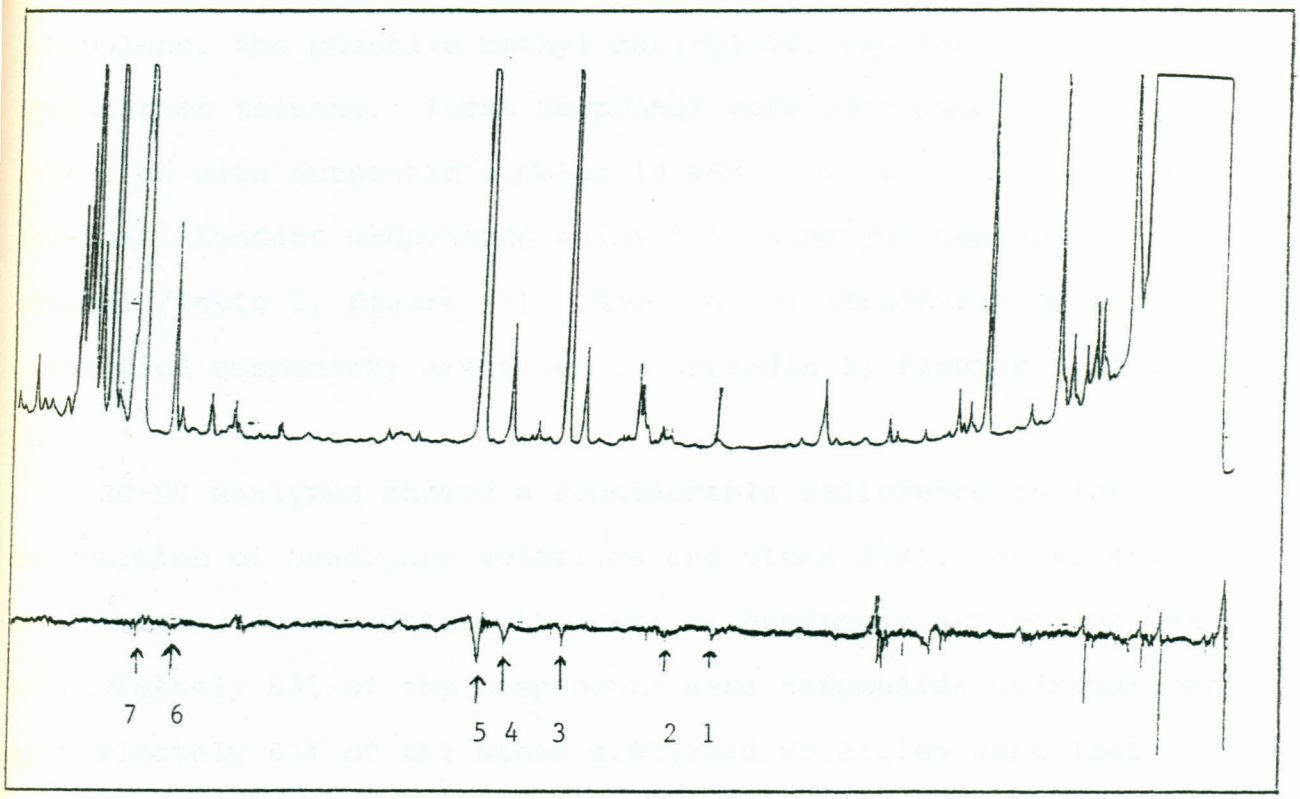


Figure 10 : GC - EAG of *M. minutiflora*.

3.4 Chemical Composition of Steam distilled Oil of *M. minutiflora*

The predominant components identified in *M. minutiflora* oil were leaf aldehyde, alcohol and ketones. This included hexanal, (E)-2-octenal, nonanal, decanal, 3-hexen-1-ol and 2-heptanone. Other compounds were sesquiterpenes ß-caryophyllene and α-humulene, the phenolic methyl salicylate, and the aromatic hydrocarbon toluene. These compounds were confirmed by GC co-injection with authentic samples in addition to GC-MS analyses. The most abundant components being leaf aldehyde hexanal and octanal (Table 8, Figure 11). Spectra and structures of the identified components are given in Appendix 5; Figures 5.01 to 5.15.

GC-MS analyses showed a considerable difference in the composition of headspace volatiles and steam distilled volatiles of *M. minutiflora*. Unlike the case of headspace extraction where approximately 83% of the components were terpenoids hydrocarbons, approximately 63% of the steam distilled volatiles were leaf aldehydes, alcohols and ketones. However, hexanal, 2-pentylfuran, methyl salicylate, decanal, ß-caryophyllene and α-humulene were common in both case. These were suspected to be either the stable compounds, abundant compounds or compounds that directly resulted from leaf tissue damage.

Table 8: Steam distilled Volatiles of *M. minutiflora*

Peak No.	Compound	Retention Time	Relative %	Method of Identification
1	Toluene	11.800	4.09	MS, RT, GC-co
2	Hexanal	12.650	26.65	MS, RT, GC-co
3	(E)-2-Hexenal	14.525	3.12	MS
4	3-Hexen-1-ol	15.000	5.96	MS, RT, GC-co
5	2-Heptanone	16.200	2.83	MS, RT, GC-co
6	Heptanal	16.625	3.06	MS, RT, GC-co
7	2-Heptenal	18.725	4.06	MS
8	2-Pentylfuran	20.625	10.73	MS
9	(E)-2-Octenal	22.676	5.05	MS, RT, GC-co
10	Nonanal	24.551	15.60	MS, RT, GC-co
11	(E)-2-Tridecenal	26.401	1.87	MS
12	Methyl salicylate	27.751	2.22	MS, RT, GC-co
13	Decanal	28.076	1.61	MS, RT, GC-co
14	ß-Caryophllene	35.776	2.03	MS, RT, GC-co
15	α-Humulene	36.726	7.49	MS, RT, GC-co

[1] based on the integration of peaks in the GC

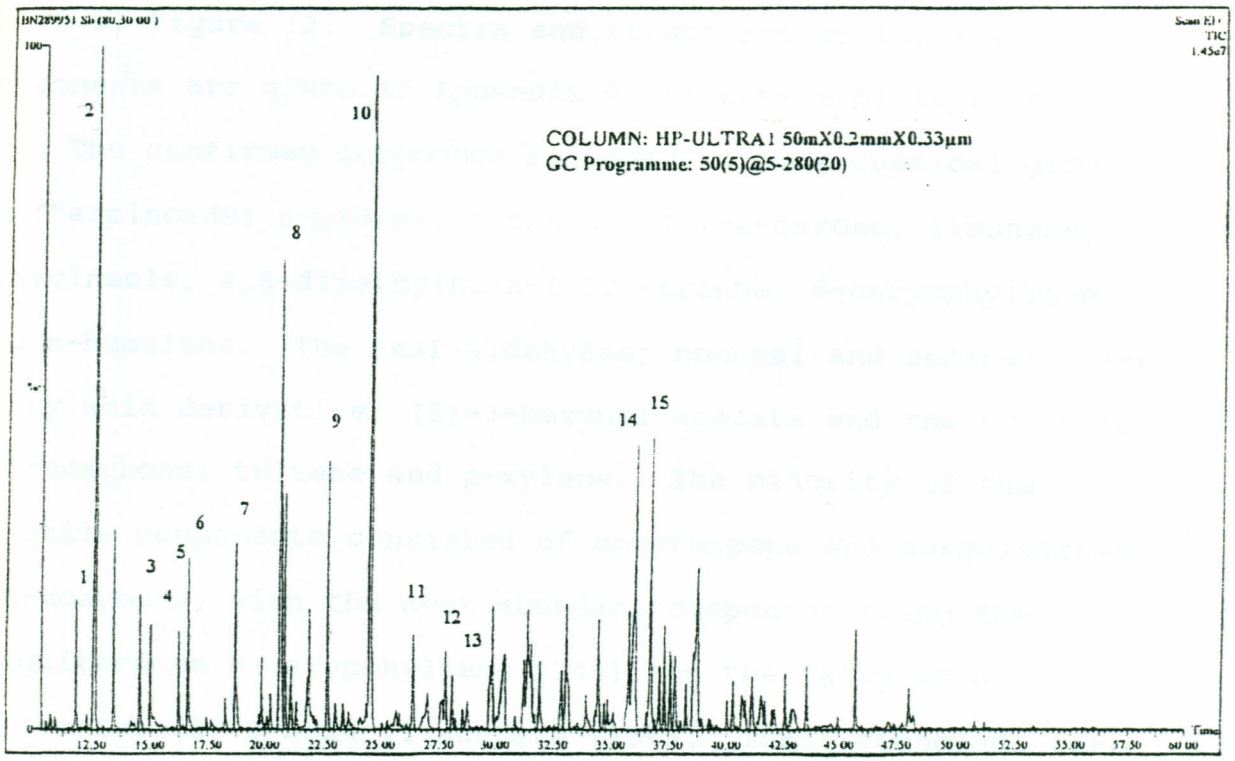


Figure 11: A Chromatogram of Steam Distilled Oil of *M. minutiflora*

3.5 Chemical Composition of Headspace Volatiles Emitted by *P. purpureum*

The blend of volatile compounds emitted by *P. purpureum* identified by GC-MS had a consistent occurrence of 16 compounds, two of which are unknown. Compounds 5 and 7 were identified by mass spectra analyses, while the rest were identified by mass spectra analysis, retention time comparison and peak enhancement with authentic samples (MS, RT, GC-Co) Table 9, Figure 12. Spectra and structures of the identified components are given in Appendix 6; Figures 6.01 to 6.15.

The confirmed compounds belonged to four chemical groups. The terpinoids; α-pinene, ß-pinene, (+)-2-carene, limonene, 1,8-cineole, 4,8-dimethylnona-1,3,7-triene, ß-caryophyllene and α-humulene. The leaf aldehydes; nonanal and decanal. The fatty acid derivative; (Z)-3-hexenyl acetate and the aromatic hydrocarbons; toluene and p-xylene. The majority of the volatile components consisted of monoterpene and sesquiterpene hydrocarbons, with the most abundant compounds being the sesquiterpene ß-caryophyllene (24%) and the Fatty acid derivative (Z)-3-hexenyl acetate (13%). These compounds were found previously in *M. minutiflora*, wheat and oat as a major components.

Very little of the usual aldehydes, etc., that result from tissue damage were observed in *P. purpureum* samples, irrespective of some unavoidable damage, at the point where the stem was cut from the base of the plant.

Table 9: Chemicals Extracted in Headspace Volatiles of *P. purpureum* using Porapak Q for 24 hrs.

Peak No.	Compounds	Retention Time	Relative [1] %	Method of Identification
1	Toluene	11.40	12.85	MS, RT ,GC-Co
2	Unknown	11.90	15.60	-
3	Unknown	14.88	3.91	-
4	P-Xylene	15.63	3.44	MS, RT, GC-Co
5	Methoxybenzene	17.28	8.84	MS
6	α-Pinene	18.68	2.06	MS, RT, GC-Co
7	Camphene	19.25	0.80	MS
8	ß-Pinene	20.33	3.71	MS, RT, GC-Co
9	(Z)-3-Hexenyl acetate	20.78	13.23	MS, RT, GC-Co
10	(+)-2-Carene	21.22	0.96	MS, RT, GC-Co
11	Limonene and 1,8-cineole*	22.27	0.43	MS, RT, GC-Co
12	Nonanal	24.43	0.77	MS, RT, GC-Co
13	4,8-Dimethylnona-1,3,7-triene	25.23	8.03	MS, RT, GC-Co
14	Decanal	27.98	0.83	MS, RT, GC-Co
15	ß-Caryophyllene	35.68	23.75	MS, RT, GC-Co
16	α-Humulene	36.55	0.80	MS, RT, GC-Co

[1] based on integration of peaks in the GC without use internal standards.

* these compounds were co-eluting.

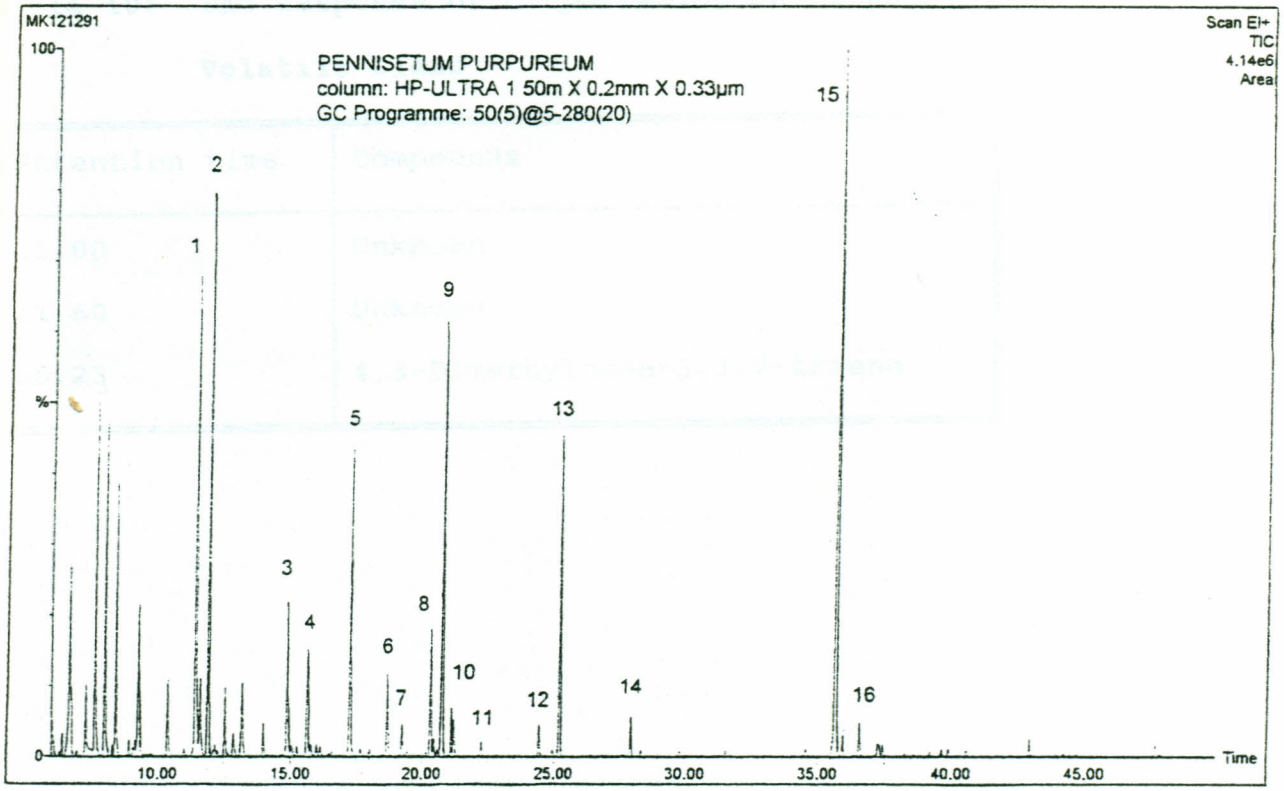


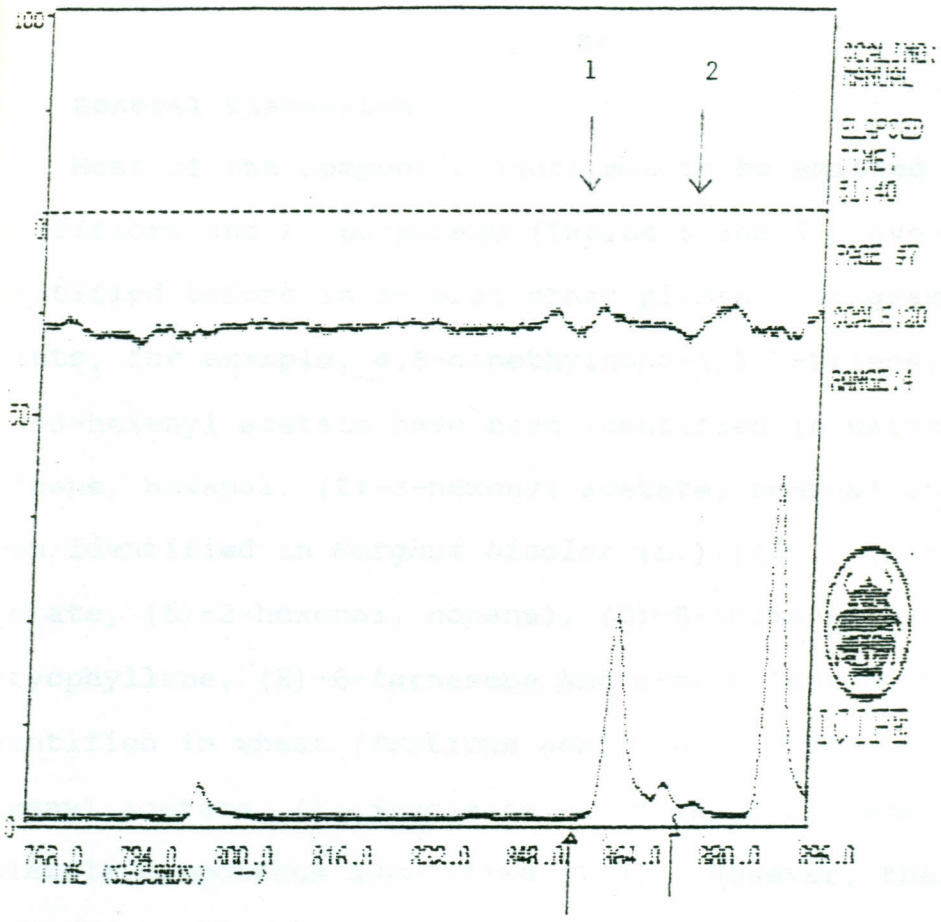
Figure 12: A Chromatogram of Air-Borne Volatiles Trapped from *P. purpureum* using Porapak Q for 24 hrs.

3.5.1 GC-EAG Analysis of *P. purpureum*

GC-EAG analysis of volatiles emitted by *P. purpureum* showed that *C. partellus* antenna responded to 3 peaks in the volatile blend, two of which were unknown (Table 10; Figure 13). The known EAG active peak was identified as 4,8-dimethylnona-1,3,7-triene, which showed a strong response.

Table 10: EAG Response of *C. partellus* on *P. purpureum* Volatile Blend

Retention time	Compounds
11.00	Unknown
11.60	Unknown
25.23	4,8-Dimethylnona-1,3,7-triene



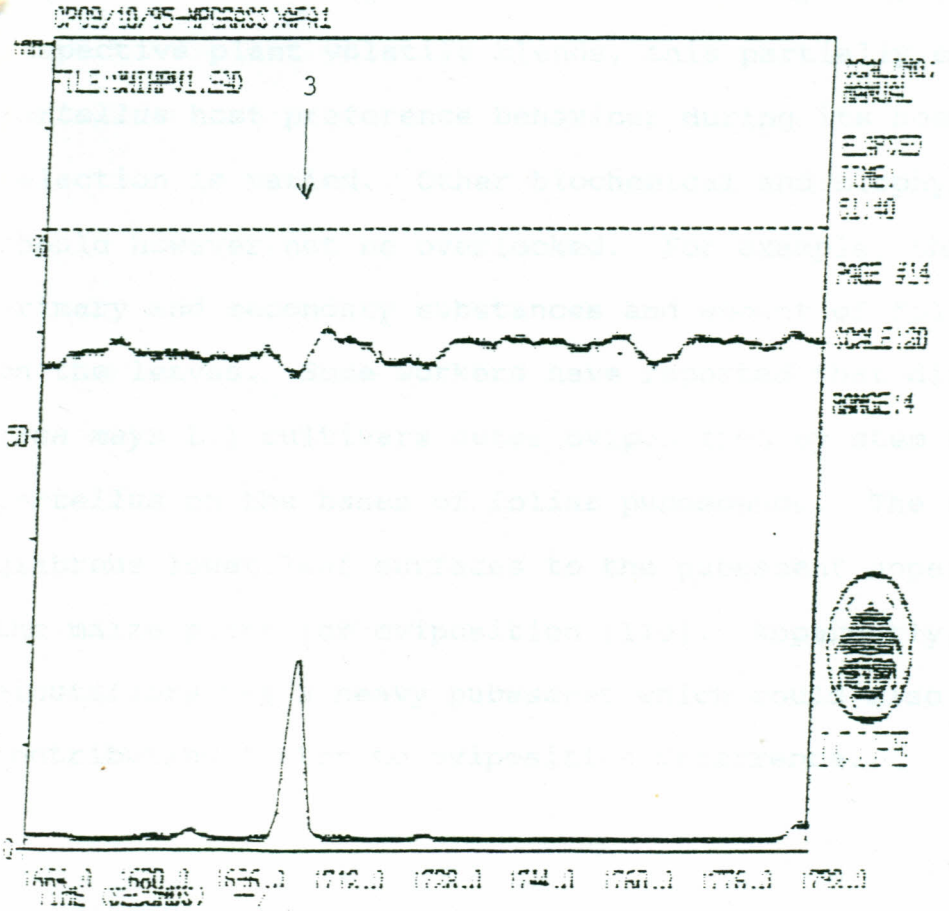


Figure 13: GC-EAG Of *P. purpureum*

3.6 General Discussion

Most of the compounds confirmed to be emitted by *M. minutiflora* and *P. purpureum* (Tables 5 and 9) have been identified before in several other plants. In graminaceous plants, for example, 4,8-dimethylnona-1,3,7-triene, myrcene and (Z)-3-hexenyl acetate have been identified in maize [47]. Toluene, hexanal, (Z)-3-hexenyl acetate, nonanal and decanal have been identified in *Sorghum bicolor* (L.) [46]. (Z)-3-hexenyl acetate, (E)-2-hexenal, nonanal, (E)-ß-ocimene, α-cubebene, ß-caryophyllene, (E)-ß-farnesene and α-muurolene have been identified in wheat (*Triticum aestivum* L.) [91]. In oats, (Z)-3-hexenyl acetate, (E)-ß-ocimene and ß-caryophyllene were the major volatile components identified [109]. However, there exists a significant difference in the chemical composition of the respective plant volatile blends, this partially explains why *C. partellus* host preference behaviour during its host plant selection is varied. Other biochemical and biophysical factors should however not be overlooked. For example, the non-volatile primary and secondary substances and amount of foliar pubescence on the leaves. Some workers have reported that different maize (*Zea mays* L.) cultivars deter oviposition by stem borer *C. partellus* on the bases of foliar pubescence. The moth prefer glabrous lower leaf surfaces to the pubescent upper surface on the maize plant for oviposition [110]. Apparently, *M. minutiflora* has a heavy pubescent which could also be a contributing factor to oviposition deterrence.

The greatest quantity of the volatile identified in both *M. minutiflora* and *P. purpureum* consisted of terpenes (82% and 55%, respectively), formed via mevalonate pathway. Unusual terpenes 4,8-dimethylnona-1,3,7-triene and 4,8,12-trimethyltrideca-1,3,7,11-tetraene, also referred to as homomonoterpenes and homosequiterpenes, result from oxidative cleavage of the sequiterpenoid nerolidol and diterpenoid geranyllinalool, respectively. Preliminary results suggest that these methylene terpenoids are released by several green plants, and may elicit behavioural responses in a variety of insects [111]. The green leaf volatiles (GLVs) hexanal, (Z)-3-hexenyl acetate and 3-hexen-1-ol commonly found in green plants were also identified. The GLVs are metabolites of the oxidative degradation of fatty acids and are often produced when enzymes are liberated due to plant tissue damage [43]. Phenolic compounds such as methyl salicylate originate from the shikimic acid pathway [112].

Some of the identified volatiles emitted by the plants under study, have previously been reported to play a role in eliciting behavioural responses in some adult phytophagous insects. Hexanal is a component of a blend of volatile compounds that were reported to increase trap catches of adult carrot fly, *Psila rosae* (F.) [88]; both α-pinene and limonene are components of plant odour that are attractive to pine weevil, *Hylobius abietics* Huckett [113, 114] and the beetle, *Ips grandicollis* Eichhoff respectively, while α-pinene and ß-pinene stimulate oviposition of the Eastern spruce budworm, *Choristoneura fumiferana* Clemens

[115]. Hexyl acetate is attractive to the cabbage rootfly, *Delia brassicae* (Bouche) [87] and α-pinene, in combination with camphene is attractive to leaf hopper, *Amrasca devastans* (Dist.) [116].

EAG results showed that 4,8-dimethylnona-1,3,7-triene through unusual terpenoid was an active compound in plants under consideration. But, the extent of activity can not be immediately concluded. This is because additional behavioural bioassay need to be carried out to determine whether the EAG active compounds elicit attraction, oviposition, repellency or neither. Previous work have revealed that electroantennogram recording may show olfactory response to various components, but in behavioural tests none of these components when applied singly are attractive. Electroantennogram recording show that the olfactory sensilla of colorado beetle to be mainly responsive to the GLVs. In behavioural tests none of these compounds, when applied singly, are attractive, whereas some of them, in minute quantities with the potted potato plants, mask the attractive host plant vapours, that is the beetles no longer show a positive response [43].

The results also showed that ß-caryophyllene was an EAG active component in *M. minutiflora* but not in *P. purpureum* volatiles. This was explained in relevance to concentration of the fraction, that is, they exist a threshold level below which the antenna does not respond. This was confirmed by comparing the peak areas, based on GC integration.

The effect of volatiles on insects is concentration or dosage dependent [117], either as individual compounds, combined components or with respect to their total amounts. Based on these and on the fact that *M. minutiflora* is a strong scented plant compared to *P. purpureum*, maize and sorghum, probably explains why *C. partellus* or tick, *Margaropus annulatus australis* (Fuller) cannot tolerate high concentrations or dosage of chemicals found constitutively in this plant.

Collection technique: The technique of collecting plant volatiles directly from air stream passed over the odour source (headspace analyses) has some major advantages over the often applied solvent extraction and/or steam distillation. The two later give no indication of how much of each identified compound is actually released into the environment and may result in enzyme catalyzed oxidation products that are not normally present in the intact plant and may mask the original volatiles. Enzymes action also degrade some of the plant volatile compounds [51]. From these experiments, volatiles collected by steam distillation of *M. minutiflora* are quite different from those collected over the headspace of the same plant. As discussed in previours work by Buttery et. al. (1984), damage to the plant material gives rise to considerable oxidative enzyme activity that break down the plant lipid and carotenoid components to a relatively large amount of volatile aliphatic aldehyde and alcohol compounds that are not present in intact plant [91]. The enzyme action might

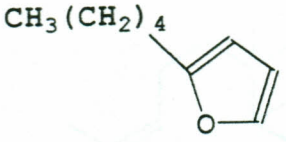
also destroy (for example, oxidise) some of the volatiles present in the intact plant.

The relatively newer technique, allows volatile compounds to be collected from living plant and thus provides more accurate assessment of the composition of volatiles emitted by plants [91, 92]. To fully understand the attraction of insect pests to their specific host plants, it would seem of primary importance to identify the compounds emitted by the intact plant. Volatiles emitted by damaged plants may also be important in the attraction of natural enemies of these insects.

Field trapping experiments compared qualitatively with laboratory headspace trapping. However, it was more prone to contamination from the neighbouring environment. Currently, a lot of work is going on towards research on better methods of field trapping.

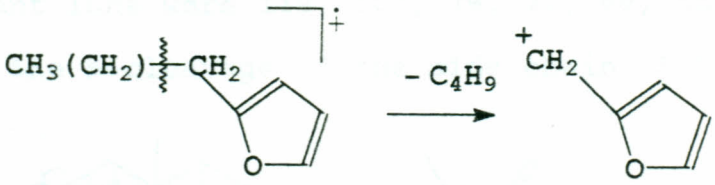
3.7 Mass Spectra Interpretation of the Unconfirmed Compounds

3.7.1 2-Pentylfuran (Appendix 4, Figure 4.04)



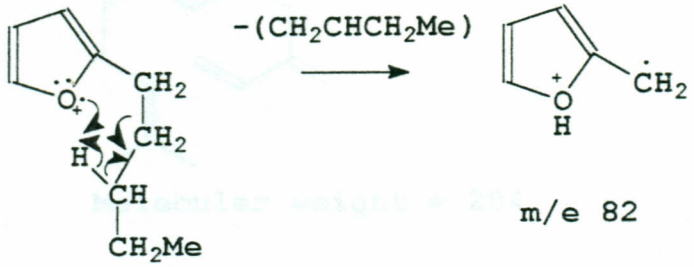
Molecular weight = 138

The mass spectrum of this fraction had a parent peak m/e 138. The base peak at m/e 81 was due to the cleavage of the bond ß to the ring.



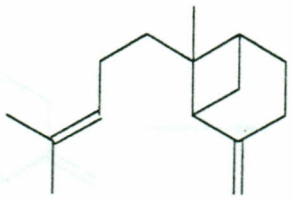
m/e 138 m/e 81

Position 2 of the furan ring was occupied by an alkyl rather than a methyl group. The alkyl group had to be pentyl because peaks at m/e 109, 95 and 81 were produced by the loss of ethyl, propyl and butyl groups from the parent ion. The peak at m/e 82 could be explained by rearrangement [118].



m/e 82

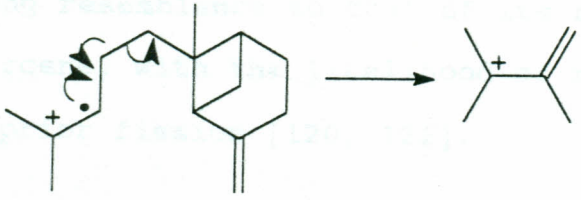
3.7.2 α-Bergamotene (Appendix 4, Figure 4.16)



Molecular weight = 204

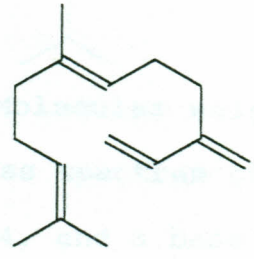
m/e 204 corresponded to the molecular ion peak, while m/e 189 and 161 were due to the cleavage of 15 (M - CH_3) and 43

($M - C_3H_7$) mass units. The base peak ion was m/e 93. Other prominent ions were 119, 107, 79, 77, 69, 55, where m/e 69 was due to the ß-cleavage of the side chain [119].



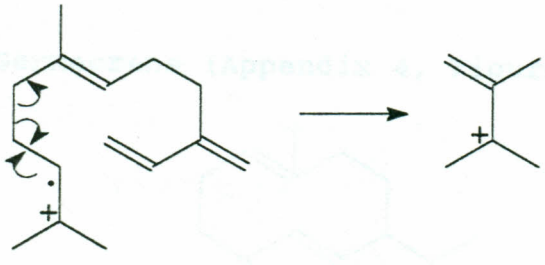
m/e 204 m/e 69

3.7.3 (E)-ß-Farnesene (Appendix 4, Figure 4.17)



Molecular weight = 204

(E)-ß-Farnesene showed a molecular ion peak at m/e 204 and a base peak at m/e 69, arising from the loss of the isopentyl end group by allylic cleavage.

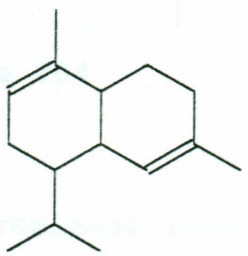


m/e 204 m/e 69

m/e 161 correspond to the loss of 43 mass unit ($M - C_3H_7$), other

prominent ions were m/e 133, 120, 107, 93, 79, 51, 41. However, it was noted that in the region below m/e 100 the spectrum of (E)-ß-farnesene with prominent ions at m/e 41, 69 and 93 showed strong resemblance to that of its monoterpene counterpart ß-myrcene, with the likelihood of rearrangement of the molecular ion prior fission [120, 121].

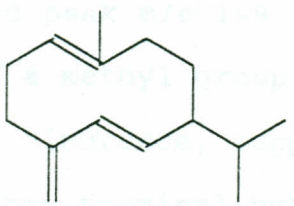
3.7.4 α-Muurolene (Appendix 4, Figure 4.19)



Molecular weight = 204

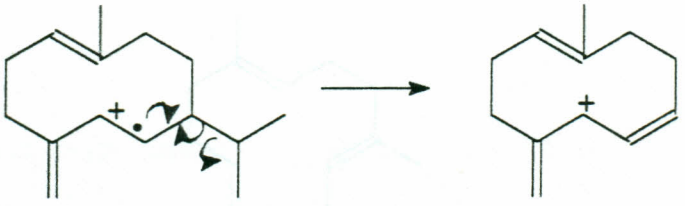
The mass spectrum of α-muurolene showed a molecular ion peak m/e 204, and a base peak m/e 105. m/e 183 and 161 resulted from the cleavage of methyl and isopropyl from the molecular ion [122]. From the spectrum it seemed as if rearrangement of the molecular ion occured before fission. Other prominent ions were m/e 147, 133, 121, 91, 79, 67, 55.

3.7.5 Germacrene (Appendix 4, Figure 4.20)



Molecular weight = 204

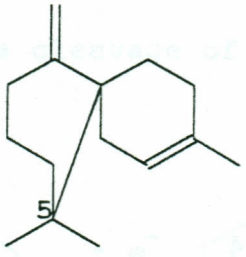
Mass spectrum of germacrene showed a molecular ion peak at m/e 204, and base peak ion at m/e 161, which corresponded to the loss of the isopropyl end of its side chain M - C_3H_7 after the ß-cleavage [119].



m/e 204 m/e 161

The other prominent ions were m/e 133, 119, 105, 91, 81, 67, 55.

3.7.6 ß-Chamigrene (Appendix 4, Figure 4.22)

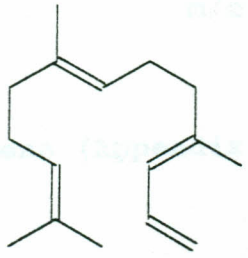


Molecular weight = 204

ß-Chamigrene showed a strong molecular ion peak m/e 204. The characteristic peak m/e 189 (M - 15), reflected the easiness of elimination of a methyl group. This was interpreted as invoked by steric hindrence, supposed to exist between the methyl group at C-5 and the terminal methylene group [123]. The other prominent ions were m/e 161, 175, 133, 121, 119, 107, 81, 67, 55,

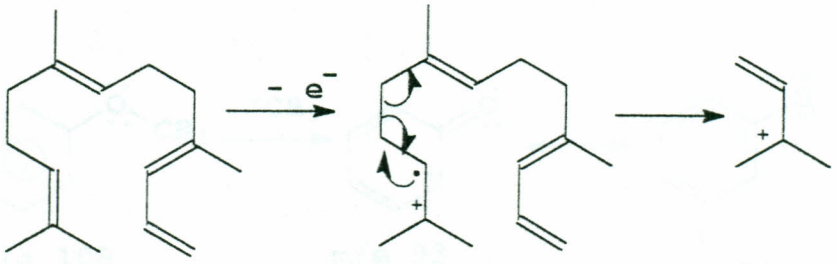
some of which could have resulted from rearrangement of the molecular ion prior fusion.

3.7.7 4,8,12-Trimethyltrideca-1,3,7,11-tetraene (Appendix 4, Figure 4.23)



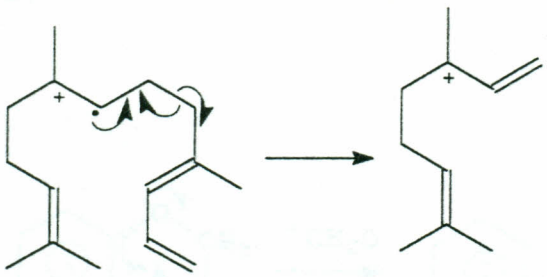
Molecular weight = 218

The molecular ion m/e 218 did not exist, this was related to the presence in the molecule of three quaternary centers as well as two doubly allylic carbon-carbon bonds, which diminished the stability of the molecular ion [122, 124]. The base peak m/e 69 resulted from the cleavage of the doubly allylic carbon-carbon bond.



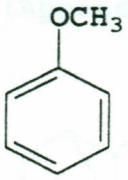
m/e 69

The other major ions were m/e 137, 119, 105, 95, 81, 55.



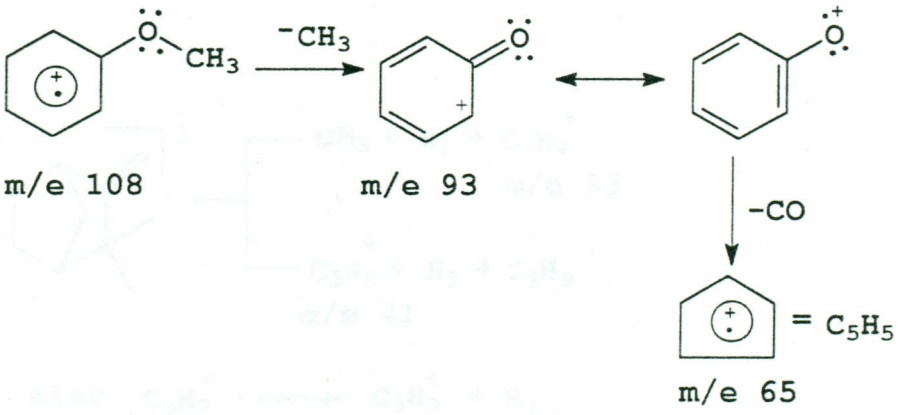
m/e 137

3.7.8 Methoxybenzene (Appendix 6, Figure 6.03)



Molecular weight = 108

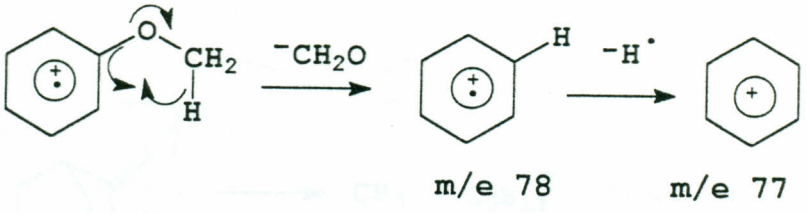
The molecular ion peak m/e 108 was also the base peak ion. Cleavage occured at the bond ß to the ring and the first formed ion decomposed further to give m/e 93 and 65 [119].



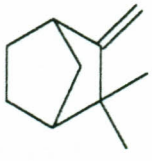
m/e 108 m/e 93 m/e 65

The characteristic aromatic peaks at m/e 78 and 77 arose as

follows;

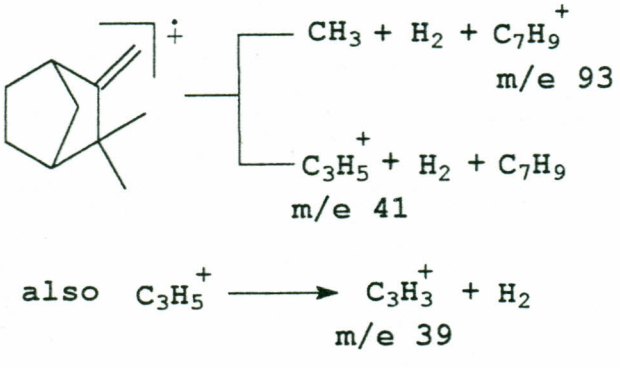


3.7.9 Camphene (Appendix 6, Figure 6.05)

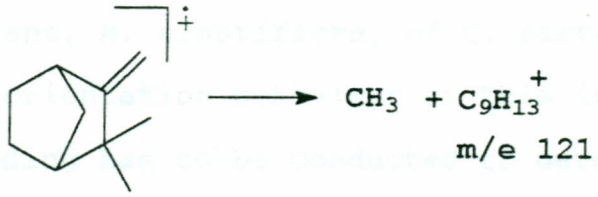


Molecular weight = 136

Camphene had a molecular ion peak at m/e 136. The base peak m/e 93, the ions 81, 41 and 39 were accounted for by elision of *gem*-dimethyl with loss of hydrogen.



The facile elimination of a methyl radical was consistent with the presence of a *gem*-dimethyl in which the methyl-carbon bonds were also allylic to the double bond [122].



The other major ions were m/e 107, 79, 67, 53.

3.8 Conclusion

Although the chemical part of the present work provided information about the qualitative composition of the volatile infochemicals released by host plant, *P. purpureum*, and non-host plant, *M. minutiflora*, of *C. partellus*, extended bioassay on the orientation behaviour of this insect for oviposition and feeding has to be conducted to determine which chemicals (quality-quantity) of the identified blend are utilized to locate host or non-host plants. However, in the screening ovipositional bioassay of *C. partellus* terpinolene have been suspected to deter oviposition [125], while 4-hydroxybenzoic acid and 4-hydroxy benzaldehyde present in the ethyl acetate extracts of undamaged *S. bicolor* have been identified as feeding stimulants of *C. partellus* larvae [126]. Such knowledge can lead to an alternative method of managing this insect pest.

3.9 Areas of Further Research

Bioassay experiments of the electrophysiologically active compounds can be carried out to determine the attractive and repulsive substances, and the dose response. From which bioassay of the identified oviposition deterrents and attractants can be carried out in the field. Similarly, the contact stimuli (non-volatiles), can be isolated, characterized and their activity on *C. partellus* evaluated. Further work could be done to determine the components responsible for tick repellency in *M. minutiflora*.

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Appendix 1

Lepidopterous Stem Borers of Maize and Sorghum Crops Recorded from Africa*.

Family	Species	
	Scientific name	Common name
Pyralidae	*Acigona ignefusalis* (Hampson)	
	Chilo aganemnon (Bleszynski)	
	Chilo diffusilineus (J. de Joannis)	
	Chilo orichalociliellus (Strand)	Coastal Stalk Borer
	Chilo partellus (Swinhoe)	Sorghum stem borer
	Eldana saccharina (Walker)	African surgarcane borer
	Maliarpha separatella (Ragonot)	
Noctuidae	*Busseola fusca* (Fuller)	Maize stalk borer
	Busseola segata (Bowden)	
	Sesamia calamistis (Hampson)	African pink stalk borer
	Sesamia cretica (Lederer)	
	Sesamia albivena (Hampson)	
	Sesamia botanephaga (Tams and Bowden)	
	Sesamia penniseti (Tams and Bowden)	
	sesamia poephaga (Tams and Bowden)	

* Seshu Reddy (1983) and Khan et al. (1991) [8, 10].

Appendix 2.

Wild Host Plants of Cereal Stem Borer Recorded from Africa*.

Host Plant	*Chilo partellus*	*Busseola fusca*	*Eldana saccharina*	*Sesamia calamistis*
Andropogon uricatus L. F	–	–	–	+
Beckeropsis uniseta (Nees) Schum.	–	–	–	+
Centrus ciliaris L.	+	–	–	+
Centrus echinatus L.	–	–	–	+
Cyperus distans L. F.	–	–	–	+
Echinochloa haploclada Stapf.	–	–	–	+
Echinochloa indica (L.) Grertn.	+	–	–	–
Hyparrhenia rufa (Nees) Stapf.	+	+	–	+
Lepturus repens (Forst.f.) R. Br.	+	+	+	+
Panicum maximum Jacq.	+	+	–	+
Pennisetum purpurenum (K.) Schumach.	+	+	–	+
Setaria barbata (Lam.) Kunth	–	+	–	+
Sorghum arundinaceum (Desv.) Stapf.	+	+	–	+
Sorghum verticilliflorum (Steud.)	+	+	–	+
Vossia cuspidate (Roxb.) W. Griff.	+	+	–	+

* Seshu Reddy (1983) and Khan et al. (1991) [8, 10]
+: recorded; –: not recorded

Appendix 3

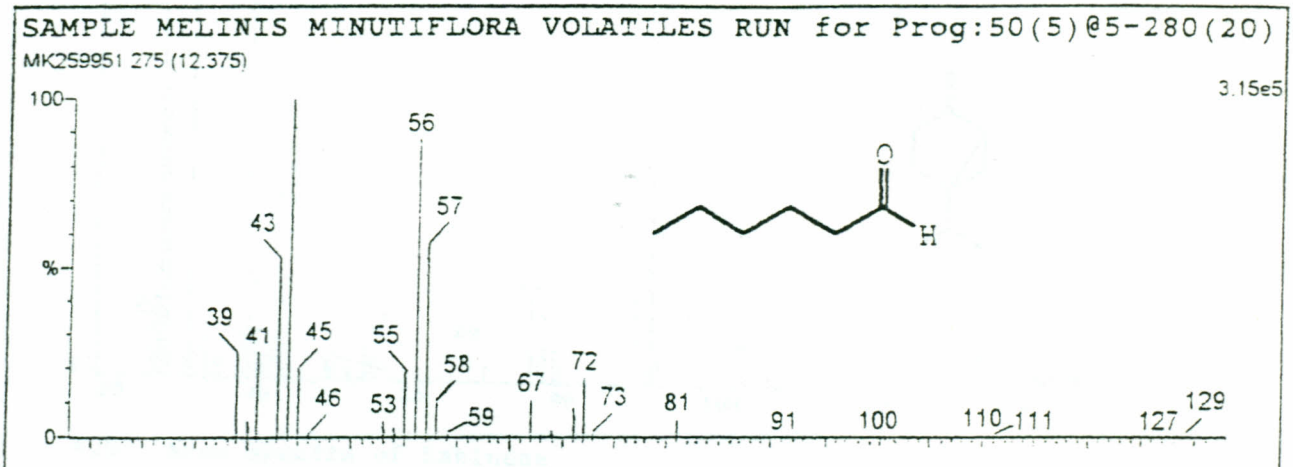
Density of *C. partellus* Larvae on Sorghum Plants in four Sorghum (Serena) and Cowpea Cropping Patterns for 1986-1988 Period* at Mbita Point (Kenya) [1].

Cropping pattern	*C. partellus* larvae/five plants (Mean ± S.E.)
Sorghum monocrop	2.38 ± 0.26
Sorghum and cowpea sown simultaneously	2.11 ± 0.19
Sorghum sown 2 weeks before cowpea	2.25 ± 0.22
Sorghum sown 2 weeks after cowpea	2.08 ± 0.19

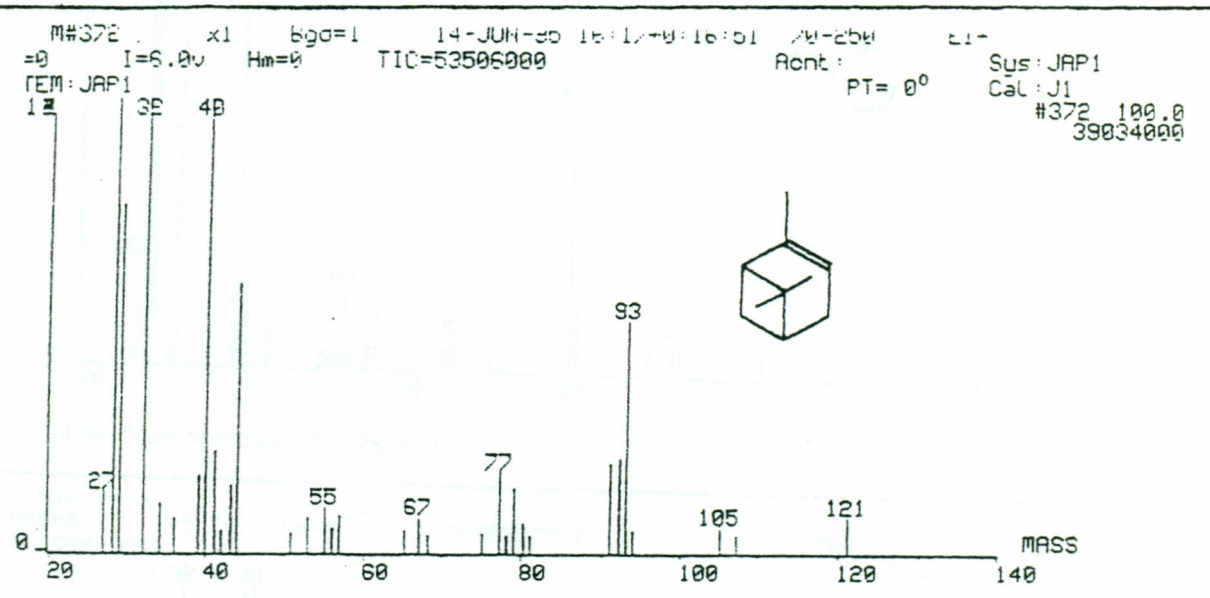
Means followed by the same letter are not significantly different P = 0.05; Duncan's Multiple Range Test (1955).

Appendix 4

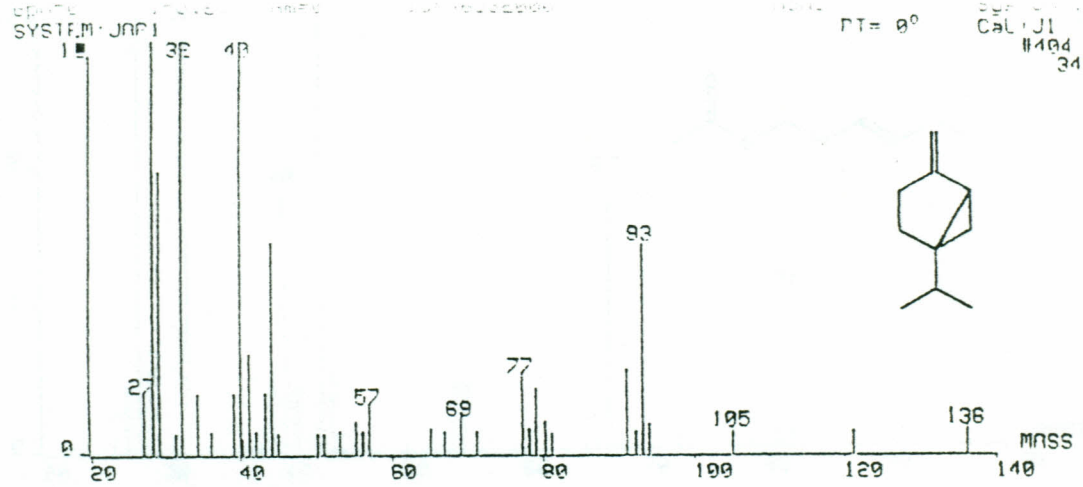
Figures Showing the Mass Spectrum and Structures of Compounds Identified in Volatile Blend Emitted by *M. minutiflora*.



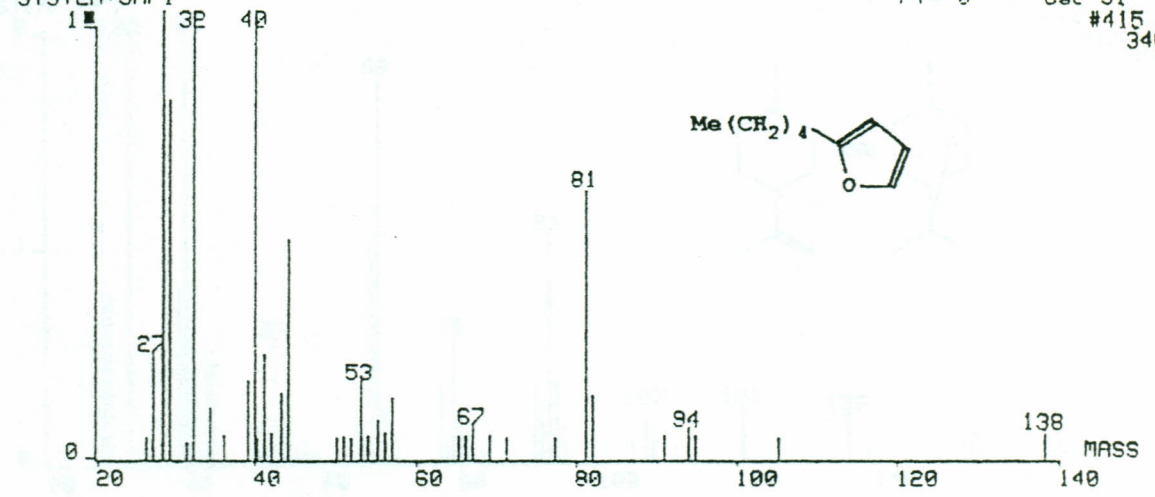
4.01 Mass Spectrum of Hexanal



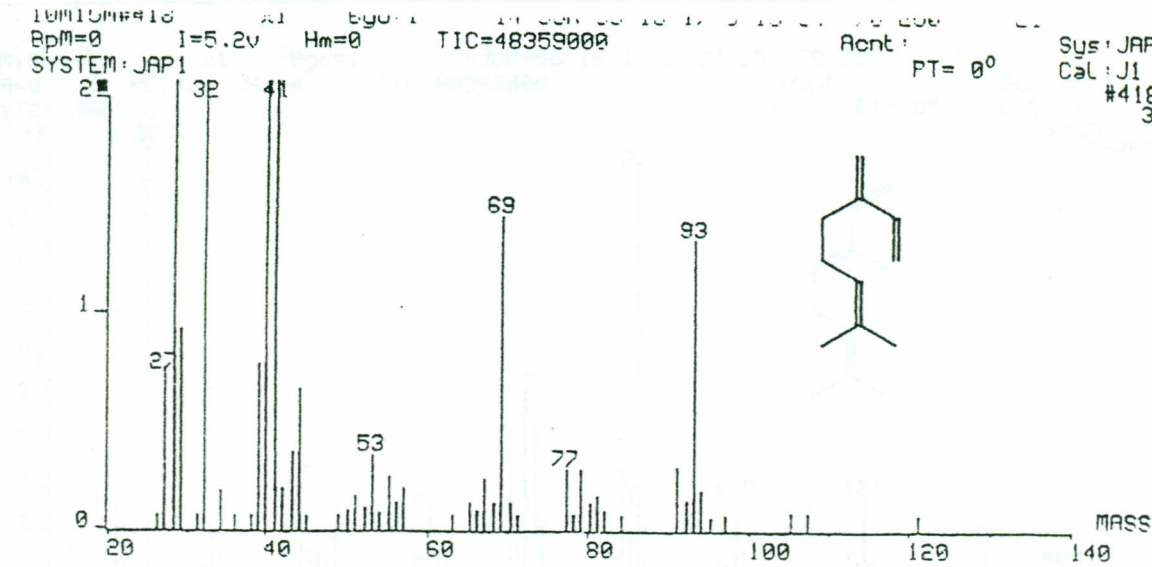
4.02 Mass Spectrum of α - Pinene



4.03 Mass Spectra of Sabinene

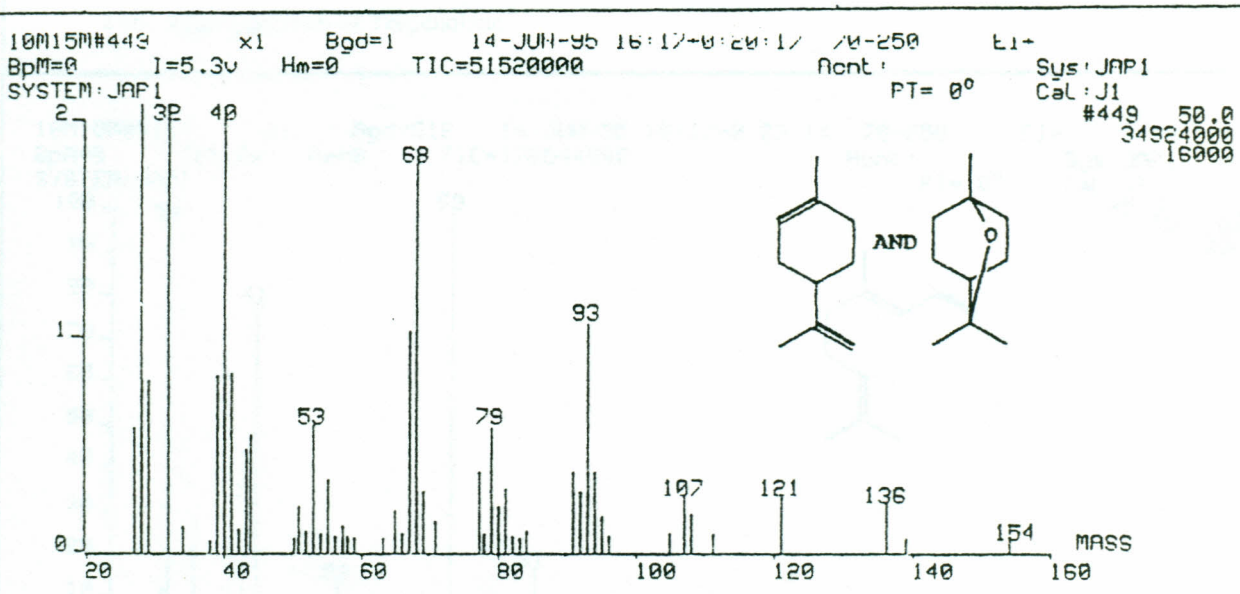


4.04 Mass Spectrum of 2-Pentylfuran

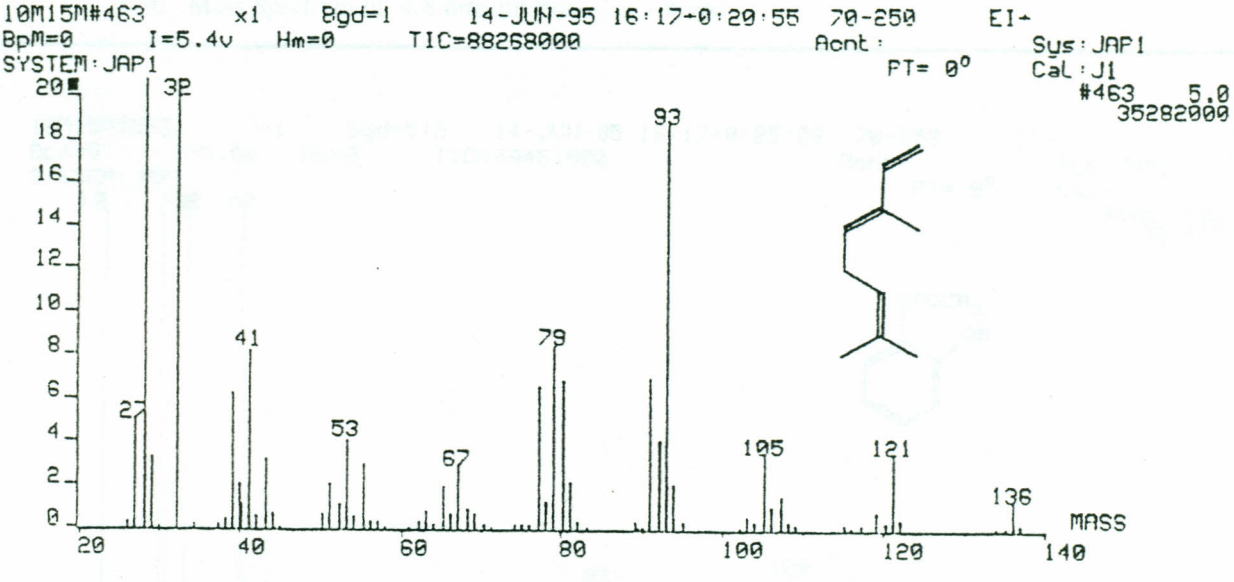


4.05 Mass Spectrum of Myrcene

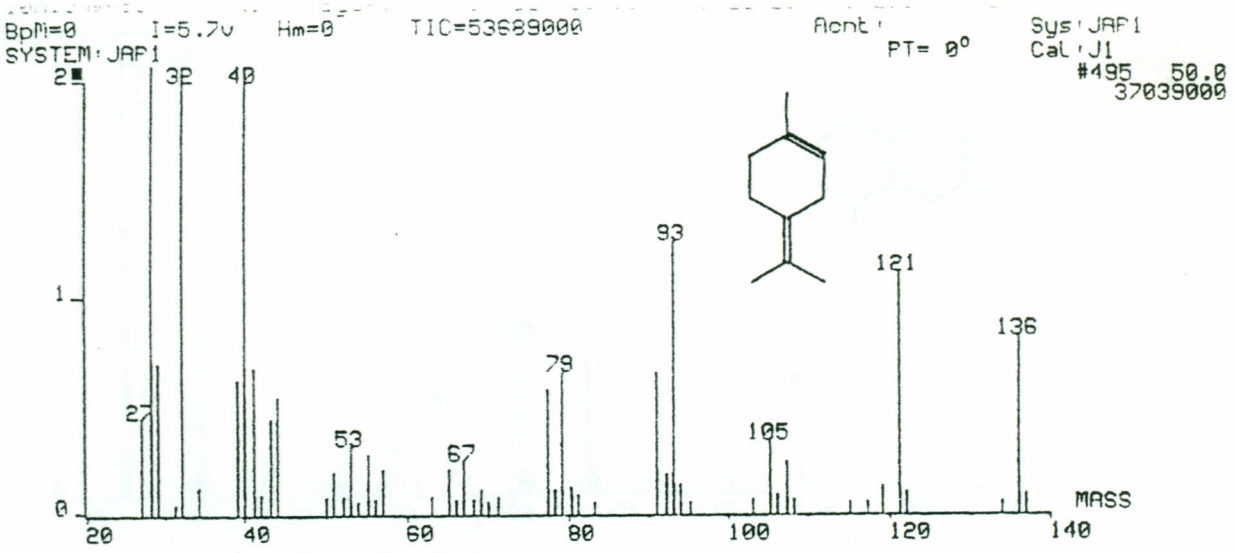
4.06 Mass Spectrum of (Z)-3-Hexenyl acetate



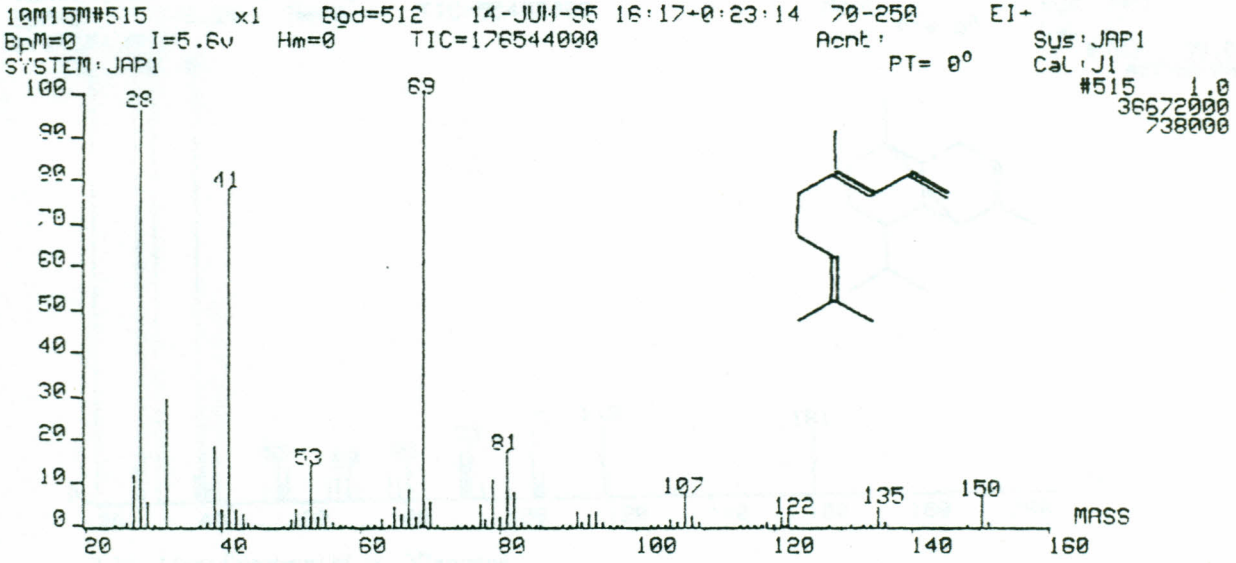
4.07 Mass Spectrum of Limonene and 1,8-Cineole



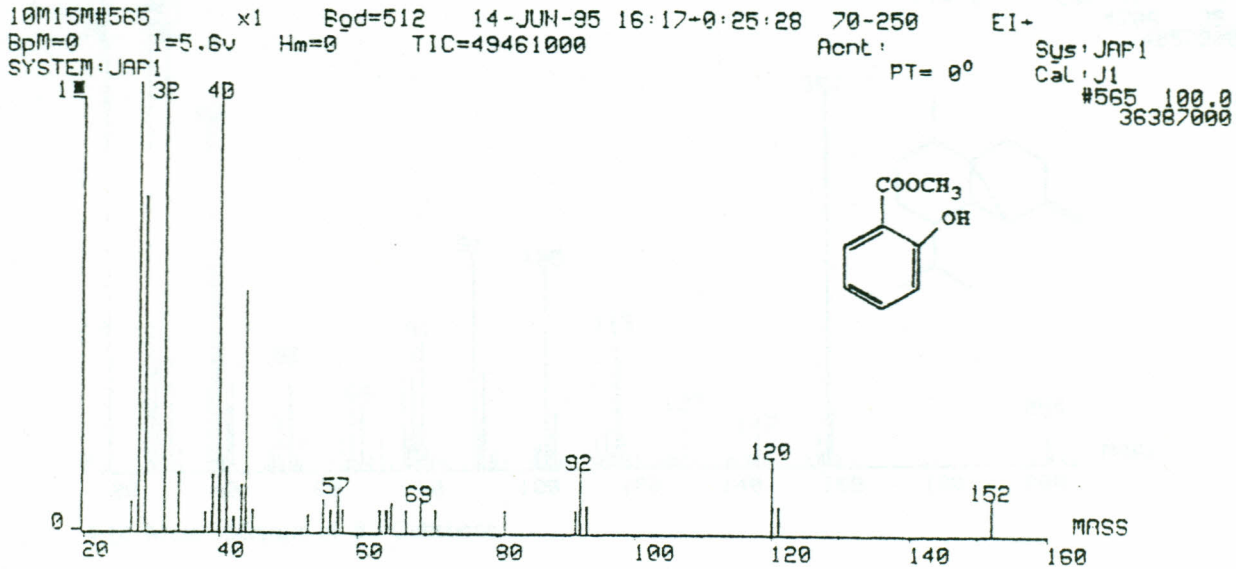
4.08 Mass Spectrum of (E) - β - Ocimene



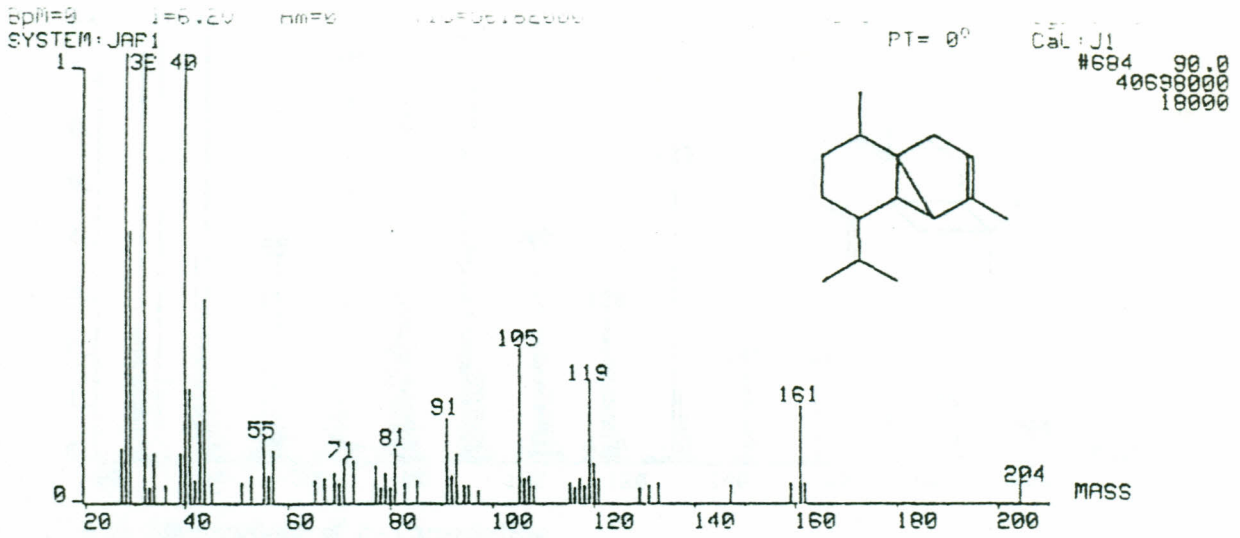
4.09 Mass Spectrum of Terpenolene



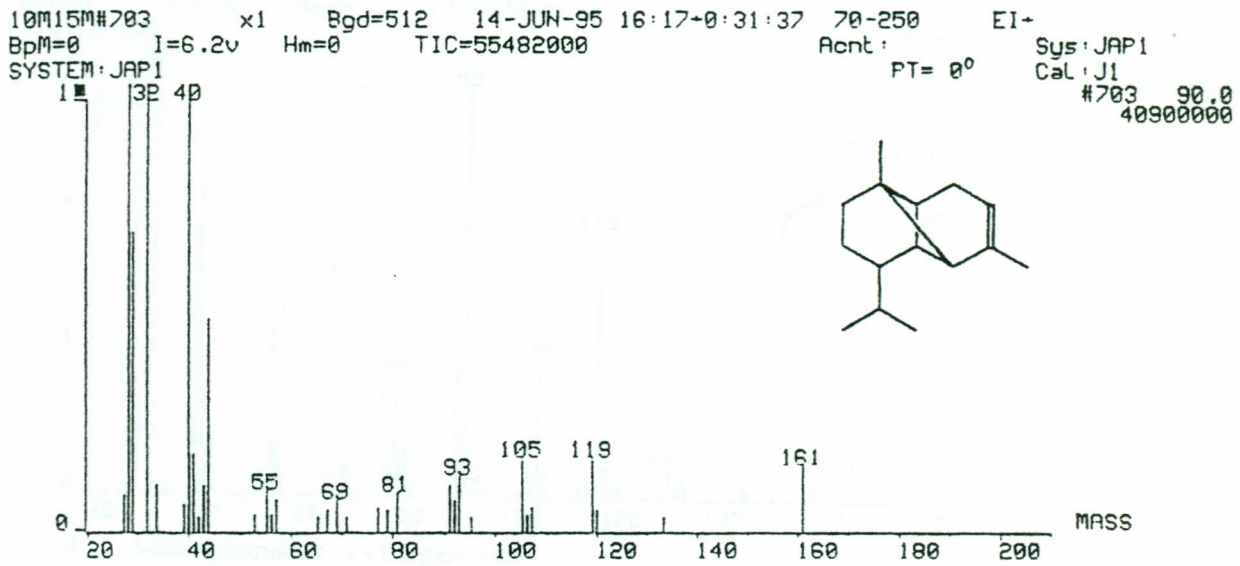
4.10 Mass Spectrum of 4,8-dimethylnona-1,3,7-triene



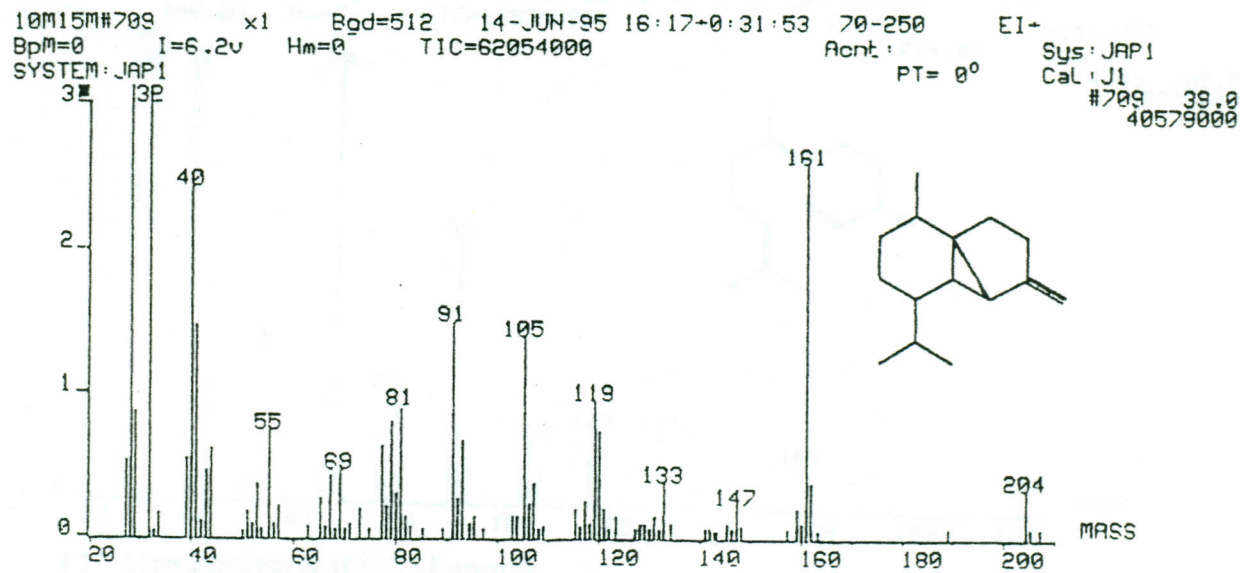
4.11 Mass Spectrum of Methyl Salicylate



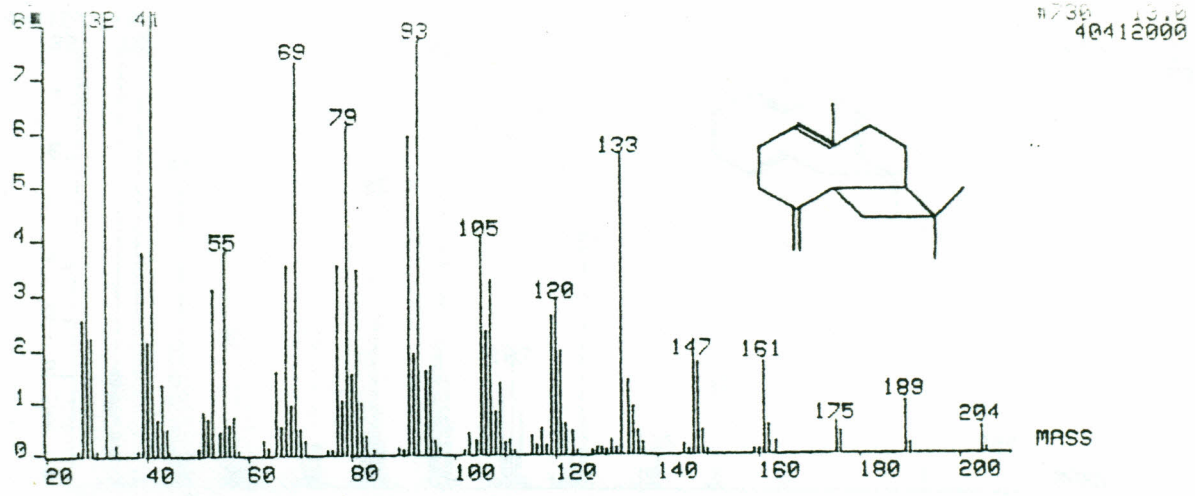
4.12 Mass Spectrum of α - Cubebene



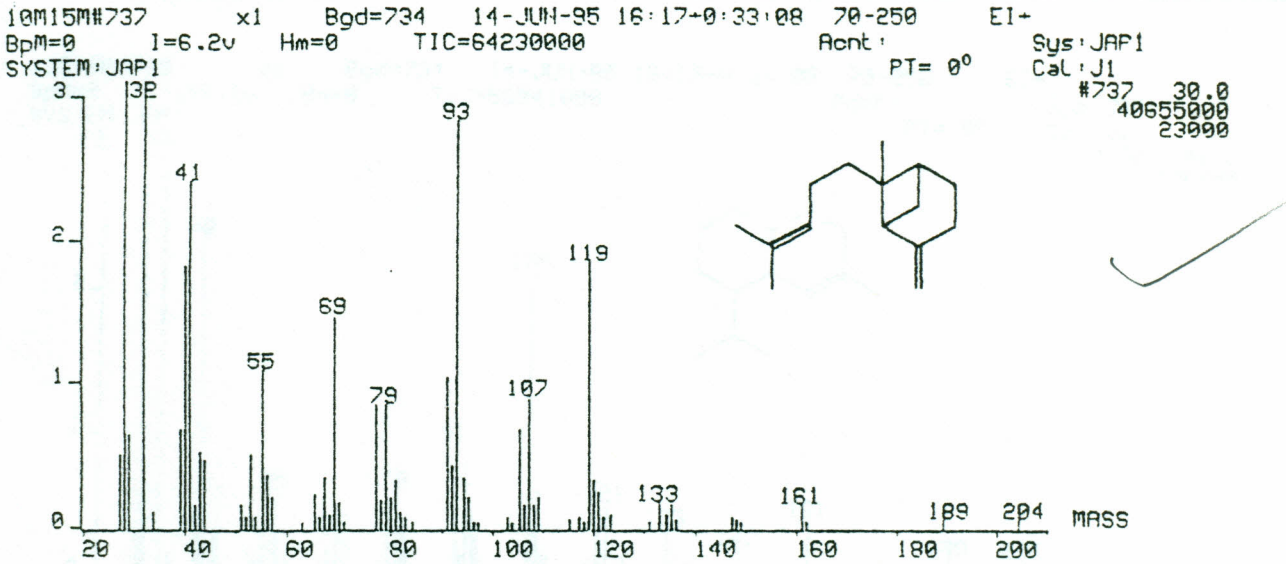
4.13 Mass Spectrum of α - Ylangene



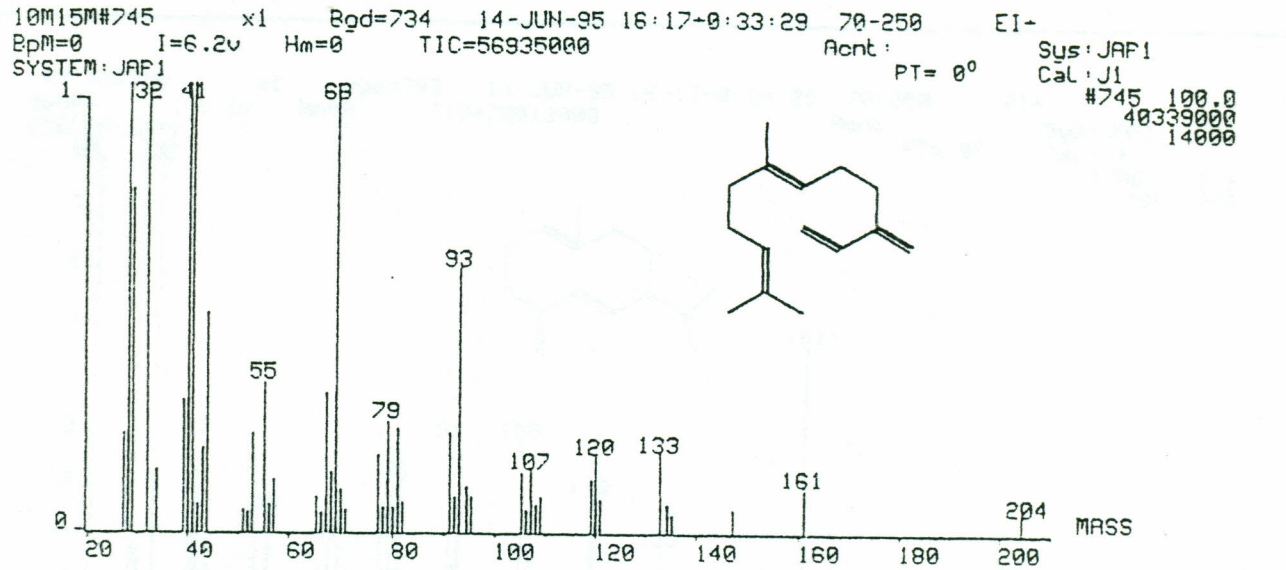
4.14 Mass Spectrum of β - Cubebene



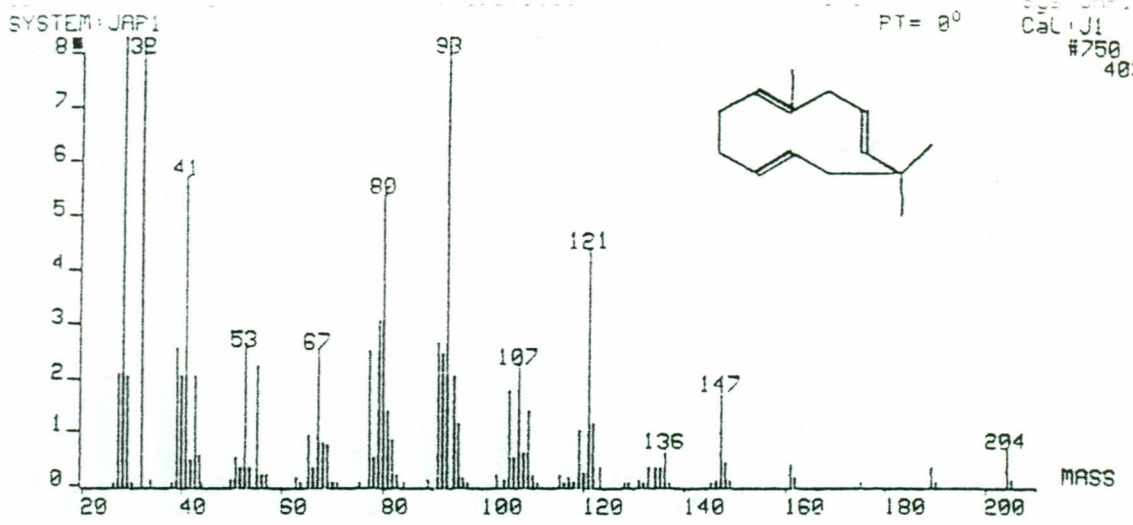
4.15 Mass Spectrum of β - Caryophyllene



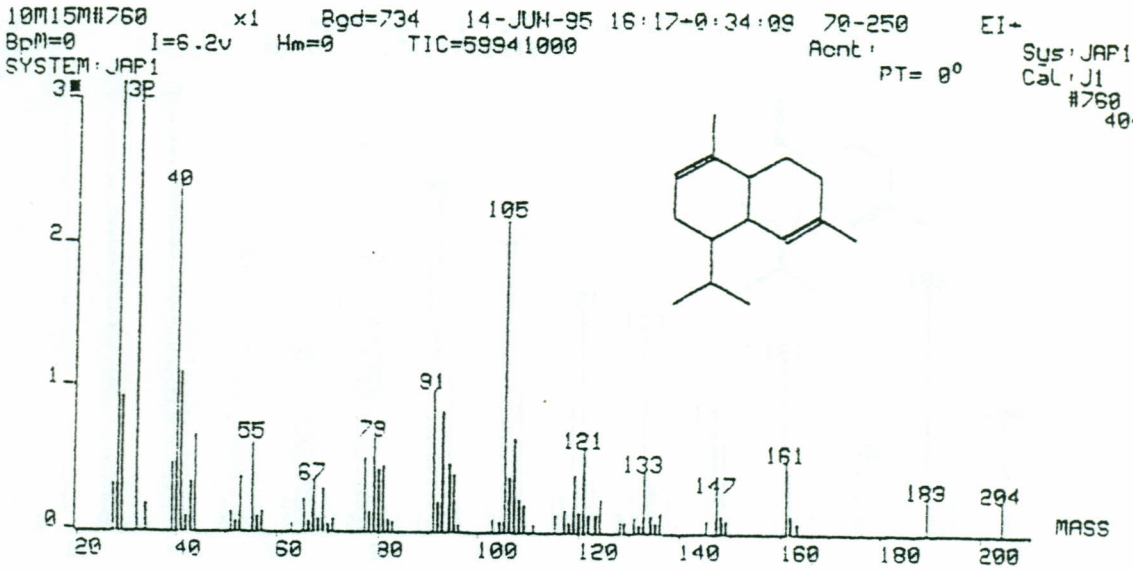
4.16 Mass Spectrum of α - Bargamotene



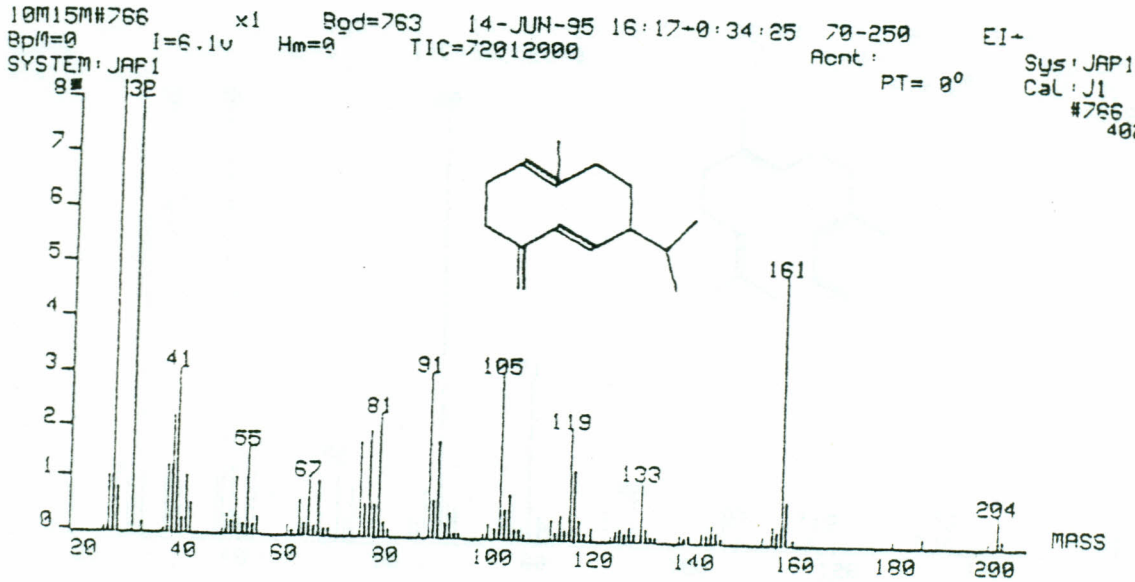
4.17 Mass Spectrum of (E) - β - Farnesene



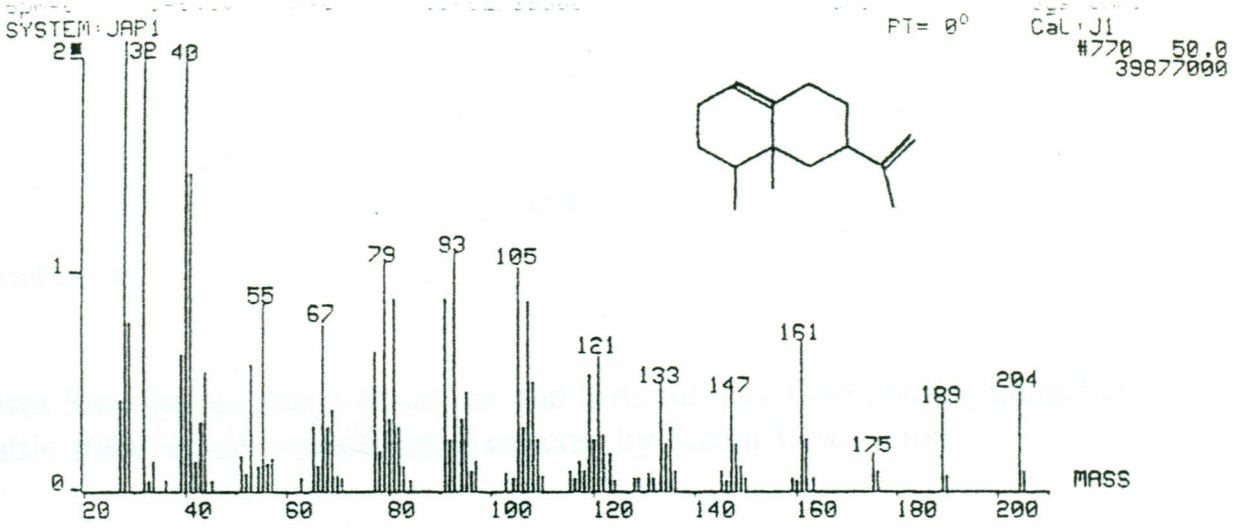
4.18 Mass Spectrum of α - Humulene



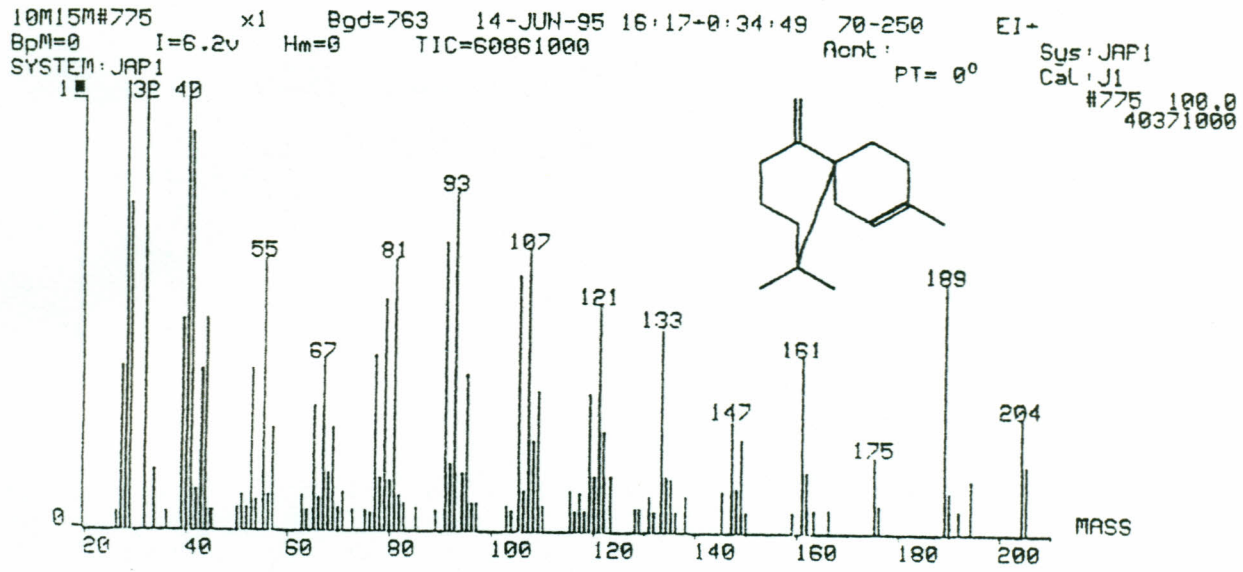
4.19 Mass Spectrum of α - Muurolene



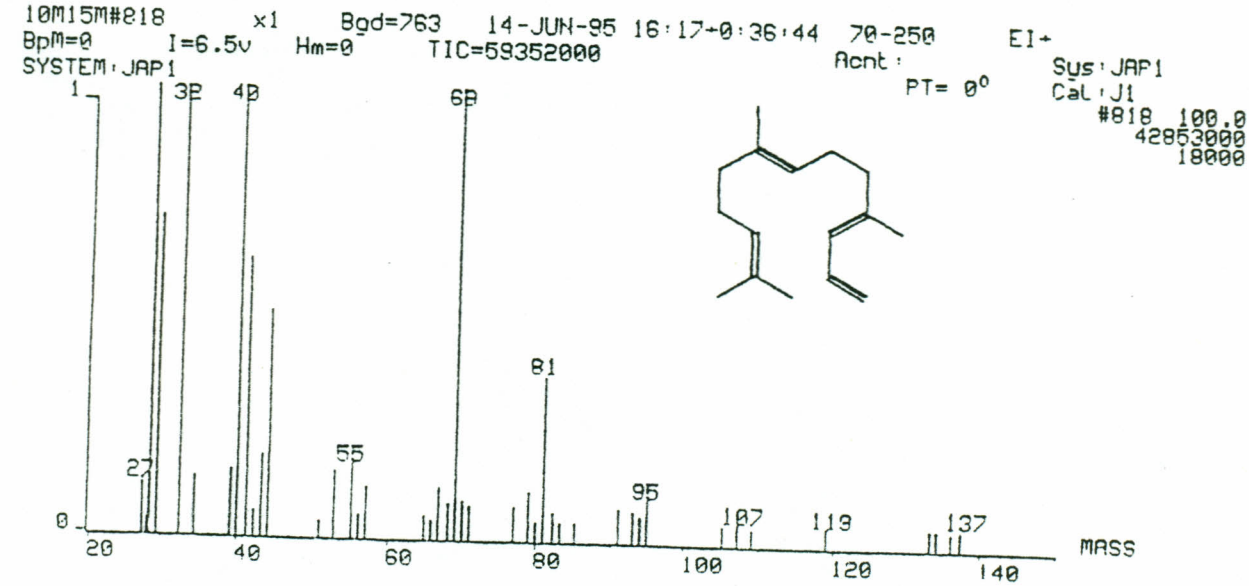
4. 20 Mass Spectrum of Germacrene



4.21 Mass Spectrum of Eremophene



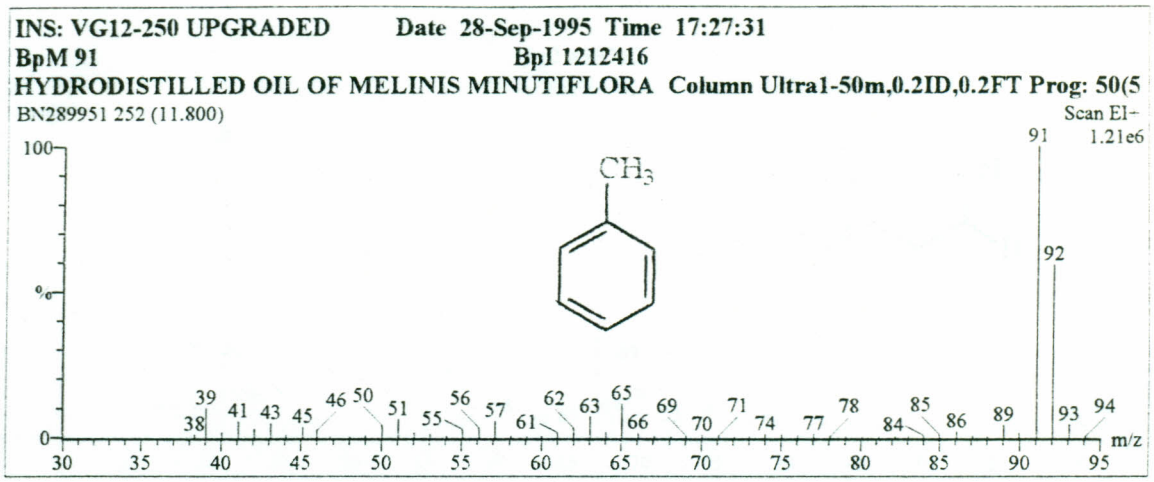
4.22 Mass Spectrum of β - Chamigrene



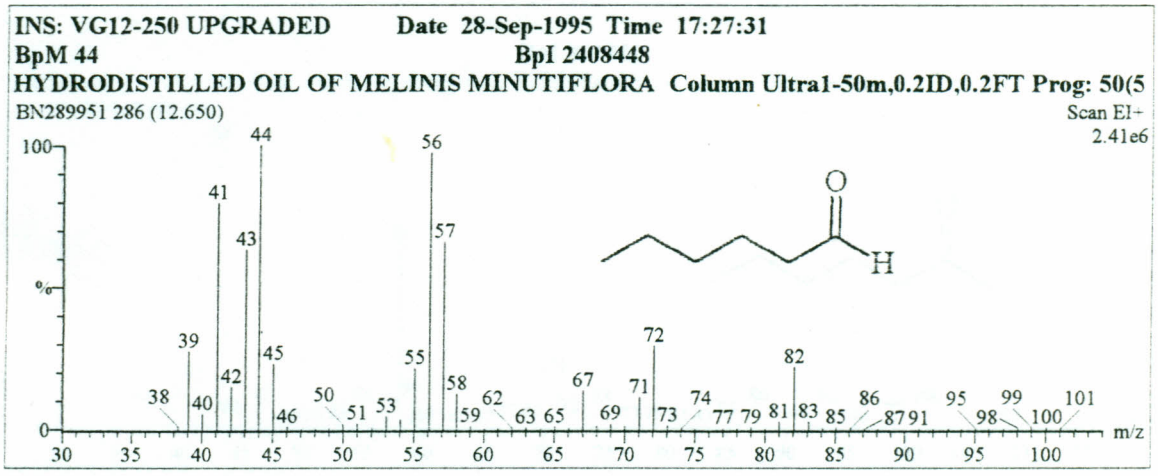
4.23 Mass Spectrum of 4,8,12-trimethyltrideca-1,3,7,11-tetraene

Appendix 5

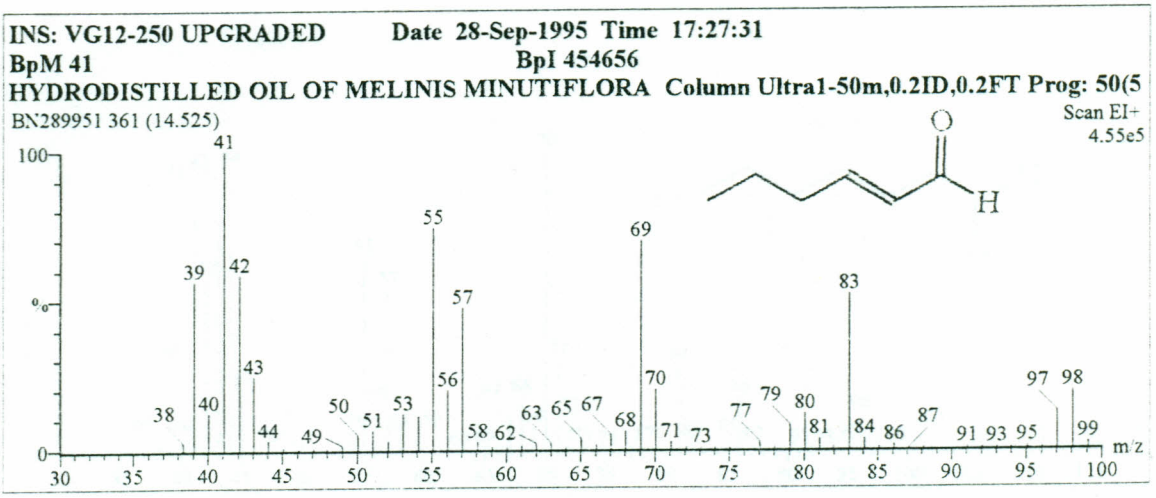
Figures Showing the Mass Spectrum and Structures of Compounds Identified in Volatile Blend of *M. minutiflora* Extracted by Steam Distillation.



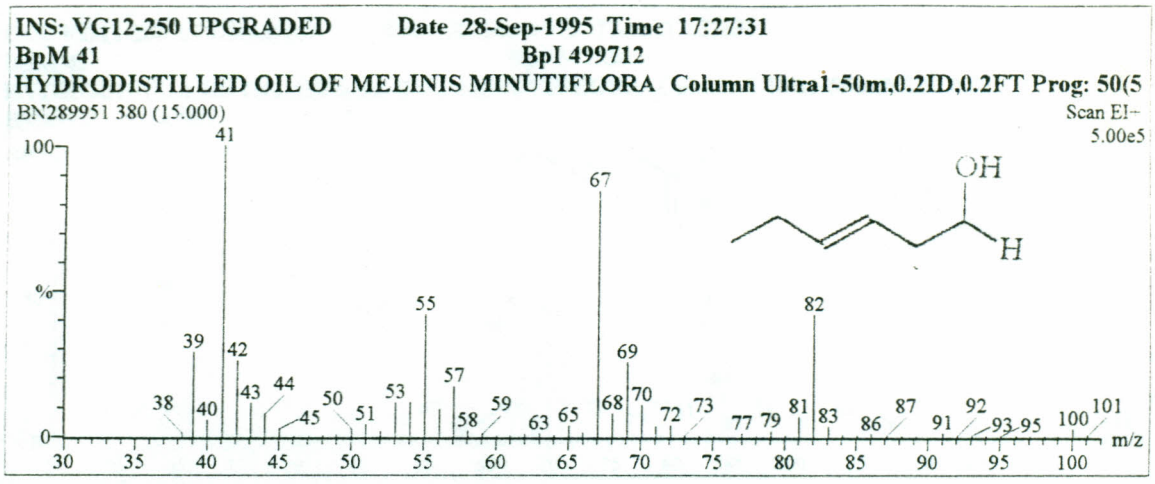
5.01 Mass Spectrum of Toluene



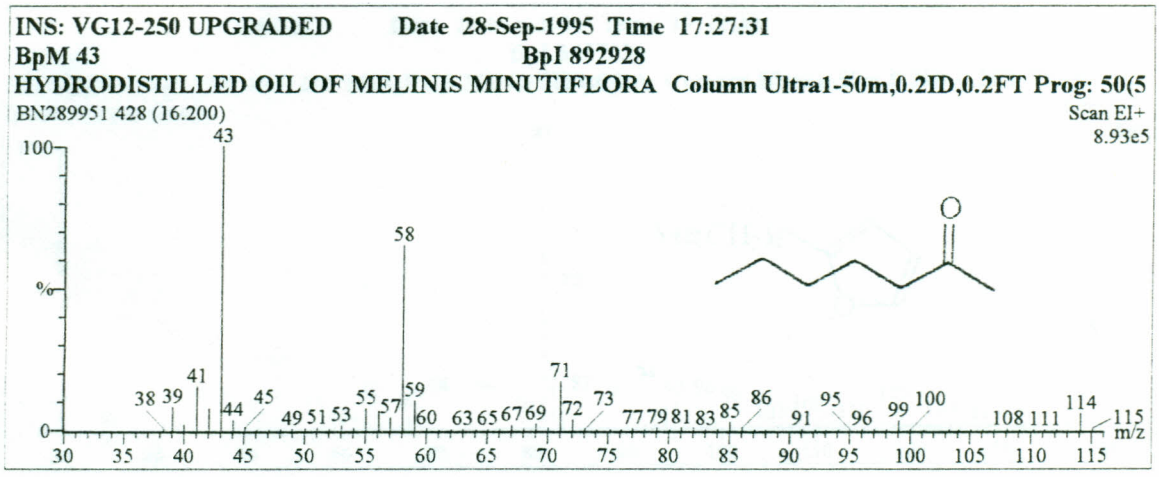
5.02 Mass Spectrum of Hexanal



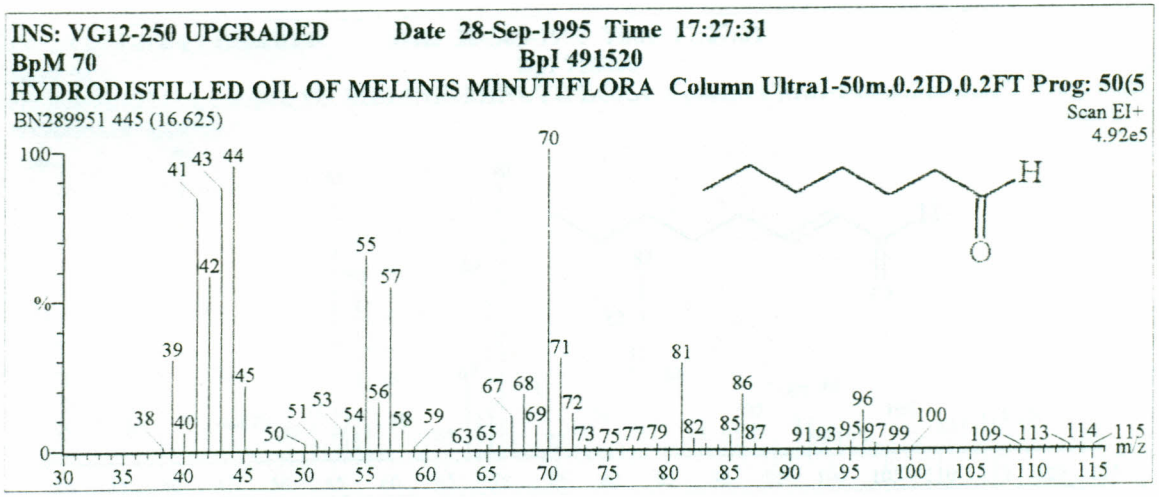
5.03 Mass Spectrum of (E) - 2 - Hexenal



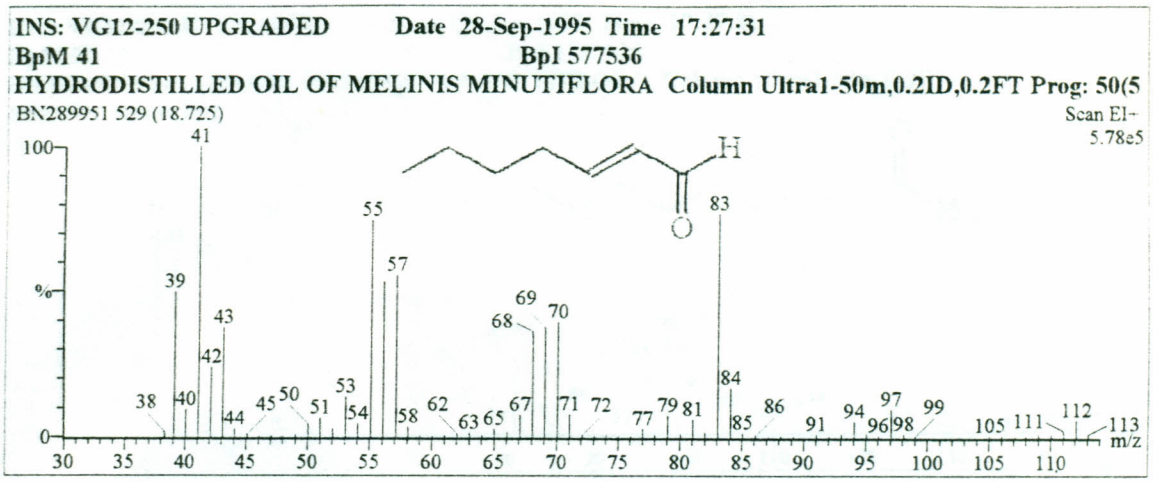
5.04 Mass Spectrum of 3-Hexen-1-ol



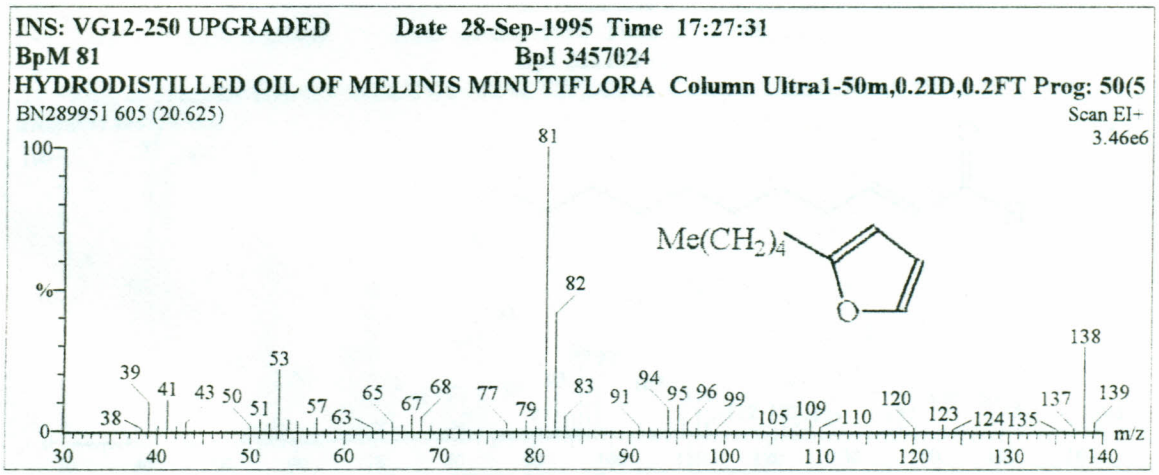
5.05 Mass Spectrum of 2-Heptanone



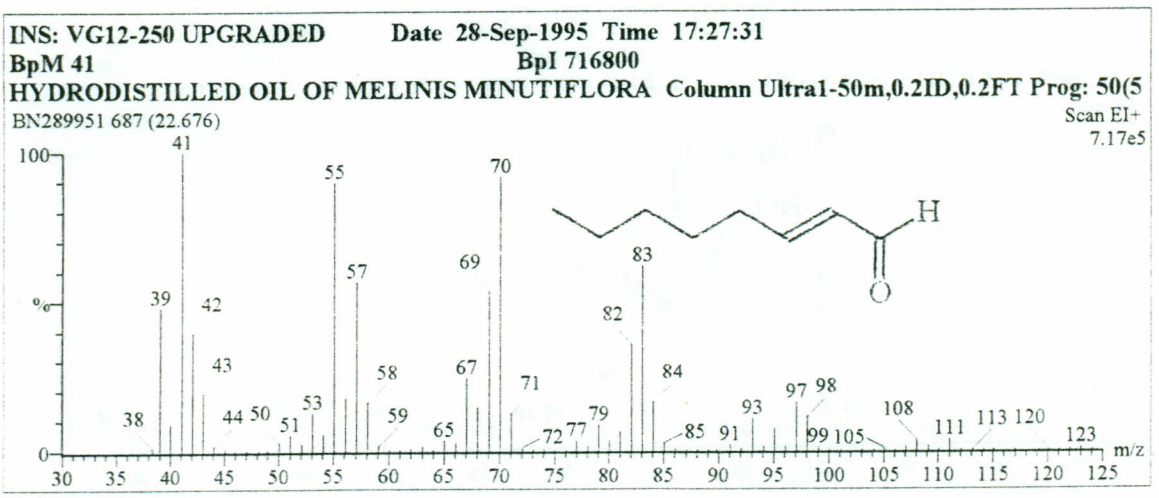
5.06 Mass Spectrum of Heptanal



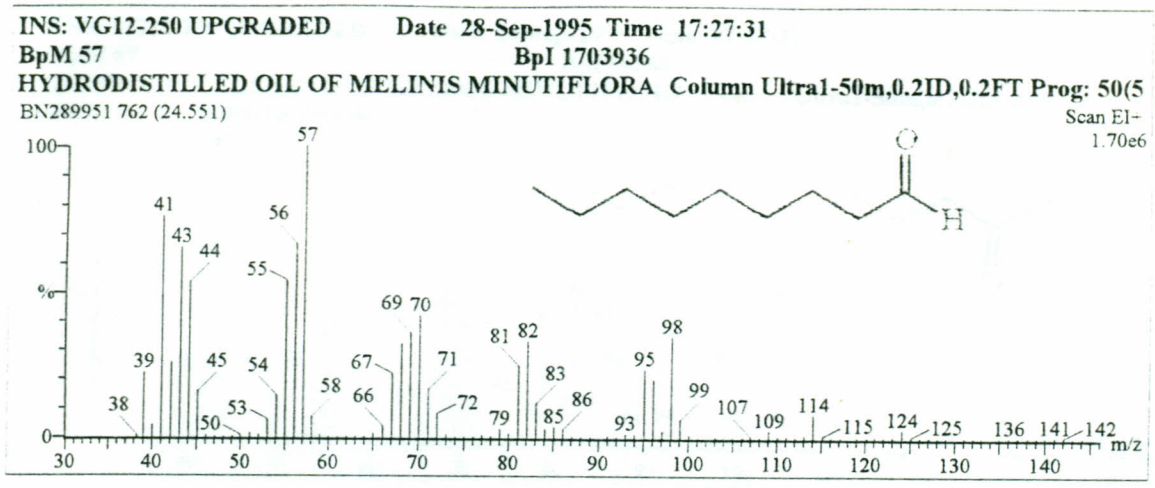
5.07 Mass Spectrum of 2-Heptenal



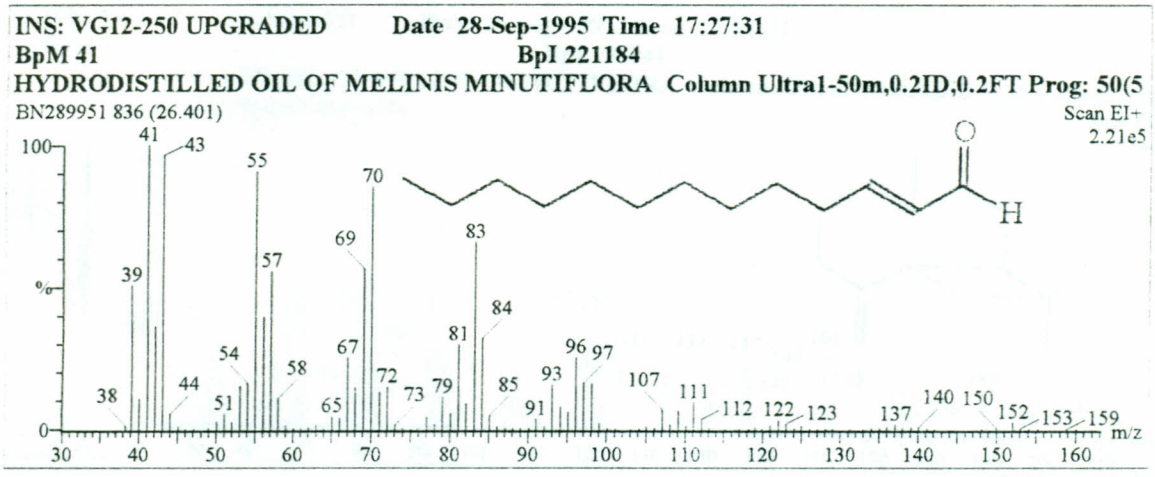
5.08 Mass Spectrum of 2-Pentylfuran



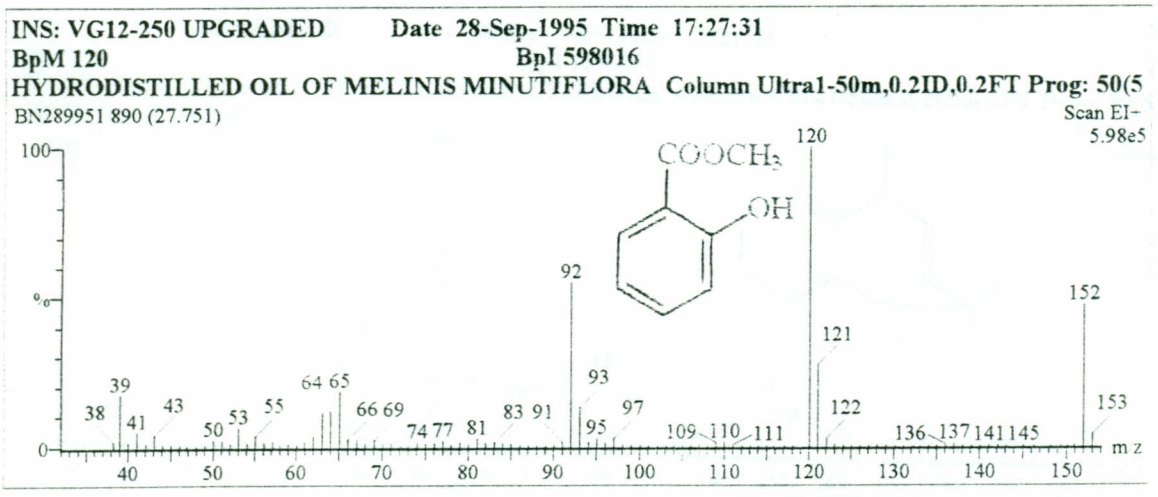
5.09 Mass Spectrum of (E)-2-Octenal



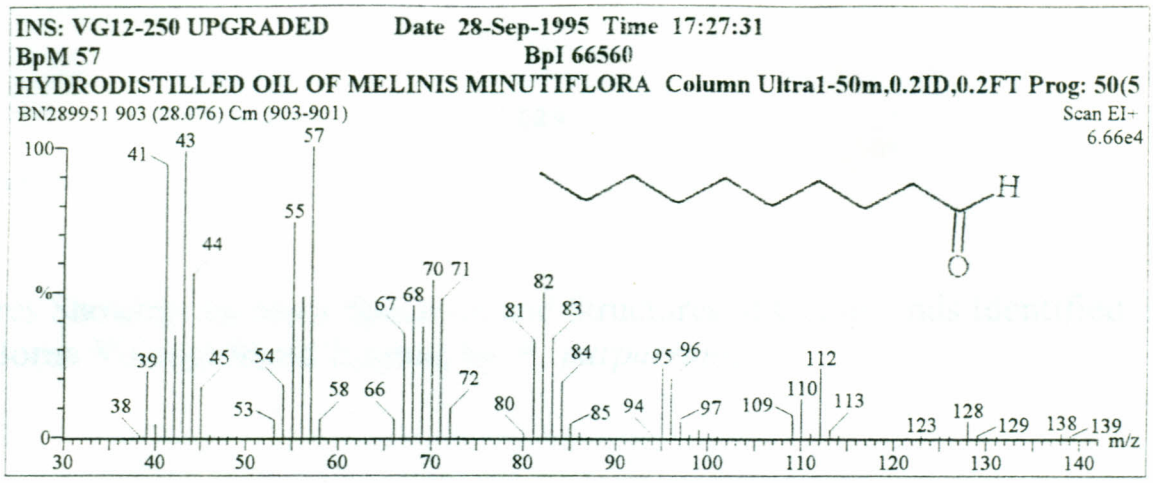
5.10 Mass spectrum of Nonanal



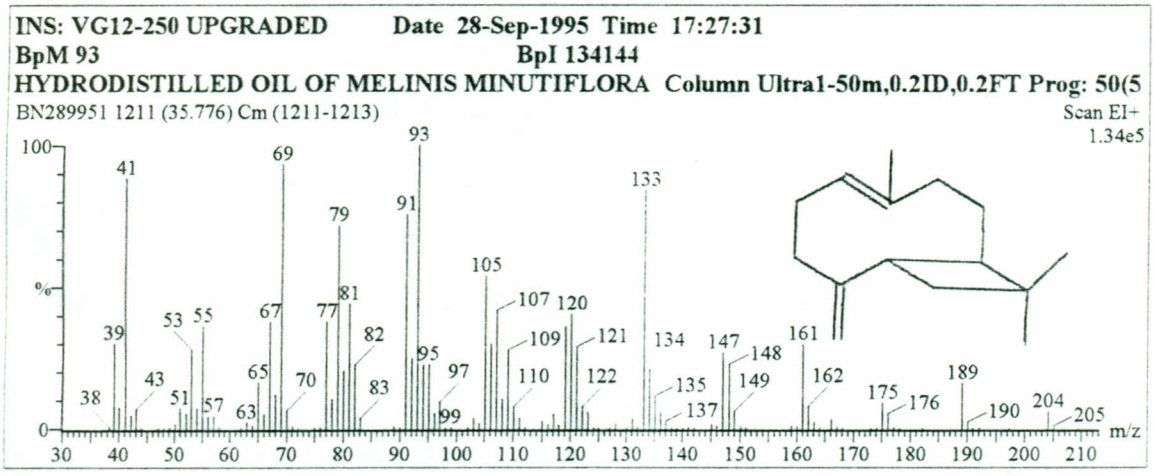
5.11 Mass of Spectrum (E)-2-Tridecenal



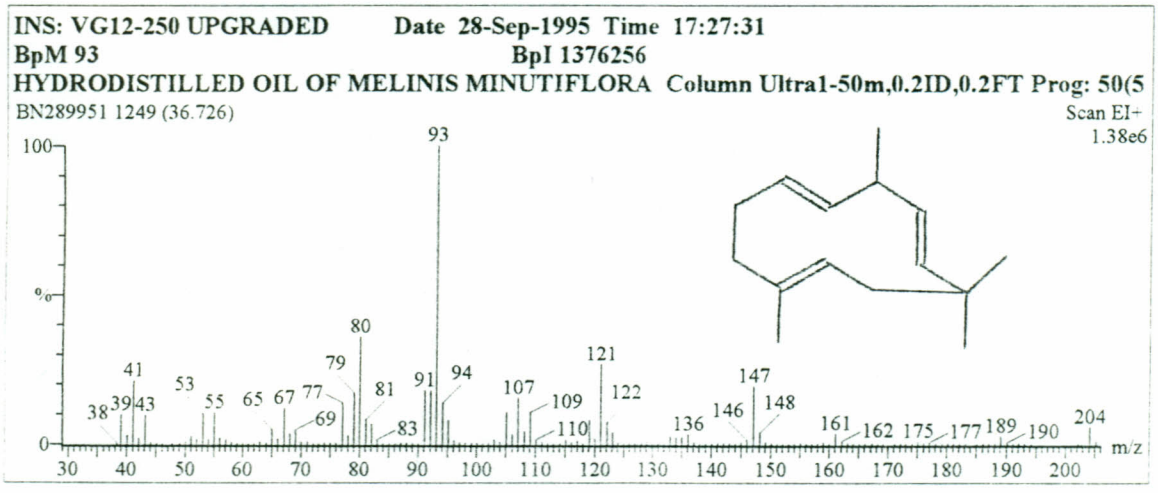
5.12 Mass Spectrum of Methyl Salicylate



5.13 Mass Spectrum of Decanal



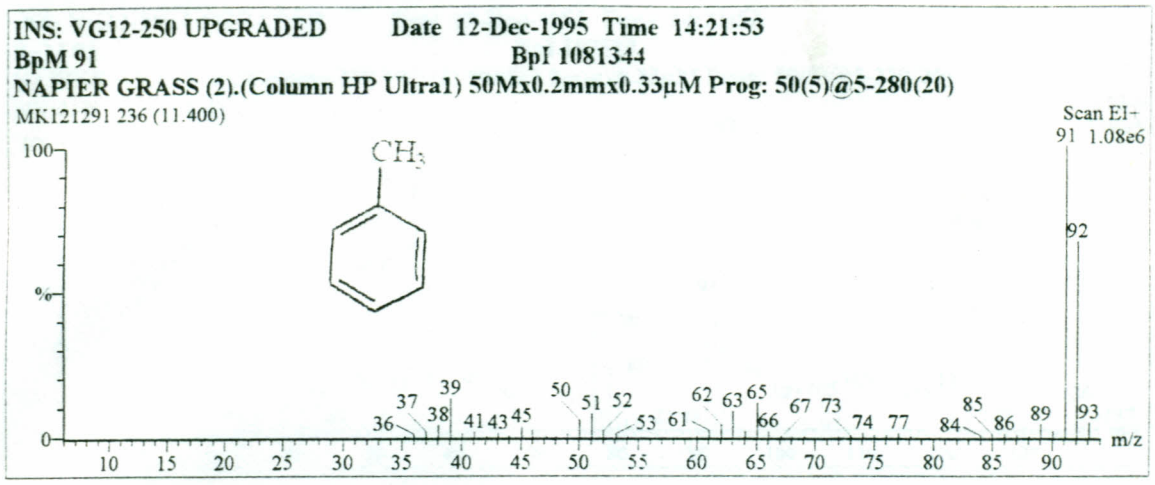
5.14 Mass Spectrum of β - Caryophyllene



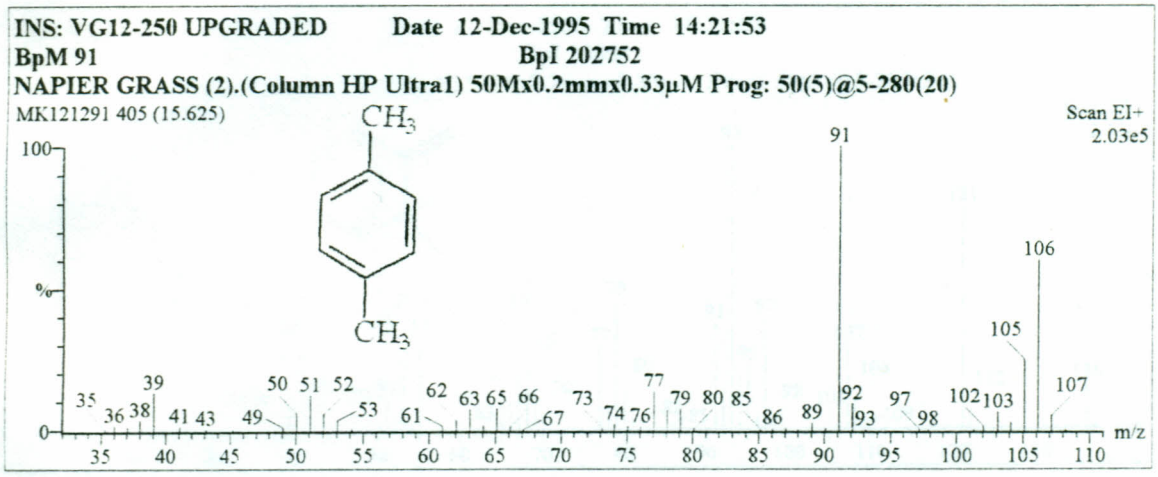
5.15 Mass Spectrum of Humulene

Appendix 6

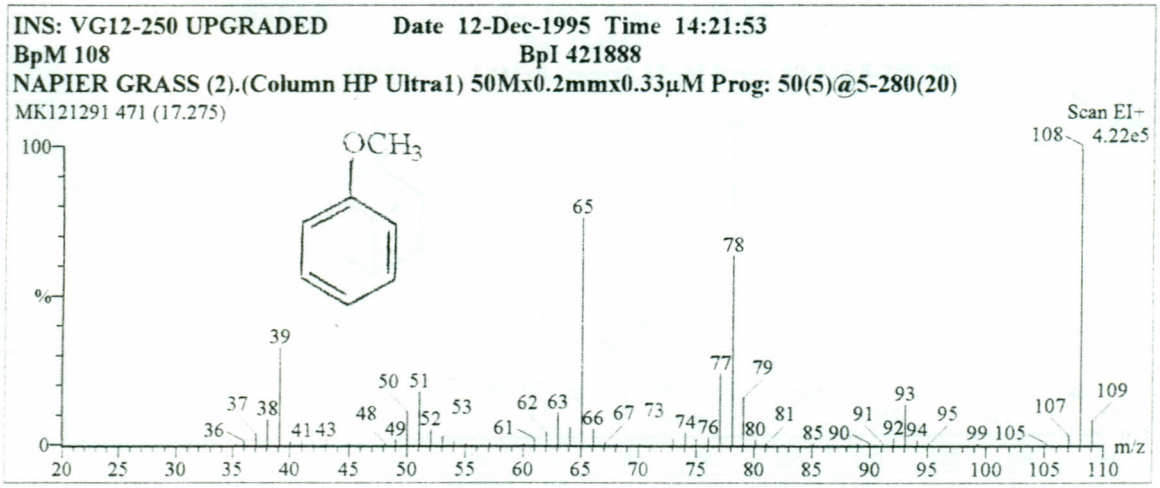
Figures Showing the Mass Spectrum and Structures of Compounds Identified in Air-Borne Volatile Blend Emitted by *P. purpureum*.



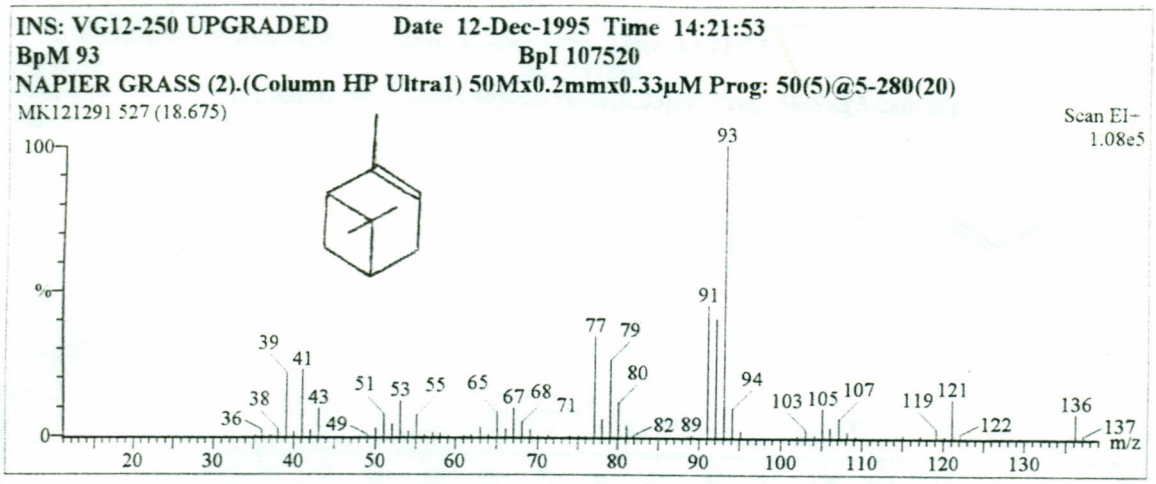
6.01 Mass Spectrum of Toluene



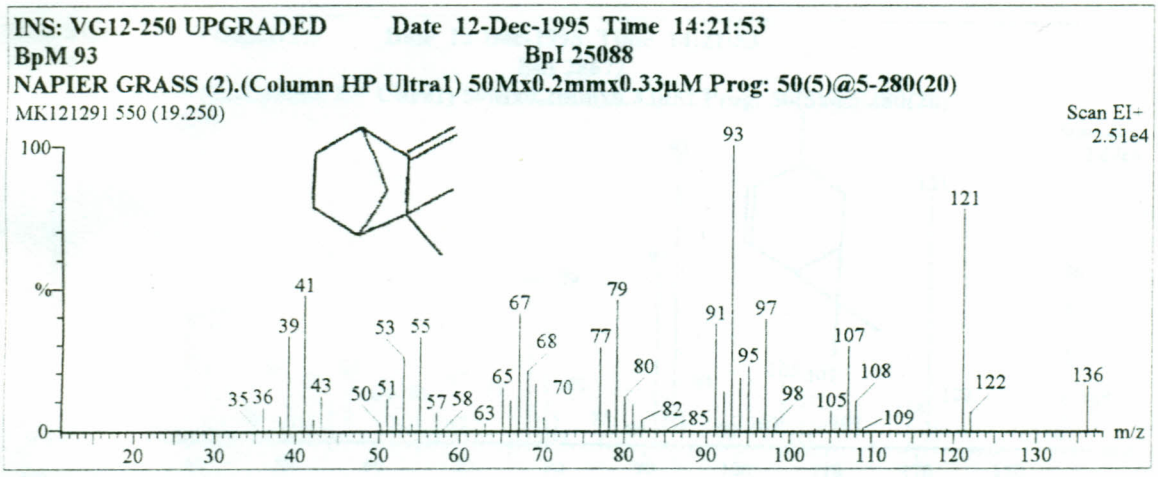
6.02 Mass Spectrum of P-Xylene



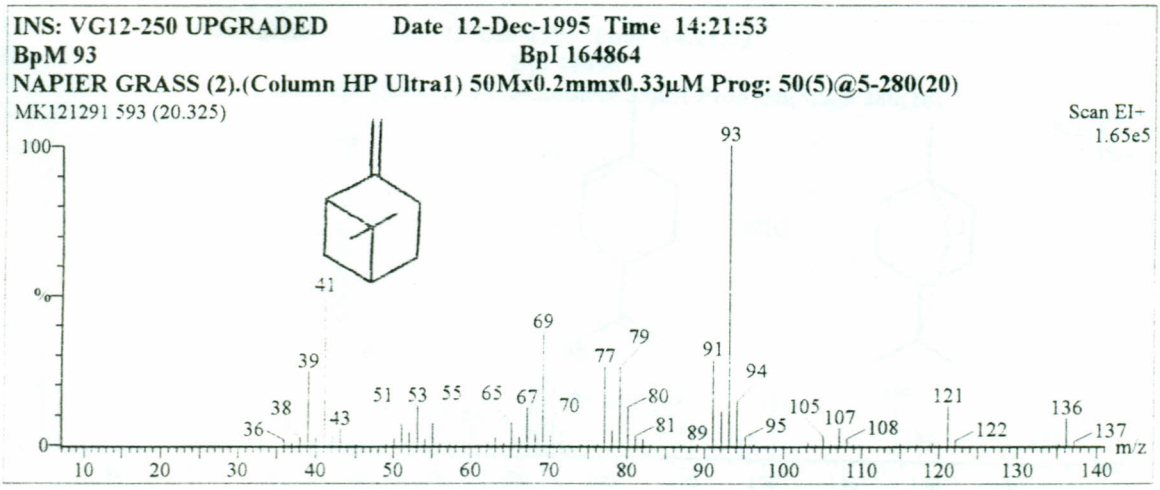
6.03 Mass Spectrum of Methoxybenzene



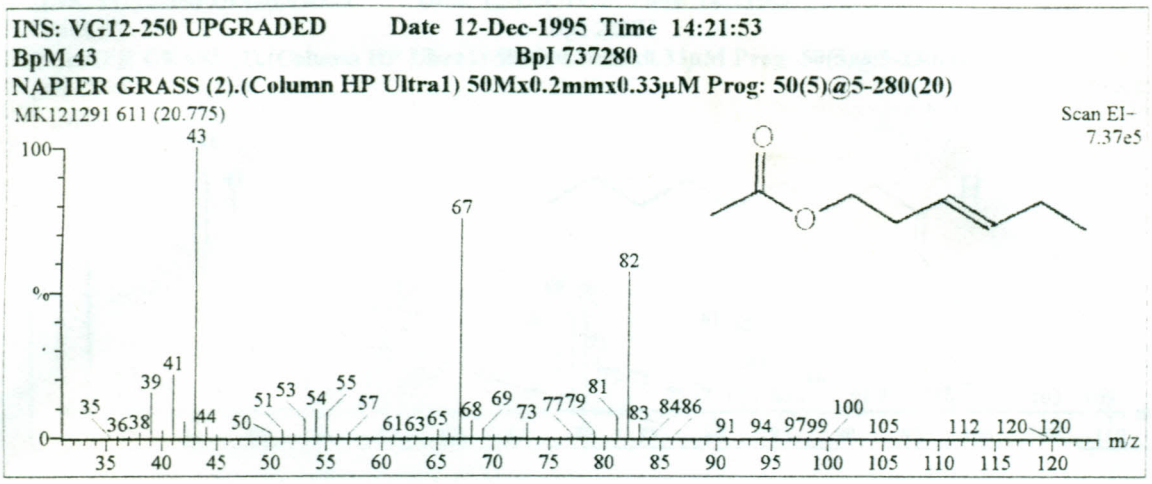
6.04 Mass Spectrum of α - Pinene



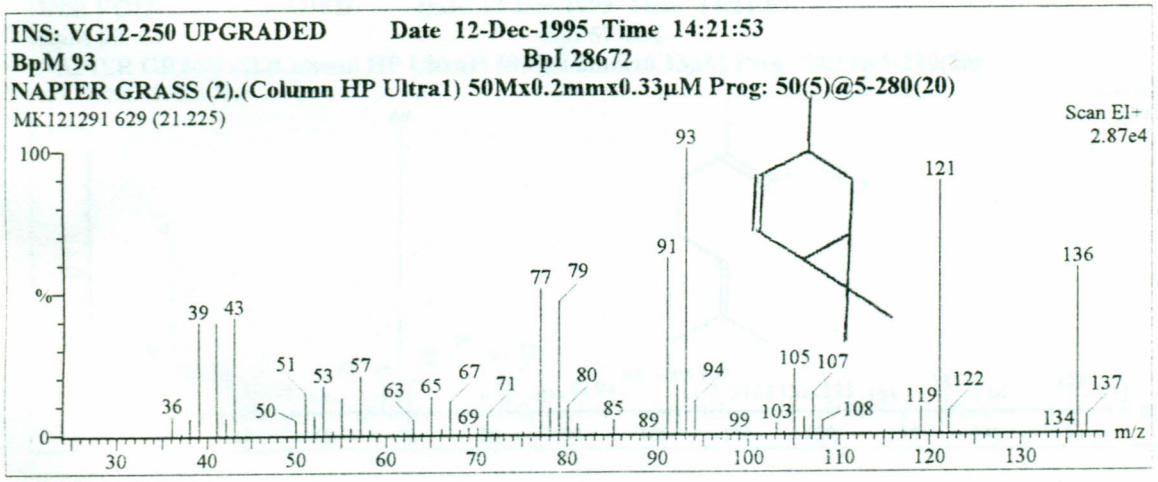
6.05 Mass Spectrum of Camphene



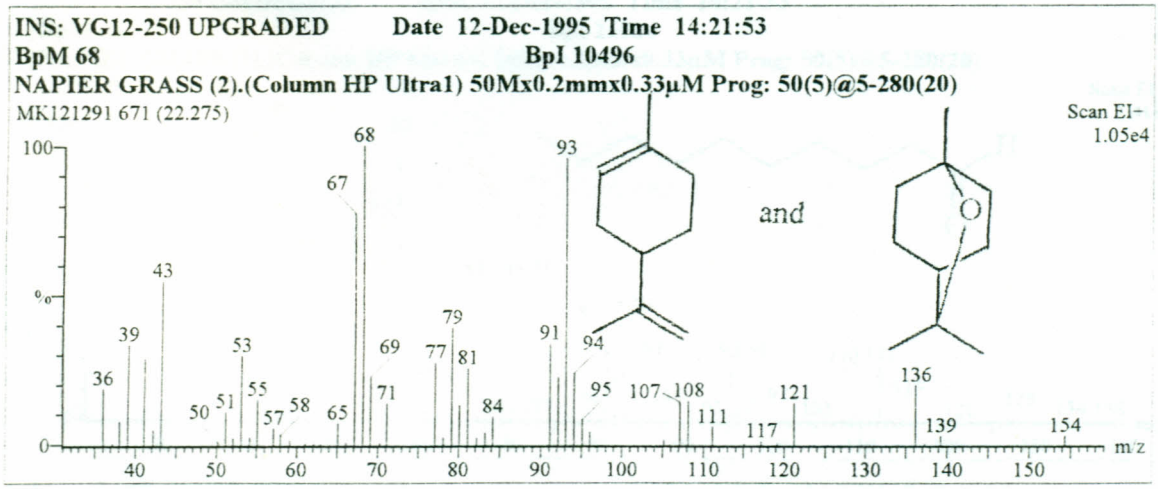
6.06 Mass Spectrum of β - Pinene



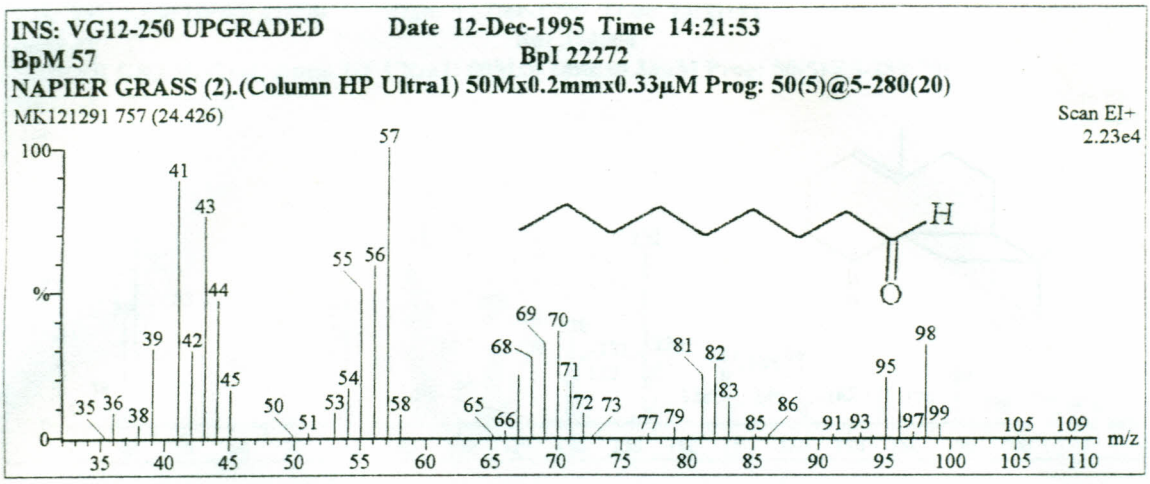
6.07 Mass Spectrum of (Z)-3-Hexenyl acetate



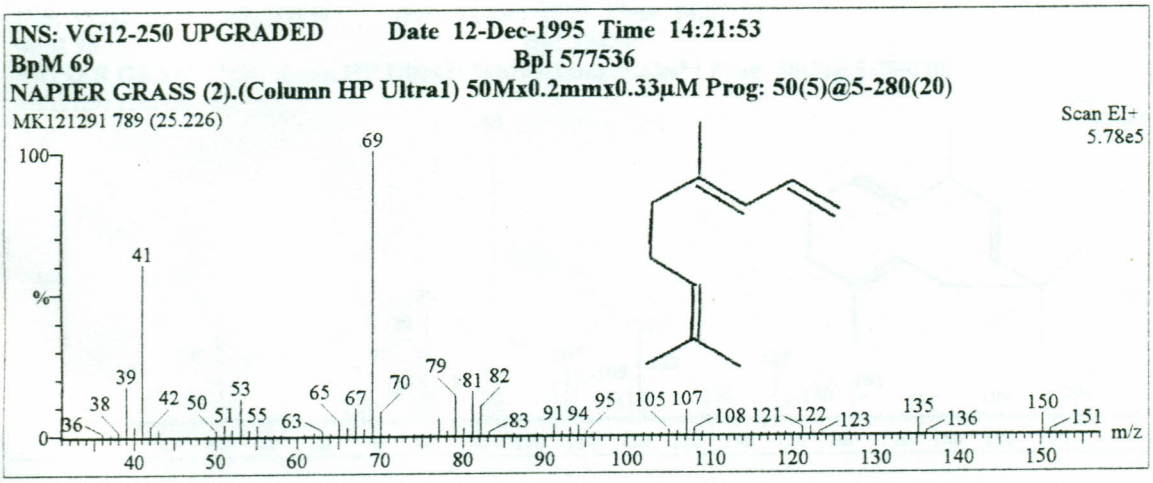
6.08 Mass Spectrum of (+)-2-Carene



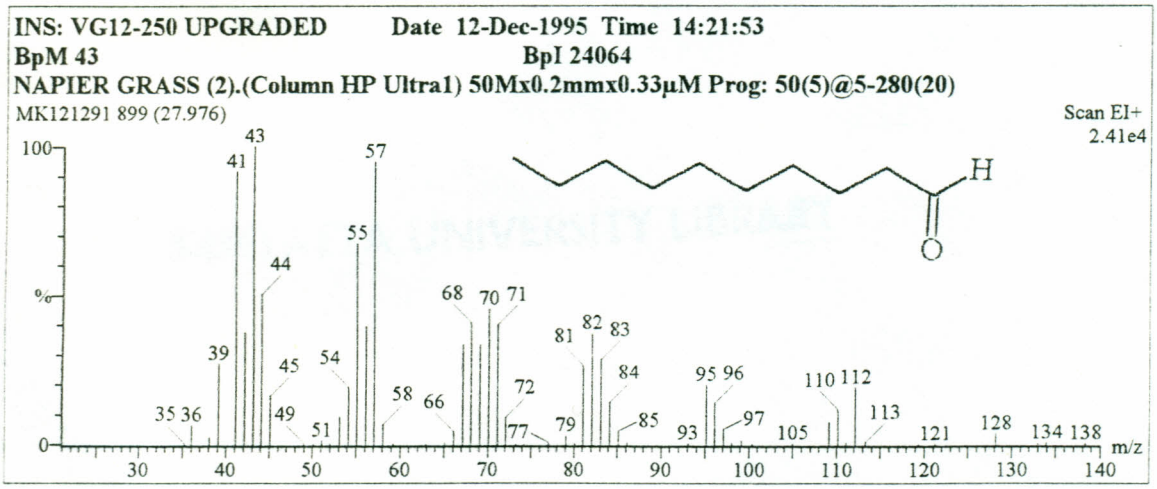
6.09 Mass Spectrum of Limonene and 1,8, Cineole



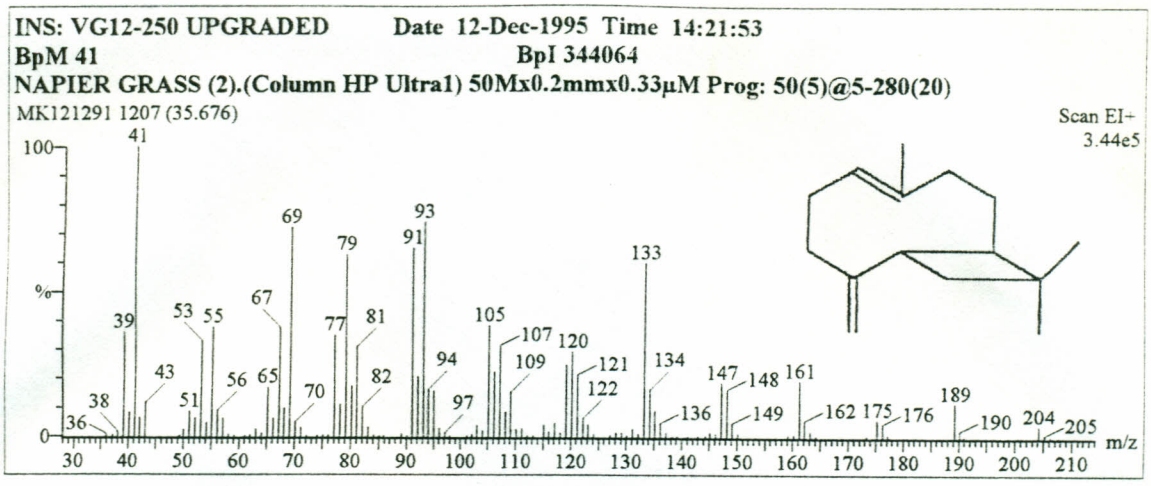
6.10 Mass Spectrum of Nonanal



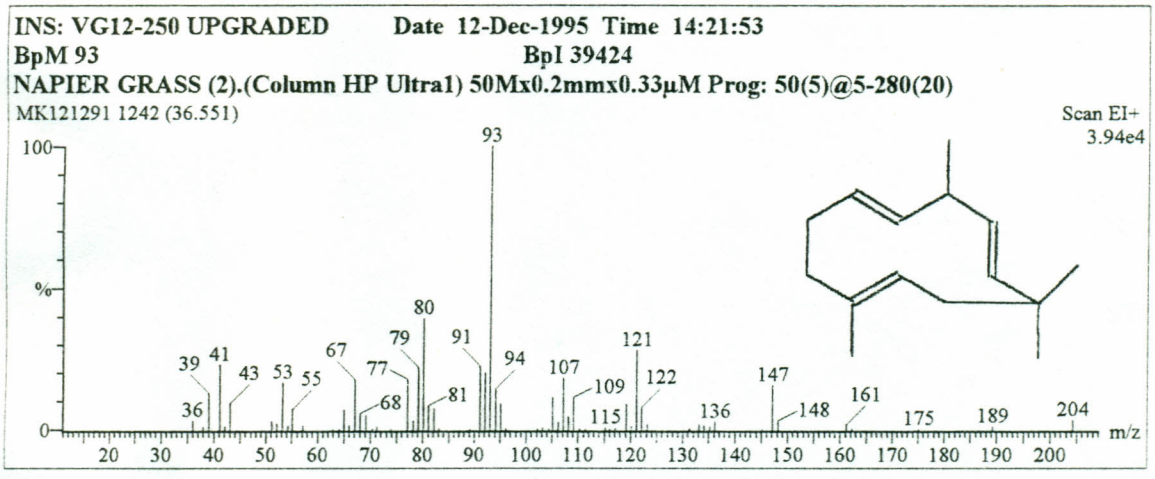
6.11 Mass Spectrum of 4,8-dimethylnona-1,3,7-triene



6.12 Mass Spectrum of Decanal



6.13 Mass Spectrum of β - Caryophyllene



6.14 Mass spectrum of Humulene