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GC-MS Analysis for Saturated Hydrocarbons From Jasmine (*Jasminum sambac L.*) Leaves Damaged by Jasmine Leaf Webworm, *Nusinoe geometralis* Guenee

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Abstract- The hexane extracts of *Jasminum sambac* L. leaves damaged by jasmine leaf webworm, *Nausinoe geometralis* (Guenee) and also healthy jasmine laeves were subjected to Gas Chromatography-Mass spectrometry (GC-MS) to determine the saturated hydrocarbons. The results revealed that both the healthy and damaged leaves had hydrocarbon compounds numbering 21 and 27 respectively. The variation in the hydrocarbon profile of healthy and damaged leaves might be related to the quality of semiochemicals these plants emit, which is important for the attraction of natural enemies in jasmine ecosystem so as to reduce further infestation by budworm. In the healthy jasmine leaves, the hydrocarbons, hentriacontane and tetracosane were detected at 25.89 mins exhibiting the largest peak area of 24610830 mm^2 octacosane, tetracosane, eicosane, nonacosane and heptacosane followed the order and emitted in enormous quantities. The natural enemy attractant, methyl salicylate and allyl isovalerate was also detected in healthy jasmine leaves. With regard to jasmine leaf web worm infested leaves, allyl isothiocyanate, allyl isovalerate, divinyl sulfide, oxalic acid potential candidates for attracting natural enemies were detected in along with an array of saturated hydrocarbons. Bis (2-ethylhexyl) phthalate, a six carbon compound was detected in maximum quantity detected with the largest peak area of 144295751 mm^2 at 38.984 mts in web worm damaged leaves.The quality and quanity of these semiochemicals emitted by the leaves might be the reason for attraction of natural enemies in the jasmine ecosystem there by further reducing the infestation of leaf webworm, as well as other pests. This feature can be exploited to enhance the efficacy of natural enemies in integrated management of jasmine pests.

Keywords: semiochemicals, synamones, saturated hydrocarbons, nausinoe geometralis, GC-MS, jasmine.

I. Introduction

Jasmine (*Jasminum sambac* L.) known in persian as yasmin *ie*. 'Gift of God' is one of the oldest fragrant flowers of India. It is traditionally as well as commercially cultivated for its sweet scented flowers. Globally, jasmine is celebrated in many countries as their national flower and utilized as decoration for ceremonies and rituals as an important part of cultural heritage. Flowers and un opened buds are used for making garlands, bouquets, in religious and ceremonial functions, perfumed hair oils, attars, soaps, wine and drinks (Thakur *et al.,* 2014). It is used for production of jasmine concrete, which is the base in cosmetic and perfumery industries and hence the phrase "no perfume without jasmine". The dried flowers are used for making the famous 'jasmine tea', scented with aroma from jasmine blossoms, which is a popular drink in South East Asian countries. An infusion of jasmine tea is beneficial in treating fever, relieving stress and anxiety. The flowers and other parts of the plant like leaf, stem, bark and root are also used for medicinal purposes (Bose and Yadav, 1989).

Flower or essential oil, jasmine find a place in useful medicines as an aphrodisiac, sedative, antiseptic, antidepressant, antispasmodic, and analgesic relieving pains and relaxing the nervous system (Ranadas *et al.,* 1985; Kanniamal and Divya, 2016). As the demand for high grade perfumes has greatly increased in recent times, there is tremendous scope for the production of concretes and oils from jasmine flowers. Also, the need for the mesmerizing jasmine flowers for diverse necessities like religious ceremonies, official and home decorations, weddings, funerals etc. is ever rising. The countries growing jasmine on a commercial scale are France, India, Italy, Moracco, Algeria, North Africa, Spain, Egypt and Israel. The area and production of total flowers in India were increasing impressively over the years. The world production of jasmine concrete is around 20 tonnes per annum, out of which India is producing and exporting about 2 tonnes (Ray *et al.,* 2014). The largest area under jasmine cultivation is in Tamil Nadu and Karnataka from where it is distributed to metropolitan cities (Nimisha and Razia, 2014). Since recent past, this commercial jasmine is affected by a number of pests like jasmine budworm (*Hendecasis duplifascialis* Hampson), the galleryworm (*Elasmopalpus jasminophagus* Hampson), the leaf webworm (*Nausinea geometralis* Guenee), the leaf roller (*Glyphodes unionalis* Hubner), the hawk moth (*Achreontia styx* Westwood), the blossom midge (*Contarinia maculipennis* Felt) and the two spotted mite

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(*Tetranychus urticae* Koch) posing serious threat to jasmine cultivation.

The existing recommendation of synthetic chemicals is only a short term solution, as the pest population increases after few months, later disproportionately requiring repeated application with high dosages, which finally became hazardous and uneconomical, leading to the endangerment of ecosystem by reducing the diversity of natural enemies. In addition, direct toxicity to human beings, animals and environment is of serious concern. It is pertinent that a change in the insect pest management strategy may form a meaningful solution to avoid the ill-effects caused by the synthetic chemical insecticides especially as environmental contaminants. Therefore, in search of safer alternatives, attention has been focused on exploration of semiochemical mediated approaches through host plant defense mechanisms. An approach of using semiochemicals in pest management is to exploit ways to chemically augment, conserve or enhance the efficacy of natural enemies in a crop ecosystem. Use of these biochemicals especially, the synomones released by host plants is of significance in biological control. Hydrocarbons present in host plants were found to act as synomones for natural enemies in different crop ecosystems. In particular, synomones play a major role by guiding the natural enemies to the potential host or prey on the plant (Hilker and Meiners, 2006). Such clues may be utilized to stimulate foraging and host selection behavior of entomophages thereby increasing their effectiveness for IPM (Ahmad *et al.,* 2004). Therefore in the present study, the hexane leaf extracts of jasmine damaged by leaf webworm and healthy leaves were analyzed through GC-MS to determine the saturated hydrocarbon profiles in them.

II. Materials and Methods

Jasmine plants were raised in an area of 20 cents at the Botanical garden premises, Tamil Nadu Agricultural University, Coimbatore during January-May 2016. All the recommended agronomic practices were followed. Healthy and leaf webworm damaged leaves were collected from the field during the period of heavy infestation by leaf webworm.

The saturated hydrocarbons were extracted from the jasmine leaves damaged by leaf webworm and healthy leaves using HPLC grade hexane as follows. The healthy and leaf webworm defoliated leaves of jasmine were plucked carefully and used for extraction.

Ten gram of leaves was immersed overnight in 100 ml of HPLC grade hexane. The filtrate was then passed through silica gel (60-120 mesh) column. The hexane solvent was allowed to evaporate and the left over residue was collected by rinsing the container with a small quantity of HPLC grade hexane (Merck) and stored in separate vials for GC-MS Analysis. Gas chromatography combined with mass spectroscopy is a preferable methodology for routine analysis of compounds.

Hexane based leaf extracts were analysed on GC-MS (Agilent Technologies 7890B GC System with 5977B MSD) mass selective detector (70eV) equipped with a 10:1 split injector. The gas chromatography is equipped with 30m fused Oven temperature programming: 60ºC (1 min hold) to 100ºC at 5ºC/min rate (1 min hold), then to 220ºC at 10º C/min rate (5 min hold) and then to 240ºC at 50ºC/min rate (8 min hold). Injector temperature was set at 275ºC. One microlitre of the extract was injected using auto sampler into the gas chromatography-mass spectroscopy (GC-MS) System for analysis injections was done in split 10:1 mode. Agilent data analysis software was used for the analysis of compounds in the extracts. Injected sample was separated into various constituents with different retention time which were detected by mass spectrophotometer. The compounds of interest were identified using standard NIST mass spectral (NIST MS 2) library. The chromatogram, a plot of intensity against retention time was recorded by the software attached to it. From the graph, the compounds were identified by comparing the data with the existing software libraries.

III. Results and Discussion

Induction of plant defense in response to herbivore involves the emission of volatile compounds called synamones that act as attractants for natural enemies of herbivores. Synamones produced by plants are reported to be very significant in eliciting host-seeking response in many natural enemies. Synamones attract predators and parasitoids, which elucidate the tritrophic interaction in a crop ecosystem (Ferry *et al.,* 2004).

Gas Chromatography mass - spectroscopy analysis of synamone extracts of healthy leaves showed the presence of 21 hydrocarbons *viz.,* cyclohexanol, 2-butanol, 2-methyl butanoic anhydride, allyl isovalerate, azetidine 1, 2 dimethyl, cyclo hexane, phenyl ethyl alcohol, methyl salicylate, heneicosane, nona decane 9 methyl, eicosane, octacosane, tetracosane, hentriacontane, eicosane, nona decane 9-methyl, pentacosane, octadecane, heptacosane, hexacosane and nonacosane (Fig 1).

But the leaf web worm damaged leaves showed the presence of 27 hydrocarbons (Table 1) *viz.,* allyl isothiocyanate, divinyl sulfide, dodecane 5-methyl, 2-amino ethanol, allyl isovalerate, azetidine, oxalic acid, dodecane, 2-penetene, dodecyl octyl ether, acetophenone, cyclohexane, cycloheptanol, 4-amino-5-(4-acetyl phenylazo) benzo furazan, iso butyl angelate, cyclo hexa siloxane,trifluoro octoxy hexadecane, butyl angelate, 2-butenoic acid, cyclohepta siloxane, benzene, benzene butanoic acid, diethylmalonic acid, cyclotetradecane, decyl trifluoroacetate, tetracosane, bis (2-ethylhexyl) phthalate, di - n- octyl phthalate and pthalic acid, di (2-propyl pentyl ester) (Fig 2).

In the healthy jasmine leaves, the hydrocarbons, hentriacontane and tetracosane (Fig 3, 4) were detected at 25.89 mins exhibiting the largest peak area of 24610830 mm^2 followed by eicosane displaying a peak area of 15511968 mm^2 in 31.462 mins. The compounds, octacosane, tetracosane and eicosane (Fig 5,6) were detected at 25.713 mins, exhibiting the third largest peak area of 13642707 mm^2. Hydrocarbons eicosane, nonacosane and heptacosane (Fig 7) were detected at 33.519 mins displaying a peak area of 9932421 mm^2.

Apart from several saturated hydrocarbons, natural enemy attractants, methyl salicylate was detected in healthy jasmine leaves at 11.92 in a peak area of 981341 mm^2 .Volatile methyl esters are common constituents of plant volatiles with important function in plant defense. Methyl salicylate, (Fig 8) a herbivore-induced volatile has been shown to attract natural enemies and affect herbivore behavior. But methyl salicylate is present in healthy jasmine leaves itself in a meager quantity.

Methyl salicylate lures examined for its effectiveness against organic soybean aphids, *Aphis glycines* Matsumura showed reduced population of aphids, with significantly greater number of syrphid flies (Diptera : Syrphidae) and green lace wings (Neuroptera: Chrysopidae) (Mallinger *et al.,* 2011). The results are in line with Du *et al.,* (1998) ; Kesseler and Baldwin, (2001) reporting that a number of herbivory induced plant volatiles have been characterized for their individual contrition to indirect defense in behavioural set ups including methyl salicylate.

Allyl isovalerate(Fig 9), a fragrant compound is also detected, in healthy jasmine leaves at 5.465 mins in a peak area of 6178643 mm^2, which findings of Zada *et al.* (2003) who found lavandulyl isovalerate in headspace volatiles of vine mealybug, *Planococcus ficus*.

With regard to jasmine leaf web worm infested leaves, allyl isothiocyanate, allyl isovalerate, divinyl sulfide, naphthalene, oxalic acid potential candidates for attracting natural enemies were detected in along with an array of saturated hydrocarbons.

The potential natural enemy attractant allyl iso-thiocyanate (Fig 10) was detected at RT of 4.307 mins at an area of 533535 mm^2 in leaf webworm webbed leaves, which is 1.073 per cent of the total compounds present. Allyl iso-thio cyanate, a naturally occurring organo-sulfur compound in mustard, radish, horseradish, is responsible for their pungent taste.

Allyl isothiocyanate serves the plant as a defense against herbivores; since it is harmful to the plant itself, it is stored in the harmless form of the glucosinolate. When the plant is damaged, the enzyme myrosinase is released and acts on a glucosinolate known as sinigrin to give allyl isothiocyanate.

Synthetic allyl isothiocyanate is used as an insecticide, bacteriocide and nematocide, and is used in certain cases for crop protection (Romanowsk and Klenk, 2005). Zabża, 1989; Titayavan and Altieri, 1990, reported that the parasitoid *Diaeretiella rapae* M'Intoshis was attracted to allyl isothiocyanate emitted by cabbage plants damaged by cabbage aphids (*Brevicoryne brassicae* L.) and increased aphid parasitism from 8.5% to 22.5%. Thus, allyl isothiocyanate release due to budworm attack in jasmine plant might attract lot of natural enemies that check the pest naturally due to tri trophic interactions. Though, allyl iso thiocyanate peak area is only 1.073 per cent of the total compounds, which is less compared to most hydrocarbons released, which means, the compound is elicited only in meager quantity, it has already proved its efficiency in attracting natural enemies in various crop ecosystems.

Moreover, divinyl sulfide (Fig 11) was detected at 4.688 mins in a peak area of 260362 mm^2. Though its emission is in meager quantity it might also play its own role in natural enemy attraction as corroborated by Ferry *et al*. (2009) who found higher cabbage fly *Delia radicum* egg predation in broccoli *Brassica oleracea* plots with dimethyl disulphide lures (2.1 eggs predated/patch of eggs).

Bis (2-ethylhexyl) phthalate, di – n- octyl phthalate, pthalic acid, di (2-propyl pentyl ester), six carbon compounds were the compounds present in maximum quantity detected with the largest peak area of 144295751 mm^2 at 38.984 mts. In general, green leaf volatiles are six carbon compounds which are very quickly produced and/or emitted upon herbivory which play an important role in plant defenses and as bis (2-ethylhexyl) phthalate (Fig 12) is also six carbon compound, produced due to leaf herbivory in jasmine ecosystem, there are chances for its potential role in natural enemy attraction. Liu *et al,* (2007) reported the presence of volatile, bis (2-ethylhexyl) phthalate in honeydew from both *B. tabaci* on cabbage and *T. vaporariorum* on cucumber and its role as kairomone in host-searching of parasitoids.

Moreover oxalic acid (Fig 13) was emitted in enormous amount in a peak area of 42578330 mm^2 at 5.962 mins. Oxalic acid and oxalates are produced and present in plants in different amounts (Korth, 2006; Nakata, 2012; Franceschi and Nakata, 2005). They provide biochemical as well as mechanical defense against insect pests and animals (Prasad and Shivay, 2017). Foliar application of acetylsalicylic and oxalic acids has the potential to encourage aphid parasitisation, parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) (Karatolos and Hatcher, 2008).

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Napthalene, (Fig 14) an aromatic hydrocarbon was detected in bud worm infested leaves at 17.493 mins with peak area of 233218 $mm^{2,}$ which was reported to be a semiochemical attracting natural enemies of stemborer in maize ecosystem (Peshwin and Pimental, 2014).

Among the other hydrocarbons, tetracosane and triacontane were detected in 29.045 mins in a peak area of 11570984 $mm^{2.}$ 17.846% of the total compounds which implies their emission in maximum quantity.

The GC-MS studies on the volatile profile emitted from leaf folder damaged leaves in the susceptible rice variety TN1 showed more of the presence of docosane, which could be responsible in attraction of *Trichomma cnaphalocroccis* Uchida and *Cotesia angustibasis* Gahan (Rathika and Nalini, 2011). Seenivasagan and Paul (2011) analyzed the extracts of cruciferous host plants of diamond back moth and revealed the presence of saturated hydrocarbons. Cauliflower leaf extract contain 12 hydrocarbons with carbon number ranging from C10-C30 in which C29 (nonacosane) was detected in highest quantity. In cauliflower extracts exclusively C10 (decane) and C12 (dodecane) hydrocarbons were identified which were not detected in other host plant extracts. The hydrocarbon C14 (tetradecane) was detected only in cauliflower and broccoli extracts, whereas C16 hexadecane was detected only in cabbage, cauliflower and broccoli extracts. C18 (octadecane) and C20 (eisocane) were detected in cabbage and cauliflower extracts. C22 (docosane) and C25 (pentacosane) were detected only in cauliflower, while C26 (hexacane) was found only on knoll-knol leaf extracts. Similarly hexane extracts of ten different varieties of tomato (*Lycopersicon esculentum* Mill.) obtained in the vegetative and flowering phase of growth contained tricosane (C23), heneicosane (C 21), pentacosane (C25) and hexacosane (C26) during the vegetative period and heneicosane (C21) and hexacosane (C26) during the flowering period (Paul *et al.,* 2008).

Comparing the peak areas of common hydrocarbon compounds *viz.,* allyl isovalerate and tetracosane, present in healthy and damaged leaves, allyl isovalerate was found to be 1.043 percent more present in leaf webworm damaged leaves, but the hydrocarbon tetracosane was 0.848 per cent more pronounced in healthy jasmine leaves.

Behaviour of a natural enemy can be manipulated potentially by enhancing their foraging ability in an ecosystem. The interface, where tritrophic interactions take place in natural condition is often the cuticle of a plant. The saturated hydrocarbons present in the epicuticular wax layer of plants have been shown to influence the foraging success of natural enemies. Therefore the hydrocarbon compounds found in the extracts of damaged leafwebworm damaged leaves have to be explored for the attraction of natural enemies to parasitize and/or to predate the herbivore or reduce the further infestation by the herbivores for efficient pest management.

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Table 1: Saturated hydrocarbon profile of the healthy and leaf webworm, *Nausinoe geometralis* damaged leaves of jasmine, *Jasminum sambac* L.

Healthy leaves			Leaf webworm damaged leaves		
RT(min)	Area(mm^2)	Name of the compound	RT(min)	Area(mm^2)	Name of the compound
4.305	3527444	Cyclohexanol	4.308	533535	Allyl isothiocyanate
4.688	3319684	2-Butanol	4.688	260362	Divinyl sulfide
5.030	10691889	2-Methyl butanoic anhydride	4.908	2214865	Dodecane 5-methyl
5.465	6178643	Allyl isovalerate	5.030	24241992	2-Amino ethanol
5.669	3556699	Azetidine 1,2 dimethyl	5.465	6480251	Allyl isovalerate
5.962	8563751	Cyclo hexane	5.670	4018056	Azetidine
7.787	1894648	Cyclo hexane (2 methyl propyl-)	5.962	42578330	Oxalic Acid
9.671	1782054	Phenyl Ethyl Alcohol	6.144	260869	Dodecane
11.924	981341	Methyl salicylate	6.545	4971755	2-Penetene
24.098	2270549	Heneicosane	7.136	125362	Dodecyl octyl ether
	2270549	Nona decane 9 methyl	8.421	65691	Acetophenone
25.713	13642707	Eicosane	11.52	83629	Cyclohexane
25.713	13642707	Octacosane	12.22	200022	Cycloheptanol
25.713	13642707	Tetracosane	12.70	91452	4-Amino-5-(4-acetyl phenylazo) benzo furazan
25.894	24610830	Hentriacontane	14.221	185482	Iso butyl angelate
25.894	24610830	Tetracosane	14.562	289982	Cyclo hexa siloxane
27.546	3828465	Eicosane	17.062	85400	Trifluoro octoxy hexadecane
27.546	3828465	Nona decane 9-methyl	17.123	464305	Butyl angelate
27.546	3828465	Hentriacontane	17.123	464305	2-Butenoic Acid
30.855	3809329	Eicosane	17.493	233218	Napthalene
30.855	3809329	Pentacosane	17.155	464305	Cyclohepta siloxane
30.855	3809329	Octadecane	17.704	145509	Benzene

31.462	15511968	Eicosane	17.817	126852	Benzene butanoic acid
33.132	4254520	Heptacosane	18.511	124684	Diethylmalonic acid
33.132	4254520	Hexacosane	19.556	140095	Cyclotetradecane
33.519	9932421	Eicosane	19.556	140095	Decyl trifluoroacetate
33.519	9932421	Nonacosane	29.045	11570984	Tetracosane
33.519	9932421	Heptacosane	29.045	11570984	Triacontane
			31.984	144295751	Bis (2-ethylhexyl) phthalate
			31.984	144295751	Di – n- octyl phthalate
			31.984	144295751	Pthalic Acid Acid, di (2-propyl pentyl ester)

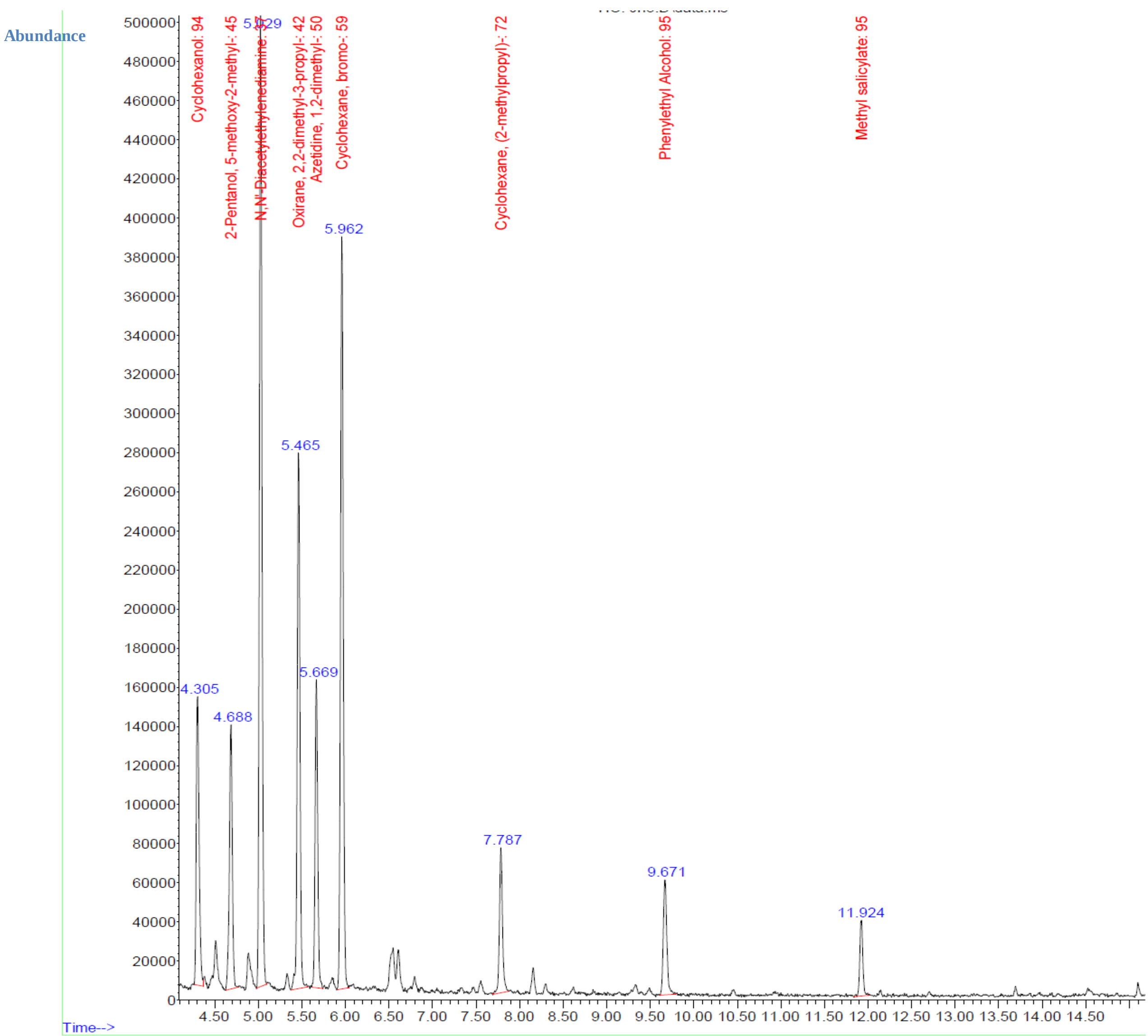

Fig. 1a: Chromatographic profiles of saturated hydrocarbons from healthy jasmine (*Jasminum sambac* L.) leaves

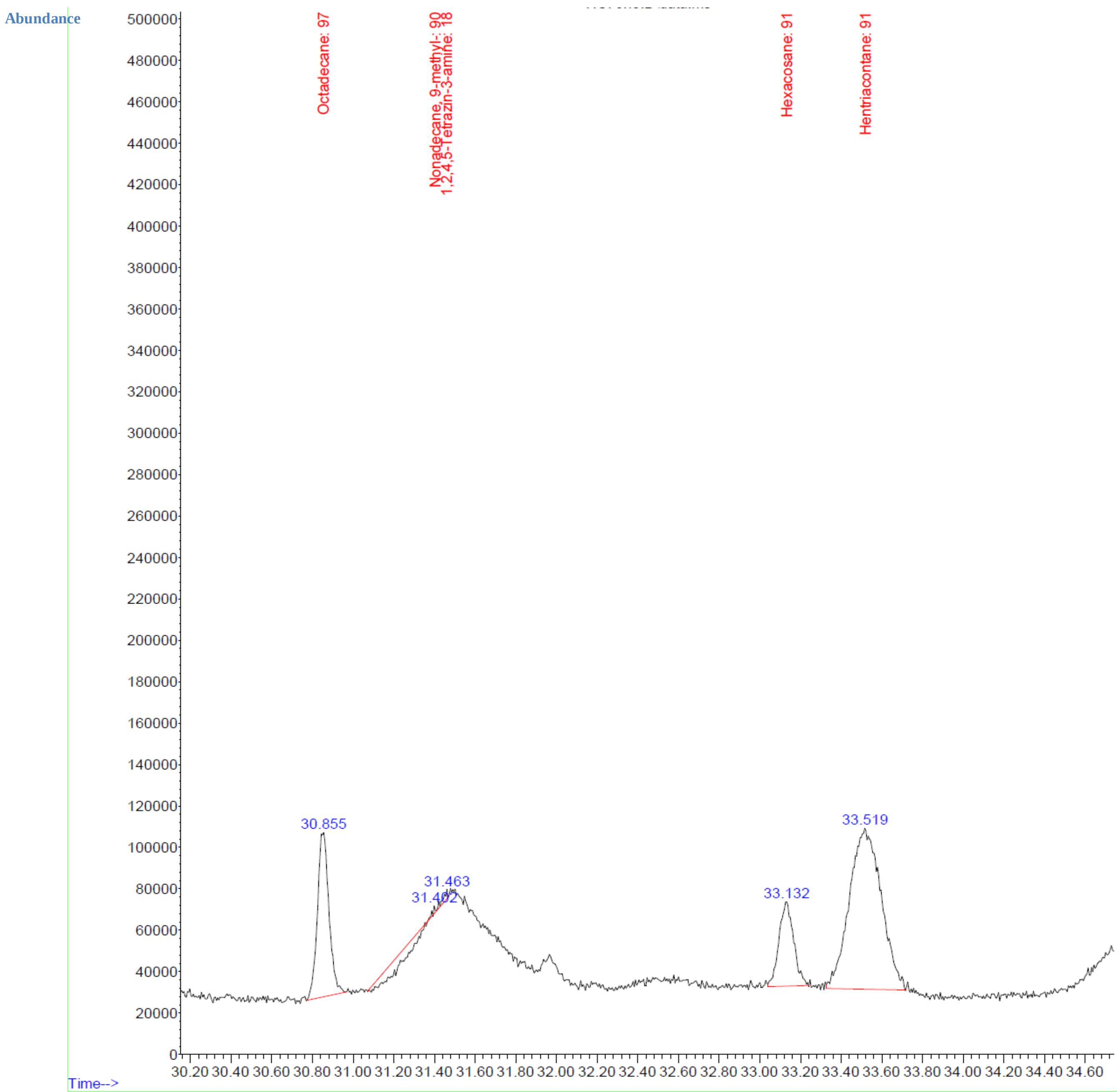

Fig. 1b: Chromatographic profiles of saturated hydrocarbons from healthy jasmine (*Jasminum sambac* L.) leaves

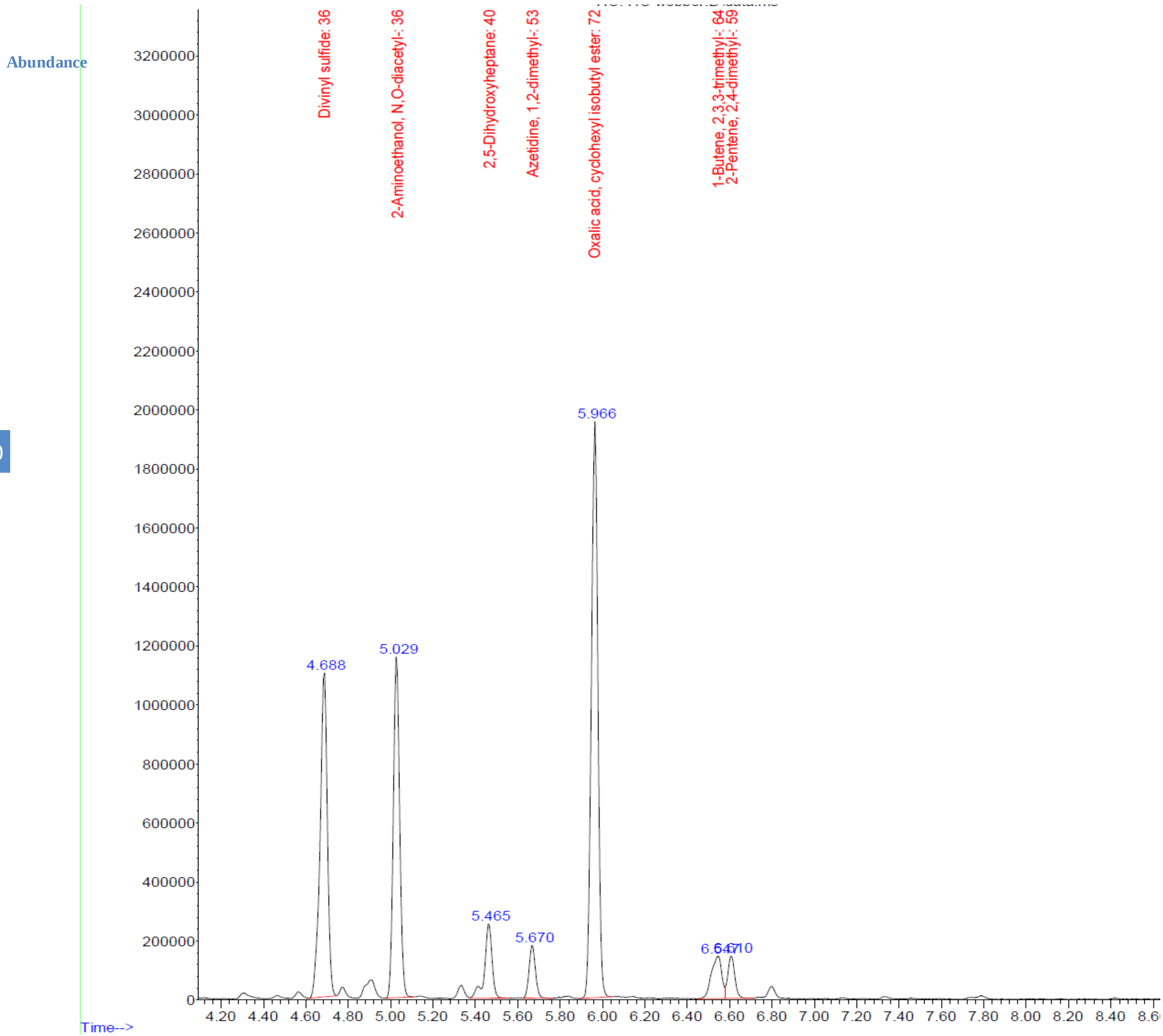

Fig. 2a: Chromatographic profiles of saturated hydrocarbons from leaf webworm, *Nausinoe geometralis* infested jasmine leaves

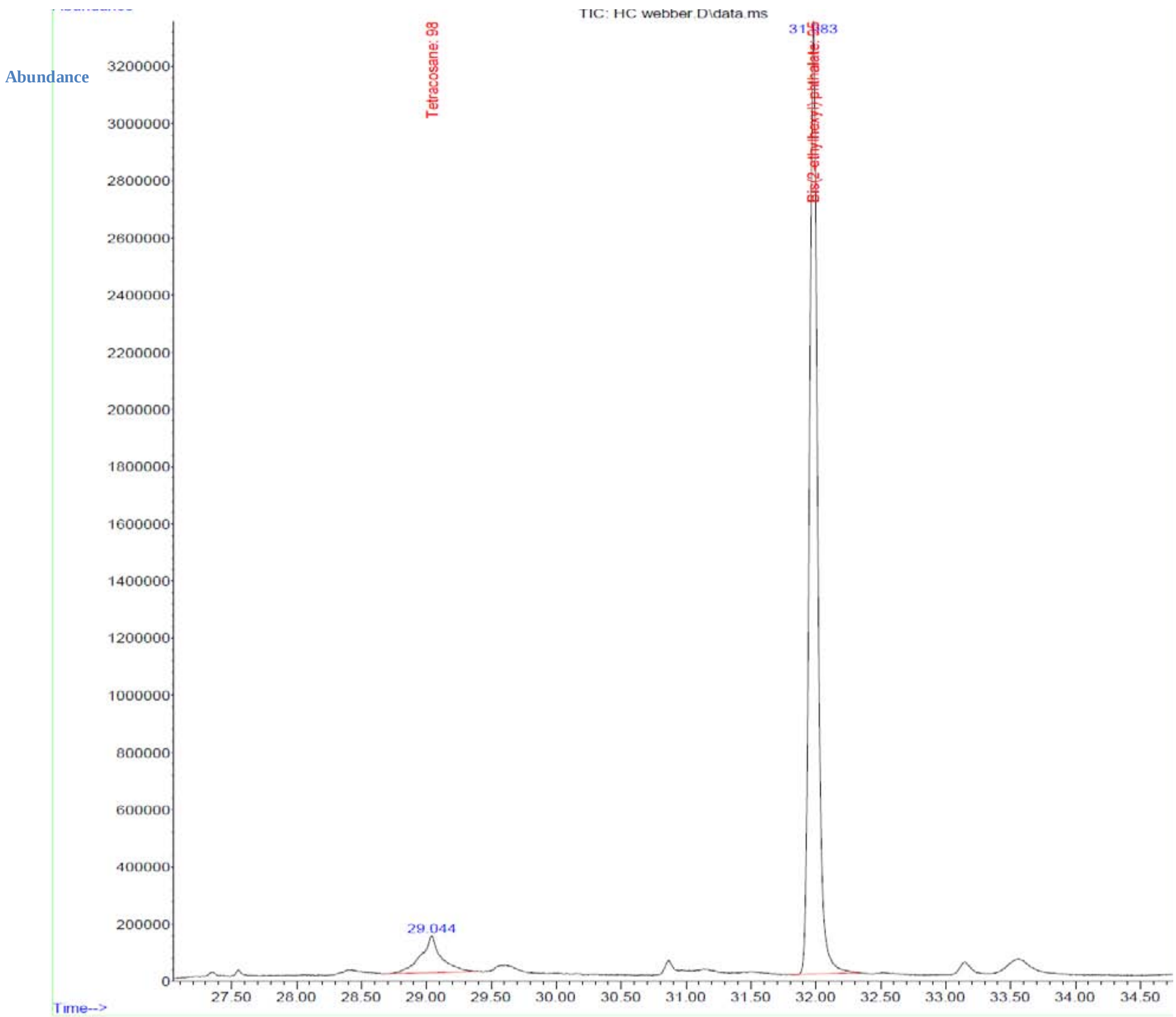

Fig. 2b: Chromatographic profiles of saturated hydrocarbons from leaf webworm, *Nausinoe geometralis* infested jasmine leaves

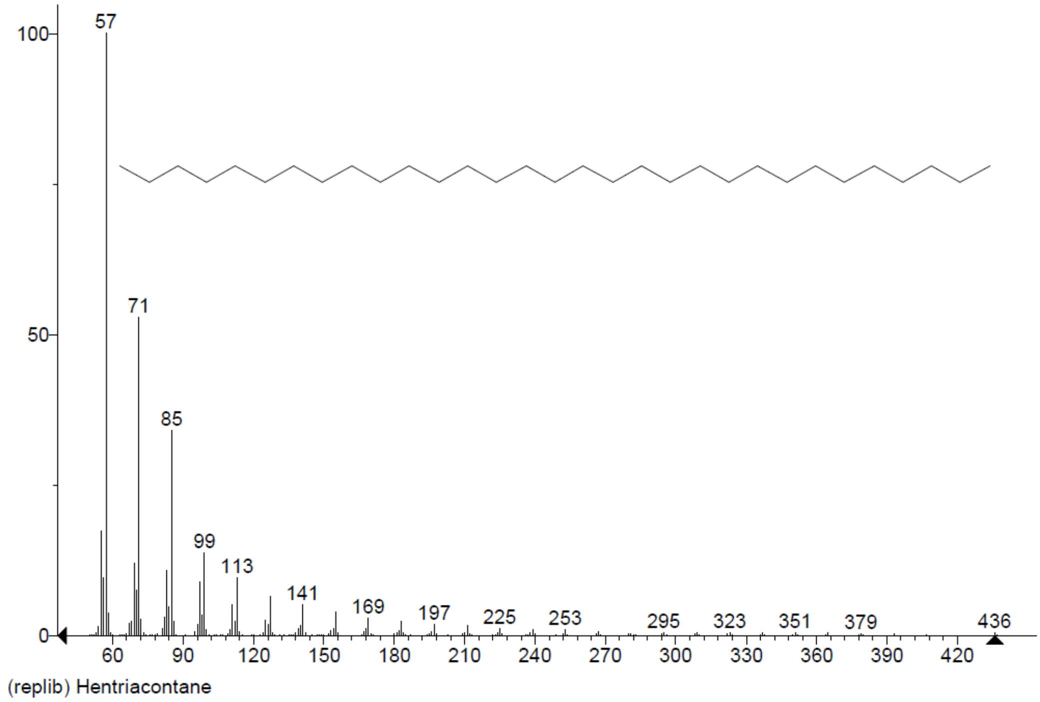

Fig. 3: Mass spectrum and structure of hentriacontane

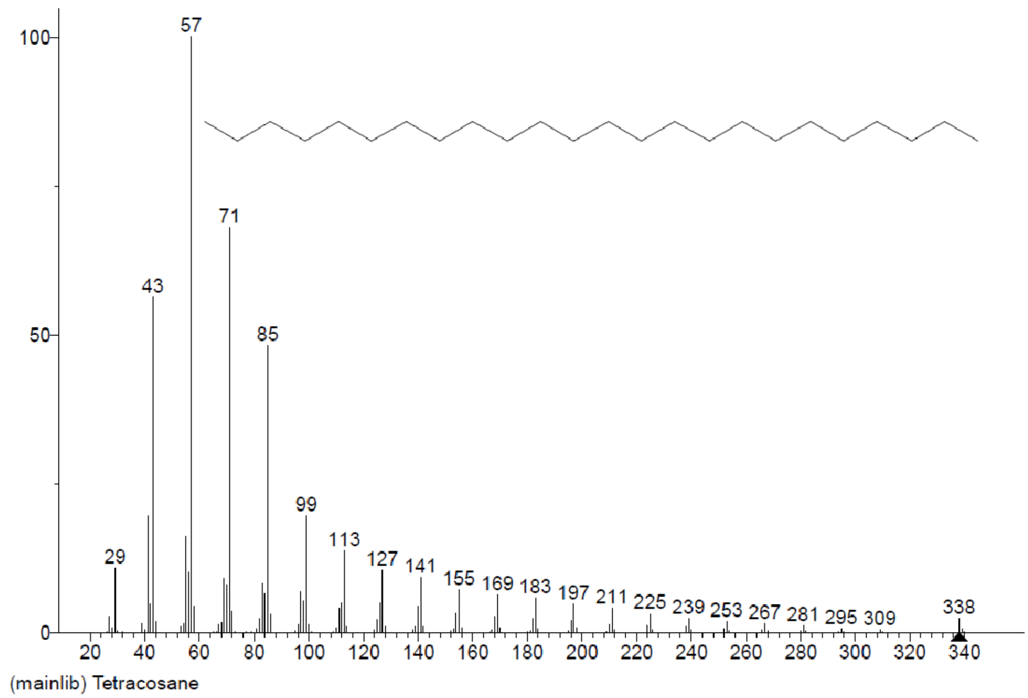

Fig. 4: Mass spectrum and structure of tetracosane

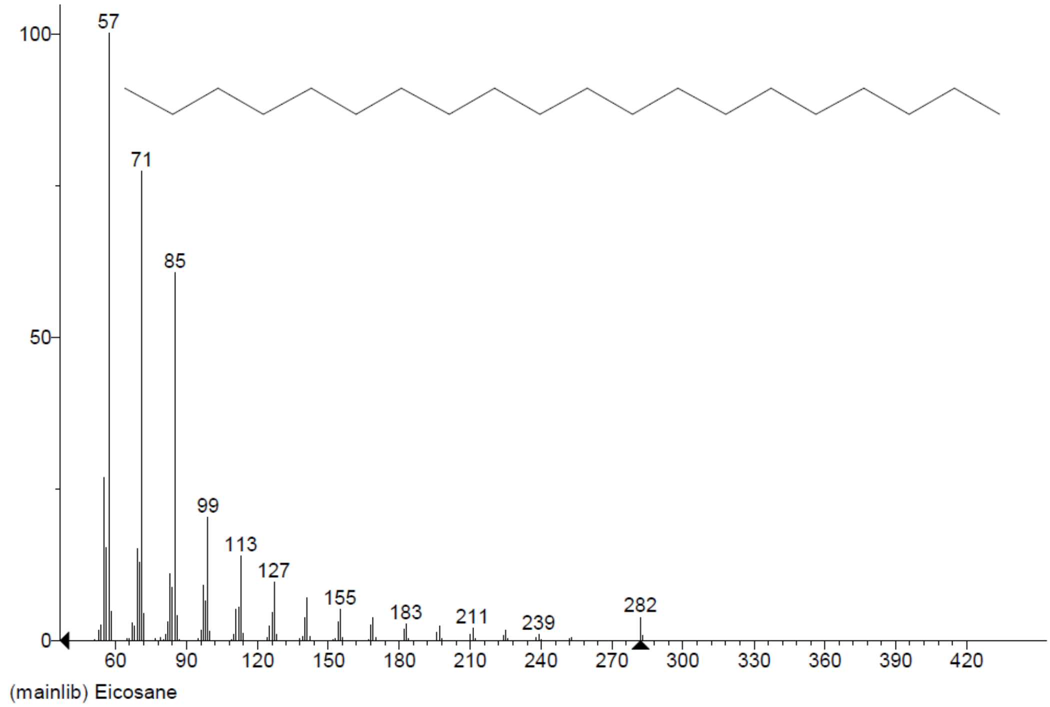

Fig. 5: Mass spectrum and structure of eicosane

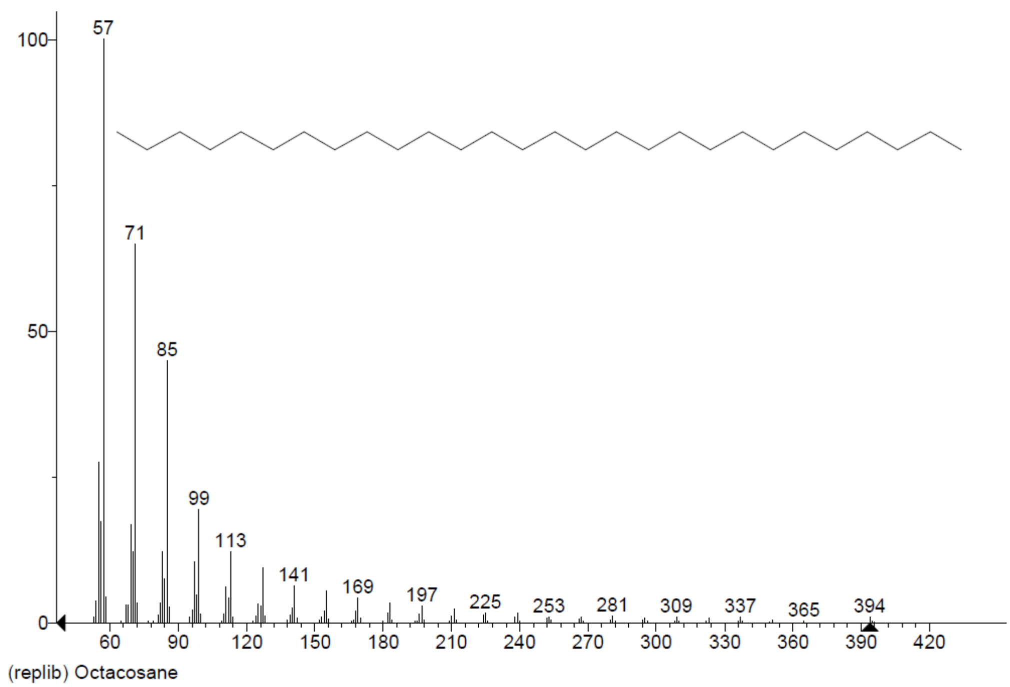

Fig. 6: Mass spectrum and structure of octacosane

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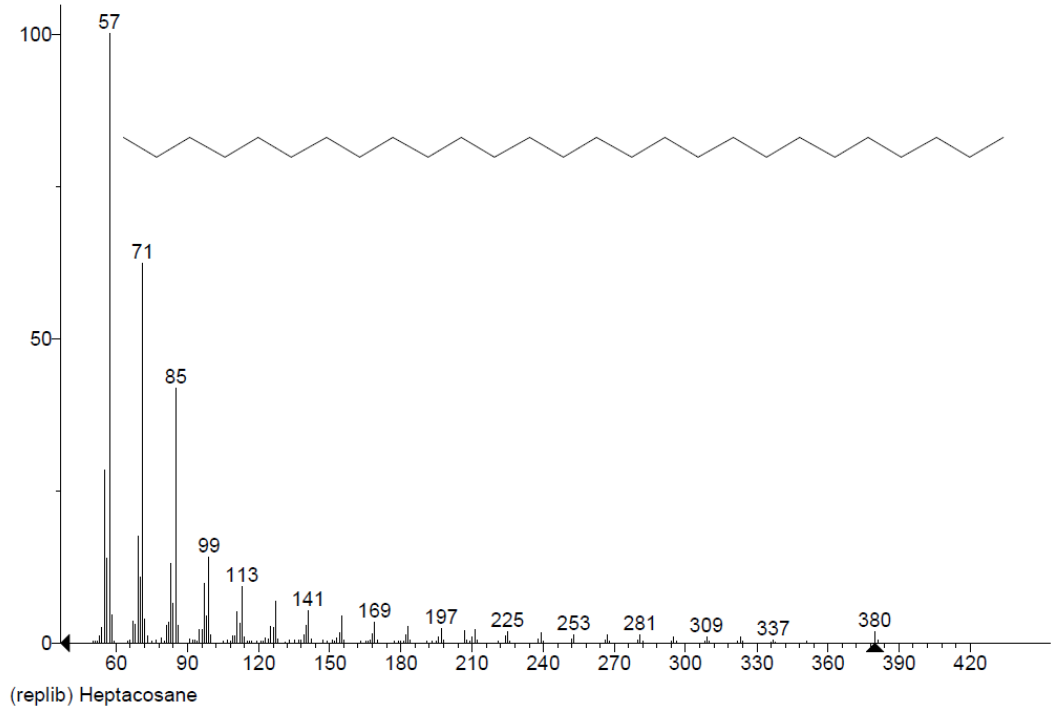

Fig. 7: Mass spectrum and structure of heptacosane

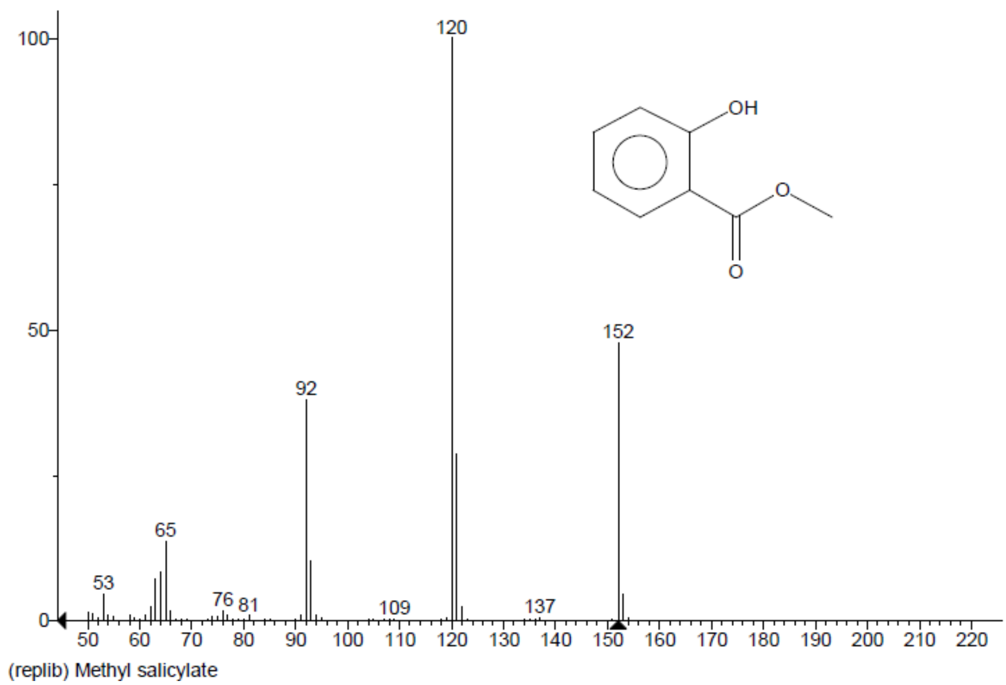

Fig. 8: Mass spectrum and structure of methyl salicylate

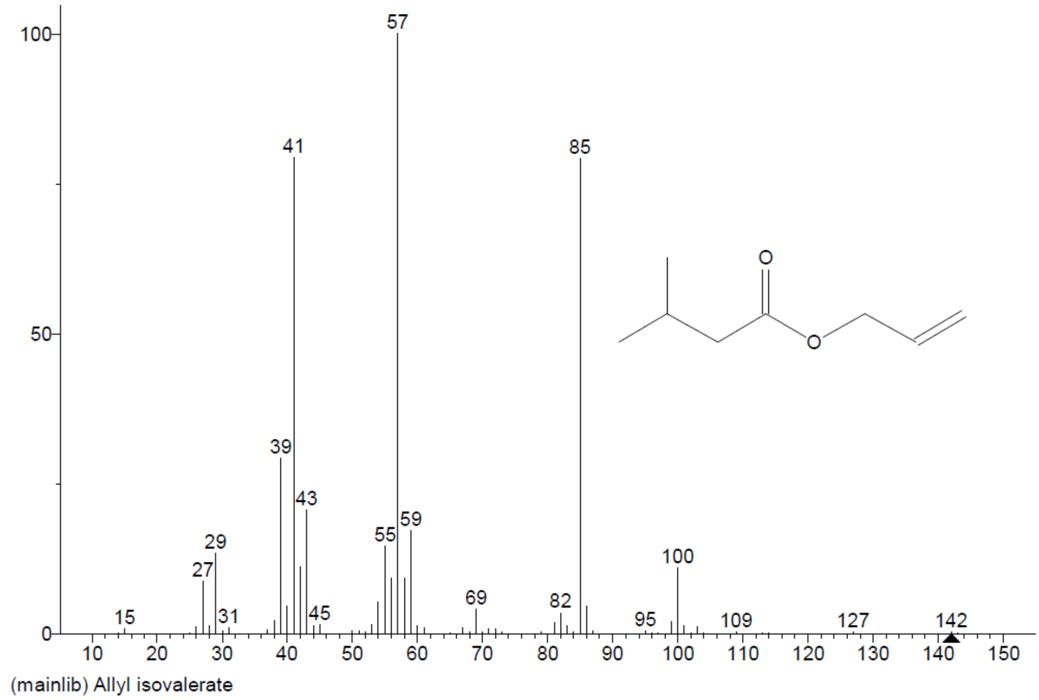

Fig. 9: Mass spectrum and structure of allyl isovalerate

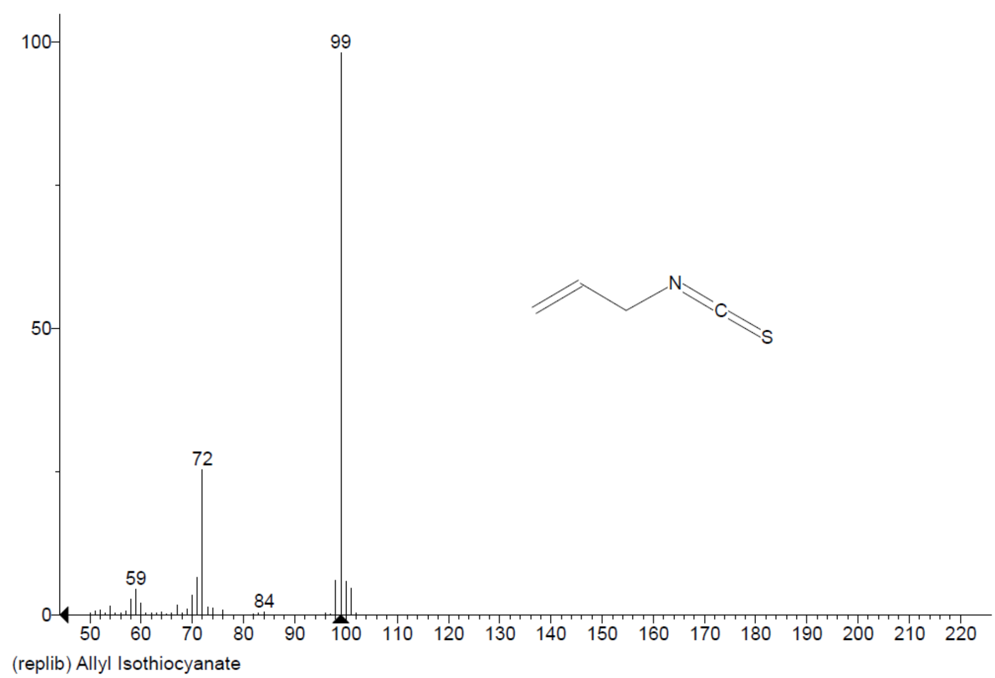

Fig. 10: Mass spectrum and structure of allyl isothiocyanate

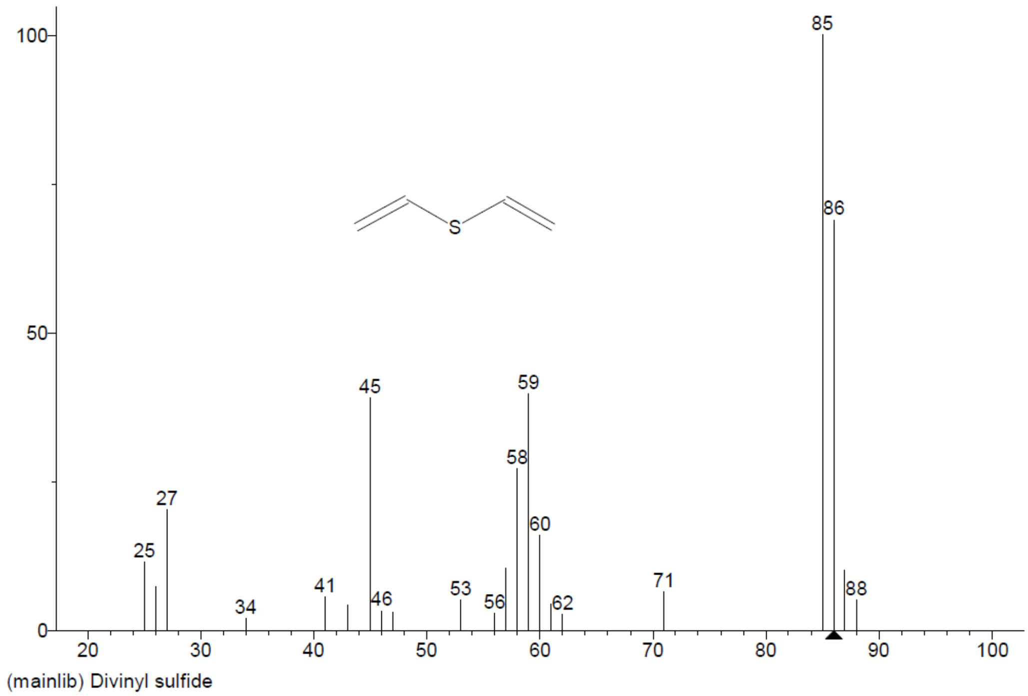

Fig. 11: Mass spectrum and structure of divinyl sulfide

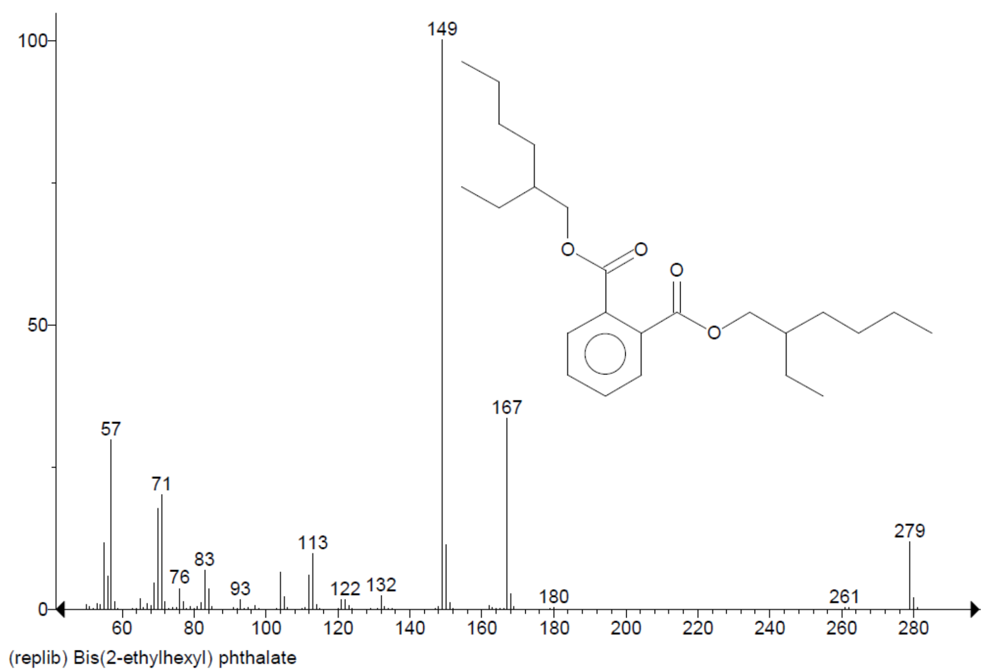

Fig. 12: Mass spectrum and structure of bis (2-ethylhexyl) phthalate

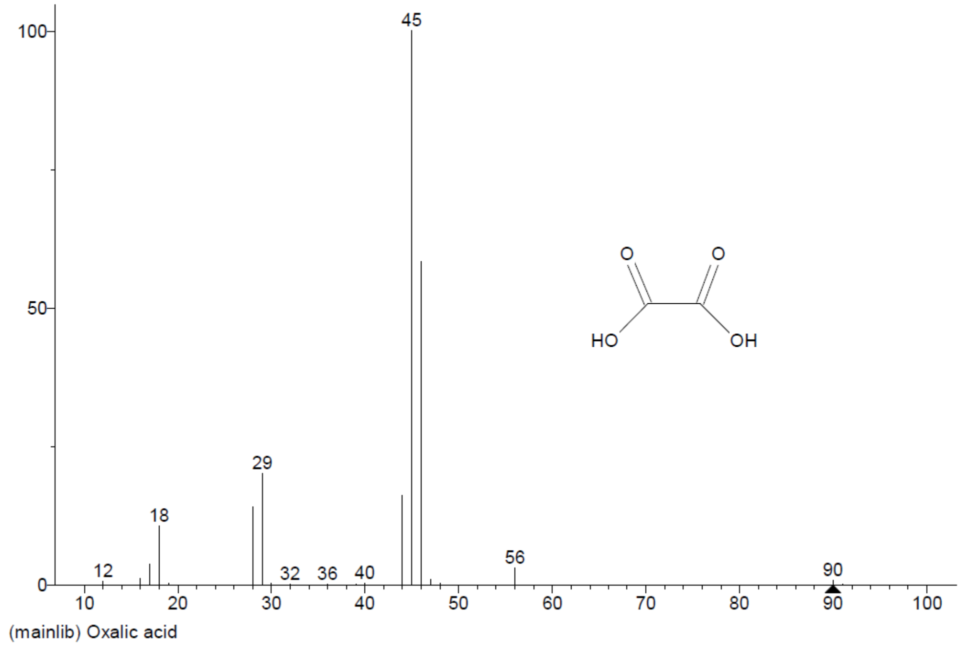

Fig. 13: Mass spectrum and structure of oxalic acid

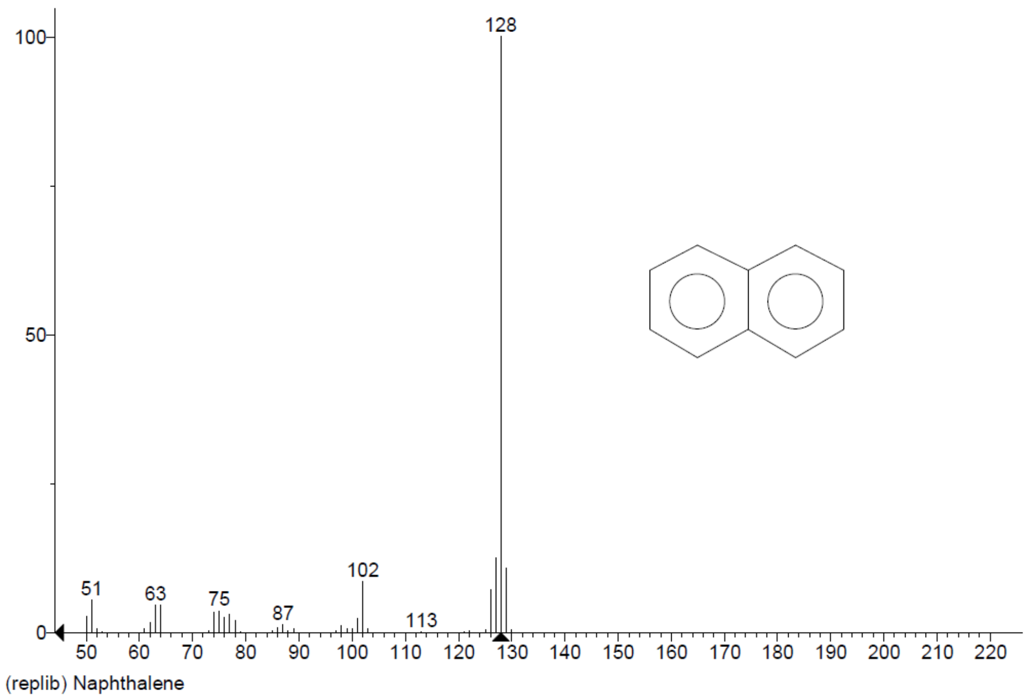

Fig. 14: Mass spectrum and structure of napthalene

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