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Identification of Saturated Hydrocarbons from Jasmine (*Jasminum sambac L.*) Buds Damaged by Blossom Midge, *Contarinia maculipennis* Felt through GC-MS Analysis

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Abstract Saturated hydrocarbons of fresh healthy jasmine buds (*Jasminum sambac* L) and infested buds damaged by blossom midge, *Contarinia maculipennis* Felt. were identified through Gas Chromatography-Mass spectrometry (GC-MS) to determine the saturated hydrocarbons. The results revealed that both the healthy and damaged buds had emitted hydrocarbon compounds numbering 24 and 34 respectively. The variation in the hydrocarbon constituents of healthy and damaged buds clearly depicts the emission of volatiles attracting beneficials witnessing tritrophic interactions in jasmine ecosystem. Linalool, the fragrant compound is detected in healthy jasmine buds at the retention time of 9.278 mins with the largest peak area of 5180722 mm^2 and in midge damaged buds in a peak area of 288967 mm^2, which might be the probable reason for the fragrance of jasmine flower. Allyl isothiocyanate, a compound responsible for plant defenses against herbivores is detected at a retention time of 4.306 mins recording the sixth largest peak area of 2461362 mm^2 in midge infested buds. Other plant defense chemicals *viz.* naphthalene, azulene, methyl salicylate, methyl anthranilate, alpha farnescene, phenol, styrene etc. were also detected in midge damaged buds. The quality and quanity of these semiochemicals emitted by the buds might be the reason for attraction of natural enemies in the jasmine ecosystem there by further reducing the infestation of midge, 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, Hendecasis duplifascialis, GC-MS, jasmine*

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1. Introduction

Jasmine is an important traditional flower, cultivated nearly throughout the tropical and subtropical parts of the world for its fragrant flowers. The flowers find place in floral decoration of marriages, to consecrate a sacred wedding ceremony, as a form of expressing love, affection, happiness and honouring the guests. The exchange of garlands made of Jasmine in marriage symbolizes the natural circle of protection and sacred bride to the spiritual life. Although more than 2,000 species are known, 40 been identified in India, and 20 species are cultivated in South India (Bhattacharjee, 1980). In India, jasmines are cultivated throughout the country. However, the largest area under Jasmine flower production is in Tamil Nadu followed by Karnataka. The annual production of flowers in India is worth more than 120 million (Dadlani, 2004). 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). *J.sambac* is ravaged by several insects *viz.*, budworm, blossom midge, leaf web worm, red spider mite and other sucking pests. Of these, budworm and blossom midge pose heavy damage as they directly attack the economic part. The adult midges lay eggs at the tip of the flower buds, when they are small and green in colour. The emerging maggots move down, reach the base of corollas and suck the sap, which results in swelling at the base of the buds and pink discolouration of buds. The infestation leads to premature drying of buds and leads to stunted growth and ultimately drying of plants. The management of jasmine pests completely relies on spraying of synthetic chemicals without right intervals, polluting the environment as well as causing detrimental effect to non- target beneficials. Therefore, it is now an urgent need to use safe but effective, biodegradable pesticides with no or less toxic effects on beneficials. 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. Plant surfaces are coated with thin layer of waxy materials that have myriad of functions. This layer is microcrystalline in structure and form the outer boundary of the cuticular membrane. It serves lot of purposes *viz.*, to limit diffusion of water and solutes while permitting controlled release of volatiles that may deter the pests or attract natural enemies and pollinators (Eigenbrode, 1996). These plants waxy materials act as synomones and play a major role by guiding the natural enemies to the potential host or prey on the plant [9]. Such clues may be utilized to stimulate foraging and host selection behavior of entomophages thereby increasing their effectiveness for IPM [1]. Therefore in the present study, the hexane bud extracts of Jasmine damaged by midges and healthy buds were analyzed through GC-MS to determine the saturated hydrocarbon profiles in them.

2. 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 midge damaged buds were collected from the field during the period of heavy infestation by midges.

The saturated hydrocarbons were extracted from the buds infested by blossom midges and healthy buds using HPLC grade hexane as follows. The healthy and midge infested discoloured buds of jasmine were plucked carefully and used for extraction. Ten gram of buds 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 bud 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 silica capillary column having 0.25 mm ID and 0.25 um film thickness run in constant flow mode (1.0 ml/min helium). Oven temperature programming: 60°C (1 min hold) to 100°C at 5°C/min rate (1 min hold), then to 220°C at 10°C/min rate (5 min hold) and then to 240°C at 50°C/min rate (8 min hold). Injector temperature was set at 275°C. One microlitre of the extract was injected using auto sampler into the gas chromatography-mass spectroscopy (GC-MS) system for analysis and injections were done in split 10:1 mode. Agilent MSD productivity chemstation for GC- MS Systems data analysis applications 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.

3. 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. Gas Chromatography mass - spectroscopy analysis of synamone extracts of healthy buds showed the presence of 24 hydrocarbons, *viz.*, cyclohexanol, cyclohexanone, 2-pentanol, 3- hexanol, 3- heptanol, 2-pentene, 5-nonanone, benzaldehyde, cyclopentanol, 3-hexenol acetate, benzyl alcohol, cyclohexane, heneicosane, linalool, phenyl methyl alcohol, methyl salicylate, alpha-farnescene, 9-tricosene, octadecane, tricosane, octacosane, tetracosane, eicosane and hentriacontane (Figure 1). Linalool was detected at the retention time of 9.278 mins with the largest peak area of 5180722 mm^2 constituting 30.240 % of the total compounds. The hydrocarbons, octadecane, tricosane and octacosane was detected at the retention time of 27.552 mins with the second largest peak area of 4970489 mm^2 constituting 7.618% of the total compounds. Hentriacontane, tetracosane and octacosane were detected at 3646033 mm^2 recording the third largest peak area at RT 30.865 mins (Table 1).

But the midge damaged jasmine buds showed the presence of 34 saturated hydrocarbons *viz.*, allyl isothiocyanate, styrene, 1,3 Pentadiene 4 methyl, benzoic acid, linalool, naphthalene, azulene, methyl salicylate, methyl anthranilate, tetradecane, alpha farnescene, naphthalene, phenol, hexadecane, heptadecane, heneicosane, octacosane, do triacontane 1-iodo, tetracosane, henetriacontane, pthalic acid isobutyloctyl ester, pentacosane, heptacosane, eicosane, indazole 4-one, 10-methyl antracene-9 carboxaldehyde, bis (2 ethyl hexyl phthalate), eicosane, tricosane, octacosane, tetracosane, hexadecane 1 - iodo, eicosane and nonacosane (Figure 2).

The results are in accordance with the findings of Kumar *et al.*, [8], who reported that the hexane leaf extracts of targeted cole crop variety White Vienna subjected to gas liquid chromatography to detect their saturated hydrocarbon profile revealed the presence of three hydrocarbons *viz.*, heneicosane (C21), docosane (C22) and nonacosane (C29).

The volatile compound linalool, is found to be present in the healthy and damaged buds of Jasmine (Figure 3). Linalool, a monoterpinol and the most common fragrance chemical is found to be present in Jasmine oil [2,3,4,17]. Linalool is detected in midge damaged buds in a peak area of 288797 mm^2 at the retention time of 9.277 mins, as against 5180722 mm^2 peak area in healthy jasmine buds implying its emission in large quantity in healthy buds. (Figure 3). This shows that linalool content is decreased in Jasmine due to midge attack. There are reports of more production of linalool due to herbivore attack. Linalool is the most abundant volatile emitted from fall army worm, *Spodoptera frugiperda* damaged rice plants [22].

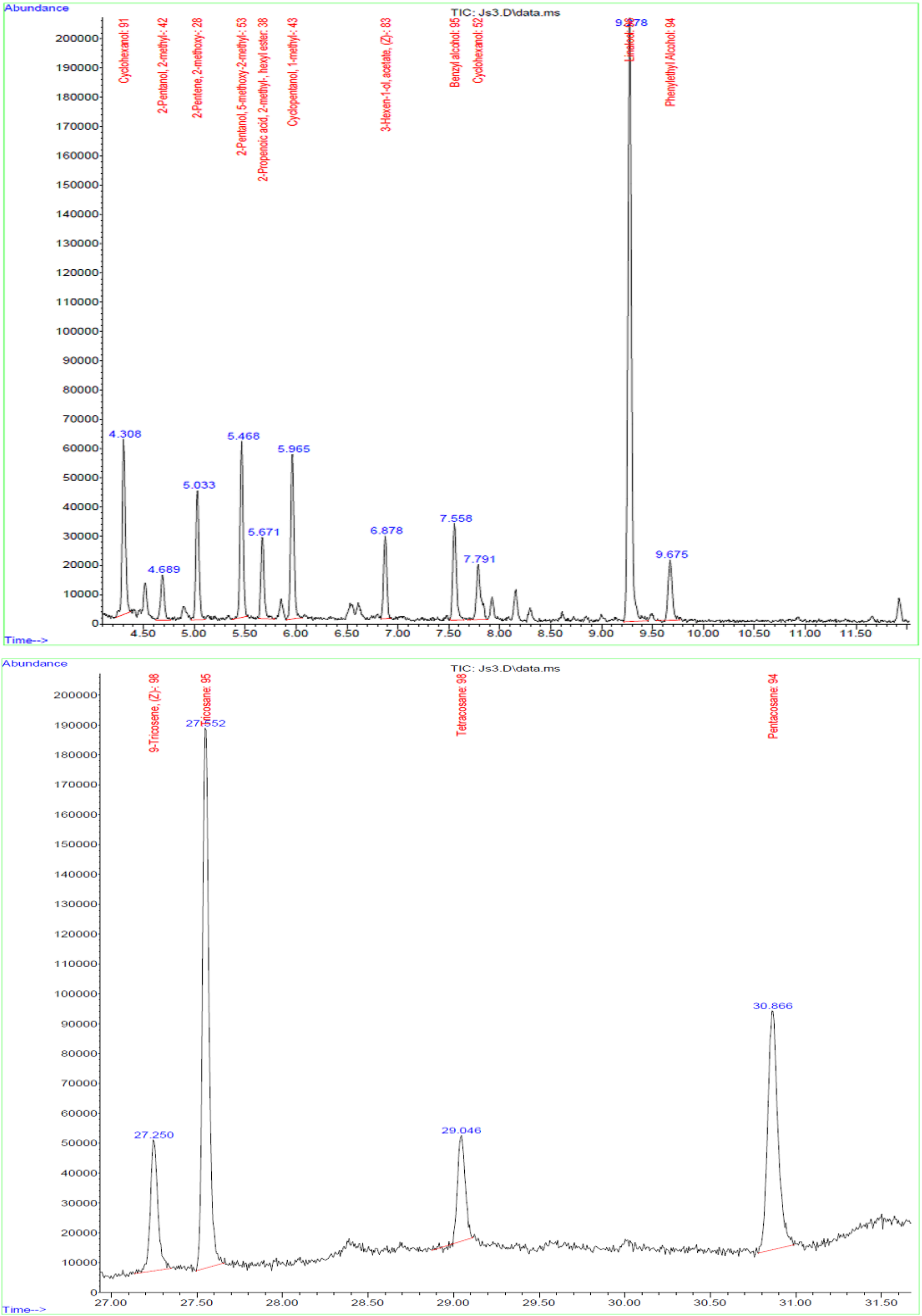

Figure 1. Chromatogram of hydrocarbons present in healthy jasmine buds

Table 1. Saturated hydrocarbon profile of the healthy and blossom midge damaged buds of Jasmine, *J.sambac*

Healthy buds			Midge damaged buds		
RT(min)	**Area(mm^2)**	**Name of the compound**	**RT(min)**	**Area(mm^2)**	**Name of the compound**
4.309	1448100	Cyclohexanol	4.306	2461362	Allyl isothiocyanate
4.519	378855	Cyclohexanone	4.461	291431	Styrene
4.690	409217	2- Pentanol	6.875	100728	1,3 Pentadiene 4 methyl
4.690	409217	3- Hexanol	9.174	274633	Benzoic Acid
4.690	409217	3- Heptanol	9.277	288797	Linalool
5.033	409217	2- Pentene	11.657	633398	Napthalene
5.671	659805	5- Nonanone	11.657	633398	Azulene
5.856	175210	Benzaldehyde	12.002	207138	Methyl salicylate
5.965	1274302	Cyclopentanol	14.849	122329	Methyl anthranilate
6.878	648174	3-Hexenol acetate	15.722	373480	Tetradecane
7.558	838689	Benzyl alcohol	17.039	372035	Alpha farnescene
7.791	574495	Cyclo hexane	17.362	3059318	Phenol
8.300	116978	Heneicosane	17.493	222876	Napthalene
9.278	5180722	Linalool	18.443	1303676	Hexa decane
9.676	693649	Phenyl methyl alcohol	19.624	122323	Heptadecane
11.926	263826	Methyl salicylate	19.773	2372226	Heneicosane
17.305	215542	Alpha-farnescene	19.773	2372226	Octocosane
27.250	1827170	9-Tricosene	20.253	1459913	Do triacontane- 1 iodo-
27.552	4970489	Octadecane	20.253	1459913	Tetracosane
27.552	4970489	Tricosane	20.253	1459913	Hentriacontane
27.552	4970489	Octacosane	20.730	2140636	Octadecane
29.046	1432970	Tetracosane	20.839	1428513	Hentriacontane
29.046	1432970	Eicosane	21.531	2880198	Pthalic acid isobutyloctyl ester
30.865	3646033	Hentriacontane	21.774	2119427	Pentacosane
30.865	3646033	Tetracosane	22.053	4547459	Heptacosane
30.865	3646033	Octacosane	22.830	1483204	Eicosane
			22.999	520662	Indazole 4-one
			23.212	845668	10-Methyl antracene-9 carboxaldehyde
			24.105	1726843	Henicosane
			24.105	1726843	Hentriacontane
			25.720	1554659	Bis (2 ethyl hexyl phthalate)
			27.251	3295502	Eicosane
			27.554	14863097	Tricosane
			27.554	14863097	Octacosane
			27.554	14863097	Tetracosane
			29.047	7789615	Hexadecane- 1 iodo
			30.868	5018550	Eicosane
			33.567	677165	Nonacosane

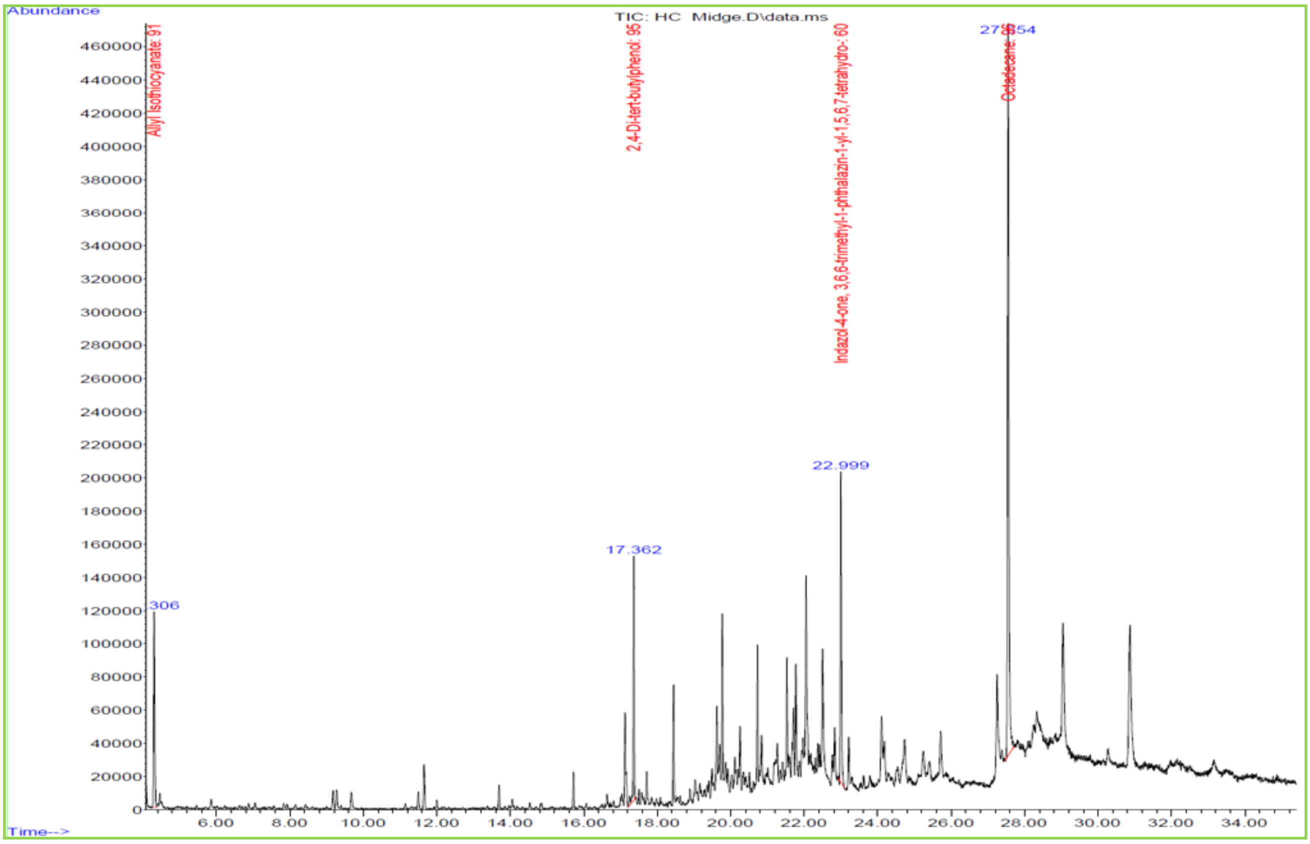

Figure 2. Chromatogram of hydrocarbons present in blossom midge damaged jasmine buds

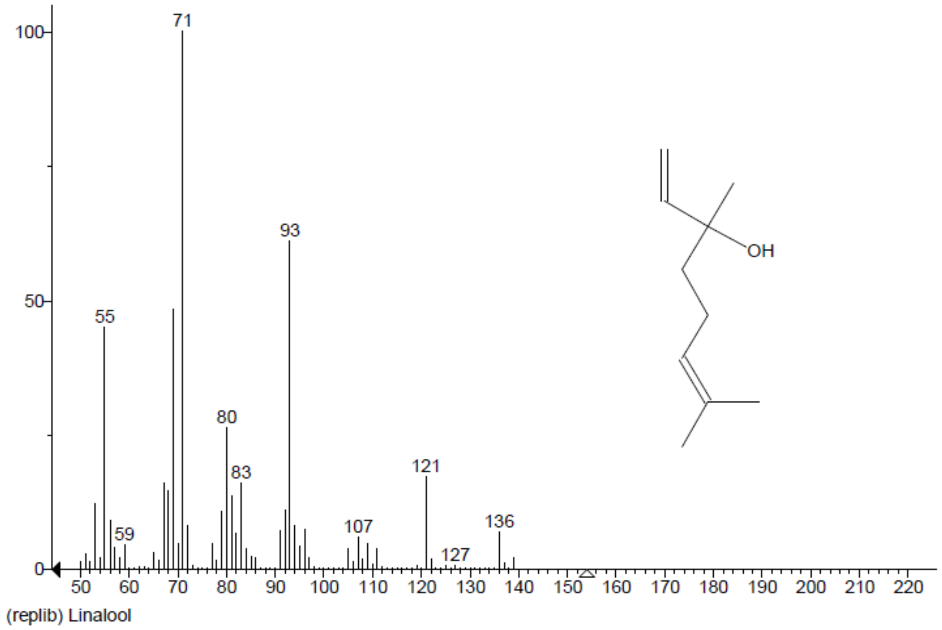

Figure 3. Mass spectrum and structure of linalool

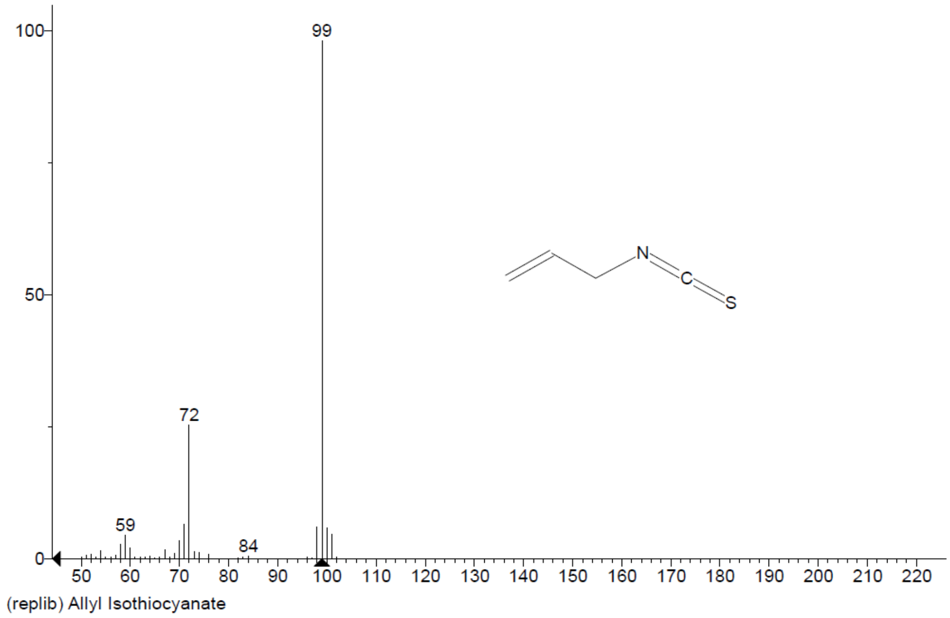

Figure 4. Mass spectrum and structure of allyl isothiocyanate

The compound allyl iso-thiocyanate was detected at RT of 4.306 mins at an area of 2461362 mm^2 in midge damaged buds, which is 5.070 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 (Figure 4). 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 [19]. Zabża, [23]; Titayavan and Altieri, [20], 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 midge attack in jasmine plant might attract lot of natural enemies that check the pest naturally due to tri trophic interactions. Allyl iso thiocyanate, had recorded sixth largest peak area and has already proved its efficiency in attracting natural enemies in various crop ecosystems.

Volatile methyl esters are common constituents of plant volatiles with important function in plant defense. Methyl salicylate, a herbivore-induced volatile has been shown to attract natural enemies and affect herbivore behavior. Methyl salicylate is detected in healthy jasmine buds itself at RT 12.002 mins and peak area of 263826 mm^2 and in midge damaged buds in a peak area of 207138 mm^2 showing the decrease in its content in midge damaged buds (Figure 5).

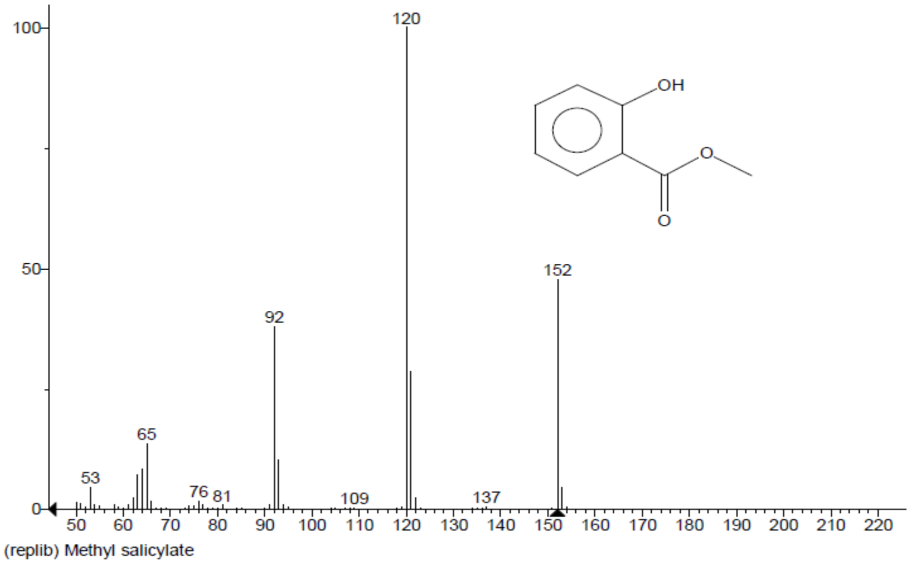

Figure 5. Mass spectrum and structure of methyl salicylate

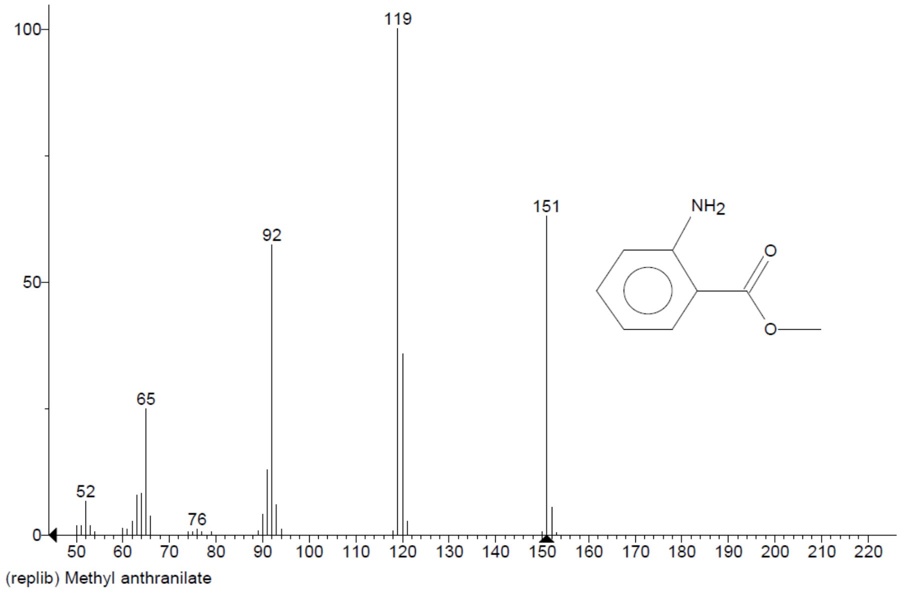

Figure 6. Mass spectrum and structure of methyl anthranilate

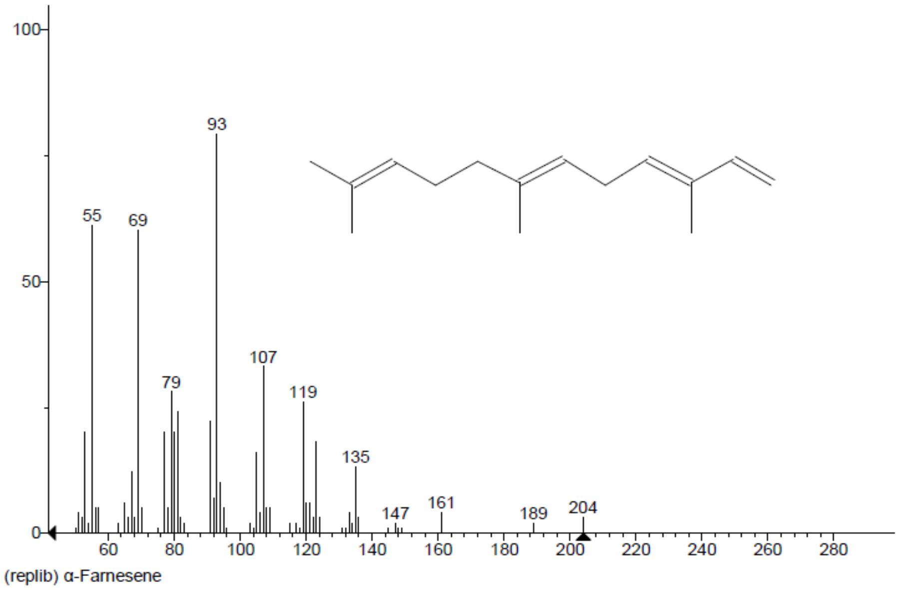

Figure 7. Mass spectrum and structure of alpha farnescene

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) [10]. The results are in line with Du *et al.*, [5]; Kesseler and Baldwin, [7] 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 and linalool. Methyl anthranilate, a potential compound known to induce plant defense was detected in midge damaged buds at RT 14.849 mins in a peak area of 122329 mm^2 (Figure 6).

Farnescene is produced by denovo biosynthesis in cotton plants when damaged by insect herbivores [11]. These compounds are likely to mediate the interactions between herbivores and their natural enemies, attracted by terpenes.

Alpha farnescene is detected in healthy jasmine buds at RT 17.305 mins and peak area of 215542 mm^2 constituting 0.330 percent and in midge infested buds in a peak area of 372035 mm^2 at the retention time 17.039 mins constituting 0.391 percent (Figure 7).

Napthalene, an aromatic hydrocarbon was detected twice in midge infested buds at RT 11.657 mins with peak area of 633398 $mm^{2,}$ and at RT 17.493 mins with peak area of 222876 mm^2 which was reported to be a semiochemical attracting natural enemies of stemborer in maize ecosystem [13] (Figure 8).

The hydrocarbons, tricosane, octacosane and tetracosane (Figure 9, Figure 10, Figure 11) recorded the largest peak area of 14863097 mm^2 at 27.554 mins of retention time and constituting 22.777% of the total compounds which implies their emission in maximum quantity. The hydrocarbon, hexadecane 1 - iodo recorded a peak area of 7789615 mm^2 at RT 29.047 mins. The hydrocarbon eicosane, was released at RT 30.868 mins with the second largest peak area of 5018550 mm^2 constituting 7.728 of total area and again at 329550 mm^2 at RT 27.251 mins. The hydrocarbon, heptacosane recorded a peak area of 4547459 mm^2 at RT 22.0153 mins showing 5.847 % of total emission. Phenol is emitted at RT 17.362 mins occupying a peak area of 3059318 mm^2.

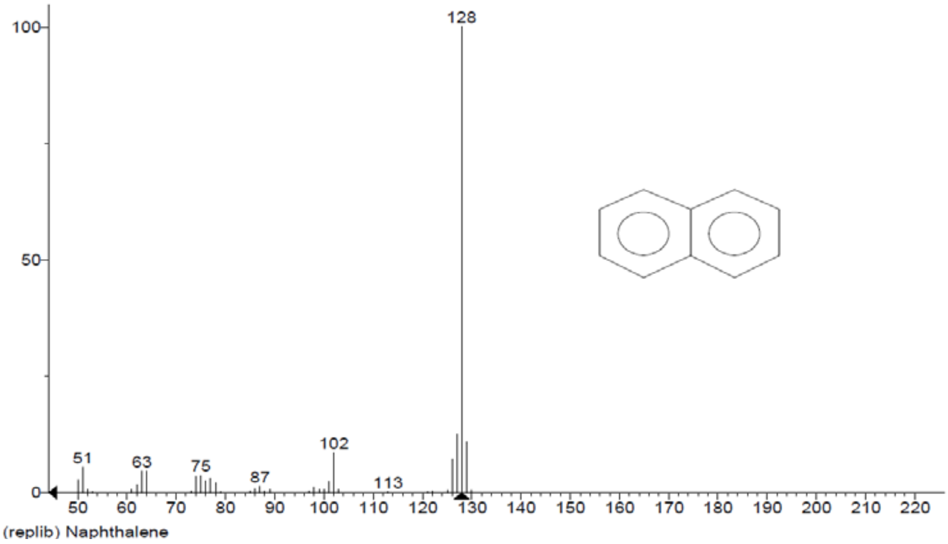

Figure 8. Mass spectrum and structure of naphthalene

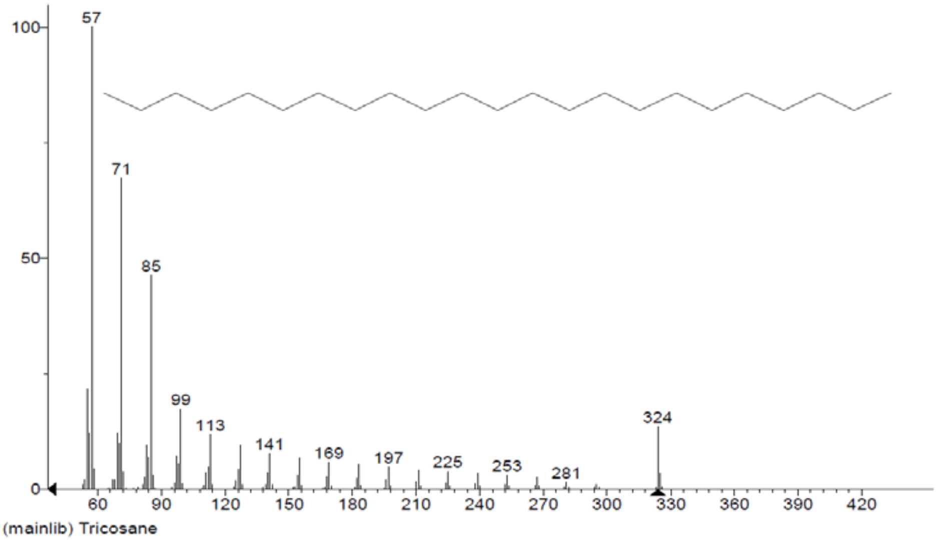

Figure 9. Mass spectrum and structure of tricosane

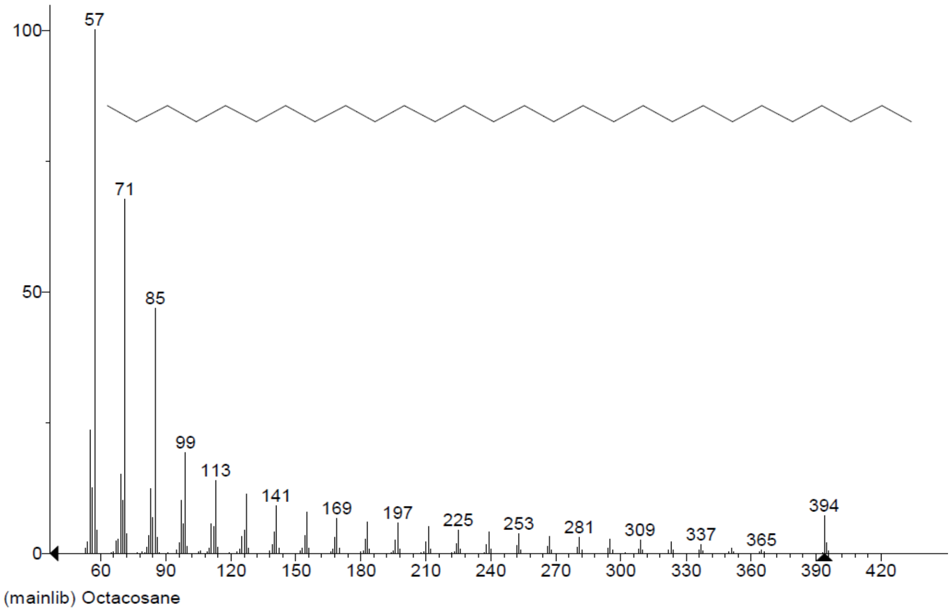

Figure 10. Mass spectrum and structure of octacosane

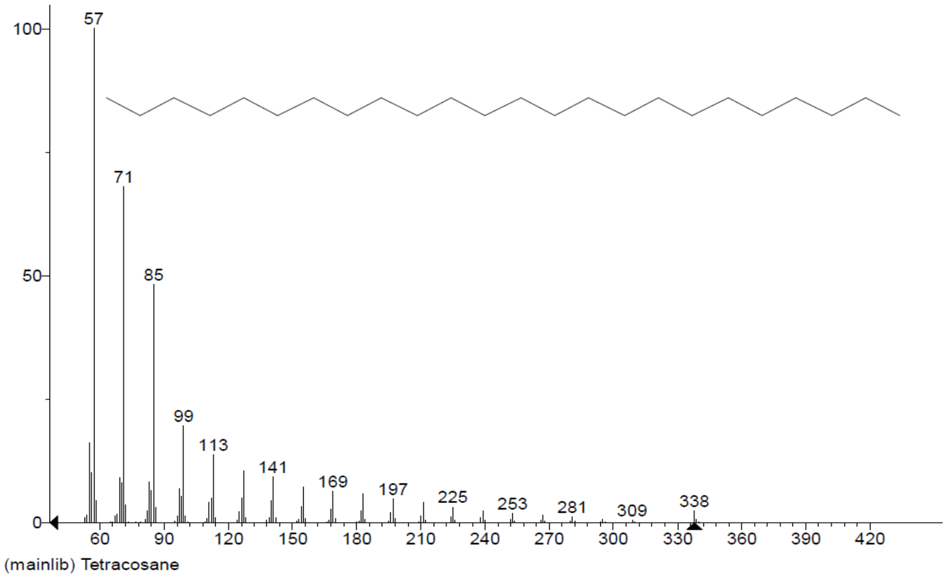

Figure 11. Mass spectrum and structure of tetracosane

The compound, pthalic acid isobutyloctyl ester was detected at a peak area of 2880188 mm^2 at RT 21.531 mins, heneicosane and octacosane at 2372226 mm^2 at 19.773 mins octadecane at 20.730 mins in a peak area of 2140636 mm^2 and pentacosane at 2119427 mm^2 in RT 21.774 mins.

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 [15].

Seenivasagan and Paul [18] 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 [12].

Comparing the peak areas of hydrocarbon compounds present in healthy and damaged buds, six compounds have more peak area in damaged than healthy buds. Alpha farnescene has recorded 215542 mm^2 peak area at RT 17.305 mins in healthy buds, but has recorded 372035 mm^2 in midge damaged buds recording 1.72 per cent increase in peak area. In healthy buds, the saturated hydrocarbons, tricosane and octacosane has recorded a peak area of 4970489 mm^2 at 27.552 mins, as against 14863097 mm^2 in midge damaged buds, recording 2.97 per cent increase.

Also, saturated hydrocarbon, tetracosane recorded 1432970 mm^2 peak area in healthy buds at RT 29.056 mins as against 14863097 mm^2 peak area in midge damaged buds recording 10.07 per cent increase. The hydrocarbon, eicosane was detected at 29.046 mins with a peak area of 1432970 mm^2 in healthy buds and was detected thrice in midge damaged buds at RT 22.830 mins, 27.251 mins and 30.868 mins in a peak area of 1483204, 3295502 and 5018550 mm^2 respectively with 1.4 and 1.5 per cent increase in release. The content of the plant defense compound, alpha farnescene present in midge damaged buds was higher than the healthy ones. Thus to summarize, midge damaged buds have more peak area when compared to the healthy jasmine buds, witnessing the increased production of hydrocarbons, which in turn attracts natural enemies.

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 midge damaged buds 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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