



RESEARCH ARTICLE

Alluring response of the parasitoid *Phanerotoma hendecasiella* Cam to synomones from *Hendecasis duplifascialis* Hampson-infested jasmine (*Jasminum sambac* L.) buds and compound identification by GC-MS

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Abstract

The jasmine budworm, *Hendecasis duplifascialis*, is a serious pest of jasmine that poses a severe threat, as it damages the economic part, the buds. The parasitoid, *Phanerotoma hendecasiella* is specific to the pest and was reported to be a potent candidate in checking the pest menace naturally. Synomones produced by plants have significance in eliciting host-seeking response in several natural enemies. With the intention to test the oriental response of *P. hendecasiella* on the saturated hydrocarbons from jasmine buds infested with jasmine bud worm, a laboratory study was conducted using an eight-arm olfactometer. The investigated components were the bud and leaf extracts of the jasmine major pests viz. budworm, blossom midge, leaf webworm and two spotted mites, along with healthy jasmine bud and leaf extracts as standard checks, hexane and water as negative and positive control. The eight arms of the olfactometer were placed with the extracts in filter paper strips. The results revealed that out of the 50 parasitoids released, 11 and 25 oriented to budworm infested bud extracts after 2 and 4 hours of treatment, while it was only one in both controls. Further, GC-MS analysis of the bud worm infested jasmine buds revealed the presence of several compounds with the ability to lure beneficial insects such as allyl iso-thiocyanate, linalool, methyl salicylate and naphthalene. The orientation of *P. hendecasiella* in budworm infested bud extracts and the existence of potent components in them flags a positive signal for the utility of the parasitoid in natural budworm suppression.

Keywords: budworm; GC-MS; jasmine; parasitoid; synomone

Introduction

Jasmine (*Jasminum sambac* L.) is the oldest flowering crop, cultivated commercially in many parts of the world for its sweet-scented flowers (1). The crop is ravaged by several pests, of which, the jasmine budworm, *Hendecasis duplifascialis* Hampson pose serious threat as they damage the flower buds by boring holes (2-6). The tiny larva makes holes on the flower buds, feeds on the inner content of the bud and makes a circular hole on the corolla tube, emerges and tunnels to move into other buds of the same shoot. Infested flowers turn violet in color and fall off (7). In case of severe infestation, adjacent flower buds are webbed together by means of silken thread. The larvae were described as green larvae with black head; boring into a number of flower buds (8) (Fig. 1). To manage the pest, jasmine growers literally pours synthetic chemical pesticides, even then the menace could not be solved. Moreover, as the globe focus on “Go Green” slogan to compete toxicity in the ecosystem, attention focuses on non-chemical pest management strategies. Biological control of crop pests exerted by the third

trophic level, natural enemies’ serves as key components of integrated pest management programs that limit the pests in any ecosystem.

Exploitation of tritrophic interactions, between the crop, pest and natural enemy, involving chemical cues, can benefit agricultural systems. When a crop is injured, several biochemical mechanisms were triggered and the plant secretes chemicals as a defense strategy that acts as cues for the natural enemies of the pest. The secreted chemicals serve as synomones and aid in natural suppression of the pest without polluting the ecosystem. The braconid, *Phanerotoma hendecasiella* Cameron (Braconidae: Hemiptera) (Fig. 2) is reported to be a larval parasitoid present in jasmine ecosystem (7, 9, 10). The parasitoid is mentioned to be a specific parasitoid of jasmine budworm by several researchers (11, 12).

In light of the above, with the intention to confirm the preference of the parasitoid, *P. hendecasiella* to the jasmine budworm in jasmine ecosystem, for its utilization in IPM strategies, the current investigation was carried out, by



Fig. 1. Symptoms of damage of budworm, *Hendecasis duplifascialis* (4).



Fig. 2. Larval parasitoid, *Phanerotoma hendecasiella* (4).

extracting the volatiles present in the jasmine budworm infested buds as well as the buds and leaves of the major pests of jasmine. In addition, the chemical profile of the best extract (bud infested bud extract) was injected in GC-MS to visualize the compounds responsible for the attraction of the natural enemies.

Materials and Methods

Extraction of the saturated hydrocarbons

The saturated hydrocarbons were extracted from the healthy and pink discolored budworm damaged jasmine buds using HPLC grade hexane. 10 g of buds were 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. For comparison, extracts were taken from the buds of blossom midge, *Contarinia maculipennis*, another major bud infester of jasmine and leaves infested with leaf webworm, *Nausinoe geometralis* and two spotted mite, *Tetranychus urticae*.

Behavioral bioassay of *P. hendecasiella* using olfactometer

The orientation behavior of the parasitoid was studied using an eight arm olfactometer according to the reports (13, 14). The eight arm olfactometer had a release chamber at the center and was connected by a pure air inlet tube. For creating pure air current, the air inlet tube was connected to a blower through an air inlet chamber fitted with a charcoal filter and an air-flow

meter. The blower unit had a battery-operated mini-fan fitted in a glass tube to generate an air current at the rate of 2 m/s. The orientation behavior of the parasitoid, towards the volatile fractions, was studied under laboratory conditions at $26 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH and 150 lux light density. The four saturated test fractions from four pests of jasmine at 50 μL each, healthy jasmine buds and leaves of jasmine and a positive control with water and a negative control with hexane were placed on filter paper strips of 30 mm x 10 mm (odour source) separately at the six arms. After permitting the solvent to evaporate for 2 min, the filter paper strip was inserted in to the connector tubes from which insects were physically excluded to avoid contamination. Fifty adults of *P. hendecasiella* were released from the top of the olfactometer through the circular entrance and the number of parasitoids opting for each volatile fractions were recorded. Observations were taken at 2 hr and 4 hr after release. An air delivery system, passed humidified and purified air through Teflon tubes into the olfactometer arms. Air flow was maintained at 7.15 mL/min using a blower fitted to the four arms of the olfactometer. Vacuum cleaning was done before and after completion. Three replicates were maintained.

Identification of hydrocarbons using GC-MS:

Gas chromatography combined with mass spectroscopy, a preferable methodology for routine hydrocarbon analysis of compounds was taken up. Saturated hydrocarbon and volatile hydrocarbon analysis was carried out in a GC MS-QP 2010 Plus (Shimadzu Kyoto, Japan) mass selective detector (70 eV) equipped with a 10:1 split injector. The gas chromatograph was armed with a 30 m fused silica capillary column having 0.25 mm ID and 0.25 μm film thickness run in constant flow mode (1.0 mL/min helium). Oven temperature is programmed at 60°C (1 min hold), then to 220°C at $10^\circ\text{C}/\text{min}$ rate (5 min hold) and then to 240°C at $50^\circ\text{C}/\text{min}$ rate (8 min hold). Injector temperature was set at 275°C . One μL of the hydrocarbons was injected using auto sampler into the GC-MS system for analysis. Injections were done in split 10:1 mode. Shimadzu GC-MS Lab solution software was used for the analysis of compounds in the volatile. Injected samples were separated into various constituents with different retention time and 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 chromatogram the compounds were identified by comparing the data with the existing software libraries.

Results and Discussion

The results on efficacy of synomonal extracts of saturated hydrocarbons on the larval parasitoid of jasmine budworm, *P. hendecasiella* is given in Table 1. The results revealed that a positive scenario, with 11 parasitoids attracted to the budworm infested extract in the olfactometer test in a 2 hr time after the treatment. However, more parasitoids oriented to the healthy jasmine bud extracts (19 no's). Another bud infester of jasmine, blossom midge infested buds recorded the congregation of 10 parasitoids. The leaf extracts from healthy jasmine leaves, leaf webworm and mite infested leaves recorded only 5, 2 and 1 parasitoids respectively at 2 HAT. It was seen that the parasitoid was more oriented towards healthy and infested bud extracts after 2 hr of treatment. However, in a period of 4 hr, budworm infested jasmine bud extracts invited maximum number of parasitoids (25 no's), while the healthy jasmine buds recorded 12 no's and midge infested bud extracts had 5 insects. In addition, the three leaf extracts tested recorded 2 parasitoids each. However, the positive and negative control recorded only one parasitoid after 2 and 4 hr of treatment.

As the olfactometer test revealed the maximum congregation of the parasitoid towards budworm infested buds, it was confirmed when the jasmine buds are infested with

budworm, the jasmine plants undergo biochemical alterations that secretes compounds with the ability to attract natural enemies, especially the larval parasitoid of the pest, *P. hendecasiella*. Therefore, the budworm infested jasmine bud extracts were injected into GC-MS to visualize its chemical profile, to check the components responsible for natural enemy attraction. The healthy jasmine bud extracts were also analyzed in GC-MS for comparison. The results of the GC-MS analysis of healthy and bud worm infested buds were provided in Table 2.

Healthy jasmine buds

The saturated hydrocarbon profile of the healthy buds of jasmine, *Jasminum sambac* L. were enumerated in Table 2. GC-MS analysis of synomone extracts of healthy buds indicated 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-farnesene, 9-tricosene, octadecane, tricosane, octacosane, tetracosane, eicosane and hentriacontane, with tetracosane and octasane detected twice. Linalool was detected at the retention time of 9.278 min with the largest peak area of 5180722 mm² constituting 7.941 % of the total compounds. The hydrocarbons, octadecane, tricosane

Table 1. Evaluation on the efficacy of synomonal extracts of saturated hydrocarbons on the attraction of *Phanerotoma hendecasiella*

Treatments	<i>Phanerotoma hendecasiella</i>		
	Number of parasitoids attracted (HAT)		Mean*
	2	4	
Healthy jasmine buds	19.00 (4.35) ^a	12.00 (3.46) ^a	15.50 (3.93) ^b
Budworm infested jasmine buds	11.00 (3.31) ^b	25.00(3.46) ^b	18.00 (4.23) ^a
Midge infested jasmine buds	10.00 (3.16) ^b	5.00 (2.23) ^b	7.50 (2.74) ^c
Healthy jasmine leaves	5.00 (2.23) ^c	2.00 (1.41) ^c	3.50 (1.87) ^d
Leaf webworm infested jasmine leaves	2.00 (1.41) ^d	2.00 (1.41) ^d	2.00 (1.41) ^e
Mite infested jasmine leaves	1.00 (1.00) ^e	2.00 (1.41) ^e	1.50 (1.22) ^{ef}
Negative control (Hexane)	1.00 (1.00) ^e	1.00 (1.00) ^e	1.00 (1.00) ^f
Positive control (Water)	1.00 (1.00) ^e	1.00 (1.00) ^e	1.00 (1.00) ^f
SE	0.1225	0.1225	0.1299
CD (0.05)	0.2627	0.2627	0.2786

Table 2. Saturated hydrocarbon profile of the healthy jasmine, *Jasminum sambac* L.

Healthy buds			
RT (min)	Area (mm ²)	Name of the compound	Group
4.309	1448100	Cyclohexanol	Alcohol that consists of an alicyclic hydrocarbon, cyclohexane
4.519	378855	Cyclohexanone	Cyclohexanone is a cyclic ketone that consists of an alicyclic hydrocarbon, cyclohexane
4.690	409217	2- Pentanol	Secondary alcohol
4.690	409217	3- Hexanol	Secondary alcohol
4.690	409217	3- Heptanol	Secondary alcohol
5.033	409217	2- Pentene	Unsaturated hydrocarbon
5.671	659805	5- Nonanone	Di-n-butyl ketone
5.856	175210	Benzaldehyde	Aromatic aldehyde
5.965	1274302	Cyclopentanol	Cyclic alcohol
6.878	648174	3-Hexenol acetate	Carboxylic ester
7.558	838689	Benzyl alcohol	Aromatic alcohol
7.791	574495	Cyclo hexane	Alicyclic hydrocarbon
8.300	116978	Heneicosane	Saturated hydrocarbon
9.278	5180722	Linalool	Cyclic monoterpenoid
9.676	693649	Phenyl methyl alcohol	Aromatic alcohol
11.926	263826	Methyl salicylate	Benzoate ester of salicylic acid
17.305	215542	Alpha-farnesene	Sesquiterpenes. Naturally occur as hydrocarbon
27.250	1827170	9-Tricosene	Simple monounsaturated hydrocarbon (muscalure)
27.552	4970489	Octadecane	Saturated hydrocarbons
27.552	4970489	Tricosane	Saturated hydrocarbons
27.552	4970489	Octacosane	Saturated hydrocarbons
29.046	1432970	Tetracosane	Saturated hydrocarbons
29.046	1432970	Eicosane	Saturated hydrocarbons
30.865	3646033	Hentriacontane	Saturated hydrocarbons
30.865	3646033	Tetracosane	Saturated hydrocarbons

and octacosane were detected at the retention time of 27.552 min with the second largest peak area of 4970489 mm² constituting 7.618 % of the total compounds. Hentriacontane, tetracosane and octacosane recorded a peak area of 3646033 mm² ranking the third largest peak area at 30.865 min (Fig. 3).

Budworm, *H. duplifascialis* infested buds

The saturated hydrocarbon profile of budworm, *H. duplifascialis* damaged buds were presented in Table 3. The synomone extracts of the budworm damaged jasmine buds analyzed in GC-MS, exhibited the presence of 35 saturated hydrocarbons viz., allyl isothiocyanate, styrene, benzoic acid, linalool, benzyl carbamate, naphthalene, azulene, methyl

salicylate, methyl anthranilate, tetradecane, alpha farnesene, naphthalene, gamma muurolene, hexadecane, hexadecane 2 methyl, carbonic acid, octadecane, heptacosane, hentriacontane, nona decane 9-methyl, eicosane, tetracosane, heneicosane, heptadecane, tricosane, octacosane, dotriacontane 1 iodo, hexa decane 1 iodo, tetracosane, tetratetracontane bis (2 ethyl hexyl phthalate), eicosane, hexadecane-1 iodo and nonacosane with heneicosane and tetracosane detected twice. The compounds present in maximum quantity were dotriacontane 1 iodo, hentriacontane and hexa decane 1 iodo, detected at 28.437 min with the largest peak area of 60743911 mm² followed by heneicosane, hexadecane-1 iodo and nonacosane, detected at 27523353

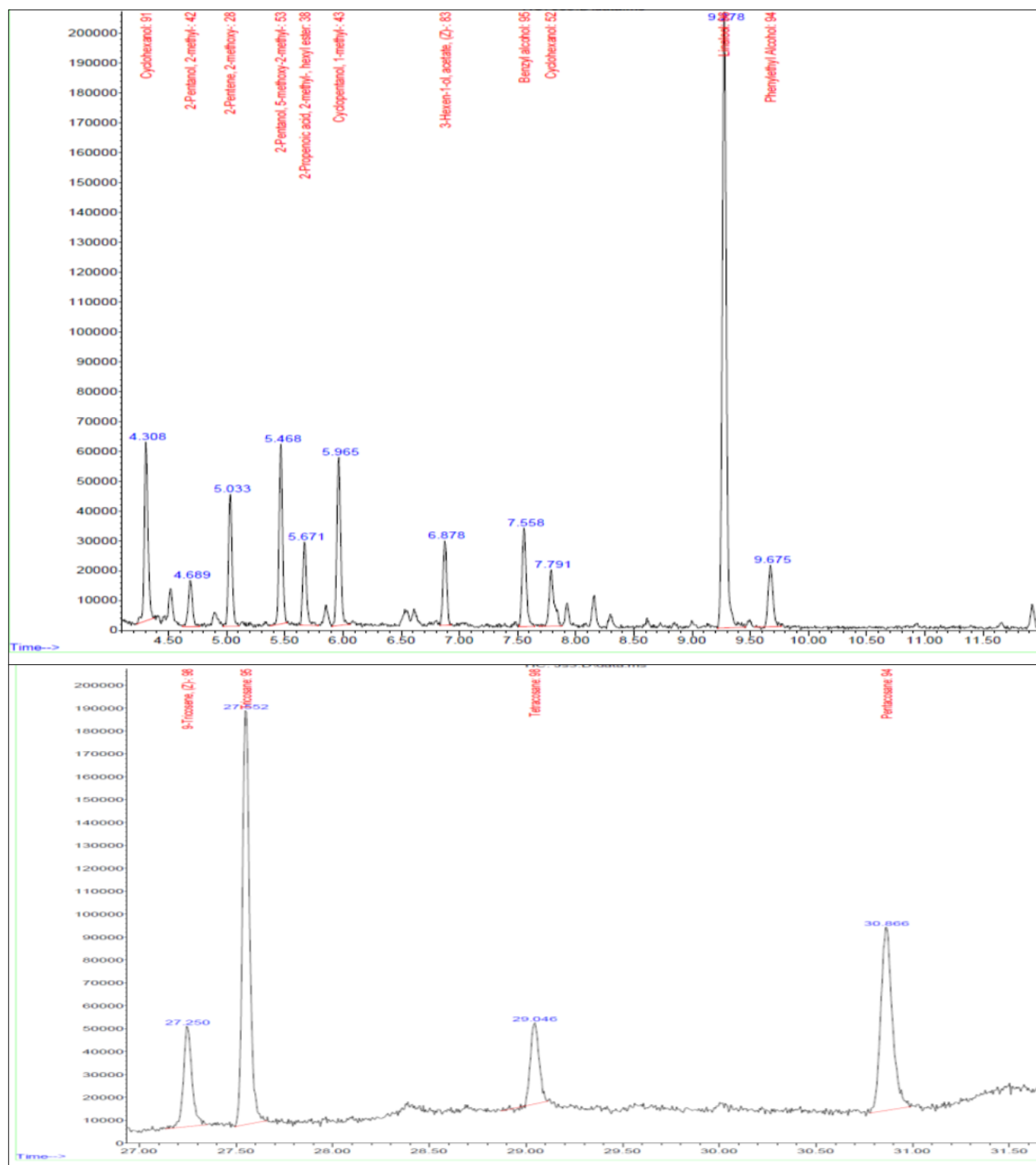


Fig. 3. Chromatographic profiles of chemical components from healthy jasmine buds.

Table 3. Saturated hydrocarbon profile of the healthy and budworm, *Hendecasis duplifascialis* damaged buds of jasmine, *Jasminum sambac* L.

Healthy buds			Budworm damaged buds		
RT (min)	Area (mm ²)	Name of the compound	RT (min)	Area (mm ²)	Name of the compound
4.309	1448100	Cyclohexanol	4.307	3650682	Allyl isothiocyanate
4.519	378855	Cyclohexanone	4.461	616209	Styrene
4.690	409217	2- Pentanol	9.174	286047	Benzoic Acid
4.690	409217	3- Hexanol	9.277	1573048	Linalool
4.690	409217	3- Heptanol	11.146	288388	Benzyl carbamate
5.033	409217	2- Pentene	11.657	432672	Naphthalene
5.671	659805	5- Nonanone	11.657	432672	Azulene
5.856	175210	Benzaldehyde	11.925	235690	Methyl salicylate
5.965	1274302	Cyclopentanol	14.849	1261857	Methyl anthranilate
6.878	648174	3-Hexenol acetate	15.722	773344	Tetradecane
7.558	838689	Benzyl alcohol	17.307	1330685	Alpha farnesene
7.791	574495	Cyclo hexane	17.493	233218	Naphthalene
8.300	116978	Heneicosane	18.315	1635542	Gamma muurolene
9.278	5180722	Linalool	18.443	3424842	Hexa decane
9.676	693649	Phenyl methyl alcohol	20.421	199105	Hexadecane 2 methyl
11.926	263826	Methyl salicylate	20.659	624871	Carbonic Acid
17.305	215542	Alpha-farnesene	20.730	2645798	Octadecane
27.250	1827170	9-Tricosene	21.048	25330057	Heptacosane
27.552	4970489	Octadecane	21.048	25330057	Hentriacontane
27.552	4970489	Tricosane	21.048	25330057	Nona decane 9-methyl
27.552	4970489	Octacosane	22.829	2007345	Eicosane
29.046	1432970	Tetracosane	22.829	2007345	Tetracosane
29.046	1432970	Eicosane	27.249	15325644	Heneicosane
30.865	3646033	Hentriacontane	27.552	19541452	Heptadecane
30.865	3646033	Tetracosane	27.552	19541452	Tricosane
			27.552	19541452	Octacosane
			28.437	60743911	Do-triacontane 1 iodo
			28.437	60743911	Hentriacontane
			28.437	60743911	Hexa decane 1 iodo
			29.004	9537561	Tetracosane
			30.861	22440473	Octadecane
			30.861	22440473	Tetracosane
			30.861	22440473	Tetracosane
			31.976	13503959	Tetratetracontane
			33.144	2274009	Bis (2 ethyl hexyl phthalate)
			33.567	27523353	Heneicosane
			33.567	27523353	Hexadecane- 1 iodo
					Nonacosane

mm². Chemical compounds with the ability to attract natural enemies' viz. allyl isothiocyanate, styrene, benzoic acid, linalool, benzyl carbamate, naphthalene, azulene, methyl salicylate, methyl anthranilate, alpha farnesene, naphthalene, γ -Muurolene were detected in budworm infested jasmine buds (Fig. 4).

An important phenomenon of nature is tritrophic interactions that involve plant, pest and its natural enemies and the mediation between them through chemical cues. The alterations in plant morphology, chemistry and resources by the pests, affects the behavior and performance of natural enemies (15). It is a modern scenario in overcoming agricultural pest management involving ecological approach, rather than using poisonous chemicals, that consequentially impact on the food chain and ecosystem. In the current investigation, the olfactometer test revealed the maximum congregation of the larval parasitoid, *P. hendecasiella* towards budworm infested buds, that confirms the bud infestation of jasmine buds by the budworm, inviting the parasitoid, *P. hendecasiella* in the jasmine ecosystem. The ability of parasitoids in locating hosts determines their success in suppressing the pest population involving chemical stimuli (16). Several such studies involving olfactometer to examine the host seeking potential of the parasitoids were investigated by several researchers. An olfactometer was used to examine the cues by which females of the parasitoid, *Spalangia endius* Walker, locate pupae of the housefly, *Musca domestica* (17). An ectoparasitoid of coleopteran stored product pest, *Anisopteromalus calandrae*

(Howard) evaluated regarding their preference in host food and various stages of the host viz. uninfested chickpea kernels, infested kernels with larvae, eggs and adults revealed that the parasitoid preferred damaged kernels with eggs and with or without larvae (16). Dual choice olfactometer assays used to determine the preference of the aphid parasitoid, *Aphelinus nigritus* and predator, *Chrysoperla rufilabris*, between plants of the same cultivar revealed that the natural enemies preferred aphid infested cultivars (18). The host location process of *Scleroderma cereicollis* and *S. domesticus* attacking xylophagous insects infesting decaying or dead or worked wood in a Y-tube olfactometer, still olfactometer and still arena, revealed their response to seasoned wood sawdust from pine and beech and host frass of the two longhorn beetles *Hylotrupes bajulus* and *Trichoferus holosericeus* (19). The behavioural reactions of *Goniozus legneri* examined in a six-arm olfactometer, revealed the frass of its host carob moth, (*Ectomyelois ceratoniae* Zeller), produced the strongest olfactory responses and the lowest response was for *Ephestia* larvae (20). The responses of insects towards their host by chemical means in an olfactometer was well experimented to substantiate the current investigation.

To elucidate the chemical cues related to the luring of the larval parasitoid, *P. hendecasiella* towards budworm infested buds, the infested bud extracts were analyzed in GC-MS, that identified 35 herbivore induced volatiles, while it was only 24 in the healthy jasmine buds without the infestation. The cyclic monoterpenoid, linalool was detected in both healthy

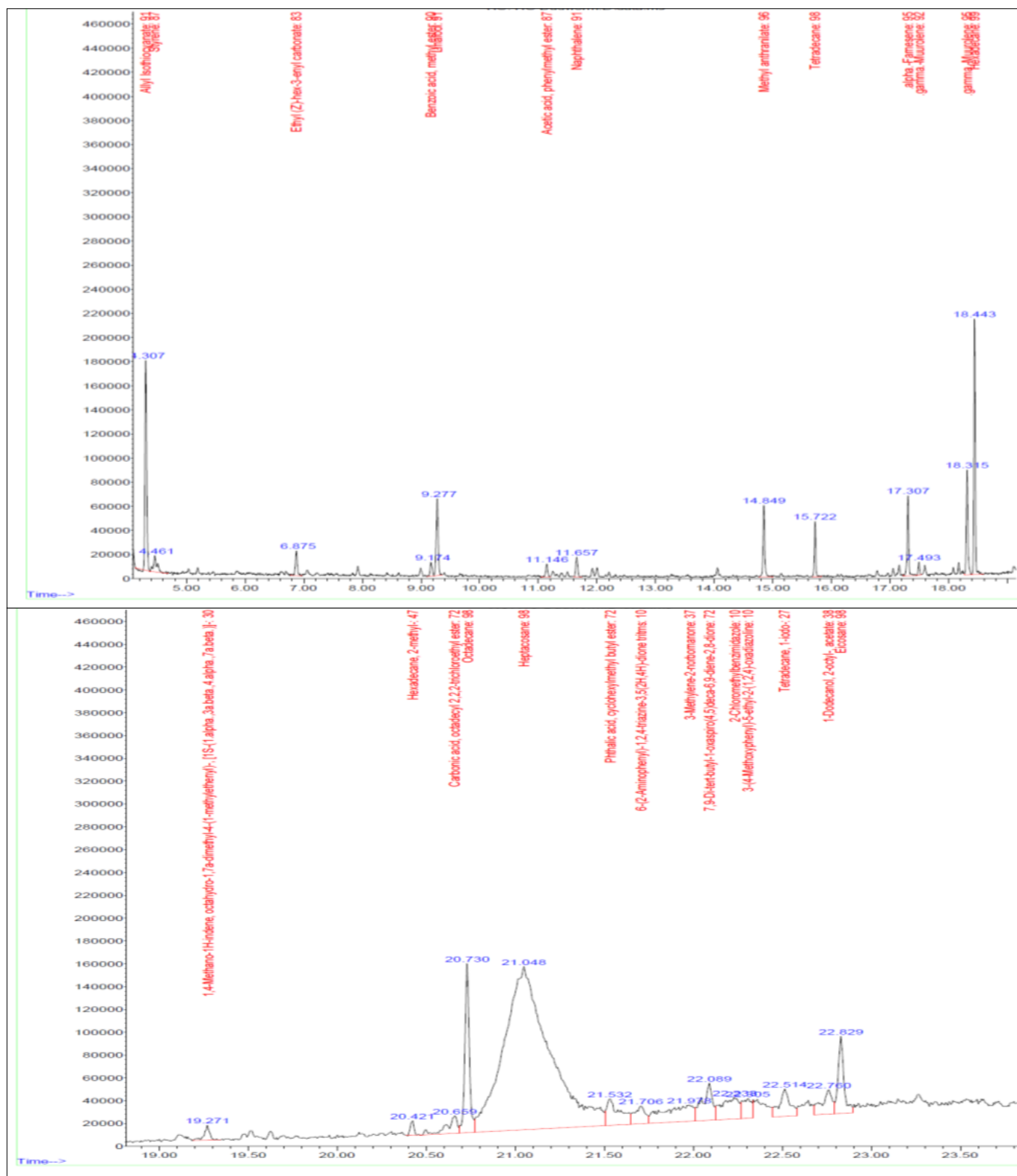


Fig. 4. Chromatographic profiles of chemical components from budworm, *Hendecasis duplifascialis* infested jasmine buds.

and infested buds at 9.27 min. However, the peak area in healthy jasmine buds were 5180722 mm² and in budworm-infested buds was 1573048 mm². Terpenoids are important plant volatiles that mediate communication between plants, pollinators and pathogens. Linalool, acyclic monoterpene, is an important flavor related volatile in plant kingdom. Flowers having linalool as dominant volatiles have broad appeal to pollinators, especially moths and bees (21). The most common fragrance chemical is found to be present in jasmine oil (22-25). Therefore, linalool present in healthy jasmine buds is justified; however, the linalool content has decreased due to budworm

infestation. Furthermore, linalool produced by plants as defense agent against insect pests and pathogens is elaborated in several research investigations (26). Linalool has a potential future use in environmentally friendly control strategies against Mediterranean fruit fly, *Ceratatis capitata* (27). It is notable that linalool occurs as two enantiomers having distinct functions. (S)-(+)-linalool mainly attracts pollinators, while (R)-(-)-linalool seems to act as insect repellents, that has been well documented accentuating its application in ecofriendly pest management (28, 29). The presence of linalool in herbivore-induced plants is supported by reports that it is the most

abundant volatile compound emitted from rice plants damaged by the fall armyworm, *Spodoptera frugiperda* (30). It is understood that the linalool in budworm infested buds have a role in acting as insect repellent. Methyl salicylate, the benzoic acid ester of salicylic acid is yet another compound present in both healthy and infested jasmine buds detected in 11.925 min in an area of 263826 and 235690 mm² respectively. It is observed that the budworm infestation reduced the emission slightly. Methyl salicylate (MeSA) is a natural plant-derived compounds, utilized as plant defence elicitor and an herbivore repellent on several crop plants (31). It was reported as a typical HIPV (Herbivore Induced Plant Volatiles) that can repel aphids and attract lady bird beetles (32, 33), specially for maize aphids (34), soybean aphids (35) and thrips (36). It was examined commercial MeSa in red maple plants and noticed a greater number of natural enemies viz. minute pirate bug (*Orius* spp.), that is a predator of thrips and aphids, fewer insect pests and less meristem damage (37). Methyl salicylate existed in jasmine buds is an encouraging factor, for the natural management of budworm and other pests of jasmine. Alpha farnesene, a naturally occurring sesquiterpene, emitted by healthy and budworm infested jasmine buds was detected at 17.30 min in 215542 mm² and 1330685 mm² respectively. It is noteworthy that the budworm infestation increased the production of the compound. Alpha farnesene, is again a notable herbivore induced volatile having the potential to manage agricultural pests (38, 39). It acts as a potential repellent for coffee borer (40) and used in managing *Asparagus* pests (41). It is a component of aggregation pheromone of the lesser mealworm, *Alphitobius diaperinus* (42), sex pheromone component of female click beetle *Selatosomus aeripennis* destructor (43) and alarm pheromone of the termite *Prorethia termitum* (44). GC-MS analysis of hydro-distillate extracts from aerial parts of the transgenic arabidopsis plants revealed biosynthesis of several novel sesquiterpenes, including beta farnesene and alarm pheromone of aphids, suggesting a potential for engineering aphid resistant strains of *Arabidopsis* (45). The existence of alpha farnesene in healthy buds and its elevated levels in budworm-infested buds pose a healthy impact on ecofriendly pest management in jasmine ecosystem.

The budworm infested jasmine buds displayed more potent components in pest management viz. Allyl iso-thiocyanate, naphthalene, azulene, methyl anthranilate and gamma muurolene. Allyl iso-thiocyanate, a naturally occurring organo-sulfur compound in mustard, radish and 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 (46). Synthetic allyl isothiocyanate is used as an insecticide, bactericide and nematocide and is used in certain cases for crop protection (47). The application of allyl isothiocyanate (AITC) has been proposed as an alternative to control stored-grain insects (48) viz. *Sitophilus zeamais*, *Rhyzopertha dominica* (Fabr.) and *Tribolium castaneum* infesting a corn grain mass and fumigant to manage the cowpea beetle, *Callosobruchus maculatus* (49). It also serves as a soil fumigant to manage soil borne pathogens and nematodes (50, 51). The existence of allyl isothiocyanate in budworm-infested buds favors natural enemies in the crop ecosystem for eco-friendly pest management in jasmine.

Styrene, a vinyl benzene compound, is detected in meagre amount in 616209 mm² area at 4.461 min in the budworm infested buds. The compound is recommended as a biocontrol agent in the protection of root-knot nematodes (52). The findings corroborate with previous study results, which detected styrene in rice stem borer infested leaves, but its potential in attracting natural enemies is not evident (53). The benzoic acid and its methyl ester, methyl benzoate detected at 9.174 min in 286047 mm², is reported as a multipurpose insecticide and fungicide with important modes of action, as a contact toxicant, a fumigant, an ovicidal toxin, an oviposition deterrent, a repellent and an attractant. Methyl benzoate is a relatively new botanical insecticide that occurs naturally as a metabolite in plants and its odour attracts insects, that could be utilized in pest management for luring natural enemies (54). More research arenas are required in this aspect; however, the presence of benzoic acid in budworm infested buds is an encouraging factor, to manage the pests of jasmine. Further, a compound benzyl carbamate was detected in 288388 mm² at 11.146 min. Carbamates are generally pesticides that are esters of carbamic acid (55). The presence of a carbamate in bud worm infested buds is a promising aspect for its prospects in pest management in jasmine ecosystem. The polycyclic aromatic hydrocarbon (PAH), naphthalene was detected twice at 11.657 and 17.493 mins in 432672 and 233218 mm². Naphthalene has been reported as potential substance against insects in several studies (56, 57). The compound was reported to be a semiochemical attracting natural enemies of stem borer in maize ecosystem (58). Azulene, a non-benzoid or naphthalene isomer and aromatic hydrocarbon compound, was detected along with naphthalene at 11.657 mins in 432672 mm². Azulene was described as an insect antifeeding compound, derived from the brown algae, *Dictyota dichotoma* (59). The existence of naphthalene and its derivatives in budworm infested bud chemical fusion, will aid in its utilization as a commercial lure, for budworm management in jasmine. A monoterpene hydrocarbon, gamma muurolene was detected in 18.31 min at 1635542 mm². The results corroborate with previous findings, which detected styrene in rice stem borer infested leaves, but its potential in attracting natural enemies is not evident (60). However, the compound is present in the essential oils of several medicinal plants with insecticidal abilities such as lemongrass (*Cymbopogon citratus*) that has insect repellent activity against, *Sitophilus granarius* Linnaeus (61). The carbonic acid was detected in 624871 mm² in 20.66 min. The compound apart from its applications in plant growth serves as an insecticide as well (62). Bis (2-ethylhexyl) phthalate, a Phthalic acid ester, was abundantly found in 13503959 mm² in 31.976 mins. Earlier studies reported the presence of volatile, bis (2-ethylhexyl) phthalate in honeydew from both *Bemisia tabaci* on cabbage and *Trialeurodes vaporariorum* on cucumber and its role as kairomone in host-searching of parasitoids (63). The compound is reported to possess larvicidal action on the destructive, *Culex quinquefasciatus* mosquitoes. Larvicidal potency on *C. quinquefasciatus* Say larvae displayed 100 % mortality of bis (2-ethylhexyl) phthalate 250 ppm after 72 hr with LC₅₀ of 67.03 ppm (64). The presence of the compound in insect repellent formulations and utilized as pesticides is well documented (65-67). 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. As bis (2-ethylhexyl) phthalate is also six carbon compound, produced due to bud herbivory in jasmine ecosystem by the budworm, provides chances for its potential role in natural enemy attraction as a bio formulation.

In addition to the several specific compounds with insect repellent and natural enemy attractant qualities in budworm infested jasmine buds, the major compounds detected were saturated hydrocarbons. The surface layers of the plant parts are coated with waxy substances that hold innumerable functions such as a diffusion of water and solutes, apart from releasing volatiles that attract natural enemies and pollinators. Those layers composed of saturated hydrocarbons are microcrystalline in structure and forms the exterior line of the cuticular membrane. They serve as synomones and play a chief role in guiding the natural enemies to the crop ecosystem to prey on the host insects (68, 69). Such cues might be utilized to stimulate the foraging and host selection behavior of entomophages, thereby, increase their effectiveness in integrated pest management (12). Hexane extracts of different varieties of tomato provoked synomonal response in *Trichogramma chilonis*. The response seemed to be associated mainly with tricosane (C23), heneicosane (C21), pentacosane (C25) and hexacosane (C26) during the flowering period (70). Plant synomones may be utilized in chemical free safe pest management to enhance foraging and host selection behavior of entomophages to formulate efficient IPM strategy (4). Approximately 15 saturated hydrocarbons were detected in budworm infested buds viz. tetradecane, hexadecane, octadecane, heptacosane, hentriacontane, nonadecane, eicosane, tetracosane, heneicosane, heptadecane, tricosane, octacosane, dotriacontane, tetratetracontane and nonacosane, with most compounds detected several times. The component detected in maximum quantity in budworm infested buds were Do-triacontane 1 iodo, Hentriacontane and Hexa decane 1 iodo, detected at 28.437 min in 60743911 mm². The following level of highest quantity was detected in Nonacosane and Hexadecane- 1 iodo again at 33.567 min in 27523353 mm², trailed by Heptacosane, Hentriacontane and Nona decane 9-methyl at 21.048 min at 25330057 mm², tracked by Octadecane, Tetracosane and Tetratetracontane at 30.861 min in 22440473 mm², chased by Heptadecane, Tricosane and Octacosane at 19541452 mm² in 21.552 min and Heneicosane at 27.24 min in 15325644 mm². The compound present in largest quantity repeatedly in the chromatographic profile, denotes its probable efficacy in natural enemy attraction. Previous research states that the hexadecane is the component of the pheromone of leek moth, *Acrolepiopsis assectella* (71). Furthermore, the related compound, *cis*-11-hexadecenal and *cis*-9-hexadecenal were the sex pheromones of the most notorious pest, *Helicoverpa armigera* (72). The compound, tetracosane, appeared twice on 22.82, 29.004 and 30.861 min in an area of 2007345, 9537561 and 22440473 mm². It was reported the presence of tetracosane in the two spotted mite infested jasmine leaves in GC-MS analysis and the natural enemy of the mite, the predatory thrips *Scolothrips sexmaculatus* being attracted to the mite infested leaf extracts in an olfactometer study (73). The compound, hentriacontane was detected twice in 21.048 and 29.004 mins in an area of 25330057 and 60743911 mm². In a study, to examine the chemical cues from the honeydew and cuticular extract of the spiraling whitefly, *T. vaporariorum*, attracted its parasitoid, *Encarsia formosa*, serving as a synomone, stated

positively with the most abundant compound in the extracts were nonacosane and hentriacontane (74). The cuticular hydrocarbons of parasitoid wasp *Leptopilina pacifica* was dominated by 9-hentriacontane, that serve as pheromone providing chemical signal (75). The compound, octadecane, appeared twice at 20.7 min and 30.861 min in 2645798 and 22440473 mm². Octadecane potentiated *T. japonicum* foraging activity against rice yellow stem borer (76). Gel formulation of octadecane (Saturated hydrocarbon) when applied 24 hr after the release of *T. chilonis* in wheat and chickpea enhanced the foraging activity against pink stem borer (*Sesamia inferens*) and pod borer (*H. armigera*) respectively, resulting in reduced symptoms and increased grain yields (77). Eicosane is the main component in the herbivore induced volatile profile of rice plants infested by variousoppers in tillering stage compared to panicle initiation and grain maturation stages (78). Furthermore, the saturated hydrocarbons tricosane, octadecane, octocosane, tetracosane, eicosane, hentriacontane, tetracontane has appeared in healthy and jasmine infested bud extracts in varying levels. The saturated hydrocarbons as a thin waxy layer in the buds of jasmine flowers, might serve to release the volatiles from plants that trail auxillary insect and other fauna as it poses positive response to larval parasitoid, *H. hendecasiella*. More research arenas in these crop-pest-auxillary fauna interactions are anticipated in jasmine for more productive responses for commercial bio formulations.

Conclusion

In a crop ecosystem, the tritrophic interaction between plants, herbivores and natural enemies are mediated by chemical cues. Synomones elicited by plants due to herbivory aid host seeking behavior of natural enemies. The cuticular layer of the plants when damaged elicits several hydrocarbons that have the potential in luring the natural enemies of the pest. Saturated hydrocarbons present in host plants were found to act as synomones for natural enemies in different crop ecosystems. Jasmine is an important traditional flower crop, infested by several pests; budworm, *H. duplifascialis*, is the major pest of jasmine infesting the buds. The knowledge on the chemistry involved in such complicated natural interaction between budworm and its parasitoid is beneficial in enhancing conservation biological control (CBC) of using population of its larval parasitoid, *P. hendecasiella*. The investigation on the tritrophic interaction between the jasmine budworm and its parasitoid, *Phanerotoma hendecasiella* mediated by chemical cues revealed a positive response for the parasitoid in its attraction to the buds infested by jasmine budworm. The GC-MS analysis of the herbivore induced synomones revealed the presence of 35 compounds most of them with latent abilities to attract diverse natural enemies, including several saturated hydrocarbons. The chemicals with potentials in natural enemy attraction such as allyl iso-thio cyanate, linalool, methyl salicylate, styrene, naphthalene, bis (2 ethyl hexyl phthalate) were present in the synomones of budworm infested buds, that paves an affirmative response for the attraction of *P. hendecasiella* in jasmine ecosystem.

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Authors' contributions

The sole author conceptualized the idea of the investigation, designed the experiment participated in the methodology, conducted formal analysis, drafted the manuscript, reviewed, edited, fine-tuned and approved the final draft.

Compliance with ethical standards

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