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Behavioural response of *C. sexmaculata* to the volatiles from *B. brassicae* and cabbage

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ABSTRACT

The behaviour and orientation modifying cues from host plants and prey insects play vital roles in the process of host selection and acceptance by predators. So, we studied the volatile organic compounds of un-infested and infested cabbage plants by cabbage aphid, *Brevicoryne brassicae,* through Gas Chromatography-Mass Spectrometry (GC-MS). Overall, 58 and 50 compounds were identified in un-infested and infested cabbage plants through DCM and DEE extract, respectively. Based on the peak area from the GC-MS analysis, the volatile organic compounds from infested cabbage were significantly greater than that from un-infested cabbage extract. In the DCM extract, volatile compounds, notably Cyclohexasiloxane tetradecamethyl, Cyclopentasiloxane decamethyl, and 3-Hexen-1-ol were released in significant amounts. In the DEE extract, 3-Hexen-1-ol, (Z)-, Cyclohexasiloxane dodecamethyl, and Cyclopentasiloxane decamethyl were also released in substantial quantities. The Y-tube olfactometer study revealed that *C. sexmaculata* adults exhibited significantly greater attraction to the volatiles emitted from cabbage infested with aphids compared to any other extract. Additionally, their response time was notably shorter when exposed to the infested cabbage with aphid extract. The results indicated that the *C. sexmaculata* could discriminate the infested cabbage and significantly responded to the plant odour, which suggested that, the synomones (volatiles from cabbage plant) and kairomones (volatiles from aphid body) played a vital role in the orientation behaviour of *C. sexmaculata* to locate the cabbage aphid.

Key Words: *Brevicoryne brassicae*, cabbage, *C. sexmaculata*, DCM extract, DEE extract, GCMS, olfactometer, rotary evaporimeter, VOC.

INTRODUCTION

Cabbage aphid, *Brevicoryne brassicae* (Linnaeus) (Aphididae: Hemiptera) is a primary pest of Brassicas worldwide (2,14). The aphid infestations resulted in a significant economic loss since they infested all the above-ground parts and destroyed crops by sucking sap from them (23). The aphid infestation resulted in stunted growth, withered flower and deformed pod, thereby causing 30-96 % yield loss (7,17). The aphids also severely affect the quality of the crops by excreting a sugary product known as ‘honeydew’ leading to the growth of a sooty mold fungus, which reduces the photosynthetic area of the leaves and the market value of the crop (4).

Plants release volatile organic compounds (VOCs) in response to insect feeding (26,31), which are essential for herbivorous insects to perceive and serve as a primary means of plant communication with their surroundings. These emitted VOCs play a crucial role in plant-insect interactions by facilitating insect communication and enhancing plant defense mechanisms (11). VOCs are significant in these interactions because they act as attractants, guiding natural predators

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to suitable hosts for feeding. These compounds can have various functions, including direct toxicity or deterring feeding (28,32), attracting predators (19), and triggering secondary plant defense responses (8).

The Coccinellidae comprises approximately 6000 described species in about 360 genera and 42 tribes (13). India offers a diverse range of coccinellids. The most widely distributed of these species are *Coccinella transversalis* (Fabricius), *Brumoides suturalis* (Fabricius), and *Cheilomenes sexmaculata*, which are known as bio-suppressive agents of aphids and occupy a special place among the aphidophagous predators due to their widespread distribution and good predating ability both in the larval and adult stages. Many species of coccinellids have effectively brought down the field population of sucking pests including aphids (5). Hence, ladybirds are being utilized in various parts of the world for augmentative biological control (29). When the aphids feed on the plant leaves, the plant releases blends of volatiles as a response to the infestation by aphids. Plants strategically release volatile compounds into the environment (20) to attract the natural predators of insects. These compounds serve as cues for insects like *C. sexmaculata* to detect and locate where the aphids are feeding on the host plant (12).

A high population of *B. brassicae* on cabbage can lead to the aggregation of a large number of beetles, whereas healthy, un-infested cabbage plants do not attract beetles due to their inability to locate them (30). With this background, we hypothesized that identifying VOCs released from the healthy and infested cabbage plants by cabbage aphid *B. brassicae* helps elucidate the orientation response of six spotted ladybird beetles towards the infested plants. Further, the time taken for the beetles to reach the odour source with the help of Y-tube olfactometer studies has field application.

MATERIALS AND METHODS

I. Plant materials

The Cabbage (*Brassica oleracea var. capitata*) seeds were sown in pots filled with soil and nutrient mixtures and later the pots kept under the greenhouse conditions. The temperature (23- 25°C) and relative humidity (60-70 %) plays an important role in the growth and development of cabbage (9). When the seedlings reached the 2- 3 leaves stage, each pot was covered with screen cages made from an anti-insect white mesh net to prevent attack by insect pests. Before the collection of leaves, the potted cabbage plants were maintained in the screen cages inoculated with aphids, *B. brassicae* (30 days after the transplanting) and the leaves were collected 15 days after the inoculation, *i.e.*, from 45 days old plants and the leaves collected from the un-infested cabbage plants were used as a control for the experimental purpose.

For comparison, we conducted volatile extraction from different sample types: Un-infested Leaves (UL) representing healthy, 45-day-old cabbage leaves without any infestation, With Aphids (WA) involving leaves infested with aphids, With-Out Aphids (WOA) where aphids were removed from the previously infested cabbage plant leaves, and Aphid Only (AO) for the extraction of volatile compounds exclusively from aphids. Dichloromethane (DCM) and Diethylether (DEE) were used separately for all the sample types. The following abbreviations were used for the extracted material: Extract DC 1 and DE 1: un-infested or healthy leaves were extracted with DCM (DC 1) and DEE (DE 1), respectively; extract DC 2 and DE 2: cabbage plant infested with aphid were extracted with DCM (DC 2) and DEE (DE 2), respectively; extract DC 3 and DE 3: infested leaves extract along with the aphids were extracted with DCM (DC 3) and DEE (DE 3), respectively;

extract DC 4 and DE 4: Aphids, *B. brassicae* were collected from infested cabbage plants with the help of clean camel hair brush and extracted with DCM (DC 4) and DEE (DE 4), respectively.

The extraction material for treatments was 50 gms of leaves with or without aphids, was collected and cut into small pieces with scissors and then submerged overnight in 150 ml each of dichloromethane (DCM) and Diethylether (DEE). In contrast, 10 g of aphids were utilized to extract AO (Aphid Only) treatment and submerged overnight in 30 ml of solvents. Then the extract was filtered using Whatman No.1 filter paper by passing through 15 g of anhydrous sodium sulphate (Na2SO4) to remove water from the extracts. Then, the extracts were decolorized by passing through the activated charcoal and concentrated under *vacuum* at 40-45 °C and 45-50 °C for DCM and DEE, respectively, using a rotary evaporimeter. The extracts were prepared to a final concentration of 1 ml, collected in different GC-MS vials, and kept in a deep freezer at -80°C. GC-MS (Gas Chromatography and Mass Spectrometry) and olfactometer studies were conducted using these concentrated extracts.

II. GCMS studies

- The main objective of the GC-MS was to separate and analyze the VOCs.
- The separation and identification of compounds from different plant extracts was carried out using GC-MS (Shimadzu QP 2010 Ultra) on an Rtx-5ms column measuring 30×0.25 mm and the NIST14 library.
- Helium gas was used as a carrier at one milliliter per minute (ml/min). A 0.2 nylon syringe filter was utilized to filter each extract for the GC-MS analysis. Then, an auto sampler injected one µl of each sample (extract).
- The ion source and injector temperatures were kept at 200°C and 230°C, respectively for complete evaporation upon injection and also it helps in the separation of the compounds.
- The identification was done by comparing the mass spectrum fragmentation pattern with the compounds kept in the MS library (25,26).

III. Olfactometer studies

A glass Y-tube olfactometer (Fig. 1) was employed to investigate the responses of *C. sexmaculata* males and females to various concentrations of extracts. The olfactometer had arms with a length of 15 cm each, a central stem measuring 10 cm in length, and an internal diameter of 3.5 cm. The experimental space was maintained at 27 ± 2°C and 65- 70 % RH, and a 40 W fluorescent lamp served as the only source of light. Each arm's average airflow per minute was 0.5 ml and the air was ventilated for 5 min before experimentation. For each concentration, 50 µl of the solution was applied to a strip of filter paper (Whatman No. 1, 2.5×0.5 cm in size), allowed to evaporate for 30 seconds, and kept within the olfactometers arm. Another arm was used as a control with the respective solvent (DCM and DEE). The concentrated solutions served as 100 % stock solutions. In preliminary experiments, we assessed the stock solution at various concentrations (1, 5, 10, 15, 25, 50, 75 and 100 percent) to establish standardized test concentrations. After evaluating the responses of the adult subjects during these pre-trials, four specific test concentrations (1, 5, 10, and 15) were selected for each treatment.

IV. Behavioral studies:

The grubs and adults of *C. sexmaculata* were collected from the cabbage fields of ICAR-Indian Agricultural Research Institute, New Delhi, India and the stock cultures were maintained on the cabbage aphids under laboratory conditions. A batch of 20 beetles were collected in each vial and starved for 8 hours. Ten male and female beetles were released separately at the end of the main

Fig 1: Y-tube olfactometer for orientation response studies

stem of the olfactometer for each replicate and repeated eight times. Thus, the behaviour of 160 beetles (80 males and 80 females) was examined, when the beetles had crossed half the length of an arm, it was recorded as a selective response to the odour and the time taken to reach the odour source was also recorded. The behaviour of the beetle is examined based on the response to the odour source. For each batch, the test solution was applied freshly. The olfactory response of each sex was recorded for 30 min continuously for each extract at different concentrations. For each trial, the tested adults and the position of the odour source were changed, and the olfactometer was also washed with ethanol after each replication (26,29).

V. Data Analysis

The data on orientation response of a dual choice test (Y-tube olfactometer) was converted to Arc sin to reduce the variation within the data and further subjected to GLM univariate analysis using Sigma Stat v2.03 (SPSS Inc., Chicago, IL). Arc sin transformation can be used for data that represents percentages. GLM univariate analysis provides regression analysis and analysis of variance for one dependent variables by one or more variables. The Orientation Response Time (ORT) of males and females to different extracts at different concentrations was compared using a two-sample t-test (WASP 1.0).

RESULTS AND DISCUSSION

A significant difference in the emitted volatiles between the cabbage infested by *B. brassicae* and the un-infested cabbage treatments were evident in the analysis. Several compounds were present in all the extracts identified by GC-MS analysis. The plants that were damaged by the *B. brassicae* (WA) could change the plant odour emission, and the released volatiles were significantly higher than those from un-infested plants (UL) and only aphid extracts (AO).

In DCM extracts, the volatile organic compounds emitted from all extracts and the identity of volatiles from the extracts confirmed that, the infestation of cabbage with aphids significantly released in high amounts, such as Cyclohexasiloxane tetradecamethyl followed by Cyclopentasiloxane decamethyl, 3-Hexen-1-ol, 3-Hexen-1-ol acetate, (E)-, compared to un-infested cabbage (Table 1 and Fig. 2). The percentage area that observed was 16.16, 15.08, 14.98 and 14.58 respectively, compared with the volatile compounds that were released from un-infested cabbage. The results show that the number of compounds released from cabbage were more after the infestation of the plants with *B. brassicae*. Similarly, the volatile organic compounds like Diethyl Phthalate, Tetradecanoic acid and 3-Hexen-1-ol, (E) released in high amount in un-infested cabbage, however when the cabbage is infested with aphids, the concentration of aforesaid compounds was reduced due to the insect-plant interaction.

Table 1. Qualitative analysis of aphid and cabbage volatiles in DCM extracts

S. No.	Chemical compound	Ret. time	UL	WOA	WA	AO
1	1,3-Dioxolane, 2-methoxymethyl-2,4,5-trimethyl	4.065	✓	X	X	X
2	Toluene	4.402	✓	✓	✓	X
3	1,5-Heptadiene, 3,4-dimethyl-	4.567	X	✓	✓	X
4	2,6-Octadiene, 4,5-dimethyl-	4.590	X	X	X	✓
5	Cyclopropanecarboxylic acid, 2-pentyl ester	5.22	✓	✓	✓	X
6	3-Hexen-1-ol, (E)-	5.264	✓	✓	✓	X
7	1,1-Dimethyl-3-chloropropanol	5.302	✓	✓	✓	✓
8	2-Hexanol, (S)-	5.409	✓	✓	✓	✓
9	Butane, 2,3-dichloro-2-methyl-	5.753	X	✓	✓	X
10	2-Pentanone, 4-hydroxy-4-methyl-	6.512	✓	✓	✓	X
11	3-Hexen-1-ol, (Z)-	6.98	✓	✓	✓	X
12	o-Xylene	7.210	✓	✓	X	✓
13	p-Xylene	7.231	X	X	✓	X
14	Thiocyanic acid, 2-propenyl ester	7.417	X	X	X	✓
15	Allyl isothiocyanate	7.814	X	✓	X	✓
16	Benzene 1,3 dimethyl	8.033	X	✓	X	✓
17	Butane, 1,3-dichloro-3-methyl-	8.015	X	X	✓	X
18	Pentane, 1-chloro-5-iodo-	8.206	X	X	✓	X
19	Pentane 1-bromo-5-chloro	8.250	X	✓	X	X
20	Thiazole, 5-methyl-	8.260	✓	✓	✓	X
21	1-Butene, 4-isothiocyanato-	11.495	X	X	X	✓
22	3-Hexen-1-ol, acetate, (E)-	12.245	✓	✓	✓	X
23	Acetic acid, cyclohexyl ester	12.275	✓	X	X	X
24	Triethyl phosphate	12.349	X	X	✓	X
25	Eucalyptol	13.246	X	✓	X	X
26	Dodecane	17.496	X	X	✓	X
27	Cyclopentasiloxane, decamethyl	17.895	✓	✓	✓	X
28	1-Tetradecene	19.672	✓	✓	✓	✓
29	Cyclohexasiloxane, dodecamethyl	24.110	X	✓	✓	X
30	Tetradecane	26.493	✓	✓	✓	✓
31	Cyano-3-methylsulfinyl propane	26.648	X	✓	X	X
32	Pentadecanal-	26.754	✓	✓	✓	X
33	cis-beta-Farnesene	28.714	X	X	X	✓

34	Cycloheptasiloxane, tetradecamethyl	29.805	✓	✓	✓	X
35	2-Butenedioic acid, dibutyl ester	30.417	X	✓	✓	X
36	Tetradecyl trifluoroacetate	31.311	X	X	✓	X
37	Cyclopropane, 1-methyl-1-(1-methylethyl)-2-nonyl-	32.705	X	✓	X	✓
38	2-Methyl-4-tributylsilyloxyoct-5-yne	32.790	X	X	X	✓
39	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	32.877	X	X	✓	X
40	n-Pentadecanol	32.898	X	X	X	✓
41	Diethyl Phthalate	32.915	✓	✓	✓	X
42	Heptadecane	33.127	X	X	X	✓
43	Tetradecanal	33.133	✓	✓	✓	X
44	2-Pentadecanone	33.539	X	X	✓	X
45	Dodecylcyclohexane	34.790	X	✓	X	X
46	Cyclooctasiloxane, hexadecamethyl	34.890	✓	✓	✓	X
47	Ethylene diacrylate	35.907	X	✓	X	X
48	Pentadecanal-	36.064	✓	✓	✓	X
49	Tetradecanoic acid	36.503	✓	X	✓	X
50	Octacosan-14-one	36.682	X	X	X	✓
51	n-Nonadecanol-1	37.921	X	X	✓	X
52	Z-5-Nonadecene	38.450	✓	X	X	X
53	Heneicosane	38.619	X	X	✓	X
54	2-Hexadecanone	38.8	✓	✓	✓	X
55	Eicosane	38.820	X	✓	X	✓
56	Cyclononasiloxane, octadecamethyl-	38.886	✓	✓	✓	X
57	1-Hexadecanol, acetate	39.128	X	X	X	✓
58	Neophytadiene	39.504	X	✓	✓	X

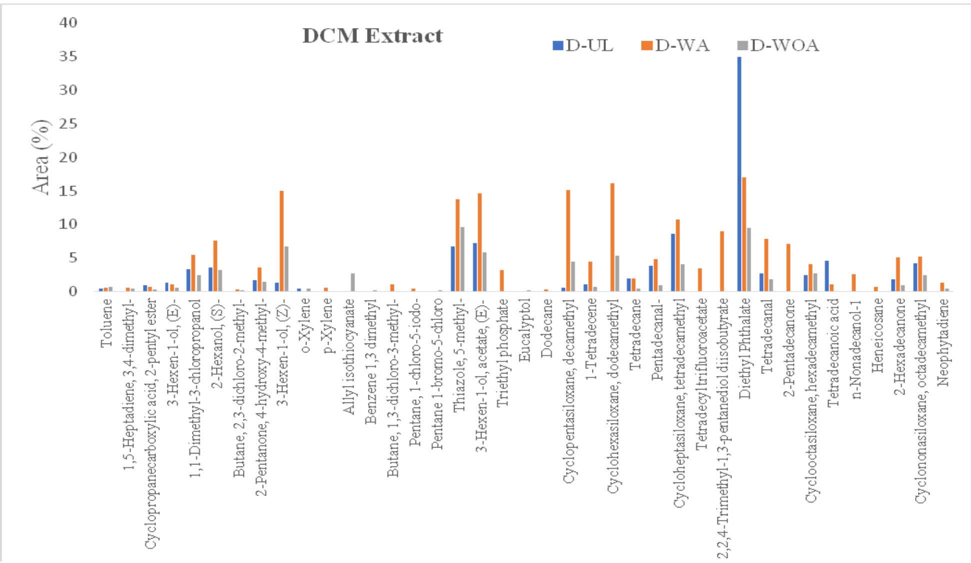

Fig. 2. Relative abundance of different volatile components in DCM extracts

Table 2. Qualitative analysis of aphid and cabbage volatiles in DEE extracts

S.No.	Chemical compound	Ret. time	UL	WOA	WA	AO
1	1,3-Dioxolane 2,2,4,5-tetramethyl-cis-	4.01	X	X	✓	X
2	Acetic acid	4.211	X	X	X	✓
3	Propanoic acid	5.052	X	X	X	✓
4	Cyclopropanecarboxylic acid, 2-pentyl ester	5.221	✓	X	✓	X
5	Cyclotrisiloxane, hexamethyl	5.675	X	X	✓	X
6	1,2-Propanediol, 3-methoxy-	5.786	X	✓	✓	X
7	Cyclohexene, 4-ethenyl	5.975	✓	✓	✓	X
8	2-Propenoic acid, 2-methyl-, ethenyl ester	6.134	X	✓	X	X
9	3-Penten-2-one, (E)-	6.15	X	✓	✓	X
10	2-pentanone, 4-Hydroxy-4-methyl	6.44	✓	✓	✓	X
11	3-Hexen-1-ol, (Z)-	6.955	✓	✓	✓	X
12	o-Xylene	7.240	X	✓	X	X
13	Allyl Isothiocyanate	7.834	X	X	X	✓
14	Styrene	7.958	X	X	✓	X
15	1-Butene, 4-isothiocyanato-	11.479	X	X	X	✓
16	trisiloxane, 1,1,1,5,5,5-hexamethyl-3-[(trimethylsilyl)oxy]-	11.875	X	X	✓	X
17	Cyclotetrasiloxane, octamethyl-	11.885	X	✓	X	X
18	Hexanoic acid	11.962	X	X	✓	X
19	Thiazole, 5-methyl-	12.28	✓	✓	✓	X
20	3-Hexen-1-ol, acetate, (E)-	12.372	✓	✓	✓	X
21	1-Hexanol, 2-ethyl-	13.285	X	X	✓	X
22	2-Propyl-1-pentanol	13.300	X	✓	X	X
23	Cyclopentasiloxane, decamethyl-	17.870	✓	✓	✓	X
24	Dodecane	19.71	✓	✓	✓	X
25	Cyclohexasiloxane, dodecamethyl-	24.012	✓	✓	✓	X
26	Cyclohexane, 1,2,4,5-tetraethyl-,	25.688	✓	X	X	X
27	Tetradecyl trifluoroacetate	26.529	X	X	✓	X
28	1-Tetradecene	26.650	✓	✓	X	X
29	Tetradecane	26.794	✓	✓	✓	X
30	Pentadecanal-	30.45	✓	X	✓	X
31	2,4-Di-tert-butylphenol	30.633	X	X	✓	X
32	3-Eicosene	32.650	✓	X	X	X
33	9-Eicosene	32.72	X	X	✓	X
34	n-Nonadecanol-1	32.919	X	✓	✓	X
35	Tridecane 2,5-dimethyl	32.95	✓	X	X	X
36	Diethyl Phthalate	33.02	✓	✓	✓	X
37	Decane, 2,8,8-trimethyl-	33.149	X	X	✓	X
38	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	33.154	X	✓	X	X
39	Cyclooctasiloxane, hexadecamethyl	34.90	✓	X	✓	X
40	Eicosane	36.055	✓	X	X	X
41	2-methyloctacosane	36.211	✓	X	X	X
42	1,2-Diphenyl-1-isocyanoethane	36.825	X	X	✓	X
43	Benzene, 1,1'-(1,2-cyclobutanediyl) bis-, trans-	37.732	X	X	✓	X
44	Tetradecanoic acid	37.996	X	X	✓	X
45	Trifluoroacetic acid, pentadecyl ester	38.43	X	X	✓	X
46	n-Nonadecanol-1	38.658	✓	✓	✓	X
47	Heneicosane	38.834	✓	X	X	X
48	Heptadecane, 2,6,10,15-tetramethyl-	38.842	X	X	✓	X
49	Cyclononasiloxane, octadecamethyl-	39.032	✓	X	✓	X
50	Neophytadiene	39.916	X	X	✓	X

On the other hand, with DEE extracts, the presence of certain VOCs such as 3-Hexen-1-ol, (Z)-, Cyclohexasiloxane dodecamethyl-, Cyclopentasiloxane decamethyl, and Styrene unequivocally indicates that cabbage infested with aphids releases a considerably higher quantity of these compounds compared to uninfested cabbage. The percentage area observed was also higher *viz*., 27.60, 22.01, 16.61 and 16.19, respectively compared to volatiles that were released from un-infested cabbage (Table 2 and Fig. 3). The volatile organic compounds such as 3-Hexen-1-ol, acetate, (E)-, Tetradecane, n-Nonadecanol- and Heneicosane released in high amount in un-infested cabbage compared to the cabbage plants infested with aphids.

Comparative analysis of extracts revealed that DC 3 extract had additional compounds like Butane 1,3-dichloro-3-methyl-, Pentane 1-chloro-5-iodo, Triethyl phosphate, Dodecane, Tetradecyl trifluoroacetate, n-Nonadecanol-1 and Heneicosane (Table 1). Similarly, extract DE 3 had additional emission of Cyclotrisiloxane hexamethyl, Styrene, trisiloxane 1,1,1,5,5,5-hexamethyl-3-[(trimethylsilyl)oxy], Hexanoic acid, 1-Hexanol, 2-ethyl Tetradecyl trifluoroacetate, 9-Eicosene and Tetradecanoic acid compared to healthy plant (un-infested leaves) volatiles (Table 2). The release of these additional compounds or alterations in their proportions following aphid infestation may play a crucial role in enhancing the attraction of natural enemies and reducing the time required to locate the odor source. Essentially, this variability in volatile emissions can be seen as a means of conveying the presence of the host plant and its prey, signifying the quality of the host. This is a recognized plant strategy for defending against herbivorous insects by enticing their parasitoids and predators. Our findings align with previous research on the emission profiles of herbivore-induced plant volatiles (HIPVs), as observed in wild tobacco (*Nicotiana attenuata*), when responding to the tobacco hornworm, *Manduca sexta* (L.). In this context, the volatile blends contained green leaf volatiles, sesquiterpenes, and 3-Hexen-1-ol acetate, all of which were responsible for attracting predator species. Manipulating these biochemically mediated tri-trophic interactions offers potential avenues to enhance the field efficiency of augmentative biological control (16). Ahmed *et al.* (1) reported that the compounds from infested broccoli were significantly more compared with the un-infested broccoli, such as D-limonene, Undecane, 3,4-dimethyl, Heptane, alpha-Pinene, Oxalic acid, Citronellol, Tridecane, n-Decanoic acid, Cyclopentane, pentyl- and n-Hexadecanoic acid.

Whereas, in the case of healthy broccoli, the VOCs were not detected. These compounds were noticed in the headspace of infested broccoli and are said to be involved in attracting beneficial insects as a response to the aphid infestation (18,25). Further, Shivaramu *et al.* (27) revealed that the volatiles of the fruiting stages of *Capsicum annum* were highly attractive to *Scirtothrips dorsalis*. Furthermore, unlike the un-infested plants, *S. dorsalis* was substantially attracted to infested plants. Xiang *et al.* (33) identified 13 compounds of volatiles of tobacco plants that affected electro physiological activity of *Phthorimaea operculella*. Among these compounds, cis-3-Hexen-1-ol showed an attractant effect on the orientation behaviors of both male and female moths. Further the compounds like nonanal, decanal, decane, and methyl hexadecanoate could increase the number of female oviposition. In the Y-tube olfactometer, female *Peristenus spretus* got attracted to m-cymene and 4-ethyl-acetophenone synthetic volatile compounds at two different concentrations (10 mg/ml and 100 mg/ml) compared with the control (mineral oil) (34).

The response of the ladybird beetle, *C. sexmaculata*, to the cabbage extract at different concentrations was conducted using the Y-tube olfactometer. The olfactometer experiments can provide a simplified view of the actual decision-making by insects and can demonstrate the impact of volatile concentration on the behavioural responses of the beetles (3). Based on the number of individuals that reached the source of the odour, the orientation or attraction of female and male

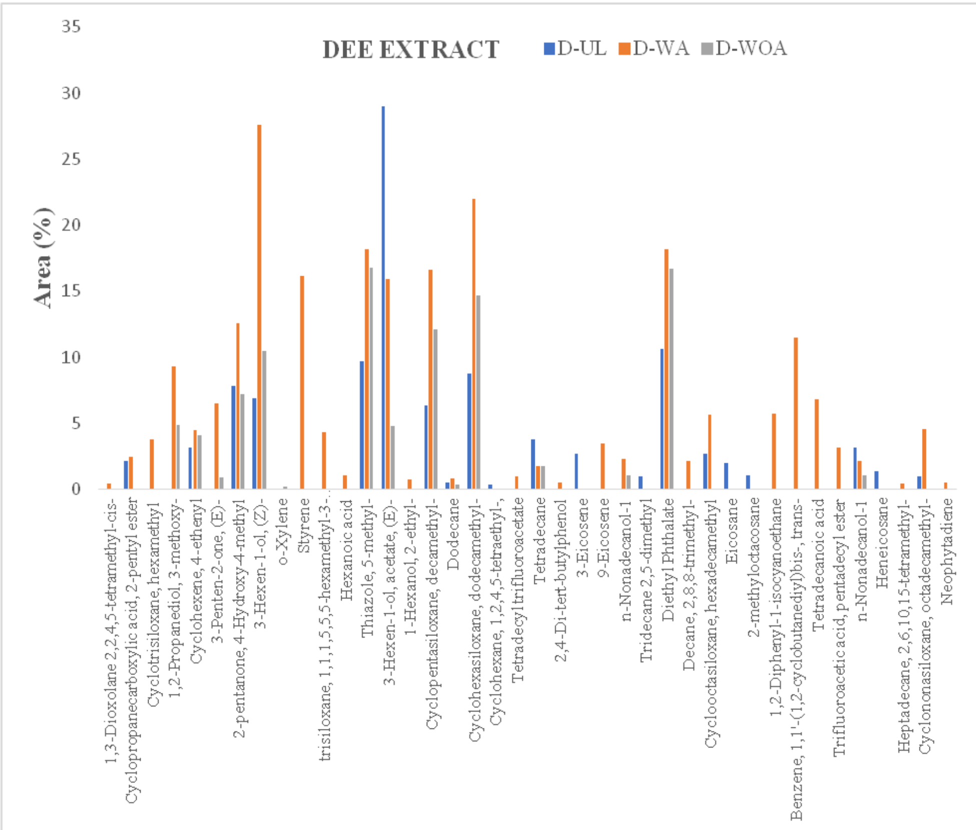

Fig. 3. Relative abundance of different volatile components in DEE extracts

individuals to various extracts was evaluated. The results showed that both sexes responded differently to various extracts at various concentrations. The diversity and variations in the volatile cues emitted from different extracts were responsible for the differential reaction. Compared with different extracts at different concentrations, the DC 3 and DE 3 extracts witnessed significantly higher orientation responses to the beetles. The male and female individual response to the extracts at various concentrations showed significantly more attraction to DC 3 and DE 3 extracts at 5 and 10 % compared to the 1 and 15 % concentrations. The percentage of male beetles attracted to the DC 3 extract at 5 and 10 % concentration was 70.00 and 63.33 %, respectively and to the DE 3 extract at 5 and 10 % concentration was 75.00 and 71.66 %, respectively (Table 3). The percentage of female beetles attracted to the DC 3 extract at 5 and 10 % concentration was 81.66 and 70.00 %, respectively, and the DE 3 extract at 5 and 10 % concentration was 73.34 and 65.00, % respectively (Table 4). As the concentration of the extract increased, the percentage response of beetles towards the odour arm was less compared with the control arm (solvent). It might be due to the higher concentration of plant volatiles or their blend, which may be repelling the predators. The female beetle's response to extracts at 1, 5, 10 and 15 % concentrations showed more attraction than the males.

Table 3. Orientation response of male *C. sexmaculata* to extracts.

Extract	1 %	5 %	10 %	15 %	F cal	*P* value
DC 1	33.33±2.10a	41.66±3.07a	35.00±3.41a	35.00±2.24a	6.15	0.3888NS
DC 2	53.33±2.10a	53.33±2.11a	58.33±3.07a	41.66±1.66b	9.47	0.000422**
DC 3	63.33±3.34a	70.00±2.58a	63.33±3.33a	46.66±2.10b	11.88	0.000109**
DC 4	43.33±2.11d	55.00±2.23b	65.00±2.24a	56.66±2.10ab	16.86	0.000001**
DE 1	38.33±1.67a	38.33±4.01a	41.66±1.67a	35.00±2.23a	1.11	0.367968NS
DE 2	50.00±2.58a	55.00±2.24a	56.66±3.34a	41.66±1.67b	7.10	0.00196**
DE 3	68.33±3.07a	75.00±2.88a	71.66±3.07a	45.00±2.23b	20.83	0.000002**
DE 4	48.33±1.67c	58.33±1.67b	68.33±1.67a	58.33±1.67b	24.00	0.00000007**

*Significant at *P*<0.05, ** Significant at *P*<0.01, *** Significant at *P*<0.001, NS-Non-significant. Values with the same letter for the respective rows are not significantly different

Table 4. Orientation response of female *C. sexmaculata* to extracts.

Extract	1 %	5 %	10 %	15 %	F cal	*P* value
DC 1	38.33±3.07a	35.00±2.23a	33.33±2.10a	43.33±2.10a	3.33	0.40182 NS
DC 2	55.00±2.23a	51.66±3.07ab	53.33±2.10ab	43.33±2.11b	4.60	0.013182*
DC 3	63.33±3.34bc	81.66±1.66a	70.00±2.58ab	46.66±2.10d	14.18	0.000000**
DC 4	46.66±2.10c	68.33±1.67a	56.66±3.33bc	55.00±2.23bc	13.65	0.000004**
DE 1	35.00±2.23a	43.33±2.10a	35.00±2.23a	43.34±2.10a	4.91	0.103 NS
DE 2	48.33±1.66ab	51.67±3.07ab	56.65±3.33a	43.34±2.10b	4.53	0.013976*
DE 3	56.66±2.10cd	73.34±3.33a	65.00±3.41bc	48.34±1.67d	15.43	0.000001**
DE 4	50.00±2.58c	65.00±2.23a	61.65±1.67a	61.65±3.07a	7.24	0.001771**

*Significant at P<0.05, ** Significant at P<0.01, *** Significant at P<0.001, NS-Non-significant. Values with the same letter for the respective rows are not significantly different.

The study also assessed the orientation response time (ORT) as an additional metric to investigate insect attraction to the volatiles. It was observed that, in the case of most extracts, there was no noteworthy disparity between the odor source and the control in terms of ORT. Although extracts like DC 1, DC 2, DE1 and DE2 at 1 % showed significant ORT, it was more in the odour arm than the control arm, which may depict that volatiles from these extracts were not attracting females effectively. When female individuals were exposed to extracts DC1, DC2, DC3, DC4, DE1, DE2, DE3 and DE4 at 5 and 10 % concentrations significant difference in ORT was observed, and time taken to reach the odour source was less than the control arm (Table 5 and 6). The orientation response time was observed less in DC 3 and DE 3 extracts at a 5 per cent concentration of 3.66 and 3.95 min, respectively, towards the odour arm. It suggests that the volatiles from these extracts effectively orient females towards the odour source. The male individuals were exposed to extracts like DC1, DC2, DC3, DC4, DE1, DE2, DE3 and DE4 at 5 and 10 % concentrations significant difference in ORT was observed, and time taken to reach the odour source was less than the control arm. In the case of male beetles, the orientation response time was less in DC 3 and DE 3 extracts at a 5 per cent concentration of 3.96 and 4.08 min, respectively, towards the odour arm (Table 5 and 6). As the percentage of concentration increased from 10 to 15 %, the time taken to reach the odour source also increased, even though there was a significant difference between the odour and control arm with respect to different extracts. The orientation response of the beetle towards the odour source at lower concentrations was due to the presence of specific volatile organic compounds at required quantity, hence these compounds helps to attract the beetles thus helps in the reduction of aphid population.

Table 5. Orientation Response Time (ORT) of *C. sexmaculata* to DCM extracts

		Male				Female			
		Mean time to reach odour source (min ± SE)							
		Odour Arm	Control Arm	T Cal	P value	Odour Arm	Control Arm	T Cal	P value
1 %	DC 1	6.12±0.17ab	5.46±0.18	2.337 NS	0.82014	6.75±0.26b	4.56±0.20	3.11*	0.0013**
	DC 2	6.51±0.15b	5.70±0.11	3.972**	3.51E-06	6.25±0.31ab	5.26±0.17	2.538*	0.0004***
	DC 3	5.86±0.11a	5.10±0.07	5.362**	3.9E-08	5.73±0.22a	6.94±0.15	-2.006 NS	1.45E-08***
	DC 4	4.83±0.15bc	4.10±0.07	4.203**	0.004591	4.05±0.20a	4.40±0.13	-1.312 NS	3.91E-05***
5 %	DC 1	6.08±0.15c	7.36±0.15	-5.296**	0.504807	5.24±5.24c	6.61±6.61	-5.683**	0.0473*
	DC 2	4.51±0.15ab	7.06±0.20	-8.975**	2.88E-07	4.13±0.15ab	7.73±0.28	-10.202**	0.0014**
	DC 3	3.96±0.18a	6.81±0.14	-11.630**	1.15E-13	3.66±3.66a	6.36±6.31	- 11.871**	5.62E-10***
	DC 4	4.80±0.16bc	7.21±0.17	-8.675**	4.56E-07	4.56±0.11bc	6.70±0.22	-7.716**	4.8E-05***
10 %	DC 1	6.50±0.12c	7.11±0.12	-3.238**	0.696254	5.25±0.09b	6.37±0.10	-6.960**	0.3167NS
	DC 2	4.60±0.20ab	7.60±0.17	-9.927**	6.96E-11	4.31±0.19a	6.75±0.17	-8.390**	1.42E-05***
	DC 3	4.53±0.16ab	7.03±0.15	-8.173**	9.08E-09	3.75±0.16a	6.45±0.19	-9.773**	0.0103*
	DC 4	4.90±0.08abc	7.95±0.10	-13.849**	4.38E-10	4.01±0.07a	7.01±0.11	-20.277**	0.0001***
15 %	DC 1	6.41±0.16b	6.03±0.12	1.691 NS	0.324609	6.23±0.16b	7.05±0.12	-4.487**	0.0027**
	DC 2	5.38±0.10a	7.81±0.15	-11.704**	3.02E-15	5.03±0.09ab	7.56±0.12	-15.261**	0.0150*
	DC 3	6.31±0.10b	7.56±0.17	-5.955**	0.016543	6.56±0.15bc	7.01±0.19	-1.577 NS	0.1852NS
	DC 4	5.53±0.20a	7.63±0.11	-8.120**	0.001636	4.51±0.18a	7.13±0.10	-12.020**	0.0006***

*Significant at P<0.05, ** Significant at P<0.01, *** Significant at P<0.001, NS-Non-significant. Values with the same letter for the respective rows are not significantly different.

Table 6. Orientation Response Time (ORT) of *C. sexmaculata* to DEE extracts

		Male				Female			
			Mean time to reach odour source (min ± SE)						
		Odour Arm	Control Arm	T Cal	P value	Odour Arm	Control Arm	T Cal	P value
1 %	DE 1	6.55±0.13b	6.00±0.13	2.551NS	0.1738NS	6.05±0.14c	5.01±0.17	4.169 **	0.2276NS
	DE 2	5.90±0.16ab	5.20±0.11	3.130*	5.05E-05***	5.73±0.10ab	4.98±0.07	5.296 **	3.05E-10***
	DE 3	5.58±0.20a	5.21±0.34	0.752 NS	1.05E-06***	5.48±0.10ab	4.06±0.05	11.180 **	0.0025**
	DE 4	5.63±0.22a	6.50±0.15	-2.753*	2.88E-06***	4.88±0.09a	5.20±0.10	-1.906 NS	1.9E-06***
5 %	DE 1	5.78±0.11bc	6.50±0.13	-3.601 **	0.5765NS	5.40±0.17c	6.46±0.16	-3.981**	0.0647NS
	DE 2	4.51±0.09a	7.98±0.17	-15.732 **	0.0005***	4.66±0.11ab	7.03±0.09	-14.443**	3.92E-09***
	DE 3	4.08±0.15a	7.58±0.12	-16.221 **	1.43E-08***	3.95±0.18a	7.81±0.17	-14.077**	5.25E-16***
	DE 4	5.51±0.09b	7.71±0.15	-9.251 **	0.0561NS	4.90±0.14bc	7.90±0.08	-15.227**	3.49E-12***
10 %	DE 1	5.90±0.27c	6.50±0.08	-1.936 NS	0.1534NS	5.60±0.13b	6.49±0.12	- 4.330**	0.2492NS
	DE 2	4.50±0.18a	7.21±0.16	-9.968 **	0.00047***	4.03±0.14a	6.20±0.17	-8.491**	1.59E-18***
	DE 3	5.43±0.17b	7.51±0.16	-8.106 **	0.000176***	5.01±0.14b	7.11±0.10	-11.639**	0.00781**
	DE 4	5.75±0.14bc	7.90±0.22	-8.022 **	0.000256***	4.25±0.10a	7.80±0.14	-16.582**	3.45E-08***
15 %	DE 1	5.86±0.20ab	7.23±0.24	-3.920 **	0.011044*	5.98±0.08bc	7.01±0.09	-7.453**	0.0978NS
	DE 2	5.81±0.12ab	7.51±0.24	-5.670 **	0.001161**	5.63±0.13bc	7.20±0.11	-7.665**	4.78E-10***
	DE 3	5.51±0.15a	7.53±0.18	-8.052 **	7.34E-06***	5.30±0.08ab	7.20±0.19	- 8.227**	0.6971NS
	DE 4	5.61±016ab	7.36±0.23	-5.966 **	0.000246***	4.70±0.18a	6.91±0.21	-7.297**	2.39E-05***

*Significant at P<0.05, ** Significant at P<0.01, *** Significant at P<0.001, NS-Non-significant. Values with the same letter for the respective rows are not significantly different.

In the investigation by Glinwood *et al.* (10), the preference of the ladybird beetle, *C. septempunctata*, for aphid-infested barley crops compared to uninfested barley was explored. Their study revealed that the hexane extract from healthy leaves was the least attractive, with only 42±0.92 % of female individuals displaying an attraction response. Notably, the research also documented that female individual exhibited a significantly higher level of attraction to infested leaves with aphid extract compared to their male counterparts (10). Similarly, Rakshith *et al.* (23) conducted a study to observe the orientation response of male and female ladybird beetles, *C. transversalis*, to various extracts. Their findings showed that the orientation response time of the beetles to reach the odor arm was shorter than that of the control arm, indicating a heightened attraction. Moreover, female individuals were observed to respond more rapidly to the odor arm than their male counterparts (23). These results collectively underscore the gender-based differences in the orientation responses of ladybird beetles to various extracts.

Among the different extracts, the highest attraction of both male and female beetles was recorded for infested leaves with aphid (WA) for both DCM and n-Hexane as extracts. The highest orientation response of beetles was observed with infested leaves with aphid extracts at a 5 % concentration of DCM (72.5±3.66 %) followed by 5 % n-Hexane extract (70.0±4.23 %). The lowest beetle response was observed with Un-infested leaf extract for a 15 % concentration of DCM (31.3±2.9) (30). The orientation behaviour of three predator's *viz*., *Eriopis connexa*, *Cycloneda sanguine* and *Harmonia axyridis*, towards the volatiles released from aphid, *Myzus persicae* on *Capsicum annum* revealed that the volatiles of prey or the host plant alone had not shown significant differences in the time required for three ladybird species to reach the source chamber. The volatiles released from the aphid-infested plant attracted the predator, *C. sanguine*, in a significantly shorter time (6). Similarly, Jaba *et al.* (15) observed that *C. sexmaculata* showed more attraction towards infested cowpea plants at high *Aphis craccivora* population densities (150) thus, exhibited a positive correlation between number of aphids and attraction of *C. sexmaculata.* Alarm pheromone, E-β-Farnasene might be responsible for the higher attraction of predatory beetle (15). The natural enemies find for aphid infestation before landing on the un-infested plant because natural enemies will first find a damaged plant and then begin searching for aphids (12).

The GC-MS analysis was instrumental in delineating the distinctions between cabbage crops infested with aphids and those that remained un-infested, shedding light on their capacity to attract natural enemies. It is believed that *C. sexmaculata* relies on the odours emitted from aphid-infested cabbage plants to detect and locate their hosts. The findings of our study consistently demonstrated a heightened preference of *C. sexmaculata* for cabbage plants that had been infested by aphids, as compared to un-infested ones, as determined through olfactometer experiments. The identification of these plant volatiles via GC-MS represents a promising and cost-effective avenue for future applications in pest monitoring, control, and early detection.

The development of synthetic formulations through the combination of species-specific VOCs offers a promising strategy within agro-ecosystems for aggregating beetles to aphid infested crops, thereby aiding in the reduction of pest populations (27). It is evident that the response of predators to VOCs is a multifaceted process in practical agricultural settings, and achieving the desired effect may require a well-balanced blend of volatiles rather than relying on single compounds. Our study has contributed to the identification of these VOCs and their individual effectiveness in beetle response through olfactometer studies. This research provides a foundation for not only understanding the efficacy of specific compounds but also for the formulation of volatile blends that can be used as lures to attract beetle populations in the field, effectively contributing to aphid population control. Future investigations should delve into how the blend of volatile compounds affects beetle attraction for pest control under varying environmental conditions, further advancing our understanding of this approach's practical application.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

REFERENCES

1. Ahmed, Q., Agarwal, M., Alsabte, A., Aljuboory, A.B. and Ren, Y. (2021). Evaluation of volatile organic compounds from broccoli plants infested with *Myzus persicae* and Parasitoids *Aphidius colemani* attraction. In *IOP Conference Series: Earth and Environmental Science* (Vol. **761**, No. 1, p. 012029). IOP Publishing.
2. Atwal, A.S. (1976). Agricultural Pests of India and South East Asia. Kalyani Publishers, Delhi, 502p.
3. Ballhorn, D.J. and Kautz, S. (2013). Induced plant defence via volatile production is dependent on rhizobial symbiosis. *Oecologia* **172**(3): 833-846.
4. Blackman, R.L. and Eastop, V.F. (2000). *Aphid on the World's crops. An Identification Guide*. Wiley, Chichester, 466 pp.
5. Chowdhuri, A.N. and Pal, S.K. (1975). Beneficial coccinellids of Simla Hills. *Science and Culture* **41**(12): 600-601.
6. Ciarla, M.V., Mareggiani, G., Heit, G. and Puhl, L. (2005). *Myzus persicae* (Homoptera: Aphididae) and *Capsicum annum* (Solanacae) volatiles: their effect on predators attraction. *Boletin de Sanidad Vegetal Plagas,* **31**: 503-507.
7. Ellis, P.R. and Singh, R. (1993). A review of the host plants of the cabbage aphid, *Brevicoryne brassicae* (Homoptera: Aphididae). IOBS/WPRS Bull **16**:192-201.
8. Erb, M., Veyrat, N., Robert, C.A., Xu, H., Frey, M., Ton, J. and Turlings, T.C. (2015). Indole is an essential herbivore-induced volatile priming signal in maize. *Nature communications* 6(1): 6273.
9. Fang, Z., Liu, Y., Lou, P. and Liu, G. (2005). Current trends in cabbage breeding. *Journal of New Seeds* **6**(2): 75-107.
10. Glinwood, R., Ahmed, E., Qvarfordt, E. and Ninkovic, V. (2011). Olfactory learning of plant genotypes by a polyphagous insect predator. *Oecologia* **166**: 637-647.
11. Guerrieri, E. and Digilio, M.C. (2008). Aphid-plant interactions: A review. *Journal of Plant Interactions* **3**(4): 223-232.
12. Hatano, E., Kunert, G., Michaud, J.P. and Weisser, W.W. (2008). Chemical cues mediating aphid location by natural enemies. *European Journal of Entomology* **105**(5): 797-806.
13. Hodek, I., Van Emden, H.F. and Honek, A. (2012). Ecology and Behaviour of the Ladybird Beetles (Coccinellidae). John Wiley & Sons, 560pp.
14. Hughes, R.D. (1963). Population dynamics of the cabbage aphid, *Brevicoryne brassicae* (L.). *Journal of Animal Ecology* **32**: 393-424.
15. Jaba, J., Haseena, B. and Ranjith, A.M. (2013). Behavioural response of Cheilomenes sexmaculata (Fabricius) to natural enemy and pest mediated semiochemicals. *Journal of Biological Control* **27**(1): 58-60.
16. Joo, Y., Schuman, M.C., Goldberg, J.K., Kim, S.G., Yon, F., Brütting, C. and Baldwin, I.T. (2017). Herbivore-induced volatile blends with both "fast" and "slow" components provide robust indirect defence in nature. *Functional Ecology* **32**(1): 136-149.

17. Khan, A.R. and Munir, M. (1986). Rapeseed and mustard family problems and prospects. In *proceedings of National Seminar on Oilseed Research and Development in Pakistan*. Agriculture Research Council, Islamabad, Pakistan, 95p.
18. Li, Y., Weldegergis, B.T., Chamontri, S., Dicke, M. and Gols, R. (2017). Does aphid infestation interfere with indirect plant defense against *Lepidopteran* caterpillars in wild cabbage? *Journal of Chemical Ecology* **43**: 493-505.
19. Liu, C.M., Matsuyama, S. and Kainoh, Y. (2019). Synergistic effects of volatiles from host-infested plants on host-searching behavior in the parasitoid wasp, *Lytopylus rufipes* (Hymenoptera: Braconidae). *Journal of Chemical Ecology* **45**: 684-692.
20. Najar-Rodriguez, A.J., Friedli, M., Klaiber, J. and Dorn, S. (2015). Aphid-deprivation from Brassica plants results in increased isothiocyanate release and parasitoid attraction. *Chemoecology* **25**: 303-311.
21. Pickel, C., Mount, R.C., Zalom, G.G. and Wilson, L.T. (1983). Monitoring aphids on Brussels sprouts. *California Agricultur*e. **26**: 24-25.
22. Pinto-Zevallos, D.M., Bezerra, R.H., Souza, S.R. and Ambrogi, B.G. (2018). Species-and density-dependent induction of volatile organic compounds by three mite species in cassava and their role in the attraction of a natural enemy. *Experimental and Applied Acarology* **74**: 261-274.
23. Rakshith, H.S., Suroshe, S.S., Chander, S.M. and Kumari, S. (2018). Behavioural response of *Coccinella transversalis* to the volatiles from *Aphis craccivora* and Cowpea. *Indian Journal of Entomology* **80**: 1331-1337.
24. Rodriguez-Saona, C., Kaplan, I., Braasch, J., Chinnasamy, D. and Andwilliams, L. (2011). Field responses of predaceous arthropods to methyl salicylate: A meta-analysis and case study in cranberries. *Biological Control* **59**: 294-303.
25. Seenivasagan, T., Sharma, K.R., Sekhar, K., Ganesan, K., Prakash, S. and Vijayaraghavan, R. (2009). Electroantennogram, flight orientation, and oviposition responses of *Aedes aegypti* to the oviposition pheromone n-heneicosane. *Parasitology research* **104**(4): 827-833.
26. Shiojiri, K., Takabayashi, J., Yano, S. and Takafuji, A. (2000). Flight response of parasitoids toward plant-herbivore complexes: A comparative study of two parasitoid-herbivore systems on cabbage plants. *Applied Entomology and Zoology* **35**(1): 87-92.
27. Shivaramu, S., Jayanthi, P.D.K., Kempraj, V., Anjinappa, R., Nandagopal, B. and Chakravarty, A.K. (2017). What signals do herbivore-induced plant volatiles provide conspecific herbivores? *Arthropod-Plant Interactions* **11**: 815-823.
28. Song, H., Dong, Z., Li, L., Lu, Z., Li, C., Yu, Y. and Men, X. (2021). Relationships among the feeding behaviors of a mirid bug on cotton leaves of different ages and plant biochemical substances. *Journal of Insect Science* **21**(1): 151-162.
29. Tank, B.D., Korat, D.M. and Borad, P.K. (2010). Determination of dominant species of predatory coccinellid in Anand region of Gujarat. *Karnataka Journal of Agricultural Sciences* **20**(3): 637-638.
30. Venkanna, Y. (2020). Predatory Behaviour of Coccinellid Beetle, *Cheilomenes sexmaculata* (Fab.) and its Predation Risk on Prey. Ph.D (Agri) Thesis submitted to Indian Agricultural Research Institute, (IARI), New Delhi.
31. Vuorinen, T., Nerg, A.M., Ibrahim, M.A., Reddy, G.V.P. and Holopainen, J.K. (2004). Emission of *Plutella xylostella* induced compounds from cabbages grown at elevated CO_2 and orientation behavior of the natural enemies. *Plant Physiology* **135**(4): 1984-1992.
32. Wu, G., Harris, M.K., Guo, J. and Wan, F. (2009). Temporal allocation of metabolic tolerance in the body of beet armyworm in response to three gossypol-cotton cultivars. *Life Sciences* **52**(12): 1140-1147.
33. Xiang, L.I., Zhang, X.G., Chun, X.I.A.O., Gao, Y.L. and Dong, W.X. (2020). Behavioral responses of potato tuber moth (*Phthorimaea operculella*) to tobacco plant volatiles. *Journal of Integrative Agriculture* **19**(2): 325-332.
34. Xiu, C., Dai, W., Pan, H., Zhang, W., Luo, S., Wyckhuys, K.A., Yang, Y. and Lu, Y. (2019). Herbivore-induced plant volatiles enhance field-level parasitism of the mirid bug, *Apolygus lucorum*. *Biological Control* **135**: 41-47.

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