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Behavioral Responses of *Coccinella septempunctata* and *Diaeretiella rapae* under the Influence of Semiochemicals and Plant Extract in Four Arm Olfactometer

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ABSTRACT

Natural enemies are more effective at controlling herbivores in diverse botanical ecosystems. Different chemical cues help to correspond in diversity of associations between prey and host plant species. Recent studies exhibited that the use of natural enemy is an ecofriendly measure to control pests. The Seven spotted ladybird beetle, *Coccinella septempunctata* play a prominent role in aphid management. It exploits several different cues released by plants to increase the efficiency of foraging. Aphid endoparasitoid, *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae) have an ability to locate its hosts by responding to odours from aphid host plants or by visual searching. The treatments with different combinations of plant extracts and semiochemicals were used for natural enemy preference experiment. The experiment was conducted with seven treatments and five replications at Glass house situated in Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi field area during Feb-April, 2015. The *Coccinella septempunctata* were collected from wheat crop plants. They remained starved for two days before Olfactometer bioassays. For *D. rapae,* mummified aphids were collected from wheat crop. Naive females were subjected to olfactometer tests. Seven different combined treatments of semiochemicals and plant extract were applied on filter paper strips at 3% concentration. The filter paper strips were placed in arms of olfactometer. The control arms were treated with n-hexane. Data pertaining to preference of *C. septempunctata* and *D. rapae* after treatment application were recorded and analysed statistically. It was found that T_6 (β-pinene + E-β-Farnesene) exhibited highest mean number entries of *C. septempunctata* (6.13%) and highest mean time spend (6.23%) as compared to two other treatments applied. The results revealed that alarm pheromone component effective kairomone for aphid predatory beetles. It was found that T_6 (β-pinene + E-β-Farnesene) exhibited highest mean number entries of *D. rapae* (7.50%) and highest mean time spend (6.39%) as compared to other treatments applied. The results revealed that release of insect derived semiochemicals can enhance visual searching and efficiency of parasitoid *D. rapae*.

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BS conducted the research and wrote the manuscript. MT provided techical support. MN and MA analysed the data.

Key words
***Coccinella septempunctata*, Treatments, Concentration, Semiochemicals, Olfactometer.**

INTRODUCTION

The four arm olfactometer was designed by Pettersson (1970). It is a volatile based instrument having central arena with food source boxes which are connected with each other through connected tubes. It is designed to study the oviposition preference behavior of insect pest and its predator via screening experiments. Volatiles are emitted from the plant parts or body of prey. Insect pests or predators are confined in the central arena to test it for food preference (Riddick *et al*., 2000).

The term 'parasitoid' for the first time was introduced by Reuter (1913). Parasitoids have the ability to respond plant odors (Moraes *et al*., 2005). The volatile profile of plant odor also play a vital role to increase parasitoid attraction (Röse *et al*., 1998; Bukovinszky *et al*., 2005). It was found that volatiles released immediately from damaged plant attract parasitoids instantaneously (Mattiacci *et al*., 2001; Hoballah and Turlings, 2005).

Responses of natural enemies towards volatiles released from aphid infested plants are often specific in terms of plant species, plant developmental stage, herbivore species and developmental stage of herbivore (Moraes *et al*., 2005; Sabelis *et al*., 2007). But sometimes, the host specificity is not universal (Shiojiri *et al*., 2001; van Poecke *et al*., 2003).

Natural enemies including Coccinellid beetles, parasitoid wasps, lacewings, and hoverflies are attracted by plant volatiles which are induced by aphid attack (Hatano *et al*., 2008). Endoparasitic wasps undergo obligatory development inside arthropod host. During the development phase, parasitoids can be influenced

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by chemical stimuli perceived from its host and the environment (Turlings *et al*., 1993; Godfray, 1994). It was found that experienced *A. ervi* and *D. rapae* exhibit a significant response to aphid induced plant volatiles as compared to naive individuals (Girling *et al*., 2006).

Natural enemies play a crucial role in pest management programs and ecological studies. Natural enemies are sensitive towards chemical cues released in multitrophic environment, with regard to host location (Poppy, 1997; Vet and Dicke, 1992). Predatory ladybeetle, *Coccinella septempunctata* (L.) is aphidiophagus and polyphagous (Pettersson *et al*., 2008; Ninkovic *et al*., 2011). It is best known aphid predator. It can consume more than 100 aphids per day (Capinera, 2008). It exploits the cues released by plants (Honek and Martinkova, 2008). The *C. septempunctata* has specialized olfactory cells in its compound eye (Pickett *et al*., 1998). The olfactory and visual cues play an important role to locate aphids (Sengonca and Liu, 1994).

The alarm pheromone is released by many aphid species, but the subfamily Aphididae releases particularly sesquiterpene EβF (Pickett and Griffiths, 1980). It is released when aphids are attacked by natural enemies. It induces avoidance behaviour among aphids (Gibson and Pickett, 1983) and increase the foraging behaviour of parasitoids (Foster *et al*., 2005). It acts as kairomone for predators such as ladybirds (Francis *et al*., 2004; Pettersson *et al*., 2008). It acts as valuable tool in aphid pest-control strategies (Roditakis *et al*., 2000). Therefore, this experiment was carried out to study the behavioural responses of *D. rapae* under the influence of seven different combinations of semiochemicals and plant extract by using four arm olfactometer.

Different chemical cues are related to diverse associations between prey and its host plant. It was found that *Coleomegilla maculate, Adalia bipuncata* and *C. septempuncata* responses were related to semiochemicals released from aphid species and their host plants (Zhu *et al*., 1999; Al-Abassi *et al*., 2000); they use chemical cues to locate their preys. Alarm pheromone component EBF is an effective kairomone for aphid predators, *i.e.* two spotted ladybeetle (Francis *et al*., 2004).

Therefore, present study was carried out to see the olfactory responses of predatory beetles towards semiochemicals and plant extract. Al-Abassi *et al* (2000) found that the semiochemicals have been intensively studied for their use in insect biocontrol programs.

MATERIALS AND METHODS

This experiment was conducted at Laboratories situated in Department of Entomology, Pir Mehr Ali Shah-Arid Agriculture University, Rawalpindi field area. The experiment was conducted comprising seven treatments with five replications.

Collection and rearing of insects

The *C. septempunctata* were collected from wheat crop plants. They were reared on 50% sugar solution. They remained starved for two days before Olfactometer bioassays.

Mummified aphids of *D. rapae* were collected from wheat crop in vials individually. On emergence, females were reared on 50% aqueous solution of honey for 2 days. Naive females were subjected to olfactometer tests.

Olfactometer bioassays

The behavioural responses of *C. septempunctata* and *D. rapae* under seven different treatments of plant extract and semiochemicals were determined by using a four-arm olfactometer (Pettersson, 1970; Kalule and Wright, 2004; Webster *et al*., 2010). The bioassay consists a pairwise treatment comparison. All bioassays for predator response were performed at 20±2°C with 0.04 W / m^2 light intensity (Young *et al*., 1987).

Treatments were applied on filter paper strips at 3% concentration. The control arms were treated with n-hexane. Filter paper strips were placed in arms of olfactometer. Two arms are kept as control and rest of two arms are kept as treatment arms. These treatments are: T_1, Turmeric; T_2, β-pinene; T_3, E-β-Farnesene; T_4, Turmeric and β-pinene; T_5, Turmeric and E-β-Farnesene; T_6, β-pinene and E-β-Farnesene and T_7, Turmeric, β-pinene and E-β-Farnesene.

Air was drawn in through the four orifices which passes in each quadrant by vacuum pump. Predator, *C. septempunctata* was released in central olfactometer chamber for 8 minutes and was allowed move freely within each region. Olfactometer was rotated at 90° after every two minutes interval. The number of entries and time spent by *C. septempunctata* in each region of olfactometer was recorded using Olfa software (Nazzi, 1996). After every 10 specimens, washed with Lipsol detergent (5% v/v; Bibby Sterilin Ltd., UK), rinsed with 80% ethanol and air dried. Data pertaining to number of entries and time spent by *C. septempunctata* in each region of olfactometer were recorded. Similar experiment was performed with *D. rapae*

Statistical analysis

Data pertaining to number of entries and time spent by *C. septempunctata* and *D. rapae* in each region of olfactometer was were analysed using Wilcoxon test. The HSD test at 5% level of significance to compare the difference between the means.

Table I.- Number of entries (Mean ± SEM) by male and female *Coccinella septempunctata* in control and treatment arm of olfactometer.

Treatment	No of entries		Wilcoxon test
	Control arm	Treatment arm	(P-value)
Male			
T_1	3.10 ± 0.23	4.90 ± 0.23	0.0004572
T_2	3.0 ± 0.26	5.90 ± 0.28	0.0001485
T_3	3.40 ± 0.22	6.80 ± 0.25	0.0001358
T_4	3.20 ± 0.25	5.40 ± 0.16	0.0001239
T_5	3.20 ± 0.25	5.80 ± 0.20	0.0001286
T_6	3.10 ± 0.23	7.20 ± 0.25	0.0001399
T_7	3.20 ± 0.25	6.0 ± 0.26	0.0001459
Female			
T_1	3.20 ± 0.20	5.20 ± 0.25	0.0002962
T_2	3.30 ± 0.15	6.0 ± 0.26	0.000115
T_3	3.30 ± 0.26	6.60 ± 0.22	0.0001383
T_4	2.90 ± 0.23	5.50 ± 0.17	0.0001247
T_5	3.10 ± 0.23	6.0 ± 0.21	0.000127
T_6	3.40 ± 0.22	7.0 ± 0.26	0.0001383
T_7	3.30 ± 0.21	5.90 ± 0.28	0.0001383

***, P<0.001; **, P<0.01; *, P<0.05, P< 0.1 and P<1. *C. septempunctata* response was measured as (Mean±SEM) number of observations in the arms of four-way olfactometer. n=80 individuals tested in each treatment.

RESULTS

Number of entries in arm of olfactometer

Male C. septempunctata

It was found that *C. septempunctata* exhibited a significant response to choose treatment arm over the control arm in all treatments tested in olfatometer bioassay. From Table I, it was found that male *C. septempunctata* exhibited the maximum significant preference towards treatment T_6 (Wilcoxon's test, T = 7.20; N = 80; P = 0.000139) as compared to other treatments applied. It was found that the treatment T_4 (Wilcoxon's test, T = 5.40; N = 80; P = 0.000127) was statistically similar to T_5 (Wilcoxon's test, T = 5.80; N = 80; P = 0.000128) which was statistically at par with T_2 (Wilcoxon's test, T = 5.90; N = 80; P = 0.000148). It was observed that *C. septempunctata* exhibited the minimum significant preference towards treatment T_1 (Wilcoxon's test, T = 4.90; N = 80; P = 0.024) as compared to other treatments applied. The preference of *C. septempunctata* towards treatment T_7 was (Wilcoxon's test, T = 6.0; N = 80; P = 0.000124) which was statistically similar to T_3 (Wilcoxon's test, T = 6.80; N = 80; P = 0.000138) (Table I).

Female C. septempunctata

It was found that *C. septempunctata* exhibited a significant response to choose treatment arm over the control arm in all treatments tested in olfatometer bioassay. From Table I, it was found that female *C. septempunctata* exhibited the maximum significant preference towards treatment T_6 (Wilcoxon's test, T = 7.0; N = 80; P = 0.000138) as compared to other treatments applied. It was found that the treatment T_2 (Wilcoxon's test, T = 6.0; N = 80; P = 0.000138) was statistically similar to T_5 (Wilcoxon's test, T = 6.0; N = 80; P = 0.000127) which was statistically at par with T_3 (Wilcoxon's test, T = 6.60; N = 80; P = 0.000138). It was observed that *C. septempunctata* exhibited the minimum significant preference towards treatment T_1 (Wilcoxon's test, T = 5.20; N = 80; P = 0.024) as compared to other treatments applied. The preference of *C. septempunctata* towards treatment T_4 was (Wilcoxon's test, T = 5.50; N = 80; P = 0.000124) which was statistically similar to T_7 (Wilcoxon's test, T = 5.90; N = 80; P = 0.000138) (Table II).

Table II.- Number of entries (Mean ± SEM) by *Diaeretiella rapae* in control and treatment arm of olfactometer.

Treatment 3% concentration	No. of entries		Wilcoxon test
	Control arm	Treatment arm	(P-value)
T_1	2.20 ± 0.25	3.90 ± 0.28	0.00109
T_2	2.30 ± 0.26	5.50 ± 0.34	0.0001494
T_3	2.40 ± 0.22	7.10 ± 0.28	0.0001383
T_4	2.50 ± 0.27	5.70 ± 0.21	0.0001086
T_5	2.10 ± 0.28	6.50 ± 0.17	0.0001301
T_6	2.60 ± 0.27	7.50 ± 0.18	0.0001254
T_7	2.0 ± 0.21	5.90 ± 0.23	0.000127

***, P<0.001; **, P<0.01; *, P<0.05; P< 0.1 and P<1. *D. rapae* response was measured as (Mean±SEM) number of observations in the arms of four-way olfactometer. n=80 individuals tested in each treatment.

Diaeretiella rapae

It was found that *D. rapae* exhibited a significant response to choose treatment arm over the control arm in all treatments tested in olfatometer bioassay. Among the seven treatments tested, *D. rapae* exhibited the maximum significant preference in treatment T_6 (Wilcoxon's test, T = 7.50, N = 80, p=0.00012), which was statistically at par with T_3 (Wilcoxon's test, T = 7.10, N = 80, p=0.00013). Whereas, the preference of *D. rapae* towards treatment T_5 was (Wilcoxon's test, T = 6.50; N = 80; P = 0.00013). It was found that the treatment T_2 (Wilcoxon's test, T = 5.50; N = 80; P = 0.00014) was statistically similar to T_4 (Wilcoxon's test, T = 5.70; N = 80; P = 0.00010) which was statistically at par with T_7 (Wilcoxon's test, T = 5.90; N = 80; P = 0.000148). Preference of *D. rapae* towards treatment T_1 was minimum (Wilcoxon's test, T = 3.90; N =

80; P = 0.00109) (Table II).

Time spent in arm of olfactometer

Male C. septempunctata

It was found that *Coccinella septempunctata* exhibited a significant response to choose treatment arm over the control arm in all treatments tested in olfatometer bioassay. From Table III, it was found that male *C. septempunctata* exhibited the maximum significant preference towards treatment T_6 (Wilcoxon's test, T = 6.13; N = 80; P = 1.083) as compared to other treatments applied. It was found that the treatment T_6 was statistically similar to T_3 (Wilcoxon's test, T = 6.05; N = 80; P = 0.00018). It was observed that *C. septempunctata* exhibited the minimum significant preference towards treatment T_1 (Wilcoxon's test, T = 3.30; N = 80; P = 0.024) as compared to other treatments applied. The preference of *C. septempunctata* towards treatment T_4 was (Wilcoxon's test, T = 4.71; N = 80; P = 0.000179) which was statistically similar to T_2 (Wilcoxon's test, T = 4.55; N = 80; P = 0.00018). The preference of *C. septempunctata* towards treatment T_7 was (Wilcoxon's test, T = 5.21; N = 80; P = 0.000179) which was statistically similar to T_5 (Wilcoxon's test, T = 5.50; N = 80; P = 0.00018) (Table III).

Table III.- Time spent (Mean ± SEM) by male and female *Coccinella septempunctata* in control and treatment arm of olfactometer.

Treatment	Time spent		Wilcoxon test (P-value)
	Control arm	Treatment arm	
Male			
T_1	2.8 ± 0.15	3.30 ± 0.06	0.02479
T_2	2.52 ± 0.06	4.55 ± 0.11	0.0001806
T_3	1.38 ± 0.06	6.05 ± 0.09	0.0001806
T_4	2.65 ± 0.10	4.71 ± 0.11	0.0001796
T_5	1.89 ± 0.09	5.50 ± 0.09	0.0001817
T_6	2.37 ± 0.03	6.13 ± 0.03	1.083e-05
T_7	2.10 ± 0.07	5.21 ± 0.04	1.083e-05
Female			
T_1	2.54 ± 0.17	3.69 ± 0.18	1.083e-05
T_2	2.21 ± 0.10	5.02 ± 0.16	0.0001817
T_3	1.34 ± 0.04	6.12 ± 0.1	0.0001806
T_4	2.33 ± 0.05	4.89 ± 0.2	1.083e-05
T_5	1.81 ± 0.09	5.02 ± 0.12	0.0001796
T_6	1.26 ± 0.04	6.23 ± 0.11	1.083e-05
T_7	1.67 ± 0.10	5.75 ± 0.13	0.0001806

***, P<0.001; **, P<0.01; *, P<0.05, P< 0.1 and P<1. *C. septempunctata* response was measured as (Mean±SEM) number of observations in the arms of four-way olfactometer. n=80 individuals tested in each treatment.

Female C. septempunctata

It was found that *Coccinella septempunctata* exhibited a significant response to choose treatment arm over the control arm in all treatments tested in olfatometer bioassay. From Table III, it was found that female *C. septempunctata* exhibited the maximum significant preference towards treatment T_6 (Wilcoxon's test, T = 6.23; N = 80; P = 1.083) as compared to other treatments applied. It was found that the treatment T_6 was statistically similar to T_3 (Wilcoxon's test, T = 6.12; N = 80; P = 0.00018). It was observed that *C. septempunctata* exhibited the minimum significant preference towards treatment T_1 (Wilcoxon's test, T = 3.69; N = 80; P = 1.083) as compared to other treatments applied. The preference of *C. septempunctata* towards treatment T_4 was (Wilcoxon's test, T = 4.89; N = 80; P = 1.083). The preference of *C. septempunctata* towards treatment T_2 was (Wilcoxon's test, T = 5.02; N = 80; P = 0.00018) which was statistically similar to T_5 (Wilcoxon's test, T = 5.02; N = 80; P = 0.000179) which was statistically at par with T_7 (Wilcoxon's test, T = 5.21; N = 80; P = 0.000181).

Table IV.- Time spent (Mean ± SEM) by *Diaeretiella rapae* in control and treatment arm of olfactometer.

Treatment 3% concentration	Time spent		Wilcoxon Test (P-value)
	Control arm	Treatment arm	
T_1	2.07 ± 0.22	3.39 ± 0.14	0.0004943
T_2	1.99 ± 0.23	4.48 ± 0.25	0.0001806
T_3	1.31 ± 0.09	6.35 ± 0.08	0.0001817
T_4	2.21 ± 0.18	4.58 ± 0.14	1.083e-05
T_5	1.69 ± 0.13	5.68 ± 0.15	0.0001817
T_6	1.12 ± 0.03	6.39 ± 0.03	1.083e-05
T_7	1.93 ± 0.21	5.16 ± 0.3	1.083e-05

***, P<0.001; **, P<0.01; *, P<0.05; P< 0.1 and P<1. *D. rapae* response was measured as (Mean ± SEM) number of observations in the arms of four-way olfactometer. n = 80 individuals tested in each treatment.

Diaeretiella rapae

It was found that *D. rapae* exhibited a significant response to choose treatment arm over the control arm in all treatments tested in olfatometer bioassay. Among the seven treatments tested, *D. rapae* exhibited the maximum significant preference in treatment T_6 (Wilcoxon's test, T = 6.39, N = 80, P = 1.083), which was statistically at par with T_3 (Wilcoxon's test, T = 6.35, N = 80, P = 0.00018). It was found that the treatment T_7 (Wilcoxon's test, T = 5.16; N = 80; P = 1.083) was statistically similar to T_5 (Wilcoxon's test, T = 5.68; N = 80; P = 0.00018). It was observed that the treatment T_2 (Wilcoxon's test, T = 4.48; N = 80; P = 0.00014) which was statistically at par with T_4 (Wilcoxon's test, T = 4.58; N = 80; P = 0.000148). The preference of *D. rapae* towards treatment T_1 was minimum (Wilcoxon's test, T = 3.39; N = 80; P = 0.00049) (Table IV).

DISCUSSION

Olfactory cues play an important role in foraging behaviour of natural enemies (Dicke *et al*., 2003) *i.e.* in ladybird foraging behaviour (Pettersson *et al*., 2005; Zhu and Park, 2005). Seagraves (2009) reported that *C. septempunctata* orient themselves towards prey using olfactory cues. Aphid cornicle secretions containing semiochemicals are attracting cues for *C. septempunctata.* Han and Chen (2002) found that seven spotted ladybird exhibited significant differences toward odor source when it was exposed to crushed 1200 tea aphids in a Y tube olfactometer. Seagraves (2009) reported that the attraction of coccinellids is related to prey density. Therefore, ladybird olfactory response by EBF is a dose dependent factor (Bhasin *et al*., 2000). Francis *et al*. (2004) found that coccinellids do not respond towards EBF when its amount is less than 2µg. Al-Abassi *et al*. (2000) found that attractivity of EBF for *C. septempunctata* decreases with increasing amount of *α*-caryophyllene.

Leroy *et al*. (2012) found that aphid associated semiochemicals, *i.e.,* [*E*]-*β*-farnesene, *α*-pinene, *β*-pinene, *Z,E*-nepetalactone and (-)-*β*-caryophyllene are potential attractants for *Harmonia axyridis*. Alarm pheromone component (*E*)- *β*-farnesene, either emitted by aphids and plants is an attractant for coccinellids, *C. septempunctata* (Al-Abassi *et al*., 2000; Ninkovic *et al*., 2001), *Adalia bipunctata* (Hemptinne *et al*., 2000), *Hippodamia convergens* (Acar *et al*., 2001) and *H. axyridis* (Verheggen *et al*., 2007; Mondor and Roitberg, 2000). Aphid alarm pheromone (*α*-pinene and *β*-pinene formulated in paraffin oil) are attractants for the Asian lady beetle. It was found that alarm pheromone can attract 70.0% of tested females in the wind-tunnel experiments. The volatile *α*-pinene can significantly attract the *H. axyridis* (Xue *et al*., 2008).

It was found that (*Z*)-3-hexenol and (*E*)-2-hexenal act as a synomone for the coccinellids *C. septempunctata* (Han and Chen, 2002). Alhmedi *et al*. (2010) found that *H. axyridis* do not show any behavourial response when (*E*)-*β*-farnesene, (*Z*)-3-hexenol and *β*-pinene is in amount of 5 µg in olfactory experiments.

Ladybirds can arrive in crop plants before aphid migrants via plant volatile chemicals (Honeˇk and Martinkova´, 2008; Ninkovic and Pettersson, 2003; Ninkovic *et al*., 2011). The continuous emission of plant volatiles affect ladybird searching behaviour. This phenomenon contributes to broader ecological significance of induced plant responses towards biotic stress (Markovic *et al*., 2014).

Vekaria and Patel (2000) found that different treatments of neem extracts were less toxic towards *D. rapae* and *C. septempunctata* as compared to chemical insecticides. Halder *et al*. (2010) tested the efficacy of chloroform, methanol extracts and oils from nayantara, *Vinca rosea* and bottle brush against *Lipaphis erysimi* and *C. septempunctata* under laboratory condition. It was found that plant extracts and oil have not exhibited mortality to *C. septempunctata* up to ten days after feeding the treated *L. erysimi*. Chakraborty and Ghosh (2010) tested the toxicity of *Bacillus thuringiensis*, *Beauveria bassiana*, malathion and Neemactin and Avermectin on ladybeetle. It was found that Neemactin and Avermectin were least toxic as compared to six insecticide formulations tested.

The results revealed that T_6 (*β*-pinene, E-*β*-Farnesene) exhibited highest mean number entries of *C. septempunctata* (6.13%) and highest mean time spend (6.23%) as compared to two other treatments applied. Results depicted that alarm pheromone is a promising biopesticide and attractant for several aphidophagous predators including *C. septempunctata*.

Hymenopteran parasitoids are important natural enemies in biological control programs of aphids in diverse crops (Araya *et al*., 2010). Previous studies revealed that parasitoids locate their hosts by semiochemicals emitted from their hosts and from the plants infested by their hosts (Zhu *et al*., 2005). Wickremasinghe and van Emden (1992) and Vet and Dicke (1992) found that a number of aphid parasitoids respond and attract towards plant volatiles in olfactometer bioassays. Aphids themselves not attractive towards all parasitoids (Micha and Wyss, 1995). Aphid release alarm pheromone from their cornicles when disturbed, which is attractive for some parasitoids (Micha and Wyss, 1996).

Micha *et al*. (2000) found that the parasitoid orientation behavior in olfactometer bioassay is influenced by the odours emitted from infested plant baits. Some parasitoids respond to aphid induced plant volatiles and some remain unresponsive to odors of host plant (Storeck *et al*., 2000; Girling *et al*., 2006). Heil (2008) reported that parasitoids have ability to distinguish between aphid infested and uninfested plants, and they can also distinguish between plants infested by different herbivores. Takemoto *et al*. (2009) found that volatiles released from *Vicia faba* infested by *Acyrthosiphon pisum* attract naive *Aphidius ervi* in a Y-tube olfactometer. Foster *et al*. (2005) reported that *D. rapae* spend up to 20 min time interval in the discs treated with E*β*F. The time spent by *D. rapae* in E*β*F treated discs increased with increase in its concentration. *D. rapae* can move towards high distances from untreated to E*β*F treated discs. Turlings *et al*. (2004) found that 90% of endoparasitoid *Cotesia marginiventris* females stay in odour treated arm. If no odour is offered in olfactometer bioassay, most of females stay in central chamber during 30 min duration. Wyckhuys and Heimpel (2007) found that response potential of aphid parasitoid *Binodoxys communis* towards certain stimuli was 59, 68, 67, 62, and 62% for odors from *Aphis glycines*, *A. oestlundi*, *A. monardae*, *A. nerii*, and *A. asclepiadi*s, respectively. In olfactometer bioassays, both male and female *A. ervi* exhibited more significant time spent in air-stream containing β-phellandrene and caryophyllene as compared to controls (George *et al*., 2013).

Our study dipicted that T_6 (*β*-pinene + E-*β*-Farnesene) exhibited highest mean number entries of *D. rapae* (7.50%) and highest mean time spend (6.39%) as compared to other treatments applied. Therefore, these semiochemicals are attractive to natural enemies, *i.e.*, predatory beetles (Han and Chen, 2002; Osawa, 2000) and parasitoids. Guerrieri *et al*. (1999) found that herbivore induced volatiles are released by plants is a systemic response. Cortesero *et al*. (2000) found that release of plant volatiles which attract parasitoid species should be enhanced through plant breeding.

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Statement of conflict of interest

The authors declare no conflict of interest.

REFERENCES

Acar, E.B., Medina, J.C., Lee, M.L. and Booth, G.M., 2001. Olfactory behaviour of convergent lady beetles (Coleoptera: Coccinellidae) to alarm pheromone of green peach aphid (Hemiptera: Aphididae). *Can. Entomol.*, **133**: 389-397. https://doi.org/10.4039/Ent133389-3

Al-Abassi, S., Birkett, M.A., Petterson, J., Pickett, J.A., Wadhams, L.J. and Woodcock, C.M., 2000. Response of the seven-spot ladybird to an alarm pheromone and an alarm pheromone inhibitor is mediated by paired olfactory cells. *J. chem. Ecol.,* **26**: 1765-1771. https://doi.org/10.1023/A:1005555300476

Alhmedi, A., Haubruge, E. and Francis, F., 2010. Intraguild interactions implicating invasive species: *Harmonia axyridis* as a model species. *Biotech. Agron. Soc. Environ.*, **14**: 187-201.

Araya, J.E., Araya, M. and Guerrero, M.A., 2010. Effects of some insecticides applied in sublethal concentrations on the survival and longevity of *Aphidius ervi* (Haliday) (Hymenoptera: Aphidiidae) adults. *Chilean J. agric. Res.*, **70**: 221-227. https://doi.org/10.4067/S0718-58392010000200005

Bhasin, A., Mordue, A.J. and Mordue, W., 2000. Electrophysiological and behavioral identification of host kairomones as olfactory cues for *Culicoides impuntatus* and *C. nubeculosis*. *Physiol. Ent*., **25**: 6-16. https://doi.org/10.1046/j.1365-3032.2000.00157.x

Bukovinszky, T., Gols, R., Posthumus, M.A., Vet, L.E.M. and van Lenteren, J.C., 2005. Variation in plant volatiles and attraction of the parasitoid *Diadegma semiclausum* (Hellén). *J. chem. Ecol.*, **31**: 461-480. https://doi.org/10.1007/s10886-005-2019-4

Capinera, J.L., 2008. *Encyclopedia of entomology*, 2nd ed. Springer, Germany, pp. 205. https://doi.org/10.1007/978-1-4020-6359-6

Chakraborty, K. and Ghosh, S.K., 2010. Incidence of *Coccinella septempunctata* in brinjal with some pesticides. *Curr. Adv. agric. Sci.*, **2**: 129-130.

Cortesero, A.M., Stapel, J.O. and Lewis, W.J., 2000. Understanding and manipulating plant attributes to enhance biological control. *Biol. Contr*., **17**: 35-49. https://doi.org/10.1006/bcon.1999.0777

Desneux, N., Decourtye, A. and Delpuech, J.M., 2007. The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Ent*., **52**: 81-106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Dicke, M., van Poecke, R.M.P. and de Boer, J.G., 2003. Inducible indirect defence of plants: From mechanisms to ecological functions. *Basic appl. Ecol*., **4**: 27-42. https://doi.org/10.1078/1439-1791-00131

Foster, S.P., Denholm, I., Thompson, R., Poppy, G.M. and Powell, W., 2005. Reduced response of insecticide-resistant aphids and attraction of parasitoids to aphid alarm pheromone; a potential fitness trade off. *Bull. entomol. Res*., **95**: 37-46. https://doi.org/10.1079/BER2004336

Francis, F., Lognay, G., Gaspar, C. and Haubruge, E., 2004. Olfactory responses to aphid and host plant volatile releases: (E)-b-farnesene an effective allomone for the predator *Adalia bipunctata*. *J. chem. Ecol*., **30**: 741-755. https://doi.org/10.1023/B:JOEC.0000028429.13413.a2

Galvan, T.L., Koch, R.L. and Hutchison, W.D., 2005. Effects of spinosad and indoxacarb on survival, development, and reproduction of the multicolored Asian lady beetle (Coleoptera: Coccinellidae).

Biol. Contr., **34**: 108-114. https://doi.org/10.1016/j.biocontrol.2005.04.005

George, D.R., King, L., Donkin, E., Jones, C.E, Croft, P. and Tilley, L.A.N., 2013. Dichotomy of male and female responses to hoverfly-driven cues and floral competition in the parasitoid wasp *Aphidius ervi* Haliday. *Biol. Contr*., **67**: 539-547. https://doi.org/10.1016/j.biocontrol.2013.08.013

Gibson, R.W. and Pickett, J.A., 1983. Wild potato repels aphids by release of aphid alarm pheromone. *Nature*, **302**: 608-609. https://doi.org/10.1038/302608a0

Girling, R.D., Hassall, M., Turner, J.G. and Poppy, G.M., 2006. Behavioural responses of the aphid parasitoid *Diaeretiella rapae* to volatiles from *Arabidopsis thaliana* induced by *Myzus persicae. Ent. Exp. Appl*., **120**: 1-9. https://doi.org/10.1111/j.1570-7458.2006.00423.x

Godfray, H.C.J., 1994. *Parasitoids, behavioral and evolutionary ecology*. Princeton University Press, Princeton, NJ, USA, pp. 83-210.

Guerrieri, E., Poppy, G.M., Powell, W., Tremblay, E. and Pennacchio, F., 1999. Induction and systemic release of herbivore-induced plant volatiles mediating in-flight orientation of *Aphidius ervi*. *J. chem. Ecol*., **25**: 1247-1261. https://doi.org/10.1023/A:1020914506782

Halder, J., Srivastava, C., Dhingra, S. and Dureja, P., 2010. Bioactivity of some plant extracts against mustard aphid, *Lipaphis erysimi* (Kalt.) and its predator *Coccinella septempunctata* (Linn.). *Pestic. Res. J*., **22**: 174-176.

Han, B. and Chen, Z., 2002. Behavioral and electrophysiological responses of natural enemies to synomones from tea shoots and kairomones from tea aphids, *Toxoptera aurantii*. *J. chem. Ecol*., **28**: 2203-2220. https://doi.org/10.1023/A:1021045231501

Hatano, E., Kunert, G., Michaud, J.P. and Weisser, W.W., 2008. Chemical cues mediating aphid location by natural enemies. *Eur. J. Ent.,* **105**: 797-806. https://doi.org/10.14411/eje.2008.106

Heil, M., 2008. Indirect defence via tritrophic interactions. *New Phytol*., **178**: 41-61. https://doi.org/10.1111/j.1469-8137.2007.02330.x

Hemptinne, J.L., Gaudin, M., Dixon, A.F.G. and Lognay, G., 2000. Social feeding in ladybird beetles: adaptive significance and mechanism. *Chemoecology*, **10**: 149-152. https://doi.org/10.1007/PL00001817

Hoballah, M.E. and Turlings, T.C.J., 2005. The role of fresh versus old leaf damage in the attraction of parasitic wasps to herbivore-induced maize volatiles. *J. chem. Ecol*., **31**: 2003-2018. https://doi.org/10.1007/s10886-005-6074-7

Honek, A. and Martinkova, Z., 2008. Why is *Coccinella septempunctata* so successful (a point of view). *Eur. J. Ent*., **105**: 1-12. https://doi.org/10.14411/eje.2008.001

Kalule, T. and Wright, D.J., 2004. The influence of cultivar and cultivar-aphid odours on the olfactory response of the parasitoid *Aphidius colemani*. *J. appl. Ent*., **128**: 120-125.

Leroy, P.D., Schillings, T., Farmakidis, J., Heuskin, S., Lognay, G., Verheggen, F.J., Brostaux, Y., Haubruge, E. and Francis, F., 2012. Testing semiochemicals from aphid, plant and conspecific: attraction of *Harmonia axyridis*. *Insect Sci*., **19**: 372-382. https://doi.org/10.1111/j.1744-7917.2011.01449.x

Markovic, D., Glinwood, R., Olsson, U. and Ninkovic, V., 2014. Plant response to touch affects the behaviour of aphids and ladybirds. *Arthropod-Pl. Interact*., **8**: 171-181. https://doi.org/10.1007/s11829-014-9303-6

Mattiacci, L., Rocca, B.A., Scascighini, N., D'Alessandro, M., Hern, A. and Dorn, S., 2001. Systemically induced plant volatiles emitted at the time of "danger". *J. chem. Ecol*., **27**: 2233-2252. https://doi.org/10.1023/A:1012278804105

Micha, S.G. and Wyss, U., 1995. The importance of plant odours for host searching of *Aphidius uzbekistanicus* (Hymenoptera, Aphidiidae), a parasitoid of the grain aphid (*Sitobion avenae*). *Gesunde Pflanzen*, **47**: 300-307.

Micha, S.G. and Wyss, U., 1996. Aphid alarm pheromone (E)-beta-farnesene: A host finding kairomone for the aphid primary parasitoid *Aphidius uzbekistanicus* (Hymenoptera: Aphidiinae). *Chemoecology*, **7**: 132-139. https://doi.org/10.1007/BF01245965

Micha, S.G., Kistenmacher, S., Mölck, G. and Wyss, U., 2000. Tritrophic interactions between cereals, aphids and parasitoids: discrimination of different plant-host complexes by *Aphidius rhopalosiphi* (Hymenoptera: Aphidiidae). *Eur. J. Ent*., **97**: 539-543. https://doi.org/10.14411/eje.2000.083

Mondor, E. and Roitberg, B., 2000. Has the attraction of predatory coccinellids to cornicle droplets constrained aphid alarm signaling behavior? *J. Insect Behav*., **3**: 321-329. https://doi.org/10.1023/A:1007754000862

Moraes, M.C.B., Laumann, R., Sujii, E.R., Pires, C. and Borges, M., 2005. Induced volatiles in soybean and pigeon pea plants artificially infested with the neotropical brown stink bug, *Euschistus heros*, and their effect on the egg parasitoid, *Telenomus podisi*. *Ent. Exp. Appl*., **115**: 227-237. https://doi.

org/10.1111/j.1570-7458.2005.00290.x

Nazzi, F., 1996. *Olfa. A computer program for collecting and analyzing behavioral data with the four-armed olfactometer*. Exeter Software, Setauket, New York.

Ninkovic, V., Abassi, S.A. and Petterson, J., 2001. The influence of aphid-induced plant volatiles in ladybird beetle searching behavior. *Biol. Contr.*, **21**: 191-195. https://doi.org/10.1006/bcon.2001.0935

Ninkovic, V., Al Abassi, S., Ahmed, E., Glinwood, R. and Pettersson, J., 2011. Effect of within-species plant genotype mixing on habitat preference of a polyphagous insect predator. *Oecologia*, **166**: 391-400. https://doi.org/10.1007/s00442-010-1839-2

Ninkovic, V. and Pettersson, J., 2003. Searching behaviour of the seven spotted ladybird, *Coccinella septempunctata*-effects of plant odour interaction. *Oikos*, **100**: 65-70. https://doi.org/10.1034/j.1600-0706.2003.11994.x

Osawa, N., 2000. Population field studies on the aphidophagous ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae): resource tracking and population characteristics. *Popul. Ecol.*, **42**: 115-127. https://doi.org/10.1007/PL00011990

Pettersson, J., Ninkovic, V., Glinwood, R., Abassi, S.A., Birkett, M., Pickett, J. and Wadhams, L., 2008. Chemical stimuli supporting foraging behaviour of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae): volatiles and allelobiosis. *Appl. Ent. Zool.*, **43**: 315-321. https://doi.org/10.1303/aez.2008.315

Pettersson, J., Ninkovic, V., Glinwood, R., Birkett, M.A. and Pickett, J.A., 2005. Foraging in complex environment-semiochemicals support searching behaviour of the seven spot ladybird. *Eur. J. Ent.*, **102**: 365-370. https://doi.org/10.14411/eje.2005.053

Pettersson, J., 1970. An aphid sex attractant in biological studies. *Ent. Scand.*, **1**: 63-73. https://doi.org/10.1163/187631270X00357

Pickett, J.A., Wadhams, L.J. and Woodcock, C.M., 1998. Insect supersense: Mate and host location by insects as model systems for exploiting olfactory interactions. *The Biochemist*, **20**: 8-13.

Pickett, J.A. and Griffith, D.C., 1980. Composition of aphid alarm pheromones. *J. chem. Ecol.*, **6**: 349-360. https://doi.org/10.1007/BF01402913

Poppy, G.M., 1997. Tritrophic interactions: Improving ecological understanding and biological control. *Endeavour*, **21**: 61-65. https://doi.org/10.1016/S0160-9327(97)01042-9

Rahmani, S. and Bandani, A.R., 2013. Sublethal concentrations of thiamethoxam adversely affect life table parameters of the aphid predator, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae). *Crop. Prot.*, **54**: 168-175. https://doi.org/10.1016/j.cropro.2013.08.002

Reuter, O.M., 1913. *Habits and instincts of insects*. Friedlander, Berlin, pp. 1-20.

Riddick, E.W., Aldrich, J.R., Deand, M.A. and Davis, J.C., 2000. Potential for modifying the behavior of the multicolored asian lady beetle (Coleoptera: Coccinellidae) with plant-derived natural products. *Annls. entomol. Soc. Am.*, **93**: 1314-1321. https://doi.org/10.1603/0013-8746(2000)093[1314:PFMTBO]2.0.CO;2

Roditakis, E., Couzin, I.D., Barlow, K., Franks, N.R. and Charnley, A.K., 2000. Improving secondary pick up of insect fungal pathogen conidia by manipulating host behaviour. *Ann. appl. Biol.*, **137**: 329-335. https://doi.org/10.1111/j.1744-7348.2000.tb00074.x

Röse, U.S.R., Lewis, W.J. and Tumlinson, J.H., 1998. Specificity of systemically released cotton volatiles as attractants for specialist and generalist parasitic wasps. *J. chem. Ecol.*, **24**: 303-319. https://doi.org/10.1023/A:1022584409323

Sabelis, M., Takabayashi, J., Janssen, A., Kant, M., van Wijk, M., Sznajder, B., Aratchige, N., Lesna, I., Belliure, B. and Schuurink, R., 2007. Ecology meets plant physiology: herbivore-induced plant responses and their indirect effects on arthropod communities. In: *Ecological communities: Plant mediation in indirect interaction webs* (eds. T. Ohgushi, T. Craig and P. Price). Cambridge University Press, Cambridge, pp. 188-217. https://doi.org/10.1017/CBO9780511542701.010

Seagraves, M.P., 2009. Lady beetle oviposition behavior in response to the trophic environment. *Biol. Contr.*, **51**: 313-322. https://doi.org/10.1016/j.biocontrol.2009.05.015

Sengonca, C. and Liu, B., 1994. Responses of the different instar predator, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), to the kairomones produced by the prey and non-prey insects as well as the predator itself. *Z. Pflanzenk. Pflanzen.*, **101**: 173-177.

Shiojiri, K., Takabayashi, J., Yano, S. and Takafuji, A., 2001. Infochemically mediated tritrophic interaction webs on cabbage plants. *Popul. Ecol.*, **43**: 23-29. https://doi.org/10.1007/PL00012011

Storeck, A., Poppy, G.M., van Emden, H.F. and Powell, W., 2000. The role of plant chemical cues in determining host preference in the generalist aphid parasitoid *Aphidius colemani*. *Ent. Exp.*

Appl., **97**: 41-46. https://doi.org/10.1046/j.1570-7458.2000.00714.x

Takemoto, H., Powell, W., Pickett, J., Kainoh, Y. and Takabayashi, J., 2009. Learning is involved in the response of parasitic wasps *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) to volatiles from a broad bean plant, *Vicia faba* (Fabaceae), infested by aphids *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae). *Appl. Ent. Zool.*, **44**: 23-28. https://doi.org/10.1303/aez.2009.23

Turlings, T.C.J., Wackers, F., Vet L.E.M., Lewis, W.J. and Tumlinson, J.H., 1993. Learning of host finding cues by hymenopterous parasitoids. In: *Insect learning: Ecological and evolutionary perspectives* (eds. D.R. Papaj and A.C. Lewis). Chapman & Hall, NY, pp. 51-78. https://doi.org/10.1007/978-1-4615-2814-2_3

Turlings, T.J., Davison, A.C. and Tamo, C., 2004. A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiol. Ent*., **29**: 45-55. https://doi.org/10.1111/j.1365-3032.2004.0362.x

van Poecke, R.M., Roosjen, M., Pumarino, L. and Dicke, M., 2003. Attraction of the specialist parasitoid *Cotesia rubecula* to *Arabidopsis thaliana* infested by host or non-host herbivore species. *Ent. Exp. Appl*., **107**: 229-236. https://doi.org/10.1046/j.1570-7458.2003.00060.x

Vekaria, M.V. and Patel, G.M., 2000. Bioeffficacy of botanicals and certain chemical insecticides and their combinations against the mustard aphid, *Lipaphis erysimi*. *Ind. J. Ent.*, **62**: 150-158.

Verheggen, F.J., Fagel, Q., Heuskin, S., Lognay, G., Francis, F. and Haubruge, E., 2007. Electrophysiological and behavioral responses of the multicolored Asian lady beetle, *Harmonia axyridis* Pallas, to sesquiterpene semiochemicals. *J. chem. Ecol*., **33**: 2148-2155. https://doi.org/10.1007/s10886-007-9370-6

Vet, L.E.M. and Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Ent.*, **37**: 141-172. https://doi.org/10.1146/annurev.en.37.010192.001041

Wabale, A.S. and Kharde, M.N., 2010. Bioefficacy of plant extracts against sugarcane woolly aphid (*Ceratovacuna lanigera* Zehntener). *Asian J. exp. biol. Sci.*, **1**: 592-595.

Webster, B., Bruce, T., Pickett, J. and Hardie, J., 2010. Volatiles functioning as host cues in a blend become non host cues when presented alone to the black bean aphid. *Anim. Behav*., **79**: 451-457. https://doi.org/10.1016/j.anbehav.2009.11.028

Wickremasinghe, M.G.V. and van Emden, H.F., 1992. Reactions of adult female parasitoids, particularly *Aphidius rhopalosiphi*, to volatile chemical cues from the host plants of their aphid prey. *Physiol. Ent.*, **17**: 297-304. https://doi.org/10.1111/j.1365-3032.1992.tb01025.x

Wyckhuys, K.A.G. and Heimpel, G.E., 2007. Response of the soybean aphid parasitoid *Binodoxys communis* to olfactory cues from target and non-target host-plant complexes. *Ent. Exp. Appl.,* **123**: 149-158. https://doi.org/10.1111/j.1570-7458.2007.00532.x

Xue, J., He, J. and Xie, Y., 2008. Attractive effect of plant volatiles on *Harmonia axyridis* (Pallas). *Chinese J. appl. environ. Biol.,* **4**: 494-498.

Young, S., David, C.T. and Gibson, G., 1987. Light measurement for entomology in the field and laboratory. *Physiol. Ent.,* **12**: 373-379. https://doi.org/10.1111/j.1365-3032.1987.tb00763.x

Zhu, J., Cossé, A.A., Obrycki, J.J., Boo, K.S. and Baker, T.C., 1999. Olfactory reactions of the twelve-spotted lady beetle, *Coleomegilla maculata* and the green lacewing, *Chrysoperla carnea* to semiochemicals released from their prey and host plant: electroantennogram and behavioral responses. *J. chem. Ecol.,* **25**: 1163-1177. https://doi.org/10.1023/A:1020846212465

Zhu, J.W. and Park, K.C., 2005. Methyl salicylate, a soybean aphid induced plant volatile attractive to the predator *Coccinella septempunctata*. *J. chem. Ecol*., **31**: 1733-1746. https://doi.org/10.1007/s10886-005-5923-8

Zhu, J.J., Obrycki, J., Ochieng, S.A., Baker T.C., Pickett, J.A. and Smiley, D., 2005. Attraction of two lacewing species to volatiles produced by host plants and aphid prey. *Naturwissenschaften*, **92**: 277-281. https://doi.org/10.1007/s00114-005-0624-2