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Short Communication

Olfactory Response of Ladybird Beetle, *Coccinella septempunctata* L. (Coccinellidae: Coleoptera) towards Aphids and their Host Plants

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Authors' Contributions
AAK and MA conceived and designed the study. AAK and AMK performed the experiments. AAK analyzed the data and wrote manuscript with the help of AMK and MA.

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ABSTRACT

The seven-spot ladybird beetle *Coccinella septempunctata* L. is a generalist predator and prefer soft bodied insects. Semiochemicals released by the plants in response to herbivorous attack influences third trophic level and natural enemies use these substances to locate their prey. Twelve-arm olfactory apparatus was used to evaluate the response of *C. septempunctata* toward aphids and different host plants of aphids. Different horticultural plants and field crops were used singly or in combination with prey as a host. An increased numbers of *C. septempunctata* was observed when host plants were added with aphids. But was no significant difference in most of the combinations except wheat with aphids (3.8889±0.4554). The fresh leaves of rose, citrus, brassica and wheat (1.2±0.2006, 1.3556±0.1416, 1.5778±0.1829, 2.1444±0.2027, respectively) attracted significantly more numbers of *C. septempunctata* as compared to control and aphids alone (0.7667±0.1204 and 0.8333±0.1153). With every next hour (3, 6 and 12 h) of observation the movement of *C. septempunctata* toward target localities was elevated. Finding of the current investigation revealed that wheat, brassica, citrus and rose infested with aphids can be used as host plants for mass rearing and culture maintenance of *C. septempunctata*.

The seven-spotted ladybird beetle *Coccinella septempunctata* L. (Coleptera: Coccinellidae) is a voracious entomophagous predator. It feed on a wide range of diets with temporal focus on aphids as an essential food for achieving maximum fecundity (Evans *et al*., 1999; Hodek and Honek, 1996). Ladybird beetles are usually considered as a beneficial insects and feed on insects like aphids, jassids, psyllids, whiteflies, scale insects, mealy bugs and phytophagous mites which are injurious to agricultural crops and forest plantations (Rafi *et al*., 2005).

Beneficial insects are sensitive to chemical aspects of the multitrophic environment, particularly with regard to host location Poppy (1997) and can learn to associate plant volatiles in presence of prey/host (Drukker *et al*., 2000). Plant leaves generally release minor quantities of volatile compounds, but when a plant is damaged by an insects, this phenomenon enhanced and captioned substances in quantity (Reddy, 2012). And the beneficial insects respond to volatiles emitted by plants significantly after damage caused by the herbivores (Turlings *et al*., 1990). To locate prey in natural habitats, entomophagous insects use numerous chemical cues emitted by host plants, alone or in association with pest (Vet and Dicke, 1992). Predators could sense the odour produced by its prey as a kairomones while finding food, however this since assumed to be stronger in adult predators as compared to their immatures (Şengonca and Liu, 1994). Ninkovic *et al*. (2001) demonstrated that predators possibly use semiochemicals to locate their prey. The volatile blends released by plants can vary widely between different combinations of plants and herbivores (Boom *et al*., 2004).

The seven-spotted ladybird, *C. septempunctata* responded positively to volatiles from the aphid, *Rhopalosiphum padi* and to infested plants of *Hordeum vulgare*. Ladybird beetles (Coccinellids) are ancient and very successful group of predatory insects belonging to

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order Coleoptera. Lady beetles can be mass-reared for use against aphids or other pests. The olfactory learning by a predatory coccinellids beetle is very effective. Predatory coccinellids can learn to associate the odour of aphid infested plants due to the presence of volatile cues released by plants in response to feeding by their herbivores (Dicke, 2009; Heil, 2008). Ladybird foraging involves navigation through a complex landscape in which suitable high quality food sources are found and exploited when they are available (Pettersson *et al*., 2005). The present study was designed to check the olfactory response of *C. septempunctata* predators either attracted toward prey (aphids) and or their host plants in association of info-chemical relation of herbivore.

Materials and methods

The studies were conducted during spring 2016 in IPM-Lab at College of Agriculture, Bahauddin Zakariya University, Bahadur Campus, Layyah (30.9648°N, 70.9399°E, 143m), Punjab, Pakistan. Olfactory response of a seven-spotted lady beetle *C. septempunctata* was evaluated toward aphids and their host plants. Theadults *C. septempunctata* were collected from insecticides free crops in the vicinity of district Layyah. Mixed population of different aphid species, *Ropasiphum madis, Sitobion avene, Diauraphis noxia* were also collected from insecticides free crops and used as insect prey. Different horticultural and field crops including; Rose, Citrus, Brassica and Wheat were used as host plants. The fresh twigs and leaves of host plants were obtained from insecticide free plants cultivated in the University farm area.

Experiment was performed by using twelve-armed olfactory apparatus fitted with an electric fan to get sufficient plant fragrance toward beetles. The transparent visionary holes on upper side of the release chamber were covered with a black sheet to avoid movement of coccinellids toward light. From insect colony, the required quantity of *C. septempunctata* were starved for 12 h prior to the initiation of trial for escalating their food searching habits. Fresh clean twigs of host plants or infested with aphids were placed in plastic jars containing cotton swab to keep them fresh for maximum time. The experiment was performed in lab conditions at 25±3 °C and 65±5 %RH. The number of beetles moved towards host plants/prey in plastic jars was counted on daily bases with the intervals of 3, 6 and 12 h after beetles released. The experimental arena was cleaned on daily basis and experiment was repeated by randomizing host plants/prey location in different jars for 30 times (30 days).

To check the normality, the data was subjected to Shapiro-Wilk Normality Test. Generalized Linear Model (GLM) procedure under Analysis of Variance (ANOVA) with Post-Hoch Tukey's at P≤ 0.05 was used to discern differences in number of beetles moved toward host plants/ prey. Similar procedure was done for analysis of hourly movement of *C. septempunctata* toward target site. The data is described as means±SE. For computation of data the analytical software Statistix 8.1® was used.

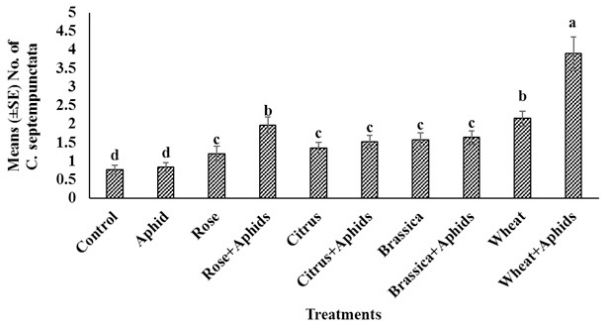

Fig. 1. Olfactory response of *C. septempunctata* towards different host plants and insect pests. All the host plants and prey showed significant difference as compared to control. Brassica and wheat in combination with aphids found more attractive to *C. septempunctata* at P ≤ 0.001.

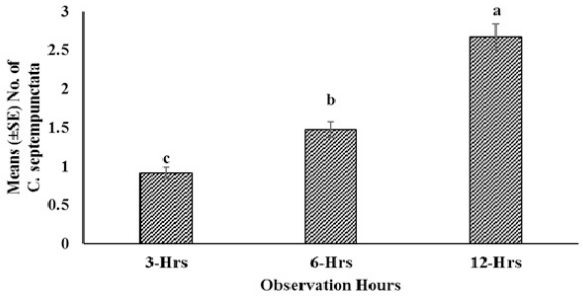

Fig. 2. Olfactory response of *C. septempunctata* in relation to observation times towards different host plants and insect pests. A significant increase in beetle's movement observed with every next observation hour at P ≤ 0.001.

Results and discussion

All the treatments showed significant difference in attraction of coccinellids as a result of olfactory response toward host plant; rose, citrus, brassica, wheat alone or infested with aphids ($F = 20, DF = 11, P < 0.001$) (Fig. 1). With every next observation hour, the incessant movement of coccinellids was also significantly increased for 3, 6 to 12 h ($F = 54.4, DF = 2, P < 0.001$) (Fig. 2). It showed predators remained active all the times to search their food and responded toward the olfaction emitted by the prey and their host plants. Maximum number of *C. septempunctata* was observed on wheat leaves

infested with aphids (3.8889±0.4554) followed by wheat alone and rose/aphids combination (2.1444±0.2027 and 1.9667±0.2123, respectively). Minimum population was counted in control and where aphids were placed alone (0.7667±1204 and 8323±1153, respectively). Rose alone, citrus and brassica alone or in combination with aphids did not showed significant difference among each other, however significantly higher coccinellids were attracted to these host plants as compared to control and aphids alone.

In current investigation a good number of coccinellids were moved to almost all host plants and aphids and they responded in active manners toward both horticultural and field crops. But in some instances like brassica, the results were surprising having significantly lesser numbers as compared to wheat. Generally, brassica is well known favorite plant for aphids but here wheat, citrus and rose leaves also proved equally attractive to aphids. Further, the behavior of coccinellids did not changed significantly toward these host plants when placed alone or in combination with aphids. These findings and previous studies (Dhaliwal *et al*., 2006) revealed that plants have semiochemicals to attract beneficial insects to safeguard themselves and these plant derived substances influence the predator's behavior to locate the herbivores. *Coccinella septempunctata* did not discriminated significantly, either citrus or rose plants were kept alone or in combination with aphids which is contradictory to the findings of Raymond *et al*. (2000) who suggested that presence of aphids and their wastes is necessary to attract starved coccinellids. And the higher numbers of coccinellids toward infested wheat are confirmation with the investigations of Raymond *et al*. (2000), Drukker *et al*. (2000), Heil (2008), Dicke (2009), Francis *et al*. (2014) they found that, the predatory coccinellids are known to be associated with odour of infested host plants and volatile cues released by plants in response to feeding by their herbivore prey. Therefore, it is concluded that the tested crops may be utilized as trap crops aside the major crops. Further, these crops may be used for mass rearing of coccinellids for augmentation against wide range of insect pests. These host plants can also be used as conservation of coccinellids with their seasonal requirements round the year. Future studies are also warranted to explore the volatile cues and semeiochemicals and can be formulated as plant origin attractants to be used in biocontrol modules in field crops.

Statement of conflict of interest

Authors have declared no conflict of interest.

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