Behavioural response of seven-spotted ladybird beetle, *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae) towards different aphids and their host plants

Abstract

The current study was investigates the olfactory response of the predatory beetle, *Coccinella septempunctata* to various host plants and their associated aphids under laboratory conditions at 27±2^{0}C and RH 65±5 %. Using a four-arm olfactometer, the beetles' attraction to healthy and aphid-infested mustard, wheat, rose, and chrysanthemum was evaluated. The results revealed that *C. septempunctata* showed a significantly higher attraction to aphid-infested plants compared to healthy plants or aphids alone. Among the treatments, mustard infested with *Lipaphis erysimi* (Kaltenbach), wheat infested with *Rhopalosiphum maidis* (Fitch), rose infested with *Macrosiphum rosae* (Linnaeus), and chrysanthemum infested with *Macrosiphoniella sanborni* (Gillette) elicited the strongest responses. These findings support the hypothesis that predatory coccinellids are more attracted to the olfactory cues emitted by aphid-infested plants, likely due to the release of semiochemicals in response to herbivore damage. This behavior enhances the effectiveness of *C. septempunctata* as a biological control agent by improving its ability to locate prey in agricultural ecosystems.

Keywords: *aphid, Coccinella septempunctata, olfactometer, response, semiochemicals*

Introduction

In India, the utilization of insecticides against insect pests is increasing day by day, resulting in the development of resistance and many harmful effects on the environment (Abrol and Shankar, 2014). Around 98% of applied pesticides reach different places other than target species, which includes non-target species, water, bottom sediments, air and food (Miller, 2004). The lack of proper technical information about the use of insecticides, their chemical content, and the amount of spray required to control the pests, enhance the pressure on people's health and the health of soil. Furthermore, the continuation of pesticide usage results in the loss of natural enemies, which in turn might result in secondary pest outbreaks (Miller, 2004). In our current situation, it is very important to develop an organic-based agriculture system that helps in increasing land productivity and environmental safety to improve the sustainable agro-ecosystem. Consequently, we need a noble and eco-friendly approach to maintain a balance between the human need for food and the sustainability of our environment. Research on safe, long-lasting, and successful pest management systems has mostly focused on usage of effective natural enemies as a replacement for harmful pesticides (Lewis *et al*., 1997).

Among the different biological control agents, coccinellids are one of the potential remedies in pest management plans with a good host range (Nelaballe and Beula, 2015). Predaceous coccinellids are more often linked to biological control than taxa of other predatory organisms (Hodek and Honek, 1996a; Jervos and Kidd, 1996). The coccinellids belong to the Coccinellidae family of the order Coleoptera. More than 6000 species of coccinellids are recorded all over the world (Vandenberg, 2002), of which described species are around 5200 (Hawkeswood, 1987). Around 400 coccinellid species have been reported from the Indian subcontinent (Poorani, 2012). About 90% of Coccinellid species are predatory in nature and they feed on phytophagous insects of agricultural, horticultural and forestry species like aphids, whiteflies, mites, psyllids, plant hoppers, coccids, pseudococcids, thrips etc., (Omkar and Pervez, 2002; Evans, 2009).

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) Generally, plant leaves release some minor quantities of volatile compounds, but if that plant is attacked or damaged by insects, this phenomenon of releasing compounds is enhanced and releases a major quantity of compounds (Reddy, 2012). These beneficial insects show a response to the volatiles that are released from the plants after the damage caused by herbivores (Turlings *et al*., 1990). Entomophagous insects use different chemical clues emitted from their host plants alone, or in host plant association with pests, to locate prey in their natural habitat (Vet and Dicke, 1992). Ninkovic *et al*., (2001) demonstrated that predators mostly use semiochemicals to find their hosts. Predators can sense the odor emitted by their prey as kairomones while searching for food, but this sense is stronger in adult predators when compared to their immature stages (Sengonca and Liu, 1994). The volatile blends/compounds released by plants vary widely between various combinations of plants and their associated herbivores (Van Den *et al.*, 2004).

So, it is imperative to investigate the response of predatory beetle, *C. septempunctata* (L.) (Coleoptera: Coccinelidae to various volatile compounds released by various plant species to determine which host plants attract this predatory beetle the most.

2. Materials and methods

Laboratory studies were conducted to study the olfactory response of predatory coccinellid beetle, *C. septempunctata* towards healthy mustard, mustard aphid (*Lipaphis erysimi* Kaltenbach), aphid infested mustard, healthy wheat, wheat aphid (*Rhopalosiphum maidis* Fitch), aphid infested wheat, healthy rose, rose aphid (*Macrosiphum rosae* L.), aphid infested rose, healthy chrysanthemum, chrysanthemum aphid (*Macrosiphoniella sanborni* Gillette), and aphid infested chrysanthemum. The experiment was carried out using a four-arm olfactometer (Pettersson, 1970; Kalule and Wright, 2004; Webster *et al*., 2010, Jagdish *et al*., 2013).To conduct the experiment, the culture of the predatory beetle, *C. septempunctata* was maintained in the laboratory.

2.1 Collection and maintenance of predatory beetles and Plant specimens

The adult beetles of *C. septempunctata* were collected from field and were kept in plastic jars that were lined with blotting paper and fed with rose aphid, *Macrosiphum rosae* on daily basis thereafter jars were covered with muslin cloth.

Wheat, mustard, rose and chrysanthemum plants were used to find the olfactory response of *C. septempunctata*. Fresh twigs and leaves of host plants and twigs infested with aphids were acquired from insecticide-free plants grown in the Crop Research Centre, Pantnagar, Uttarakhand

2.2 Olfactometer bioassay studies

The behavioral responses of *C. septempunctata* towards different hosts were determined by using a four-arm olfactometer. The outside apertures of the treatments were tightly covered with a lid after the treatments were placed into the arms of the olfactometer. To produce uniform flow, a tiny axial flow fan (DC 10V, 3.5 cm dia) was mounted at each of the four distal openings of the arms. Each arm's odour resource was retained at the distal end. This setup allowed the beetles to receive airflow bathing the olfactory cues from the odour source and travel upwind in response to the volatiles. To prevent insects from moving towards the light, the translucent upper side of the release chamber was covered with a cloth. Twenty beetles of each species were deprived of food for 12 hrs prior to the start of the study to intensify their meal satiety. Treatments used placed in different arms include healthy twigs of a particular crop in one arm, twigs infested with aphids of that crop in one arm, and only aphids related to that crop in one arm and the control is treated with n-hexane because of its odourless nature (Siddique, 2019). The number of beetles moving towards host plants/prey in an olfactometer were counted every 30 minutes for three hours and the experimental arena was cleaned on regular basis.

Statistical analysis

To distinguish variations in the number of beetles moving towards prey and their host plants, the Duncan's multiple range test (DMRT) under Analysis of Variance (ANOVA) with $P \leq 0.05$ was utilized. The analytical program SPSS version 16.0 was used to compute the data.

3. Results and discussion

3.1 Response of *Coccinella septempunctata* towards different host plants and their aphids

The predatory beetle, *C. septempunctata* responded positively and significantly to various treatments viz., healthy mustard, *L. erysimi*, *L. erysimi* infested mustard, healthy wheat, *R. maidis*, *R. maidis* infested wheat, healthy rose, rose aphid *M. rosae*, *M. rosae* infested rose, healthy chrysanthemum, *M. sanborni* and *M. sanborni* infested chrysanthemum as presented in Table 1 and Fig. 1.

3.2 Response of *C. septempunctata* towards mustard and mustard aphids

Among all the different treatments, a significantly higher response of beetles was observed towards *L. erysimi* infested mustard with a percent orientation of 30. This was followed by 25 percent on healthy mustard and 10 percent on *L. erysimi* alone. No response of beetles was towards the control arm. This clearly suggested that these predatory beetles preferred the olfactory experiences provided by mustard over clean air.

3.3 Response of *C. septempunctata* towards wheat and wheat aphids

Among all the different treatments, a significant higher response of beetles was observed towards *R. maidis* infested wheat with a percent orientation of 25. This was by 20 percent on healthy wheat and 10 percent on *R. maidis* alone. The average percent per cent orientation towards the control arm was only 5 percent. This clearly suggested that these predatory beetles preferred the olfactory experiences provided by wheat over clean air.

3.4 Response of *C. septempunctata* towards rose and rose aphids

Among all the different treatments, a significant higher response of beetles was observed towards *M. rosae* infested rose with a percent orientation of 30. This was followed by 20 percent on healthy rose and 10 percent on *M. rosae* alone. The average percent orientation towards the control arm was only 5. This clearly suggested that these predatory beetles preferred the olfactory experiences provided by rose over clean air.

3.5 Response of *C. septempunctata* towards chrysanthemum and chrysanthemum aphids

Among all the different treatments, a significantly higher response of beetles was observed towards *M. sanborni* infested chrysanthemum with a percent orientation of 30. This was followed by 25 percent on healthy chrysanthemum and 10 percent on *M. sanborni* alone. The percent orientation towards the control arm was 0. This clearly suggested that these predatory beetles preferred the olfactory experiences provided by chrysanthemum over clean air.

Table 1: Olfactory response of *Coccinella septempunctata* to different treatments

C. septempunctata	**Percent orientation of *C. septempunctata***			
	Mustard	**Wheat**	**Rose**	**Chrysanthemum**
Control	0^{a} (0.00)	5^{a} (12.92)	5^{a} (12.92)	0^{a} (0.00)
Healthy twig	25^{c} (30.00)	20^{c} (26.56)	20^{c} (26.56)	25^{c} (30.00)
Aphids alone	10^{b} (18.43)	10^{b} (18.43)	10^{b} (18.43)	10^{b} (18.43)
Infested twig	30^{c} (33.21)	25^{d} (30.00)	30^{d} (33.21)	30^{d} (33.21)

Numbers followed by different letters within the same columns are significantly different (p>0.05)

Data presented in parentheses are angular transformed value

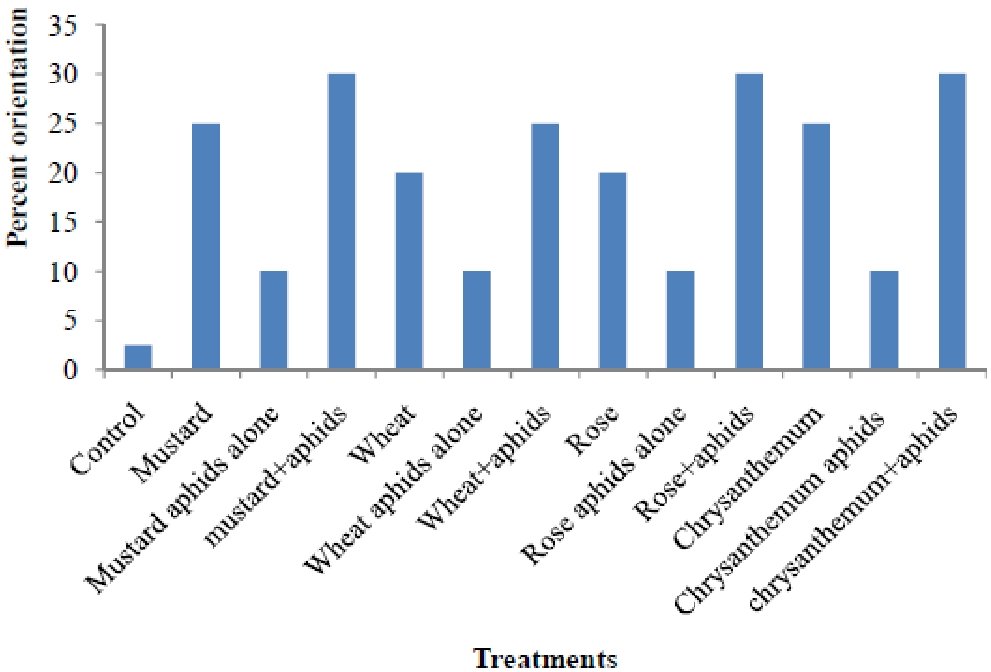

Fig 1. Olfactory response of the *Coccinella septempunctata* towards different host plants and their aphids

In the current study, a maximum number of coccinellids were attracted to nearly all host plants and aphids, and they responded strongly to both field and ornamental crops. When compared to treatments with only host plants or only aphids, the treatment with both host plants and aphids (host plants infested with aphids) attracted more coccinellids. The present findings are in accordance with the findings of (Dhaliwal *et al*., 2006), who observed that plants produce semiochemicals in response to herbivore attack to attracts beneficial insects in order to protect themselves, and that these plant-derived substances impact predator behavior in order to identify the pests. Khan *et al*. (2017) reported that a greater number of *C. septempunctata* were attracted towards host plants infested with aphids rather than healthy plants. Higher numbers of *C. septempunctata* towards infested wheat are confirmed by investigations of Drukker *et al*. (2000), Dicke (2009), Francis *et al*. (2004), Heil (2008) and Raymond *et al*. (2000). Glinwood *et al.* (2011) reported that predatory coccinellids link the odour of aphid-infested plants with the presence of prey.

Conclusion

The study findings support the hypothesis that predatory coccinellids are more attracted to the olfactory cues emitted by aphid-infested plants, likely due to the release of semiochemicals in response to herbivore damage. This behavior enhances the effectiveness of *C. septempunctata* as a biological control agent by improving its ability to locate prey in agricultural ecosystems.

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1.

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