**Behavioural response of *Coccinella septempunctata* (Linnaeus) to Herbivore-induced plant volatiles**

**Abstract**

The study aimed to investigate the attractiveness of specific plant volatiles to adult *Coccinella septempunctata* beetles using a Y-tube olfactometer. The results revealed that the majority of the adult beetles exhibited a preference for stimuli containing methyl salicylate, (-)-trans-caryophyllene, and cis-3-hexenyl acetate. These compounds elicited a significantly higher response compared to other tested stimuli. The findings suggest that these specific plant volatiles play a crucial role in attracting *C. septempunctata* beetles. This insight contributes to our understanding of the ecological dynamics governing predator-prey interactions and foraging behavior in ladybirds. Furthermore, the identified attractive compounds have potential applications in pest management strategies by harnessing natural predator attraction toward specific plant volatiles. Overall, this study provides valuable insights into the chemical ecology of *C. septempunctata* and highlights the practical implications for utilizing natural attractants in integrated pest management approaches.

**Keywords:** *Coccinella septempunctata*, Y-tube olfactometer, plant volatiles, methyl salicylate, (-)-trans-caryophyllene, cis-3-hexenyl acetate

**Introduction**

As we understand habitat management as a tool in integrated insect pest management, we recognize the voluminous and multidimensional chemical information present within and among all trophic levels of an agroecosystem. These chemical signals are utilized extensively as means of communication among the individuals of same species and with other species, thereby serving as a vital network interweaving the various components and trophic levels of the ecosystem (Price *et al.,* 1980). A multitude of scientific workers have contributed tremendous amount of knowledge and understanding into the identification of semiochemicals and their utilization for the manipulation of pest species population dynamics, either directly, or indirectly, through the improvement in effectiveness of natural enemies (Cortesero and Lewis, 1983). The highly specific bioactivity of semiochemicals makes them an attractive option for environmentally safe alternative pest control option (Dicke *et al.,* 1990). Plant originated volatile compounds are utilized by pest insects as cues for feeding and oviposition (Colazza *et al.,* 2004). These compounds can be exploited to manipulate pest population dynamics. These volatile compounds may not be essential for species survival, especially if the insect pest is oligophagous or polyphagous (Shorey 1977). These chemicals are produced in very low quantities so to they can be only used as a cue by the insects to cause a potential change in the behavior. However, some of these compounds have been effectively used as attractants for monitoring or in combination with poison baits (Levinson et al., 1990 and Steiner *et al.,* 1961).

Compared to the large body of information on parasitoids, less information is available on those chemical cues that guide predators during location and acceptance of oviposition sites (Steidle and van Loon 2003). Earlier studies have revealed that Coccinellids use various cues to locate their host (Obata, 1997 and Hermon *et al.,* 1988). For example, hoverflies rely on various chemical blends when searching for an oviposition site. These blends consist of plant and insect semiochemicals such as (E)-β-farnesene (EβF), the main component of the alarm pheromone of most aphid species (Nault *et al.*, 1984 and Francis *et al.,* 2005a). This sesquiterpene has been found to act as a kairomone for several aphid predators, including *Episyrphus balteatus* De Geer larvae, *Harmonia axyridis* (Pallas) adults, and *Adalia bipunctata* (Linn.) larvae and adults (Francis *et al.,* 2004, 2005b and Verheggen *et al.,* 2007a).

The purpose of this study was to investigate and screen the volatiles of host plant and prey by electroantennogram (EAG) and these EAG active compounds was further evaluated in Y-tube olfactometer to identify their attractancy or repellency. The results obtained with this study could be used to manipulate the behaviour *C. septempunctata* which would ultimately increase the effectiveness of predators in *Brassica* ecosystem.

**Materials and methods**

**Test insects**

***Lipaphis erysimi* (Kaltenbach):** Initial culture of aphids was collected from mustard fields Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi (India). The aphid infested twigs were fixed in conical flask with non-absorbent cotton. The desired quantity of water was poured in the conical flask to ensure the water requirement of the twigs are fulfilled and then transferred into a BOD incubator where constant temperature (20ºC) and RH (65 ± 5%) was maintained. When the aphids multiplied in sufficient quantity then they were utilized for maintenance of *C. septempunctata*.

***Coccinella septempunctata*:** Initially the adult beetles were collected from *Brassica* fields (Identification?). These beetles were then transferred to a jar (20×10 cm) and provided with sufficient aphids. The temperature of rearing room was kept at 25±2ºC with 60±5% RH and 16:8 (L:D) throughout rearing periods. The rearing protocol followed was as described by Sarwar and Saqib (2010). Females laid eggs at the bottom of the jar on tissue paper from which they were transferred to another jar (20×10 cm) for further development. The newly emerged grubs were separated and put into individual vials (2.5×1.2 cm) with sufficient aphids to avoid cannibalism. The grubs in individual vials were checked daily and aphids were provided till pupation. The newly emerged adults were fed with aphids and further used for EAG and olfactometer studies.

**Chemicals:** Six organic volatile compounds (OVCs) [methyl salicylate (98%), cis-3-hexenyl acetate (99%), (-)-trans-caryophyllene (99%), (R)-(+)-limonene (95%), citronellal (98%), citronellol (98%)] were purchased from Sigma-Aldrich (USA). Five different concentrations (0.0001, 0.001, 0.01, 0.1 and 1 %) of each compound were prepared by serial dilution and these were further used for EAG and olfactometer studies.

**Y-tube olfactometry:** A Y-tube glass olfactometer (20×20×20 cm length with 1.5 cm diameter) was used for present investigation. The temperature of room was maintained at 26±2ºC and 65±5% RH. A 40-w florescent bulb was used as a light source. The 1% concentration was used for this study as it was found higher attractive to parasitoids (Kumar and Paul, 2023, 2024) and the same concentration was tested against *C. septempunctata*. Individual insect was released through the opening of olfactometer and 10 minutes were provided to parasitoids and 5 minutes to beetles to reach stimuli source. The time spent by individual insects in the olfactometer arm was recorded. The number of insects attracted to either stimuli source was also recorded. Ten individuals were considered as one replication and were repeated six times with different individuals. The location of stimuli source was changed alternatively for every release. After every released the setup was washed with ethanol and left for drying in oven at 200ºC.

**Statistical analysis:** The EAG response obtained with OVCs of from parasitic wasps and beetles were analyzed using ANOVA (analysis of variance) and means of treatment were separated by Fisher’s LSD test (p < 0.05). The difference between time spent in treatment and control was subjected to paired t-test (P < \*0.05, \*\*0.01 and \*\*\*0.001) by Xlstat software version 2016. The cumulative number of both wasp and beetle response separately was subjected to chi-square test for goodness of fit using SAS 4.3.

**Results and Discussions:** We found similar results with *C. septempunctata*, where the time spent by beetles in treatment arm (OVCs) were significantly more as compared to control (n-hexane) (t = 3.41, df = 5, p = 0.018). The beetles were noticed to spend more time in arm containing stimuli of methyl salicylate (t = 8.02, df = 5, p = 0.0004), cis-3-hexenyl acetate (t = 7.26, df = 5, p = 0.0007), (-)-trans-caryophyllene (t = 3.50, df = 5, p = 0.017), and (R)-(+)-limonene (t = 3.46, df = 5, p = 0.017) (Fig. 1). The dual choice experiment conducted in Y-tube olfactometer revealed that higher percentage of adult beetles were attracted to methyl salicylate (71.60 ± 1.00), (-)-trans-caryophyllene (65.00 ± 0.71) and cis-3-hexenyl acetate (61.60 ± 1.00) (Fig. 2).

Chemical cues of host plant and host insects are important sources of information that mediates trophic interaction within species or across the taxonomic groups including insect predators and parasitoids (Carde and Miller, 2004, Dicke and Grostal, 2001). Within species these cues help in locating host, attracting opposite sex for mating, and inform others for danger. However, the member of different species can exploit these volatile cues to locate their host (Wyatt, 2003). Although, volatiles emitted from plant in presence of herbivore are more reliable than volatiles of uninfested plants and aphids alone.

The results we obtained with olfactometer studies confirmed the attractiveness of these compounds to adults of *C. septempunctata*. Maximum percentage of beetles were attracted to methyl salicylate, (-)-trans-caryophyllene and cis-3-hexenyl acetate. We also noticed significant differences between the time spent by adult beetles in treatments (viz., methyl salicylate, (-)-trans-caryophyllene, cis-3-hexenyl acetate) and control (n-hexane) (Fig. 1). Many species of coccinellid beetles are known to use methyl salicylate as olfactory cue to find or locate their prey. For instance, adult coccinellids were found to be attracted to methyl salicylate baited trap in the field (Zhu and Park, 2005 and Gadino *et al.,* 2012). Similarly, Rodriguez-Saona *et al.,* (2011) also noticed more attraction of coccinellids beetles to baited trap cards with methyl salicylate in the field. More recently, in a Y-tube olfactometer experiment 71% of adult beetles, *Hippodamia* variegate (Goeze) were found attracted to methyl salicylate. Since we do not have any supportive information on (-)-trans-caryophyllene, cis-3-hexenyl acetate then we cannot draw any conclusion regarding this. But both of the compounds were found to attract the beetles. We also found the ladybird beetles were attracted towards (R)-(+)-limonene. In a previous study, Chenier and Philogene (1989) found the attraction of conifer beetles towards stimuli of limonene. A significant new finding in our studies indicated that citronellal and citronellol showed some repellent activity towards *C. septempunctata* at 10 mg/mL doses. Similarly, the adult beetle was noticed to spend more time by moving here and there in olfactometer arm to sense or learn the stimuli of citronellal and citronellol. The vapor of both compounds interacts with antennal sensilla and modified the choice of beetles which leads the maximum adult away from the treatments.

**Conclusions:** Our finding suggests that the adults of *C. Septempunctata* were able to detect and recognize a wide range of compounds. Citronellal and citronellol had a some repellent effects on beetles where methyl salicylate, cis-3-hexenyl acetate, (-)-trans-caryophyllene and (R)-(+)-limonene was the attractant to both the species. These compounds could be used to increase effectiveness of parasitoids and predators by manipulating their behaviour.

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Time spent (min)

**Figure 1.** Time spent (± SE) for *C. septempunctata* adult into uninfested OVCs (treatment) and n-hexane (control). An asterisk (\*) above the letter indicates a significant difference between male and female.



**Figure 2.** Response of *C. septempunctata* to OVCS in Y-tube olfactometer.[Values are mean ± SE (n=6). Significant differences at *p<*\*0.01 and \*\*0.001, respectively in Chi-square test for 50:50 distribution in a 2×2 contingency table compared to control stimulus].