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Electroantennogram Bioassay of *Coccinella septempunctata* (Linnaeus) to Herbivore-induced plant volatiles

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Abstract

In the study on *Coccinella septempunctata*, the predator's antennal sensitivity to six synthetic compounds was investigated using electroantennogram techniques. These compounds were selected based their biological activities. The results revealed that (-)-trans-caryophyllene, citronellal, and citronellol were the most attractive compounds to the predator whereas methyl salicylate, cis-3-hexenyl acetate, and (R)-(+)-limonene were the least attractive compounds. These findings hold potential for manipulating the behavior of C. septempunctata by using these attractive compounds to enhance its effectiveness as a biological pest control agent. Such manipulation could contribute to more sustainable agricultural practices by reducing reliance on chemical pesticides. The study underscores how understanding insect sensory perception can lead to innovative pest management strategies. Overall, this research sheds light on specific synthetic compounds that can attract Coccinella septempunctata, offering insights into potential applications for enhancing biological pest control methods and improving crop management practices. These findings have implications not only for pest management but also for broader agricultural sustainability efforts.

Keywords: Aphids; Coccinella septempunctata; Electroantennography; Herbivore-induced plant volatiles; Olfactometry

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Introduction

Agricultural ecosystems depend heavily on effective pest management strategies to ensure crop productivity and sustainability. One key component of these strategies involves the use of biological control agents, such as predatory insects, to manage pest populations in a natural and sustainable manner. Among these predators, Coccinella septempunctata, or the seven-spotted ladybird beetle, is a prominent species known for its voracious appetite for various pest insects such as aphids and scale insects [1]. As such, understanding the factors that attract C. septempunctata to specific environments becomes crucial in enhancing its efficiency as a biological control agent within integrated pest management systems [2].

In recent years, there has been increasing recognition of the role that plant volatiles play in mediating interactions between herbivorous insects, their natural enemies (predators and parasitoids), and host plants within agro ecosystems [3]. Volatile organic compounds (VOCs) are emitted by plants in response to environmental stress or herbivore attack and can act as chemical cues for foraging predators such as ladybird beetles. Consequently, understanding how plant volatiles influence predator behavior has emerged as an essential area of study within the field of chemical ecology with significant implications for sustainable pest management practices [4]. Previous research has demonstrated that various plant volatiles can impact the foraging behavior and attraction of predatory insects like parasitoids and other insect predators [5]. However, there is limited specific research focused on identifying which VOCs specifically attract Coccinella septempunctata beetles.

To address this gap in knowledge regarding ladybird beetle attraction to volatile compounds emitted by plants present within agricultural landscapes we aim to investigate which specific VOCs elicit strong attraction from adult Coccinella septempunctata using a electroantennogram – an apparatus widely utilized for assessing antennal insect responses to volatile compounds [6,7,8,9]. Utilizing this methodological approach will allow us to evaluate whether certain VOCs are responsive or not towards this beneficial predator but also elucidate their preferences towards different doses of odors. By identifying those VOCs that strongly effects the antennal responses of Coccinella septempunctata beetles through our investigation using electroantennogram, tests could potentially shed light on novel methods aimed at enhancing biological control through promoting natural predator attraction towards specific plant

volatiles prevalent within agricultural landscapes ultimately contributing valuable insights into improving existing pest management strategies through harnessing ecological interactions between pests & natural enemies mediated by plant volatile emissions.

Materials and methods

Lipaphis erysimi (Kaltenbach): The initial culture of aphids was collected from mustard fields at the Division of Entomology, ICAR-Indian Agricultural Research Institute in New Delhi, India. The twigs infested with aphids were carefully placed in conical flasks and secured with non-absorbent cotton. A specific amount of water was added to the flask to ensure that the twigs' water requirements were met. The prepared flasks were then transferred to a BOD incubator where a constant temperature of 20ºC and relative humidity (RH) of 65 ± 5% were maintained.

Coccinella septempunctata: The adult beetles were initially collected from Brassica fields and transferred to a 20×10 cm jar with enough aphids for sustenance. The rearing room was maintained at a temperature of 25±2ºC, 60±5% relative humidity, and a light-dark cycle of 16:8 (L:D) throughout the rearing period, in accordance with the protocol described by Sarwar and Saqib (201). Females laid eggs at the bottom of the jar on tissue paper, which were then transferred to another 20×10 cm jar for further development. Upon hatching, the newly emerged grubs were individually placed into vials (2.5×1.2 cm) containing sufficient aphids to prevent cannibalism. These grubs were monitored daily and provided with aphids until they pupated. The newly emerged adults were fed aphids and subsequently used for EAG (Electroantennography) 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.

Electrophysiology: The EAG recordings for C. septempunctata were conducted using a range of OVC concentrations. The experimental setup and protocol utilized in this study were previously described by [6,7,8,9] and [10,11]. Newly emerged adult parasitic wasps and beetles (aged 2-4 days) were anesthetized in a refrigerator, after which their heads were removed with a sharp scalpel. The antennae were then excised, with the terminal segments removed before being placed between electrodes for recording.

For the chemical assay, 10 µL of each compound's concentration was applied to filter paper strips (1.5×.5 cm) and allowed to evaporate the solvent for 10 seconds. Subsequently, the filter paper strip containing the compound was placed inside a Pasteur pipette using forceps. Each recording session began with the application of air as compound followed by n-hexane (solvent), after which the specific compound was presented. At least one minute was given between two compounds to allow for recovery of the antenna.

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

The antennal sensitivity of C. septempunctata beetles was found dose-depended and higher response was obtained at 1% concentration (Fig. 1). Adult beetles had strong sensitivity to (-)-trans-caryophyllene (1.53±0.14mV), citronellal (1.35±0.01) and citronellol (1.31±0.09). Adult beetles were found to be equally sensitive to (R)-(+)-limonene and cis-3-hexenyl acetate (Table 1). A significant difference was noticed between EAG response of OVCs and n-hexane (control) (Fisher's LSD, $p < 0.05$).

The EAG recordings of C. septempunctata in response to a range of OVC concentrations have provided valuable insights into the olfactory perception of this insect species. The utilization of newly emerged adult beetles for the EAG recordings ensured that the responses obtained were representative of the adult stage and not affected by age-related factors. The experimental setup and protocol, consistent with previous studies [8,9] as well as [10,11] ensured methodological consistency and comparability with prior research. Overall, these methodological considerations

contributed to robust findings regarding C. septempunctata sensitivity to OVCs at various concentrations (Figure 1). The results obtained from this study hold implications not only for understanding the olfactory behavior of C. septempunctata but also for potential applications in pest management strategies or environmental monitoring efforts.

Table 1. EAG response (mV) of *C. septempunctata* to OVCs.

S. No.	Compounds	*C. septempunctata*
1.	(R)-(+)-limonene	1.06±0.08[c]
2.	Methyl salicylate	1.01±0.10[c]
3.	Cis-3-hexenyl acetate	1.07±0.11[c]
4.	(-)-trans-caryophyllene	1.53±0.14[a]
5.	Citronellal	1.35±0.01[b]
6.	Citronellol	1.31±0.09[b]
7.	Air	0.01±0.001[e]
8.	Hexane	0.20±0.01[d]

Mean (±SE) with common letter in column are not significantly different (Fisher's LSD, $P < 0.01$).

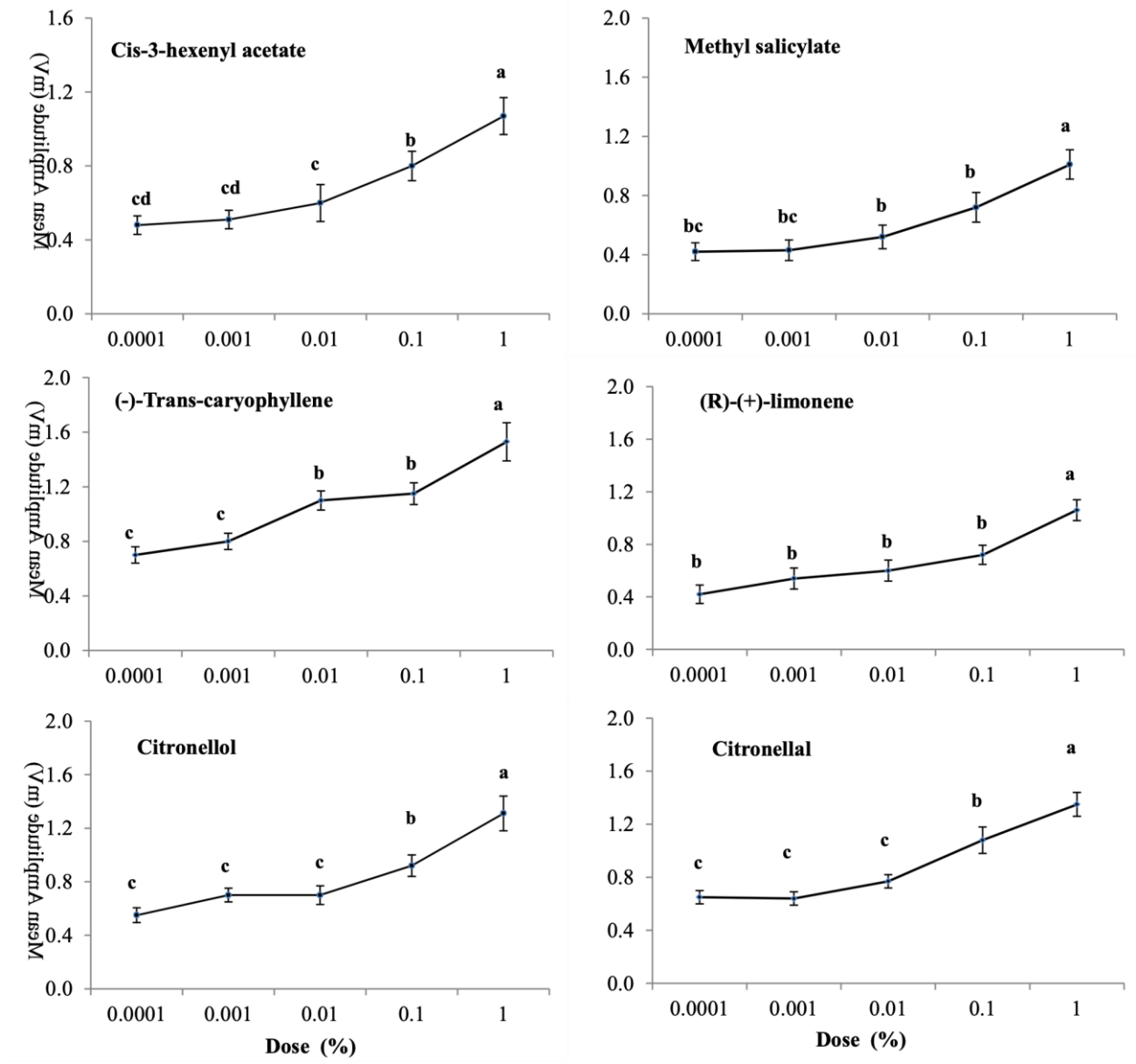

Figure 1. EAG responses profile of *C. septempunctata* to different concentrations.

It was previously demonstrated that C. septempunctata beetle was attracted to HIPVs [12]. In the present investigation, we found strong antennal sensitivity of beetles towards (-)-trans-caryophyllene, citronellal and citronellol. It is the first report on the EAG response of C. septempunctata to these compounds. The higher sensitivity of beetles to these compounds is due the volatility and the ability of these compounds to stimulate the olfactory receptors of antenna. It is also possible that adult beetle have specific sensilla on their antenna for above compounds. In a recent electroantennogram study, the adults of ladybird beetle, Hippodamia convergens (Guérin-Méneville) were found to be sensitive to methyl salicylate [13]. Earlier, [12] noticed the antennal response of C. septempunctata to aphid-induced volatile i.e. methyl salicylate. Many studies have indicated the positive response of methyl salicylate in the antenna of aphid predators including Chrysopa phyllochroma Wesmael [14] and Chrysopa lucasina Lacroix [15]. In contrast to these studies, we obtained lesser EAG response among beetles with methyl salicylate as compared to (-)-trans-caryophyllene, citronellal and citronellol. The differences in results might be due the difference in geographical conditions, test insects (C. septempunctata) and use of different doses of compounds. Cis-3-hexenyl acetate and (R)-(+)-limonene are OVCs that influence the antennal receptors of insect's predators [16]). In our study, antenna of C. Septempunctata was found to be equally sensitive to both the compounds.

Conclusions

Our finding suggests that the antennae of both C. Septempunctata were able to detect and recognize a wide range of compounds. Citronellal and citronellol was repellent to both wasps and 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.

References

[1] Pretty, J., and Pervez Bharucha, Z. 2015. Integrated pest management for sustainable intensification of agriculture in Asia and Africa. Insects, 6(1), 152-182.

[2] Sharma, A., Sandhi, R. K., & Reddy, G. V. (2019). A review of interactions between insect biological control agents and semiochemicals. Insects, 10(12), 439.

[3] Turlings, T. C., & Erb, M. (2018). Tritrophic interactions mediated by herbivore-induced plant volatiles: mechanisms, ecological relevance, and application potential. Annual review of entomology, 63(1), 433-452.

[4] Morrell, K., & Kessler, A. (2017). Plant communication in a widespread goldenrod: keeping herbivores on the move. Functional Ecology, 31(5), 1049-1061.

[5] Girling, R. D., Stewart-Jones, A., Dherbecourt, J., Staley, J. T., Wright, D. J., & Poppy, G. M. (2011). Parasitoids select plants more heavily infested with their caterpillar hosts: a new approach to aid interpretation of plant headspace volatiles. Proceedings of the Royal Society B: Biological Sciences, 278(1718), 2646-2653.

[6] Kumar, J., Paul, B., & Nebapure, S. M. (2021). Electroantennogram responses of *Earias vittella* (fabricius) to volatiles of cotton plant. Journal of Experimental Zoology India, 24(2).

[7] Kumar, J., & Paul, B. (2022). Effects of plant volatiles on electrophysiology of *Bracon bravicornis* (Wesmael)(Hymenoptera: Braconidae). Journal of Entomological Research, 46(1), 60-66.

[8] Kumar, J., & Paul, B. (2023). Electrophysiological and behavioral response of *Mallada basalis* (Walker) to volatiles of host plant and its prey. Journal of Entomological Research, 47(1), 94-99.

[9] Kumar, J., & Paul, B. (2024). Effects OF plant volatiles on the electrophysiological and behavioural response of aphid's parasitoid (*Dieretiella rapae*). Plant archives, 24(1): 991-997.

[10] Kumar J, Paul B, Nebapure SM (2018) Electrophysiological response of Dysdercus cingulatus (Fabricius) (Hemiptera: Pyrrhocoridae) to cotton plant extracts and other host related compounds. https://doi.org/10.1007/s40011-018-0992-y

[11] Kumar J, Paul B, Shivashankara, Kumari S (2017) Differential electroantennogram response of male and female of Dysdercus cingulatus to okra plant volatiles. J Entomol Zool Stud 5:197-201.

[12] Zhu JW, Park KC (2005) Methyl salicylate, a soybean aphid-induced plant volatile attractive to the predator Coccinella septempunctata. J Chem Ecol 31: 1733-46.

[13] Salamanca, J., Souza, B., Lundgren, J. G., & Rodriguez-Saona, C. (2017). From laboratory to field: electro-antennographic and behavioral responsiveness of two insect predators to methyl salicylate. Chemoecology, 27, 51-63.

[14] Xu XX, Cai,XM, Bian L, Luo ZX, Xin ZJ, Chen ZM (2015) Electrophysiological and behavioral responses of Chrysopa phyllochroma (Neuroptera: Chrysopidae) to plant volatiles. Environ Entomol 44: 1425–1433.

[15] Hegde M, Oliveira JN, Costa JG, BleicherE, Santana EG, Bruce JA, Caulfield J, Dewhirst SY, Woodcock CM, Pickett JA,Birkett MA (2011) Identification of semiochemicals released by cotton, Gossypium hirsutum, upon infestation by the cotton aphid, Aphis gossypii. J Chem Ecol 37: 741-750.

[16] Raina R, Joseph M, Sen A (2004) Electroantennogram responses of Chrysoperla carnea (Stephens) to volatiles. Indian J Exp Biol 42: 1230-1240.

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