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Synomonal and Kairomonal Mediated Tritrophic Interactions between Brinjal Cultivars, Mealybug *Coccidohystrix insolita* Green and Natural Enemy *Chrysoperla zastrowi sillemi* Esben-Peterson

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Abstract: The orientation response of *Chrysoperla zastrowi sillemi* Esben-Peterson adults towards the synomonal compounds of different mealybug infested brinjal cultivars were evaluated using a multi-armed olfactometer and the results revealed that the cultivar Udit attracted the highest number of natural enemies while the cultivar Pusa Uttam attracted the lowest number. The differences in compounds and their concentration in the volatile profile of the cultivars may lead to the highest preference of *C. zastrowii sillemi* to Udit and the lowest preference to Pusa Uttam. The relative response of *C. zastrowi sillemi* adults to the synomonal extracts of healthy and mealybug infested brinjal cultivars in a Y- tube olfactometer were evaluated and the results revealed that *C. zastrowi sillemi* adults showed more preference towards the synomonal compounds of mealybug infested plants than that of healthy synomonal extracts. The relative response of *C. zastrowi sillemi* towards the kairomonal compounds of mealybug *Coccidohystrix insolita* Green was also evaluated in a Y tube olfactometer and the results revealed that the highest mean number of adult lacewings were attracted to the kairomonal compounds of mealybug compared to control n-hexane. The present study revealed that the difference in composition of hydrocarbons in volatile blend determined the selectivity of natural enemy, *C. zastrowii sillemi.*

Keywords: Infochemicals, Olfactometer, Volatile hydrocarbons, Brinjal cultivars, Orientation response, Semio-chemicals, Intra specific communication, Ecosystem

Info-chemicals are involved in intraspecific and interspecific communication, and the latter plays a vital role in maintaining tritrophic interactions. The info-chemical mediated tritrophic interactions in the ecosystem mainly operates through allelochemicals which include allomones, kairomones and synomones (Arimura et al 2009). When plants are infested by herbivores, they emit plant volatiles that serve as signals to natural enemies, indicating the presence of herbivores. This communication benefits the plant by attracting the natural enemies of pests, while also assisting the natural enemies in locating suitable host organisms (Sheikh et al 2017). Tritrophic interactions within an ecosystem can exhibit varying dynamics based on the host plant species or the genotypes present within those host plants (Valencia-Cuevas et al 2018). The herbivorous insects are able to discriminate its host and non-host plants and even they can distinguish their host plants of different quality. The conspecific and hetero-specific interactions in an ecosystem coupled with environmental factors may modify the host searching capability of insects by altering the quality of host (Ninkovic et al 2019). Similarly, the carnivorous insects are responding to the volatile cues emanated from the plants infested with their prey and are able to distinguish the cues emanated from mechanically damaged plants and insect infested plants (Yoon et al 2010). The difference in the volatile blend of these synomones recruit specific natural enemies to the host plant. Likewise, the kairomonal compounds emanated from the prey species also act as a volatile cue for guiding natural enemies to the target pest (Turlings and Erb 2018).

The brinjal mealybug, *Coccidohystrix insolita* Green is prevalent in the Indian subcontinent and even distributed in other parts of the oriental region, Afro-tropical and Palearctic region (Ben- Dov 2013). The management of mealybug is a difficult task due to the polyphagous nature, wide distribution, cryptic habitat and resistance development to pesticides. However, mealybugs are confronted by a diverse array of natural enemies that play a vital role in the regulation of the pest population. Worldwide, there have been documented about 118 species of predators and 79 species of parasitoids associated with mealybugs. Specifically within Kerala, 20 species of predators and 11 species of parasitoids have been recorded (Shylesha and Mani 2016, Mohan and Anitha 2023). So there is utmost importance in understanding the different species interactions in the ecosystem for developing a sustainable pest management strategy. The understanding of various levels of interactions that take place in between different trophic levels can lead to the development of biological control methods that will not only yield fruitful results in pest suppression but also encourage the abundance and effectiveness of the entomophagous insect guild in natural as well as man-made ecosystems. So in this study, the relative response of the natural enemy *Chrysoperla zastrowi sillemi* Esben-Peterson towards the synomonal compounds of different brinjal cultivars infested by mealybug *C. insolita* was evaluated. The relative response of natural enemies towards infested and un-infested plant synomonal compounds and its response to mealybug kairomonal compounds were also tested.

MATERIAL AND METHODS

The experiments was conducted at Department of Agricultural Entomology, College of Agriculture, Vellayani, Trivandrum (N 8^{0}25'46.6788”, E 76^{0} 59'15.02016”) during the year 2019-2020. The laboratory experiments were conducted at a temperature of 27 ^{0}C and relative humidity of 65-70 %.

Extraction of Synomones

Un-infested brinjal cultivars: Ten cultivars of brinjal were maintained in insect -proof nylon cage separately inside the net house. The synomonal compounds were extracted from healthy leaf samples. Leaf sample (10g) was taken from each plant and immersed overnight in 100 mL of HPLC grade distilled hexane in glass bottles. The hexane extract was filtered through a Whatman No. 1 filter paper and anhydrous sodium sulphate (1g) was added and kept for 2h for dehydration. The hexane extract was subjected to column chromatography and was passed through the silica gel of 60 to 120 mesh size. The eluted compound was collected and distilled at a temperature of 60 to 70^{0}C in a rotary vacuum flash evaporator. The leftover residue was collected by rinsing the flask with HPLC grade hexane in a small glass vial. The compounds were stored at -80 ^{0}C in a low-temperature cabinet (Trang 2008).

Infested with the mealybug *C. insolita*: Ten cultivars of brinjal were maintained under an insect-proof nylon cage in a net house. Twenty adult mealybugs were carefully inoculated into each plant using a soft camel brush at one month after transplanting. Twenty days after inoculation, the synomonal compounds were extracted from the mealybug infested leaf samples as per the procedure mentioned for extraction of synomonal compounds from un-infested brinjal cultivar.

Maintenance of natural enemy culture: A nucleus culture (eggs) of the predator, *C. zastrowi sillemi* was purchased from NBAIR, Bangalore and reared in the laboratory. The eggs (100 nos) were mixed with 0.75 cc of sterilized eggs of *Corcyra cephalonica* Stainton in a plastic container. The emerged larvae were transferred separately in to small plastic boxes and reared on the diet containing eggs of *C. cephalonica*. Brown paper was provided for facilitating cocoon formation in the plastic boxes. The cocoons were collected and placed in a glass jar for adult emergence. The emerged adults were maintained with a diet containing 50 % honey and castor pollen. The adult chrysopids were used for further studies.

Response of *C. zastrowi* sillemi to synomonal compounds of brinjal: The response of the natural enemy, *C. zastrowi sillemi* towards synomonal compounds of brinjal was evaluated by multi-armed olfactometer bioassay and Y tube olfactometer bioassay

Multi-armed olfactometer assay: Relative response of the predator *C. zastrowi sillemi* towards the synomonal compounds of various brinjal cultivars was evaluated in a multi-armed olfactometer. The olfactometer made up of glass with a central portion of 15 cm diameter and arm length of 10 cm and diameter of 2.5 cm was used for the study. The olfactometer was kept at a temperature of 27 ^{0}C and relative humidity of 65-70 % under a 40 W fluorescent lamp. The average airflow through each arm was maintained at 10 L/h. Synomonal compounds (50 µl) of each brinjal cultivar was taken in a Whatman filter paper of size 2 cm * 1 cm and placed in different arms of the olfactometer. N-hexane (0.5 mL) in a similar strip of filter paper was considered as control and placed in the remaining arm. Ten freshly emerged adult chrysopids were released at the central arena of the olfactometer and observed the response of the chrysopids at every 5 minutes for a period of 30 minutes. The experiment was repeated ten times and data regarding the relative preference of chrysopids towards synomonal extracts were recorded and analyzed.

Y-tube olfactometer assay: Based on the previous experiment, the most preferred and the least preferred brinjal cultivar to *C. zastrowi sillemi* were selected for a Y-tube olfactometer assay. The Y-tube olfactometer was made up of glass with 28 cm base length and arm length of 13 cm with 2.5 cm diameter. The olfactometer was kept at a temperature of 27^{0}C and relative humidity of 65 to 70 % under a 40 W fluorescent lamp. The synomonal extracts of mealybug infested and un-infested plants were tested to find out the relative response of *C. zastrowii sillemi* to the volatiles emanated from the plants. Synomonal extracts (50 µl each) were taken in separate filter paper bits and placed inside the two arms of the olfactometer. The individual adult chrysopids was released at one end of the base tube and given 4 minutes to walk towards the end of the olfactometer arm. The choice made by the chrysopids was recorded as it crossed about 4 cm in an olfactometer arm after the division of the base tube and remained for about 20 second in the odor source. After testing 5 chrysopids, the olfactometer was washed with ethanol, rinsed using distilled water and dried in a hot air oven and the positions of odor sources were exchanged to avoid any bias in the experiment. The experiment was repeated with another 5 chrysopids and altogether considered as a single replication. The olfactometer assay was replicated 10 times.

Extraction of kairomones from *C. insolita*: The kairomonal compounds emanated from the mealybug body was extracted by immersing mealybug (1g) in 10 mL of HPLC grade hexane. The hexane extract was placed in a shaking water bath at a temperature of 28 ^{0}C for 2 h and later at a temperature of 50 ^{0}C for 20 minutes. The hexane extract was filtered through a Whatman No. 1 filter paper and passed through a silica gel column of 60 to 120 mesh size. The eluted compounds were distilled at 60 to70 ^{0}C in a rotary vacuum flash evaporator and the leftover residue was collected by rinsing the flask with HPLC grade hexane into a small glass vial. The compound was stored at $^{-}$80 ^{0}C in a low-temperature cabinet (Trang 2008).

Response of *C. zastrowi* sillemi to kairomonal compounds of *C. insolita*: The response of *C. zastrowii sillemi* adults towards the kairomonal compounds of *C. insolita* was evaluated in a Y-tube olfactometer. The olfactometer was kept at a temperature of 27 ^{0}C and relative humidity of 65 to 70 % under a 40 W fluorescent lamp. The kairomonal compound (50µl) was taken in a Whatman filter paper bit of size 2 cm * 1 cm and placed in one arm while n-hexane was taken as the control in another arm of the olfactometer. The experiment was conducted as per the procedure mentioned in Y tube olfactometer bioassay.

Statistical analysis: The data was analyzed by paired t-test and anova using GRAPE software.

RESULTS AND DISCUSSION

The multi armed olfactometer experiment to identify the relative preference of natural enemy *C. zastrowi sillemi*, to the synomonal compounds of different brinjal cultivars revealed that the cultivar Udit attracted the highest number of natural enemies (2.87) which was statistically superior to all other treatments (Table 1). However, significantly lowest number of *C. zastrowi sillemi* were oriented towards the cultivar, Pusa Uttam (0.28). The variation in preference shown by the natural enemies may be related to the difference in volatile compounds emanating from the host cultivar. Hanumantharaya (2006) conducted a six-armed olfactometer study to evaluate the response of the predator, *C. carnea* towards the synomonal extracts of cotton and sunflower genotypes revealed that the preference shown by the predator towards the cultivar DHH-543 and KBSH-1 was due to the difference in the volatile profile of the cultivars. Similarly, Trang (2008) also reported that the parasitoid *Apanteles angaleti* Mues exhibited a higher preference towards the synomonal compounds of cotton genotype RS 2013 in a six-armed olfactometer study as the volatile profile of the cultivar made it more attractive to the parasitoid compared to other genotypes. Kumar et al. (2017) observed that synomones of the sugarcane cultivar, CO- 0238 attracted the highest number of parasitoid, *Cotesia* sp. and they suggested that the long-range cues emanating from the infested host plants played a significant role in guiding the parasitoids to the host plant.

The relative preference of natural enemy to *C. zastrowi sillemi* adults towards infested and healthy brinjal synomonal compounds were evaluated in a Y shaped olfactometer . *C. zastrowi sillemi* showed more preference towards the synomonal compounds of mealybug infested plants (5.20) than that of healthy synomonal extracts (2.90). The relatively high preference of natural enemies towards mealybug infested plant synomonal extracts may due to the presence of more volatile compounds in it compared to the healthy plant synomones. As a result of insect herbivory, a cascade of events take place in the plants which ultimately led to the higher production of plant volatiles that act as a reliable long-distance cue for natural enemies. The present findings are in consonance with Manna et al (2024). Jagdish (2008) and Xiu et al (2019) also demonstrated that coccinellid predators showed a higher preference towards the aphid infested plant synomonal compounds compared to healthy plant odors. Ahmed et al (2021) observed that aphid infested broccoli plants emitted a higher concentration of volatile organic carbons than un-infested plants. The variation in the volatile profile of healthy and mealybug infested plants may lead to the differential response of natural enemies to the synomonal compounds.

Table 1. Response of *Chrysoperla zastrowi sillemi* to synomonal compounds of brinjal cultivars

Cultivars	Number of *C. zastrowii* attracted
Haritha	2.26
Neelima	0.47
Ponni	0.83
Pink Long	0.67
Udit	2.87
Green Long	0.47
Pusa Purple Long	0.45
Pusa Kaushal	0.53
Pusa Uttam	0.28
Pusa Shyamla	0.42
n-hexane	0.38
CD (p=0.05)	0.24

Table 2. Response of *Chrysoperla zastrowi sillemi* towards synomonal compounds of healthy and mealy bug infested brinjal cultivar and kairomonal compounds of *Coccidohystrix insolita*

Treatments	Synomones from healthy plants	Synomones from mealybug infested plants	Kairomones from mealybug, *C. insoilta*	n-hexane (control)
Mean	2.90	5.20	5.90	2.80
Standard deviation	0.876	1.135	1.101	1.033
t test value (0.01)	3.25**		3.25**	

The highest mean number of adult lacewings were attracted to the arm containing kairomonal compounds of mealybug (5.90) compared to the arm containing n-hexane (2.80) in tube olfactometer study (Table 2). The higher preference of natural enemies towards the kairomonal compounds may be due to the volatiles present in it which act as olfactory cue for natural enemies. Urbina et al (2018) recorded that *Cryptolaemus montrouzieri* (Mulsant) exhibited significant response to the kairomones produced by different mealybugs. Fand et al. (2020) observed that the bacterial volatiles of the mealybug honeydew acts as a kairomone source to the parasitoid *Anagyrus dactylopii* (Howard).

CONCLUSION

The variation in preference shown by the *C. zastrowii sillemi* towards different brinjal cultivars may be related to the difference in volatile compounds emanating from the cultivar. Particularly, *C. zastrowii sillemi* exhibited more preference towards mealybug infested plant synomonal compounds rather than un-infested plants. The variation in the volatile profile of healthy and mealybug infested plants may lead to the differential response of natural enemies to the synomonal compounds. Likewise, mealybug kairomones attracted more *C. zastrowii sillemi* than the n hexane (control) which may be also due to the variation of hydrocarbon profile of the kairomones emanated from the mealybug.

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