A novel prospect for an oviposition attraction of *Chrysoperla carnea* (Steph.) (Neuroptera: Chrysopidae) for its population upsurge

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Abstract

**Background:** Attraction and oviposition preference of the green lacewing, *Chrysoperla carnea* (Steph.) (Neuroptera: Chrysopidae), in response to prey availability in an ovipositional site was studied. The study aimed to investigate whether an adult attraction of *C. carnea* to oviposition on the substrate was affected by aphid juice (AJ) of fresh brassica aphid, *Brevicoryne brassica*.

**Result:** In laboratory bioassay, the number of eggs laid by female was significantly higher on AJ-treated area of substrate than control, indicating that oviposition was influenced by the AJ application. Attraction period of AJ lasted for 4 days. In Y-maze olfactometer studies, video tracking software ANY-maze® indicated that *C. carnea* spent significant more time in the novel arm provided with AJ, showing an attraction. In the greenhouse study, AJ spray attracted a significantly higher number of male and female and considerably increased the number of eggs laid on brassica plant.

**Conclusion:** Fresh AJ may be a potential attractant for raising population of this predatory species at a particular location without being involved in rearing and augmentation process. This finding is of special interest and may be of benefit in biological control. As it is likely that the AJ spray could be used to enhance *C. carnea* population attraction to the desire field.

**Keywords:** *Chrysoperla carnea*, *Brevicoryne brassica*, Aphid juice, Attraction period, Tracking

**Background**

Green lacewings are naturally important in most agricultural systems because of their ease to hunt the aphids, whiteflies, mites, and some other small, soft bodied insects (Golmohammadi et al. 2021). They frequently have specialized mechanisms for searching, choosing, and consuming their prey that signify them good candidate for augmentation and conservation biological control (Moradi et al. 2019). Prey is more important for nutrition of their predaceous larvae and as well as their adult for reproduction (Pitwak et al. 2016).

Lacewings are attracted to vegetation differently; some of them prefer herbaceous plants and other species choose to live at a vertical plant (Thöming et al. 2020). Several other reasons were reported to be affected related to the prey host or host plant selected by the prey (Cuello et al. 2019), orientation and floral structure (Koczor et al. 2017), volatiles produced by the prey and prey host plants (Alcalá Herrera et al. 2019).

Some of the studies been evaluated the consequences of ovipositional site preference of *Chrysoperla* sp. with...
Specifically, laboratory and greenhouse studies were conducted to test the hypothesis whether ovipositional preference of female chrysoperla and the development of the offspring on aphid-infested sites (Salamanca et al. 2015). The ovipositional location is obviously important to the chrysoperla, and many other studies have shown that reasons such as prey type, predation risk, prey density, and prey age may all affect the ovipositional behavior (Imam 2020).

It is clear from our previous study that different aphid species may provide different food qualities to chrysoperla predators and some might be toxic and arrest their development, or leads to their mortality (Sohail et al. 2020). Further, aphidophagous predators are highly dependent on the aphids as an important for oviposition (Riddick 2020).

In field crops as brassica and wheat, insecticidal application is mostly obliged by the unavailability of natural enemies to minimize aphids’ populations. Sometimes, its populations are poorly managed by natural enemies due to their late arrival and/or early dispersal (Zhao et al. 2015). Late appearance to an aphid-infested area may reflect the prey density, preference, or delayed detection; however, early departure may signify deterrence due to some reason or fear of prey scarcity. Hence, the early attraction of natural enemies and their retention in the desired field could efficiently manage the aphid population and minimize the dependence on insecticides.

Artificial sprays have been used to attract chrysoperla predators in harmony with prey in desired field crops and orchards (Kunkel and Cottrell 2014). Researchers found that either tryptophan or caryophyllene mixed into artificial food sprays can attract adults of *Chrysoperla sp.* (McEwen et al. 1994). Adults of *Chrysoperla* use volatile to locate their prey, mating site, as well as sites suitable for oviposition (Kunkel and Cottrell 2014).

In a previous study, influence of two aphid species (*Aphididae: Homoptera*), i.e., oleander aphid, *Aphis nerii* and brassica aphid, *Brevicoryne* brassicae was examined on mortality and biological parameters of *C. carnea* and found the evidence of preference for *B. brassicae* with successful biology (Sohail et al. 2020). The present study aimed to test the hypothesis whether *B. brassicae* body juice enhances an ovipositional attraction of *C. carnea*. Specifically, laboratory and greenhouse studies were conducted to test: (1) oviposition preference of female *C. carnea* to AJ under optimum laboratory conditions; (2) attraction period of AJ for *C. carnea*; (3) attraction of a female using Y-tube olfactometer; and (4) attraction and ovipositional preference toward AJ in the greenhouse. As the novelty of this study is concern, attraction of *C. carnea* for an egg laying through AJ has not been studied at all and video tracking was used to investigate preferential behavior using software ANY-maze®.

**Methods**

All experiments were conducted from February to May 2021, in Biological Control Laboratory at 25 ± 2 °C, 65 ± 10% RH and 12L/12D photoperiod and in a greenhouse at 28 ± 2 °C, 55 ± 10% RH and 14L/10D photoperiod at Plant Protection Division, Nuclear Institute of Agriculture (NIA), Tandojam (25° 25′ 17.3″ N 68° 32′ 29.3″ E).

**Insects**

*Chrysoperla carnea* adults were captured from Tandojam (location: 25° 24′ 50.4″ N 68° 30′ 59.3″ E) during October 2020, as commonly found in sugarcane, cotton, and mango orchard. Specialized LED-based light traps were installed in various points to attract lacewing trichromatic vision (Gagliò et al. 2017). The collected adults were placed in rearing cages (23.5 × 23.5 × 20.5 cm) made of crystal acrylic sheets (1 cm gauge). Adults were reared on an artificial diet consisting of honey, sugar, yeast, and distilled water (1:2:1:2), replaced daily and supplied ad libitum (Sohail et al. 2019). Rearing cages were provided black linen cloth at the top for egg laying, after which eggs were harvested with a razor blade. Harvested *C. carnea* eggs were transferred in black cover cloth and fed on frozen Angoumois grain moth (*Sitotroga cerealella* Olivier) eggs (Pasini et al. 2018). On hatching of first instars, larvae with frozen *Sitotroga* eggs (0.6 g/ tube) were sealed in polypropylene transparent straw/tubes (2 inches long) with 3 pin aeration holes, to avoid cannibalism (Sohail et al. 2018) (Fig. 1). On pupation, the pupal portion was cut and separated from tubes and transferred on glass Petri plates (6 cm diameter) for emergence (Fig. 1). The life stages were maintained at 25 ± 1 °C and 60 ± 5% RH and 15:9 (L/D) for further studies. Fresh *B. brassica* samples were collected from mustard plants (*Brassica campestris*) grown in the open field (25° 25′ 18.5″ N 68° 32′ 33.5″ E) near Sindh Agriculture University, Tandojam.

**Oviposition preference**

*Chrysoperla carnea* females were tested for possible preference of oviposition. Two choices between aphid juice (AJ)-treated area and untreated/control (treated with distilled water) area were given for oviposition in the egg-laying apparatus (ELA). AJ was prepared with fresh aphids collected from an open field of mustard plants (variety S9). Ten grams of aphids was homogenized using mortar and pestle and made the volume up to 100 ml with distilled water. The mixture was centrifuged at 10,000 rpm (using Universal 320R, Hettich®) for 20 min...
Supernatant was used as AJ for further experiments. ELA was made up of a 4-L glass jar (locally made by ToyoNasic®) provided with black cloth (batiste cotton) on the top, as substrate for oviposition. AJ was applied on the black cloth/substrate in four different patterns: (1) half circle pattern (HCP); (2) quadrant pattern (QP); (3) chess pattern (CP); and (4) alternate line pattern (ALP) (Fig. 2). Four different ELA were used for each of the pattern, and each pattern received 300 µl of AJ, spread on the measured surface by the camel hair brush. Two pairs of *C. carnea* (5 days old) were released in each of ELA and provided with artificial diet. Attraction period was measured by counting the number of eggs laid on both treated (AJ) and control (distilled water) area daily for 7 days. All eggs were removed, and substrate was changed after each count. The entire experiment was replicated four times.

**Attraction period**

Longevity of AJ was assessed against the *C. carnea* oviposition. ELA (same as in the previous experiment) was used along with the quadrant pattern of the substrate. Substrate was received 300 µl of AJ. Three pairs of 5-day-old *C. carnea* were released in ELA and provided with artificial diet. Attraction period was measured by counting the number of eggs laid on both treated (AJ) and control (distilled water) area daily for 7 days. All eggs were removed, and substrate was changed after each count. The entire experiment was replicated 10 times.

**Y-tube olfactometer bioassay**

Olfactory bioassay was intended to track *C. carnea* female in a dual-choice Y-maze glass olfactometer in the presence of AJ (Fig. 3). Only gravid females (had swollen abdomen with eggs) were used in olfactometer study. After each test, the female was replaced by new one and olfactometer was washed with detergent and then oven-dried. The experiment was conducted at room conditions, i.e., 25 ± 2 °C, 65 ± 10% RH. Y-maze olfactometer was made up of three arms, i.e., one release arm and two novel arms. Novel arms were provided with wells for treatment application. One novel arm was provided with AJ (5 µl) in its well, while other one got the distilled water. All arms were closed with 80 mesh sieve to avoid either escape of larva or to stick on the treatments. A single *C. carnea* female was released through
the release arm, and its behavior was observed for up to 5 min. Totally, six females were assessed during this experiment. Female behavior was recorded by the digital camera (Nikon® D-5200, with Nikon lens 10–24 mm f/3.5–4.5, Tokyo, Japan) fitted at a height of 40 cm above the arena. Video recordings were analyzed using video tracking software (ANY-maze® 6.1, Stoelting Co., USA). The Y-maze arena apparatus was set to record readings of only novel arms. Path tracking, distance traveled, speed, and time spent in each novel arm were recorded. The novel arm, where female spent more than half of the total novel arm time, was considered attractive, and the other arm that retained the female less than half of the novel arm time was marked as non-attractive.

**Greenhouse study**

Greenhouse study was conducted to determine the attraction and oviposition preference for adult *C. carnea* for AJ-treated plants. Mustard plants were grown in a greenhouse with a total area of 54.1 m³ (4.3 m L × 3.4 m W × 3.7 m H). Greenhouse was divided into three plots, and each plot (1 m W × 3 m L) possessed three rows of five plants (total of 15 plants/plot), with a spacing of 45 cm between plants and 25 cm between rows (Fig. 4). Four plants (two plants for each treatment, i.e., AJ and control) were randomly selected in each plot (total of 12 plants). Each plant received 5 ml of treatment solution (either AJ or distilled water). AJ (concentration, 100 mg of aphid/1 ml of distilled water) was applied to selected plants, 2 h before the releasing of *C. carnea* adults. Prior to release, 40 adults (20 males and 20 females) of *C. carnea* were placed in a small crystal acrylic rearing cage (23 cm L × 23 cm H × 24 cm W) with an open top that was covered with black cloth (batiste cotton). The cage was then placed in the released zone of the greenhouse. In the afternoon (16:00 h), cover was removed from the cage, allowing *C. carnea* adults to
exit the cage. Ovipositional preference was measured by counting the number of eggs on each targeted plant daily for 7 days. All eggs were removed daily using forceps after each count. Attraction of *C. carnea* was measured by counting the number of adults on the targeted plants for a total of 7 days. The entire experiment (i.e., trails) was replicated three times, each done in a different section (each section contains three plots as mentioned above) of the greenhouse.

**Statistical analysis**

Oviposition preference and attraction period in the laboratory and as well as in the greenhouse were analyzed using paired *t* test (*p* > 0.005). All paired *t* tests were performed using statistical software “SPSS,” version 16.0. Parameters of olfactory bioassay and their comparisons were analyzed by analysis of variance and Tukey’s test (*p* > 0.005) using software “JMP,” version 15.2.1.

**Results**

**Oviposition preference**

Results revealed that *C. carnea* was readily attracted to and laid more eggs on the substrate treated with AJ. Section(s) of the substrate treated with AJ in each pattern received significantly [HCP (*t*= 3.58, *p*= 0.007); QP (*t*= 2.35, *p*= 0.047); CP (*t*= 2.48, *p*= 0.038); and ALP (*t*= 2.75, *p*= 0.025)] high oviposition rate than that of not treated with AJ (Fig. 5). This indicates the strong oviposition preference of *C. carnea* female to the substrate applied with AJ.

**Attraction period**

Attraction period of AJ was assessed toward oviposition preference of *C. carnea* female using ELA with QP substrate in captivity. AJ was found significantly more attracted for 4 days after application (Fig. 6). The second day after application, an effectiveness of AJ was started to decline significantly till the 4th day, as the decline over time in attraction was comparable for most of the days. Although AJ-treated substrate received more eggs than control, nonsignificant difference was found in the last 3 days.

**Y-tube olfactometer bioassay**

Olfactometer track (Fig. 7) showed the significance attraction of female *C. carnea* to the AJ. Females spent significantly (*p*= 0.019) more time in the novel arm provided with the AJ than in the novel arm with no AJ, showing a strong attraction of *C. carnea* toward AJ (Fig. 8). Likewise, the distance traveled in novel arm with AJ was recorded significantly (*p*= 0.028) more than the other novel arm (Fig. 9), while nonsignificant difference (*p*= 0.019) was found in maximum speed of the female in both novel arms (Fig. 10), indicating that the AJ did not enhance the speed of the female.

*Fig. 5* Graph presents the oviposition preference of *Chrysoperla carnea* female in all substrate patterns: HCP half circle pattern, QP quadrant pattern, CP chess pattern, ALP alternate line pattern. Data are mean ± SE with paired *t* test: *p* ≤ 0.05
In the greenhouse, a significant attraction \((t=4.89, p=0.008)\) of *C. carnea* adults was observed toward AJ. The number of *C. carnea* adults was 3.4 times more attracted to plants treated with AJ than on the plants with no AJ, indicating that an attraction of lacewing adults to brassica plants was influenced by the application of AJ. Likewise, plants treated with AJ significantly \((t=4.05, p=0.016)\) received more numbers of lacewing eggs than the plants with no AJ. This oviposition preference was 2.68-folds greater than the plants with no AJ, indicating that an oviposition preference is also influenced by the preference of AJ (Fig. 11).

**Discussion**

The present results depicted that AJ was attractive for adults of *C. carnea* and also found influential for their oviposition. These results are analogous with previously reported studies suggesting an attraction and oviposition preference of green lacewings to the plant with aphid presence. For example, *Chrysoperla rufilabris* (Burmeister), *Chrysopa nigricornis* (Burmeister), and *C. comanche* (Banks) preferred pecan tree, *Carya illinoensis* (Wangenh.) on aphid appearance (Kunkel and Cottrell 2014). Petersen and Hunter (2002) reported that female lacewings are more likely to attract and lay eggs on plants with aphids and honeydew than on clean foliage.

Female lacewings need to assess soundings to oviposit in a suitable environment. One way to achieve those behavior is through the chemical cues, which often have different information levels and can be related to the presence of prey (Hilker and McNeil 2008). These chemical cues could come from three potential sources that
are responsible of indicating the prey abundant environment: (1) plant volatiles emitted by aphid-infested plants, act as synomones in prey searching behavior (Simpson et al. 2011); (2) substance emanated for prey body; in our case, the brassica aphid *B. brassicae*; and (3) other excretions of its prey body (i.e., honeydew) that act as kairomones, while prey searching behavior (Goldansaz et al. 2004). Plant volatiles have not been studied in the present experiments. Substance emitted by prey/herbivores themselves may attract predators for colonization for their next progeny (Salamanca et al. 2018), especially with *C. carnea*, when the imago was not a predatory

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**Fig. 8** Time duration of *Chrysoperla carnea* female spent in Y-tube olfactometer is presented in this graph. Black dots are denoting the values of time in second. The dotted line is presenting box plots and displaying means time duration. All black solid vertical lines in box plots indicate ±SEM. Black circles at right are presenting the treatment mean comparisons by using Tukey HSD.

**Fig. 9** Distance traveled by *Chrysoperla carnea* female in Y-tube olfactometer is presented in this graph. Black dots are denoting the values of distance in meters. The dotted line is presenting box plots and displaying means distance traveled. All black solid vertical lines in box plots indicate ±SEM. Black circles at right are presenting the treatment mean comparisons by using Tukey HSD.
stage. Laboratory and greenhouse results showed that substances from fresh aphid body juice were attractive to *C. carnea* female providing clue that these substances may have influence in this attraction.

Among the substance released by aphid, (E)-β-farnesene (alarm pheromone) is known to attract the predators to the site where prey is colonized (Francis et al. 2004). *C. carnea* found responsive to (E)-β-farnesene (Zhu et al. 1999). Verheggen et al. (2009) isolated (E)-β-farnesene from headspace of a crushed individual of pea aphid, *Acyrthosiphon pisum*. Crushed aphids emit the higher amount of volatile substances that could be sufficient to be perceived by natural enemies than whole unstressed aphids (Francis et al. 2004).
Aphids only emit (E)-β-farnesene, when they are disturbed by external stimulus (Salamanca et al. 2015). This indicates that their removal from the host plant and crushing may create stress which leads to the production of volatile substance. It creates a probability for AJ to be responsible for the attraction of C. carnea in the laboratory and greenhouse. The same attraction was observed in hoverfly for A. fabae and A. pisum, as both produce (E)-β-farnesene (Francis et al. 2005).

Zhu et al. (1999) reported that both male and female C. carnea responded and attracted most strongly to (E)-β-farnesene and 2-phenylethanol in the field test. 2-phenylethanol is a sex pheromone released by the oviparae (virgin female) of aphid through metathoracic tibiae for mate finding (Zhu et al. 2005). Females of several lacewings species are also found attracted to this pheromone substance and achieve a higher level of fecundity after preying aphid with 2-phenylethanol (Hooper et al. 2002). So, AJ may possess the sufficient amount of 2-phenylethanol that could be one of the reasons to attract both male and female and also suppose that it may be used by C. carnea as a gesture for attracting food or oviposition sites.

Feeding habit of adult C. carnea, palyno-glycophagous, may also be one of the reasons of attraction to AJ. As in C. carnea, non-predatory lacewing adults feed on pollen, nectar, and honeydew (Koczor et al. 2019). Quality and quantity of honeydew consumed by the female lacewing elicited the oviposition response and egg fertility (Frechette and Coderre 2000). Although it is a food source to lacewing, it may have isomers of tryptophan that are supposed to act as kairomones for adult lacewings, as aphids habitually feed on the phloem sap of the plant which is dominant with sucrose and provide the C skeleton for amino acids, lipids, and protein synthesis (Sun et al. 2016). High concentration of sucrose reduces performance of aphid as it dilutes other nutrients like proteins, amino acids, and other organic acids; as a consequence, aphids must consume more phloem sap and excrete excessive amount of sucrose from alimentary canal as honeydew (Pescod et al. 2007). Thus necessary amount of honeydew or its composition is still present in the alimentary canal of aphid which may be responsible for the attraction of lacewing through AJ. McEwen et al. (1993) reported that C. carnea adult showed a positive response to aphid honeydew and increases retaining time with higher turning frequency. This may be a strong reason for lacewing adults to remain in the area and a subsequent increase in egg laying. This study is supportive to the obtained results of Y-tube olfactometer bioassay, where adult spent more time in the novel arm provided with the AJ.

Conclusion
Results suggest AJ to be a potent attractant to both adult male and female of C. carnea. These findings may be benefit in conservation of natural enemies in the desired area and may offer more efficient control of sucking pest complex (particularly aphids’ species) through predation of C. carnea and related taxa of different life history and traits. The present study provided the basic understanding of attraction and oviposition preference of C. carnea with better practical implementation. Further studies related to AJ and its chemical analysis may shed more light to produce an economical and commercially available lure to suppress the sucking pest population below economic threshold level.
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