Kairomonal effect of *Corcyra cephalonica* Stainton on *Trichogramma chilonis* Ishii and *Chrysoperla zastrowi sillemi* (Esben-peterson)

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**ABSTRACT**

Studies have been taken up to evaluate the concentration (0.1 % or 1000 ppm) of the hexane extracts of male and female whole body and larval wash of host *Corcyra cephalonica* Stainton on *Helicoverpa armigera* (Hub.) eggs against *Trichogramma chilonis* Ishii and *Chrysoperla zastrowi sillemi* (Esben-Peterson) for their kairomonal effect *in vitro* condition. Treating irradiated eggs of *Helicoverpa armigera* (Hubner) with hexane extract of adult female whole body of *C. cephalonica* recorded the parasitization of 15.94 per cent by *T. chilonis* on third day after inoculation which increased from 54.50 to 78.11 per cent on fifth and seventh day after inoculation as compared to 6.87, 18.94 and 31.97 per cent when the eggs were treated with hexane alone on third, fifth and seventh days after inoculation, respectively. Maximum emergence (70.31%) was observed with *C. cephalonica* female whole body extract followed by male whole body extract (58.64%). The highest predation by *C. zastrowi sillemi* on hexane extract of *C. cephalonica* female whole body treated eggs of *H. armigera* was 72.61% whereas it was 33.12 per cent in hexane treated eggs.

**KEY WORDS:** *Chrysoperla zastrowi sillemi*, *Corcyra cephalonica*, *Helicoverpa armigera*, semiochemical, *Trichogramma chilonis*

**INTRODUCTION**

Semiochemicals that convey information between organisms as signalling chemicals play a critical role in enabling insects to find food, mates and a suitable location for their progeny. Number of chemicals released from hosts, host secretions, hosts by-products and associated organisms influence the behaviour of natural enemies. Foraging female insect parasitoids use these chemical cues extensively to locate, identify and exploit their host in different eco-system (Beevers et al., 1981; Alhmedi et al., 2010; Penaflor et al., 2012). Many types of stimuli influence the habit location and host selection behaviour of parasitoids and predators among which the semiochemicals play a major role (Baker, 1982; Kumar and Ambrose, 2014; Joachim and Weisser, 2015). Similarly, host insects also contain saturated long chain hydrocarbons on their body surfaces. The surface hydrocarbon composition is observed to be species specific in insects (Lockey and Metcalfe, 1988). These saturated long chain hydrocarbons that are present on the surface of host plants and host insects have been reported to elicit synomonal and kairomonal responses in *Trichogramma* spp. The behavioural responses of *Trichogramma* spp. to synthetic hydrocarbons has been reported by Grenier et al. (1993). The host insects contain characteristic hydrocarbons, fatty acids and proteins present in their body or byproduct, which act as stimulants or
arrestants to the parasitoids to intensify their search in the near vicinity of the host (Tumlinson et al., 1992).

Saturated long chain hydrocarbons present on the body surface of *Spodoptera litura* (Fab.) and *Earias vitella* (Fab.) moths have been reported to elicit kairomonal response in *Trichogramma* spp. (Padmavathi and Paul, 1997; Maruthadurai et al., 2011 and 2011). In order to evaluate the role of kairomones released by host insect on foraging activities by *T. chilonis* and *C. zastrowi sillemi*, laboratory bioassay were conducted with the hexane extracts (1000 ppm) of male and female whole body and larval wash of host *C. cephalonica* to demonstrate the kairomonal interaction among the parasitoid, predator and the host.

**MATERIALS AND METHODS**

Laboratory studies were carried out at Bio-control laboratory, Agricultural College and Research Institute, Madurai during 2014 – 2015 to study the kairomonal effect of *C. cephalonica* to natural enemies.

**Insect cultures:** Larvae of *H. armigera* collected from field were reared separately in multi-cavity tray containing chickpea flour based semi-synthetic diet. Old diet was replaced with fresh ones in alternate days. Pre-pupae were collected in vermiculite for pupation. Pupae collected from culture were placed in adult emergence cage measuring 30 x 30 x 30 cm. Five pairs of newly emerged adults were transferred to plastic buckets of seven litre capacity maintaining the sex ratio of 1:1 for oviposition. Adults were fed with 10 per cent sugar solution enriched with multivitamin drops. The mouth of the bucket was covered with sterile muslin cloth which served as oviposition substrate. The buckets were kept in a dark place at 25° C with 75% RH. Muslin cloth along with eggs was collected from third-day onwards and used for experiment (Parthiban et al., 2014).

*C. cephalonica* was reared in the laboratory as per the method suggested by Navarajanpaul (1973). The emerged *C. cephalonica* adults were collected in the morning and allowed inside an oviposition cage of 21 x 25 cm size, with a wire mesh at bottom and lateral sides for ventilation. Adults were provided with 50 per cent honey solution as food. Eggs were collected at the bottom on a blotting paper kept in tray and cleaned with sieves or egg separator. The cleaned eggs were sprinkled over broken cumbu grains, at the rate of one cc per 2.5 kg of grains, fortified with ten grams of yeast in a plastic basin (45 x 30 x 10 cm) and covered with khada cloth. Care was taken to maintain the culture free of storage mites and diseases by mixing 5.0 g of wettable sulphur 80 WP and streptomycin sulphate 0.5 per cent, respectively. The emerged adults were collected and used again for culturing both host (*C. cephalonica*) and parasitoid (*T. chilonis*). The culture was maintained at 26 ± 2° C, R.H. 75 ± 5% and photoperiod 16:8 h scoto/photo regime.

The egg parasitoid, *T. chilonis* was mass cultured on the eggs of *C. cephalonica* as per the method described by Prabhu (1991). The fresh *C. cephalonica* eggs were collected in the early morning and sterilized under UV radiation of 15 Watts for 20 minutes at a distance of 15 cm to avoid the emergence of *C. cephalonica* larvae. The sterilized eggs were then pasted on paper cards of 21 x 30 cm size containing thirty, 7 x 2 cm rectangles. These egg cards were placed in polythene bags along with nucleus card at 6:1 ratio for parasitization by the egg parasitoids at 26 ± 2° C, R.H. 75 ± 5% and
After parasitisation, the egg cards were cut into bits and the three days old, cent per cent parasitized eggs (eggs appearing black and plumpy) were used for the experiments.

Mass rearing of *C. zastrowi sillemi* was done with *C. cephalonica* eggs as feed, following the method described by Swamiappan (1996). Grubs of *C. zastrowi sillemi* were reared in galvanized iron (GI) basins (28 cm dia) at 250 larvae per basin covered with khada cloth. The eggs of *C. cephalonica* were provided as feed for the grubs in the laboratory. About 2.5 cc of *C. cephalonica* eggs per basin were provided on alternate days. After five feedings, the larvae pupated into white coloured round silken cocoon. The cocoons were collected and transferred into one litre plastic container with wire mesh window for the emergence of adults at 26 ± 2°C, R.H. 75 ± 5% and a photoperiod 16:8 h scoto/photo regime. The adults were collected and transferred to GI troughs (30 cm dia. x 12 cm ht), wrapped inside with brown sheets for collecting the eggs. The trough was covered with nylon cloth and kept firm with the help of a rubber band. Over the cloth covering, two bits of foam sponge (2.5 cm²) dipped in water were kept besides an artificial protein rich diet in the form of semi solid paste was smeared. This diet consisted one part of yeast powder + one part of fructose + one part of honey + one part of Protinex®. Water was mixed to make it just a paste. The adults laid eggs on the brown sheet wrapped inside the trough. The adults were collected daily and allowed into fresh rearing troughs with fresh feed at 26 ± 2°C, R.H. 75 ± 5% and photoperiod 16:8 h scoto/photo regime. From the old troughs the brown paper sheets along with *C. zastrowi sillemi* eggs were removed.

Extraction of kairomone: The whole body washes from adult male, female and larvae of moth of *C. cephalonica* were prepared as per the method described by Ananthakrishnan *et al.* (1991). Freshly emerged, healthy, 0-24 hrs old moths of male and female were collected and kept in a deep freezer (REMI model) at –20°C for 15 min for immobilization. Subsequently, 10 g of moths, third instar larvae and larval frass were weighed and soaked in 100 ml of distilled hexane (HPLC grade) for 24 hrs andShacked in water bath (Genuine model) at 28°C for two hours followed with 20 minutes at 50°C. These were filtered through Whatman No.1 filter paper (Yasuda 1997). The hexane fraction was subsequently concentrated by vacuum evaporation at 40°C (LARK model). The extracts were stored at –20°C in deep freezer till further use for bioassay studies. A concentration of 0.1% (1000 ppm) of the extract of host insect was prepared after dilution with hexane and used throughout the experiment.

Bioassay: Bioassay studies of whole body wash and larval exuding kairomones of host insects were carried out at 26 ± 2°C and 75 ± 5% R.H. and photoperiod 16:8 h scoto/photo regime. The procedure adopted was similar to the one described by Lewis *et al.* (1975). Clean, healthy, 0-24 hrs old eggs of *H. armigera* sterilized under UV light for 45 minutes were washed twice in hexane to remove any trace of scales or kairomones present on the surface of eggs. These eggs were pasted with pure white gum on dull coloured cardboard, measuring 7 x 2 cm at the rate of average of 80 - 100 eggs per piece (egg card). Kairomone extracts (1000 ppm) of *C. cephalonica* (male moths, female moths and larvae extracts) used to treat the hexane washed eggs, separately and shade dried. Each egg card was considered as one replication and each treatment was replicated seven times.
Control was maintained with hexane alone.

Egg card taken in a glass tube (7.5 x 2.5 cm) was introduced with freshly emerged *T. chilonis* adults (6:1). Per cent parasitization was observed on 3rd, 5th and 7th days after introduction. Similarly, one second instar of *C. zastrowi sillemi* was released in a vial with hexane washed *H. armigera* eggs (80-100 nos.) and per cent predation was calculated 24 hr after release (Elanchezhyan *et al.*, 2009; Murali baskaran, 2013).

**Statistical analysis:** Data obtained from the bioassay of body washes of host insects were subjected to ANOVA (Analysis of Variance). Before analysis, data on per cent parasitism were transferred by arcsine transformation. In order to know the interaction between treatments, data from laboratory bioassay were subjected to factorial CRD (Completely Randomized Design) analysis and the means obtained were separated by LSD (Least Significant Difference) (Gomez and Gomez, 1984).

**RESULTS AND DISCUSSION**

The efficacy of hexane extracts of *C. cephalonica* on parasitism corroborated that (Table 1) highest mean percentage parasitism of 49.52 by *T. chilonis* was recorded in hexane extract of female whole body wash of *C. cephalonica* (1000 ppm) followed by 42.81 percentage in male whole body wash. Among the host insect wash larval extract recorded the lowest mean percentage parasitism of 34.39, whereas the control (hexane) recorded the least mean parasitism (19.26). When the interaction between the different washes were analyzed, it was found that the female body wash of *C. cephalonica* recorded the highest mean parasitization level of *T. chilonis* on eggs of *H.armigera*, recording 15.94, 54.50 and 78.11 per cent on 3rd, 5th and 7th day after introduction of parasitoids, respectively which was significantly different from hexane extract of male whole body (12.04, 46.85 and 69.54%), and larval extract (11.20, 37.42 and 54.85%) while it was 6.87, 18.94 and 31.97 per cent parasitization in hexane alone treated eggs for the same period.

Similarly, highest mean per cent emergence (70.31%) was recorded in female body wash of *C. cephalonica* followed by male body wash (58.64%) (Table 1). The lowest mean emergence was recorded in larval extract (42.16%) and the lowest mean per cent emergence was recorded in control (31.05%).

Predatory activity by *C. zastrowi sillemi* was enhanced from 33.12 (hexane treated eggs of *H. armigera*) to 72.61 per cent (Table 2), 24 hr after treatment when treated with hexane extract of female whole body, followed by hexane extract of male whole body (62.87%), and larval extract (46.33%).

The outcome of the present study indicate that kairomonal compounds from *C. cephalonica* female whole body wash increased parasitization when applied over target sites. This findings is in agreement with the earlier reports in this direction in a number of host-parasitoid systems (Elzen *et al.*, 1984; Nordlund *et al.*, 1984; Nordlund, 1987; Shu *et al.*, 1990). This supports the present findings where the whole body washes of female *H. armigera* and *C. cephalonica* moths recorded the highest PAI, parasitism and emergence by *T. chilonis* (Anathakrishnan *et al.*, 1991). The present result was endorsed with the findings of Singh *et al.* (2002) who stated that an analysis of *H. armigera* whole body wash for possible kairomonal substances using gas chromatography indicated the presence of fifteen saturated
hydrocarbons were identified among which heneicosane and hexacosane were the major ones. Rest of the saturated hydrocarbons found were heptadecane, nonadecane, hexadecane and pentadecane and tricosane this hydrocarbons may be reason for enhanced parasitism, emergence and predation. The significance of these kairomonal substances in behavioural manipulation of entomophagous insects was earlier emphasized and reviewed by Lewis et al. (1976). Paul et al. (2002) explicated that pentacosane and hexacosane recorded very high parasitoid activity index and parasitism for *T. brasiliensis* and *T. exiguum* indicating high kairomonal activity. Srivastava et al. (2008) found that kairomones from male *S. litura* and female *S. exigua* showed the highest parasitoid activity index (PAI) and parasitism by *T. chilonis*. Padmavathi and Paul (1998) categorized these hydrocarbons as favourable for *Trichogramma* sp. which support the present findings. The whole body washes of *C. cephalonica* female showed higher parasitism by *T. brasiliensis* and *T. japonicum* as compared to that of male moth (Paul et al., 1997).

Attraction of *T. chilonis* was more towards female body wash of *Chilo partellus* (Swinhoe), *Sesamia inferens* Walker and *Sitotroga cerealella* Oliver as compared to male body wash (Padmavathi and Paul, 1997). Srivastava et al. (2008) recorded more number of favourable hydrocarbons in *S. litura* male and *S. exigua* female and this is in agreement with present findings. Paramasivan et al. (2004) identified more number of favourable hydrocarbons in female body wash of *C. partellus* as compared to male.

The whole insect body of *E. vittella* was found to increase PAI and per cent parasitism by *Trichogramma* spp. which may be attributed to the presence of various saturated hydrocarbons in the range of C13 to C30 with varying quantities (Mahesh et al., 2012). Presence of single chain hydrocarbons like dotriacontane and nonadecane would have been responsible for the enhanced predatory activity of *C. carnea*, as suggested by Singh and Paul (2002).

Bakthavatsalam and Singh (1999) exemplified scales and abdominal tip extracts of *C. cephalonica* and *H. armigera* elicited good behavioural response in *C. zastrowi sillemi* larvae. Hegde et al. (2000) noticed the grub of *C. zastrowi sillemi* to spend the longest time (0.98 min.) near wax droplets smeared with *H. armigera* scale extract, followed by *H. armigera* egg extract (0.54 min.) and abdominal tip extract (0.34 min.). Laboratory observations on parasitism rates by *T. chilonis* in response to *C. cephalonica* female whole body wash, treatments reveal the importance of kairomones from female moths. Similar observations have been made by Nordlund et al. (1976) on *T. pretiosum* Riley support our study on the role of kairomones in improving parasitism. Larvae of the generalist predator *C. zastrowi sillemi* have specific preference to certain hydrocarbons and other chemicals at a particular concentration. Such preferential behaviour of the larvae may be utilized for their activity of manipulation in the release programmes to enhance their host searching activity.

**CONCLUSION**

The present study indicates that kairomone compounds from *C. cephalonica* female whole body wash in the manipulation of parasitoid activity, which could play a major role in future biological control programmes.
Table 1: Per cent parasitism and emergence of *Trichogramma chilonis* on eggs of *Helicoverpa armigera*, as influenced by hexane extracts of *Corcyra cephalonica*

<table>
<thead>
<tr>
<th>Insect samples</th>
<th>% parasitization by <em>T. chilonis</em> after*</th>
<th>Mean</th>
<th>% emergence of <em>T. chilonis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd day</td>
<td>5th day</td>
<td>7th day</td>
</tr>
<tr>
<td>Male whole body</td>
<td>12.04 (20.30)b</td>
<td>46.85 (43.20)b</td>
<td>69.54 (56.50)b</td>
</tr>
<tr>
<td>Female whole body</td>
<td>15.94 (23.53)a</td>
<td>54.50 (47.58)a</td>
<td>78.11 (62.11)a</td>
</tr>
<tr>
<td>Larval extract</td>
<td>11.20 (19.55)b</td>
<td>37.42 (37.71)c</td>
<td>54.85 (47.78)c</td>
</tr>
<tr>
<td>Control (Hexane)</td>
<td>6.87 (15.19)c</td>
<td>18.94 (25.80)d</td>
<td>31.97 (34.43)d</td>
</tr>
</tbody>
</table>

SEd 0.3871 0.2582 0.2664 0.2593 0.2540
CD (P=0.05) 0.8927 0.5954 0.6144 0.5979 0.5858

*Each value is the mean of seven replications
Figures in parentheses are arcsine transformed values
In a column, means followed by the common letter(s) are not significantly different by LSD (P=0.05)

Table 2: Per cent predation by *Chrysoperla zastrowi sillemi* on eggs of *H. armigera*, as influenced by hexane extracts of *C. cephalonica*

<table>
<thead>
<tr>
<th>Insect samples</th>
<th>% predation by <em>C. zastrowi sillemi</em> after 24 h*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male whole body</td>
<td>62.87 (52.46)b</td>
</tr>
<tr>
<td>Female whole body</td>
<td>72.61 (58.44)a</td>
</tr>
<tr>
<td>Larval extract</td>
<td>46.33 (42.90)c</td>
</tr>
<tr>
<td>Control (Hexane)</td>
<td>33.12 (35.14)g</td>
</tr>
<tr>
<td>Mean</td>
<td>53.73 (47.13)</td>
</tr>
</tbody>
</table>

SEd 0.2560
CD (P=0.05) 0.5904

* Each value is the mean of seven replications
Figures in parentheses are arcsine transformed values
In a column, means followed by the common letter(s) are not significantly different by LSD (P=0.05)
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[MS received 19 May 2015; MS accepted 28 June 2015]