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Enhancing *in vivo* foraging activities of *Trichogramma chilonis* Ishii and *Chrysoperla zastrowi sillemi* (Esben-Peterson) on eggs of *Corcyra cephalonica* Stainton through kairomonic activity of *Helicoverpa armigera* (Hubner)

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ABSTRACT: Bioassay of hexane extracts (1000 ppm) of male and female whole body, frass and larval wash of host *Helicoverpa armigera* (Hubner) against *Trichogramma chilonis* Ishii and *Chrysoperla zastrowi sillemi* (Esben-Peterson) revealed their kairomonal activities under in vitro condition. Treating irradiated eggs of *Corcyra cephalonica* Stainton with hexane extract of adult female whole body of *H. armigera* (1000 ppm) recorded the parasitization of 17.34 per cent by *T. chilonis* on third day after inoculation which increased from 50.64 to 64.28 per cent on fifth and seventh day after inoculation and they were 7.94, 21.76 and 32.58 per cent when the eggs were treated with hexane alone on third, fifth and seventh days after inoculation, respectively. Maximum emergence (48.16%) was observed with H. *armigera* female whole body extract followed by male whole body extract (39.33%). The highest predation by *C. zastrowi sillemi* on hexane extract of *H. armigera* female whole body treated eggs of *C. cephalonica* was recorded (61.13%) whereas it was 37.85 per cent in hexane treated eggs.

KEY WORDS: *Chrysoperla zastrowi sillemi*, *Corcyra cephalonica*, *Helicoverpa armigera,* kairomone, *Trichogramma chilonis, foraging activities*

INTRODUCTION

Natural enemies detect chemical cues that are emanating from the host insects which help in their host location. 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 (Penaflor *et al*., 2012; Parthiban *et al*., 2015). Many types of stimuli influence the habit location and host selection behaviour of parasitoids and predators among which the semiochemicals play a major role (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. 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

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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.

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. (Maruthadurai *et al*., 2011). In order to evaluate the role of kairomones released by host insect on parasitism and predation by *T. chilonis* and *C. zastrowi sillemi* laboratory bioassay were conducted with the hexane extracts (1000 ppm) of male and female whole body, frass and larval wash of host *H. armigera* to explain the kairomonal interaction between 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 to 2016 to study the kairomonal effect of *H. armigera* to natural enemies. 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 protocol suggested by Navarajanpaul (1973). The egg parasitoid, *T. chilonis* was mass cultured on the eggs of *C. cephalonica* as per the method described by Prabhu (1991). Mass rearing of *C. zastrowi sillemi* was carried out with *C. cephalonica* eggs as feed, as per the method described by Swamiappan (1996).

The whole bodywash from adult male, female, larvae and frass of moth of *H. armigera* was 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 and shaked 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. 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 studies of whole body wash, larval and frass 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 *C. cephalonica* 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 0.05 cc eggs per piece (egg card). Kairomone extracts (1000 ppm) of *H. armigera* (male moths, female moths, larvae and frass 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 eight 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, five second instar of *C. zastrowi sillemi* was released in a vial with hexane washed *C. cephalonica* eggs (700-750) and per cent predation was calculated 24 hr after release (Murali Baskaran, 2013).

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

The results on parasitism corroborated that the highest mean per cent parasitism (44.09%) by *T. chilonis* was recorded in hexane extract of female whole body wash of *H. armigera* (1000 ppm) followed by 36.16 percentage in male whole body wash. Among the host insect washes larval and frass extract recorded the lowest mean percentage parasitism of 26.68 and 23.26, respectively, whereas the control (hexane) recorded the least mean parasitism (20.76). When the interaction between the different washes were analysed, it was found that the female body wash of *H. armigera* recorded the highest mean parasitization level of *T. chilonis* on eggs of *C. cephalonica*, recording 17.34, 50.64 and 64.28 per cent on 3rd, 5th and 7th day after introduction of parasitoids, respectively which was significantly different from hexane extract of male whole body (11.52, 41.05 and 55.92%), larval extract (8.89, 31.22 and 39.94%) and frass extract (9.12, 23.83 and 36.83%) while it was 7.94, 21.76 and 32.58 per cent parasitization in hexane alone treated eggs (Table 1).

Similarly, the highest mean per cent emergence (48.16%) was recorded in female body wash of *H. armigera* followed by male body wash (39.33%) (Table 2). The lowest mean emergence was recorded in frass extract (25.94%) among the different washes followed by larval extract (27.83%) and the lowest mean per cent emergence was recorded in control (22.15%).

Predatory activity of *C. zastrowi sillemi* was enhanced from 37.85 per cent (hexane treated eggs of *C. cephalonica*) to 61.13 per cent (Table 3), 24 hr after treatment when treated with hexane extract

Table1. Parasitism by *Trichogramma chilonis* on eggs of *Corcyra cephalonica*, as influenced by hexane extracts of *Helicoverpa armigera*

Insect samples	% parasitization by *T. chilonis* after*			Mean
	3rd day	5th day	7th day	
Male whole body	11.52(19.84)[b]	41.05(39.85)[b]	55.92(48.40)[b]	36.16(36.97)[b]
Female whole body	17.34(24.61)[a]	50.64(45.37)[a]	64.28(53.30)[a]	44.09(41.67)[a]
Frass extract	9.12(17.57)[c]	23.83(29.22)[d]	36.83(37.36)[d]	23.26(28.83)[d]
Larval extract	8.89(17.34)[c]	31.22(33.97)[c]	39.94(39.20)[c]	26.68(31.10)[c]
Control (Hexane)	7.94(16.36)[d]	21.76(27.81)[e]	32.58(34.81)[e]	20.76(27.11)[e]
SEd	0.3845	0.2561	0.2432	0.2611
CD (P=0.05)	0.8567	0.5705	0.5419	0.5818
CV	2.46	0.89	0.70	0.97

*Mean of eight replications; Figures in parentheses are arcsine transformed values

In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05)

Table 2. Emergence of *T. chilonis* on eggs of *C. cephalonica* as influenced by hexane extracts of *H. armigera*

Insect samples	% emergence *
Male whole body	39.33 (38.84)[b]
Female whole body	48.16 (43.95)[a]
Frass extract	25.94 (30.62)[d]
Larval extract	27.83 (31.84)[c]
Control (Hexane)	22.15 (28.08)[e]
Mean	32.68 (34.86)
SEd	0.2559
CD (P=0.05)	0.5702
CV	0.90

*Mean of eight replications; Figures in parentheses are arcsine transformed values

In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05)

Table 3. Predation by *Chrysoperla zastrowi sillemi* on eggs of *C. cephalonica*, as influenced by hexane extracts of *H. armigera*

Insect samples	% predation after 24 h*
Male whole body	54.37 (47.51)b
Female whole body	61.13 (51.43)a
Frass extract	41.27 (39.97)d
Larval extract	43.93 (41.51)c
Control (Hexane)	37.85 (37.97)e
Mean	47.71 (43.68)
SEd	0.2394
CD (P=0.05)	0.5334
CV	0.67

*Mean of six replications; Figures in parentheses are arcsine transformed values

In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05)

of female whole body, followed by hexane extract of male whole body (54.37%), larval extract (43.93%) and frass extract (41.27%).

DISCUSSION

Parasitoids detect chemical cues that are emanating from the host insects which help in their host location. These semiochemicals which are often found in the host insect or their by-products act as arrestants or stimulants to the parasitoids to intensify their search in the near vicinity of the host (Tumlinson *et al*., 1992). These findings are in agreement with the report of Lewis *et al*. (1972) who confirmed the presence of host searching stimulant for *T. evanescens* Westwood in scales left by ovipositing corn ear worm moth, *Heliothis zea* (Boddie). Moth scales of *H. zea* and tricosane acted as releaser for the parasitoids, *T. pretiosum* and *T. acheae* and doubled the rates of parasitization by them on *H. zea* eggs over that of unstimulated parasitoids. Saturated long chain hydrocarbons present on the body surface of *H. armigera* and *C. cephalonica* moths were reported to elicit kairomonal response on *Trichogramma* spp. (Padmavathi and Paul, 1997). However, egg wash of *Chilo partellus* (Swinhoe) was reported to increase the parasitoid activity index and per cent parasitism of *T. chilonis* than female and male whole body wash (Paramasivam *et al*., 2004).

In the present study, other than whole body hexane wash, larval and frass extracts of *H. armigera*, could also elicit kairomonal effect towards the parasitoid on the eggs of *C. cephalonica*. But in general, larval and frass extracts of lepidopteran insects were reported to evoke the response of the larval parasitoids as suggested by several workers including, Hu and Chen (1987) and Parthiban *et al*. (2015). The result is in conformity with the findings of Singh *et al*. (2005) who stated that an analysis of *H. armigera* whole body wash for possible kairomonal substances using gas chromatography confirmed the presence of fifteen saturated hydrocarbons, which include, heneicosane and hexacosane. Rest of the saturated hydrocarbons were heptadecane, nonadecane, hexadecane and pentadecane and tricosane which might 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) proved beyond 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*.

Attraction of *T. chilonis* was more towards female body wash of *Chilo partellus* (Swinhoe), *Sesamia inferens* Walker and *Sitotroga cerealella* Oliver compared to male body wash (Padmavathi and Paul, 1997). The whole insect body of *E. vittella* was found to increase parasitoid activity index and per cent parasitism by *Trichogramma* spp. which may be attributed to the presence of various saturated hydrocarbons in the range of C_{13} to C_{30} 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.). 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.

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