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Research Article

Kairomonal effect of host body washing on the egg parasitoid *Trichogramma brasiliensis* (Ashmead) (Hymenoptera: Trichogrammatidae)

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ABSTRACT: Bioassay with hexane extracts of male and female whole body wash of host insects *viz*., *Earias vittella* and *Spodoptera litura* with *Trichogramma brasiliensis* revealed their kairomonal activities. Whole body extracts of male and female moths were analysed separately by gas chromatography for determining their hydrocarbon profile, which showed the presence of straight chain saturated hydrocarbons ranging from C_8 to C_{35}. The concentration of these hydrocarbons ranged from 0.02 µg/g to 2579 µg/g of insect extract. Both the host insects showed variation in number and concentration of these chemicals, which were responsible for influencing the parasitoid activity, parasitism and emergence. The foraging activity of the parasitoid as indicated by parasitoid activity index (PAI) was highest (12.00) in *S. litura* male body extract at concentration C_4 (1000 ppm) followed by (10.83) in *E. vittella* female body extract at C_5 (10,000 ppm) by *T. brasiliensis*. Maximum percentage parasitism of (45.55) and emergence (12.50) was observed at concentration C_4 in *S. litura* male body extract followed by *E. vittella* female body extract. Highest overall response was recorded in egg cards treated with whole body wash of male and female of *S. litura* and *E. vittella,* respectively, which may be attributed to the presence of more number of favourable saturated hydrocarbons *viz*.; heneicosane, tricosane, pentacosane, hexacosane, octacosane, and nonacosane, as revealed by GC. These favourable hydrocarbons at appropriate concentration of body extract of *E. vittella* female and *S. litura* male could be used for enhancing parasitization by *T. brasiliensis*.

KEY WORDS: semiochemical, kairomone, *Earias vittella, Spodoptera litura. Trichogramma brasiliensis*.

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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 ecosystem (Beevers *et al*., 1981). Among the different chemical stimuli involved in host selection by a natural enemy, kairomones appear to be the most important. The host insects emit characteristic hydrocarbons, fatty acids and proteins present in their body or by-product, which act as stimulants or arrestants to the parasitoids to intensify their search in the near vicinity of the host (Tumlinson *et al*., 1992). It has been reported that the host-searching stimulant for *Trichogramma evanescens* Westwood was present in the scales left by ovipositing corn earworm moth, *Heliothis zea* (Boddie) (Lewis *et al*., 1972). Saturated long chain hydrocarbons present on the body surface of *Helicoverpa armigera* (Hubner) and *Corcyra cephalonica* Stainton moths have been reported to elicit kairomonal response in *Trichogramma* spp. (Ananthakrishnan *et al*., 1991; Padmavathi and Paul, 1997). Among the egg parasitoids, the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) has received greater attention because of its polyphagous nature against many potential lepidopterous pests (Nagarkatti and Nagaraja, 1979). *T. brasiliensis* is extensively used to manage bollworm complex *viz*., *H. armigera*, *Earias vittella* (Fab.), *E. insulana* (Boisduval), *Spodoptera litura* (Fab.) and *Pectinophora gossypiella* (Saunders) (Ballal and Singh, 2003; Kumar *et al*., 2009). In order to evaluate the role of kairomones released by host insect on parasitism by *T. brasiliensis*, a laboratory bioassay was conducted with the whole body wash of male and female of *E. vittella* and *S. litura* and their saturated hydrocarbon profiles were elucidated by gas chromatography to explain the kairomonal interaction between the parasitoids and the host.

MATERIAL AND METHODS

(a) *E. vittella*

The culture of the host insect, *E. vittella* was maintained at 28±2°C and 60±5 per cent R.H. The larvae of *E. vittella* were reared on okra as well as artificial diet developed by Gupta *et al*. (2005a). The eggs laid on the fruits were removed with wet camel hairbrush and kept for hatching. Immediately after hatching the larvae were transferred on to the fresh okra fruit (after making incision with the help of a knife on okra fruit) kept in rearing jar. Male and female cocoons of *E. vittella* were segregated based on the pupal characters. Male cocoons were identified with the presence of a well-developed knob at the antero-dorsal end which is absent in female cocoons (Mahapatro and Gupta, 1999).

(b) *S. litura*

Larvae of *S. litura* were reared on artificial diet developed by Gupta *et al*. (2005b). Hatched larvae were transferred to approximately 10g of diet kept in sterilised glass vials (7.5 cm x 2.5 cm), which were secured with sterile cotton plugs. The male and female of *S. litura* were separated based on the markings on the wings of adults and on pupa (Gautam, 2008). The males have a blue-grey band from the apex to the inner margin of each fore wing which was absent in female.

(c) Egg parasitoids

Cultures of the parasitoid were maintained in glass vials of 10 cm x 2.5 cm size (Gautam, 2008). Cards having approximately 1000 parasitised eggs that have turned black were kept in each vial for adult emergence. Honey (50%) was offered as adult food in the form of fine streaks on the inner wall of the glass vial. The adult parasitoids were offered an egg card (12 x 2.5 cm) containing 8000-10,000 numbers of, 0-24 h old, healthy and UV sterilized eggs of *C. cephalonica* for parasitisation. After 24 h, the egg cards were removed and kept for the development of parasitoids in fresh glass vials. Parasitised eggs turned black on the 4^{th} day after parasitisation. A small segment of the egg card containing approximately 1000 parasitised eggs was cut from each egg card and used for further rearing of the parasitoids.

Extraction of kairomone

The whole body wash from adult of male and female moths of *E. vitella* and *S. litura* was prepared as per the method described by Ananthakrishnan *et al*. (1991). Freshly emerged, healthy, 0-24 h old moths of male and female were collected and kept in a deep freezer at –20°C for 15 min for immobilization. Subsequently, 10 g of moths were weighed and soaked in 100 ml of distilled hexane for 24 hrs and shaked in water bath (Haake, SWB 20) at 28°C for two hours followed with 20 minutes at 50°C. These were filtered through Whatman No.1 filter paper. anhydrous sodium sulphate was added @ 1g/10g and kept for dehydration. After removal of sodium sulphate the filtrate was passed through silica gel (60-120 mesh) column. The extracts were distilled at 60-70°C and the residues left at the bottom of the glass jar collected by rinsing with small quantity of HPLC grade distilled hexane. The solvent hexane was removed by evaporation and the resultant extract was diluted to the required concentration by using HPLC grade hexane. The extracts were stored at –20°C in deep freezer till further use for GC analysis and bioassay studies.

Bioassay

Bioassay studies of whole body wash of host insects were carried out at 26° ± 2°C and 65 ± 5% R.H. in Borosil® Petri dishes (50 x 15 mm) on a laboratory table with a single 40 W overhead fluorescent tube as source of light. The procedure adopted was similar to the one described by Lewis *et al.* (1975).

Clean, healthy, 0-24 hr 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. They were then pasted equidistantly on $4cm^2$ Whatman No.1 filter paper piece at the rate of 30 eggs per piece (hereafter referred to as egg card). The kairomone extracts were serially diluted to desired concentration *viz*., 1, 0.1, 0.01, 0.001, and 0.0001 per cent and applied at the rate of 50 µl. A control was maintained where only hexane was used. Five such egg cards with different concentrations of extracts along with one control card were arranged equidistantly in the experimental arena, which consisted of a 150 mm dia petridish, the base of which was covered with Whatman No. 1 filter paper disc of the same diameter. One such petridish was considered as one replication and each extract was replicated six times. Sufficient numbers of healthy and well fed 0-24 hr old *T. brasiliensis* adults were transferred to a glass vial and anaesthetized using etherized carbon dioxide for 15 seconds as described by Gautam (2008). Ten healthy, fast reviving females were separated based on antennal dimorphism and released at the centre of each Petri dish containing treated egg cards. The parasitoids were allowed to search in the experimental arena for a total period of 45 minutes from the time of release. Number of parasitoids that visited each egg card were counted at 5 minutes interval and the total number visited for each card is called parasitoid activity index (PAI). After 45 min, the parasitoids were removed carefully from each card and these cards were kept individually in homeopathic vials for development at 26° ± 1°C and 65 ± 5% R.H. Per cent parasitism and total emergence were recorded on 6^{th} and 10^{th} day, after parasitisation respectively, as described by Paramasivan *et al*. (2004).

Identification of hydrocarbons

Whole body wash of male and female host insects were analysed through gas chromatography (Varian 430) fitted with flame ionization detector for the presence of straight chain saturated hydrocarbons. The capillary column temperature was maintained from 100-260°C @ 10°C/min. The injector and detector temperatures were maintained at 320°C. Nitrogen was used as a carrier gas with a flow rate of 20 ml/min. and one µl of each body wash was injected for analysis.

Retention time (Rt) of each peak of unknown compound (sample) was compared with that of known hydrocarbons standards (Sigma Aldrich, USA) having concentration of 1000 ppm. The concentration of the saturated hydrocarbons, present in the body washes was calculated in µg/g of leaf or insect extract (Singh, 2003) with the help of following formula:

female body wash (4.64). Among the concentration, irrespective of hosts and sexes, the concentration C_4 (1000 ppm) recorded the highest mean PAI with a mean value of (8.67) and the lowest mean PAI was recorded in control (3.00). When the interaction between whole body wash of host insects and concentration was analysed, it revealed that the concentration C_4, recorded the highest mean PAI of 12.00 in *S. litura* male body wash and the lowest PAI of 2.00 was recorded in *E. vittella* male body wash at C_1.

In case of parasitism, the highest mean percentage parasitism of 24.90 by *T. brasiliensis* was recorded in *S. litura* male body wash followed by 23.15 of female *E. vittella* (Table 2). The lowest mean percentage parasitism of 11.29 was observed in *E. vittella* male body wash followed by 12.87 in female of *S. litura*, irrespective of concentration. Among the concentration irrespective of washes, the concentration C_4 (1000 ppm) showed the highest mean percentage parasitism (27.78) whereas the control recorded the least mean parasitism (7.91). When the interaction between the different washes and concentration were analysed, it was found that the male body wash of *S. litura* recorded the highest mean percentage parasitism (45.55) at C_4. Least percentage parasitism (5.55) was recorded in male body wash of *E. vittella* at C_1.

$$\text{Concentration of Hydrocarbon in Sample (µg/g of insect extract)} = \frac{\text{Area of unknown saturated hydrocarbons} \times \text{Dilution of sample}}{\text{Area of standard saturated hydrocarbons} \times \text{Insect extract (g)}} \times \text{Concentration of standard saturated hydrocarbon}$$

Statistical Analysis

Data obtained from the bioassay of whole body washes of male and female host insect were subjected to two way analysis of variance, in completely randomized design using OPSTAT software (O. P. Sheoran, HAU, Hisar) for the comparison of treatments. Before analysis, data on parasitoid activity index, emergence and per cent parasitism were transferred to square root transformation + 0.5 and arc-sine transformation, respectively.

RESULTS AND DISCUSSION

Foraging activity of *T. brasiliensis* as revealed by mean parasitoid activity index (PAI) was the highest (7.17) in the case of *S. litura* male body wash, irrespective of the concentration followed by *E. vittella* female body wash (6.94) (Table 1). The lowest mean PAI was recorded in *E. vittella* male body wash (3.92) followed by *S. litura*

Similarly, highest mean emergence of (7.42) was recorded in male body wash of *S. litura* followed by *E. vittella* female body wash (7.22). The lowest mean emergence was recorded in *S. litura* female body wash (3.50) irrespective of concentrations (Table 3). Among the concentrations, irrespective of body washes and sexes, the highest mean emergence was noticed at C_4 (8.54) and the lowest mean emergence was recorded in control (2.33). When the interaction between whole body washes of host insects and concentration was analysed, it was found that *S. litura* male body wash showed the highest mean emergence of (12.50) at C_4 and (11.33) at C_5, whereas in the female body wash of *S. litura* highest mean emergence was noticed at C_5 (6.50). In case of *E. vittella*, highest mean emergence of (12.00) was recorded in female body wash at C_5 and the lowest mean emergence (2.00) was observed in male body wash at C_1.

Table 1. Effect of body washes of host insects on the parasitoid activity index (PAI) of *Trichogramma brasiliensis*

Body wash Concentration (ppm)	Host insect *Earias vittella*		*Spodoptera litura*		Mean PAI
	Male	Female	Male	Female	
C1	2.00 (1.55)	5.67 (2.46)	4.33 (2.14)	3.67 (2.02)	3.92 (2.04)
C2	2.33 (1.54)	4.33 (2.12)	7.17 (2.75)	5.83 (2.49)	4.92 (2.23)
C3	5.83 (2.49)	8.50 (2.80)	6.83 (2.70)	2.50 (1.52)	5.92 (2.38)
C4	7.50 (2.78)	9.17 (3.09)	12.00 (3.52)	6.00 (2.47)	8.67 (2.97)
C5	3.17 (1.82)	10.83 (3.35)	9.00 (3.04)	7.33 (2.77)	7.58 (2.74)
Control (Hexane)	2.67 (1.69)	3.17 (1.83)	3.67 (2.02)	2.50 (1.59)	3.00 (1.78)
Mean	3.92 (1.98)	6.94 (2.61)	7.17 (2.69)	4.64 (2.15)	
		S.E (m) ±	CD $P \leq 0.05$		
Treatment		0.087	0.244		
Concentration		0.107	0.299		
Interaction (T × C)		0.214	0.599		

Values are mean of six observations.

Figures in the parentheses are square root transformed values.

C_1 = 1ppm; C_2 = 10 ppm; C_3 = 100 ppm; C_4 = 1000 ppm; C_5 = 10,000 ppm

It was observed that kairomonal effect, as evidenced by enhanced parasitoid activity index, parasitism and emergence by *T. brasiliensis* was more in the whole body wash of male *S. litura* and female *E. vittella* as compared to male and female of *E. vittella* and *S. litura,* respectively. This may be due to presence of hydrocarbons *viz.;* heneicosane, tricosane, pentacosane, hexacosane, octacosane and nonacosane in the body wash (Table 4). Padmavathi and Paul (1998) categorized these hydrocarbons as favourable for *Trichogramma* spp. which support the present findings. The whole body wash 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 for female body wash of *Chilo partellus* (Swinhoe), *Sesamia inferens* Walker and *Sitotroga cerealella* Oliver as compared to male body wash (Padmavathi and Paul, 1997). This was in conformity with the present findings where the female whole body wash of *E. vittella* showed higher parasitism and emergence by *T. brasiliensis*. Srivastava *et al*. (2008) found that kairomones from male *S. litura* and female *S. exigua* showed highest parasitoid activity index (PAI) and parasitism from *T. chilonis*. This supports the present findings where the whole body wash of male *S. litura* recorded highest PAI, parasitism and emergence by *T. brasiliensis*.

The gas chromatogram of the male and female body extracts of two host insects revealed the presence of saturated hydrocarbons ranging from C_8 to C_{35}. Better kairomonal interaction observed in the male body wash of *S. litura* and female body wash of *E. vittella* could be due to the presence of more number of favourable hydrocarbons in the extracts. Paramasivan *et al*. (2004) identified more number of favourable hydrocarbons in female body wash of *C. partellus* as compared to male. Srivastava *et al*. (2008) recorded more number of favourable hydrocarbons in *S. litura* male and *S. exigua* female and this was in agreement with present findings.

Table 2. Effect of body washes of host insects on percentage parasitization of *Trichogramma brasiliensis*

Body wash Concentration (ppm)	Host insect *Earias vittella*		*Spodoptera litura*		Mean PAI
	Male	Female	Male	Female	
C1	5.55 (13.31)	17.22 (24.17)	15.55 (22.95)	7.78 (15.71)	11.52 (19.04)
C2	8.89 (16.65)	12.77 (20.73)	27.78 (31.39)	14.44 (21.68)	15.97 (22.61)
C3	14.44 (21.51)	33.33 (34.88)	17.77 (24.31)	9.44 (14.09)	18.75 (23.70)
C4	23.33 (28.52)	26.11 (30.20)	45.55 (42.44)	16.11 (23.34)	27.78 (31.12)
C5	9.44 (14.39)	40.55 (39.39)	32.22 (34.48)	23.33 (28.43)	26.39 (29.17)
Control (Hexane)	6.11 (14.06)	8.89 (17.03)	10.55 (17.95)	6.11 (13.81)	7.91 (15.71)
Mean	11.29 (18.08)	23.15 (27.73)	24.90 (28.92)	12.87 (19.51)	
		S.E (m) ±	CD $P \leq 0.05$		
	Treatment	1.105	3.097		
	Concentration	1.353	3.793		
	Interaction (T×C)	2.706	7.586		

Values are mean of six observations.

Figures in the parentheses are angular transformed values

C_1 = 1ppm; C_2 = 10 ppm; C_3 = 100 ppm; C_4 = 1000 ppm; C_5 = 10,000 ppm

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Table 3. Effect of body washes of host insects on percentage parasitization of *Trichogramma brasiliensis*

Body wash Concentration (ppm)	Host insect *Earias vittella*		Host insect *Spodoptera litura*		Mean PAI
	Male	Female	Male	Female	
C1	2.00 (1.55)	5.00 (2.29)	4.67 (2.24)	1.17 (1.32)	3.17 (1.85)
C2	2.67 (1.65)	3.50 (1.95)	6.50 (2.61)	4.33 (2.18)	4.25 (2.10)
C3	4.50 (2.22)	10.83 (3.30)	6.33 (2.59)	3.00 (1.72)	6.17 (2.46)
C4	7.67 (2.85)	9.67 (3.10)	12.50 (3.60)	4.33 (1.96)	8.54 (2.88)
C5	3.50 (1.88)	12.00 (3.52)	11.33 (3.41)	6.50 (2.63)	8.33 (2.86)
Control (Hexane)	1.83 (1.51)	2.33 (1.53)	3.17 (1.91)	2.00 (1.55)	2.33 (1.63)
Mean	3.69 (1.94)	7.22 (2.62)	7.42 (2.73)	3.56 (1.90)	
		S.E (m) ±	CD $P \leq 0.05$		
	Treatment	0.085	0.239		
	Concentration	0.104	0.293		
	Interaction (T×C)	0.209	0.586		

Values are mean of six observations.

Figures in the parentheses are angular transformed values

C_1 = 1ppm; C_2 = 10 ppm; C_3 = 100 ppm; C_4 = 1000 ppm; C_5 = 10,000 ppm

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Table 4. Hydrocarbon profile of whole body washes of *Earias vittella* and *Spodoptera litura*

Name of hydrocarbon	*E. vittella* (µg/g)		*S. litura* (µg/g)	
	Male	Female	Male	Female
Favourable				
Heneicosane (C_{21})	–	–	27.01	–
Docosane (C_{22})	0.90	0.31	–	–
Tricosane (C_{23})	–	–	15.59	–
Pentacosane (C_{25})	–	–	6.86	5.36
Hexacosane (C_{26})	–	0.58	11.49	–
Octacosane (C_{28})	–	–	6.58	–
Nonacosane (C_{29})	0.77	0.02	1.15	–
Unfavourable				
Pentadecane (C_{15})	–	–	2579.87	–
Heptadecane (C_{17})	–	–	–	–
Eicosane (C_{20})	–	–	39.54	–
Tetracosane (C_{24})	–	–	22.53	–
Others				
Nonane (C_9)	–	2.31	–	1.40
Dodecane (C_{12})		76.80	–	100.67
Tridecane (C_{13})	37.37	–	–	52.07
Hexadecane (C_{16})	2.63	–	–	3.51
Nonadecane (C_{19})	9.96	24.97	843.14	7.72
Triacontane (C_{30})	–	0.25	1.42	–
Hentriacontane (C_{31})	–	0.31	6.00	–

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