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Response of the egg parasitoid *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) to kairomones from three host insects

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ABSTRACT: Kairomones emanating from males and females of three different host insects, *viz.*, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) and *Chilo auricilius* (Dudgeon) (Crambidae: Lepidoptera) that tend to influence the parasitic potential of *Trichogramma chilonis* Ishii was studied. Whole body extracts of male and female moths were analysed separately by gas liquid chromatography for determining their hydrocarbon profile, which showed the presence of straight chain saturated hydrocarbons ranging from C_9 to C_{50}. Different host insects showed variation in number and concentration of these chemicals, which were responsible for influencing parasitoid activity and parasitism. The concentration of these hydrocarbons ranged from 4 ppm to 1215280 ppm. The foraging activity of the parasitoid as indicated by parasitoid activity index (PAI) was highest (14.63) in *S. exigua* female body extract at highest concentration (60, 0000 ppm). Per cent mean parasitism rates recorded for *S. litura* male and *S. exigua* male and female body extract were significantly different from that for the body extract of *C. auricilius*. Both kairomonal interactions observed in the male body extract of S. *litura* could be due to the presence of appreciably very high amounts of favourable hydrocarbons docosane and heneicosane and comparatively high amount of octosane and pentacosane as compared to its female body extract. Similarly, female body extract of *S. exigua* also showed comparatively higher amounts of favourable hydrocarbons tricosane, docosane, pentacosane, heneicosane and hexacosane as compared to its male body extract. Based on the hydrocarbon profile of body washings of males and females of the three host insects and quantity of favourable and unfavourable hydrocarbons present in individual extract body, it may be concluded that heneicosane and hexacosane exerted significantly higher level of kairomonal effect on the parasitoid as compared to other hydrocarbons. The response elicited by the male extract body of S. *litura* was significantly higher, whereas the body extract of male *C. auricilius* elicited the least response from *T. chilonis*. These favourable hydrocarbons at the appropriate concentration of body extract of spent adults of *S. litura* males and *S. exigua* females could be used for enhancing parasitization by *T. chilonis*.

KEY WORDS: *Chilo auricilius*, host body extract, kairomones, *Spodoptera exigua*, *S. litura*, *Trichogramma chilonis*.

INTRODUCTION

Modifying insect behaviour in pest management programme through the utilization of nontoxic semiochemicals is recognized as a promising alternative to conventional approaches. Foraging female entomophagous use chemical cues extensively to locate identify and exploit their hosts. Examination of interspecies chemical communication responsible for parasitoid -host relationship suggest that the chemical signals are highly specific and direct (Lewis *et al.*, 1982; Noldus and Van Lenteren, 1985; Padmavathi and Paul, 1997; Reddy *et al.*, 2002). Certainly, when a parasitoid specializes on one or a few closely related species, and especially when those host species are polyphagous, a host specific kairomone would be the most efficient way for the parasitoids to find their hosts among a variety of plant environments. The host insects contain characteristic hydrocarbons, fatty acids and proteins present in their body, which are responsible for such responses (Shu *et al.*, 1990; Frenoy *et al.*, 1991). The egg parasitoid *Trichogramma chilonis* is widely distributed in the Indian subcontinent and is responsible for large scale mortality of a variety of lepidopterans occurring in several crops and efficiently controls them. Plant surfaces also contain such hydrocarbons and in the case of the lepidopteran host of *Trichogramma* spp.; the hydrocarbons mainly originate from the host wing scales. Padmavathi and Paul (1997) reported that the attraction of *T. chilonis* was more for female body wash of *Chilo partellus*, *Sesamia inferens* and *Sitotroga cerealla* as compared to male body wash. Use of synomones and kairomones will help in increasing the effectiveness of natural enemies in biological control programmes. An attempt was made in the present study to determine the role of kairomones emanating from three host insects, *viz.*, *S. litura*, *S. exigua* and *Chilo auricilius* on the egg parasitoid *T. chilonis*.

MATERIAL AND METHODS

The nucleus culture of the host insects used in the present studies were obtained from the Biological Control Laboratory of the Division of Entomology, Indian Agricultural Research Institute, New Delhi. The host insect for the egg parasitoid *Corcyra cephalonica* (Stainton) was reared on crushed and sterilized sorghum grains in a closed type rearing system as described by Paul and Sreekumar (1998). The cultures of *S. litura* and *S. exigua* were reared on artificial diet (Paul, 1990) at 25±2°C and 65±5% R.H. Neonate larvae were transferred to approximately 10 g of diet kept in sterilized glass vials (7.5 cm x 2.5 cm). After second instar, the larvae were transferred to individual vials (7.5 cm x 2.5 cm) and offered approximately 10 g of the diet. After pupation, the pupae were collected from the diet and treated with 0.2 % sodium hypochlorite solution for three minutes, washed thoroughly in distilled water and kept for emergence. The larvae of *C. auricilius* were multiplied on semisynthetic diet, as per Varma and Avasthy (1973) with certain modifications. The culture was maintained at 25± 2°C and 65± 5 % RH.

The whole body extract of male and female moths (three replications) were prepared as described by Ananthakrishnan *et al.* (1991) with certain modifications. Male and female pupae were segregated and kept separately for emergence. Fresh adults (0-24 hrs old) were collected and kept in a deep freezer at -20°C for immobilization. About 6 g of the moths of each group were weighed and placed separately in stoppered bottles, 30 ml of distilled HPLC grade hexane was added to each bottle and the bottles were shaken in a waterbath (Haake, SWB20) at 28°C for 2 hours and later at 50°C for 20 minutes. The hexane fraction was dried over sodium sulphate and passed through a silica column to remove impurities. Hexane was completely distilled off on a waterbath. The resultant extract was diluted to the required concentration using HPLC grade hexane and used both for GLC and bioassay studies.

The whole body extract of host insects after extraction were analysed by Gas Liquid Chromatography (Hewlett packard Model 5890 A), fitted with a flame ionisation detector (FID) and 3 % OV-17 glass column (2 M i.d.2mm), packed on chromosorb Q. The column temperature was 80°C to 250°C @ of 10°C/min. Injector and detector temperature was 250°C. Nitrogen was used as a carrier gas with a flow rate of 30 ml/ min and the injection volume used was 3 µl. The hydrocarbons present in the body extract were identified by comparing with the standards obtained from Sigma, USA and grouped as favourable, unfavourable and others based on earlier studies (Padmavathi and Paul, 1998; Paul *et al.*, 2002; Yadav *et al.*, 2003). The concentration of unknown saturated hydrocarbons was calculated using the following formula:

$$\text{Concentration of unknown saturated hydrocarbons} = \frac{\text{Area of unknown hydrocarbons}}{\text{Area of standard saturated hydrocarbons}} \times \text{Concentration of standard Saturated hydrocarbons}$$

Bioassays were carried out under laboratory conditions (26± 2°C and 65± 5% RH) in glass petri dishes of 15 cm diameter as described by Padmavathi and Paul (1998). In each petri dish, the base of which was covered with a Whatman No 1 filterpaper, six 4 cm² filter paper pieces (egg cards) each containing thirty, UV sterilized, 0-24 h old *C. cephalonica* eggs washed in hexane were placed equidistantly on the periphery, which formed the experimental arena. Body extracts were applied at different concentrations on five egg cards at the rate of 50 µl/card.

Ten healthy, 0-24 h old, anaesthetized, fast reviving *Trichogramma* females were released at the centre of each petri dish. The parasitoids were allowed to search in the experimental arena for a total period of 45 minutes (9 observations) from the time of recovery. The number of parasitoids that visited the cards were counted at five minutes interval and the total number that visited each card constituted the Parasitoid Activity Index (PAI). After 45 minutes, the parasitoids were removed carefully from each egg card and these cards were kept individually in glass vials for development at 26± 1°C and 65± 5 % RH. The parasitoid activity index and per cent parasitism were recorded and the data were subjected to statistical analysis as described by Gomez and Gomez (1986) after square root transformation. The data on Per cent females were subjected to arcsine transformation. Bioassay of individual whole body wash of host insects were subjected to two-way analysis of variance in (ANOVA) completely randomized design (CRD). In order to know the interaction between the treatments and concentration, the data were subjected to two-factorial ANOVA analysis. For comparing the various treatments, the means of original data were used.

RESULTS AND DISCUSSION

Bioassays with different concentrations of whole body extract of host insects indicated species-specific response by *T. chilonis* (Table 1). Foraging activity of the parasitoid as evidenced by parasitoid activity index (PAI) was highest (14.63, in *S. exigua* female body wash at highest concentration (60,0000 ppm), whereas it was the lowest in *S. litura* (Table 1). The response observed to whole body extracts of *S. litura* male and *S. exigua* female and male were significantly higher than that to the body washings of *S. litura* female and *C. auricilius* male and female. Concentration C_4 (30,0000 ppm) recorded the highest mean PAI of 7.73 and lowest PAI of 3.5 was recorded in control (hexane), irrespective of the host and sexes. Male *S. litura* elicited the highest PAI and parasitism whereas the body extracts of male *C. auricilius* recorded the lowest PAI and parasitism (Table 1).

The highest mean per cent parasitism was recorded in *S. litura* male body extract (60.83) at the lowest concentration C_5 (20,0000 ppm), whereas the lowest mean per cent parasitism was found in female body wash of *S. litura*, at the highest concentration 60,0000 ppm.(C_1). Per cent mean parasitism rates recorded for *S. litura* male and *S. exigua* male and female body extracts were significantly different from that for body extracts of *C. auricilius* female and male (Table 2). The highest per cent mean parasitism was observed in C_3(40,000 ppm, 31.25), whereas the lowest per cent mean parasitism was observed at C_2(16.04, at 50,000 ppm), irrespective of the host insects and sex.

Chemical identification of saturated hydrocarbons present in body extracts of host insects:

Table 1. Effect of whole body extract of different host insects on Parasitoid Activity Index of *Trichogramma chilonis*

Concentration	Host Insect						Mean
	Spodoptera litura		*Spodoptera exigua*		*Chilo auricilius*		
	Female	Male	Female	Male	Female	Male	
C_1	0.63 (0.95)	8.50 (2.54)	14.63 (3.58)	7.63 (2.53)	2.38 (1.41)	2.38 (1.41)	6.02 (2.07)
C_2	3.38 (1.59)	7.13 (2.38)	3.38 (1.66)	4.00 (1.93)	4.13 (1.92)	1.25 (1.14)	3.88 (1.77)
C_3	7.00 (2.39)	9.75 (2.72)	8.25 (2.38)	9.50 (2.98)	6.88 (2.24)	2.38 (1.32)	7.29 (2.34)
C_4	2.62 (1.36)	7.50 (2.46)	11.25 (2.87)	4.50 (1.88)	1.50 (1.28)	2.13 (1.19)	4.92 (1.84)
C_5	10.13 (2.79)	14.38 (3.53)	9.00 (2.56)	8.13 (2.46)	3.38 (1.64)	1.38 (1.17)	7.73 (2.36)
Control (Hexane)	5.00 (1.98)	3.38 (1.60)	4.50 (1.76)	4.88 (2.13)	1.88 (1.28)	1.63 (1.19)	3.55 (1.66)
Mean	4.75 (1.82)	9.45 (2.73)	9.30 (2.61)	6.75 (2.36)	3.65 (1.70)	1.90 (1.25)	

	S.E(m)±	CD at 5%
Treatment	0.19	0.54
Concentration	0.19	0.54
Interaction (TxC)	0.48	1.32

Mean of eight observations

Figures in parentheses are square root transformed values.

C_1 = 60,0000 ppm C_2 = 50,0000 ppm C_3 = 4 0,0000 ppm C_4 = 30,0000 ppm C_5 = 20,0000 ppm

The hydrocarbon profile of the male and female body extracts of the three host insects indicated the presence of saturated hydrocarbons ranging from C_{16} to C_{50} with individual concentrations ranging from 4 ppm to 1215280 ppm (Table 3). Six hydrocarbons which were earlier reported as favourable, *viz.*, heneicosane, docosane, tricosane, pentacosane, hexacosane and octacosane were found with the individual concentration ranging from 11ppm to 361640 ppm. Four other hydrocarbons, *viz.*, pentadecane, heptadecane, eicosane and tetracosane that were reported as unfavourable in earlier studies were also detected and their concentration ranged from 43 ppm to 840320 ppm. Certain other hydrocarbons with unknown kairomonal activity were also detected and their individual concentration was between 4 ppm and 1215280 ppm.

Different workers have reported the effect of kairomonal compounds from several host insects on *Trichogramma* sps (Alborn *et al.*, 1997; Ananthakrishnan *et al.*, 1991; Jennings and Jones, 1986; Zaborski *et al.*, 1987). In whole body extract of different host insects as well as in their scales many hydrocarbons have been identified with kairomonal activity (Ananthakrishnan *et al.*, 1991; Gross *et al.*, 1984; Padmavathi and Paul, 1997; Paul *et al.*, 1997; Shu and Jones, 1988).

The results indicated that the kairomonal effect, as evidenced by enhanced parasitoid activity index (PAI) and parasitism, was significantly higher

Table 2 Effect of whole body extract of different host insects on Parasitism of *Trichogramma chilonis*

Concentration	Host Insect						Mean
	Spodoptera litura		*Spodoptera exigua*		*Chilo auricilius*		
	Female	Male	Female	Male	Female	Male	
C_1	0.42 (4.86)	36.67 (33.03)	57.50 (50.32)	29.58 (31.23)	12.08 (16.01)	14.17 (16.76)	25.07 (25.37)
C_2	17.92 (19.50)	37.08 (33.83)	15.0 (18.49)	9.17 (14.35)	10.42 (14.91)	6.67 (11.37)	16.04 (18.74)
C_3	36.25 (33.28)	40.83 (36.64)	37.08 (32.32)	36.67 (35.66)	27.50 (26.60)	9.17 (12.85)	31.25 (29.56)
C_4	11.25 (13.57)	33.33 (30.87)	50.42 (43.94)	19.58 (21.87)	7.08 (12.66)	12.92 (15.10)	22.43 (23.00)
C_5	37.92 (35.05)	60.83 (50.54)	40.83 (28.42)	29.58 (28.44)	12.92 (15.89)	2.08 (7.03)	30.69 (27.56)
Control (Hexane)	15.42 (18.41)	25.83 (23.67)	19.17 (19.66)	30.42 (30.55)	7.92 (11.51)	12.50 (14.29)	18.54
Mean	19.86 (20.78)	63.75 (34.76)	36.67 (32.19)	25.83 (27.02)	12.99 (16.26)	9.59 (12.90)	

	S.E(m)±	CD at 5%
Treatment	3.60	10.04
Concentration	3.05	8.48
Interaction (TxC)	8.06	2.45

Mean of eight observations

Figures in parentheses are angular transformed values.

C_1 = 60,0000 ppm C_2 = 50,0000 ppm C_3 = 4 0,0000 ppm C_4 = 30,0000 ppm C_5 = 20,0000 ppm

in the body extract of male *S. litura* and female *S. exigua* as compared to male and female body extract of *C. auricilius*. The hydrocarbon profile of body extracts of male and female of the three host insects and the quantity of favourable and unfavourable hydrocarbons present in the different body extracts studied by GLC indicated the presence of very high quantity of favourable hydrocarbons in the male body wash of *S. litura* as compared to all other body washes. The gas liquid chromatograms of the male and female body extracts of three host insects revealed the presence of hydrocarbons ranging from C_9 to C_{50}. Better kairomonal interaction observed in the male body extracts of *S. litura* could be due to the presence of appreciably high amounts of favourable hydrocarbons docosane and heneicosane and comparatively higher amounts of octacosane and pentacosane in the male body extract of *S. litura*, as compared to its female body extract. Similarly, female body extract of *S. exigua* showed comparatively higher amounts of favourable hydrocarbons like tricosane, docosane, pentacosane, heneicosane and hexacosane as compared to its male body extract. The unfavourable hydrocarbons tetracosane and eicosane were also present in comparatively low amount in female body extract, as compared to male.

The body extract of *C. auricilius* revealed the presence of comparatively fewer number of hydrocarbons as compared to the other two host insects, which may be the reason for its least

Table 3. Hydrocarbon profile of body extracts of different host insects

Name of Hydrocarbon	Name of Host Insects					
	Spodoptera litura Male	*Spodoptera litura* Female	*Spodoptera exigua* Male	*Spodoptera exigua* Female	*Chilo auricilius* Male	*Chilo auricilius* Female
Favourable Heneicosane (C_{21})	289360	376	336	420	183	337
Docosane (C_{22})	361640	86	55	59	99	297
Tricosane (C_{23})	11	52	473	54	87	273
Pentacosane (C_{25})	50	18	33	43	67	329
Hexacosane (C_{26})	764	924	472	876	38	514
Octacosane (C_{28})	384	220	555	485	-	-
Unfavourable						
Pentadecane (C_{15})	-	660	-	-	-	-
Heptadecane (C_{17})	228	480	252	585	43	195
Eicosane (C_{20})	840320	120	116	83	132	261
Tetracosane (C_{24})	81	60	232	114	45	370
Others						
Hexadecane (C_{16})	207	32	736	45	36.5	74
Octadecane (C_{18})	5	900	600	766	40	190
Nonadecane (C_{19})	230	440	308	124	65	123
Heptacosane (C_{27})	23484	4	376	15	6	-
Nonacosane (C_{29})	135000	43	160	220	47	51
Triacontane (C_{30})	126	14	68	112	-	-
Dotriacontane (C_{32})	-	366	598	2120	-	-
Tetratriacontane (C_{34})	8240	8040	101480	192912	28180	-
Hexatriacontane (C_{36})	-	3365	1496	1172	-	-
Octatriacontane (C_{38})	124960	940	344	1348	-	-
Tetracontane (C_{40})	310720	860	652	1768	-	-
Tetratetracontane (C_{44})	1215280	-	2300	1926	-	-
Hexatetracontane (C_{46})	-	-	-	-	-	-

response (Table 3). The female body extract of *C. auricilius* showed a better response than that of male. Paul *et al.* (1997) found that the whole body extract of female moth of *C. cephalonica* evoked higher parasitism by *T. brasiliensis* and *T. japonicum* as compared to that of male moth. Padmavathi and Paul (1997) reported that the attraction of *T. chilonis* was more for female body extract of *C. partellus*, *S. inferens* and *S. cerealella* as compared to male body extract. Better response to the female body wash of *C. auricilius* could be due to the presence of comparatively higher amounts of favourable hydrocarbons tricosane, docosane, pentacosane, heneicosane and hexacosane in female body extract, as compared to the male body wash. The hydrocarbon profile of the body extracts of these three host insects and their kairomonal effect on *T. chilonis* have been evaluated for the first time.

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