**Research Article** 





# Influence of rice stem borer sex pheromones on the behaviour of its egg parasitoids

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**ABSTRACT**: The egg parasitoids *Trichogramma japonicum* Ashmead (Hymenoptera: Trichogrammatidae), *Tetrastichus schoenobii* Ferriere (Hymenoptera: Eulophidae) and *Telenomus dignus* (Gahan) (Hymenoptera: Scelionidae) are key biotic factors that regulate stem borer population. Augmentation and conservation of these parasitoids is an essential component of Integrated Pest Management (IPM) strategies for stem borers. Similarly, use of sex pheromone technology either by monitoring or mass trapping is also gaining momentum in managing rice stem borers. The synergistic effect of indigenous blends of sex pheromone (YSB and YSB-PSB) on the behaviour of egg parasitoids of stem borer was studied through olfactometer assay. Among the three egg parasitoids tested *T. dignus* exhibited greater response for a longer period, to its host YSB sex pheromone compound (Z)-11 hexadecenal + (Z)-9 hexadecenal at 0.5  $\mu$ l treatment dose with an attraction index (AI) per cent of 19.36 followed by *T. japonicum* (10.76) and *T. shoenobii* (6.35). The treatment doses at 1.0 and 2.0  $\mu$ l resulted in lesser orientation. Similarly, for individual compound, (Z)-11 hexadecenal, (Z)-11-hexadecenol, (Z)-11hexadecenyl acetate, and (Z)-9-hexadecenal, irrespective of treatment doses and parasitoid species, resulted in lesser or poor attraction. Results revealed that host synthetic YSB pheromone at lower concentration attracted and retained egg parasitoids for a longer period and elicited as a synomone and kairomone kind of source.

KEY WORDS: Olfactometer, Parasitoids, pheromone, Telenomus dignus, Tetrastichus schoenobii, Trichogramma japonicum

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# INTRODUCTION

The rice ecosystem is biologically active and dynamic system having a wide range of beneficial fauna and the natural enemies. This biodiversity plays a significant role in the natural regulation of rice pests. Components of rice IPM should have minimum interference on the activity of natural enemies of the crop pest. One such technique is the insect sex pheromone, a promising tool for the management of rice stem borers because of their natural occurrence, lack of toxicity, high bioactivity, species specificity, long potency and compatibility with other IPM components (Krishnaiah et al., 2004, 2008; Ravi et al., 2008). It is a well established technique in rice for monitoring and mass trapping of stem borers. Egg parasitoids play a major role in regulation of stem borer population and can cause up to 90 per cent parsitisation in the field. Inundative release of bio agents, particularly Trichogramma japonicum Ashmead, as pest management strategy is also becoming popular in managing rice stem borers to avoid indiscriminate use of synthetic pesticides.

In general, foraging entomophages use chemical cues to locate, identify and exploit their hosts. Examination of inter species chemical communication responsible for parasitoid-host relationship suggest that the chemical signals are highly specific and direct (Lewis et al., 1975; Noldus and Van Lentern, 1985; Padmavathi and Paul, 1997; Reddy et al., 2002). When a parasitoid specializes on one or a few closely related species, a host specific kairomone would be most efficient way for the parasitoid to find its host. The host insects contain characteristic hydrocarbons, fatty acids and protein present in their body, which are responsible for such responses (Shu et al., 1990; Frenoy et al., 1991). Ganeswara Rao (1996) reported attraction of T. japonicum to host wing scales, hairs of the anal tuft of host females and hairs of the host egg masses when it was applied to filter paper bits. Similarly, for Trichogramma chilonis Ishii (Hymenoptera: Trichogrammatidae), the attraction was more for female body wash of Chilo partellus (Swinhoe) (Lepidoptera: Pyralidae), Sesamia inferens (Walker) (Lepidoptera: Noctuidae). and Sitotroga cerealla (Olivier) (Lepidoptera: Gelechiidae) as compared to male body wash (Padmavathi and Paul, 1997). Use of synomone and kairomone will help in increasing the effectiveness of natural enemies in biocontrol programmes. Sex pheromone of host insects could also act as attractant for parasitoids. In order to assess the potential of Yellow Stem Borer (YSB) and YSB- Pink Stem Borer (PSB) sex pheromone as an attractant for egg parasitoids, an attempt was made in the present study to know the influence of sex pheromone of *Scirpophaga incertulas* (Walker) (Lepidoptera: Pyralidae) on the behaviour of key egg parasitoids of rice stem borer by olfactometer assay.

# MATERIALS AND METHODS

Influence of the indigenous sex pheromone of the host insect, S. incertulas on the behaviour of its egg parasitoid namely Telenomus dignus (Gahan), Tetrastichus schoenobii Ferriere and T. japonicum found in the rice ecosystem was studied through olfactometer assay under laboratory conditions in Tamil Nadu Rice Research Institute, Aduthurai, India. The orientation behaviour of the respective egg parasitoids was studied using 'Y' tube glass olfactometer. The 'Y' tube olfactometer consists of 600 mm long test arm and two numbers of bait arms of (15 mm) length. Air flow in the bait chamber was maintained at 0.4 m/s in each arm. The air flow unit (Boyu air pump -model U9900) has provision for air flow control with the charcoal filter attachment in the inlet was used for the delivery of pure air. The air flow was measured using portable anemometer (Prove Instruments Inc. Taiwan).

The *T. japonicum* used in the study were obtained from the Biocontrol Laboratory, ADAC & RI, Trichy and the parasitoids, *T. dignus* and *T. schoenobii* were collected from parasitized YSB egg masses from the field. Female parasitoids of less than 48h old, mated and chemically inexperienced were used in all the experiments. The internal state of the parasitoid was kept constant before using them in olfactometer assay. Since the parasitoids were observed to show phototropic behaviour in the olfactometer the experiment was conducted in uniform dim light.

Filter paper bits (5mm x 2mm) impregnated with 0.5, 1.0 and 2.0  $\mu$ l of individual compounds (Z)-11-hexadecenal, (Z)-9-hexadecenal and combination blends of (Z)-11hexadecenal and (Z-)-9-hexadecenal (3:1 ratio) designated as indigenous YSB sex pheromone and the multiple species trapping blend comprised of (Z)-11-hexadecenal, (Z)-11hexadecenol, (Z)-11-hexadecenyl acetate, and (Z)-9hexadecenal (1:0.56:0.51:0.50 ratio) as indigenous YSB-PSB sex pheromone was studied for the response. Filter paper impregnated with hexane (HPLC grade) was used as control. In each experiment a total of 20 females were used with four replications. The orientation response of the parasitoids to the pheromone compounds was scored by recording number of parasitoids entering into the bait arm. The experiment was carried out uniformly between 10.00 and 13.00 h in a room maintained at  $26\pm2^{\circ}$ C with RH of  $50\pm10\%$ . After completion of individual experiment the entire unit was washed with acetone and oven dried. The parasitoids once used in the assay were discarded after the completion of the experiment. This was done to prevent influence of the previous exposure on the stimulus.

Response of the parasitoid to the pheromone cue was assessed using Attraction Index (AI) (Ravi and Palaniswami, 2002).

In the above formula the number responding to control refer to number of female that enter the test arm without any odour source.

### **RESULTS AND DISCUSSION**

#### Impact of sex pheromone on Trichogramma japonicum

Host pheromone blend at 0.5µ1 exhibited a greater response in T. japonicum. The attraction index for the parasitoid to the host pheromone was greater (AI = 10.76) (Table 1). The individual compound (Z)-11 hexadecenal elicited greater response in T. japonicum adults (AI = 6.56) and the AI value was 2.36 for (Z)-9 hexadecenal. The indigenous YSB and PSB combination blend elicited minimum influence on the T. japonicum (AI=0.26). Based on the response elicited, the indigenous YSB blend was found to have more influence on orientation of T. japonicum. The efficacy percentage of the lure on YSB varied from 44.83 to 66.67 per cent respectively. The present study is in conformation with the findings of Ganeswara Rao (1996) who reported that 0.5 mg of binary blend (Z)-11 hexadecenal + (Z)-9 hexadecenal elicited behavioural response in the females of T. japonicum. The possible reason could be that the washing contained the pheromone components as one among the sources which elicited the response.

The *T. schoenobii* also showed a greater attraction towards the binary blend (Z)-11 hexadecenal + (Z)-9 hexadecenal and the mean number of parasitoids attracted towards the bait chamber was 7.00 (Table 2). In terms of attraction index the YSB lure had an AI value of 6.35

AI = \_\_\_\_\_

No. of females released-No. of females responding to control

Pheromone compound	Exposure Dose (µl)								
	0.5			1.0			2.0		
	No.	AI	EP	No.	AI	EP	No.	AI	EP
(Z)-9- hexadecenal	3.20	2.36°	14.04	2.60	2.60	14.94	2.20	1.31°	18.97
(Z)-11- hexadecenal	7.20	6.56 <sup>b</sup>	31.58	4.60	4.60	26.44	3.80	2.99 <sup>b</sup>	32.76
(Z)-11 hexadecenal + (Z)-9 hexadecenal (3:1)	11.20	10.76ª	66.67	9.00	9.00	58.44	5.20	4.46 <sup>a</sup>	44.83
(Z)-11 hexadecenal + (Z)-9-hexadecenal+ (Z)-11-hexadecenol, (Z)-11-hexadecenyl acetate									
(1:0.50:0.56:0.51)	1.20	0.26 <sup>d</sup>	9.38	1.20	1.20	10.91	0.40	0.58°	3.45
Hexane	0.0	0.00 <sup>d</sup>	0.0	0.0	0.0	0.0	0.0	0.0 <sup>d</sup>	0.0
CD $(P = 0.05)$		2.27			2.12			1.15	

#### Table 1: Response of Trichogramma japonicum females to host pheromone compounds (Olfactometer assay)

No. - Mean number responded

AI – Attraction Index

EP – Efficacy percentage

Table 2: Response of Tetrastichus schoenobii females t	o its host pheromone compounds (olfactometer assay)
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Pheromone compound	Exposure Dose (µl)								
	0.5			1.0			2.0		
	No.	AI	EP	No.	AI	EP	No.	AI	EP
(Z)-9- hexadecenal	3.00	2.15°	18.52	2.20	1.31 <sup>b</sup>	15.07	1.00	0.05	11.63
(Z)-11- hexadecenal	5.00	4.25 <sup>b</sup>	30.86	4.80	4.04 <sup>a</sup>	32.88	3.00	2.15	34.88
(Z)-11 hexadecenal + (Z)-9 hexadecenal (3:1)	7.00	6.35ª	41.18	6.60	5.93ª	45.21	4.20	3.41	48.84
(Z)-11 hexadecenal + (Z)-9-hexadecenal+ (Z)-11-hexadecenol, (Z)-11-hexadecenyl acetate									
(1:0.50:0.56:0.51)	1.20	0.26 <sup>d</sup>	8.00	0.80	0.16 <sup>c</sup>	5.48	0.40	0.58	4.65
Hexane	0.0	0.0 <sup>d</sup>	0.0	0.20	0.79 <sup>b</sup>	1.37	0.00	0.0	0.00
CD ( $P = 0.05$ )		1.74			2.63			NS	

No. - Mean number responded

AI – Attraction Index

EP – Efficacy percentage

followed by (Z)-11 hexadecenal (AI = 4.25) and (Z)-9 hexadecenal (AI = 2.15). The indigenous YSB-PSB combination blend had minimal influence (AI= 0.26) on *T. schoenobii* as in the case of *T. japonicum*. The data on efficacy percentage (EP) also revealed a similar trend.

The mean number of *Telenomus dignus* adults attracted to the pheromone source (Z)-11 hexadecenal + (Z)-9 hexadecenal was greater (AI=19.36) compared to the individual component. Again (Z)-11 hexadecenal elicited greater response (AI=15.59) in this species too. In terms of efficacy percentage, the YSB blend (Z)-11 hexadecenal + (Z)-

9 hexadecenal (3:1) (EP = 40.08%) was effective followed by (Z)-11 hexadecenal (EP= 32.64%) and (Z)-9 hexadecenal (EP = 8.26%). The EP value (17.77%) was comparatively low for the YSB-PSB combination blend (Table 3). These is in line with the findings of Norduland *et al.* (1983) who reported that *Telenomus remus* Nixon actively searched for the host in the presence of the host sex pheromones.

# Influence of pheromone molecules on egg parasitoid activity

The efficiency of an egg parasitoid in a biological control program is highly dependent upon the

Pheromone compound	Exposure Dose (µl)								
	0.5			1.0			2.0		
	No.	AI	EP	No.	AI	EP	No.	AI	EP
(Z)-9- hexadecenal	4.00	3.16d	8.26	3.60	2.78d	7.83	4.40	3.62d	9.28
(Z)-11- hexadecenal	15.80	15.59b	32.64	15.00	14.65b	32.61	15.40	15.17b	32.49
(Z)-11 hexadecenal + (Z)-9 hexadecenal (3:1)	19.40	19.36a	40.08	19.00	18.95a	41.30	19.20	19.16a	40.51
(Z)-11 hexadecenal + (Z)-9-hexadecenal+ (Z)-11-hexadecenol, (Z)-11-hexadecenyl acetate									
(1:0.50:0.56:0.51)	8.60	8.03c	17.77	8.00	7.4c	17.39	8.40	7.82c	17.72
Hexane	0.60	0.37e	1.24	0.40	0.58e	0.87	0.00	0.0	0.00
CD $(P = 0.05)$		2.22			1.44			0.85	

#### Table 3: Response of *Telenomus dignus* females to host pheromone molecules (Olfactometer assay)

No. - Mean number responded

AI – Attraction Index

EP – Efficacy percentage

semiochemical-mediated interrelationships of the environment, the plant, and the phytophagous insect (Vinson, 1975). The importance of semiochemicals in many host-parasitoid interactions was evident through this study.

Interestingly, the sex pheromone blends and their individual molecule were found to elicit considerably good orientation response in all the three parasitoids species at 0.5  $\mu$ 1 dose. The response of the parasitoids to the host sex pheromone was well evidenced by antennal drumming and insertion of ovipositor eliciting high attraction index values. The *T. dignus* showed maximum response to the sex pheromone blend and the parasitoids were retained for longer period in the sex pheromone arena. Orientation response of the parasitoids to the YSB sex pheromone was evidenced by the maximum attraction index (AI = 19.36). Other parasitoids *T. japonicum* (AI=10.76) and *T. schoenobii* (AI=6.35) were also found to show behavioural response for the host pheromone.

The synergistic role of insect sex pheromones on the egg parasitoids observed in the present study was significant. In a similar work on the egg parasitoid of stem borer against Nordlund *et al.* (1983) and Noldus and Van Lenteren (1985) also reported direct behavioural response of the host sex pheromone in the egg parasitoids of stem borer *T. remus* and *Trichogramma evanescens*.

The observations in the present study is significant in the light of the existing biological control recommendation against rice stem borer, wherein the inundative release of *T. japonicum* against YSB is advocated. Deployment of sex pheromone in the rice field can play a significant role in increasing the host searching behaviour of *T. japonicum*. The above study revealed the role of YSB indigenous sex pheromones as a synomone (adaptively advantageous to both the emitter as well as receiver) and kairomone (adaptively advantageous to the receiver in rice ecosystem) hence, from these studies it was proven that YSB indigenous sex pheromone has a synergistic role in stem borer management.

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