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Kairomones, their optimum concentrations, and application techniques to enhance the parasitization efficiency of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae)

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ABSTRACTS: **Studies to identify effective kairomones, their optimum doses and appropriate application techniques with a view to increase the parasitizing efficiency of *Trichogramma chilonis* Ishii were conducted during 2005-06. Fresh hexane extract of *Corcyra cephalonica* (1%) + hexacosane (0.3%) and *C. cephlonica* scale extract (1%) + nonacosane (0.3%) were most attractive and recorded highest egg parasitization (77.25% in both) when applied in impregnated rubber septa. The second effective kairomone was *C. cephalonica* scale extract (1%) + pentacosane (0.3%). Kairomone impregnated septa as dispensers proved better over paper strips. No significant difference in parasitizing efficiency was recorded between ochre and orange septa kairomone dispensers.**

KEY WORDS: Hexacosane, kairomones, nonacosane, pentacosane, rubber septa, scales extract, *Trichogramma chilonis*, tricosane

INTRODUCTION

Trichogramma chilonis Ishii (Hymenoptera: Trichogrammatidae) is an effective egg parasitoid of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and releases of this parasitoid, either alone or in combination with other biocontrol agents effectively controlled the bollworm incidence in cotton (Rahman *et al.*, 2003; Balakrishnan *et al.*, 2004; Panchbhai *et al.*, 2004) and borer pest of sugarcane (Singhal *et al.*, 2001). However, success of *T. chilonis* in the biocontrol of *H. armigera* under field conditions depends up on its host acceptance and searching efficiency in a highly complex crop environment. Host derived kairomones play key role in improving efficiency of natural enemies (Brown *et al.*, 1970; Lewis *et al.*, 1975a & b). Haskell *et al.* (1981) reported intensification of searching behaviour of *Trichogramma* spp. when *Heliothis* scales extract was sprayed on eggs or food plants and the chemical was identified as 13-methyl hentriacontane. *Trichogramma* females were reported to use kairomones as sign to recognize and parasitize a host egg. The scales found on lepidopteran eggs carried the chemical stimulus that attracted Trichogrammatids (Noldus, 1989). Kairomones such as tricosane, pentacosane,

hexacosane, docosane and nonacosane, which are components of the scales of lepidopteran moths, were observed to increase the parasitising efficiency of Trichogrammatids (Padmavathi and Paul 1998; Paul *et al.*, 2002). Renou *et al.* (1992) observed that the use of a mixture with heneicosane, tricosane, pentacosane, heptacosane and nonacosane increased the upwind regression in the case of *Trichogramma brassicae* Bezdeko. Tandon (2001) highlighted future prospects of kairomones in enhancing the efficiency of natural enemies for the success of biological control crop pests. Paramasivam and Paul (2005) attempted kaolinite clay as carrier with leaf extracts of flowering phase of maize, sunflower and egg wash of *Chilo partellus* (Swinhoe) and observed that the semiochemical dust formulation recorded highest parasitism by *T. chilonis* in field.

The present studies were carried out to identify effective kairomonal compound, optimum concentration and appropriate application technique to increase the parasitizing efficiency of *T. chilonis* and the results are presented in this article.

MATERIALS AND METHODS

The studies were conducted in the Entomophagous Insect Behaviour Laboratory, Project Directorate of Biological Control during 2005-06.

Insect cultures

The culture of the egg parasitoid, *T. chilonis* was maintained on *Corcyra cephalonica* (Stainton) eggs in the Entomophagous Insect Behaviour Laboratory and two day old adult females were used in all the experiments carried out in this study. However, fresh eggs and scales of *C. cephalonica* were obtained from Mass Production Laboratory, Project Directorate of Biological Control and used in the experiments.

Moth Scales extraction and preparation of kairomonal formulations

Kairomone formulations were prepared by extracting 1 g fresh scales of *C. cephalonica* in 100 ml of hexane in a water bath at a temperature of 30° and 60°C for 30 minutes, respectively. The extract was filtered through a Whatman No.1 filter paper and 100 mg of tricosane (Sigma Aldrich Chemicals) (0.1%) was mixed thoroughly. Exactly 0.4 ml of this kairomone solution was impregnated in a rubber septum (Welfax polymers, Bangalore) under a fume hood and immediately transferred to a polythene bag and sealed. The same formulation was sprayed on Whatmann filter paper bits @ 0.4 ml for 3 bits and after drying, the paper bits were kept into polythene bags and sealed.

Kairomonal formulations were prepared with tricosane, pentacosane, nonacosane and hexacosane at 0.1, 0.2 and 0.3 per cent concentration along with *C. cephalonica* scales (1%) and impregnated in the rubber septa as detailed above and used to find out the efficiency of these compounds.

Wind tunnel bioassay

The bioassays were conducted in a wind tunnel made of transparent, non-adsorbent, non-odorant acrylic sheets of 4mm thickness with a trap chamber (25cm diam) and a test chamber (25 cm diam) connected through a tunnel of 15 cm diam. The length of the wind tunnel was 100 cm. One kairomone-impregnated septum was kept at a distance of 50 cm from the test chamber along with a small bit of card containing 50 UV radiated fresh eggs of *C. cephalonica*. One hundred adults of *T. chilonis* were released in the test chamber and a wind flow of 25 cm per second was maintained through the trap chamber to the test chamber. After 60 minutes, the egg cards were collected and kept in a small vial for observation. The parasitization, which is directly related to the number of adults visiting the egg cards, was counted once the eggs turned black. A treated control where the filter paper bits (3 in number) treated with the above kairomone was maintained and along with an untreated control where there was no treatment given. The concentration of the tricosane was increased to 200 mg to find out the response of *T. chilonis*. Five replications were maintained for each treatment. The

per cent eggs parasitized were computed from the eggs parasitized. The percentages were transformed into arcsine values and the data were subjected to analysis of variance.

Another experiment was conducted with the *C. cephalonica scale* extract (1%) fortified with compounds like hexacosane, pentacosane, nonacosane and tricosane, at 0.1, 0.2 and 0.3 per cent as per the above procedure given above.

Influence of septa colour on parasitization efficiency

In another experiment, two types of septa, namely, ochre and coral orange were used to study the effect of colour of septa on the attractancy of *T. chilonis*. The procedure followed was same as described earlier.

RESULTS AND DISCUSSION

In the first experiment, the kairomone-impregnated septa (at 0.1 % and 0.2%) were compared with the kairomone sprayed filter papers. Both the treatment containing impregnated septa with 0.1 and 0.2 per cent tricosane recorded highest parasitization compared to filter paper bits impregnated with tricosane (0.1%) (Table 1).

Table 1. Comparison of kairomones application techniques

Treatment	Per cent parasitization
1. Tricosane (0.1%) impregnated in septa	49.60 (44.69)
2. Tricosane (0.2%) impregnated in septa	41.00 (39.25)
3. Tricosane (0.1%) sprayed on filter paper	2.40 (9.23)
4. Untreated control	0.00 (0.0)
C D (p = 0.05)	11.36

Figures in parentheses are arcsine-transformed values.

In the second experiment, the application of kairomones through rubber septa was compared to find out the efficacy of the different compounds in the attraction of *T. chilonis*. Among the compounds tested, nonacosane and hexacosane at higher concentration of 0.3 per cent recorded highest parasitism, namely, 77.25 per cent in both the cases (Table 2). Nonacosane at lower concentrations (0.1 & 0.2%) evoke less response, which was reflected by lower parasitism i.e., 42.75 and 42.38 per cent, respectively. Hexacosane at 0.1 and 0.2 per cent concentrations was least effective and statistically on par with control. Pentacosane at lower concentrations (0.1 & 0.2%) recorded higher parasitism. In case of tricosane, maximum parasitism was observed in 0.3 per cent concentration, which was significantly higher than other two concentrations. Keeping in view concentration and kairomone, hexacosane (0.3%) and nonacosane (0.3%) were most efficient, followed by pentacosane (0.2%). Gross *et al.* (1984) recorded increased parasitism of *Heliothis zea* (Boddie) eggs by *Trichogramma pretiosum* Riley when hexane extract of moth scales at the rate of one ml/ 30 cm was applied.

There was no significant difference between two colour septa, namely, coral orange and ochre in terms of per cent parasitization (Table 3).

Our studies indicated that kairomonal compounds present in the *C. cephalonica* moth scales fortified with nonacosane (0.3%), hexacosane (0.3%) pentacosane (0.2%) and tricosane (0.3%) increased significantly the egg parasitism efficiency of *T. chilonis*. Earlier investigation made by Ananthakrishnan *et al.* (1991) also indicated the role of *C. cephlonica* moth scale extract in enhancing parasitizing efficiency of *T. chilonis*. Gross *et al.* (1984) recorded increase in the parasitism of *H. zea* eggs by *T. pretiosum* on kairomone (hexane extract of *H. zea* moth scales) treated leaves. Boo and Yang (2000) made similar observations when studying the effect of male moth scale extract of *H. assulta* (Guenee) on increasing efficiency of *T. chilonis* and reported that the extract contained fractions of saturated hydrocarbons. In our earlier studies we have observed that *C. cephalonica* scales fortified with the tricosane recorded higher parasitism by *T. chilonis* under field

Table 2. Efficiency of different compounds on the parasitism efficiency of *T. chilonis*

Compound	Per cent parasitization at concentration			
	1% + 0.1%	1% +0.2%	1% + 0.3%	Mean
1. Scale + Tricosane	46.25 (42.55)	46.25 (42.55)	53.00 (46.72)	48.50 (43.94)
2. Scale + pentacosane	55.75 (48.32)	63.50 (52.84)	51.00 (45.58)	56.75 (48.92)
3. Scale + nonacosane	42.75 (40.76)	42.38 (40.54)	77.25 (61.69)	54.03 (47.66)
4. Scale + hexacosane	19.75 (25.96)	19.52 (25.76)	77.25 (61.69)	38.84 (37.80)
5. Control	21.50 (27.55)	21.06 (27.26)	1.00 (2.88)	14.52 (19.23)
Mean	37.20 (37.03)	38.54 (37.79)	51.90 (43.71)	
CD (p = 0.05)				
For concentrations	3.98			
For compounds	5.14			
For compounds and concentrations	8.91			

Figures in parentheses are aresine-transformed values.

and laboratory conditions (unpublished work of the authors).

Table 3. Parasitism of *T. chilonis* with ochre and coral orange red septa impregnated kairomone

Compound	Per cent parasitization	
	Ochre	Coral orange
1. Tricosane (0.1%)	33.84	32.63
2. Pentacosane (0.1%)	46.62	46.20
3. Hexacosane (0.1%)	4.76	4.99
T test	NS	

Several pheromone compounds have been impregnated into different types of septa and used effectively for the management of crop pests (Cork, 2004). However, studies on the development of kairomonal formulation for field use are very limited. In the present study, it was observed that rubber septa are better dispensers for kairomones in comparison to paper strips. Perhaps rubber septa have better retention of kairomones and controlled release, while paper strips loose the cue fast. Kant *et al.* (1998) recorded that cork and rubber septa gave higher catches of *H. armigera* than cigarette filters or filter. Similarly, Ventura *et al.* (2005) also reported that rubber septa dispensers impregnated with plant kairomone (Floral volatiles-1, 4-dimethoxybenzene) attracted more *Diabrotica speciosa* (Germar) beetles than control (dental wick). Knight and Light (2005) developed a halobutyl rubber septa impregnated with ethyl (E-2)- 2, 4, decadienpate at different doses, which successfully trapped codling moth *Cydia pomonella* L.

The ochre and coral orange colour of rubber septa did not effect attraction of *T.chilonis* adult females to kairomone source in the present study, thereby; no significant difference in per cent egg parasitism was noticed. However, the chemical present in rubber septa imparting different colours may effect the degradation of kairomones, and ultimately efficiency.

Presently, though the rubber septa gave better results, it is necessary to find out the effect of different types of septa with suitable colours for effective utilization under field conditions. The shelf life of the formulation need to de improved with the use of suitable stabilizers.

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