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Active Dispersal of *Trichogramma chilonis* Ishii and *T. pretiosum* Riley Towards Host Eggs Under Confined Condition and Their Parasitisation Efficiency

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The active movement of two trichogrammatids *viz*., *Trichogramma chilonis* and *T. pretiosum* towards eggs of *Leucinodes orbonalis* Guenee and *Corcyra cephalonica* Stainton was assessed in 2cm diameter PVC tubes of 1, 2, 3, and 4 meters. The trichogrammatids moved up to 3m towards the eggs of *L. orbonalis* and *C. cephalonica*. *T. chilonis* showed more active movement than *T. pretiosum*. At a distance of 1m, 50.05 per cent of *T. chilonis* was recaptured on *L. orbonalis* egg cards which got subsequently reduced on the increase in the length of PVC tubes, which was 14. 64 per cent at 2m and 1.19 per cent at 3m and became nil in a length of 4m, while the dispersal behaviour of male and female parasitoid was compared. The female wasps were more active than male wasps and dispersed to a maximum distance of 3m in both the species. The study also showed that the preference of trichogrammatids towards the natural host (*L. orbonalis*) was more when compared to the factitious host (*C. cephalonica*). The recapture of *T. chilonis* and *T. pretiosum* at release distance of 1m was 50.05 and 28.58 per cent in *L. orbonalis* respectively whereas, *C. cephalonica* eggs attracted 41.25 and 9.66 per cent parasitoids respectively. Among *T. chilonis*, *T. japonicum* and *T. pretiosum*, *T. pretiosum* showed a higher parasitisation of 90.00 per cent and emergence of 91.03 per cent on one day old eggs of *L. orbonalis*. The study also showed that the age of host was a matter in parasitisation as well emergence of trichogrammatids. The increase in host age was negatively associated with parasitisation as well as emergence of trichogrammatids irrespective of species.

Key words: *Trichogramma chilonis*, *T. pretiosum*, *Leucinodes orbonalis*, Active dispersal, Parasitisation

Trichogramma spp. (Hymenoptera: Tricho grammatidae), egg parasitoids are used against wide range Lepidopterous pests and are fruitfully used in inoculative as well as inundative biological control programmes all over the world. Trichogrammatids have a low active flight capacity. A thorough understanding of searching behaviour of trichogrammatids can is vital for the optimisation of biological control programmes. The major means of short distance dispersal in *Trichogramma* spp. are; walking and short jumps. Female wasps randomly search for hosts by using the host kairomones such as moth scales and sex pheromones which enable them to locate eggs (Romeis *et al*., 2005).

The factor which has a clear influence on the efficiency of released biocontrol agent is their dispersal and host finding behaviour (Bigler, 1994). The dispersal behaviour of *Trichogramma* is still a controversy, as to whether walking or flying. Dispersal of this minute parasitoid in open field is influenced by plant architecture, wind speed, direction and total leaf area of the crop. In fields of maize, wasps were able to parasitise egg masses of *Ostrinia nubilalis* within a radius of eight metres. Low rate of dispersal of about three metres was also reported in many *Trichogramma* species. The distance of dispersal of these wasps varies with species and crop. In cotton, *T. minutum* Riley dispersed to 4-8m, in apple, *T. dendrolimi* Matsumura and *T. embryophagum* (Hartig) could move a distance of 3m in one day (Suverkropp *et al*., 2009), and strong winds in a fixed direction can favour the dispersal of trichogrammatids in field (Bigler *et al*., 1990). The usual method used to determine dispersal behaviour of *Trichogramma* is by recording data on parasitism, but many a times this data will not guarantee the absence or presence of wasp and the number of wasps present (Suverkropp *et al*., 2009).

In order to find out the actual distance that can be moved by *T. chilonis* and *T. pretiosum* without the influence of external factors, and their parasitisation efficiency against *L. orbonalis* the laboratory experiments were conducted and the results are reported.

Material and Methods

T. pretiosum and *T. chilonis* parasitoids used in the experiment were reared on UV treated *Corcyra cephalonica* eggs at 25±2°C and 60±5% RH in Bio-control laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

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Eggs of *Leucinodes orbonalis* and *C. cephalonica* were obtained from pure cultures maintained in the laboratory at 25±2°C and 65±5% RH. These eggs were cleaned and sterilized by keeping them under ultra violet light for 20 minutes.

The experiment was conducted under controlled conditions at 25±2°C and 60±5% relative humidity, poly vinyl chloride (PVC) tubes of diameter 2 cm at four lengths such as 1, 2, 3 and 4 meters. On one end of the tube 100 to 150 freshly emerged adult wasps were released and on the other end a glue pasted paper card with 100 host eggs or without host eggs was kept. Both the ends of tubes were covered with black cotton cloths after release of adult trichogrammatids. A constant air flow of 1 ml minute^{-1} was supplied through air delivery system (Champion aquarium air pump) on the side where the eggs were placed and kept for four days without disturbance. For both the species experiments were conducted separately and replicated thrice. On the fourth day, observations on number of adult wasp (male and female) trapped in the glue pasted paper were made. The male and female wasps were differentiated based on their difference in characters of antennae.

The laboratory experiment to find out the parasitisation efficiency of *T. pretiosum* and *T. chilonis* against the eggs of *L. orbonalis* were carried out at 25±2°C and 65±5% RH. In this experiment, eggs of *L. orbonalis* were taken from laboratory cultures and pieces of cotton cloth each containing 30 eggs were used. The adults of different *Trichogramma* spp. were collected from the laboratory cultures at the rate of 6:1 by aspirator and introduced into test tubes which consisted one-day, two-day and three-day old sterilized eggs of *L. orbonalis*. The treatments were replicated thrice in Completely Randomized Design (CRD).

Daily observations were made using hand magnifier (15X) up to the incubation period (7 days) of the eggs of *L. orbonalis* to count the number of eggs parasitized by parasitoid. At the same time when the colour of eggs of *L. orbonalis* was changed into black, the cloths containing parasitized eggs of *L. orbonalis* was placed in new cleaned test tubes and carefully closed and tied. The observations were continued until the emergence of parasitoids. The number of parasitoids emerged from each treatment was counted to work out the emergence rate.

Results and Discussion

Active dispersal of Trichogramma spp.

The rate of recapture of *T. chilonis* and *T. pretiosum* wasps in the glue pasted paper card with host eggs in different distances are furnished in Table 1. In the case of *T. chilonis,* a maximum of 50.05 per cent of wasps moved towards *L. orbonalis* eggs and recaptured at 1m distance, while the percentage of recapture of wasps at 2m and 3m distances were 14.64 and 1.19 respectively. The percentage of recapture at a distance of 1m was 41.25 for *C. cephalonica* eggs while the recapture of wasps at 2m and 3m distance was 13.40 and 0.34 per cent respectively. At 4m distance, no wasp was recaptured irrespective of the host eggs. In the glue pasted paper kept without any host eggs, the recapture was 2.11 per cent at 1m distance. No wasps were recaptured at 2, 3, and 4 meters when no host eggs were kept.

Table 1. Active movement of *T. chilonis* and *T. pretiosum* towards host eggs

Host	Percentage of wasps recaptured at a distance of							
	1m		2m		3m		4m	
	TC	TP	TC	TP	TC	TP	TC	TP
Corcyra cephalonica eggs	41.25	9.66	13.40	3.45	0.34	0.15	0.00	0.00
Leucinodes orbonalis eggs	50.05	28.58	14.64	9.03	1.19	0.95	0.00	0.00
No eggs	2.11	0.27	0.00	0.00	0.00	0.00	0.00	0.00

TC= *Trichogramma chilonis*, TP= *Trichogramma pretiosum*

Table 2. Gender ratio of *T. pretiosum* attracted to cues from host eggs

Host	Sex ratio (M:F) of recaptured wasps at a distance of							
	1m		2m		3m		4m	
	TC	TP	TC	TP	TC	TP	TC	TP
Corcyra cephalonica eggs	1:1.38	1:6.4	1:3.58	1:14	-	-	-	-
Leucinodes orbonalis eggs	1:4.24	1:4.5	1:7.43	1:18.5	-	-	-	-
No eggs	-	1.0: 1.0	-	-	-	-	-	-

TC= *Trichogramma chilonis*, TP= *Trichogramma pretiosum*

In case of *T. pretiosum* a maximum of 28.58 per cent of wasps moved towards *L. orbonalis* eggs and recaptured at 1m distance, while the percentage of recapture of wasps at 2m and 3m distance was 9.03 and 0.95 respectively. The recapture at a distance of 1m was 9.66 for *C. cephalonica* eggs and the recapture of wasp at 2m and 3m distance was 3.45 and 0.15 per cent respectively. At 4m distance no wasp was recaptured irrespective of the host eggs. In the glue pasted paper kept without any host eggs,

the recapture was 0.27 per cent at 1m distance. No wasp was recaptured at 2m, 3m and 4m where no host eggs were kept.

The recapture of wasps at 1m distance was the maximum for *T. chilonis* and *T. pretiosum* irrespective of the host eggs and there was no recapture at 4 m. The range of recapture of wasps in the experiment was 0- 50.05 and 0- 28.58 respectively for *T. chilonis* and *T. pretiosum*.

Table 3. Parasitisation efficiency of *Trichogramma chilonis* on eggs of *Leucinodes orbonalis*

	Age of eggs	Parasitism* (%)	Emergence*(%)	Sex ratio (M:F)
With release of T. *chilonis*	One day old	65.56[a] (54.163)	81.37[a] (64.612)	1:1.82
	Two day old	45.56[b] (42.450)	68.25[b] (55.712)	1: 1.52
	Three day old	21.11[c] (27.349)	49.17[c] (44.522)	1: 3.50
Without release of T. *chilonis*	One day old	0.00[d] (0.523)	0.00[d] (0.523)	-
	Two day old	0.00[d] (0.523)	0.00[d] (0.523)	0-
	Three day old	0.00[d] (0.523)	0.00[d] (0.523)	-
SEd		1.80	1.75	
CD(0.05)		3.92	3.83	

*Mean of three replicates; figures in parentheses are arcsine transformed values; in column, means followed by the common alphabet(s) are not significantly different by LSD (P=0.05)

A three factorial evaluation showed that there was significant difference between the species, host eggs and distance moved by the wasps. The percentage recapture showed a clear decrease of recapture over the increase in distance. The recapture on a release distance of 1 m was 50.05 and 28.58 respectively for *T. chilonis* and *T. pretiosum* towards the eggs of *L. orbonalis*. This per cent recapture got reduced to zero at four metre distance.

This is a maiden attempt to find out the active dispersal of *T. chilonis* and *T. pretiosum* under confined condition. This experiment was largely devoid of external factors (except for lowest air flow @ 1ml min^{-1}; slow air flow does not have any impact in dispersal); the movement of the parasitoids was mainly due to chemical cues emanating from the host eggs which is evident with the negligible movement of parasitoids when no eggs were provided in the tube. *T. chilonis* and *T. pretiosum* were highly active at shorter distances and the activity subsequently declined sharply and later found to be absent at a distance of four metres. The present findings are similar to that of the observation made by Ayvaz *et al*. (2008) in the experiment with *T. evanescens* under confined condition. Chapman *et al*. (2009) also observed similar negative correlation of decrease in parasitism over increase in release distance in case of *T. ostriniae* on *Ostrinia nubilalis*.

Percentage of *T. chilonis* recaptured was more than *T. pretiosum* at 1m, 2m and 3m distances, indicating that *T. chilonis* was a more actively moving species than *T. pretiosum*. Similar observations were made by Sharma and Aggarwal (2015) who reported that *T. chilonis* activity was more when compared to *T. japonicum* on eggs of *C. cephalonica*. The parasitoids were preferentially attracted more towards eggs of *L. orbonalis* over *C. cephalonica.* This is in accordance with Takada *et al*. (2001) who reported that *Trichogramma* parasitoid preferred its natural host for oviposition than that of the factitious host.

In all the experiments more number of female wasps were attracted towards the host egg than the males. Males were recaptured only up to 2m while the females were recaptured at 3m distance (Table 2). This is similar to the observation made by Stern *et al*. (1965) who reported that females dispersed longer distance than male in *T. semifumatum*. This behavioural difference in movement by males and females is linked with mate searching and oviposition. The movement of males ends with mating, however in females, it continues up to searching the suitable host for oviposition.

Parasitisation efficiency of Trichogramma spp

The efficiency in parasitisation on different aged eggs of *L. orbonalis* by the egg parasitoid *T. chilonis* and its emergence rate are summarized in Table 3. It showed a high preference towards the freshly laid eggs. The maximum parasitisation showed by *T. chilonis* was 65.56 per cent on one day old eggs followed by two days eggs with 45.56 per cent and least was on three days old eggs with 21.11 per cent.

The emergence rate of *T. chilonis* varied with age of host eggs. The maximum emergence was from eggs of one day old (81.37 per cent) followed by two days old (68.25 per cent) and minimum on three days old eggs (49.17 per cent). The sex ratio of emerged adults revealed that the females were more than males.

The efficiency in parasitisation on different aged eggs of *L. orbonalis* by the egg parasitoid *T. pretiosum* and its emergence rate are summarized in Table 4. The data revealed that the parasitoid preferred one day old eggs. The maximum parasitisation observed was 90.00 per cent in one day old eggs followed by 82.22 per cent in two days old eggs and 73.33 per cent in three days old eggs.

Table 4. Parasitisation efficiency of *Trichogramma pretiosum* on eggs of *Leucinodes orbonalis*

	Age of eggs	Parasitism* (%)	Emergence* (%)	Sex ratio (M:F)
With release of *T. pretiosum*	One day old	90.00[a] (71.58)	91.03[a] (72.74)	1: 1.96
	Two day old	82.22[b] (65.08)	67.56[b] (55.31)	1: 2.42
	Three day old	73.33c (58.98)	31.75[c] (34.29)	1:4.00
Without release of *T. pretiosum*	One day old	0.00[d] (0.52)	0.00[d] (0.52)	-
	Two day old	0.00[d] (0.52)	0.00[d] (0.52)	-
	Three day old	0.00[d] (0.52)	0.00[d] (0.52)	-
SEd		1.21	1.35	
CD(0.05)		2.64	2.95	

*Mean of three replicates; figures in parentheses are arcsine transformed values; in column, means followed by the common alphabet(s) are not significantly different by LSD (P=0.05)

The emergence rate of *T. pretiosum* from the parasitized eggs was also decreased with increase in the host age. The per cent emergence was maximum from one day old eggs (91.03 per cent) followed by two days old eggs (67.56 per cent) and three days old eggs with least emergence of 31.75 per cent. Here also the emergence rate of females was higher than male.

The parasitisation efficiency varies among *T. pretiosum* and *T. chilonis* and the age of *L. orbonalis* eggs. It was observed that the parasitisation by *T. pretiosum* was maximum with 90.00 per cent on one day old eggs while it was 65.56 per cent by *T. chilonis*. Similar trend was observed in case of emergence rate of parasitoids. *T. pretiosum* showed maximum emergence rate of 91.03 per cent from one day old eggs of *L. orbonalis* followed by *T. chilonis* (81.37%). The study also showed that parasitisation and emergence rate varies according to the age host eggs. One day old eggs showed high parasitisation followed by two days and three days old eggs by all parasitoids.

The studies conducted by Niranjana and Sridhar (2014), Niranjana *et al*. (2015) and Niranjana *et al*. (2018) resulted in similar results that, the parasitism and emergence of parasitoids got reduced upon increasing the host age and also reported that *T. pretiosum* had highest parasitisation of 91.99 and emergence of 87.52 per cent on one day old eggs of *L. orbonalis*. Hanapur and Nandihalli (2003) also reported 64.00 per cent parasitisation on eggs of *L. orbonalis* in field condition by *T. chilonis* which is in corroboration with the results of present laboratory experiment. The study speculates that host preference and nutritional status of the egg (freshly laid eggs contains more nutrients than developed eggs) are the probable cause for the difference in the parasitisation by different species of trichogrammatid wasps.

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