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In-vitro host searching movement behavior of *Trichogramma* species

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Abstract
The present experiment was conducted at the Biocontrol laboratory, SKS CARS, Surgi, Rajnandgaon (C.G.) during the years 2023-24 and 2024-25 to evaluate the host searching movement behavior of *Trichogramma japonicum* in vertical, horizontal, and diagonal directions. Results revealed that both species travelled up to 2.00 meters in all directions, beyond which no parasitization nor adult emergence was observed. In vertical movement, *T. chilonis* exhibited a higher parasitization (78.6%) as compared to *T. japonicum* (72.4%), while *T. japonicum* showed greater adult emergence (128.00%). Similar trends were observed in horizontal and diagonal movements, with *T. chilonis* consistently achieving higher parasitism rates, and *T. japonicum* showing slightly better emergence percentages.

Keywords: Dispersal, emergence, *in vitro*, parasitization, *trichogramma*

Introduction

Trichogramma species makes up one of the most commonly used groups of natural enemies for biological control programs worldwide, as the major success in using *Trichogramma* to control economically important lepidopteran pests. The biology and ecology of these wasps have been intensively studied to identify traits that contribute to successful biological control (Zang *et al.*,2021) [17]. These parasitoids are typically reared in factitious host eggs, the most common of which belong to Lepidoptera such as *Ephestia kuehniella* (Zeller), *Corcyra cephalonica* (Stainton) (Pyralidae), *Sitotroga cerealella* (Olivier) (Gelechiidae), or silkworms, but large-scale multiplication remains costly. Several authors have determined that feeding of adult *Trichogramma* species with honey or sugar in the laboratory influence parasitoids longevity and fecundity (Leatemia *et al.*, 1995, Abd El-Hafez *et al.*, 1999, Gurr and Nicol, 2000, Karimi and Hatami, 2010) [8, 1, 4, 6]. Field fitness of such parasitoids mass reared for biological control is generally evaluated by assessing parasitization rates of pest eggs in the target crop (Kolliker-Ott *et al.*, 2004) [7] and efficient dispersal is a key factor in the host searching leading to high parasitization. Dispersal capacity and host searching ability in the field are important quality attributes in biological control, but are difficult to estimate in the laboratory (Kolliker-Ott *et al.*, 2004) [7].

Dispersal ability of a parasitoid is fundamental for determining effectiveness in control because, based on this information, the number of points for release per unit area can be determined so that distribution is as uniform as possible (Pinto and Parra 2002, Parra 2010, Pereira *et al.*, 2010, Pastori *et al.*, 2013) [13, 10, 11]. The present study aimed to test the dispersal ability and parasitization performance of *Trichogramma japonicum* and *Trichogramma chilonis* in laboratory conditions. We chose to evaluate dispersal ability as a parameter because of its importance to field and storage efficacy, and due to the fact that it is still poorly studied in the laboratory (Honda *et al.*, 1999) [5]. The data obtained in this study may be of use in estimating the number of parasitoids needed to accomplish parasitization in field and storage conditions.

Materials and Methods

Three different movement directions were studied, namely

1. Vertical
2. Horizontal and
3. Diagonal

Under this experiment, testing of vertical, horizontal and diagonal movements of *Trichogramma* spp. were conducted by using transparent poly vinyl chloride (PVC) pipes of 6mm. diameter, cut into different lengths such as 1, 2, 3, 4 and 5 meters. PVC pipes of the above lengths were taken and inserted vertically, horizontally and diagonally into the lid of the plastic container (A) of 100 ml capacity provided with a parasitized trichocard of 100 eggs placed in it and closed with the lid. At the other end of the pipe (with different test lengths), another plastic container (B) was connected with the lid into three different direction *i.e.*, vertical, horizontal and diagonal having a sentinel card with 100 eggs in each direction. The experiment was replicated five times per test length by changing the lengths of the pipe.

Observations were recorded after 48 hours of the emergence of *Trichogramma* from the parasitized cards of container A to see the vertical distance traveled to reach container B.

Results and Discussion

Testing the vertical movement of *Trichogramma* spp.

a) Per cent parasitation

The result from the experiment showed that, *T. japonicum* and *T. chilonis* travelled up to 2.0 meter vertically and per cent parasitization by the two different species of *Trichogramma*, differed significantly. Maximum percentage of parasitization was shown by *T. chilonis* (78.6%) followed by *T. japonicum* (72.4%). At 3.0 m, 4.0 m and 5.0 m, no parasitization were observed.

b) Adult emergence percentage

The results from the experiment showed that, *T. japonicum* and *T. chilonis* travelled up to 2.0 meter vertically and adult emergence percentage by two different species of *Trichogramma*, differed significantly. Maximum emergence percentage was shown by *T. japonicum* (128.00%) followed by *T. chilonis* (106.00%). At 3.0 m, 4.0 m and 5.0 m no emergence was observed.

Table 1: Per cent parasitation of *Trichogramma* spp. in vertical direction

S. No.	Vertical movement (In meters)	Mean percentage of *Corcyra* eggs parasitized	
		T. japonicum	***T. chilonis***
1	1.00	72.4	78.6
		(58.54)	(62.48)
2	2.00	68.4	80.2
		(56.07)	(63.86)
3	3.00	0.00	0.00
		(0.00)	(0.00)
4	4.00	0.00	0.00
		(0.00)	(0.00)
5	5.00	0.00	0.00
		(0.00)	(0.00)
CD at 5%		5.57	3.6
SE(m)±		1.87	1.21

(Figures in parentheses are arc sin transformed values)

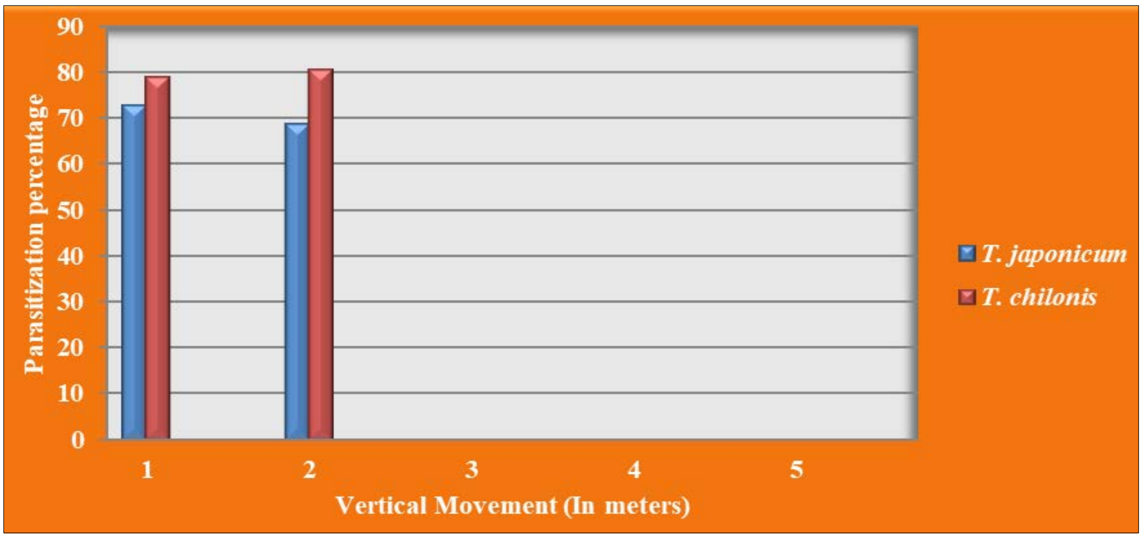

Fig 1: Per cent parasitiation of *Trichogramma* spp. in vertical direction

Table 2: Adult emergence percentage of *Trichogramma* spp. in vertical direction

S. No.	Vertical movement (In meters)	Mean percentage of Adult emergence	
		T. japonicum	***T. chilonis***
1	1.00	128*	106*
		1.28**	1.06**
		(6.46)	(5.91)
2	2.00	114*	101*
		1.14**	1.01**
		6.14	5.78
3	3.00	0.00*	0.00*
		0.00**	0.00**
		(0.00)	(0.00)
4	4.00	0.00*	0.00*
		0.00**	0.00**
		(0.00)	(0.00)
5	5.00	0.00*	0.00*
		0.00**	0.00**
		(0.00)	(0.00)
CD at 5%		0.56	0.133
SE(m)±		0.19	0.04

(Figures in parentheses are arc sin transformed values)
(Values with * mark are the per cent emergence after multiplication with 100)
(Values with ** mark are the per cent emergence without multiplication with 100)

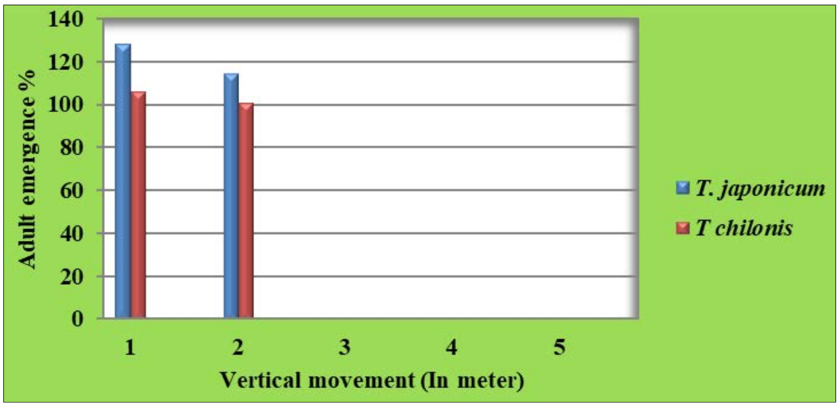

Fig 2: Adult emergence percentage of *Trichogramma* spp. in vertical direction

2. Testing the horizontal movement of *Trichogramma* spp.

a) Per cent parasitation

The result from the experiment showed that, *T. japonicum* and *T. chilonis* travelled up to 2.00 meter horizontally and per cent parasitization by two different species of *Trichogramma*, differed significantly. Maximum percentage of parasitization was shown by *T. chilonis* (75.8%) followed by *T. japonicum* (65.8%). At 3.00 m, 4.00 m and 5.00 m. no parasitiation was observed.

b) Adult emergence percentage

The result from the experiment showed that, *T. japonicum* and *T. chilonis* travelled up to 2.00 meter horizontally and adult emergence percentage by two different species of *Trichogramma*, differed significantly. Maximum emergence percentage was shown by *T. japonicum* (110.00%) followed by *T. chilonis* (108.00%). At 3.0 m, 4.00 m and 5.00 m no emergence was observed.

Table 3: Per cent parasitiation of *Trichogramma* spp. in horizontal direction

S.No.	Horizontal movement (In meters)	Mean percentage of *Corcyra* egg parasitized	
		T. japonicum	***T chilonis***
1	1.00	65.8	75.8
		(54.33)	(61.23)
2	2.00	64.4	78.0
		(53.6)	(62.19)
3	3.00	0.00	0.00
		(0.00)	(0.00)
4	4.00	0.00	0.00
		(0.00)	(0.00)
5	5.00	0.00	0.00
		(0.00)	(0.00)
CD at 5%		5.77	4.49
SE(m)±		1.94	1.51

(Figures in parentheses arc sin transformed values)

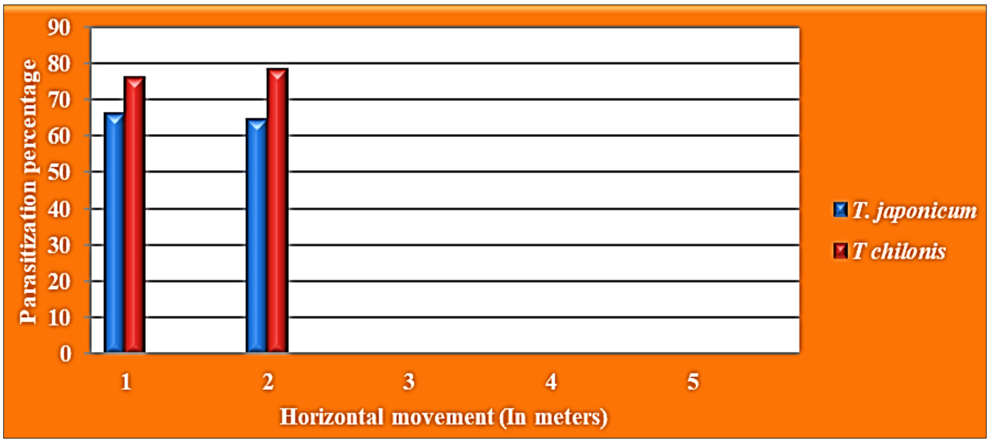

Fig 3: Percent parasitiation of *Trichogramma* spp. in horizontal direction

Table 4: Adult emergence percentage of *Trichogramma* spp. in horizontal direction

S.No.	Horizontal movement (In meters)	Mean percentage of adult emergence	
		T. japonicum	*T. chilonis*
1	1.00	110*	108*
		1.10**	1.08**
		(6.02)	(5.96)
2	2.00	110*	106*
		1.10**	1.06**
		(6.02)	(5.91)
3	3.00	0.00*	0.00*
		0.00**	0.00**
		(0.00)	(0.00)
4	4.00	0.00*	0.00*
		0.00**	0.00**
		(0.00)	(0.00)
5	5.00	0.00*	0.00*
		0.00**	0.00**
		(0.00)	(0.00)
CD at 5 %		0.159	0.147
SE(m)±		0.054	0.05

(Figures in parentheses are arc sin transformed values)
(Values with * mark are the per cent emergence after multiplication with 100)
(Values with ** mark are the per cent emergence without multiplication with 100)

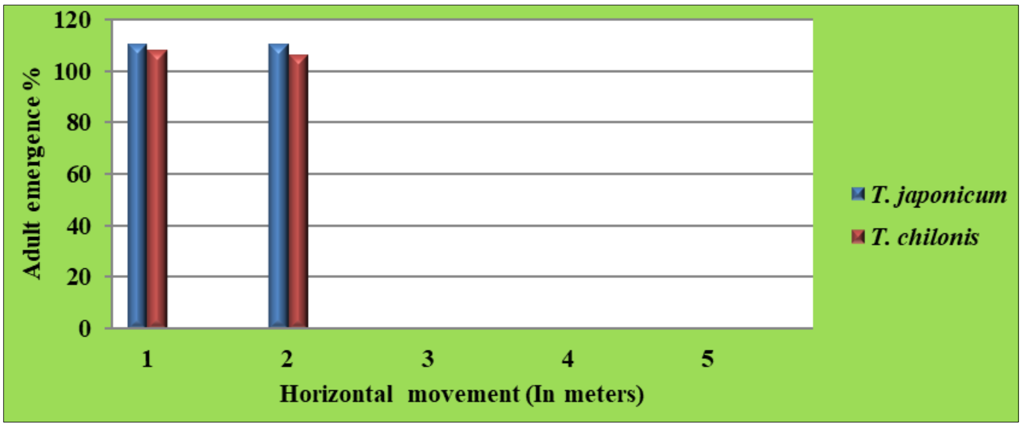

Fig 4: Adult emergence percentage of *Trichogramma* spp. in horizontal direction

3. Testing the diagonal movement of *Trichogramma* spp.

a) Per cent parasitiation

The result from the experiment showed that, *T. japonicum* and *T. chilonis* travelled up to 2.00 meter diagonally and per cent parasitization by two different species of *Trichogramma*, differed significantly. Maximum percentage of parasitization was shown by *T. chilonis* (76.6%) followed by *T. japonicum* (67.00%). At 3.00 m, 4.00 m and 5.00 m, no parasitiation was observed.

b) Per cent adult emergence

The result from the experiment showed that, *T. japonicum* and *T. chilonis* travelled up to 2.00 meter diagonally and adult emergence percentage by two different species of *Trichogramma*, differed significantly. Maximum emergence percentage was shown by *T. japonicum* (111.00%) followed by *T. chilonis* (107.00%). At 3.00m, 4.00 m and 5.00 m, no emergence was observed.

Table 5: Per cent parasitiation of *Trichogramma* spp. in diagonal direction

S. No.	Diagonal movement (In meters)	Mean percentage of *Corcyra* egg parasitized	
		T. japonicum	*T chilonis*
1	1.00	67.00	76.6
		(55.02)	(61.18)
2	2.00	65.4	70.4
		(54.17)	(57.29)
3	3.00	0.00	0.00
		(0.00)	(0.00)
4	4.00	0.00	0.00
		(0.00)	(0.00)
5	5.00	0.00	0.00
		(0.00)	(0.00)
CD at 5%		4.99	4.50
SE(m)±		1.68	1.51

(Figures in parentheses are arc sin transformed values)

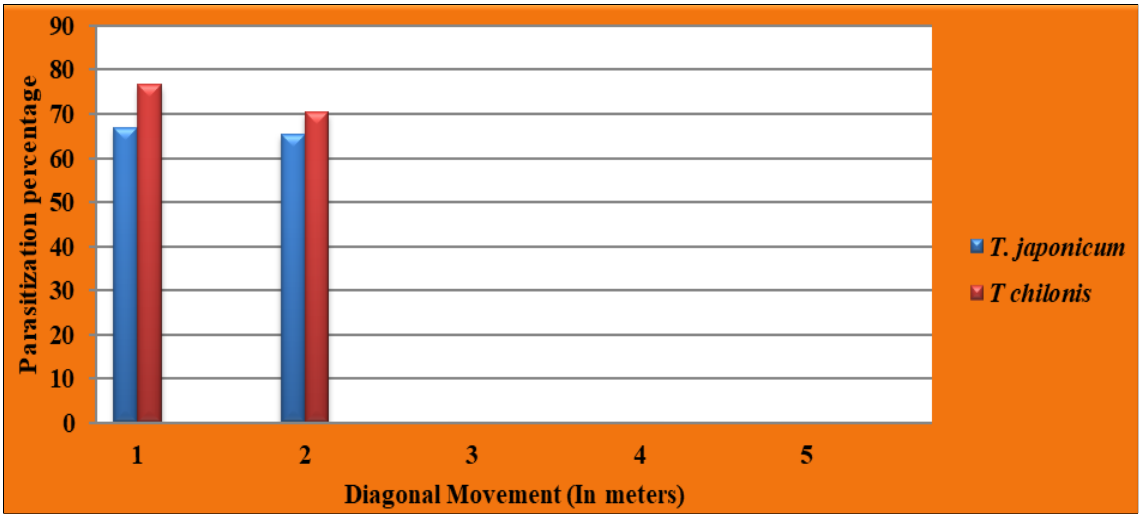

Fig 5: Per cent parasitiation of *Trichogramma* spp. in diagonal direction

Table 6: Adult emergence percentage of *Trichogramma* spp. in diagonal direction

S. No.	Diagonal movement (In meters)	Mean percentage of Adult emergence	
		T. japonicum	*T chilonis*
1	1.00	111*	107*
		1.11**	1.07**
		(6.05)	(5.93)
2	2.00	112*	115*
		1.12**	1.15**
		(6.08)	(6.13)
3	3.00	0.00*	0.00*
		0.00**	0.00**
		(0.00)	(0.00)
4	4.00	0.00*	0.00*
		0.00**	0.00**
		(0.00)	(0.00)
5	5.00	0.00*	0.00*
		0.00**	0.00**
		(0.00)	(0.00)
CD at 5 %		0.17	0.34
SE(m)±		0.06	0.11

(Figures in parentheses are arc sin transformed values)
(Values with * mark are the per cent emergence after multiplication with 100)
(Values with ** mark are the per cent emergence without multiplication with 100)

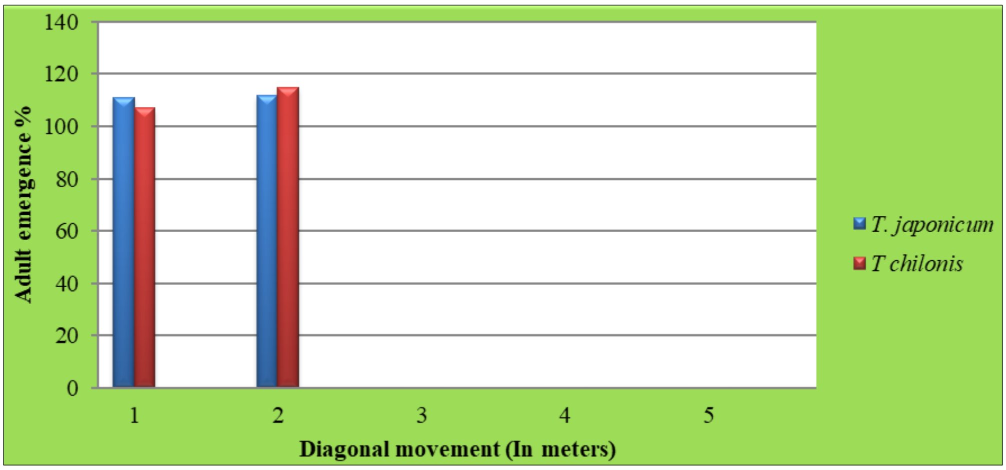

Fig 6: Adult emergence percentage of *Trichogramma* spp. in diagonal direction

The present findings are in total agreement with Mukhearjee (2021) who tested the vertical and horizontal movement of *Trichogramma japonicum*, *T. chilonis* and *T. pretiosum* under laboratory conditions using transparent PVC pipes of 7.00 mm diameter with different lengths *i.e.* 1m, 2m, 3m and 5m, and revealed that all the three species of *Trichogramma* traveled up to 2 meters in both the directions *i.e.* vertically as well as horizontally with the help of the chemical cues released from the egg.

Similar observations were made by Ranjith *et al.* (2018) [14] who assessed the active movement of two Trichogrammatids *viz*., *Trichogramma chilonis* and *T. pretiosum* towards eggs of *Leucinodes orbonalis* and *Corcyra cephalonica* in 2 cm 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*.

The present findings are also in line with Chapman *et al.* (2009) [2] also observed decrease in parasitism over increase in release distance in case of *T. ostriniae* on *Ostrinia nubilalis*. Similar results with the present studies were also reported by Sharma and Aggarwal (2015) [15] stating that the dispersal or host searching capacity of *T. chilonis* and *T. japonicum* was negatively correlated with the distance between host eggs and parasitoid released point and parasitization rate was more near the release point (1-3 m) with maximum parasitism at 1m by both the parasitoid species which is in total agreement with the present results.

The present studies are in alignment with Sharma and Aggrawal (2015) who also stated that highest percentage of parasitism was observed at 1m from parasitoid release point and was significantly better than other distance treatment. The parasitisation showed declining trend with increase in distance from release point. Higher parasitism was recorded at shorter distance *i.e.*, up to 3m and there was no parasitism beyond 9m. Among the egg parasitoid species, *T. chilonis* was recorded with higher parasitism, ranging from 0.00 to 18.10% at different distance treatments in comparison to *T. japonicum*, where parasitisation ranged from 0.00 to 15.49%. However, both the species showed better parasitism at shorter distance which is in complete match with the present findings.

Distance had a clear proportional impact on dispersal probability of the parasitoid was stated by Geetha and Balakrishnan (2011) [3]. They also found that the mean dispersal rate, as indicated by the per cent parasitism, was quite high at 1m (73.78%) which decreased to 7.67 at 30 m is in line with the present results.

Similarly, in cotton significantly higher parasitisation (5.92 to 17.58%) was recorded up to a distance of 1m from the point of release of *T. chilonis* than the increased distances (Wadhwa and Gill, 2006) [16] also supports the present results.

Conclusions

In the present studies, the response of both species of *Trichogramma* tested in three directions (vertical, horizontal, and diagonal), showed maximum parasitization at 2.00 meters, with a clear drop in activity beyond that. *T. chilonis* consistently showed higher parasitization rates than *T. japonicum* across all movement directions. *T. japonicum* had slightly higher emergence rates than *T. chilonis* in vertical and horizontal movement tests, while the emergence rates were very close to each other in the diagonal movement test.

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