

Original Research Article

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Identification of suitable *Trichogramma* sp. and working-out dosages for management of Brinjal Shoot and Fruit Borer under laboratory condition

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ABSTRACT

Keywords

Brinjal, *L. orbonalis*, *Trichogramma*, Parasitism, Dosage.

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The shoot and fruit borer, *Leucinodes orbonalis* has a countrywide distribution and has been categorized as the most destructive and most serious pest causing huge losses in brinjal. In the present investigation, six species of *Trichogramma* were used for selection of most suitable species against shoot and fruit borer, *L. orbonalis*, the per cent parasitism ranged between 3.60 to 93.20 per cent and the highest per cent parasitism was observed in *T. evanescens* (93.20 %), which was *on par* with *T. chilonis* 92.00 per cent. In a dosages experiment, the highest per cent parasitism of 74.1 per cent was recorded in the dosage having 15 adults/sq. m (150000 adults/ha) and lowest parasitism of 44.2 per cent was recorded in dosages of 5 adults/sq. m (50000 adults/ha). Among the two species highest parasitism was recorded in the *T. chilonis* (68.0 %) compared to *T. evanescens* (55.9 %) and 100,000 was found to be optimum dosages.

Introduction

Brinjal is grown throughout the country, all round the year (Choudhary, 1970) and is believed to be cultivated in India for the last 4,000 years. South East Asian countries account for almost 50 per cent of world's area under brinjal cultivation. Brinjal assumes special significance among vegetables in the hot wet season when other vegetables are in short supply and it is the only vegetable that is available at an affordable price for rural and urban poor. So, it is known as a poor man's crop.

Brinjal is attacked by a plethora of insect and mite pests, starting from seedling stage to

senescence. A survey carried out by the Asian Vegetable Research and Development Centre (Anon., 1995) indicated that the brinjal shoot and fruit borer (BSFB), *Leucinodes orbonalis* Guenée, leaf hopper, *Amrasca biguttula biguttula* Ishida and epilachna beetle, *Henosepilachna* (*Epilachna*) *vigintioctopunctata* Fabricius are the most destructive pests on brinjal in Asia. Independently, in the entire South Asian region, the shoot and fruit borer has been identified as the primary limiting factor in brinjal production. Occasionally, brinjal is severely infested by mites, *Tetranychus* sp., aphids, *Aphis gossypii* Glover and whiteflies

including *Bemisia tabaci* Guenée and *Trialeurodes* sp. In Himachal Pradesh, 27 different insect species and one mite species are reported to be associated with brinjal crop (Patil and Mehta, 2008), shoot and fruit borer, *L. orbonalis* (Lepidoptera: Pyralidae) is observed to be the key pest throughout Asia (Purohit and Khatri, 1973; Kuppuswamy and Balasubramanian, 1980; Allam *et al.*, 1982; Regupathy *et al.*, 1997).

The shoot and fruit borer is reported to be parasitized by number of parasitoids (Krishnamoorthy and Mani, 1998), among them, *Trathala flavor-orbitalis* was recorded as one of the important parasitoids causing considerable larval parasitization.

The information on seasonality of natural enemies is scanty, particularly their availability in sprayed and unsprayed conditions. However, no attempts have ever been made to explore, mass multiply and utilize parasitoids under field conditions for extensive control of BSFB in India. No attempt has been made to determine molecular genetic diversity among different populations. Such information based on existence of haplotypes could pinpoint the availability or effectiveness of natural enemies and devise control programmes. The number of polymorphic markers was higher indicating the diversity among the populations (Lynch and Milligan, 1994; Williams *et al.*, 1990).

Materials and Methods

Identification of suitable species of *Trichogramma* spp.

In this experiment, the glass tube having size 70 sq. cm (20 × 3.5 cm) was used for screening of six different species of trichogrammatids against the eggs of BSFB (Table 1). The species selected were based on

their record of parasitizing BSFB eggs under the field conditions in different regions or world used for field evaluation.

Fifty *Leucinodes* eggs laid on black cloth were pasted on 6 × 2 cm paper card with fine layer gum and was placed in the glass tube separately for screening of six different species of trichogrammatids and fine streak of 50 per cent honey was provided as adult food. Parasitoids (@ 1 females/50 eggs of *Leucinodes orbonalis*) were released in each glass tube 70 sq. cm (20 × 3.5 cm) for parasitism by covering the mouth of the tube by double layered black cloth. The parasitized cards were collected and placed in another glass tube after 24 h of parasitism. After 4 or 5 days, the parasitized eggs turned black and black eggs were counted and recorded per cent parasitism using the formula and sex-ratio was recorded separately for all six different species of trichogrammatids. The per cent parasitism was calculated by using the formula:

$$\% \text{ Parasitism} = \frac{\text{Number of black eggs with emergence hole}}{\text{Number of host eggs provided for parasitism}} \times 100$$

From the above experiment, the species which gave the highest per cent parasitism was selected for cage study to workout dosage and finally select most efficient species for field evaluation. The study was conducted in insect rearing cage (1 sq. m) made up nylon mesh with front opening zip. The two selected *Trichogramma* species, *T. chilonis* and *T. evanescens* were tested at three different dosages, viz., @ 50,000, 100,000 and 150,000/ha (Table 3). The eggs of *L. orbonalis* were used and parasitoids in the three dosages were released at the rate 5, 10 and 15 adults for cage of 1 sq. m size. The eggs offered for parasitism was at the rate of 250 eggs/treatment and the experiment was replicated 10 times.

To check the parasitism at different treatment dosages, the *Leucinodes* eggs were counted and pasted on the 6 × 2 cm paper card with fine layer gum and was placed inside in the top of the cage and fine streak of 10 per cent honey was provided as adult food. Parasitoids (@ 5, 10 and 15 adults/250 eggs of *Leucinodes*) were released in cage having size of 1 sq. m. The parasitized cards were collected and placed in glass tube after 24 h of exposure in the cage.

After 4 or 5 days the parasitized eggs that turned black were counted and recorded for per cent parasitism was recorded using the formula as described above and sex-ratio was recorded separately for two different species of *Trichogramma*. From this experiment, most promising species was selected that gave highest parasitism and dosages was also decided. The selected species was used for field trial at most effective dosage. The data is analyzed by using the two-way ANOVA for interpretation of final selection of species and dosage based on CD values.

Results and Discussion

Screening of various trichogrammatids against brinjal shoot and fruit borer, *L. orbonalis* in test tube

The six species of trichogrammatids like *Tr. bactrae*, *Tr. armigera*, *T. achaeae*, *T. japonicum*, *T. chilonis* and *T. evanescens* were used for screening of *Leucinodes* eggs for selecting the most suitable species for further experiments (Table 1).

Per cent hatching

The per cent hatching ranged between 5.2 to 93.6 per cent and the highest per cent hatching was observed in *T. japonicum* (93.6 %), followed by *Tr. bactrae* (74.6 %) and *Tr. armigera* (46.4 %). The least hatching was observed in *T. chilonis* released vials (5.20

%), indicating its suitability for the host (CD = 3.49; $P = 0.05$) (Fig. 1; Table 2).

Per cent parasitisation

The screening of different trichogrammatid species revealed that the per cent parasitisation ranged between 3.60 to 93.20 per cent and the highest was observed in *T. evanescens* i.e., 93.20 per cent, which was on par with *T. chilonis* i.e., 92.00 per cent, followed by *T. achaeae*, *Tr. armigera*, *Tr. bactrae*, whereas *T. japonicum* gave lowest per cent of parasitisation of 3.60 per cent (CD = 3.19, $P = 0.05$) (Fig. 1; Table 2).

Per cent adult emergence

The exposure of *L. orbonalis* eggs for trichogrammatid species the adult emergence ranged from 7.60 to 95.60 per cent and the highest per cent adult emergence was observed in *T. chilonis* i.e., 95.60 per cent, which was on par with *T. evanescens* i.e., 94.40 per cent, followed by *T. achaeae*, *Tr. armigera*, *Tr. bactrae* and least in case of *T. japonicum* i.e., 7.60 per cent (CD = 4.63; $P = 0.05$) (Fig. 1; Table 2).

Per cent female emergence

The per cent female emergence ranged from 2.80 to 65.20 per cent, the highest emergence was recorded for *T. chilonis* i.e., 65.2 per cent, which was on par with *T. evanescens* i.e., 62.4 per cent, followed by *T. achaeae*, *Tr. armigera*, *Tr. bactrae* and the least was by *T. japonicum* i.e., 2.80 per cent (CD = 0.353; $P = 0.05$) (Fig. 1; Table 2).

Screening of trichogrammatids at different dosages against brinjal shoot and fruit borer, *L. orbonalis* eggs under caged condition

From the above screening experiments, two species of trichogrammatids, viz., *T. chilonis*

and *T. evanescens* were selected for determination of their optimum dosages for field evaluation.

Per cent parasitisation

The two factor ANOVA analyses revealed that there is a significant difference between the dosages used and the species selected with respect to per cent parasitisation. The highest per cent parasitism of 74.1 per cent was recorded in the dosage having 15 adults/sq. m (150000 adults/ha) and lowest was recorded in dosage having 5 adults/sq. m (50000 adults/ha) with parasitisation being 44.2 per cent. But the per cent mean parasitisation among the two species was highest in *T. chilonis* (62.50 %) as compared to *T. evanescens* (57.70 %) and it differed significantly (CD = 4.81; P = 0.01) (Table 3).

Per cent adult emergence

The results revealed that there was a significant difference between the dosages used and the species selected with respect to per cent adult emergence.

The highest per cent adult emergence of 72.85 per cent was recorded in the dosage having 15 adults/sq.m (150000 adults/ha) and lowest was observed, in case of dosage having 5 adults/sq.m i.e., 43.10 per cent (50000 adults/ha).

The per cent adult emergence among the two species was highest in *T. chilonis* (61.4 %) as compared to *T. evanescens* which showed 56.6 per cent (CD = 4.81; P = 0.01) (Table 3). In general highest emergence was recorded at the highest dosage.

Table.1 Different species of trichogrammatid cultures maintained under laboratory condition and used for initial screening test

Sl. No.	Species	Taxonomic position	
		Order	Family
1	<i>Trichogrammatoidea bactrae</i> Nagaraja	Hymenoptera	Trichogrammatidae
2	<i>Trichogrammatoidea armigera</i> Nagaraja	Hymenoptera	Trichogrammatidae
3	<i>Trichogramma achaeae</i> Nagaraja and Nagarkatti	Hymenoptera	Trichogrammatidae
4	<i>Trichogramma japonicum</i> Ashmead	Hymenoptera	Trichogrammatidae
5	<i>Trichogramma chilonis</i> Ishii	Hymenoptera	Trichogrammatidae
6	<i>Trichogramma evanescens</i> Westwood	Hymenoptera	Trichogrammatidae

Table.2 Screening of various trichogrammatids against eggs of *L. orbonalis* under laboratory condition

Species	% Hatching of <i>L. orbonalis</i> eggs*	% Parasitisation of <i>L. orbonalis</i> eggs*	% Adult emergence of Trichogrammatids*	% Females emergence of Trichogrammatids*
<i>Tr. bactrae</i>	76.40 (61.02) ^b	20.80 (27.04) ^d	18.20 (25.22) ^d	9.20 (18.36) ^d
<i>Tr. armigera</i>	49.60 (44.76) ^c	47.60 (43.62) ^c	44.40 (41.78) ^c	30.80 (33.66) ^c
<i>T. achaeae</i>	23.80 (29.12) ^d	74.40 (59.66) ^b	67.00 (54.94) ^b	48.00 (43.85) ^b
<i>T. japonicum</i>	93.60 (75.44) ^a	3.60 (10.70) ^e	7.60 (15.96) ^e	2.80 (9.49) ^e
<i>T. chilonis</i>	5.20 (12.89) ^e	92.00 (73.61) ^a	95.60 (79.60) ^a	65.20 (53.93) ^a
<i>T. evanescens</i>	5.40 (13.32) ^e	93.20 (75.04) ^a	94.40 (76.78) ^a	62.40 (52.21) ^a
S. Em±	1.19	1.09	1.58	1.20
CV (%)	6.79	5.07	7.23	7.67
CD @ 1 %	4.73	4.33	6.27	4.78

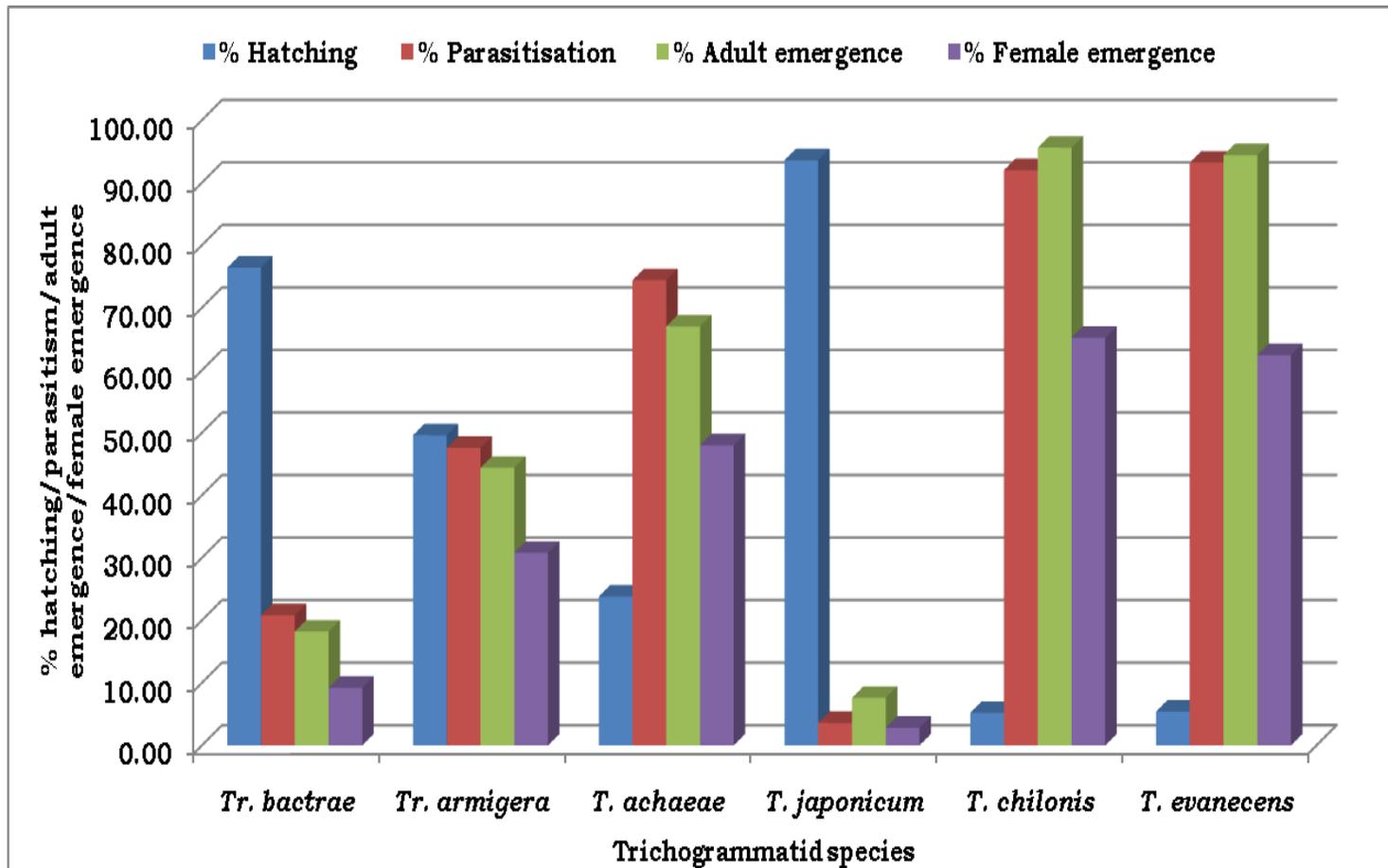
Note: *Mean of five replications; Figures within the parentheses are arc sine transformations; in a column means followed by same letter(s) are not significantly different as per DMRT; * Significant at 1 %; NS= Non-Significant; BSFB eggs were provided @ 50 eggs/female parasitoid.

Table.3 Screening of trichogrammatids at different dosages on *L. orbonalis* eggs under laboratory cage conditions (1 sq. m)

Dosage/ha	Per cent parasitism*		Mean factor	Per cent adult emergence*		Mean factor	Per cent desiccation*		Mean factor	Per cent female emergence*		Mean factor
	<i>T. chilonis</i>	<i>T. evanescens</i>	B factor	<i>T. chilonis</i>	<i>T. evanescens</i>	B factor	<i>T. chilonis</i>	<i>T. evanescens</i>	B factor	<i>T. chilonis</i>	<i>T. evanescens</i>	B factor
50000	43.80 (41.20)	44.60 (41.90)	44.20 (41.60)	42.70 (40.60)	43.60 (41.30)	43.10 (40.90)	1.08 (5.81)	0.96 (5.23)	1.02 (5.52)	21.60 (27.39)	22.60 (28.24)	22.10 (27.82)
100000	68.00 (55.90)	55.90 (48.60)	61.90 (52.30)	67.10 (55.40)	54.90 (48.00)	61.00 (51.70)	1.12 (5.64)	1.00 (5.36)	1.06 (5.50)	34.00 (35.56)	28.40 (31.98)	31.20 (33.77)
150000	75.60 (60.50)	72.60 (58.80)	74.10 (59.70)	74.40 (59.70)	71.30 (58.00)	72.85 (58.90)	0.80 (4.48)	1.24 (5.85)	1.02 (5.16)	37.30 (37.45)	36.20 (36.94)	36.75 (37.20)
Mean A factor	62.50 (52.60)	57.70 (49.80)		61.40 (51.90)	56.60 (49.10)		1.00 (5.31)	1.07 (5.48)		30.97 (33.97)	29.07 (32.39)	
	A factor	B factor	AXB	A factor	B factor	AXB	A factor	B factor	AXB	A factor	B factor	AXB
S. Em±	-	1.80	-	-	1.70	-	-	-	-	-	0.95	-
CD @ 1 %	-	5.46	-	-	5.30	-	-	-	-	-	3.96	-
F- test	NS	*	NS	NS	*	NS	NS	NS	NS	NS	*	NS

Note: *Mean of ten replications; Figures within the parentheses are arc sine transformations; * Significant at 1 %; NS= Non-Significant; BSFB eggs were provided @ 250 eggs/5, 10, 15 female parasitoids.

Fig.1 Screening of various trichogrammatids against eggs of Brinjal shoot and fruit borer, *L. orbonalis* under laboratory condition



Per cent desiccation

The results revealed that there were non-significant differences between the dosages evaluated and the species selected with respect to per cent desiccation, by two factor ANOVA analyses (Table 3).

Per cent female emergence

The results revealed that there was a significant difference between the dosages used and two species with respect to per cent female emergence by two factor ANOVA analysis. The highest per cent female emergence recorded was 36.75 per cent in the dosage having 15 adults/sq.m (150000 adults/ha) and lowest per cent female emergence of 22.10 per cent was recorded when dosage was 5 adults/sq.m (50000 adults/ha) (Table 3). Nevertheless, the per cent female emergence was highest in case of *T. chilonis* as compared to *T. evanescens*.

Screening of various trichogrammatids against brinjal shoot and fruit borer, *L. orbonalis* in test tube condition

The selection of most promising species or strains of *Trichogramma* has been advocated as first step in achieving successful control program (Hassan, 1989). In order to select most promising species for evaluation against brinjal shoot and fruit borer, *L. orbonalis*, six species of trichogrammatids like *Trichogrammatoidea bactrae*, *Tr. armigera*, *Trichogramma achaeae*, *T. japonicum*, *T. chilonis* and *T. evanescens* were used in the present study.

In the present study, the per cent parasitism was ranged between 3.60 to 93.20 per cent, at least two species, *T. evanescens* and *T. chilonis* parasitized significantly higher number of *L. orbonalis* eggs (> 92.0) compared to other species. The per cent

parasitism recorded for *T. evanescens* was 25.3 to 2488 times and for *T. chilonis*, per cent parasitism was 23.5 to 2455 times higher compared to other four species tested, which indicated usefulness of these two species. In an earlier studies in the Philippines, Alpuerto (1984) reported that The results were showing more parasitization when compared with the study reported by Alpuerto (1984) from Philippines that parasitism by *T. chilonis* ranged from 34.6 to 51.3 per cent on *L. orbonalis* eggs and Nafus and Schreiner (1986) were of the opinion that *T. chilonis* is the most important natural enemy attacking the pyralid pests. *Trichogramma* viability is a feature closely related to the parasitoid host resemblance (Bezerra and Parra, 2004). In *Trichogramma* production, quality control considers practicality as acceptable when the rate of borne adults exceeds 85 per cent (Navarro, 1998). Thus, the species used in the present study expressed low or high affinity to the host eggs based on parasitism obtained, which is result of host acceptance. Gonçalves *et al.*, (2003), while assessing the quality of *T. pretiosum* reared in *Sitotroga cerealella* eggs, observed viability levels above 89 per cent, which represents host acceptance and species affinity.

The per cent parasitism in the present study was similar in the two *Trichogramma* species, with values above 92.0 per cent. Considering that the quality control on *Trichogramma* production requires parasitism of more than 90 per cent (Navarro, 1998), the two species meet the criterion. It is attributed that host adaption plays significant role in acceptance of host eggs, thereby selection of promising species (Pereira *et al.*, 2004), however, in the present study *L. orbonalis* eggs were offered to all species without any adaptation, thus in the present experiment on selection resulted in identifying two species, *viz.*, *T. evanescens* and *T. chilonis* as most apt for further evaluation against *L. orbonalis*.

Screening of trichogrammatids at different dosages against brinjal shoot and fruit borer, *L. orbonalis* eggs under caged condition

From screening experiment, two species of trichogrammatids, viz., *T. chilonis* and *T. evanescens* were selected, based on their aptness for parasitizing *L. orbonalis* eggs, to work out dosages in a 1 sq. m cage and three dosages tried were @ 50000, 100000 and 150000 parasitoids per hectare against *L. orbonalis* for field evaluation.

The results revealed that there is a significant difference between the dosages used and the species selected with respect to per cent parasitism. The highest per cent parasitism, i.e., 74.1 per cent was recorded in the dosage @ 150000 adults/ha) and lowest of 44.2 percent was recorded, where dosage @ 50000 adults/ha was used. Between the two species the per cent parasitism was higher in *T. chilonis* compared to *T. evanescens*, i.e., 68.0 and 75.6 per cent, and 55.9 and 72.6 per cent in dosages @ 100000 and 150000/ha, respectively. In earlier field studies, Sangappa (1999) opined that release of *T. chilonis* @ 2.0 and 1.5 lakh per ha starting from 30 days after transplanting at an interval of 20 days were found effective in reducing the *L. orbonalis* incidence at shoot and fruit bearing stage of the crop and recorded 152.05 q per ha and 144.5 q per ha healthy fruit yield, respectively, while in other studies, the dosages of *T. chilonis* @ 5,00,000 adults per ha was reported effective in suppressing the pest and also recorded higher increase in yield (57 % over control) (Anon., 2001).

The present studies resulted in not only identification of most suitable species, i.e., *T. chilonis*, but also an effective dosages @ 100000/ha for obtaining higher suppression of the pest on brinjal.

In conclusion, six species of *Trichogramma* were used for selection of most suitable species, the per cent parasitism ranged between 3.60 to 93.20 per cent and the highest per cent parasitism was observed in *T. evanescens* (93.20 %), which was *on par* with *T. chilonis* 92.00 per cent. In a dosages experiment, the highest per cent parasitism of 74.1 per cent was recorded in the dosage having 15 adults/sq. m (150000 adults/ha) and lowest parasitism of 44.2 per cent was recorded in dosages of 5 adults/sq. m (50000 adults/ha). Among the two species highest parasitism was recorded in the *T. chilonis* (68.0 %) compared to *T. evanescens* (55.9 %) and 100,000 was found to be optimum dosages.

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