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Comparative study on the efficacy of nine *Trichogramma* species (Hymenoptera: Trichogrammatidae) on the eggs of *Leucinodes orbonalis* (Lepidoptera: Crambidae)

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Abstract
The parasitism efficiency through sexual and parthenogenesis reproduction and emergence rate of nine different egg parasitoids belong to the Genus *Trichogramma viz*., *T. pretiosum*, *T. embryophagum*, *T. achaeae*, *T. japonicum*, *T. brassicae*, *T. mwanzai*, *T. chilonis*, *T. dendrolimi* and *T. evanescens* on the eggs of Brinjal Shoot and Fruit Borer, *Leucinodes orbonalis* was studied in laboratory condition during three different seasons, *Kharif*, 2013 (August to September 2013), *Rabi*, 2013 (November to December 2013) and summer, 2014 (March to April 2014). The results revealed that the parasitoids, *T. pretiosum* and *T. embryophagum* had the highest parasitism efficacy against one-day old eggs of *L. orbonalis* during *Rabi*, 2013 (27°C ± 5°C) with 92.01 and 90.02 per cent respectively. The study evidenced the superiority of *T.embryophagum* with maximum emergence rate (90.22 per cent) and greatest ability to parasitize an average of 11.21 eggs of *L. orbonalis* through parthenogenesis reproduction.

Keywords: emergence rate, *Leucinodes orbonalis,* parasitism efficacy, parthenogenesis, *Trichogramma* spp.

1. Introduction
The Eggplant Shoot and Fruit Borer, *Leucinodes orbonalis* Guenee is the most serious and destructive pest of *Solanum melongena* (common name: brinjal) with a yield reduction of 60 – 80 % [1, 2] and distributing throughout South Asian countries including India and Sri Lanka. As its larvae inhabits inside the plant shoots or fruits by forming tunnels, the management of this pest becomes so difficult, on the other hand adversely affecting the marketability of its fruit yield [3]. It is fairly possible when the pesticides are sprayed before the neonate larvae bore inside the shoots or fruits of eggplant. However, as the neonate larvae enter the fruits or shoots of brinjal within a few hours, the control is only possible by the frequent application of toxic insecticides [3]. Therefore, presently farmers rely exclusively on the application of pesticides to control *L. orbonalis*, produce blemish-free eggplant fruits and get maximum yield. The indiscriminate use of insecticides against *L. orbonalis* in India increased the level of contamination in eggplant and exceeded the Maximum Residue Level (MRL) [4].
Apart from this the indiscriminate uses of toxic, broad-spectrum pesticides are killing the natural enemies of *L. orbonalis*, which were giving satisfactory control of the pest before the use of insecticides became widespread [5]. Parasitoids are susceptible, when they come in direct contact with these products. In such circumstances blanket application of pesticides without understanding the behaviour of the parasitoid may adversely affect the beneficial capacity of the parasitoid. Thus the trend now moves toward the development of Bio-intensive Integrated Pest Management (BIPM) and as the present study on the efficacy of different species of egg parasitoid *Trichogramma* spp. on the egg of *L. orbonalis* was one of the components of it, will help to formulate an effective BIPM against *L. orbonalis*.

2. Materials and Methods
2.1 Parasitism and emergence rate of *Trichogramma spp.*
Forty-five pieces of cotton cloth each containing thirty eggs from an each uniformed one-day, two-day and three-day old eggs of *L. orbonalis* were obtained from laboratory culture. These egg containing cotton clothes were separately kept in test tubes of 2 cm diameter and tightly covered by pieces of cotton cloths. Twenty-five numbers of adult insects from each different

Trichogramma spp. namely; *T. pretiosum*, *T. embryophagum*, *T. achaeae*, *T. japonicum*, *T. brassicae*, *T. mwanzai*, *T. chilonis*, *T. dendrolimi* and *T. evanescens* were separately collected from the laboratory cultures by aspirator and introduced each species into five test tubes as five in each where each test tube consisted each of one, two and three-day old eggs of *L. orbonalis*. Untreated controls were also set by keeping different aged groups of eggs of *L. orbonalis* without introduction of egg parasitoids. Each treatment was replicated as five and arranged in Completely Randomized Design (CRD) and conducted during three different seasons *viz*., *Kharif*, 2013 (30.6±1.3°C) *Rabi*, 2013 (29.7±0.8°C) and summer, 2014 (36.2±1.3°C) at the Bio-control laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, India.

The parasitized eggs of *L. orbonalis* by all different *Trichogramma* spp., which were turned to black colour, were counted and transferred to another cleaned test tube and rigidly covered. The emergence rate was worked out by counting the number of adult *Trichogramma* spp. emerged out of parasitized eggs of *L. orbonalis*.

2.2 Parasitism ability of *Trichogramma* spp. by parthenogenesis reproduction

A single parasitized egg from each of parasitized one, two and three-day old eggs of *L. orbonalis* by different *Trichogramma* spp. was cautiously cut with the small piece of cloth and kept in cleaned test tubes separately and covered by cloth rigidly. After the emergence of parasitoid, a small piece of cloth with 35 one-day old eggs of *L. orbonalis* was provided to each test tube containing a single female parasitoid of different *Trichogramma* spp. to find the parasitism ability by parthenogenesis reproduction, which emerged from parasitized different age groups of *L. orbonalis* eggs. An untreated control was arranged by keeping a piece of cloth consisting 35 one-day old eggs of *L. orbonalis* in a test tube without introduction of any *Trichogramma* spp. Each treatment was replicated five times and arranged in CRD and repeated in three different seasons as mentioned above.

2.3 Statistical analysis

The data obtained through the study were analyzed by two-way ANOVA. Multiple comparisons were done by Duncan's Multiple Range Test (DMRT). All the comparisons were considered significant when $p < 0.05$.

3. Results and Discussion

3.1 Parasitism efficacy and emergence rate of *Trichogramma* spp.

The statistical analysis (two-way ANOVA and three-way with replications, Tables 1 and 2) showed that there was significant difference among different species of *Trichogramma* in selecting eggs of *L. orbonalis* for parasitization under laboratory conditions. It was observed that *T. pretiosum* and *T. embryophagum* were superior and showed maximum parasitism and emergence rate against the eggs of *L. orbonalis*. The egg parasitoids, *T. acheae*, *T. japonicum*, *T. brassicae* and *T. mwanzai* expressed also remarkable efficacy against the eggs of *L. orbonalis*. The study also proved the inefficacy of *T. chilonis*, *T. dendrolimi* and *T. evanescens* against the eggs of *L. orbonalis*.

Table 1: Parasitism efficiency of different *Trichogramma* spp. against *L. orbonalis* during three different seasons

***Trichogramma* spp.**	**Age of eggs of *L. orbonalis/days***	**Parasitism Efficacy***		
		***Kharif* 2014**	***Rabi* 2014**	**Summer 2015**
T. pretiosum	One	86.67^{a}	92.22^{a}	72.22^{a}
	Two	64.44^{b}	72.22^{b}	48.89^{b}
	Three	25.56^{de}	32.22^{def}	15.56^{e}
T. embryophagum	One	80.00^{a}	90.00^{a}	68.89^{a}
	Two	60.00^{b}	70.00^{b}	44.44^{bc}
	Three	22.22^{e}	34.44^{d}	22.22^{de}
T. achaeae	One	68.89^{b}	78.89^{b}	32.22^{cd}
	Two	32.22^{d}	56.67^{c}	14.44^{ef}
	Three	15.56^{g}	23.33^{e}	3.33^{hi}
T. japonicum	One	48.89^{c}	57.78^{c}	23.33^{de}
	Two	18.89^{e}	33.33^{de}	14.44^{efg}
	Three	6.67^{h}	12.22^{g}	3.33^{hi}
T. brassicae	One	32.22^{d}	41.11^{d}	17.78^{e}
	Two	21.11^{e}	37.78^{d}	8.89^{fgh}
	Three	5.56^{h}	14.44^{fg}	4.44^{h}
T. mwanzai	One	11.11^{gh}	13.33^{g}	7.78^{gh}
	Two	8.89^{h}	7.78^{g}	0.00^{i}
	Three	0.00^{i}	2.22^{i}	0.00^{i}
T. chilonis	One	0.00^{i}	0.00^{i}	0.00^{i}
	Two	0.00^{i}	0.00^{i}	0.00^{i}
	Three	0.00^{i}	0.00^{i}	0.00^{i}
T. dendrolimi	One	0.00^{i}	0.00^{i}	0.00^{i}
	Two	0.00^{i}	0.00^{i}	0.00^{i}
	Three	0.00^{i}	0.00^{i}	0.00^{i}
T. evanescens	One	0.00^{i}	0.00^{i}	0.00^{i}
	Two	0.00^{i}	0.00^{i}	0.00^{i}
	Three	0.00^{i}	0.00^{i}	0.00^{i}
Control	One	0.00^{i}	0.00^{i}	0.00^{i}
	Two	0.00^{i}	0.00^{i}	0.00^{i}
	Three	0.00^{i}	0.00^{i}	0.00^{i}
CV (%) CD (0.05)		19.59	16.07	31.19

Trichogramma spp.	3.56	4.00	
Age of eggs of *L. orbonalis*	1.95	2.19	
Interaction	6.16	6.93	

*Values are mean of five replications.
Values are transformed to arcsine transformation. In each column, means with similar alphabets do not vary significantly at P=0.05 by DMRT.

Table 2: Emergence rate of different *Trichogramma* spp. from parasitized eggs of *L. orbonalis* during three different seasons

***Trichogramma* spp.**	**Age of eggs of *L. orbonalis***	**Emergence Rate (Per cent)***		
		***Rabi* 2014**	***Rabi* 2014**	***Rabi* 2014**
T. pretiosum	One	82.11^{a}	87.89^{a}	39.65^{a}
	Two	80.59^{a}	83.04^{a}	30.92^{a}
	Three	72.70^{a}	82.70^{a}	11.11^{b}
T. embryophagum	One	81.83^{a}	90.07^{a}	36.21^{a}
	Two	76.14^{a}	77.81^{ab}	25.04^{a}
	Three	67.41^{a}	68.93^{a}	12.96^{b}
T. achaeae	One	72.11^{ab}	87.38^{a}	27.31^{b}
	Two	62.50^{ab}	76.25^{ab}	12.22^{b}
	Three	46.83^{c}	63.43^{ab}	0.00^{d}
T. japonicum	One	55.95^{c}	62.55^{ab}	19.76^{b}
	Two	45.93^{c}	63.53^{ab}	9.52^{d}
	Three	27.78^{c}	37.78^{c}	0.00^{d}
T. brassicae	One	65.74^{a}	71.83^{b}	21.43^{b}
	Two	56.39^{bc}	68.95^{ab}	6.67^{d}
	Three	33.33^{bcd}	60.71^{ab}	0.00^{d}
T. mwanzai	One	36.67^{bcd}	55.56^{c}	8.33^{d}
	Two	30.00^{d}	33.33^{c}	0.00^{d}
	Three	0.00^{e}	33.33^{c}	0.00^{d}
T. pretiosum	One	82.11^{a}	87.89^{a}	39.65^{a}
	Two	80.59^{a}	83.04^{a}	30.92^{a}
	Three	72.70^{a}	82.70^{a}	11.11^{b}
T. embryophagum	One	81.83^{a}	90.07^{a}	36.21^{a}
	Two	76.14^{a}	77.81^{ab}	25.04^{a}
	Three	67.41^{a}	68.93^{a}	12.96^{b}
T. achaeae	One	72.11^{ab}	87.38^{a}	27.31^{b}
	Two	62.50^{ab}	76.25^{ab}	12.22^{b}
	Three	46.83^{c}	63.43^{ab}	0.00^{d}
Control	One	0.00^{e}	0.00^{d}	0.00^{d}
	Two	0.00^{e}	0.00^{d}	0.00^{d}
	Three	0.00^{e}	0.00^{d}	0.00^{d}
CV (%)		29.30	35.27	64.52
CD(0.05)				
Trichogramma spp.		11.58	15.00	9.04
Age of eggs of *L. orbonalis*		7.58	9.83	5.92
Interaction		20.07	25.99	15.66

*Values are mean of five replications.
Values are transformed to arcsine transformation. In each column, means with similar alphabets do not vary significantly at P=0.05 by DMRT

In contrast to the present study, an author stated that *T. chilonis* proved its efficacy against *L. orbonalis* [6]. Further it was observed from a study that although a combination of parasitoids performed well in parasitizing 54 – 64% of eggs of *Acherontia styx* in brinjal in Andhra Pradesh, India, 80% of this was achieved by *T. chilonis* [7].

Contradictory to the present study few authors identified that the inundative release of *T. chilonis* at the rate of 2.5 to 10 lakh adults per ha reduced the fruit borer damage on field level at different areas in India [7,8]. Further few findings revealed the reduction in the infestation of *L. orbonalis* was achieved when the *T. chilonis* was applied along with endosulfan @ 350 g ai/ha [9] and Nimbecidine® and NSKE [10].

Apart from this, the efficacy of *T. japonicum* against *L. orbonalis* [11] and few *Trichogramma* species *viz*., *T. chilonis, T. achaeae, T. pretiosum* and *T. brasiliensis* against the chilli fruit borer (*Heliothis armigera*) [12] were also revealed by certain authors.

3.2 Influence of aging of host's eggs on the parasitism efficacy

It was revealed from the study that almost all efficient egg parasitoids of *L. orbonalis* preferred one-day old eggs of *L. orbonalis* than two and three-day old ones. The parasitization rate of efficient egg parasitoids of *L. orbonalis* was reduced with the senescence of eggs of *L. orbonali.* It was also noted form a study that it may lead to prevent the competition by already developed embryo of host to the developing embryo of parasitoid [13]. The finding clearly stated that monitoring the prevalence of *L. orbonalis* adult moth at field level and knowledge on the biology of *L. orbonalis* are essential before going for a field release of egg parasitoids. If the egg parasitoids are released on incorrect time that will lead to low or even no parasitization and worsening the expenses.

The foraging behavior of egg parasitoids was studied by several workers and stated that the egg parasitoids; *Telenomus remus* [14] and *T. cacoeciae* [13] were also preferred freshly laid one or two-day old eggs of its host for parasitization. Though the *Trichogramma* parasitoids reject the host eggs older than 5 days for parasitism, the venom injected at the time of the stinging ultimately prevents the development of host embryo [15].

It was also pointed out that proper timing and number of releases of *T. evanescens* significantly reduced the infestation of bollworm in cottons [16].

3.3 Parthenogenesis reproduction

The parasitism by parthenogenesis was exhibited by all effective parasitoids studied during this period however; *T. embryophagum* had the highest capacity to parasitize parthenogenetically followed by *T. pretiosum* (Table 3). The ability of parasitism by parthenogenesis may be advantageous in the process of field release of egg parasitoids, because even if the female egg parasitoids failed in finding the male insect, at certain levels the parasitization may occur under field conditions.

A study stated that the parthenogenesis reproduction is the common phenomenon observed in arthropods including *Trichogramma* wasps induced by the widespread bacterium *Wolbachia* [17].

Table 3: Parthenogenetically parasitized eggs of *L. orbonalis* by a single insect of *Trichogramma* spp. at three different seasons

***Trichogramma* spp.**	**Age of eggs of *L. orbonalis* in days**	**Parthenogenetically parasitized eggs of *L. orbonalis* (Nos.)***		
		***Kharif* 2014**	***Rabi* 2014**	**Summer 2015**
T. pretiosum	One	9.00^{b}	9.33^{b}	4.00^{ab}
	Two	9.33^{b}	8.67^{b}	3.33^{ab}
	Three	8.67^{b}	8.33^{b}	4.00^{ab}
T. embryophagum	One	10.67^{a}	11.67^{a}	5.00^{a}
	Two	10.33^{a}	11.67^{a}	4.67^{a}
	Three	10.33^{a}	11.33^{a}	4.67^{a}
T. achaeae	One	7.33^{c}	8.67^{b}	3.33^{bc}
	Two	7.33^{c}	8.33^{b}	3.00^{bc}
	Three	7.00^{c}	8.33^{b}	2.67^{bc}
T. japonicum	One	7.33^{c}	8.33^{b}	3.33^{c}
	Two	6.67^{c}	8.00^{b}	2.33^{c}
	Three	7.33^{c}	7.67^{bc}	2.67^{c}
T. brassicae	One	6.33^{d}	7.33^{c}	3.67^{bc}
	Two	6.00^{d}	7.00^{c}	3.33^{bc}
	Three	6.33^{d}	7.33^{c}	2.67^{bc}
T. mwanzai	One	4.67^{e}	5.33^{d}	3.67^{cd}
	Two	4.33^{e}	5.00^{d}	1.67^{d}
	Three	4.33^{e}	5.33^{d}	2.00^{d}
Control	One	0.00^{f}	0.00^{e}	0.00^{e}
	Two	0.00^{f}	0.00^{e}	0.00^{e}
	Three	0.00^{f}	0.00^{e}	0.00^{e}
CV (%)		6.27	4.74	14.14
CD (0.05)				
Trichogramma spp.		0.15	0.17	0.28
Age of eggs of *L. orbonalis*		0.10	0.11	0.18
Interaction		0.26	0.29	0.48

*Values are mean of five replications.

Values are transferred to square root (√(X+0.5)) transformation. In each column, means with similar alphabets do not vary significantly at P=0.05 by DMRT.

3.4 Influence of climatic seasons on the reproduction and emergence rate of parasitoids

The climatic seasons prevailed in the study area highly influenced the biology of egg parasitoids. The parasitism by sexual and parthenogenesis reproduction and emergence rate were extremely reduced in summer season (36.2±1.3°C). The statistical analysis (two-way ANOVA, Table 4) evidenced that the season *Rabi*, 2013 (29.7±0.8°C) enhanced the biology of efficient egg parasitoids of *L. orbonalis* by increasing parasitism and emergence rate followed by the season *Kharif*, 2013 (30.6±1.3°C).

Table 4: Influence of climatic seasons on the reproduction and emergence rate of *Trichogramma* spp.

***Trichogramma* spp.**	**Seasons**	**Parasitism by sexual reproduction (per cent)**	**Emergence rate (Per cent)**	**Parasitism by parthenogenesis (Nos.)**
T. pretiosum	*Kharif* 2014	86.67^{ab}	82.11^{a}	9.00^{bcd}
T. embryophagum		80.00^{bc}	81.83^{a}	10.67^{ab}
T. achaeae		68.89^{cd}	72.11^{ab}	7.33^{de}
T. japonicum		48.89^{ef}	55.95^{c}	7.33^{de}
T. brassicae		32.22^{gh}	65.74^{bc}	6.33^{ef}
T. mwanzai		11.11^{jk}	36.67^{d}	4.67^{ghi}
Control		0.00^{l}	0.00^{e}	0.00^{k}
T. pretiosum	*Rabi* 2014	92.22^{a}	87.89^{a}	9.33^{bc}
T. embryophagum		90.00^{ab}	90.07^{a}	11.67^{a}
T. achaeae		78.89^{bc}	87.38^{a}	8.67^{bcd}
T. japonicum		57.78^{de}	62.55^{bc}	8.33^{cd}
T. brassicae		41.11^{fg}	71.83^{bc}	7.33^{de}

T. mwanzai		13.33^{ijk}	55.56^{c}	5.33^{fg}
Control		0.0^{l}	0.00^{e}	0.00^{k}
T. pretiosum	Summer 2015	72.22^{cd}	39.65^{d}	4.00^{hij}
T. embryophagum		68.89^{cd}	36.21^{d}	5.0^{f0gh}
T. achaeae		32.22^{gh}	27.31^{d}	3.33^{j}
T. japonicum		23.33^{hi}	19.76^{d}	3.33^{j}
T. brassicae		17.78^{ij}	21.43^{d}	3.67^{ij}
T. mwanzai		7.78^{k}	8.33^{d}	3.67^{i}
Control		86.67^{ab}	0.00^{e}	0.60^{k}
CV (%)		15.05	21.74	7.44
CD (0.05)				
Trichogramma spp.		5.53	8.82	0.17
Seasons		3.62	5.77	0.11
Interaction		9.59	15.27	0.29

In each column, means with similar alphabets do not vary significantly at P=0.05 by DMRT

A study showed that the fecundity ability of an egg parasitoid *T.fasciatum* could be controlled by the environmental temperature and humidity [18]. A field study also stated that the emergence rate of *T.pretiosum* was drastically reduced when the temperature increased beyond 37°C [19]. In addition the longevity and fecundity were declined when the *T.brassicae* was exposed to a heat shock by 44°C [20]. Further a study revealed that *T.chilonis* parasitized the eggs of *Plutella xylostella* when the temperature range was 24 – 28°C [21]. Additionally a result specified that the developmental period and the emergence rate of *T.dendrolimi* were decreased with increasing temperatures from 26 to 32°C [22].

Besides increased mortality and premature mortality were observed in *Trichogramma* sp. when the temperature exceeded its optimum [23]. Furthermore few studies recorded that 32°C lowest the emergence rate in certain *Trichogramma* spp. and 35°C delayed the development period in *T.pretiosum* [25] and no progeny development in *T. cacoeciae* [26]. It was also noted from the studies that temperature played a significant effect on the parasitism rate of the egg-parasitoid *T. evanescens* [27, 28]. These findings are par with the present study.

4. Conclusions

The egg parasitoids, *Trichogramma pretiosum* and *T. embryophagum* were found as the most efficient egg parasitoids with maximum parasitism and emergence rate against one-day old eggs of *L. orbonalis.* The season, *Rabi* 2013 (November to December 2013) with a temperature of 29.7±0.8°C favored the biology of efficient *Trichogramma* spp. on the eggs of *L. orbonalis*. It was observed that almost all-efficient egg parasitoids studied during this period, preferred freshly laid eggs of *L. orbonalis* for parasitism. Aging of host eggs reduced the parasitism rate of *Trichogramma* spp.

The parthenogenesis mode of reproduction was observed in the egg parasitoid *L. orbonalis*. A maximum of 11.6 eggs of *L. orbonalis* was parasitized by a single female adult of *T. embryophagum* during the season, *Rabi* 2013.

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