



## PERFORMANCE OF *Trichogramma* EGG PARASITOID SPECIES ON CASTOR SEMILOOPER *Achaea janata* AND THEIR CONSERVATION IN POMEGRANATE ECOSYSTEM

<sup>\*a</sup>Elango, K., <sup>b</sup>Sridharan, S., <sup>c</sup>Saravanan, P.A. & <sup>d</sup>Balakrishnan, S.

<sup>a,b,c</sup>Department of Agricultural Entomology, <sup>d</sup>Department of Spices and Plantation Crops  
Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

\*Corresponding author email: elaento@gmail.com

### ABSTRACT

*Achaea janata*, a lepidopteran castor semilooper insect pest, is one of the economically important pests affecting castor plant growth-developmental process. Economic injury level caused by *Achaea janata* can be controlled through both biological and chemical measures. Despite the higher egg parasitization potential of *Trichogramma*, little is characterized against *Achaea janata*, which is susceptible to egg parasitization. Our aim was to test the parasitization potential of different *Trichogramma* egg parasitoid species viz. *Trichogramma chilonis*, *T. japonicum*, *T. acheaea*, *T. pritiolum*, *T. embryophagum*, *T. evanescens*, *T. dendrolimi* and *T. brassicae* against eggs of *Achaea janata*, under controlled conditions. Interestingly, a significant difference in parasitism (0.00–100 %) among the eight different *Trichogramma* spp. was noticed. *T. acheaea* parasitized the highest number of *Achaea janata* eggs and had more progeny ( $9 \pm 0.81$  adults /egg) than the other *Trichogramma* spp. The insecticide-tolerance and egg parasitization of *T. acheaea* and *T. chilonis* was tested against six different insecticides viz. imidacloprid 17.8 SL (0.00712%), thiamethoxam 25% WG (0.005%), chlorantraniliprole 18.5 SC (0.0037%), fipronil 5% SC (0.01%), spinosad 45% SC (0.0125%) and Azadirachtin 1% EC (0.02%) having varied formulation. Interestingly, *T. chilonis* provided 79.5 - 83.9% parasitization in insecticidal treatment. In conclusion, *T. acheaea* and *T. chilonis* exhibited better performance on *A. janata* eggs than the other six *Trichogramma* spp. and could be considered as our most suitable *Trichogramma* candidate for control of *A. janata*.

**KEYWORDS:** *Achaea janata*, *Trichogramma*, conservation, Azadirachtin, pomegranate

### INTRODUCTION

The castor semilooper *Achaea janata* (Noctuidae; Lepidoptera) major pest of castor and also other hosts including rose, pomegranate, ber, mango and citrus. Earlier days it is a minor pest in pomegranate but now days the farmers are turned into normal planting to high density planting to get high yield. So in high density planting due to microclimate the minor pest became a major pest. It is a leaf eater and in severe cases, the larvae completely defoliate the plants, leaving behind only the midribs of leaves and stems. Egg parasitoids of the genus *Trichogramma* are keystone natural enemies of many lepidopterous pests in agriculture and forestry (Li, 1994). As a consequence, efforts to develop biological control methods in IPM programs were initiated through the release of egg parasitoids and use of pheromones. However, release and integration issues that are considered important for the development of a successful IPM include important factors such as the pesticide choice and the timing of application (Smith, 1996). In fact, *Trichogramma* is generally sensitive to pesticides and most chemical products can be immediately toxic (contact toxicity). Moreover, contact with pesticides at the less susceptible life stage (parasitoids within hosts) can cause prolonged development time of immature stages and reduced emergence from parasitized eggs (Franz *et al.*, 1996). Accordingly, this is important to determine the

compatibility of parasitoids with pesticides for pest management programs. However, if the egg parasitoids are to be used in biological control, it is necessary to evaluate their searching ability and their tolerant level to insecticide exposure (Consoli *et al.*, 2009). Therefore, the objectives of this study were to assess the ability of *Trichogramma* species searching host eggs, and the level of tolerance for insecticide spray /residues to understand the potential use of *Trichogramma* egg parasitoids as a bio-control agent of castor semilooper in pomegranate ecosystem

### MATERIALS & METHODS

#### Test insect

Larvae of *A. janata* collected from pomegranate seedling raised in TNAU orchard, Coimbatore was used to establish the stock culture. Field collected larvae were kept in an iron rearing cage (60 X 60 X 60 cm). Castor leaves were provided as a food for larvae. During the process of rearing, inactive sluggish larvae and pupae which exhibited disease symptoms and slow growing as well as malformed individuals were removed. After pupation, the pupae kept in Petri plate were transferred to rearing cages for adult emergence. Five pairs of healthy adults were released in rearing cage provided 10% sugar solution in a penicillin vial with cotton wick. Fresh castor leaves placed in conical flask containing water was provided as a source of egg laying. The cage was covered with black coloured

sterile muslin cloth, which served as an oviposition chamber. Sugar solution (10%) was given as adult food in a suitable sterile glass vials and absorbent cotton. The sugar solution was replaced periodically. After two to three days castor leaves were removed and observed for oviposition. After oviposition, the eggs collected were used for testing parasitoid potential of *Trichogramma* egg parasitoid and the neonates were provided with young castor leaves up to their pupation. The pupae were then placed in iron rearing cages for adult emergence and other procedure were followed for mass culturing as described earlier. The whole setup was maintained in the culture room under controlled temperature (25 to 27 °C) with relative humidity (RH) of 70 ±5 percent. This culture was used for further laboratory studies

**Potential of *Trichogramma* sp. against castor semilooper**

Laboratory experiment was conducted during 2015-16 in Department of Agricultural Entomology, TNAU to determine the parasitization potential of *Trichogramma* sp on the eggs of *A. janata*. Fresh eggs were collected from mass culture maintained in biocontrol laboratory. The eggs of castor semilooper along with leaf bits were kept in test tubes. The egg parasitoids viz., *Trichogramma chilonis*, *T. japonicum*, *T. acheaea*, *T. pritiolum*, *T. embryophagam*, *T. evanescens*, *T. dendrolimi*, *T. brassicae* was obtained from bio control laboratory were tested against castor semilooper eggs. Egg parasitoids collected with help of aspirator were left in the ratio (6:1) inside the test tube (15 x 2.5 cm) and covered with muslin cloth secured with rubber band. The test tube released with adult parasitoid as

well as allowed for parasitization. The parasitized eggs were identified by the change in the colour of the egg from white to black. After 7 days, *Trichogramma* parasitoids emerged from the parasitized eggs was recorded with the help of image analyzer. Based on the number of parasitized eggs, the percent parasitization was worked out.

**Conservation studies for Egg parasitoid**

Laboratory experiment was conducted to assess the bio safety of most efficacious insecticides and botanicals against egg parasitoid *Trichogramma chilonis* Ishii. The treatments included were imidacloprid 17.8SL (0.00712%) (Confidor®) thiamethoxam 25% WG (0.005%) (Actara®), chlorantranil prole 18.5 SC (0.0037%) (Coragen®), fipronil 5% SC (0.01%) (Regent®), spinosad 45% SC (0.0125%) (Tracer®), Azadiractin 1% EC (0.02%) and untreated check.

**Effect on adult emergence**

The bioassay method described by Jalali and Singh (1997) was adopted for *T. chilonis*. Three days old parasitized egg cards were cut into 1cm<sup>2</sup> bits and sprayed with the insecticides using hand atomizer. For untreated check, parasitized egg card was sprayed with distilled water. The treatments were replicated three times and the treated egg cards were shade dried for 10 min and then kept in test tubes (15 x 2.5cm) for adult emergence. The number of parasitoids that emerged from each treatment was recorded after 48 h of treatment based on the emergence hole of the egg. The percent emergence was worked out using the formula

$$\text{Per cent adult emergence} = \frac{\text{No.of.wasp emerged (eggs with emergence slit)}}{\text{Total number of eggs in 1 cm}^2} \times 100$$

**Effect on Parasitization**

Fresh, sterilized *Corcyra cephalonica* Stainton eggs on egg card treated with respective treatments was exposed to adult parasitoids at 6:1 ratio and then parasitization of eggs was observed on 7 days after the release of adult

parasitoids. The parasitized eggs were identified by the change in the colour of the egg from white to black. Based on the number of parasitized eggs, the percent parasitization was worked out

$$\text{Per cent parasitization} = \frac{\text{Number of parasitized eggs}}{\text{Total number of } \textit{Corcyra cephalonica} \text{ eggs}} \times 100$$

**Toxicity to adult parasitoid**

The bioassay method described by McCutchen and Plapp (1988) for *C.carnea* was adopted for this study. The treatment solutions were prepared using water and acetone in the ratio 20:80. Glass scintillation vials of 10 ml capacity were evenly coated with 0.5 ml of respective treatments dissolved in acetone and dried by rotating the

tube horizontally. *T. chilonis* adults were released into the vials at the rate of 30 numbers per vial and provided with honey solution. The vials covered with muslin cloth secured with rubber band and observations on the mortality of the adults were made on 1, 3 and 6 hours after treatment. Percent mortality was worked out as given below:

$$\text{Per cent mortality} = \frac{\text{Number of dead wasp}}{\text{Total number of wasp}} \times 100$$

**Statistical analysis**

The data collected under laboratory experiments in completely randomized design were analyzed using analysis of variance (ANOVA) using AGRES 3.01 and

AGDATA software. Data in the form of percentages were transformed to arcsine values and those in numbers were transformed to  $x+0.5$  and analyzed. The mean values of

the treatments were compared using DMRT at 5 per cent level of significance

## RESULTS

### Potential of *Trichogramma sp* egg parasitoid against castor semilooper

Efficiency of *Trichogramma* egg parasitoid tested against castor semi looper eggs was presented in table 1. Among

all *Trichogramma* species tested, *T.chilonis* and *T.achaea* showed 100 ±0.00% parasitization on castor semilooper eggs followed by *T. pretiosum* and *T. japonicum* with 90 ± 3.16% parasitization. Regarding adult emergence from castor semilooper eggs, *T. achaea* was found superior with adult parasitoid emergence of 9 ±0.81 adults per egg followed by *T.chilonis* with 7.6 ±0.69 adults per egg.

**TABLE 1.** Performance of *Trichogramma* species on the parasitization of castor semilooper eggs

S.No	Egg parasitoids species	Percent parasitization	No. of adult emergence / Egg
		*Mean ± SD	*Mean ± SD
1	<i>Trichogramma chilonis</i>	100 ± 0.00	7.6 ± 0.69
2	<i>Trichogramma japonicum</i>	90 ± 3.16	6.2 ± 2.29
3	<i>Trichogramma acheae</i>	100 ± 0.00	9.0 ± 0.81
4	<i>Trichogramma embryophagum</i>	80 ± 4.21	5.9 ± 3.17
5	<i>Trichogramma evanescense</i>	80 ± 4.21	5.0 ± 3.49
6	<i>Trichogramma dendrolimi</i>	70 ± 4.83	3.1 ± 3.31
7	<i>Trichogramma brassicae</i>	70 ± 4.83	3.9 ± 3.38
8	<i>Trichogramma pretiosum</i>	90 ± 3.16	6.7 ± 3.40

\*Mean of 30 eggs; SD – Standard deviation

**TABLE 2.** Bio safety of newer insecticides and plant product to *T. chilonis*

Treatments	Dosage (g or ml per litre)	Parasitization* (%)	Adult emergence* (%)	Mortality*(%)		
				1 HAT	3 HAT	6 HAT
T <sub>1</sub> - Imidacloprid 17.8 SL (0.00712%)	0.4	69.23 (56.71) <sup>c</sup>	60.12 (50.84) <sup>c</sup>	83.33 (65.90) <sup>a</sup>	97.77 (81.41) <sup>b</sup>	100 (89.37) <sup>a</sup>
T <sub>2</sub> -Thiamethoxam 25 WG (0.005%)	0.2	70.07 (56.83) <sup>c</sup>	74.57 (59.71) <sup>cd</sup>	74.44 (59.63) <sup>b</sup>	100 (89.37) <sup>a</sup>	100 (89.37) <sup>a</sup>
T <sub>3</sub> -Chlorantraniliprole 18.5 SC(0.0037%)	0.2	70.28 (56.96) <sup>c</sup>	79.25 (62.90) <sup>bc</sup>	63.33 (52.73) <sup>c</sup>	93.33 (75.03) <sup>c</sup>	100 (89.37) <sup>a</sup>
T <sub>4</sub> -Fipronil 5% SC (0.01%)	2	53.51 (47.01) <sup>d</sup>	71.79 (57.91) <sup>d</sup>	76.66 (61.11) <sup>b</sup>	97.77 (81.41) <sup>b</sup>	100 (89.37) <sup>a</sup>
T <sub>5</sub> - Spinosad 45% SC (0.0125%)	0.2	77.97 (62.00) <sup>c</sup>	79.52 (63.09) <sup>bc</sup>	76.66 (61.11) <sup>b</sup>	95.55 (77.82) <sup>bc</sup>	100 (89.37) <sup>a</sup>
T <sub>6</sub> -Azadirachtin 10000 ppm (0.02%)	2	86.26 (68.24) <sup>b</sup>	83.95 (66.38) <sup>b</sup>	58.88 (50.11) <sup>c</sup>	92.22 (73.80) <sup>c</sup>	98.88 (83.92) <sup>a</sup>
T <sub>7</sub> -Untreated control	--	93.66 (75.41) <sup>a</sup>	91.62 (73.17) <sup>a</sup>	0.00 (0.00) <sup>d</sup>	0.00 (0.00) <sup>d</sup>	0.00 (0.00) <sup>b</sup>

\*Mean of three observations; Values in the parentheses are sinc transformed values.

Means followed by the common letter (s) are not significantly different at P=0.05 level by DMRT

### Insecticides effect on *Trichogramma chilonis* adult emergence

The impact of efficacious insecticidal treatments on the adult emergence from parasitized eggs of *T. chilonis* was studied. The effect of different treatments viz., imidacloprid 17.8 SL (0.00712%), thiamethoxam 25 WG (0.005%), chlorantra niliprole 18.5 SC (0.0037%), fipronil 5% SC (0.01%), spinosad 45% SC (0.0125%), and azadirachtin 10000 ppm (0.02%) on the adult emergence of *T. chilonis*. The results revealed that imidacloprid 17.8 SL (0.00712%) showed an adult emergence of 60.1 per cent which was the lowest as compared to rest of the treatments. Treatment with fipronil 5% SC (0.01%) showed an adult emergence of 71.7 percent. Spinosad 45% SC (0.0125%), azadirachtin 10000 ppm (0.02%) and untreated check recorded an adult emergence 79.52, 83.95 and 91.62 per cent, respectively.

### Insecticides effect on parasitization of *Trichogramma chilonis*

The results of insecticidal treatments on parasitization of *T. chilonis* showed maximum parasitization in untreated check (93.6%). Spinosad 45% SC (0.0125%) and azadirachtin 10000 ppm (0.02%) were found statistically on par with each other with respective parasitization of 79.5 and 83.9%. Similarly, the % parasitization recorded in chlorantraniliprole 18.5 SC (0.0037%) (79.2%) and thiamethoxam 25 WG (0.005%) (74.5%) were on par with each other.

### Insecticides effect on *Trichogramma chilonis* adult mortality

Different insecticidal treatments were evaluated for their toxicity to adult *T. chilonis* (Table 2). The results revealed that imidacloprid 17.8 SL (0.00712%) had maximum mortality 83.3 of percent at 1 HAT followed by

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thiamethoxam 25 WG (0.005%) and fipronil 5% SC (0.01%) which showed 74.4 and 76.6 per cent mortality, respectively and on par with each other. Chlorantraniliprole 18.5 and azadirachtin 10000Ppm (0.02%) showed less mortality of 63.3 and 58.8% at 1 hour after treatment. At 3 HAT thiamethoxam 25 WG (0.005%) showed 100 per cent mortality of *T. chilonis* adults.

Imidacloprid 17.8 SL (0.00712%) and fipronil 5% SC (0.01%) on par with each other showed 97.7 per cent mortality at 3 HAT respectively. Imidacloprid 17.8 SL (0.00712%), chlorantraniliprole 18.5 SC (0.0037%), Fipronil 5% SC (0.01%) and Spinosad 45% SC (0.0125%) showed 100 per cent mortality at 6 Hours after treatment.



a *T. chilonis*



b. *T. japonicum*



c. *T. Achaea*



d. *T. pretiosum*



e. *T. embryophagum*



f. *T. evanscense*

**PLATE 1:** *Trichogramma* species on the parasitization of castor semilooper eggs

**DISCUSSION**

Egg parasitoids, *T. chilonis* and *T. achaea* showed highest percent parasitisation followed by *T. pretiosum* and *T. japonicum*. Maximum adult emergence was observed in *T. chilonis* and *T. achaea*. This study strongly concluded that *T. chilonis* and *T. achaea* are effective egg parasitoid to controlling castor semilooper Similarly, Krishnamoorthy

(2014) stated that among all egg parasitoids, *Trichogramma chilonis* was promising as a natural and augmentation control agent for several lepidopteran pests. Among the pesticides screened for their bio efficacy, the best performed insecticides chlorantraniliprole 18.5 SC and thiamethoxam 25 WG, spinosad and azadirachtin 10000 ppm were tested against commonly used biocontrol

agents to assess its selectivity. The results showed that all the treatments had lesser effects on the emergence of *Trichogramma chilonis* Ishii. Likewise, the parasitisation rate by *T. chilonis* was also not affected with the above treatments. The present study finds the support from Hussain *et al.* (2012) who worked on the toxicity of newer insecticides against egg parasitoid *T. chilonis* and suggested that highest emergence of *T. chilonis* was observed in chlorantraniliprole treated egg cards in comparison with other insecticides. Similarly Thiruvani (2014) showed that the thiamethoxam 25 WG did not cause any adverse effect on the adult emergence of egg parasitoid, *T. pretiosum*. The present results agree to the findings of Ughade *et al.* (2002) who reported that spinosad was moderately safer to *T. chilonis*. Numerous laboratory and field studies have revealed that *Trichogramma* wasps is highly susceptible to most of the broad spectrum insecticides. This is the reason that various attempts to suppress pest population by biological control measures have often failed because of deleterious effects of chemical on the beneficial insects (Borgemeister *et al.*, 1993).

### CONCLUSION

Among the *Trichogramma* species evaluated for parasitisation of *A. janata* eggs, *T. chilonis* and *T. achaea* showed highest percent parasitisation, followed by *T. pretiosum* and *T. japonicum*. Maximum adult emergence was observed in *T. chilonis* and *T. achaea*. This study strongly concluded that *T. chilonis* and *T. achaea* are effective egg parasitoid to controlling castor semilooper. Chlorantraniliprole 18.5 SC (0.0037%) and thiamethoxam 25 WG (0.005%), spinosad 45% SC (0.0125%) and azadirachtin 10000 ppm (0.02%) had lesser effects on the emergence of *Trichogramma chilonis* indicating their bio safety. Using both of this insecticides and egg parasitoids will give perfect pest management and higher yield in the pomegranate ecosystem.

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