

# Field efficacy of *Trichogramma chilonis* reared on different factitious hosts for the management of sugarcane stem borers

Yadavalli LALITHA<sup>1</sup>, Omprakash NAVIK<sup>1</sup>, Richa VARSHNEY<sup>1</sup>, Veeranna Nagappa PATEL<sup>2</sup>, Chandish Ramakrishna BALLAL<sup>1</sup>

<sup>1</sup>ICAR-National Bureau of Agricultural Insect Resources, Hebbal, Bengaluru, Karnataka, India

<sup>2</sup>Zonal Agricultural Research Station, Mandya, Karnataka, India

## Abstract

The egg parasitoid, *Trichogramma chilonis* Ishii (Hymenoptera Trichogrammatidae), is an important biocontrol agent of lepidopteran pests and is generally mass-reared on the eggs of *Corcyra cephalonica* (Stainton) (Lepidoptera Pyralidae). In this study, eggs from two factitious hosts, eri silkworm (ESW), *Samia cynthia ricini* Drury (Lepidoptera Saturniidae), and *C. cephalonica*, were used for mass rearing *T. chilonis*. The field efficacy of *T. chilonis* reared from the two different hosts was evaluated against sugarcane borers, *Chilo infuscatellus* Snellen (Lepidoptera Crambidae) and *Chilo sacchariphagus indicus* (Kapur) (Lepidoptera Crambidae). Weekly releases of *T. chilonis* reared from ESW and *C. cephalonica* eggs showed a similar parasitism rate and were effective in reducing crop damage by both sugarcane borers. We found no significant difference between the parasitism levels by *T. chilonis* reared from ESW (10.53%) and *C. cephalonica* (7.81%). A higher percentage of millable canes was obtained when parasitoids from either kind of eggs were released in a field compared to no release of parasitoids. There was no significant difference in the percentage of millable canes obtained from the fields with *T. chilonis* reared from ESW (77.99%) or *C. cephalonica* (70.23%). Producing *T. chilonis* from ESW eggs reduced rearing costs by 50% compared to using *C. cephalonica* eggs. Consequently, the production of *T. chilonis* using large-sized ESW eggs is more suitable for on-farm production and *C. cephalonica* could be used for commercial insectaries or research institutes.

**Key words:** factitious host, *Corcyra cephalonica*, *Samia cynthia ricini*, field efficacy, *Trichogramma chilonis*, sugarcane borers.

## Introduction

Sugarcane, *Saccharum officinarum* L., is an important industrial crop cultivated in more than 90 countries of tropical and subtropical regions for sugar and ethanol production (Chinnadurai, 2017; FAO, 2020). India is the second-largest producer of sugarcane in the world and the crop is cultivated in over 4.73 million hectares with a production of 376.91 million tons and productivity of 79.60 t/ha (FAO, 2020). In India, insect pests are a serious constraint in sugarcane production (Sharma *et al.*, 2017). Worldwide, over 1500 insect pest species attack sugarcane (Long and Hensley, 1972) and 220 species in India (David *et al.*, 1986) are known to attack various growth stages of sugarcane (Avasthy and Tiwari, 1986; Mahesh *et al.*, 2016). Of these, sugarcane stem borers are of major concern as they reduce the crop yield, besides affecting the quality of juice/sugar recovery in the sugar factories (Easwaramoorthy, 1983). The sugarcane early stem borer, *Chilo infuscatellus* Snellen and the internode borer, *Chilo sacchariphagus indicus* (Kapur) (Lepidoptera Crambidae), are the key pests that cause serious damage from the early stages until the harvest of sugarcane (Easwaramoorthy and Nandagopal, 1986; Sharma *et al.*, 2020). Damage by *C. infuscatellus* causes losses of 22-33% and *C. sacchariphagus indicus* 10-35% from internode formation to harvest (Yalawar *et al.*, 2010).

Chemical insecticides usually are not effective against the stem borers, *C. infuscatellus* and *C. sacchariphagus indicus*, owing to their internal tissue feeding habit and the plant canopy structure that densely covers the field (Ananthanarayana and David, 1986; Mahesh *et al.*, 2016). In addition, chemical control drastically affects

non-target organisms and poses detrimental effects to the environment and humans (Crowder *et al.*, 2010). Biological control is the alternative pest management option that offers sustainable sugarcane production by minimizing the infestation levels of sugarcane borers (Srikanth *et al.*, 2016). Amongst various biocontrol agents, egg parasitoids of the genus *Trichogramma* have been widely used in the biological control of lepidopteran pests in agriculture and forestry (Smith, 1996; Zang *et al.*, 2021) and successful control of the pests in the field has been reported in several countries (Xu *et al.*, 2020). Augmentative releases of *Trichogramma* spp. are made to suppress infestations by stem borers. The ease of rearing *Trichogramma* spp. on factitious hosts and their short generation time make mass production a feasible and affordable technology compared to producing other parasitoids (Nagaraja, 2013).

Mass rearing of parasitoids is crucial to produce in millions for field releases and this process involves the production of various factitious hosts which either reared on artificial diets or natural diets (Parra and Coelho, 2022). Therefore, selection of the most suitable factitious host for the production of high-quality *Trichogramma* spp. is an essential prerequisite to establishing an effective mass-rearing process (Iqbal *et al.*, 2020). Eggs of many factitious hosts have been used for rearing various *Trichogramma* spp. including, *Sitotroga cerealella* (Olivier) (Lepidoptera Gelechiidae), *Antheraea pernyi* (Guerin-Meneville) (Lepidoptera Saturniidae), *Corcyra cephalonica* (Stainton) (Lepidoptera Pyralidae), and eri silkworm (ESW) *Samia cynthia ricini* Drury (Lepidoptera Saturniidae) (Greenberg *et al.*, 1998). However, for cost-effective production of *Trichogramma*, an efficient

alternative factitious host is needed to produce a large number of parasitoid in shorter period for field releases. Nevertheless, in India, *Trichogramma chilonis* Ishii (Hymenoptera Trichogrammatidae) is generally mass-reared on small-sized eggs of the factitious hosts, *C. cephalonica* and *S. cerealella*. The small eggs of *C. cephalonica* support only one parasitoid per egg which makes production expensive. Additionally, long-term storage (more than 2-3 weeks) of *C. cephalonica* eggs at low temperatures can decrease their suitability for mass rearing *T. chilonis* (Huang *et al.*, 2013; Xu *et al.*, 2020). Conversely, the large eggs of ESW are considered suitable factitious hosts for mass rearing *Trichogramma* spp. with practical benefits, such as a high reproduction rate with 20-60 adults per egg (Lalitha *et al.*, 2010; Wang *et al.*, 2014). Further, the large ESW eggs are convenient to handle and amenable to long-term storage and transportation, thus reducing the production costs compared to smaller eggs (Wang *et al.*, 2014). Furthermore, the ESW eggs were suitable for mass rearing *Trichogramma dendrolimi* Mastumura and *Trichogramma ostriniae* (Peng et Chen) and releases of these parasitoids in corn resulted in control of the Asian corn borer *Ostrinia furnacalis* (Guenee) (Lepidoptera Crambidae) in China (Shen *et al.*, 1986; Wang *et al.*, 2014).

The biological attributes of *Trichogramma* spp. reared on suitable factitious hosts are essential for their successful field performance (Greenberg, 1991; van Lenteren *et al.*, 2003; Nagaraja, 2013). An earlier study on the biological attributes of *T. chilonis* reared on ESW or *C. cephalonica* eggs indicated that the former was a significantly superior host (Lalitha *et al.*, 2010). Therefore, our objective in the current study was to evaluate the field performance of *T. chilonis* reared from eggs of the two factitious hosts for the management of sugarcane stem borers.

## Materials and methods

### Sources of *T. chilonis* and factitious hosts

*T. chilonis* (Accession No. NBAII-MP-TRI-13) was obtained from the Mass Production Unit, ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, Karnataka, India. The parasitoid colony had been maintained using eggs of the rice moth, *C. cephalonica*, under controlled laboratory conditions of  $27 \pm 2$  °C and  $65 \pm 5$  % RH with a 14:10 L:D photoperiod. The initial culture of *C. cephalonica* was obtained from the Mass Production Unit, ICAR-NBAIR, Bengaluru, as a factitious host for rearing *T. chilonis*. The ESW cocoons for mass rearing *T. chilonis* were collected from the Department of Sericulture, Bangalore University, Bengaluru, Karnataka, India.

### Mass rearing of *S. cynthia ricini* and *C. cephalonica*

The ESW cocoons were kept in a  $30 \times 30 \times 30$  cm insect rearing cage lined internally with black long cloth for adult emergence. The adults were allowed to emerge and mate in the cage. Female ESW laid an average of 300 eggs with 85% hatch. Freshly laid eggs oviposited on the cloth were dislodged, washed with distilled water, air-

dried, and kept for hatching under controlled environmental conditions at  $27 \pm 2$  °C and  $65 \pm 5$  % RH with a 14:10 L:D photoperiod. The larvae were reared in a tray (Excel small, rectangular multipurpose organizing and storage plastic trays) on a natural diet of fresh and clean castor leaves provided three times per day until they pupated. Rearing trays were cleaned daily. About 40% of the larvae produced female moths. As in the previous generation, cleaned cocoons were placed in an insect rearing cage for adult emergence. The eggs for mass rearing *T. chilonis* were stored at 9 °C in a refrigerator for 12 days before being used. A total of 42-50 days was required to obtain ESW eggs for rearing the egg parasitoids (Lalitha *et al.*, 2010).

The rice moth larvae were reared on a broken sorghum-based diet (Lalitha and Ballal, 2015). The artificial diet contained 2.5 kg of broken sorghum, 75 g of coarsely powdered groundnut seeds, 5 g of yeast, and 75 mg of streptomycin sulphate. Approximately 4000 *C. cephalonica* eggs (0.25 cc) were sprinkled on the thoroughly mixed diet and kept in 45 cm long, 30 cm wide, and 20 cm high boxes made with 12 mm thick fine quality plywood. Six circular 2.5 cm diameter holes were cut through the lid of each box and covered both sides with brass mesh (150 µm pore size). The *C. cephalonica* rearing boxes were placed in an iron rack until moths emerged. Each rearing box produced 6 to 14 cc (average 12 cc) of moths (Lalitha and Ballal, 2015) with 50-85% emergence, depending on the season. Moths were collected using a semi-mechanized device made from a vacuum cleaner attached to a plastic cylinder and transferred directly into oviposition cages. The moths were fed honey diluted 50% with water plus vitamin E (Evion® 400 mg; 2 capsules/litre of 50% diluted honey) in a cotton swab. The loose eggs were collected from the oviposition cages, cleaned, and used for parasitoid rearing. The *C. cephalonica* females each produced 300-350 eggs, development from egg to adult took 40-45 days, and the moths emerged from larval rearing boxes up to 120-125 days from the day of sprinkling eggs on the diet.

### Rearing *T. chilonis* on factitious hosts

*T. chilonis* was reared on UV-irradiated *C. cephalonica* eggs (15w UV light for one hour) and uniformly glued on to the surface of  $17.5 \times 11$  cm paper tricho-cards ( $12 \times 8$  cm of eggs). These egg cards were exposed to female *T. chilonis* in transparent, 2 mil thick (~0.05 mm),  $40 \times 30$  cm polyethylene bags at 30 eggs per female until the adults died. Adult parasitoids were fed fine streaks of honey diluted 50% with water (Jalali *et al.*, 2003). To rear *T. chilonis*, ESW eggs were stored at 9 °C for 12 days, treated with potassium hydroxide (0.1%) for one minute, and air-dried. Initially, for 12 generations of small-scale rearing, treated ESW eggs were glued on to  $3 \times 2$  cm paper cards and exposed for parasitism in a ratio of one female per egg in a 55 ml glass tube ( $15 \times 2.5$  cm). Subsequently, to mass rear *T. chilonis* ESW, host eggs were glued on to  $17.5 \times 11$  cm paper cards in a  $12 \times 8$  cm area. These paper cards with ESW eggs were exposed to *T. chilonis* females in transparent, 2 mil thick (~0.05 mm),  $40 \times 30$  cm polyethylene bags at 3 females per egg. The opening of each bag was fastened with a rubber band. The females were provided with fine

streaks of honey diluted 50% with water as food. These cards were maintained at  $27 \pm 2$  °C and  $65 \pm 5\%$  RH with a 14:10 L:D photoperiod for eight days before being used. The cards with ESW or *C. cephalonica* eggs parasitized by *T. chilonis* were used for field releases.

### Field site and parasitoid releases

A field trial was conducted for three consecutive years in irrigated sugarcane fields in Madla, Mandya district of Karnataka, India ( $12^{\circ}35'57''\text{N}$   $76^{\circ}48'42''\text{E}$ ). The sugarcane cultivar CO 62175 was planted in three fields each with an area of 4000 m<sup>2</sup>. The sugarcane setts were planted in red loamy soil with a spacing of  $90 \times 30$  cm following the ridges and furrow method (Shukla *et al.*, 2017). A recommended application of fertilizer (300:100:200 NPK kg ha<sup>-1</sup>) was made and weeds were managed as required. These sugarcane fields were separated by a minimum distance of 500 m and the space between them was occupied by other field crops, such as sugarcane and rice.

The experiment was conducted in a randomized complete block design with three treatments: T1) release of *T. chilonis* reared on ESW eggs; T2) release of *T. chilonis* reared on *C. cephalonica* eggs, and T3) no release of parasitoids. Each field was divided into five equal blocks as replicates. Weekly releases of *T. chilonis* were initiated 30 days after the sugarcane was planted. A total of approximately 20,000 adults of *T. chilonis* reared on eggs of either ESW or *C. cephalonica* were released at 20 points (4 points/ block) in separate fields. Before release, the egg cards of ESW and *C. cephalonica* parasitized by *T. chilonis* were cut into 20 pieces ( $4 \times 1.5$  cm) to uniformly release the parasitoids. Parasitized eggs of ESW and *C. cephalonica* (100 eggs/host) were checked for parasitoid emergence to estimate the number of *T. chilonis* adults that emerged from the parasitized eggs prior to release. From each parasitized ESW egg, 7-34 adult *T. chilonis* emerged ( $20.01 \pm 2.55$  egg) and 1-3 adults emerged from the eggs of *C. cephalonica* ( $1.07 \pm 0.11$  adults/ egg). For the release, one piece of an egg card of the tricho-card was stapled to the lower surface of a leaf at four equally distributed locations in each block. The *T. chilonis* adults emerged from ESW parasitized eggs on the 20 pieces of egg cards were (200 parasitized eggs/ card  $\times$  20 parasitoids/egg = 4,000 parasitoids  $\times$  5 block = 20,000 parasitoids). The *T. chilonis* adults from *C. cephalonica* parasitized eggs were released at each of 20 equally distributed points in a separate field (1000 parasitized eggs/card  $\times$  4 cards/block  $\times$  one parasitoid/egg = 4000 parasitoids  $\times$  5 blocks = 20,000 parasitoids). A total of twelve releases were made at weekly intervals. During the field trial, no insecticides were sprayed in the treatment or control plots.

### Pest incidence, field parasitism and sugarcane injury

The incidence of *C. infuscatellus* indicated as percent dead hearts was recorded from 20 randomly selected canes from each block at fortnightly intervals commencing from 15 to 75 days after initiating the first release (45 to 105 days after planting). *C. sacchariphagus indicus* incidence, intensity and infestation index were assessed after discontinuing the parasitoid releases. The pest incidence was assessed by counting the infested canes in each clump at 20 spots in each block for each treatment.

Twenty canes in each block were randomly selected for destructive sampling to record the pest incidence and intensity based on exit holes made by pests in each treatment, and the infestation index was calculated using the following formulae (Geetha *et al.*, 2009):

$$\text{Pest incidence (\%)} = \frac{(\text{Number of injured canes})}{(\text{Total number of canes observed})} \times 100$$

$$\text{Pest intensity (\%)} = \frac{(\text{Number of injured internodes})}{(\text{Total number of internodes observed})} \times 100$$

$$\text{Infestation index} = \frac{(\text{Percent pest incidence}) \times (\text{Percent intensity})}{100}$$

For assessing field parasitism by *T. chilonis*, sentinel cards ( $4 \times 1.5$  cm) holding approximately 100 unparasitised eggs of *C. cephalonica* were used both in the release and untreated control fields. These sentinel cards were stapled onto the lower surface of sugarcane leaves at six spots in the treatment and control plots after parasitoid releases. The sentinel cards were collected just prior to the next release and carried to the laboratory. These cards were kept individually in glass vials ( $15 \times 2.5$  cm) and held at  $27 \pm 2$  °C and  $65 \pm 5\%$  RH with a 14:10 L:D photoperiod for adult parasitoid emergence. The *T. chilonis* emergence rates were calculated for sentinel *C. cephalonica* eggs recovered from the treated and untreated control fields. All of the parasitoids that emerged from the *C. cephalonica* eggs in both the treated and control fields also were identified. In addition, the percentage of millable canes from each field was estimated at the time of harvest.

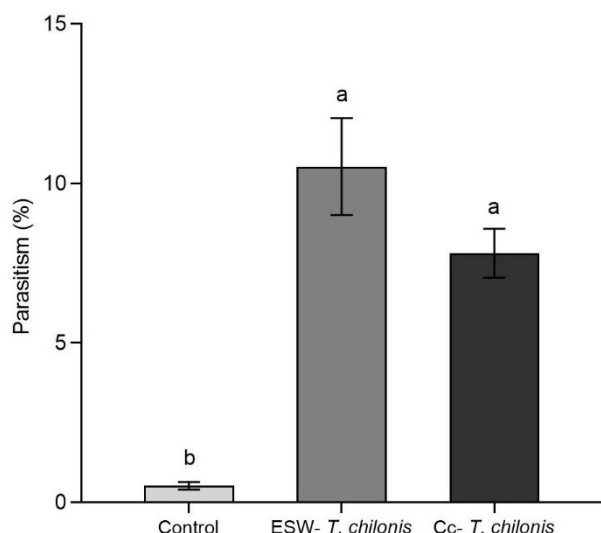
### Statistical analysis

Statistical analysis was carried out using SAS Analytics software (SAS Institute, 2011). The data on sugarcane borer incidence, pest intensity, and field parasitism by *T. chilonis* were subjected to arcsine transformation before analysis. The transformed data were analysed by two-way ANOVA using PROC GLM, and means  $\pm$  SE were presented. The difference between treatments was compared when ANOVA was significant using Tukey's post hoc significance test at a significance level of 5%.

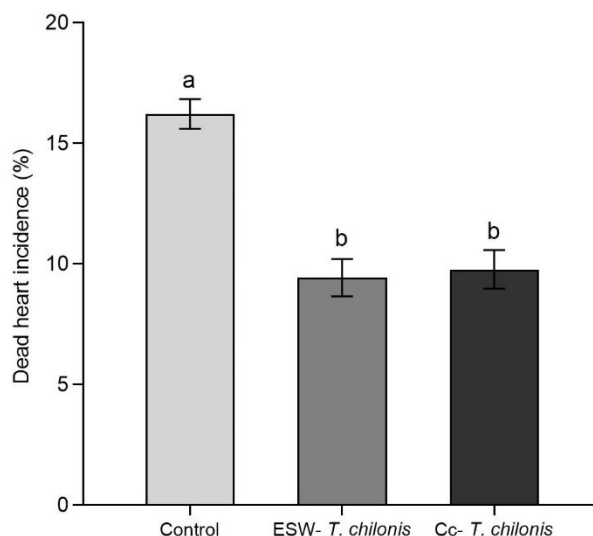
## Results

### Field efficacy of *T. chilonis* reared from different hosts

There was no significant difference in the percent parasitism of *C. cephalonica* eggs on sentinel cards by *T. chilonis* reared from eggs of ESW or *C. cephalonica* ( $F = 65.13$ ;  $df = 2, 171$ ;  $p < 0.0001$ ) (figure 1). The mean parasitism by *T. chilonis* reared from ESW and *C. cephalonica* eggs was  $10.53 \pm 1.52\%$  and  $7.81 \pm 0.77\%$ , respectively. The parasitism of ESW-reared *T. chilonis* varied from 8.08 to 13.72%, while the parasitism of *T. chilonis* reared on *C. cephalonica* eggs varied from 6.64 to 9.39%. The parasitism of *T. chilonis* reared from different host eggs was significantly different between the years ( $F = 3.36$ ;  $df = 2, 171$ ;  $p = 0.0372$ ) and the higher parasitism was recorded during second year by *T. chilonis* reared from eggs of ESW (13.73%) and *C. cephalonica* (9.39%). The interaction between treatments and years had no-significant effect on the parasitism of *T. chilonis* ( $F = 0.47$ ;  $df = 4, 171$ ;  $p = 0.756$ ).



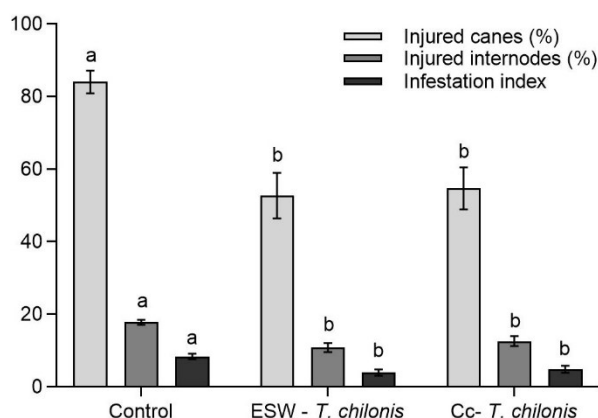
**Figure 1.** Mean ( $\pm$  SE) percent parasitism of *C. cephalonica* eggs on sentinel cards by *T. chilonis* in sugarcane. Control = no releases of *T. chilonis*; ESW- *T. chilonis* = releases of *T. chilonis* reared on ESW eggs; Cc- *T. chilonis* = releases of *T. chilonis* reared on *C. cephalonica* eggs.



**Figure 2.** Mean ( $\pm$  SE) percentage of dead heart caused by *C. infuscatellus* in different treatments. Control = no releases of *T. chilonis*; ESW- *T. chilonis* = releases of *T. chilonis* reared on ESW eggs; Cc- *T. chilonis* = releases of *T. chilonis* reared on *C. cephalonica* eggs.

#### Effect of *T. chilonis* releases on pest damage incidence

Significantly lower cane damage by *C. infuscatellus* was recorded in the fields where *T. chilonis* reared from the eggs of ESW and *C. cephalonica* were released ( $F = 26.73$ ;  $df = 2, 216$ ;  $p < 0.0001$ ) (figure 2). The parasitoids reared from ESW and *C. cephalonica* were equally effective in reducing the incidence of dead heart caused by *C. infuscatellus*. Similarly, a significantly lower number of injured canes ( $F = 16.40$ ;  $df = 2, 81$ ;  $p < 0.0001$ ), injured internodes ( $F = 18.71$ ;  $df = 2, 81$ ;  $p < 0.0001$ ) and infestation index ( $F = 9.47$ ;  $df = 2, 81$ ;  $p = 0.0002$ ) were recorded for *C. sacchariphagus indicus* in the fields where releases were made with *T. chilonis* reared from the two different host eggs. *T. chilonis* reared from different host eggs showed similar efficacy in reducing the damage caused by *C. sacchariphagus indicus* in sugarcane (figure 3). A significantly lower incidence of cane injury, percentage of injured internodes and infestation index were recorded for sugarcane during the second year compared to the first and third years (table 1).



**Figure 3.** Mean ( $\pm$  SE) injured canes (%), injured internodes (%) and infestation index of *C. sacchariphagus indicus* in different treatments. Control = no releases of *Trichogramma*; ESW- *T. chilonis* = releases of *T. chilonis* reared on ESW; Cc- *T. chilonis* = releases of *T. chilonis* reared on *C. cephalonica* eggs.

**Table 1.** Mean ( $\pm$  SE) percentage of injured canes and internodes, and the infestation index of *C. sacchariphagus indicus* in sugarcane fields following releases of *T. chilonis* reared from two different hosts.

Treatment	Year 1			Year 2			Year 3		
	Injured canes (%)	Injured internodes (%)	Infestation index	Injured canes (%)	Injured internodes (%)	Infestation index	Injured canes (%)	Injured internodes (%)	Infestation index
ESW- <i>T. chilonis</i>	66.00 $\pm$ 7.33 a	5.18 $\pm$ 0.75 a	3.91 $\pm$ 0.93 a	20.00 $\pm$ 7.30 a	1.23 $\pm$ 0.56 a	0.60 $\pm$ 0.36 a	72.00 $\pm$ 9.98 a	7.90 $\pm$ 1.93 a	7.24 $\pm$ 2.04 a
Cc- <i>T. chilonis</i>	68.00 $\pm$ 6.80 a	7.70 $\pm$ 1.81 ab	6.14 $\pm$ 2.07 ab	20.00 $\pm$ 7.30 a	1.12 $\pm$ 0.48 a	0.54 $\pm$ 0.29 a	76.00 $\pm$ 4.99 a	9.76 $\pm$ 1.08 a	7.80 $\pm$ 1.26 a
Control	92.00 $\pm$ 3.27 b	10.60 $\pm$ 0.91 b	9.58 $\pm$ 0.67 b	76.00 $\pm$ 6.53 b	7.50 $\pm$ 1.14 b	6.54 $\pm$ 1.05 b	84.00 $\pm$ 4.99 a	10.98 $\pm$ 1.60 a	9.40 $\pm$ 1.78 a
df	2, 27	2, 27	2, 27	2, 27	2, 27	2, 27	2, 27	2, 27	2, 27
F	5.01	5.85	6.00	16.91	20.21	25.27	0.50	1.63	0.96
p	0.01	0.007	0.007	<0.0001	<0.0001	<0.0001	0.61	0.21	0.39

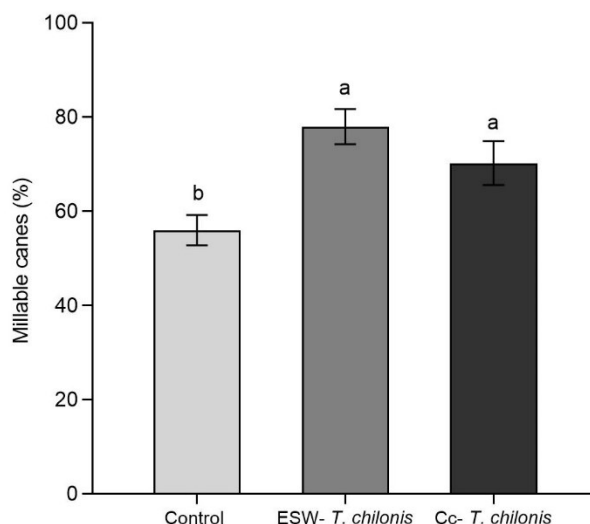
Different letters indicate significant difference between the different treatments using Tukey's test ( $P < 0.05$ ).

## Millable canes and cost benefit analysis

The percentage of millable canes was significantly different between the treated and untreated fields ( $F = 8.94$ ;  $df = 2, 81$ ;  $p = 0.0003$ ). However, there was no significant difference in the percentage of millable canes obtained from the fields where releases were made of *T. chilonis* reared on host eggs of ESW or *C. cephalonica* (figure 4). No significant difference was recorded in the percentage of millable canes between the three years of field trials ( $F = 2.14$ ;  $df = 2, 81$ ;  $p = 0.112$ ). The interaction between treatments and years also had no significant effect on the percentage of millable canes ( $F = 0.40$ ;  $df = 4, 81$ ;  $p = 0.807$ ). The cost of *T. chilonis* reared from ESW eggs was lower than for the regular laboratory host, *C. cephalonica*, used in parasitoid production. The total production cost of 20,000 parasitoids was reduced by over 50% when *T. chilonis* was mass-reared on ESW eggs (table 2).

## Discussion

*Trichogramma* spp. are important biocontrol agents for the management of various lepidopteran pests, utilised through augmentative biological control or as a component of supporting Integrated Pest Management (IPM) programmes (Smith, 1996; Parra and Coelho, 2022). The success of these egg parasitoids is linked to selection of suitable factitious host eggs and their large scale rearing to produce millions of parasitoids (Nagaraja, 2013; Parra, and Coelho, 2022). In this study, we have used *S. cynthia ricini* and *C. cephalonica* eggs as factitious hosts in the mass rearing of *T. chilonis*. The ESW was easily reared on castor leaves and the large-sized eggs supported the development of an average of 20 parasitoids per egg, while the eggs of *C. cephalonica* reared on an artificial diet sustained the development of 1 to 3 parasitoids per egg. Greenberg *et al.* (1998) reported that ESW eggs support the development of an average of 25 *Trichogramma* spp. adults per egg with the emergence of 27-60 parasitoids per egg. However, most commercial insectaries in India mass produce *Trichogramma* spp. on *C. cephalonica* eggs because of the availability of a standardized rearing system and artificial diet (Lalitha *et al.*, 2010; Nagaraja, 2013). The mass rearing facilities require a fully equipped laboratory with scientific knowledge available and a moderate infrastructure (Wang *et al.*, 2014). The simple rearing protocols developed for producing ESW eggs (Lalitha *et al.*, 2010) requires only a laboratory with minimum mass rearing inputs. Therefore, ease of rearing and the large ESW eggs produced more *T. chilonis* parasitoids in less time and at a lower cost in comparison to



**Figure 4.** Mean ( $\pm$  SE) percentage of millable canes obtained from sugarcane fields that received *T. chilonis* reared from two different host eggs. Control = no releases of *T. chilonis*; ESW- *T. chilonis* = releases of *T. chilonis* reared on ESW eggs; Cc- *T. chilonis* = releases of *T. chilonis* reared on *C. cephalonica* eggs.

*C. cephalonica*. Furthermore, use of the ESW is advantageous because the eggs can be stored for a longer period and remain viable for producing *T. chilonis*. Wang *et al.* (2014) reported that the use of ice for quick freezing ESW eggs helps to store them for 6 months and they remain viable for production of *Trichogramma*. The *Trichogramma* parasitized eggs can be stored at 3-5 °C for 50 days.

In the current study, we evaluated the performance of *T. chilonis* reared from eggs of the ESW and compared with the performance of parasitoids reared from *C. cephalonica* eggs. The effectiveness of *Trichogramma* spp. is dependent on their establishment in the field and parasitism of target host eggs (Du *et al.*, 2018; Sharma *et al.*, 2020). Sentinel egg cards are placed in the field to assess the prevailing populations of released parasitoids (Sharma *et al.*, 2020). In our study, the presence of parasitized eggs on sentinel cards placed in sugarcane fields verified the presence of significant populations of the released parasitoids reared from both factitious hosts. We found no significant difference in the parasitism of *T. chilonis* obtained from either of the factitious hosts. The parasitism of *T. chilonis* on unparasitized sentinel *C. cephalonica* eggs has been reported in several studies, with recovery ranging from 1.86 to 66.48% in sugarcane (Thirumurugan *et*

**Table 2.** Production cost of *T. chilonis* reared from host eggs of ESW and *C. cephalonica* (Cc).

Species	Diet & host eggs cost (\$) <sup>1</sup>	Parasitoid rearing cost (\$)	Total cost (\$)	Total parasitoids/tricho-card (No.) <sup>2</sup>	Cost/20,000 parasitoids (\$)
ESW- <i>T. chilonis</i>	0.29	0.19	0.48	64,000	0.15
Cc- <i>T. chilonis</i>	0.27	0.07	0.34	20,000	0.34

<sup>(1)</sup> Cost of castor leaves and cost of nucleus egg culture of ESW; <sup>(2)</sup> Parasitoids emerged from one tricho-card (ESW eggs/ tricho-card = 3200; *C. cephalonica* eggs/ tricho-card = 20000); Tricho-card size: 17.5 × 11 cm; surface area used for host eggs on cards 12 × 8 cm.

al., 2006; Geetha *et al.*, 2009; Sharma *et al.*, 2020). Furthermore, the field releases of *T. chilonis* reared from both factitious hosts significantly reduced the percentage of dead heart incidence by *C. infuscatellus* and canes and internodes by *C. sacchariphagus indicus*. We recorded a relatively low infestation of *C. sacchariphagus indicus* during the second year due to the higher parasitism by *T. chilonis* to the target pest. This shows that the *T. chilonis* reared from ESW eggs are effective in parasitizing the host eggs and reducing crop damage. Earlier studies also suggested the suitability of ESW eggs for mass rearing *T. dendrolimi* and *T. ostriniae* (Wang *et al.*, 2014), and the releases of these parasitoids were effective in controlling Asian corn borer, *O. furnacalis*, on corn in China (Feng *et al.*, 1977; Shen *et al.*, 1986; Wang *et al.*, 2014). Further, multiple releases of *T. chilonis* reared from the routine host, *C. cephalonica*, were also effective in reducing the crop damage by stem borers of sugarcane in different parts of India (Thirumurugan *et al.*, 2006; Geetha *et al.*, 2009; Sharma *et al.*, 2020), and these parasitoids were mass-reared and released by following standardized production and release methods (Nagaraja, 2013; Lalitha and Ballal, 2015).

The weekly releases of *T. chilonis* reared from eggs of either host produced a higher percentage of millable canes than in the non-released field. We found no significant difference in the percentage of millable canes obtained from fields that received parasitoids reared from ESW or *C. cephalonica* eggs. The higher percentage of millable canes obtained in the parasitoid released field was due to the higher parasitism of target pest eggs by *T. chilonis*. Similar results were reported on improved cane yield following inundative releases of *T. chilonis* against borer pests of sugarcane (Rachappa and Naik, 2000; Geetha *et al.*, 2009; Geetha and Balakrishnan, 2010; Visalakshi *et al.*, 2016; Prabakaran *et al.*, 2020). In this study, the increase in millable canes obtained in the field where parasitoids reared on ESW eggs were released showed the potential of mass rearing *T. chilonis* on large host eggs at the farm-level, as the ESW eggs are a by-product of silk production. Therefore, the farm-level production of *T. chilonis* on ESW eggs may be encouraged to produce a large number of parasitoids in less time and at a lower production cost to obtain sustainable cane yield. Augmentative releases of *T. chilonis* in sugarcane, rather than applying insecticides, can maintain stem borers below the action threshold and conserve natural enemy biodiversity (Snyder, 2019).

## Conclusions

This study demonstrates the utilization and suitability of ESW eggs as a factitious host for mass rearing *T. chilonis* for the management of sugarcane borers. The large-sized eggs are a by-product of silk production and can be utilized to produce more parasitoids in less time with minimum inputs and lower production costs than on *C. cephalonica* eggs. *T. chilonis* reared from ESW were efficient in parasitizing the target pest and reducing crop damage with an improved millable cane yield, and were comparable to the parasitoids reared from the laboratory host,

*C. cephalonica*. This suggests the suitability of ESW eggs for farm level production of *T. chilonis*, and the *C. cephalonica* eggs are more viable for the production of parasitoids at commercial insectaries or institutions. Future research should focus on large-scale production of parasitoids on ESW eggs and their validation as biocontrol agents to support Integrated Pest Management to reduce the crop damage.

## Acknowledgements

The authors thank the Director, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India providing the research facilities. The authors are also thankful to Nanjundaswamy and Mahadevappa for providing sugarcane fields for experimental trials and the field assistants, Ravichandra, Suresh, Raja and Jayamma, for assisting during the field trial.

## References

- ANANTHANARAYANA K., DAVID H., 1986.- Chemical control, pp. 423-435. In: *Sugarcane entomology in India* (DAVID H., EASWARAMOORTHY S., JAYANTHI R., Eds).- Sugarcane Breeding Institute, Coimbatore, India.
- AVASTHY P. N., TIWARI N. K., 1986.- The shoot borer (*Chilo infuscatellus* Snell), pp. 68-92. In: *Sugarcane entomology in India* (DAVID H., EASWARAMOORTHY S., JAYANTHI R., Eds).- Sugarcane Breeding Institute, Coimbatore, India.
- CHINNADURAI C., 2017.- Potential health benefits of sugarcane, pp. 1-11. In: *Sugarcane biotechnology: challenges and prospects* (MOHAN C., Ed.)- Springer, Cham, Switzerland.
- CROWDER D. W., NORTHFIELD T. D., STRAND M. R., SNYDER W. E., 2010.- Organic agriculture promotes evenness and natural pest control.- *Nature*, 466: 109-123.
- DAVID H., EASWARAMOORTHY S., JAYANTHI R., 1986.- *Sugarcane entomology in India*.- Sugarcane Breeding Institute, Coimbatore, India.
- DU W.-M., XU J., HOU Y.-Y., LIN Y., ZANG L.-S., YANG X., ZHANG J.-J., RUAN C.-C., DESNEUX N., 2018.- *Trichogramma* parasitoids can distinguish between fertilized and unfertilized host eggs.- *Journal of Pest Science*, 91: 771-780.
- EASWARAMOORTHY S., 1983.- Estimation of damage and losses caused by sugarcane pests, pp. 5-17. In: *Sugarcane pest management in India* (BALASUBRAMANIAN M., SOLAYAPPAN A. R., Eds).- Tamil Nadu Co-operative Sugar Federation, Madras, India.
- EASWARAMOORTHY S., NANDAGOPAL V., 1986.- Life tables of internode borer, *Chilo sacchariphagus indicus* (K.) on resistant and susceptible varieties of sugarcane.- *Tropical Pest Management*, 32: 221-228.
- FAO, 2020.- *FAOSTAT*.- Statistics database, Food and Agriculture Organization, Sugarcane area and production. [online] URL: <https://www.fao.org/faostat/en/#data/QC>
- FENG G. K., CHOU Y. L., CHANG K. S., NIEH S. H., 1977.- Studies of the control of European corn borer by using *Trichogrammatid* egg parasites.- *Acta Entomologica Sinica*, 20: 253-258.
- GEETHA N., BALAKRISHNAN R., 2010.- Dispersal pattern of *Trichogramma chilonis* Ishii in sugarcane field.- *Journal of Biological Control*, 24: 1-7.
- GEETHA N., SHEKINAH E., RAKKIYAPPAN P., 2009.- Comparative impact of release frequency of *Trichogramma chilonis* Ishii against *Chilo sacchariphagus indicus* (Kapur) in sugarcane.- *Journal of Biological Control*, 23: 343-351.

- GREENBERG S. M., 1991.- Evaluation techniques for *Trichogramma* quality, pp. 138-145. In: *Proceedings of the 5<sup>th</sup> workshop of the IOBC global working group "Quality control of mass reared arthropods"* (BIGLER F., Ed.).- Wageningen, Netherlands.
- GREENBERG S. M., NORDLUND D. A., KING E. G., 1998.- A review of production of factitious hosts for the use in mass rearing of *Trichogramma* spp. in the former Soviet Union, the United States, Western Europe, and China.- *Journal of Entomological Sciences*, 33 (1): 15-32.
- HUANG S. S., ZANG L. S., RUAN C. C., 2013.- Parasitization ecology, mass production, and application of *Trichogramma*.- Science Press, Beijing, China.
- IQBAL A., HOU Y.-Y., CHEN Y.-M., ALI A., MONTICELLI L. S., DESNEUX N., ZANG L.-S., 2020.- Impact of *Trichogramma* parasitoid age on the outcome of multiparasitism in the factitious host eggs of Chinese oak silkworm, *Antheraea pernyi*.- *Journal of Pest Science*, 93: 1347-1357.
- JALALI S. K., RABINDRA R. J., RAO N. S., DASAN C. B., 2003.- Mass production of trichogrammatids and chrysopids. p. 16. *Technical Bulletin No. 33*.- Project Directorate of Biological Control, Bangalore, Karnataka, India.
- LALITHA Y., BALLAL C. R., 2015.- Influence of seasons and inoculum dosages on the production efficiency of *Corcyra cephalonica* Stainton.- *Journal of Biological Control*, 29: 25-30.
- LALITHA Y., JALALI S. K., VENKATESAN T., SRIRAM S., 2010.- Production attributes of *Trichogramma* reared on eri silkworm eggs vis-à-vis *Corcyra* eggs and economics of rearing system. pp. 47. In: *Blueprint for the future of arthropod rearing and quality assurance. International 12<sup>th</sup> workshop of the IOBC global working group on arthropod mass rearing and quality control (IOBC-AMRQC)*, 18-22 October 2010. International Atomic Energy Agency (IAEA), Vienna International Centre, Vienna, Austria.
- LONG W. H., HENSLEY S. D., 1972.- Insect pests of sugarcane.- *Annual Review of Entomology*, 17: 149-176.
- MAHESH P., SRIKANTH J., CHANDRAN K., SINGARAVELU B., 2016.- Resistance of *Saccharum* spp. against *Chilo sacchariphagus indicus* (Kapur) (Lepidoptera: Crambidae) in India.- *Experimental Agriculture*, 54 (1): 83-95.
- NAGARAJA H., 2013.- Mass production of trichogrammatid parasitoids. pp. 174-190. In: *Biological control of insect pests using egg parasitoids* (SITHANANTHAM S., BALLAL C. R., JALALI S. K., BAKTHAVATSALAM N., Eds).- Springer, New Delhi, India.
- PARRA J. R. P., COELHO A. JR., 2022.- Insect rearing techniques for biological control programs, a component of sustainable agriculture in Brazil.- *Insects*, 13 (1): 105.
- PRABAKARAN M., SITHANANTHAM S., SANJAYAN K. P., KRISHNA MOHAN R., PARIMALARANGAN S., NAIDU S. E., 2020.- Impact of *Trichogramma* release regimes and combination with mass trapping on sugarcane internode borer (*Chilo sacchariphagus indicus*) management.- *Journal of Biological Control*, 34 (2): 119-123.
- RACHAPPA V., NAIK L. K., 2000.- Dispersion of *Trichogramma chilonis* Ishii in sugarcane ecosystem.- *Karnataka Journal of Agricultural Sciences*, 13: 867-871.
- SAS INSTITUTE, 2011.- *SAS version 9.3 system options: reference, 2<sup>nd</sup> edition*.- SAS Institute, Cary, NC, USA.
- SHARMA S., KOONER R., ARORA R., 2017.- Insect pests and crop losses. pp. 45-66. In: *Breeding insect resistant crops for sustainable agriculture* (ARORA R., SANDHU S., Eds).- Springer, Singapore.
- SHARMA S., SHERA P. S., KAUR R., SANGHA K. S., 2020.- Evaluation of augmentative biological control strategy against major borer insect pests of sugarcane -a large-scale field appraisal.- *Egyptian Journal of Biological Pest Control*, 30: 1-10.
- SHEN X. C., WANG K. Z., MENG G., 1986.- Field experiment of inoculative releases of *Trichogramma* spp. in the early season in Henan Province.- *Chinese Journal of Biological Control*, 2: 152-154.
- SHUKLA S. K., SHARMA L., AWASTHI S. K., PATHAK A. D., 2017.- Sugarcane in India (package of practices for different agro-climatic zones).- *AICRP(S), Technical Bulletin*, 1: 1-64.
- SMITH S. M., 1996.- Biological control with *Trichogramma*: advances, successes, and potential of their use.- *Annual Review of Entomology*, 41 (1): 375-406.
- SNYDER W. E., 2019.- Give predators a complement: conserving natural enemy biodiversity to improve biocontrol.- *Biological Control*, 135: 73-82.
- SRIKANTH J., EARWARAMOORTHY S., JALALI S. K. 2016.- A 100 years of biological control of sugarcane pests in India.- *Commonwealth Agricultural Bureau Reviews*, 11: 1-32.
- THIRUMURUGAN A., JOSEPH M., SUDHAGAR R., GANESAN N. M., 2006.- Improving efficacy of *Trichogramma chilonis* against shoot borer, *Chilo infuscatellus* (Snellen) in sugarcane ecosystem of tropical India.- *Sugar Tech*, 8: 155-159.
- VAN LENTEREN J. C., HALE A., KLAPWIJK J. N., SCHELT J., STEINBERG S., 2003.- Guidelines for quality control of commercially produced natural enemies, pp. 265-304. In: *Quality control and production of biological control agents: theory and testing procedures* (VAN LENTEREN J. C., Ed.).- CABI Publishing, Wallingford, UK.
- VISALAKSHI M., BHAVANI B., RAO S. G., 2016.- Field evaluation of *Trichogramma chilonis* (Ishii) for the management of shoot borers in sugarcane.- *International Journal of Bio-resource and Stress Management*, 7: 915-918.
- WANG Z. Y., HE K. L., ZHANG F., LU X., BABENDREIER D., 2014.- Mass rearing and release of *Trichogramma* for biological control of insect pests of corn in China.- *Biological Control*, 68: 136-144.
- XU W., WEN X.-Y., HOU Y.-Y., DESNEUX N., ALI A., ZANG L.-S., 2020.- Suitability of Chinese oak silkworm eggs for the multigenerational rearing of the parasitoid *Trichogramma leucaniae*.- *PLoS ONE*, 15 (4): e0231098.
- YALAWAR S., PRADEEP S., AJITH KUMAR M. A., HOSAMANI V., RAMPURE S., 2010.- Biology of sugarcane internode borer, *Chilo sacchariphagus indicus* (Kapur).- *Karnataka Journal of Agricultural Sciences*, 23 (1): 140-141.
- ZANG L.-S., WANG S., ZHANG F., DESNEUX N., 2021.- Biological control with *Trichogramma* in China: history, present status and perspectives.- *Annual Review of Entomology*, 66: 463-484.

**Authors' addresses:** Yadavalli LALITHA (corresponding author: ylalitha31121961@gmail.com), Omprakash NAVIK, Richa VARSHNEY, Chandish Ramakrishna BALLAL, ICAR-National Bureau of Agricultural Insect Resources, Post Bag No. 2491, H.A. Farm Post, Bellary Road, Hebbal, Bengaluru - 560024, Karnataka, India; Veeranna Nagappa PATEL, Zonal Agricultural Research Station, V.C. Farm, Mandya - 571405, Karnataka, India.

Received June 13, 2022. Accepted September 9, 2022.