

## Original Research

DOI : <http://doi.org/10.22438/jeb/44/4/MRN-4085>

## Evaluation of comparative parasitic efficiency and biology of hymenopteran egg parasitoids, *Trichogramma chilonis* and *T. japonicum* on *Corcyra cephalonica* and *Helicoverpa armigera*

S. Roy<sup>1</sup>, B.K. Mishra<sup>1</sup> and K. Roy<sup>2\*</sup> <sup>1</sup>Department of Entomology, Shiksha O Anusandhan, Deemed to be University, Bhubaneswar-751 029, India<sup>2</sup>Department of Entomology, F./Ag., Bidhan Chandra Krishi Viswavidyalaya, Mohanpur-741 252, India\*Corresponding Author Email : [roy.kusal@bckv.edu.in](mailto:roy.kusal@bckv.edu.in)\*ORCID: <https://orcid.org/0000-0002-5179-2373>

Received: 05.01.2022

Revised: 10.08.2022

Accepted: 02.01.2023

## Abstract

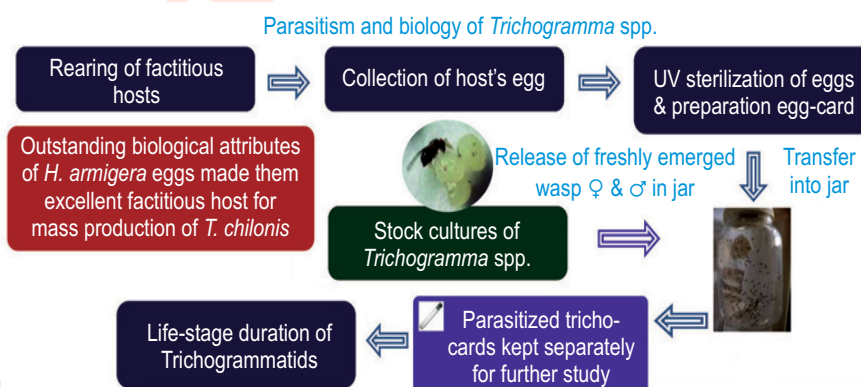
**Aim:** To evaluate the comparative parasitic efficiency and biology of Trichogrammatid wasps on noble laboratory host *Corcyra cephalonica* as well as on *Helicoverpa armigera*, the cosmopolitan, polyphagous and precarious insect pest of many cultivated crops.

**Methodology:** The biology of *Trichogramma chilonis* and *T. japonicum*, their sex ratio, and parasitic efficiency were studied. Host insects were reared on artificial diet to obtain their eggs for initiating the experiment. Egg-card pieces were prepared with the irradiated eggs of respective host insects. Egg-cards were introduced into the tubes containing freshly emerged adult parasitoids. Parasitized egg-cards (Trichocards) were kept separately in plastic tube to study the biological attributes and percent parasitization of the parasitoids. Egg cards of different host eggs taking eggs of different age groups were prepared to study the effect of age of host eggs on the biological efficiency of the parasitoids.

**Results:** Irrespective of *Trichogramma* species, the duration (d) of eggs to adult period was more when reared on the eggs of *H. armigera* than on the eggs of *C. cephalonica*. Maximum life stage duration of *T. chilonis* and *T. japonicum* from egg to adult was 8.2 days and 10.2 days, respectively, when reared on *H. armigera* eggs. There was maximum parasitization (84.8%) of fresh eggs of *C. cephalonica* by *T. chilonis* as compared to the egg parasitization of 73.4% by *T. japonicum*. Likewise, extreme parasitization of fresh eggs of *H. armigera* was recorded as 62.4% when parasitized by *T. chilonis* against 59.7% parasitization by *T. japonicum*.

**Interpretation:** According to the study, *H. armigera* eggs have the finest biological and parasitic features in terms of the highest adult longevity, sex ratio, mean progeny female<sup>-1</sup>, and mean adult egg<sup>-1</sup>, making them the best for mass production of the egg parasitoids, *T. chilonis*.

**Key words:** *Corcyra cephalonica*, Host eggs, *Helicoverpa armigera*, Parasitization, *Trichogramma*



**How to cite :** Roy, S., B.K. Mishra and K. Roy: Evaluation of comparative parasitic efficiency and biology of hymenopteran egg parasitoids, *Trichogramma chilonis* and *T. japonicum* on *Corcyra cephalonica* and *Helicoverpa armigera*. *J. Environ. Biol.*, **44**, 630-638 (2023).

## Introduction

The spread of insecticide resistance and environmental problems linked with chemical control, has led to intensified research on alternative control methods; for example, biological control. Egg parasitoids of the genus *Trichogramma* (Westwood) (Hymenoptera: Trichogrammatidae) are widely used in biological control of large number of lepidopteran pests (Guo et al., 2019; Qu et al., 2020; Zang et al., 2021). *Trichogramma* consists of approximately 620 species and about 80 genera of insect egg-parasitoids (Vinson et al., 2015) and present in varied habitat across the world (Romeis et al., 2005; Knutson, 2005; Sumer et al., 2009). Due to ease of replication, host suitability, degree of adaptation to the local agro-ecosystem and compatibility with other pest management methods, a few *Trichogramma* species are widely used to control lepidopteran pests on cash crops (Cherif et al., 2019; Mansour and Biondi, 2021; Zang et al., 2021). They can eliminate pests during the egg stage and therefore, preclude crop damage caused by the larval stage. *Trichogramma* spp. have several attributes that make them potentially useful as biological control agents.

A short life cycle of 8-10 days and a high reproductive potential, i.e., a female *Trichogramma* wasp can lay about 50-100 eggs in 6-7 days of active egg laying period (Park et al., 2000) with maximum of 60-90% of female progenies contributed them to build up a huge population rapidly and made affordable compared to other parasitoids (Parra and Zucchi, 2004). In recent years, over 32 million ha of agricultural and forest lands across 40 countries in the world has been treated annually with *Trichogramma* spp. to control insect pests (Li, 1994; Zang et al., 2021). The ecological and biological characteristics of *Trichogramma* spp. were studied around the world (Zang et al., 2021). In biological control programme involving parasitoid release, it is essential to demonstrate its potential to suppress a pest population. For a polyphagous parasitoid, the suitability of the factitious hosts may vary greatly. Characteristics of host-egg such as volume, chorion thickness, nutritional content, age and egg distribution can affect the rate of parasitism as well as the number, quality and sex ratio of parasitoids (Hoffmann et al., 1995; Greenberg et al., 1996; Pomari et al., 2012). *Trichogramma* parasitism levels vary substantially depending on the habitat, plant or plant structure where the host eggs are found (Romeis et al., 2005).

In addition to the direct suitability of a factitious host, there is also a potential for an evolutionary response by the parasitoid being reared for many generations on an alternate host. Laboratory selection for physiological, morphological, or behavioral characteristics that are adaptive when the factitious host is used may cause a parasitoid to perform less well on the target host (Hoffmann et al., 2001). Several species of *Trichogramma* are available for controlling different crop pests, the most common being *T. chilonis* and *T. japonicum*. A deeper comprehension of the development and behaviour of parasitoids on various factitious hosts is necessary to investigate the possibility of parasitoids serving as biological control agents for

crop pests (Chailleux et al., 2012; Li et al., 2019). The study was undertaken with an aim to evaluate the comparative parasitic efficiency and biological attributes of two Trichogrammatid wasps, *Trichogramma chilonis* (Ishii) and *Trichogramma japonicum* (Ashmead) on *Corcyra cephalonica* (Stainton), the noble laboratory host cum common stored-grain pest, and on *Helicoverpa armigera* (Hübner), the cosmopolitan and precarious insect pest of many cultivated crops.

## Materials and Methods

Laboratory experiments were carried out in the Biological Control Laboratory of the Department of Entomology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar to evaluate the eggs of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) and *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) as hosts of the egg parasitoids *Trichogramma chilonis* Ishii and *Trichogramma japonicum* Ashmead. Biology of the parasitoids, their sex ratio, and parasitic efficiency were studied in detail in the laboratory at  $27\pm 2^{\circ}\text{C}$  and  $70\pm 3\%$  RH. The eggs of *C. cephalonica* and the cultures of *T. chilonis* and *T. japonicum* were procured from the Bio-control Laboratory of the Department of Entomology, OUAT to initiate the experiment. Larvae of *H. armigera* were collected from arhar (*Cajanus cajan*) crop raised at the Central Farm of OUAT, Bhubaneswar, Odisha. *C. cephalonica*, *H. armigera*, and the Trichogrammatids (hymenopteran egg-parasitoids) were reared in the laboratory following the procedure described by Parra (2008).

**Rearing of *C. cephalonica*:** Overnight laid *Corcyra* eggs (not exposed to UV light) were procured from the Bio-control Laboratory of the Department of Entomology. Wooden box measuring  $15\times 30\times 45$  cm provided with ventilator holes and a lid to close the box was used for rearing *Corcyra*. Bold grains of maize meant for human consumption were milled to make 3-4 pieces of each grain and about 2.5 kg grain were added to each box. The box was charged with 0.5cc of *Corcyra* eggs and streptomycin sulphate (0.5g for 2.5kg of grains) to prevent mould growth. The larvae that hatched within 3-5 days started to feed. At this stage, light webbings were noticed on the surface. As the larvae grew up, they moved down. During this period, the larvae were allowed to grow undisturbed in the box. They pupate inside opaque whitish cocoon surrounded by webbed grains. Approximately, after 40 days of charging, the moths started emerging. They were either aspirated with mechanical moth collector or collected with specimen tubes. The entire operation was carried out in a tent of mosquito net to prevent the moths from escaping. The collected moths were transferred to the specially designed oviposition cage. The eggs collected were cleaned to remove scales and broken limbs. The eggs obtained were used to continue the succeeding generation.

**Rearing of *H. armigera*:** Mature larvae of *H. armigera* were collected from the arhar (*Cajanus cajan*) crop raised at the Central Farm of OUAT, Bhubaneswar, Odisha. Sterilized vials measuring  $10\text{ cm} \times 2\text{ cm}$  were arranged in plastic trays. Single larva was introduced in each vial, fed with pre-soaked Kabuli

chana (*Cicer arietinum*) and closed with a cotton plug. The vials were cleaned everyday and the larva was fed with fresh food. After 6<sup>th</sup> instar, when the full-grown larvae were ready to pupate, they were placed loosely on a petridish and kept inside a cage, provided required temperature ( $27.3 \pm 2.6^\circ\text{C}$ ) and humidity ( $74 \pm 2\%$ ) were maintained. After 8-10 days, when adult moth emerged then they were transferred to glass jar which was covered with black cloth, fastened with rubber band. The moths were fed with 50% honey solution. Female moths laid eggs all over the inner surface of the cloth cover. Cloth strip along with the eggs was surface sterilized with 0.15 % sodium hypochlorite for 1 min and thereafter washed with water. After 3-5 days of incubation, larva hatched out; the neonates were fed with the fruits of lady's finger. A week later, the larvae were transferred individually into the vials and fed with pre-soaked Kabulichana. Larval period was completed within 12-14 days and the same procedure was followed to maintain the host insect culture.

**Biology of *T. chilonis* and *T. japonicum* on different host eggs:** Egg-card pieces (15 × 10 cm) were prepared from the drawing sheets (70 × 56 cm). One cubic cm of *C. cephalonica* and *H. armigera* eggs were spread uniformly on the glued surface of the card and labelled. Irradiated (irradiation was done for half an hour under 15 watt UV light) egg-card of each host insect was introduced separately into the glass jar containing freshly emerged parasitoid adults (females and males). Parasitized egg-card (Trichocard) cut into pieces having ~100 parasitized eggs per card, labelled properly and placed inside a plastic tube (make: Tarson) whose top was secured with a muslin cloth and kept for adult emergence. A total of forty tubes (20 each for every host insect) were maintained for two parasitoids to study different biological parameters. The trichocards turned black after 4-8 days due to parasitization by the parasitoids. The cards were removed from the tubes at an interval of 6 hrs and observed under a stereoscopic bionocular zoom to record the developmental stages of the parasitoid. The egg-larval period was determined looking at the blackening of the egg colour. The observation on pupal period, percent mean number of progenies per female, mean number of adult emergences, total life period from egg to adult, adult longevity and sex ratio were recorded visually.

**Percent parasitization and viability:** To work out the degree of parasitization and emergence of *T. chilonis* and *T. japonicum*, parasitized egg-cards (Trichocards) as described above were used. Immediately after getting black eggs of different hosts, the total number of eggs it contained and number of eggs parasitized were counted. Later, cards were allowed for adult emergence inside the polythene bag (40cm × 20cm) kept in insect rearing cage. After the emergence of adult, the number of adults emerged from the cards was counted. The percent parasitization and percent emergence was calculated by simple arithmetic formulae.

**Effect of age of host eggs on the biological efficiency of parasitoids:** Egg-cards of different host eggs were prepared taking eggs of different age groups, viz., fresh (0hr), 24, 48, 72 and 96 hr after irradiation. Cards containing 100 UV-irradiated eggs

were placed in plastic tubes (12×3.5cm) containing 20 pairs of one-day-old male and female of *T. chilonis* and *T. japonicum*. Ten such tubes were maintained and arranged in plastic trays for both parasitoids species. BOD incubator was set at  $27 \pm 2^\circ\text{C}$  and  $70 \pm 3\%$  RH and the observations on parasitization, adult emergence and percentage of female and adult longevity were recorded on each age group of eggs for all the host insects. The experiment was arranged with 10 replications for each respective age group of eggs. During the experiment, the adults of *T. chilonis* and *T. japonicum* were fed with 50% honey solution for all the treatments.

**Statistical analyses:** Data were analyzed following Completely Randomized Design (CRD) for working out the ANOVA after making suitable transformation for uniform distribution of data (Gomez and Gomez, 1984) in OPSTAT (Sheoran et al., 1998). The significant effect of treatments was measured by F-test to compute LSD at  $p < 0.05$ . Duncan's Multiple Range Test at  $p < 0.05$  was performed using MSTATC software for pair-wise comparison of the treatment means as well as to reduce the Type I error. Graph was made in MS Excel 2013 version.

## Results and Discussion

The egg-larval period of *T. chilonis* was significantly different ( $p < 0.05$ , df-18, F-stat- 86.6, LSD- 0.11) in different host eggs, ranging from the highest duration of  $5.1 \pm 0.03$  SEM d in *H. armigera* eggs to a minimum duration of  $4.7 \pm 0.04$  SEM d in *C. cephalonica* eggs (Fig. 1). The pupal period also varied significantly ( $p < 0.05$ , df-18, F-stat- 32.6, LSD- 0.19) among the host eggs. It ranged from a minimum of  $3.2 \pm 0.03$  SEM d in *C. cephalonica* eggs to a maximum of  $3.7 \pm 0.08$  SEM d in *H. armigera* eggs (Fig. 1). The maximum total life period of *T. chilonis* from egg to adult was recorded in *H. armigera* ( $8.2 \pm 0.05$  SEM d) which was higher than *C. cephalonica* ( $7.8 \pm 0.04$  SEM d) (Fig. 1). The longevity of females differed statistically ( $p < 0.05$ , df-18, F-stat- 64.1, LSD- 0.07) between hosts being,  $3.7 \pm 0.03$  SEM d and  $3.4 \pm 0.02$  SEM d in *H. armigera* and *C. cephalonica*, respectively (Fig. 2). In contrast, the longevity of male varied between 2.7-2.8 d, irrespective of the hosts. This indicated that females live longer than males. Sex ratio was also highest in *C. cephalonica* eggs (1:3.2) than *H. armigera* eggs (1:2.3).

The mean number of progeny female<sup>-1</sup> of *T. chilonis* was significantly high ( $p < 0.05$ , df-18, F-stat- 4318.1, LSD- 0.10) in *H. armigera* eggs ( $24.8 \pm 0.04$  SEM) whereas, it was low in *C. cephalonica* eggs ( $21.7 \pm 0.03$  SEM) (Fig. 3). Likewise, the development of number of adults of *T. chilonis* per egg of *Helicoverpa* ( $3.2 \pm 0.03$  SEM) was significantly higher ( $p < 0.05$ , df-18, F-stat- 103.2, LSD- 0.10) than the production of number of adults of *T. chilonis* per egg of *Corcyra* ( $2.7 \pm 0.04$  SEM) (Fig. 3). It has been observed that the egg-larval period of *T. japonicum* was significantly ( $p < 0.05$ , df-18, F-stat- 158.6, LSD- 0.12) maximum ( $5.9 \pm 0.05$  SEM d) on *H. armigera* eggs than on *C. cephalonica* eggs ( $5.2 \pm 0.03$  SEM d) (Fig. 1). Thus, the significant ( $p < 0.05$ , df-18, F-stat- 378.8, LSD- 0.10) maximum total life period (egg to

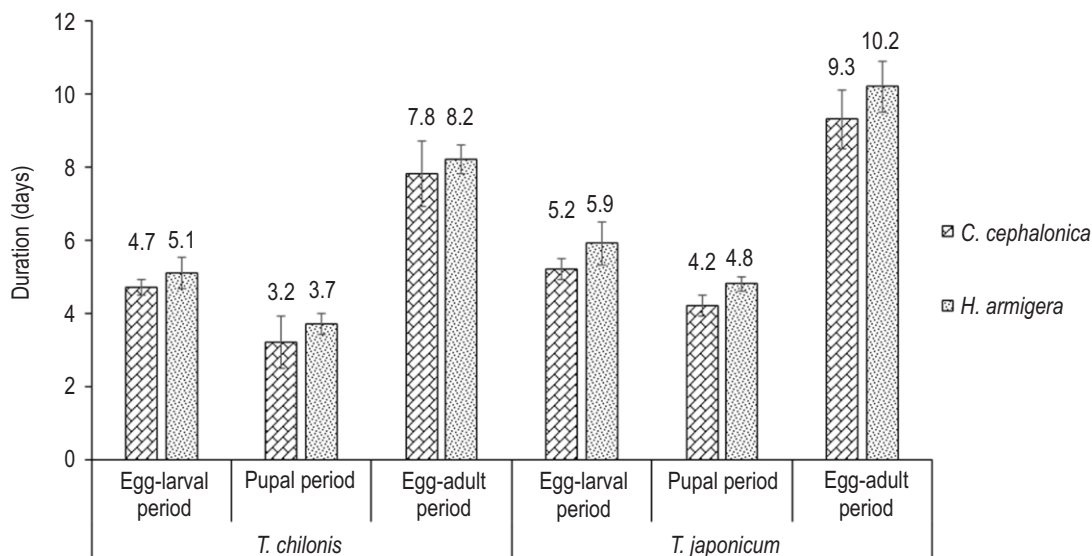


Fig. 1: Duration of egg-larval, pupal and egg-adult period of *T. chilonis* and *T. japonicum* reared on different factitious host eggs. Error bars are SEM.

adult) of *T. japonicum* was recorded as  $10.2 \pm 0.04$  SEM d in *H. armigera* and  $9.3 \pm 0.03$  SEM d in *C. cephalonica* (Fig. 1). At the same time, the longevity of females differed ( $p < 0.05$ , df-18, F-stat- 172.8, LSD- 0.12) between hosts being,  $4.8 \pm 0.05$  SEM d and  $4.1 \pm 0.03$  SEM d in *H. armigera* and *C. cephalonica*, respectively (Fig. 2). On the contrary, the longevity of males ranged between 3.1-3.3 days irrespective of the hosts. The study indicated that the females live longer than the males. Sex ratio was observed to be 1:3.2 and 1:2.3 in *C. cephalonica* and *H. armigera*, respectively. This reveals that the sex ratio of the parasitoid did not vary greatly between, *C. cephalonica* and *H. armigera*. The mean number of progeny per female was statistically higher ( $p < 0.05$ , df-18, F-stat- 815.2, LSD- 0.14) in *H. armigera* ( $22.6 \pm 0.06$  SEM) than *C. cephalonica* ( $20.8 \pm 0.04$  SEM) (Fig. 3). Only 71.4% of adults emerged from *C. cephalonica* eggs due to small size whereas 76.6% adults emerged from *H. armigera* eggs. *Corcyra* eggs being smaller, harboured the offsprings for short time. *Corcyra* eggs are smaller than *Helicoverpa* eggs (Anantanawat et al., 2016). This can affect the number of *Trichogramma* that can develop inside the egg, as smaller eggs may only be able to support a few *Trichogramma* larvae.

In the present study, the mean progeny per female was higher in the eggs of *H. armigera* than *C. cephalonica*. Similar kind of trend about the suitability of different host eggs for *T. pretiosum* and *Trichogrammatoidea lutea* was observed (Greenberg and Podbereszkya, 1983; Mawela et al., 2013). Bai et al. (1992) compared the quality of *T. pretiosum* adults reared from different hosts and concluded that it depends on the host's size from which it has emerged. In general, most *Trichogramma* species prefer to oviposit on medium-to large-sized eggs (Schmidt, 1994). The size of host egg influences the emergence of parasitoids. The smaller the eggs, the greater will be the

depletion of nutrients for embryonic development of parasitoid and subsequently, its biology and performance. The eggs of *H. armigera* and *C. cephalonica* had an approximate volume of  $0.08 \text{ mm}^3$  and  $0.036 \text{ mm}^3$ , respectively (Anantanawat et al., 2016; Consoli et al., 1999). This may be the reason for sheltering more number of progeny of *Trichogramma* spp. in *H. armigera*. Apart from the egg volume, its surface characteristics, chorion design, layers, etc., govern the parasitoid development. The chorion surface of *C. cephalonica* has a rugged texture (Consoli et al., 1999) however, the top of the egg of *H. armigera* is smooth, otherwise the surface contains approximately 24 longitudinal ribs (Bhatt and Patel, 2001), which may lead to the greater preference and development of progeny of *T. chilonis* in *H. armigera* eggs in the present study.

The maximum parasitization of *C. cephalonica* eggs by *T. chilonis* (84.8%) and *T. japonicum* (73.4%) was observed in fresh eggs, followed by one-day-old eggs where per cent parasitization was recorded 80.7 and 71.2, respectively (Table 1). The degree of egg parasitization by the Trichogrammatid wasp was inversely related with the age of host eggs. The adult emergence of *T. chilonis* and *T. japonicum* was maximum (74.8% and 71.4%) from fresh eggs of *C. cephalonica*, followed by 24hr, 48hr, 72hr and 96hr old eggs (Table 1). No significant difference was noted between fresh and one-day-old eggs with respect to adult emergence, but it differed significantly between eggs of other age group. The per cent emergence of adult decreased significantly as the age of the host egg was more than 24 hr. The production of female wasp species and longevity of adult (including male and female) was not significantly affected by the various age-groups of *C. cephalonica* eggs (Table 1). A general trend of gradual decline in female wasp production and adult longevity with the increase of age of host eggs was observed. Parasitization of *H.*

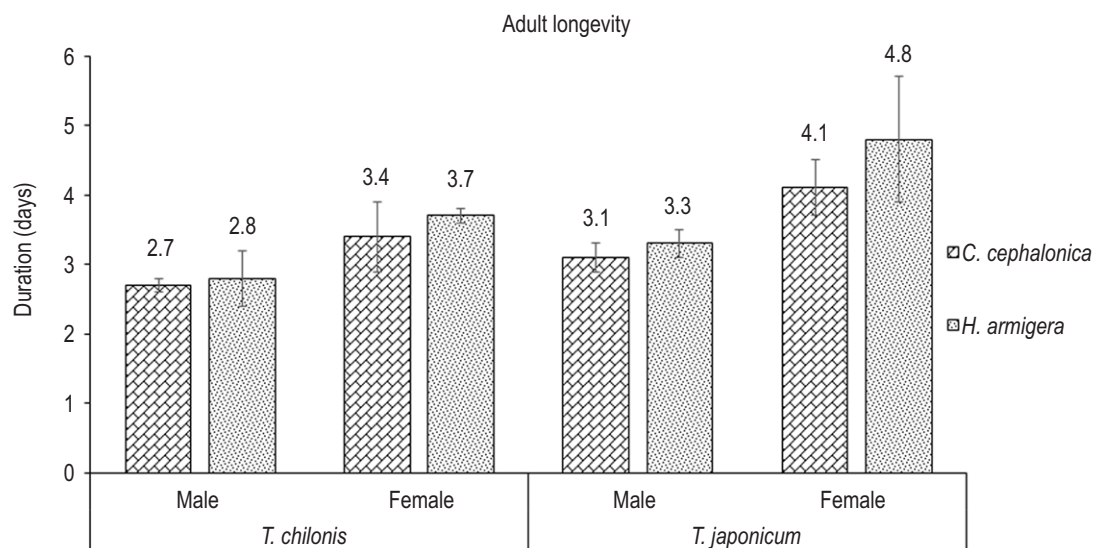


Fig. 2: Longevity of adult male and female of *T. chilonis* and *T. japonicum* reared on different factitious host eggs. Error bars are SEM.

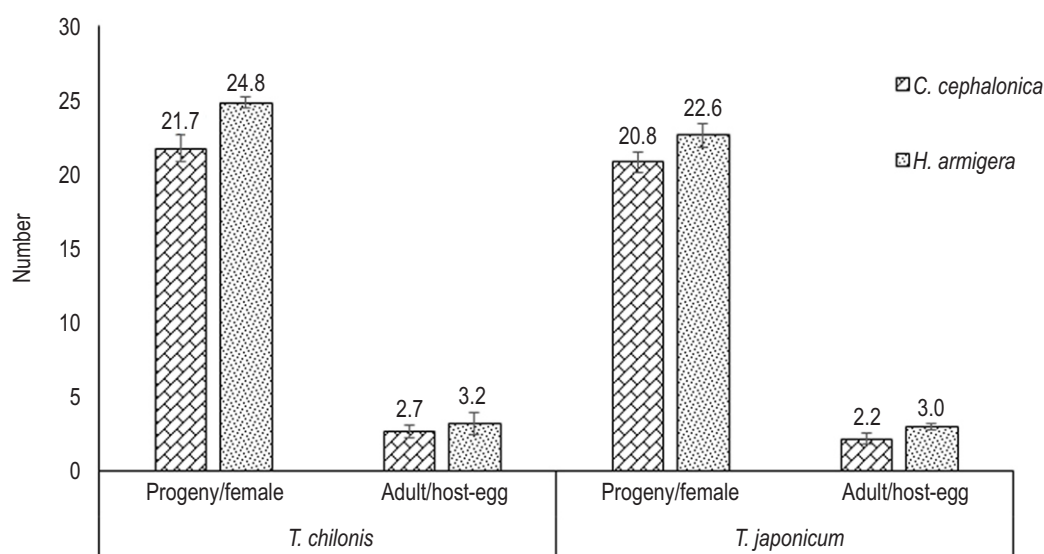


Fig. 3: Number of progeny per female of *T. chilonis* and *T. japonicum* reared on different factitious host eggs and number of adult *T. chilonis* and *T. japonicum* produced per host egg. Error bars are SEM.

*armigera* eggs by *T. chilonis* (62.4%) and *T. japonicum* (59.7%) was maximum in freshly laid eggs (Table 2). The fresh, 24 hr and 48 hr old eggs of *H. armigera* were statistically at par with regard to the parasitization by *T. chilonis*, however, the rate of parasitization by the wasp species significantly varied for 72 and 96 hr old eggs. A dramatic change in egg parasitization by *T. japonicum* was observed with a shift from fresh to 24 hr old eggs. The per cent emergence of *T. chilonis* (86.5) and *T. japonicum*

(76.6) adult was maximum when fresh *H. armigera* eggs were used as host for parasitic wasps. It decreased as the age of eggs increased. Production of female wasps to the maximum extent was observed when fresh eggs of *H. armigera* were used as host, though the age of eggs had no significant influence on female wasp production. Adult longevity of *T. chilonis* and *T. japonicum* was maximum when fresh host eggs were used,  $4.7 \pm 0.62$  d and  $4.8 \pm 0.82$  d, respectively (Table 2). The longevity of wasps

Table 1: Effect of age of host (*C. cephalonica*) eggs on the biological attributes of *T. chilonis* and *T. japonicum*

| <i>T. chilonis</i> |     |                           |                          |                          |                         |                 |     |                          |                          | <i>T. japonicum</i>       |                          |                 |     |                          |                           |                           |                          |                 |     |
|--------------------|-----|---------------------------|--------------------------|--------------------------|-------------------------|-----------------|-----|--------------------------|--------------------------|---------------------------|--------------------------|-----------------|-----|--------------------------|---------------------------|---------------------------|--------------------------|-----------------|-----|
| Age of eggs (h)    | N   | % Parasitization          | Adult emergence %        | Female (%)               | Adult longevity (d)     | Age of eggs (h) | N   | % Parasitization         | Adult emergence %        | Female (%)                | Adult longevity (d)      | Age of eggs (h) | N   | % Parasitization         | Adult emergence %         | Female (%)                | Adult longevity (d)      | Age of eggs (h) | N   |
| Fresh              | 100 | 84.8 ± 3.84 <sup>a</sup>  | 74.8 ± 3.92 <sup>a</sup> | 62.4 ± 2.81 <sup>a</sup> | 4.1 ± 0.41 <sup>a</sup> | Fresh           | 100 | 73.4 ± 1.96 <sup>a</sup> | 71.4 ± 2.18 <sup>a</sup> | 53.8 ± 2.10 <sup>a</sup>  | 4.7 ± 1.31 <sup>a</sup>  | 24              | 100 | 71.2 ± 2.36 <sup>a</sup> | 70.1 ± 3.42 <sup>a</sup>  | 50.7 ± 1.86 <sup>ab</sup> | 4.2 ± 0.90 <sup>a</sup>  | 48              | 100 |
| 24                 | 100 | 80.7 ± 2.91 <sup>a</sup>  | 72.9 ± 1.43 <sup>a</sup> | 61.1 ± 1.23 <sup>a</sup> | 3.8 ± 0.32 <sup>a</sup> | 24              | 100 | 69.8 ± 1.90 <sup>a</sup> | 61.5 ± 2.10 <sup>b</sup> | 51.3 ± 2.79 <sup>ab</sup> | 4.1 ± 0.91 <sup>a</sup>  | 48              | 100 | 59.7 ± 2.30 <sup>b</sup> | 55.3 ± 3.71 <sup>bc</sup> | 50.6 ± 2.09 <sup>ab</sup> | 3.8 ± 0.62 <sup>ab</sup> | 72              | 100 |
| 48                 | 100 | 74.1 ± 4.52 <sup>ab</sup> | 63.5 ± 2.71 <sup>b</sup> | 59.7 ± 2.13 <sup>a</sup> | 3.7 ± 0.41 <sup>a</sup> | 48              | 100 | 43.6 ± 3.61 <sup>c</sup> | 40.9 ± 2.58 <sup>d</sup> | 50.3 ± 1.86 <sup>ab</sup> | 3.8 ± 0.10 <sup>ab</sup> | 72              | 100 |                          |                           |                           |                          | 96              | 100 |
| 72                 | 100 | 55.9 ± 3.48 <sup>c</sup>  | 51.6 ± 4.29 <sup>c</sup> | 58.9 ± 3.10 <sup>a</sup> | 3.7 ± 0.93 <sup>a</sup> | 72              | 100 |                          |                          |                           |                          | 96              | 100 |                          |                           |                           |                          |                 |     |
| 96                 | 100 | 49.8 ± 2.93 <sup>c</sup>  | 49.2 ± 3.08 <sup>c</sup> | 55.6 ± 1.8 <sup>ab</sup> | 3.6 ± 0.81 <sup>a</sup> | 96              | 100 |                          |                          |                           |                          |                 |     |                          |                           |                           |                          |                 |     |

Values are mean ± S.D.; Mean followed by different alphabets in a column are significantly different according to DMRT at  $p < 0.05$

Table 2: Effect of age of host (*H. armigera*) eggs on the biological attributes of *T. chilonis* and *T. japonicum*

| <i>T. chilonis</i> |     |                           |                           |                           |                          |                 |     |                           |                          | <i>T. japonicum</i>      |                          |                 |     |                           |                           |                          |                          |                 |     |
|--------------------|-----|---------------------------|---------------------------|---------------------------|--------------------------|-----------------|-----|---------------------------|--------------------------|--------------------------|--------------------------|-----------------|-----|---------------------------|---------------------------|--------------------------|--------------------------|-----------------|-----|
| Age of eggs (h)    | N   | % Parasitization          | Adult emergence %         | Female (%)                | Adult longevity (d)      | Age of eggs (h) | N   | % Parasitization          | Adult emergence %        | Female (%)               | Adult longevity (d)      | Age of eggs (h) | N   | % Parasitization          | Adult emergence %         | Female (%)               | Adult longevity (d)      | Age of eggs (h) | N   |
| Fresh              | 100 | 62.4 ± 1.92 <sup>a</sup>  | 86.5 ± 5.62 <sup>a</sup>  | 55.5 ± 0.92 <sup>a</sup>  | 4.7 ± 0.62 <sup>a</sup>  | Fresh           | 100 | 59.7 ± 3.48 <sup>a</sup>  | 76.6 ± 4.92 <sup>a</sup> | 51.2 ± 1.06 <sup>a</sup> | 4.8 ± 0.82 <sup>a</sup>  | 24              | 100 | 50.1 ± 4.47 <sup>b</sup>  | 72.2 ± 4.08 <sup>a</sup>  | 50.7 ± 0.80 <sup>a</sup> | 4.0 ± 0.72 <sup>a</sup>  | 48              | 100 |
| 24                 | 100 | 57.8 ± 2.24 <sup>a</sup>  | 79.3 ± 4.81 <sup>a</sup>  | 54.4 ± 0.86 <sup>a</sup>  | 4.0 ± 0.18 <sup>a</sup>  | 24              | 100 | 43.5 ± 4.08 <sup>bc</sup> | 64.5 ± 3.02 <sup>b</sup> | 51.0 ± 0.08 <sup>a</sup> | 3.9 ± 0.36 <sup>ab</sup> | 48              | 100 | 39.6 ± 3.92 <sup>cd</sup> | 59.8 ± 3.10 <sup>bc</sup> | 50.1 ± 1.03 <sup>a</sup> | 3.2 ± 0.89 <sup>bc</sup> | 72              | 100 |
| 48                 | 100 | 52.7 ± 3.08 <sup>ab</sup> | 72.9 ± 3.90 <sup>ab</sup> | 52.8 ± 1.18 <sup>a</sup>  | 3.9 ± 0.16 <sup>a</sup>  | 48              | 100 | 33.2 ± 2.20 <sup>cd</sup> | 48.1 ± 2.96 <sup>d</sup> | 51.3 ± 0.80 <sup>a</sup> | 3.2 ± 0.61 <sup>bc</sup> | 72              | 100 |                           |                           |                          |                          | 96              | 100 |
| 72                 | 100 | 46.3 ± 1.09 <sup>bc</sup> | 62.5 ± 1.28 <sup>c</sup>  | 50.7 ± 2.79 <sup>ab</sup> | 3.7 ± 0.09 <sup>ab</sup> | 72              | 100 |                           |                          |                          |                          | 96              | 100 |                           |                           |                          |                          |                 |     |
| 96                 | 100 | 33.3 ± 1.76 <sup>d</sup>  | 50.6 ± 0.98 <sup>d</sup>  | 51.2 ± 0.23 <sup>bc</sup> | 3.6 ± 0.06 <sup>bc</sup> | 96              | 100 |                           |                          |                          |                          |                 |     |                           |                           |                          |                          |                 |     |

Values are mean ± S.D.; Mean followed by different alphabets in a column are significantly different according to DMRT at  $p < 0.05$

gradually dwindled with increase in the age of host eggs. There was non-significant difference in adult longevity between fresh and 24 hour old eggs. A successful completion of life cycle of a parasitic wasp inside a host egg depends on ample supply of nutrients. A fresh egg can suitably supply more nutrients than older eggs essentially for completing the development of a parasitoid. In the present study, no significant difference in parasitization between fresh and one-day-old eggs was observed, but parasitization decreased gradually as the age of eggs increased. A reduced parasitism of Trichogrammatid wasp on 2-day-old *C. cephalonica* eggs and 3-day-old *H. armigera* eggs was observed when compared to one day old eggs (Laurentis et al., 2019). Performance of parasitoid may be influenced by the age of host eggs (Pak et al., 1986). Since host eggs undergo morphological and physiological changes in the course of their embryonic development that may interfere with their acceptance by the female wasp (Godin and Boivin, 2000; Colazza et al., 2010). When age of egg is more than 24hr, it is possible that the embryo is already undergoing embryonic development and decreases the amount of food available for the development of wasp larva inside the egg (Jarjees and Merritt, 2004). Reduced availability of nutrients in the precarious host eggs when they are older than 24hr may lead to the reduced parasitization and adult emergence of Trichogrammatid wasps. Zahid et al. (2007) reported a significant decrease in parasitization of eggs of *S. cerealella* by *T. chilonis* with an increase in the age of host egg. Schuld et al. (1999) documented an insignificant difference in the adult emergence of *T. chilonis* when offered with *Plutella xylostella* eggs of different age-group. The production of female wasps and longevity of adult was not influenced significantly by the age of host eggs. Hoffman et al. (2001) recorded insignificant changes in the sex ratio of the progeny of the parasitoid, *T. ostrinae* and the percentage of females was around 60% in all age-group of eggs. The age of the host did not affect the sex ratio of *T. nerudai* parasitizing *S. cerealella* and *T. chilonis* (Botto et al., 2004).

Preference of the parasitoids and fitness of their progeny largely depended upon the species, size, age and nutritional quality of the precarious host (Du et al., 2018). Trichogramma's field performance in controlling the target pest may be anticipated by their *in vitro* performance (Coelho et al., 2016). The present study evaluated host dependent variations in the relative parasitic efficiency and biology of *T. chilonis* and *T. japonicum*, the prospective biological control agent in India that are released inundatively to manage lepidopteran pests of crops. Efficiency in parasitization is one of the key factors in selecting the best parasitoid for field release. In the present study, a variation in the host and age induced parasitization and biological attributes of *T. chilonis* and *T. japonicum* could affect their successful use. One of the key indicators of productivity of biocontrol agent is their ability to parasitize their hosts, and this ability can vary depending on the hosts used for growing the biological control agents. Irrespective of the Trichogrammatid wasp and host species, maximum parasitization of fresh eggs was always observed. Parasitization reduced progressively with the age of host eggs. In a field study, Sharma and Aggarwal (2015) observed that *T. chilonis* had higher parasitism (18.10%) of lepidopteran pests of Basmati rice in

comparison to *T. japonicum* (15.49%). Irrespective of the hosts percent parasitization of *T. chilonis* was more than *T. japonicum* in the present study.

The eggs of *H. armigera* were the best for mass production of *T. chilonis* as they exhibited the best biological and parasitic traits with respect to the highest adult longevity, sex ratio, mean progeny per female and mean adult per egg as compared to the other host eggs. *T. chilonis* performed well when fresh host-eggs were used. Host eggs of 0 and 24hr can be better utilized for the mass production of egg parasitoids. Though both *T. chilonis* and *T. japonicum* could parasitize the eggs of *H. armigera*, the former should be preferred as its parasitization is substantially higher than the later. The findings of this study demonstrated the feasibility of using *T. chilonis* in controlling *H. armigera* in addition to utilization of its egg for mass rearing of parasitoid.

### Acknowledgments

The authors gratefully acknowledge the help rendered by the Head, Department of Entomology, College of Agriculture, OUAT, Bhubaneswar for providing necessary laboratory facilities for conducting the experiments.

**Authors' contribution:** **S. Roy:** Conducted the experiment, assembled data and drafted the manuscript; **B.K. Mishra:** Conceptualized and framed the idea of the experiment and checked the drafted manuscript; **K. Roy:** Made statistical analysis of raw data, prepared graphical presentation, edited and fine-tuned the draft manuscript.

**Funding:** Financial assistance has been provided by OUAT, Odisha for carrying out the research work and SOA, Bhubaneswar, Odisha for bearing the publication cost.

**Research content:** The research content of the manuscript is original and has not been published elsewhere.

**Ethical approval:** Not applicable.

**Conflict of interest:** The authors declare that there is no conflict of interest.

**Data availability:** Not applicable.

**Consent to publish:** All authors give their consent to publish the article in *Journal of Environmental Biology*.

### References

- Anantanawat, K.J., R. Glatz and M.A. Keller: Effect of induced tolerance to Bt toxin on the egg size of *Helicoverpa armigera* and parasitism by *Trichogramma pretiosum*. *Physiol. Entomol.*, **41**, 267–273 (2016).
- Bai, B., R.F. Luck, L. Forster, B. Stephens and J.A.M. Janssen: The effect of host size on quality attributes of the egg parasitoid, *Trichogramma pretiosum*. *Entomol. Exp. Appl.*, **64**, 37–48 (1992).
- Bhatt, N.J. and P.K. Patel: Biology of chickpea pod borer, *Helicoverpa armigera*. *Indian J. Entomol.*, **63**, 255–259 (2001).

- Botto, E.N., C. Horny, P. Klasmer and M. Gerding: Biological studies on two neotropical egg parasitoid species: *Trichogramma manerudai* and *Trichogramma* sp. (Hymenoptera: Trichogrammatidae). *Biocontrol. Sci. Technol.*, **14**, 449-457 (2004).
- Chailleux, A., N. Desneux, J. Seguret, H.D.T. Khanh, P. Maignet and E. Tabone: Assessing European egg parasitoids as a mean of controlling the invasive South American tomato pinworm *Tuta absoluta*. *Plos ONE*, **7**, e48068 (2012).
- Cherif, A., R. Mansour, S. Barhoumi-Attia, L. Zappalà and K. Grissa-Lebdi: Effectiveness of different release rates of *Trichogramma macacoeciae* (Hymenoptera: Trichogrammatidae) against *Tuta absoluta* (Lepidoptera: Gelechiidae) in protected and open field tomato crops in Tunisia. *Biocontrol Sci. Technol.*, **29**, 149-161 (2019).
- Coelho, A. Jr., P.F. Rugman-Jones, C. Reigada, R. Stouthamer and J.R.P. Parra: Laboratory performance predicts the success of field releases in inbred lines of the egg parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). *Plos ONE*, **11**, 256-257 (2016).
- Colazza, S., E. Peri, G. Salerno and E. Conti: Host searching by egg parasitoids: exploitation of host chemical cues. In: *Egg Parasitoids in Agrosystems with Emphasis on Trichogramma* (Eds. F.L. Cònsoli, J.R.P. Parra and R.A. Zucchi). Springer, New York, USA, pp. 97-147 (2010).
- Consoli, F.L., E.W. Kitajima and J.R.P. Parra: Ultrastructure of the natural and factitious host eggs of *Trichogramma galloi* Zucchi and *Trichogramma mapretiosum* Riley (Hymenoptera: Trichogrammatidae). *Int. J. Insect Morphol. Embryol.*, **28**, 211-229 (1999).
- Du, W. M., J. Xu, Y.Y. Hou, Y. Lin, L.S. Zang, X. Yang, J.J. Zhang, C.C. Ruan and N. Desneux: *Trichogramma* parasitoids can distinguish between fertilized and unfertilized host eggs. *J. Pest Sci.*, **91**, 771-780 (2018).
- Godin, C. and G. Boivin: Effects of host age on parasitism and progeny allocation in Trichogrammatidae. *Entomol. Exp. Appl.*, **97**, 149-160 (2000).
- Gomez, K.A. and A.A. Gomez: *Statistical Procedures for Agricultural Research*. 2<sup>nd</sup> Edn., John Wiley & Sons, New York, USA (1984).
- Greenberg, S.M. and L.V. Podbereskaya: The comparative evaluation of *Trichogramma* quality reared on different hosts. In: (Ed. G.V. Gisenko). *Plant Prote.*, **30**, 39-41 (1983).
- Greenberg, S.M., D.A. Nordlund and E.G. King: Mass production of *Trichogramma* spp.: Experience in the former Soviet Union, China, the United States and Western Europe. *Biocontrol News Inform.*, **17**, 51-60 (1996).
- Guo, X.J., N. Di, X. Chen, Z. Zhu, F. Zhang, B. Tang, H. Dai, J. Li, R. Guo and S. Wang: Performance of *Trichogramma pintoi* when parasitizing eggs of the oriental fruit moth *Grapholita molesta*. *Entomol. Gen.*, **39**, 239-249 (2019).
- Hoffmann, M.P., D.L. Walker and A.M. Shelton: Biology of *Trichogramma mastrinae* (Hymenoptera: Trichogrammatidae) reared on *Ostrinia nubilalis* and survey for additional hosts. *Entomophaga*, **40**, 387-402 (1995).
- Hoffmann, M.P., P.R. Ode, D.L. Walker, J. Gardner, S. Van Nouhuys and A.M. Shelton: Performance of *Trichogramma mastrinae* (Hymenoptera: Trichogrammatidae) reared on factitious hosts, including the target host, *Ostrinia nubilalis*. *Biol. Cont.*, **21**, 1-10 (2001).
- Jarjees, E.A. and D.J. Merritt: The effect of parasitization by *Trichogramma australicum* on *Helicoverpa armigera* host eggs and embryos. *J. Invertebr. Pathol.*, **85**, 1-8 (2004).
- Knutson, A.: A guide to the use of *Trichogramma* for biological control with special reference to augmentative releases for control of bollworm and budworm in cotton. Texas Agricultural Extension Service. B-6071, 5-98, 42 pages (2005).
- Laurentis, V.L., G.R. Dagmara, N.A. Santos, V.F.P. Carvalho, A.M. Vacari, S.A.D. Bortoli, R.C.S. Veneziani, G.D.C. Inácio and B.G. Dami: Performance of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) on eggs of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Sci. Rep.*, **9**, 1156 (2019).
- Li, L.Y.: Worldwide use of *Trichogramma* for biological control on different crops: A survey. In: *Biological Control with Egg Parasitoids* (Eds.: E. Wajnberg and S.A. Hassan). CAB International, Wallingford, Oxon, UK, pp. 37-51 (1994).
- Li, X.Y., Q. Lei, H.Q. Hua, H.F. Song, S. Wang, R. Ramirez-Romero, H.J. Dai, J.T. Li and Y.X. Li: Impact of host suitability on oviposition preference toward fertilized and unfertilized host eggs in two *Trichogramma* parasitoid species. *Entomol. Gen.*, **39**, 313-323 (2019).
- Mawela, K.V., R. Kfir and K. Krüger: Effect of temperature and host species on parasitism, development time and sex ratio of the egg parasitoid *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatidae). *Biol. Control*, **64**, 211-216 (2013).
- Mansour, R. and A. Biondi: Releasing natural enemies and applying microbial and botanical pesticides for managing *Tuta absoluta* in the MENA region. *Phytoparasitica*, **49**, 179-194 (2021).
- Pak, G.A., H.C.E.M. Buis, I.C.C. Heck and M.L.G. Hermans: Behavioural variations among strains of *Trichogramma* spp.: Host-age selection. *Entomol. Exp. Appl.*, **40**, 247-258 (1986).
- Park, Y.K., H.P. Lee and K.S. Lee: Effect of temperature on the biology of *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) reared on a factitious host, *Antheraea pernyi* (Lepidoptera: Saturniidae) egg. *J. Asia-Pacific Entomol.*, **3**, 65-70 (2000).
- Parra, J.R.P. and R.A. Zucchi: *Trichogramma* in Brazil: Feasibility of use after twenty years of research. *Neotrop. Entomol.*, **33**, 271-281 (2004).
- Parra, J.R.P.: Mass rearing of natural enemies. In: *Encyclopedia of Entomology* (Ed.: J.L. Capinera). 2<sup>nd</sup> Edn., Vol. 4, Springer, Berlin/Heidelberg, Germany, pp. 2301-2305 (2008).
- Pomari, A.F., A.F. Bueno, R.C.O.F. Bueno and A.O. Menezes Junior: Biological characteristics and thermal requirements of the biological control agent *Telenomus remus* (Hymenoptera: Platygasteridae) reared on eggs of different species of the genus *Spodoptera* (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.*, **105**, 72-81 (2012).
- Qu, Y., X. Chen, L.S. Monticelli, F. Zhang, N. Desneux, H. Dai, R. Ramirez-Romero and S. Wang: Parasitism performance of the parasitoid *Trichogramma dendrolimi* on the plum fruit moth *Grapholitha funebrana*. *Entomol. Gen.*, **40**, 385-395 (2020).
- Romeis, J., D. Babendreiera, F.L. Wäckers and T.G. Shanower: Habitat and plant specificity of *Trichogramma* egg parasitoids—underlying mechanisms and implications. *Basic Appl. Ecol.*, **6**, 215-236 (2005).
- Schmidt, J.M.: Host recognition and acceptance by *Trichogramma*. In: *Biological Control with Egg Parasitoids* (Eds.: E. Wajnberg and S.A. Hassan). CAB International, Wallingford, U.K., pp. 113-135 (1994).
- Schuld, M., G. Madel and R. Schmuck: Impact of *Vairimorpha* sp. (Microsporidia: Burnellidae) on *Trichogramma chilonis* (Hymenoptera, Trichogrammatidae), a hymenopteran parasitoid of the cabbage moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *J. Invertebr. Pathol.*, **74**, 120-126 (1999).
- Sharma, S. and N. Aggarwal: Dispersal ability and parasitisation performance of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) in organic Basmati rice. *J. Environ. Biol.*, **36**, 1345-1348 (2015).

- Sheoran, O.P., D.S. Tonk, L.S. Kaushik, R.C. Hasija and R.S. Pannu: Statistical Software Package for Agricultural Research Workers. In: Recent Advances in Information Theory, Statistics & Computer Applications (Eds.: D.S. Hooda and R.C. Hasija). Department of Mathematics Statistics, CCS HAU, Hisar, pp.139-143 (1998).
- Sumer, F., A.S. Tuncbilek, S. Oztemiz, B. Pintureau, P. Rugman-Jones and R. Stouthamer: A molecular key to the common species of *Trichogramma* of the Mediterranean region. *BioControl*, **54**, 617-624 (2009).
- Vinson, S.B., S.M. Greenberg, T.X. Liu, A. Rao and L.F. Volosciuk: Biological Control of Pests using *Trichogramma*: Current Status and Perspectives. Northwest A&F University Press, Yangling, China. ISBN: 9787568300582, 496 pages (2015).
- Zahid, M., A. Farid, A. Sattar and I. Khan: Effects of parasitoids and host egg age on parasitism by *Trichogramma chilonis* (Ishii). *Suranaree J. Sci. Technol.*, **14**, 381-384 (2007).
- Zang, L.S., L. Sheng, S. Wang, F. Zhang and N. Desneux: Biological control with *Trichogramma* in China: History, present status and perspectives. *Annu. Rev. Entomol.*, **66**, 463-484 (2021).