



## **Determination of Parasitism Efficacy and Development of Effective Field Release Technique for *Trichogramma* spp. (Trichogrammatidae: Hymenoptera)**

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### **Authors' contributions**

This work was carried out in collaboration between all authors. Author SNA designed the study and wrote the protocol. Author ZJC conducted the field and laboratory research works, wrote the first draft of the manuscript and performed statistical analysis. Authors CKD, MAM and AA reviewed the experimental design and all subsequent drafts of the manuscript. All authors read and approved the final manuscript.

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### **ABSTRACT**

To determine the parasitism efficacy of *Trichogramma* spp. on the host eggs of *Sitotroga cerealella* Olivier (Pyralidae: Lepidoptera) and *Corcyra cephalonica* Stainton (Pyralidae: Lepidoptera) and to develop the effective field release technique for *Trichogramma* spp., the study was conducted at the IPM Laboratory and Research field of Entomology Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh during the period from July to December 2013. Higher parasitism on

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the eggs of *S. cerealella* and *C. cephalonica* was recorded from *Trichogramma chilonis* Ishii (Trichogrammatidae: Hymenoptera) (85.5%, 93.8% respectively), compared to those of *Trichogramma evanescens* West (Trichogrammatidae: Hymenoptera) (83.4%, 92.7% respectively). Higher percent adult emergence was recorded from the eggs parasitized by *T. evanescens* compared to those by *T. chilonis*. The field release techniques for *T. evanescens* and *T. chilonis* on parasitizing the eggs of *S. cerealella*, *C. cephalonica* and *Leucinodes orbonalis* Guenee (Crambidae: Lepidoptera) were also assessed by using paper strip method (released during pupation) and adult release method in both micro-plot and open field conditions. In micro-plot, the results showed that *T. evanescens* parasitized 75.5% of host eggs (mean of three host eggs) by adult release method and 38.83% only by paper strip method. In case of open field condition, *T. chilonis* parasitized 78.6% of host eggs by adult release method and 40.2% only by paper strip method. The results indicate that as a field release technique of *T. chilonis*, the adult release method is superior to paper strip method.

**Keywords:** Parasitism; bio-control agent; natural enemies; augmentation; pest regulation.

## 1. INTRODUCTION

In Bangladesh, the egg parasitoids, *Trichogramma* spp. (Trichogrammatidae: Hymenoptera) and the larval parasitoid, *Bracon hebetor* Say (Braconidae: Hymenoptera) have been being widely used in high value vegetable crops since 2003, as a component of Integrated Pest Management (IPM) approaches [1-3]. Similarly, the *Trichogramma* spp. are also being used in the world due to easier mass rearing techniques in insectaries and higher parasitism rates on many important crop insect pests [4,5]. Researchers have already developed some improved laboratory rearing techniques for *Trichogramma* spp., which are capable of producing quality parasitoids [6], but more effective rearing technique still needs to be developed. Actually the poor quality of mass reared *Trichogramma* often results in control failures as different species and strains of the genus prefer different eggs, crop habitats with different searching abilities and tolerance levels to weather conditions [7]. The mass rearing of natural enemies for use in integrated pest management requires the availability of factitious hosts, capable of maintaining the potentialities of biological control agents for many successive generations, and that can be reared at minimal costs [8]. The parasitism efficacy of parasitoids depends on their rearing techniques in the laboratory and releasing techniques in the field. In order to develop the effective mass rearing technique, we need to find out the effective host eggs and optimal egg parasitism rate. The efficacy of mass rearing techniques can be improved by selecting the most effective and adapted species or strain for the specific crop or pest situation. Local strains that are collected from target area should be the first choice for

evolutionary studies, although sometimes exotic species/strains may also be more effective and should be evaluated. In fact, the species and strain(s) parasitizing or killing the greatest number of eggs are considered to be higher potential bio-control insect, as for achieving grand success at field level [9].

*Trichogramma* is important for plant protection because of its wide spread natural occurrence and its success as biological control agent by mass releasing. After mass rearing it is essential to release the parasitoids in the field for better parasitism. To maximize the field parasitism it is recommended that the growers and other stakeholders continue release the parasitoids as many locations as possible. There are various field release techniques for *Trichogramma*. In many countries of the world, Tricho cards are used to release the parasitoids [10]. The cards are cut into bits neatly along the grids with least damage to the eggs and stapled beneath the foliage in the upper canopy level. Recently, scientists are beginning to advocate the release of cards @ 1/5 m row length. In recent years, Bangladesh Sugarcane Research Institute, Pabna and Bangladesh Agricultural Research Institute, Gazipur have been partially successful to control various borer pests of sugarcane and vegetable crops by using paper strip. The scientists of the above two institutes have used paper strip at pupal stage of the *Trichogramma* spp. But they were unable to minimize the natural predation of parasitoids pupae using paper strips. In fact, natural predation of released parasitized eggs in the field is a common phenomenon of paper strip method. In this method, about 40-50% of the parasitized eggs by *Trichogramma* spp. were found to be eaten by different predators after their field release.

Therefore, the study was carried out to determine the parasitism efficacy of *Trichogramma chilonis* Ishii and *Trichogramma evanescens* West using *Sitotroga cerealella* Olivier and *Corcyra cephalonica* Stainton as host eggs and to find out the effective field release method to widely use as a component of IPM packages in Bangladesh and or elsewhere of the world where applicable.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Host Eggs in the Laboratory

The rearing techniques of the host species, *S. cerealella* and *C. cephalonica* were practiced in the IPM laboratory at a temperature of  $25\pm 3^{\circ}\text{C}$  and RH of 70-80%. The hosts were mass reared by using wheat grain as a diet in a specialized mass rearing chamber (Photo 1). In case of *C. cephalonica*, 100 adults (sex ratio 1:1) were kept in a plastic container of the rearing chamber at the first day. The top portion of the container was covered with 32 mesh net. Some wire nets were placed inside the plastic container to lay eggs. At the second day, fresh eggs were collected on a fresh paper by shaking and putting down the top of the container. While for the collection of *S. cerealella* eggs, 1000 adults were collected from the *S. cerealella* mass rearing chamber and kept them in a glass cylinder. In the subsequent days, the eggs laid on the wall of the cylinder were brushed and sieved to collect fresh eggs.



Photo 1. Mass rearing chamber of *Sitotroga cerealella* & *Corcyra cephalonica*

### 2.2 Maintenance of *Trichogramma* spp. Colony

The colonies of the species, *T. chilonis* and *T. evanescens* were maintained in the IPM

laboratory, Entomology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh. These two parasitoid species were reared on the eggs of host species, *S. cerealella* and *C. cephalonica*, which were simultaneously being reared in the laboratory.

### 2.3 Parasitism of Host Eggs by *Trichogramma* spp.

The parasitism capacity of *T. chilonis* and *T. evanescens* was analyzed by using the freshly laid eggs of *S. cerealella* and *C. cephalonica*. For the parasitism study, one paper strip (Photo 2) containing 100 eggs of each host species and five pairs of *Trichogramma* spp. were placed together in individual test tube (15 cm x 3 cm). Strips with pupae which were almost ready to emerge were collected from the reared colony stock of *Trichogramma* in the IPM laboratory in the form of a strip which contained 100 pupae of *Trichogramma* in *S. cerealella* or *C. cephalonica* eggs. In this way, 12 strips with host eggs (6 strips of *S. cerealella* and 6 strips of *C. cephalonica*) were parasitized by *T. chilonis* and *T. evanescens*.

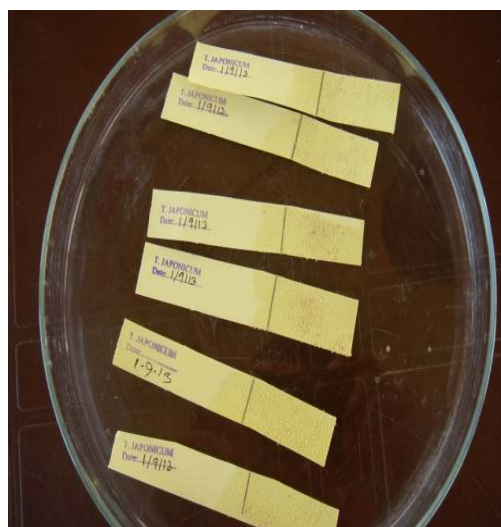


Photo 2. Paper strip of *Sitotroga cerealella* for parasitism test

### 2.4 Field Release Techniques of *Trichogramma* spp.

The parasitism efficacy of *Trichogramma* spp. on three different host eggs, viz. *S. cerealella*, *C. cephalonica* and *L. orbonalis* (eggplant shoot and fruit borer) were evaluated by releasing *T. chilonis* and *T. evanescens* using paper strip

method (released during pupation) and adult release method in micro-plot and open field conditions.

## 2.5 Micro-plot Conditions

Five host-egg strips (each strip supporting 200 host eggs of each insect pest) were clipped on the eggplant leaves of 4 plants in the field. The plants were then covered with fine mesh siphon net. Paper strips supporting 500 parasitized host eggs by *T. evanescens* and *T. chilonis* were placed in micro-plot. On the other hand, adult parasitoids were released in micro-plot having the same number of host egg strips. After 3 days, the host egg strips were collected from the field and kept in test tubes. One micro-plot was considered one replication. Three replicates were used for two treatments.



Photo 3. Microplot condition

## 2.6 Open Field Conditions

Five host egg strips (each strip supporting 200 host eggs of each insect pest) were clipped on the eggplant leaves of 4 plants in the field. Paper strips supporting 500 parasitized host eggs of *T. evanescens* and *T. chilonis* were placed in that field. The distance between the release of parasitized eggs and the host eggs was 10 meters. Adult parasitoids were released in another field having the same number of host egg strips. After 3 days, the host egg strips were collected from the field and kept in test tubes. One micro-plot was considered one replication. Three replicates were used for two treatments.

## 2.7 Experimental Design and Data Analysis

In case of host eggs parasitism by *Trichogramma* spp., each test tube was considered one replication. This experiment was laid out in a

Completely Randomized Design (CRD). All the data of host eggs parasitism, micro-plot and open field conditions were analyzed following MSTAT-C program.

## 3. RESULTS AND DISCUSSION

### 3.1 Parasitism Efficacy of *Trichogramma* spp.

The parasitism efficacy of *T. chilonis* and *T. evanescens* on the host eggs of *S. cerealella* and *C. cephalonica*, percent adult emergence and adult longevity (days) are shown in Table 1 and described in the following sub-headings.

### 3.2 Parasitism Efficacy of *Trichogramma* spp. on Two Host Eggs

There was a variation between the egg parasitism rate of *T. evanescens* and *T. chilonis* on both the host eggs, while it was less pronounced either in *Sitotroga cerealella* or in *Corcyra cephalonica*. The results indicate that *T. chilonis* is more efficient parasitoid than *T. evanescens*. Many reports indicate that not only the type of host eggs, but also the factors such as intra- and interspecific interactions with biotic components [11], climatic conditions [12] and other factors can interfere with the performance of parasitoids as naturally occurring bio-control agents. The parasitism rate of *Trichogramma pretiosum* Riley (Trichogrammatidae: Hymenoptera) was approximately 80% against the eggs of *Anagasta kuehniella* Zeller (Pyralidae: Lepidoptera) [13]. The adult female parasitoid depends on the size of the rearing host egg in which the insect develops [14]. There are reports of increased size being associated with increased fitness in parasitoids, measured as success in locating hosts [15] and with increased fecundity [16], although that was not found in case of fecundity of these tested parasitoids.

### 3.3 Rate of Adult Emergence

The highest adult emergence (92.4%) was recorded from the parasitized eggs of *S. cerealella* by *T. evanescens* which was the same when *T. chilonis* parasitized the eggs of *C. cephalonica*. The adult longevity varied from 4.1 to 4.4 days irrespective of parasitoid and host eggs. Some authors reported that the emergence rate of *Trichogramma* adults varied with the size and type of the host egg, number of parasitoids that develop per egg, development period in host

**Table 1. Parasitism efficacy and adult emergence of two different *Trichogramma* spp. on host eggs of *S. cerealella* and *C. cephalonica***

Host	Parasitoid	Egg parasitism (%) (Mean ± SE)	Adult emergence (%) (Mean ± SE)	Adult longevity (days) (Mean ± SE)	No. of parasitoid emerged/egg
<i>Sitotroga cerealella</i>	<i>T. evanescens</i>	83.4±1.3	92.4±0.5	4.2±0.4	1
<i>Corcyra cephalonica</i>	<i>T. chilonis</i>	85.5±0.6	91.8±0.8	4.1±0.3	1
<i>Sitotroga cerealella</i>	<i>T. evanescens</i>	92.7±0.7	92.9±0.2	4.4±0.3	1
<i>Corcyra cephalonica</i>	<i>T. chilonis</i>	93.8±0.8	92.4±0.4	4.4±0.2	1

eggs and temperature [17]. Overall, the results manifest no significant differences among the biological parameters that were observed in individuals reared upon two host eggs. The performance of the parasitoid, *Trichogramma ostrinia* Pang and Chen (Trichogrammatidae: Hymenoptera) on the eggs of three factitious hosts, *Ostrinia nubilalis* Hubner (Crambidae: Lepidoptera), *Sitotroga cerealella* Olivier (Pyralidae: Lepidoptera), *Trichoplusia ni* Hubner (Noctuidae: Lepidoptera) was evaluated and found *S. cerealella* as an intermediate host for rearing the parasitoid as compared to the other hosts [18]. Similarly, the emergence and survival of the adults of *T. chilonis* by using host eggs of *C. cephalonica* was determined and showed that besides *S. cerealella* rearing on other hosts gave desired results, as found *S. cerealella* and *C. cephalonica* to be equally good for the rearing of *T. chilonis* and *T. evanescens* [19].

### 3.4 Field Release Techniques

The parasitism rates of *T. evanescens* and *T. chilonis* observed by using the paper strip and adult release methods are compared in micro-plot and open field conditions. The results indicated that after 48 hours of the release, *T. evanescens* parasitized 38.83% of the host eggs (mean parasitism of three host eggs) in the micro-plot in case of paper strip release method (released during pupation). In contrast, the mean parasitism by *T. evanescens* was 75.5% in case of adult parasitoid release method in the micro-plot condition. Similar trend of parasitism by *T. evanescens* was observed in open field condition. However, the parasitism in the open field was lower than that of the micro-plot in both the methods of parasitoid release. After 48 hours of the release of *T. chilonis*, it parasitized 40.2% of the host eggs in case of paper strip release method and 78.6% in case of adult parasitoid release method in micro-plot condition. Likewise *T. evanescens*, similar trend of parasitism by *T. chilonis* was observed in the open field

condition. It is revealed from the overall results that host egg parasitism was 1.94 folds higher in adult parasitoid release than parasitized eggs on paper strips in micro-plot condition. Similar trend was also observed in open field, where egg parasitism was 2.28 folds higher in adult parasitoid release than parasitized eggs on paper strips. Different field release techniques for *Trichogramma chilonis* Ishii (Trichogrammatidae: Hymenoptera), an egg parasitoid on sugarcane internode borer (*Chilo sacchariphagus indicus* (Kapur)) were assessed [20]. They conducted field trials during the two consecutive seasons of 1994–95 and 1995–96 at Sugarcane Research Station, Cuddalore, India. Among the techniques tested, release of the parasitoid through closed cup mechanism and loose egg exposure recorded higher percent emergence as against the conventional release technique of open card release tied to a sugarcane plant. The higher emergence of parasitoids in different techniques over the conventional method might be attributed to the safety afforded against sunlight, moisture and predators. In this study, the adult release method in both micro-plot and open field conditions increased the parasitism as well as protected the parasitoids from adverse environmental conditions and natural predation. In contrast, many of the parasitized eggs were eaten by different predators where and when the paper strips were put in micro-plot and open field conditions.

### 4. CONCLUSION

From the present results, it is evident that the host eggs of both *S. cerealella* and *C. cephalonica* could be used to successfully rear the two egg parasitoid species, *Trichogramma chilonis* and *T. evanescens* in any augmentative biological control at the field. Although the parasitism rate was lower except for *T. chilonis* when parasitized eggs of *C. cephalonica*, but the rate of emergence of *Trichogramma* was higher. After rearing when

the parasitoids were released in the field by the adult release and paper strip methods, the adult release method had performed better as compared to those of paper strip method due to no risk of predation in adult release method. When adults were released directly in the field, they parasitized higher number of host eggs within 48 hours as compared to those of the paper strip method. The adult release method is easier, quicker and more effective and thus could be used in mass release in the field.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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