

# Effect of Treating Eggs of the Tomato Leaf Miner, *Tuta absoluta* with *Bacillus thuringiensis* on Biological Characteristics of Three *Trichogramma* Species under Laboratory Conditions

Ghazwan Alsaedi, Ahmad Ashouri\* and Reza Talaei-Hassanloui

Department of Plant Protection, College of Agriculture and Natural Resources,  
University of Tehran, Karaj, Iran

\* Corresponding author: E-mail: ashouri@ut.ac.ir

**Abstract** – In this study we investigated parasitism rate, adults emergence rate, number of females and adult longevity of three parasitoids, *Trichogramma brassicae*, *T. evanescens* and *T. embryophagum* when treated with/without *Bacillus thuringiensis* (Bt) reared on the tomato leaf miner *Tuta absoluta* eggs under controlled conditions. According to the obtained results, significant differences were observed among parasitism percentages of *Trichogramma* species parasitism rates, also the highest parasitism rate 54% was obtained for *T. embryophagum* when treated with Bt reared in the *T. absoluta* eggs. However the highest mortality for eggs parasitized by *T. evanescens* when they were treated with/without Bt and with water reared on the *T. absoluta* eggs and only *T. evanescens* were 85.85, 85.27 and 92.42% respectively. In addition, the adult emergence rate of *T. embryophagum* was significantly more than other species when treated with Bt reared on the *T. absoluta* eggs 62.96% followed by *T. brassicae* with Bt reared on the *T. absoluta* eggs 59.87%. So number of females was high in the *T. brassicae* compared with other species. Also longevity of *T. embryophagum* was significantly more than other species. Consequently, this is the first study to investigate *T. embryophagum* for parasitizing eggs of *T. absoluta* and results of present study suggested that *T. embryophagum* with Bt could be more efficient for biocontrol of *T. absoluta*.

**Keywords** – *Tuta Absoluta*, Natural Enemies, *Trichogramma* Species, Parasitism Rate, Mortality, Adults' Emergence Rate, Number of Females, Adult Longevity, *Bacillus Thuringiensis*.

## I. INTRODUCTION

The tomato leaf miner *Tuta absoluta*, is one of the most destructive pests of tomato worldwide [11], [8]. It is considered a typical invasive species because of its capacity to develop very quickly on tomato cultivations and to spread rapidly in new areas causing economically relevant damage [11], [8]. Since the time of its initial detection, the pest has caused serious damages to tomato in invaded areas [16]. However it is currently considered a key agricultural threat to European, Middle East, North African tomato production and part of Asia.

Wasps of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are the principal egg parasitoids of lepidopteron agricultural pests [30], [25], [24]. Due to simplicity of rearing of these wasps in many countries, *Trichogramma* wasps were commercially applied as mass releases in over 32 million hectares against more than 30

key pest worldwide [36], [3].

Otherwise *Bacillus thuringiensis* is the most important microorganism with entomopathogenic activity against certain insect orders. It is ubiquitous, gram-positive and spore-forming bacterium which produces insecticidal crystal proteins during sporulation. Natural isolates of *B. thuringiensis* have been used as a biological pesticide since the 1950s for the control of certain insect species among the orders Lepidoptera, Coleoptera and Diptera as an alternative to chemical pesticides. Furthermore it's the majority of pathogenic strains possess activity against larvae of this order, among other things no damage to the environment.

Moreover, Alsaedi et al. [2] suggest there will be good opportunities and new options for putting *T. brassicae*, *T. evanescens* and *T. embryophagum* within sort of valuable assets of any of the IPM strategies against *T. absoluta* for generations to come in order to reduce the use of chemicals. Otherwise Aziz et al. [4] conducted experiment to evaluate the effect of bacteria *B. thuringiensis* in characters of *T. absoluta*, results showed that using bacteria *B. thuringiensis* due to significant increase on killing ratio of larval instars and make it reached to 65.92, 66.55, 64.98, 70.78% for 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instars, respectively, compared with control treatment which have 0% of mortality ratio.

The biological characteristics, thermal requirements and parasitism capacity of parasitoid wasp had been studied on eggs of different lepidopteron [29]. Furthermore this egg parasitoid wasp has been widely used to control *T. absoluta* [31], [17], [15], [21], [7], [11], [22], [33]. *T. brassicae* is used worldwide for the control of Lepidoptera pests. It acts as an egg parasitoid of the carob moth, *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae). Kuskea et al. [20] evaluated the effects of inundative releases of *T. brassicae* against the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae). Also this egg parasitoid may attack *T. absoluta* [26].

In Iran, *T. brassicae* is the most widespread species followed by *T. embryophagum* [13], [23]. In the current study, parasitism rate and some biological characteristics of *T. brassicae*, *T. evanescens* and *T. embryophagum* were investigated on the eggs of *T. absoluta*, aside from to achieve successful biological control programme by *Trichogramma* and *B. thuringiensis*, therefore it is necessary to select the species which possess high

performance on target pest proportionate to the ambient conditions.

In this study we investigated parasitism rate and some biological characteristics of *T. brassicae*, *T. evanescens* and *T. embryophagum* when treated with/without *B. thuringiensis* reared in the tomato leaf miner *T. absoluta* eggs under controlled conditions.

## II. MATERIALS AND METHODS

### Plants and Insects

Tomato plants (*Solanum lycopersicum* L.) were grown in a greenhouse (25±5°C, 70±5% RH, 16L: 8D). Five weeks old plants were used in the experiments. The plants were grown in small pots (8 cm height, 9 cm diameter) in mixture of soil and perlite (1:1). Tomato plants were introduced into cages with approximately 30 *T. absoluta* females and removed 24 h later, tomato plants with eggs were kept at the same conditions in the growth chambers until used for experiments. Mated females of *T. absoluta* were obtained by pairing a virgin male and a virgin female moth, one day after mating *T. absoluta* females were used in the experiments. *T. brassicae*, *T. evanescens* and *T. embryophagum*, were reared on eggs of the moth *Ephestia kuehniella* at the University of Tehran, in a climate chamber (25°C, 70±5% RH, L16:D8). Only mated, 24 hour old, wasps were used in the experiments. All experiments were accomplished at the Ecology and Behavior Lab, Department of Plant Protection, University of Tehran (Karaj, Iran).

### Efficacy of *Trichogramma* species on eggs of *T. absoluta* in laboratory

At the beginning of experiments, 20 eggs of *T. absoluta* were placed into a card 6 cm and then put in glass vial (10 cm in height), then one newly emerged female wasp of *Trichogramma* sp. was transferred into vial for parasitism. The exposed eggs were maintained in a growth chamber with 25°C, relative humidity of 70±5% and a photoperiod of 16L: 8D h. Honey solution (10%) was supplied in a thin layer as a food source for feeding female wasps. Twenty replications were run for each species of wasps, data were recorded daily and rate parasitism, adult emergence percentage, number of female and longevity were assessed.

### Formulation of Bt

The Bt formulation used in the experiments was *B. thuringiensis* subsp. *kurstaki* (10<sup>9</sup> spor/g) WP, Biolep, Biorun Company, Iran.

### Efficacy of *B. thuringiensis* on eggs *T. absoluta* in laboratory assay

*B. thuringiensis* subsp. *kurstaki* at concentration of 10<sup>6</sup> spor/ml of water was used in treatments. The eggs were sprayed with suspension of *B. thuringiensis* and then offered to *Trichogramma* species. Twenty replicates were tested. The collected adult wasps of various *Trichogramma* species were separately released on eggs of *T. absoluta* that sprayed with *B. thuringiensis* for 24 h. The exposed eggs were kept in a growth chamber with 25°C, relative humidity of 70±5% and a photoperiod of 16L: 8D h, data were recorded daily and parasitism rate, adults emergence

rate, number of females and adult longevity were assessed.

### Statistical Analysis

For statistical analysis, each mean value is given with its standard error. The differences in parasitism percentage, adult emergence, number of female and adult longevity among three *Trichogramma* species were compared using one way ANOVA. If significant differences were detected, comparisons were run using the Duncan's test ( $p < 0.05$ ). Statistical analysis was performed using the software SPSS Statistics for Windows Version 21 (SPSS 2012).

## III. RESULTS

The biological characteristics of *T. brassicae*, *T. evanescens* and *T. embryophagum* are presented in figure 1, 2, 3, 4 and 5. The results in figure 1 showed significant differences among parasitism percentages of *T. brassicae*, *T. evanescens* and *T. embryophagum* ( $F = 2.756$ ;  $df = 8$ ;  $p < 0.007$ ), however the highest parasitism rate was obtained for *T. embryophagum* with Bt was 54%, also the lowest parasitism rate was obtained for *T. evanescens* with Bt was 24.75%. Also the results in figure 2 indicated significant differences among mean mortalities of three parasitoid species ( $F = 15.461$ ;  $df = 8$ ;  $p < 0.000$ ), therefore the highest mortality for eggs parasite by *T. evanescens* with/without Bt and with water were (85.85, 85.27, 92.42%) respectively, while the lowest mortality for *T. brassicae* and *T. embryophagum* with Bt were 40.12, 37.03% respectively. Otherwise percentage emergence of adult wasps in *T. embryophagum* with Bt was significantly more than other species that showed in figure 3 were relatively high 62.96% followed by *T. brassicae* with Bt was 59.87% ( $F = 11.643$ ;  $df = 8$ ;  $p < 0.000$ ). However number of females (female/total) was high in the *T. brassicae* without Bt and with water were 94.73 and 91.66% respectively, in comparison with other species, which was significantly different from the number of females of *T. embryophagum* ( $F = 3.587$ ;  $df = 8$ ;  $p < 0.001$ ).

The number of females (female/total) of three *Trichogramma* species is shown in figure 4. The results in figure 5 showed the highest adult longevity in *T. embryophagum* with/without Bt were 8, 7.55 day respectively, on eggs of *T. absoluta*, also 7 days on eggs of *T. absoluta* sprayed with water for *T. embryophagum* which is significantly higher than the other two species ( $F = 13.119$ ;  $df = 8$ ;  $p < 0.000$ ).

## IV. DISCUSSION

In this study findings on parasitism rate of three *Trichogramma* species were tested under laboratory conditions, therefore our results showed that although all *Trichogramma* species did accept *T. absoluta* eggs under laboratory conditions but one species of *Trichogramma* species, *T. embryophagum* appeared to be promising with 54% parasitism when treated with Bt vs. 45.5% for parasitism without Bt.

Also our results were approach with Chailleux et al. [9] when investigated Twenty-nine *Trichogramma* species-strains on *T. absoluta* eggs under laboratory conditions

and one strain of the *T. euproctidis* species appeared promising (68.7% parasitism vs. 35.4% for parasitism for *T. achaeae*). Furthermore, we found a positive relationship between the number of parasitized eggs when treated with Bt were significantly different with Vaez et al. [32] suggested that functional response parameters were affected by Bt treatment that parasitic wasps developed in Bt-treated hosts were weaker than normal ones, because of sub-lethal effect of Bt was investigated on *T. brassicae* females searching host eggs laid by *Helicoverpa armigera* treated by LC<sub>20</sub> of the bacterium.

Zouba et al. [37] showed that *T. absoluta* eggs are better accepted by *T. bourarachae* females; and the rate of egg parasitism by *T. bourarachae* (65.64%) were significantly higher compared to *T. cacoeciae* (57.05%). Moreover, studies suggest that larger host eggs can positively influence parameters such as percentage of parasitism, number of parasitoids emerged per egg, longevity, and sex ratio [35], [5], [18]. Also *Trichogramma* parasitism rate varied with physical and chemical barriers and by the type and characteristics of host eggs such as their size, hardness, scales and kairomones [28], [6], [23], [2].

The species of *T. embryophagum* presented acceptable adult emergence rate. Furthermore the emergence rates showed higher values than those of other *Trichogramma* species. Indeed, the emergence rate of *Trichogramma* adults can also vary with the size and quality of the host egg, number of parasitoids that develop per egg, development period in host eggs and temperature [12], [27]. Also adult longevity of *T. embryophagum* on eggs of *E. kuehniella* has been reported as 12.37 days by Haghani [19]. However Akbari et al. [1] recorded parasitism percentages 10.75, 9.30, 9.75 for *T. brassicae*, *T. embryophagum* and *T. pinto* investigated on eggs of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) under controlled conditions. These differences show that the host species can influence adult longevity of parasitoid wasps. Also, reduction in host fecundity may show inverse effects of bio insecticides on yolk deposition process and this may reduce both the quality and quantity of the eggs deposited by host as well as their size [32], Therefore amongst the sub-lethal effects of microbial insecticides on hosts themselves are prolonged larval development, decreased adult body size, reduced pupal weight and reduced fecundity, moreover, these events may lead to smaller parasitoids with lower fecundity, survival and searching ability [10], [34], [14].

## V. CONCLUSION

This study showed that parasitoids *Trichogramma* species have parasite eggs of *T. absoluta* as one of herbivores. However, it suggests no direct effective of Bt occurs on *Trichogramma* species and referring to the order of their application the integration of these two organisms especially *T. embryophagum* with Bt may produce desirable results and new options for putting these parasitoids within the sort of valuable assets of any of the IPM strategies against *T. absoluta* for generations to come in order to reduce the use of chemicals and, consequently, improve food safety and environment quality.

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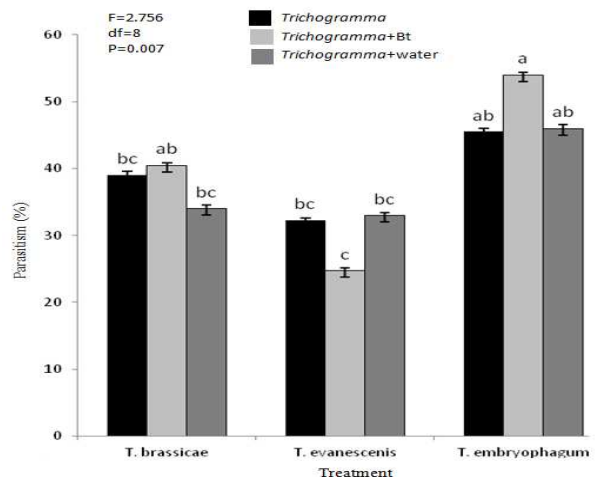


Fig. 1. The parasitism of *T. brassicae*, *T. evanescens* and *T. embryophagum* when treated with/without Bt, with water reared in the *T. absoluta* eggs in laboratory

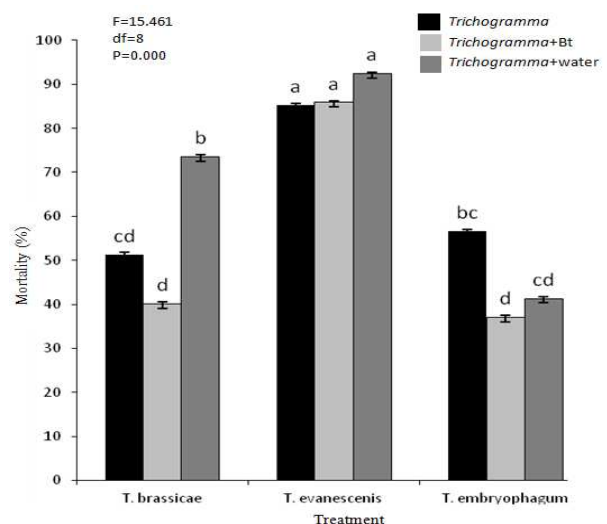


Fig. 2. The Mortality of *T. brassicae*, *T. evanescens* and *T. embryophagum* when treated with/without Bt, with water reared in the *T. absoluta* eggs in laboratory

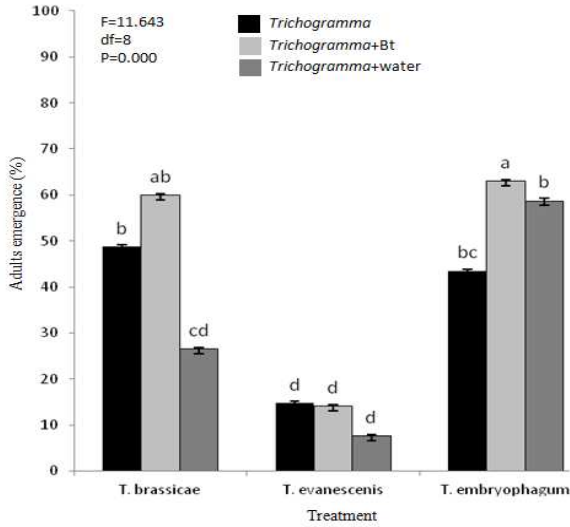


Fig. 3. The adults emergence of *T. brassicae*, *T. evanescens* and *T. embryophagum* when treated with/without Bt, with water reared in the *T. absoluta* eggs in laboratory.

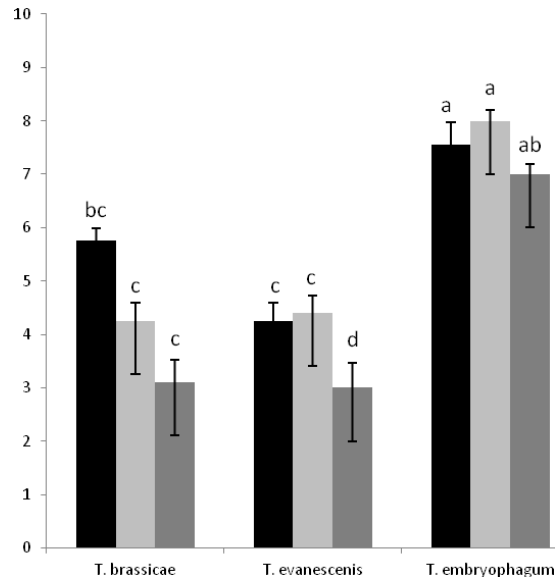


Fig. 5. The Longevity of *T. brassicae*, *T. evanescens* and *T. embryophagum* when treated with/without Bt, with water reared in the *T. absoluta* eggs in laboratory.

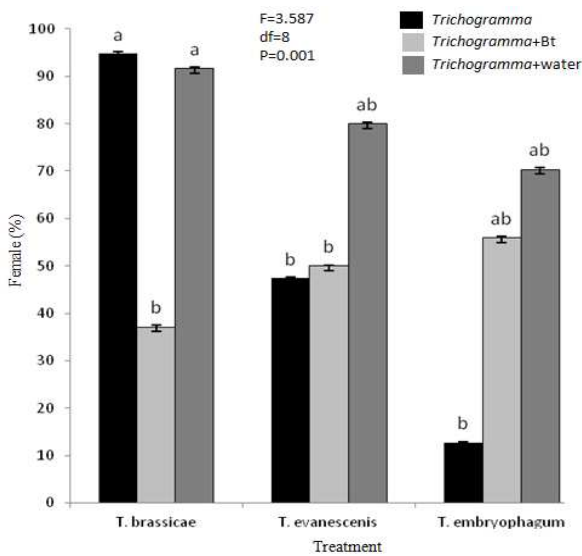


Fig. 4. Female of *T. brassicae*, *T. evanescens* and *T. embryophagum* when treated with/without Bt, with water reared in the *T. absoluta* eggs in laboratory.