

Assessment of Two *Trichogramma* Species with *Bacillus thuringiensis* var. *krustaki* for the Control of the Tomato Leafminer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in Iran

Ghazwan Alsaedi, Ahmad Ashouri*, Reza Talaei-Hassanloui

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

Email: *ashouri@ut.ac.ir

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Abstract

In this study, we investigated the efficiency of *T. brassicae* and *T. embryophagum* in combination with bacterial suspension of *B. thuringiensis* against the tomato leafminer *T. absoluta* eggs in cage inside greenhouse (semi field) experiments. Four treatments were used (*T. brassicae* or *T. embryophagum* + *T. absoluta*) (T1), (*B. thuringiensis* + *T. absoluta*) (T2), (*T. brassicae* or *T. embryophagum* + *B. thuringiensis* + *T. absoluta*) (T3) and control (T4). The lowest number of *T. absoluta* mines (6.1, 0.5 mine per plant) were recorded in T3 for *T. brassicae* and *T. embryophagum* were significantly lower than those of all other treatments which were followed by T1 and T2, while the highest number of mines per plant (50.70) were recorded in control (T4). In addition, the parasitism rate, adults' emergence, the number of females and adult longevity of two parasitoids were investigated. According to the obtained results, the highest parasitism rate was obtained for *T. embryophagum* when treated with Bt reared in the *T. absoluta* eggs (31.18%). However, no significant differences were detected between *T. brassicae* and *T. embryophagum* in mortality and adult emergence rates were found when they were treated with/without Bt reared in the *T. absoluta* eggs in cage inside greenhouse. Also, the longevity of *T. embryophagum* was significantly better than *T. brassicae* $p = 0.000$. This is the first study to investigate *T. embryophagum* in cage inside greenhouse for parasitizing the 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 brassicae*, *Trichogramma embryophagum*, Biological Characteristics,

1. Introduction

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop next to potato. In 2013, world production of tomatoes was reduced to 163.4 million tonnes, with Iran accounting for 3.8% of the total [1]. This crop is attacked by a wide range of pests. Invasive species represent a major threat to crops [2] [3] [4] and agricultural pests can reduce yield and increase production costs related to their management [5]. The tomato leaf miner, *T. absoluta* (Lepidoptera: Gelechiidae), has been a long-time pest of open field and greenhouse tomato in South America [6] [7] [8].

This key pest started moving and invading towards the eastern regions, within being first reported from Spain in 2006. Today, *T. absoluta* represents a major threat to both natural and agronomic ecosystems in most countries of South America, Europe, the Middle East and some part of the pest status of *T. absoluta* has been argued to arise mainly from its high fecundity, high mobility, migratory potential as well as its high propensity to rapidly develop resistance against different classes of pesticides [1]. The larvae of *T. absoluta* mine the leaves producing large galleries and burrow into the fruit, causing a substantial loss of tomato production in protected and open field cultivations. Damage may reach to 100% of the yield if no control strategy is taken. *T. absoluta* can complete between 10 - 12 generations per year in appropriate climatic conditions and each female can lay between 250 - 300 eggs during her life time. A variety of methods, including monitoring, mass trapping as well as the use of chemical, cultural, and biological factors have been developed for control of *T. absoluta*, each of which may contribute partially to reduce damage on cultivations.

For example, the use of synthetic insecticides for control of *T. absoluta* is questionable with respect to the mine-feeding nature of the larvae [9]. On the other hand, as tomato is consumed as raw product in many countries, the use of synthetic pesticides has provoked concern about the harmful effects of these compounds on human health. Additionally, wide application of chemical pesticides is expected to threaten the survival and normal activity of non-target organisms, particularly natural enemies and pollinators more than the target pest itself, thus negatively affecting on production by reducing the efficiency of pollinators and biological control agents.

Biological control, as an alternative to chemical control, has been attempted in several South American and European countries [10] [11]. Currently, two important groups of biological control agents, *i.e.* entomopathogenic bacteria and oophagous parasitic wasps have been considered as efficient agents for control of *T. absoluta*. The soil dwelling bacterium, *Bacillus thuringiensis* var. *kurstaki*, for example, have exhibited satisfactory efficacy against *T. absoluta* larval infestations in Spanish outbreaks. Delayed application of this bacterium in combina-

tion with the use of resistant tomato cultivars has been argued to cause higher larval mortality, because the larvae seem to become more susceptible to the pathogen after a longer period of feeding on the resistant crop. The egg parasitic wasps of the genus *Trichogramma*, are another important group of biological control agents that have provided promising results for control of *T. absoluta* under greenhouse conditions. *T. achaeae*, for example, has been identified as a suitable species for biological control of *T. absoluta* in South American. Under greenhouse conditions, the release of 30 adult female wasps per plant (i.e. 75 adults per m²) every 3 - 4 days during August and September of 2008 was reported to reduce the damage by 91.74% in the southeast of Spain [12]. Several other native species of *Trichogramma* have been also evaluated for controlling *T. absoluta* in many crops worldwide [10]-[17]. Given the host specificity of *B. thuringiensis* var. *Krستaki* against lepidopteran larvae, this bacterium is an ideal agent to be used in combination with other biological agents, such as *Trichogramma* wasps, in integrated management of *T. absoluta*. It has been reported that a combine application of *T. pretiosum* and *B. thuringiensis* resulted in the reduction of fruit damage by 2% in South America.

T. brassicae and *T. embryophagum* are among the most widespread species of *Trichogramma* in Iran [18] [19]. Before decision making about the combined use of biological agents, it is necessary to evaluate their unwanted side effects on each other's in order to select for the best combination from available agents and so, prevent increased production costs.

In the current study, the efficiency of *T. brassicae* and *T. embryophagum* alone and in combination with *B. thuringiensis* was investigated against the tomato leafminer *T. absoluta* under greenhouse conditions. Additionally, the parasitism rate and some other biological characteristics of *T. brassicae* and *T. embryophagum* were investigated in presence of *B. thuringiensis*, in order to explore any detrimental effects of the entomopathogenic bacterium on these egg parasitoids.

2. Materials and Methods

2.1. Plants and Insects

Tomato plants (*Solanum lycopersicum* L.) were grown in a greenhouse (25°C ± 5°C, 70% ± 5% RH, 16L:8D), seeds were originated from the University of Tehran, Department of Plant Protection, Ecology and Behavior Lab (Karaj, Iran). Five week old plants were used in the experiments.

All used insects were collected from the University of Tehran. Mated females of *T. absoluta* were obtained by pairing a virgin male and a virgin female moth. The eggs of *T. absoluta* were obtained by placing adult males and females approximately 30 adult inside oviposition cages (50 cm × 50 cm) containing tomato plants offered as a host plant for egg laying and removed 24 h later, tomato plants with eggs were kept at the same conditions in the growth chambers until used for the experiments. Colonies of *T. brassicae* and *T. embryophagum* (Hymenoptera: Trichogrammatidae), were reared on eggs of the Mediterranean

flour moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae) in the University of Tehran, in a climate chamber ($25^{\circ}\text{C} \pm 5^{\circ}\text{C}$, $70\% \pm 5\% \text{RH}$, L16:D8). Only mated 24 hour old wasps were used in the experiments.

2.2. Formulation of Bt

The Bt formulation used in the experiments was *B. thuringiensis* sbsp. *kurstaki* (10^9 spore/g) WP, Biolep, Biorun Company, Iran.

2.3. Test Facilities

The experiment was conducted in a $6 \times 10 \text{ m}^2$ research greenhouse located at the University of Tehran, Department of Plant Protection (Karaj, Iran). Experimental cages ($95 \times 70 \times 120 \text{ cm}$) were screened with anti-thrips polyethylene mesh, and accessed by a separate door secured with a zipper.

2.4. Experimental Design

This experiment was conducted with four treatments (including control), each containing five tomato plants (30 cm in height) were placed inside each of the screened cages and one pair of adult *T. absoluta* per plant was released in each cage immediately after planting. In treatment 1 (T1), namely *Tuta* + *Trichogramma*, 20 adult *Trichogramma* females per plant was released in the cages one day following *T. absoluta* mating. In treatment 2 (T2), namely *Tuta* + Bt, 25 ml per plant of the Bt suspension (with a concentration of 10^6 spore/ml) was sprayed on plant surfaces two times, once at the beginning of the experiment before *T. absoluta* release and the other one week later. In treatment 3 (T3), namely *Tuta* + *Trichogramma* + Bt, the release of *Trichogramma* wasps and the spraying of Bt suspension was simultaneously conducted exactly like what described for treatment 1 and 2, respectively. Finally, in treatment 4 (T4, *i.e.* the control), the plants were sprayed two times with water, with no release of *Trichogramma* wasps and no Bt treatment.

2.5. Sampling

Sampling was conducted for two weeks, 2 times. The presence of pest eggs was sampled separately. In the first treatment, 15 leaves for each replicate were selected at random from the upper, middle, and bottom third of tomato plants experimented in each treatment after 5 days from *Trichogramma* sp. release. Data were recorded two times once; at the fifth day after the beginning of the experiment and the other after two weeks. The parasitism rate, adult emergence rate percentage, number of females and adult longevity were assessed. Second treatment sampling was conducted for two weeks, 15 leaves selected at random from the upper, middle, and bottom third of the plant tomato plants experimented in each treatment. In the third treatment, 15 leaves for each replicate were selected at random from the upper, middle, and bottom third of tomato plants experimented in each treatment after 5 days from *Trichogramma* sp. release. Data were recorded twice; first record after 5 days from 1st week and the second

record after 2nd week. The rate of egg parasitism, adult emergence percentage, number of females and adult longevity were assessed. In the fourth treatment, 15 leaves (total 150 leaves) recently expanded were selected at random from the upper, middle, and bottom third of the tomato plants experimented in each treatment, in order to determine the preferred plant section for oviposition of *T. absoluta*. The leaves were collected, labeled, and transported to the laboratory and then examined under a stereoscopic microscope to determine whether eggs had hatched or had been killed. In addition, three leaves, selected along each of the five selected plants were examined for the presence of *T. absoluta* larval mines from a randomly selected upper, middle and low in all treatments.

3. Statistical Analysis

For statistical analysis, each mean value is given with its standard error. The differences in parasitism rate, adult emergence, number of females and adult longevity between two Trichogramma species were compared using one way analysis of variance (ANOVA). When significant differences were detected, further comparisons were run using the Duncan' test ($p \leq 0.05$). Univariate general linear model (GLM) analysis was performed for comparison of the number of mines per plant. Statistical analysis was performed using the software SPSS Statistics for Windows Version 21 (SPSS 2012).

4. Results

The average number of *T. absoluta* larval mines on tomato leaves treated with Trichogramma species as well as Bt suspension have been summarized in **Figure 1**. Significant difference was observed in the number of larval mines of plants treated with *T. brassicae* and *T. embryophagum* alone or in combination with *B. thuringiensis* ($F = 78.385$; $df = 5$; $p \leq 0.001$). Additionally there was significant difference in the number of larval mines between two weeks in nearly all experimental treatments ($F = 4.524$; $df = 1$; $p \leq 0.050$). In first treatment (T1), an average of 18.2 and 12.10 mines/plant of *T. absoluta* were recorded during the first week when *T. brassicae* and *T. embryophagum* were alone released respectively. A significant increase in the number of mines per plant was recorded in the second week after treatment. When *B. thuringiensis* was used in combination with each of *T. brassicae* and *T. embryophagum* the number of larval mines was recorded as 10.50 and 2.80 mines/plant during the first week, respectively. However, in contrast to the application of Trichogramma alone, the integration of Bt and Trichogramma resulted in a significant decrease in the average number of larval mines in the second week, such that an average number of 6.10 and 0.50 mines/plant was recorded for *T. brassicae* and *T. embryophagum* respectively. Indeed, the mean number of *T. absoluta* mines in the treatment with parasitoid releases and *B. thuringiensis* was significantly lower than in the control (T4) (**Figure 1**). In particular, the best results in relation to *T. absoluta* control were obtained in the treatment where *T. embryophagum* was released in combination with *B. thuringiensis* application. The biological characteristics of *T. brassicae*

and *T. embryophagum* have been presented in Figure 2-6. As the Figure 2, the results shows significant differences were observed among parasitism percentages of *T. brassicae* and *T. embryophagum* in presence of *B. thuringiensis* ($F = 4.617$; $df = 3$; $p \leq 0.010$), with the highest parasitism rate was obtained for *T. embryophagum* with Bt was 31.18%. Otherwise, there was no significant difference

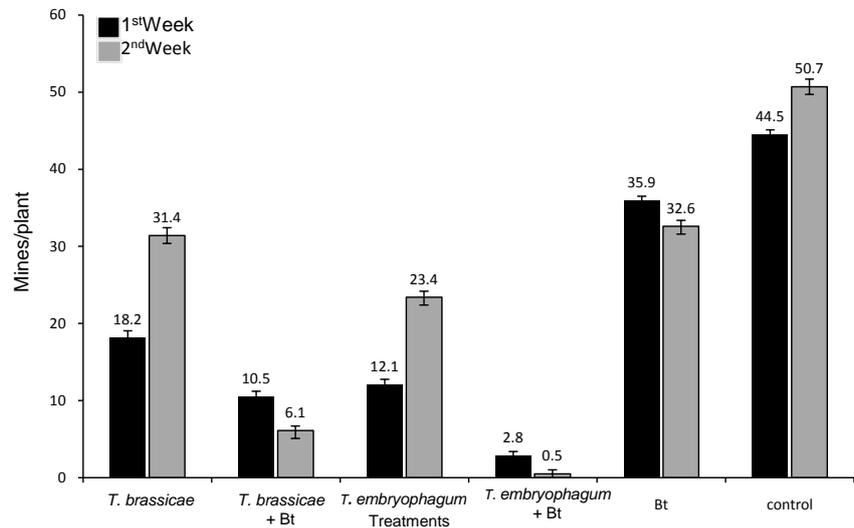


Figure 1. Mean number of *Tuta absoluta* mines per plant according to release *T. brassicae* and *T. embryophagum* in comparison to the control, in the first and second week (Treatments: $F = 78.385$, $df = 5$, $p = 0.000$) (Weeks: $F = 4.524$, $df = 1$, $p = 0.036$).

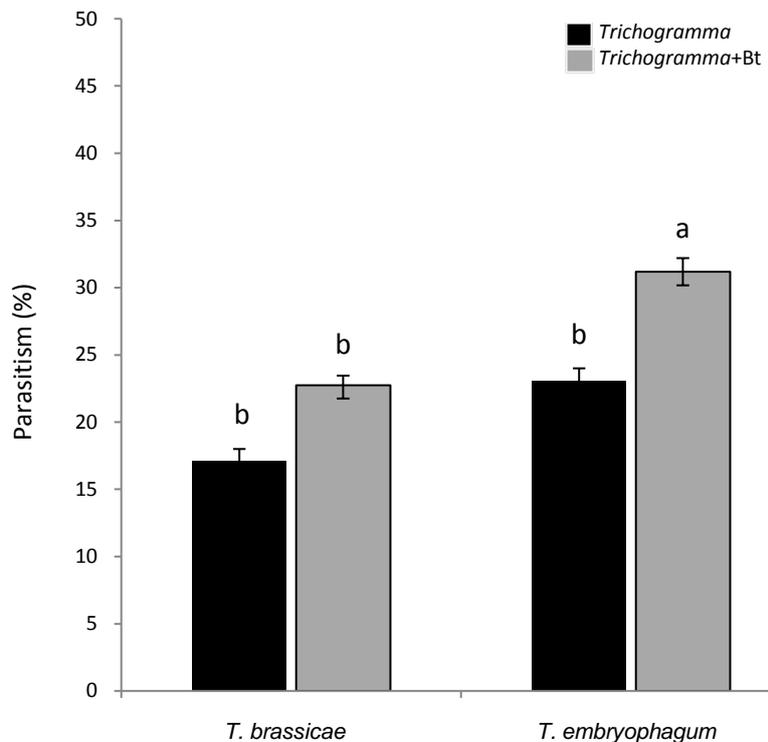


Figure 2. The parasitism of *T. brassicae* and *T. embryophagum* when treated with/ without Bt reared in the *T. absoluta* eggs incage inside greenhouse ($F = 4.617$, $df = 3$, $p = 0.008$).

in parasitism rate of other treatments. Additionally, we found no significant differences among mortality of two parasitoid species in presence or absence of Bt treatment ($F = 1.365$; $df = 3$; $p \leq 0.269$). In this experiment, an average mortality of 21.92 and 37.25% was recorded for *T. brassicae* in presence and absence of *B. thuringiensis*, respectively, while the mortality of *T. embryophagum* was recorded as 24.80, 26.84% in presence and absence of *B. thuringiensis* respectively (Figure 3). Similarly, there was no significant difference in the emergence of adult rate of both wasp species in presence or absence of *B. thuringiensis* (78.07 and 62.74% for *T. brassicae* and 75.18 and 73.85% for *T. embryophagum* in presence and absence of *B. thuringiensis*, respectively) ($F = 1.408$; $df = 3$; $p \leq 0.256$) ($p = 0.256$) (Figure 4). The ratio of females in untreated plants was significantly higher than that of Bt treated ones in both *Trichogramma* species (68.54% and 48.83% for *T. brassicae* and 67.54% and 43.63% for *T. embryophagum* in absence and presence of Bt respectively) ($F = 7.782$; $df = 3$; $p \leq 0.001$) (Figure 5). The number of females of two *T. richogramma* species is shown in Figure 5. Finally, we found significant difference in the longevity of adult parasitic wasps in presence or absence of Bt ($F = 12.504$; $df = 3$; $p \leq 0.000$). The longevity of *T. embryophagum* adults reared on Bt treated eggs of *T. absoluta* (6.4 d) was significantly higher than that of untreated plants (5.6 d) (Figure 6). Although, the longevity of *T. brassicae* was not affected by Bt treatment, it was significantly lower than *T. embryophagum* (Figure 6).

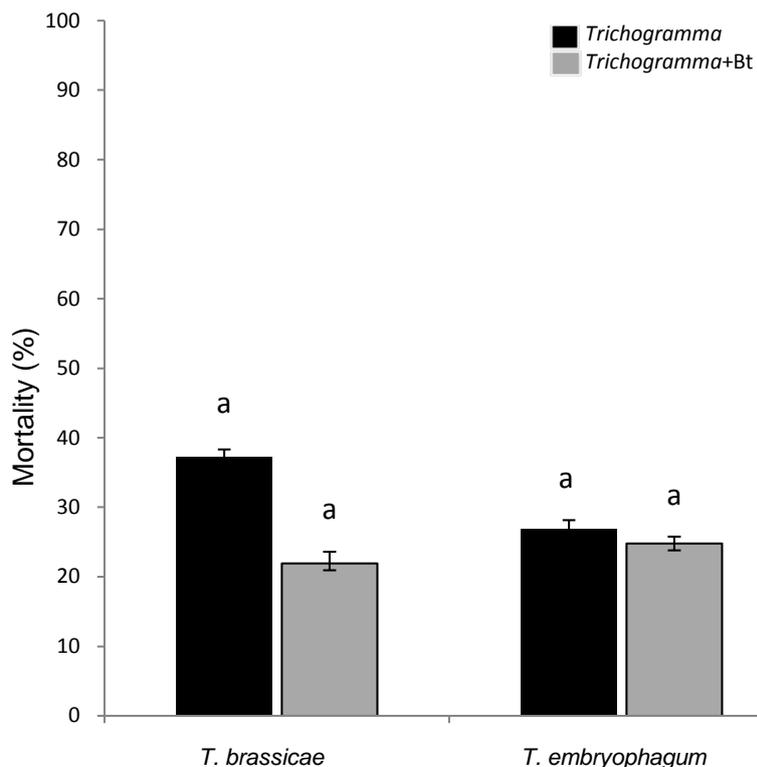


Figure 3. The Mortality of *T. brassicae* and *T. embryophagum* when treated with/without Bt reared in the *T. absoluta* eggs incage inside greenhouse ($F = 1.365$, $df = 3$, $p = 0.269$).

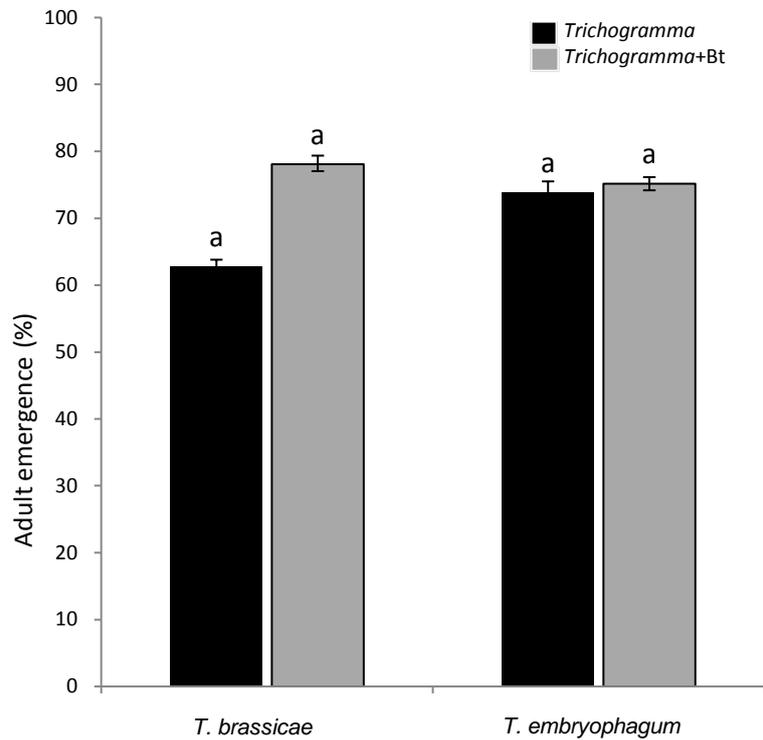


Figure 4. The adult emergence of *T. brassicae* and *T. embryophagum* when treated with/without Bt reared in the *T. absoluta* eggs in cage inside greenhouse ($F = 1.408$, $df = 3$, $p = 0.256$).

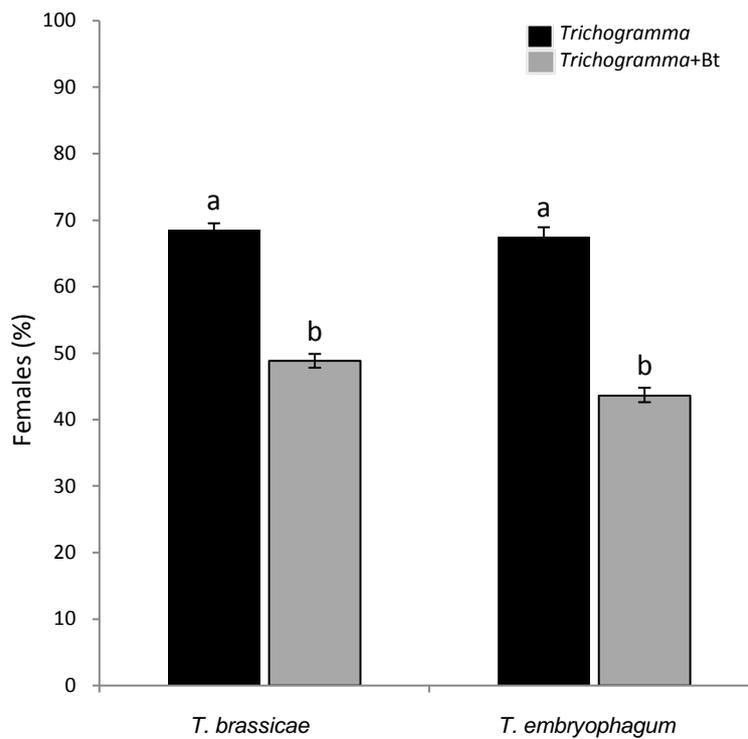


Figure 5. Females of *T. brassicae* and *T. embryophagum* when treated with/without Bt reared in the *T. absoluta* eggs in cage inside greenhouse ($F = 7.782$, $df = 3$, $p = 0.000$).

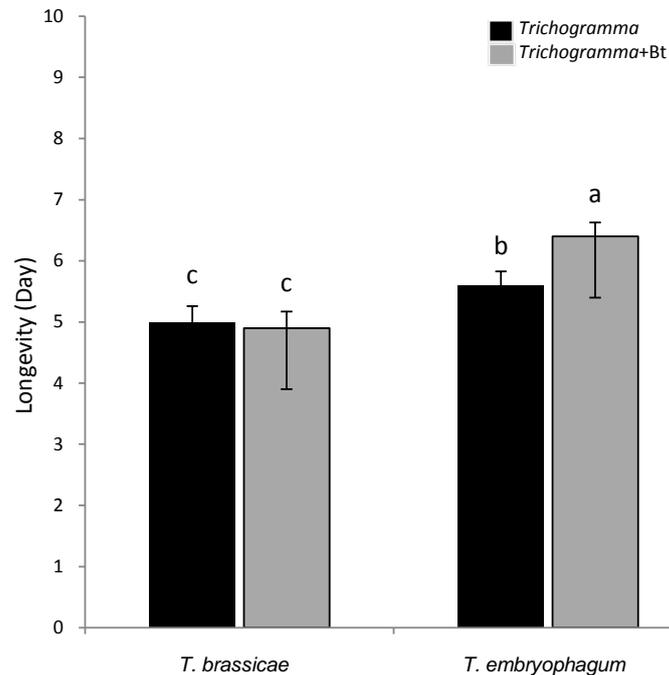


Figure 6. Longevity of *T. brassicae* and *T. embryophagum* when treated with/without Bt reared in the *T. absoluta* eggs in cage inside greenhouse (F = 12.504, df = 3, $p = 0.000$).

5. Discussion

The present study showed an efficiency of *T. brassicae* and *T. embryophagum* released in combination with *B. thuringiensis* in controlling the damage of *T. absoluta*, when compared to the untreated control plants, *Trichogramma* sp. release and spray with *B. thuringiensis* only. The decrease of eggs was higher than the larvae in the first treatment when *T. brassicae* and *T. embryophagum* were released in the first week, which was consistent with. Results reported by [20]. However, significant differences between the two species were consistently observed in biocontrol activity against *T. absoluta*, where *T. embryophagum* performed slightly well than *T. brassicae* in the first week when released without *B. thuringiensis*, and better in the second week when released with *B. thuringiensis*. These results are in agreement with earlier investigations that demonstrated that the integration of these two organisms (*Trichogramma* sp. + *B. thuringiensis*) may produce desirable results in *T. absoluta* control [9] [21]. Also, compared with the control treatment, the decrease of mines numbers of *T. absoluta* was higher in *Trichogramma* sp. + *B. thuringiensis* treatments. The average number of larval mines per plant of mines per plant in *Trichogramma* sp. + *B. thuringiensis* treatments was significantly lower than that of *Trichogramma* release without Bt treatment.

Consistent with the previous findings, the data collected on 1st week after the treatment indicated that the lowest number of *H. armigera* larvae per plant was recorded in treatment (Trichocard having 300 parasitized eggs in combination with Neem extract) and treatment (Trichocard having 300 parasitized eggs in

combination with 45 *Chrysoperla* 2nd instar larvae and neem extract) recorded 0.50 and 0.60 respectively [22].

In this study, the parasitism rate of two *Trichogramma* species were tested in cage experiments inside greenhouse. Results showed that both *T. richogramma* species accepted *T. absoluta* eggs as host and the species of *T. embryophagum* exhibited promising (31.18%) parasitism when treated in combination with Bt vs. 23.05% of parasitism without Bt. Our result were in line with results obtained by Chailleux *et al.* [23] that investigated twenty-nine *Trichogramma* species-strains on *T. absoluta* eggs under laboratory conditions and recorded the highest rate of egg parasitism (35.4%) for *T. achaeae*. Furthermore, we found a significant increase in the number of parasitized eggs when the tomato plants were simultaneously treated with Bt. These results were significantly different by Vaez *et al.* [21] suggested that the functional response parameters of *Trichogramma* wasps were affected by Bt treatment such that those parasitic wasps which developed inside Bt-treated hosts were weaker than normal ones. They attributed these observations to the sub-lethal effects of Bt on *T. brassicae* females searching host eggs laid by *H. armigera* treated by LC₂₀ of the bacterium. Moreover, studies have suggested that larger host eggs can positively influence parameters such as percentage of parasitism, number of parasitoids emerged per egg, longevity, and sex ratio [24] [25] [26]. The parasitism rate of *Trichogramma* wasps have been argued to vary with physical and chemical barriers as well as by the type and characteristics of host eggs such as their size, hardness, scales and kairomones [17] [19] [27] [28]. Similarly, 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 [29] [30]. Adult longevity of *T. embryophagum* reared on eggs of *Ephestia kuhniella* Zeller has been reported as 12.37 days by [31]. These differences show that the host species may also influence adult longevity of parasitoid wasps. Also, reduction in host fecundity may show inverse effects of bioinsecticides on yolk deposition process and this may reduce both the quality and quantity of the eggs deposited by host as well as their size [21], 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 [32] [33] [34].

6. Conclusions

The results will undoubtedly be supported by the current scientific and applied research conducted with parasitoids and entomopathogens, which will help in managing *T. absolutea* in those areas or crop cycles. In this scenario, augmentative and conservation strategies intending to increase the role of these natural enemies in *T. absoluta* control could become the cornerstone in the reconfiguration of integrated pest management in the tomato crop.

This study showed that different species of *Trichogramma* wasps have parasi-

tized eggs of *T. absoluta* as one of their herbivores. Additionally, we found no direct effect of Bt treatment on parasitism rate and some other biological characteristics of *Trichogramma* species. These findings may justify the combination of these two biocontrol organisms, especially *T. embryophagum* as a promising strategy for integrated management of *T. absoluta* under greenhouse conditions.

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