



## Research Article

# Integration of *Trichogramma evanescens* Westwood and *Bacillus thuringiensis* subsp. *kurstaki* for controlling lepidopterous pests in tomato fields

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**ABSTRACT:** The efficacy of the egg parasitoid *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) and *Bacillus thuringiensis* subsp. *kurstaki* for controlling *Helicoverpa armigera* (Hubner) and *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae) in tomato fields was investigated. Single and combined treatments of *T. evanescens* and the bioinsecticide were tested. Control methods were carried in plant protection research station, at Qaha, Qalyubia Governorate during summer plantations, 2008. The efficacy of these control methods was recorded on the basis of mean number of larvae/plant and reduction percentage of the larvae during six weeks from each application. Based on reduction percentages in the number of *H. armigera* and *C. chalcites* larvae, the efficacy of the tested treatments could be descendingly arranged as follows *T. evanescens* + *B. thuringiensis*, *B. thuringiensis* and *T. evanescens*. The corresponding values were 90, 82.9, and 81.2 % for the above treatments respectively, against *H. armigera* and 86.3, 59.3 and 57.5% for the above treatment respectively, against *C. chalcites*. The result also shows that there was no negative interaction between *B. thuringiensis* application and *T. evanescens* releases. By comparing the ratio of damaged caused to tomato fruits by all lepidopterous larvae complex feeding, mean weight of healthy fruits of yield and mean weight of single healthy and infected fruit after the above mentioned treatments, the integrated treatment (*T. evanescens* + *B. thuringiensis*) was the most effective followed by *B. thuringiensis* and *T. evanescens*.

**KEY WORDS:** *Helicoverpa armigera*, *Chrysodeixis chalcites*, parasitoid, bioinsecticide, tomato

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## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) belonging to Solanaceae is an important and remunerative vegetable crop grown around the world for fresh market and processing (Salunkhe *et al.*, 1987). Egypt is considered as one of the important tomato producers in the world (WPTC, 2011). Tomato is the main vegetable crop in Egypt. The tomato-growing area in 1989 was 32% of the total vegetable-growing area and the tomato production represented 35% of total vegetable production (Zaki, 1992).

The American bollworm, *Helicoverpa armigera* (Hübner) known as “tomato fruit worm” is a polyphagous pest that feeds on over 200 host plants and wild species including at least 45 families (Sidde Gowda *et al.*, 2002; Ibrahim, 2012). The production and productivity of the tomato crop is greatly hampered by the fruit borer, *H. armigera* which causes damage to developing fruits and results in yield loss ranging from 20 to 60% (Tewari and

Krishnamoorthy, 1984; Lal and Lal, 1996). Due to wider host range, multiple generations, migratory behavior, high fecundity and existing insecticide resistance this insect became a difficult pest to tackle (Hussain *et al.*, 1991; Ahmed *et al.*, 2000).

*Chrysodeixis chalcites* (Esper) is a highly polyphagous pest that causes important damage by feeding on the foliage and fruit of a variety of vegetable and ornamental crops, both in field and greenhouse production. It is considered a “major pest” and “one of the most serious lepidopteran pests” in many countries (CABI, 2013; Polaszek *et al.*, 2012) also known as the tomato looper is a serious pest native to Mediterranean and tropical regions.

Pest problem is main limiting factor for tomato cultivation as this is attacked by different insect pests such as *Helicoverpa* and *Chrysodeixis* etc. Due to its high fecundity, polyphagous nature and quick adaptation against insecti-

cides, control of this pest with any single potent toxicant for a long time is quiet difficult and rather impossible (Ghosh *et al.*, 2012).

*Trichogramma evanescens* West. (Hymenoptera: Trichogrammatidae) is a native egg parasitoid in Egypt. *Trichogramma* parasitoids have already been successfully used in biological control of various lepidopteran agricultural pests (Prattisoli, *et al.*, 2005; Smith, 1996). They are easy to rear (Mansour, 2010) and to release in open fields or protected crops.

*Bacillus thuringiensis* is a biological control agent and is used extensively in integrated pest management programs especially for the control of Lepidoptera, Coleoptera and human disease insect vectors (Wei, *et al.*, 2003). Taghizadeh (2006) evaluated the effect of *Bt* on cotton bollworm *Helicoverpa armigera* Hubner and results showed that *Bt* can control pests as a biological agent and can be accepted as an alternative biopesticide for synthetic insecticides like endosulfan.

The objective of this study is to evaluate the efficacy of releasing *T. evanescens*, *Bacillus thuringiensis* subsp. *kurstaki* and their integration to control *Helicoverpa armigera* (Hubner) and *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae) in tomato fields and calculate the quantity of production and percent of injury for each treatment during Summer plantation 2008 at plant protection research station, at Qaha, Qalyubia Governorate.

## MATERIALS AND METHODS

Field trials were carried out during summer 2008 at plant protection research station, Qaha, Qalyubia Governorate to evaluate the effectiveness of the egg parasitoid *Trichogramma evanescens* and microbial insecticide *Bacillus thuringiensis* (Dipel 2x) as biological control agents of *Helicoverpa armigera* and *Chrysodeixis chalcites* (Esper) in tomato.

Tomato seedlings var. super strain B were transplanted into the open field on 2<sup>nd</sup> of April in a randomized complete block design with four treatments replicated three times.

Each plot was 8 rows wide and 12m long separated by 12m. to discourage *T. evanescens* movement between them. Planting, cultivation, irrigation and other cultural practices were done in accordance with local recommendations and imidacloprid (Admire 20% SC) at 125ml / 100L was applied at weekly intervals for six weeks starting immediately after transplanting to control the cotton white fly. When  $\leq 50\%$  of the plants had open flowers the following treatments were applied:

## Treatments for field experiments

Three treatments (A, B, C and untreated) served to carry out different experiments to compare efficacy of the tested three treatments for controlling *H. armigera* and *C. chalcites* namely *B. thuringiensis*, *T. evanescens* and *B. thuringiensis* + *T. evanescens* and control, respectively.

Treatment A: Plots received six applications of *Bacillus thuringiensis* var *kurstaki* preparation of Dipel 2X (wetable powder, Abott, orth Chicago 1L 3200 international units of potency per mg.) at a rate of 50g / 100L. were applied weekly from 20 May through 24 June.

Treatment B: Plots received releases of *T. evanescens* to control eggs of *H. armigera* and *C. chalcites*. The parasitoid was mass reared on *Sitotroga cerealella* eggs, releases were made from paper cards bearing the parasitized eggs (1500 eggs/ card) was obtained from Plant Protection Research Institute, Agriculture Research Center, ARC and carried to the field in an ice box. Two paper cards were installed on two plants 4 M. apart along the center of each release plot (plot was 8 rows wide and 12m long). Releases were made at weekly intervals for 6 weeks starting 20<sup>th</sup> of May.

Treatment C: Plots received six weekly applications of *T. evanescens* releases as shown in treatment B simultaneously plus *Bacillus thuringiensis* as shown in treatment A starting 20<sup>th</sup> of May.

Untreated (Control): Plots were left free of any applications as control. Larval counts were made before each application by shaking 5 plants selected randomly for each plot onto a collecting cloth, all pink and red fruit were harvested from two inside rows of each plot and the numbers of healthy and damaged fruits were recorded to calculate the percentage of fruit damaged by lepidopterous pests.

## Statistical analysis

The statistical analysis (ANOVA) and simple correlation of the obtained data were performed by using SAS program (SAS institute, 1988), the post hoc test was done by DMRT.

## RESULTS AND DISCUSSION

The experimental trials were implemented to evaluate the role of different methods in controlling *Helicoverpa armigera* and *Chrysodeixis chalcites* (Esper) larvae infested tomato in summer plantation during 2008. The experimental results are indicated in Table 1, revealed the mean number /plant and % reduction of *H. armigera* on tomato plants before and after treatment with *Trichogramma evanescens*, *Bacillus thuringiensis* and their combined effect.

Before treatment the mean number of *H. armigera* larva on tomato plants were 1.40, 0.93 and 1.06 larvae / plant on May 20<sup>th</sup> in the above mentioned experimental

plots respectively, while in the untreated plots the mean number was 0.86 larvae/plant.

After treatment, the population of *H. armigera* larvae decreased gradually till reached 0.70, 0.20 and 0.13 larvae/plant during 4<sup>th</sup> week of June, while in untreated plots the mean number increase gradually till reached 7.46 larvae/plant during the same week. The obtained results in the same table indicated that the average of reduction were 81.2, 82.9 and 90% for the above mentioned treatments respectively, the integrated treatment (*T. evanescens* + *B. thuringiensis*) with high significant difference was the most effective followed by *B. thuringiensis* while *T. evanescens* was the least effective.

On the other hand, before treatment the mean number of *C. chalcites* larvae on tomato plants were 6.50, 4.00 and 5.26 larvae/plant in May 20<sup>th</sup> in experimental plots treated with *T. evanescens*, *B. thuringiensis* and their combined effect respectively, while in the untreated plots the mean number was 8.13 larvae/plant.

After treatment, the population of *C. chalcites* larvae decreased gradually till reached 3.00, 1.80 and 0.20 larvae/plant during 4<sup>th</sup> week of June, while in untreated plots the mean number increased gradually till reached 13.40 larvae/plant during the same week. The results in the same table indicated that the average of reduction was 57.5, 59.3 and 86.3% for the above mentioned treatments respectively, the integrated treatment (*T. evanescens* + *B. thuringiensis*) with high significant difference was the most effective fol-

lowed by *B. thuringiensis* while *T. evanescens* was the least effective.

### Comparisons on basis of the controlling index and potency levels

In the present work, comparisons among the tested control methods are based on both control index method developed by Sun (1950) and the potency levels expressed as number of folds frequently used in this respect.

On the ground of control index illustrated in Table 1, the efficacy of treatments during the 6<sup>th</sup> week after treatments of *B. thuringiensis* and *T. evanescens* recorded 98.9 and 95.5% respectively as effective to the integrated treatment (*T. evanescens* + *B. thuringiensis*) against *H. armigera* during summer plantation 2008.

In table (2) the efficacy of treatments during 6<sup>th</sup> week after treatments of *B. thuringiensis* and *T. evanescens* recorded 74.41 and 73.59% respectively as effective to the integrated treatment (*T. evanescens* + *B. thuringiensis*) against *C. chalcites* during summer plantation 2008.

Concerning the potency level expressed as number of folds (Table 1), the efficacy of the tested treatments, i.e. *B. thuringiensis* and *T. evanescens* recorded 1.0 and 1.0 times, respectively as effective as the integrated treatment (*T. evanescens* + *B. thuringiensis*) against *H. armigera* during summer plantation 2008. The same results were also obtained with *C. chalcites* for the mentioned treatments.

% General reduction in the tested treatment

**Table 1. Mean number and reduction of *Helicoverpa armigera* infesting tomato plants before and after treatment by *Trichogramma evanescens*, *Bacillus thuringiensis* and *T. evanescens* + *B. thuringiensis* during summer season 2008**

Treatments	Account before treatments	Weekly mean number of larvae / plant account after treatments						Mean
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	
<i>T. evanescens</i>	1.40	001.20	001.09	000.93	000.80	000.80	000.70	81.20 b
	Reduction (%)	047.30	077.70	086.80	089.00	092.10	094.20	
	Control index*	068.30	088.80	092.30	094.70	095.30	095.50	
	Potency level**	001.00	001.00	001.00	001.00	001.00	001.00	
<i>B. thuringiensis</i>	0.93	000.80	000.60	000.60	000.50	000.40	000.20	82.90 b
	Reduction (%)	047.20	081.50	087.20	089.60	094.10	097.50	
	Control index*	068.10	093.10	092.80	095.30	097.40	098.90	
	Potency level**	001.00	001.05	001.00	001.00	001.00	001.00	
<i>T. evanescens</i> + <i>B. thuringiensis</i>	1.06	000.53	000.46	000.33	000.33	000.26	000.13	90.00 a
	Reduction (%)	069.30	087.50	094.00	094.00	096.60	098.60	
	Control index*	100.00	100.00	100.00	100.00	100.00	100.00	
	Potency level**	001.50	001.13	001.10	001.10	001.00	001.00	
Control	0.86	001.40	003.00	004.33	004.46	006.26	007.46	
<b>L.S.D (P=0.05)</b>								06.43

\* Control index = ----- × 100  
 % General reduction in the most promising treatment  
 % General reduction in the tested treatment

\*\* Potency level = -----  
 % General reduction in the least effective treatment

Effect of lepidopterous larvae on yield, mean weight and

*sis*, and their integrated (*T. evanescens* + *B. thuringiensis*) treatments, while the average weight of fruit in untreated plots was 79.04 g. The mean weight of damaged fruits were 88.16, 94.7 and 110.1 g. for the same treatments, respectively, while untreated was 54.22g.

**Table 2. Mean number and reduction of *Chrysodeixis chalcites* infesting tomato plants before and after treatment by *Trichogramma evanescens*, *Bacillus thuringiensis* and *T. evanescens* + *B. thuringiensis* during summer season 2008**

Treatments	Account before treatments	Weekly mean number of larvae / plant account after treatments						Mean
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	
<i>T. evanescens</i>	6.50	005.33	004.60	003.46	003.26	003.26	003.00	57.50 b
	Reduction (%)	025.90	048.30	062.40	066.60	069.70	071.90	
	Control index *	040.09	057.30	072.14	074.16	073.60	073.59	
	Potency level**	001.00	001.00	001.08	001.05	001.00	001.00	
<i>B. thuringiensis</i>	4.00	002.66	002.60	002.40	002.20	002.00	001.80	59.30 b
	Reduction (%)	039.90	052.50	057.60	063.35	069.80	072.70	
	Control index *	061.76	062.28	066.59	070.55	073.71	074.41	
	Potency level**	001.54	001.09	001.00	001.00	001.00	001.01	
<i>T. evanescens</i> + <i>B. thuringiensis</i>	5.26	002.06	001.13	001.00	000.80	000.46	000.20	86.30 a
	Reduction (%)	064.60	084.30	086.50	089.80	094.70	097.70	
	Control index *	100.00	100.00	100.00	100.00	100.00	100.00	
	Potency level**	002.49	001.75	001.50	001.42	001.36	001.36	
Control	8.13	009.00	011.13	011.50	012.20	013.46	013.40	
<b>L.S.D. (0.05)</b>								06.06

single fruit weight

The results in Table 3 revealed the ratio of damaged caused to tomato fruits by lepidopterous larvae complex feeding after treatment were 34.26, 26.07 and 5.72% for *T. evanescens*, *B. thuringiensis* and their integrated (*T. evanescens* + *B. thuringiensis*) treatments, respectively, while was 74.5% in untreated plots with high significant difference with other three treatments. It is obvious that the application of integrated treatment (*T. evanescens* + *B. thuringiensis*) reduced the ratio of damaged fruits to 5.72% lower than *B. thuringiensis* while *T. evanescens* was least effective.

Data in Table 3 also concluded the mean weight of healthy fruits of yield were 2961.8, 4323.00, 5324.8 and 641.7 gram for *T. evanescens*, *B. thuringiensis*, their integrated (*T. evanescens* + *B. thuringiensis*) treatments and untreated plots, respectively whereas the mean weight of damaged fruits recorded 1295.3, 1158.2, 342.2 and 1179.3 gram for the same treatments and untreated plots, respectively.

The obtained data also showed the mean weight of single healthy and infected fruit (g.) after treatments. The average weights of healthy fruit were 100.38, 103.8, and 116.47 g., respectively for *T. evanescens*, *B. thuringien-*

Statistical analysis of the results revealed that the maximum quantity of damaged fruits due to fruit borer was noticed in untreated plots while the maximum quantity of healthy fruits resulted from plots received the integrated treatment (*T. evanescens* + *B. thuringiensis*) followed by *B. thuringiensis* and the release of *T. evanescens* was least effective.

Our studies reveal that the use of bioinsecticide has enhanced the effectiveness of *T. evanescens* in controlling lepidopterous pests in tomato fields during summer plantation 2008. These results may indicate several facts. First, the tested bioinsecticide (*B. thuringiensis*) are not toxic to *T. evanescens*. Egg parasitoids have been found highly compatible with *B. thuringiensis*, as the eggs of the host insect are not the target stage for the microbe. The release of *T. evanescens* in a Bt-based IPM program in tomato, improved profitability of this program when compared with the use of conventional insecticides (Trumble and Alvarado-rodriguez, 1993). Second, dust treatment of Bt did not retard the searching parasitoids. Finally, there is an opportunity for further, large scale integration of the biocontrol agents under field conditions.

**Table 3. Mean season yield and percent of fruits injury caused by lepidopterous pests after treatment with *Trichogramma evanescens*, *Bacillus thuringiensis* and their combined during 2008 summer plantation season**

Treatment	Mean number of fruits			Mean weight of Total fruits (gm)		Single fruit weight (gm)		
	Healthy	Damaged	Total	Ratio of Damaged	Healthy	Damaged	Healthy	Damaged
<i>T. evanescens</i>	0029.34 b	0015.28 b	0044.60 a	0034.26 b	2961.80 b	1295.30 a	0100.38 a	0088.16 b
<i>B. thuringiensis</i>	0041.26 a	0012.70 b	0048.70 a	0026.07 c	4323.00 a	1158.20 a	0103.80 a	0094.70 ba
<i>T. evanescens</i> + <i>B. thuringiensis</i>	0045.60 a	0003.09 c	0054.01 a	0005.72 d	5324.80 a	342.20 b	0116.47 a	0110.10 a
Untreated	0008.23 c	0024.11 a	0032.34 b	0074.50 a	0641.70 c	1179.30 a	0079.04 b	0054.22 c
F value	0032.37	0015.18	0005.90	00097.90	0031.90	0029.43	0005.70	0011.41
L.S.D.	0008.60	0006.54	0011.14	0008.50	1058.30	0238.49	0019.20	0020.50

\*The values have the same letters horizontally are not significantly different at 0.05% level.

Our results are also in accordance with the results of (Mandour *et al.*, 2012) who concluded that the integration of *T. evanescens* and biocides enhanced the control of potato tuber moth in storage over that of single treatment (*T. evanescens* alone or biocide alone). Also our findings confirmed with the results of (Khan and Kumar, 2005) who studied the bio-efficacy of *Trichogramma* spp. against leaf folder in rice ecosystem. They reported that all the doses used in the inundative releases of egg parasitoids were found effective over the control.

*Bacillus thuringiensis* was proven to give no detrimental effects on adult survival and egg parasitism of the wasp based on contact toxicity test. The combined treatment of both biological control agents significantly decreased the crops damage rate, though none of the biological control agents alone showed any significant control efficacy (Hwang *et al.*, 2010). On the other hand, finding of this study are disagreement with finding of Movahedi *et al.* (2014) which showed that there was no difference between Bt spray treatment and release of *Trichogramma* plus Bt spray against *Ostrinia nubilalis* in corn fields, so the combination of these two factors did not increase the efficiency of each other.

Effective eco-friendly integrated pest management (IPM) by using a combination of both microbial agents and natural enemies can reduce the use of chemical pesticides, inhibit development of resistance to pests and is more favorable in terms of safer and more stable production of agricultural products.

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