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Effectiveness of Different Bio-techniques for Controlling the Pin Worm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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ABSTRACT

A study to evaluate the use of the egg-parasitoid, *Trichgramma evanescens* (West.) releases alone and/or in combination with certain bio-insecticides for controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under field conditions was carried out at the experimental farm of the Environmental Studies and Research Institute, Sadat City University, Minufiya Governorate, Egypt during 2013 and 2014 tomato growing summer plantation. Results indicated that early practices, at seedling stage, of the tested control techniques achieved highest potential, best foliage and fruit protection, and played a vital role for suppression *T. absoluta* damage comparing to the same applications at either flowering or fruiting stages. Results revealed significant and positive efficacy by practicing 12 releases of *T. evanescens* (at the rate of 80.000 parasitoid/fed.) at seedling stage, which reduced the damage at both foliage and fruiting stages by the lowest mean numbers (1.31±0.195, 1.51±0.027, 0.4±0.018 and 0.43±0.018) and (1.06±0.098, 1.39±0.027, 0.37±0.015 and 0.31±0.012) of infested leaves, mines/leaf, surviving larvae and infested stems in both seasons, respectively. Also, a significant fruit reduction (0.70±0.012 and 0.61±0.015) was recorded at seasons 2013 and 2014, respectively. On the other hand, this treatment gave the lowest mean values (53.89±0.36 and 50.66±0.61 kg/ton) of loss of fruit infestation relative to the highest healthy fruit yield (10.18±0.023 and 10.5±0.02 ton/fed) in both seasons, respectively. Also, obtained results indicated that the technique of 4 releases of *Trichogramma* + 4 applications of Protecto + Spinosad bait performed the best efficiency plant protection comparing to other tested treatments practiced at both flowering and fruiting stages in both seasons.

Key words: *Tuta absoluta,* Control, *Trichogramma evanescens,* Protecto, Spinosad bait, Radiant.

INTRODUCTION

The tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an invasive pest, native to South America that rapidly invaded Southern Europe and North Africa (Desneux *et al.*, 2011). The first record of this pest in Egypt was by late 2009. In 2010, it reached middle-Egypt and recently has well established in most of the Egyptian Governorates (Goda *et al.,* 2015). The pest was recognized as a very injurious and most destructive insect pest affecting the tomato crop during its whole cycle, in both greenhouses and/or open fields, causing a great economic loss in tomato fruit yield, that may reach (100%) with the absence of control strategies (Picanco *et al.*, 2007 and Silva *et al.*, 2011). *T. absoluta* has a high reproductive potential and short life-cycle that increases the risk of developing resistance by insecticidal applications (Pereyra and Sanchez, 2006). Moreover, increasing population levels of the pest leads growers to extend the use of insecticidal applications, that has many negative side-effects on natural enemies in tomato crops (Desneux *et al.*, 2007). But the internal living and feeding habits of *Tuta* larvae and its ability to produce several broods each year has encouraged depending upon the insecticides (may reach 8 to 25) sprays (Temerak, 2011). However, pesticides have shown partial success because of the general endophytic behavior of the larval instars and the rapid selection of resistant populations (Siqueira *et al.*, 2000 and Silva *et al.*, 2011). Therefore, several sustainable control methods and integrated pest management (IPM) programs have been recently evaluated (Batalla-Carrera *et al.*, 2010; Mollá *et al.*, 2011 and Vacas *et al.*, 2011).

Biological pest control can play an essential role in natural pest suppression, thus enhancing the natural balance may achieve sustainable agricultural systems. The egg parasitoid, *Trichogramma* spp. (Hymenoptera: Trichgrammatidae) has performed one of the best tactics for controlling different lepidopteran pests worldwide, which preserves the endemic natural enemy complex, reduces the need for additional treatments to control secondary pests, and reduces the environmental pollution associated with insecticides (Smith, 1996).

The present study aimed to evaluate the use of *Trichgramma evanescens* (West.) releases alone and/or in combination with recommended bio-insecticides for controlling *T. absoluta* under field conditions.

MATERIALS AND METHODS

Experimental design

Experiments were conducted at the experimental farm of the Environmental Studies and Research Institute, Sadat City University, Minufiya Governorate, Egypt to compare different numbers and/or times of releases of *T. evanescens* alone and/or in combination with different bio-insecticides against *T. absoluta* on tomato crop. An area of about two feddans (8400^{m2}) was divided into 13 equal parts (about 350m2 each). Each part contained three plots. A randomized complete block design, with 3 replicates, was followed. Seedlings of tomato were cultivated at the summer plantation during the two seasons, 2013 and 2014. All plots received the recommended agricultural practices, except using pesticides. Twelve treatments were applied, separated by 300 m from each other to prevent migration of *T. evanescens* from one part to the other. Planting date was 23rd of February in both seasons.

Materials used

The egg parasitoid, *Trichogramma evanescens*: The parasitoid was obtained from the International Company for Bio agriculture, Egypt. Releasing cards included about 2000 parasitized eggs/ card. Each card had 2-ages, to allow the successive adult emergence of *Trichogramma* along a period of one week post card installed in the field. The releasing cards were hung on the plant's stem at 7 day intervals.

The bio-insecticide, Protecto: is a commercial product formulation contains (32x10^{6} IU/mg) of *Bacillus thuringiensis* subsp. *kurstaki)* (WP) 10%.

The Bio-insecticide, Spinosad bait: Conserve 0.024% CB, contains, 0.024% spinosad plus food attractions (5 ml/liter), used as full traps.

The Bio-insecticide Radiant: Spinotram 12SC%, is the 2nd new generation of the spinosyn group, with the same mode of action as spinosad. It is a mixture of two active spinosyn (spinosyn J & L). It is a trademark of Dow Agro Sciences.

Treatments and technique

At seedling stage: (10 days post planting):

T1: Twelve releases of *Trichogramma* at a rate of (80.000 parasitoid/fed.), at 7 day intervals.

T2: Eight releases of *Trichogramma* at a rate of (80.000 parasitoid/fed) + 4 applications of radiant at a rate of (60 ml/fed.), at 10 day intervals.

T3: Eight releases of *Trichogramma* at a rate of (80.000 parasitoid/fed.), at 7 day intervals + 4 applications of Protecto (WP) 10%, at a rate (300 gm/fed.), at 10 day intervals.

T4: Six releases of *Trichogramma* at a rate of (80.000 parasitoid/fed.), at 7 day intervals + 4 applications of Protecto (WP) 10%, at a rate (300 gm/fed.), at 10 day intervals + spinosad bait, Conserve 0.024% CB, contains, 0.024% spinosad plus food attractions, (5 ml/liter), used as traps between rows (one trap/ 10 m) at 15 day intervals.

At flowering stage:

T5: Nine releases of *Trichogramma* at a rate of (80.000 parasitoid/fed.), at 7 day intervals.

T6: Six releases of *Trichogramma* at a rate of (80.000 parasitoid/fed.), at 7 day intervals + 4 applications of radiant, at a rate of (60 ml/fed.), at 10 day intervals.

T7: Six releases of *Trichogramma* at a rate of (80.000 parasitoid/fed.), at 7 day intervals + 4 applications of Protecto (WP) 10%, at a rate (300 gm/fed.), at 10 day intervals.

T8: Four releases of *Trichogramma* at a rate of (80.000 parasitoid/fed.), at 7 day intervals + 4 applications of Protecto (WP) 10%, at a rate (300 gm/fed.), at 10 day intervals + spinosad bait: Conserve 0.024%, (5 ml/liter), used as full traps between rows one trap/ 10 m, at 15day intervals.

At fruiting stage:

T9: Six releases of *Trichogramma* at a rate of (80.000 parasitoid/fed), at 7 day intervals.

T10: Six releases of *Trichogramma* at a rate of (80.000 parasitoid/fed), at 7 day intervals + 4 applications of radiant, at a rate of (60 ml/feddan), at 10 day intervals.

T11: Six releases of *Trichogramma* at a rate of (80.000 parasitoid/fed.) at 7 day intervals + 4 applications of Protecto (WP) 10%, at a rate (300 gm/fed.), at 10 day intervals.

T12: Four releases of *Trichogramma* at a rate of (80.000 parasitoid/fed.), at 7 day intervals + 4 applications of Protecto (WP) 10%, at a rate (300 gm/fed.), at 10 day intervals + spinosad bait: Conserve 0.024% CB, contains, 0.024% spinosad plus food attractions, 5 ml./liter placed as full traps between rows one trap/10 m at 15day intervals.

T13: Untreated as a control treatment that didn't receive any of the formerly mentioned treatments.

Assessment

For foliage infestation, sampling included; infested leaves, mines/leaf, surviving larvae and infested stems throughout the experimental period as well as infested fruits, fruit yield losses due to infestation under different tested treatments. Therefore, randomized samples of (10 compound leaves and 5 stems) /5 plant/ replicate were collected weekly prior to every treatment until the end of each season. Direct count technique (5 random tomato plants/ replicate/ treatment) was practiced to estimate the mean number of infested fruit (El-Awady *et al.*, 2008). Fruit infestations were represented by mean numbers of (infested and healthy) fruits, weights of (infested and healthy) fruits as well as losses in yield due to infestation (kg/ton), tomato fruit yield (ton/fed.).

Statistical analysis

All data were statistically analyzed using SPSS program V.16. The LSD values were used to determine the most significant among the means of treatments. Differences were considered significant at $p < 0.05$ level.

RESULTS AND DISCUSSION

The performance of different bio-control treatments against the tomato borer on different vegetative parts of the tomato plant as well as evaluation of their efficiency at harvest stage during the two successive seasons of study was presented in tables (1, 2 and 3). Degrees and intensity of pest infestation were represented by mean numbers of (infested leaves, mines/leaf, surviving larvae and infested stems) foliage infestation and infested fruits, losses in yield due to infestation (kg/ton), tomato fruit yield (ton/fed.) at harvest stage. The two seasons' results revealed highly significant differences between the control and the tested treatments for all studied traits. Generally, the intensity of infestation and degree of damage varied sharply, according to the different treatments and time of application. In addition, results showed that 12 releases of *T. evanescens* at seedling stage at the rate of (80.000 parasitoid/fed.) performed best efficacy for reducing the pest damage as it received the lowest mean numbers (1.13±0.195, 1.51±0.027, 0.4±0.018 and 0.43±0.018) in season 2013 and (1.06±0.098, 1.39±0.027, 0.37±0.015 and 0.31±0.012 in season 2014 for infested leaves, mines/leaf, surviving larvae and infested stems, respectively (Tables 1 & 2). Furthermore, significant fruit reduction (0.70±0.012 and 0.61±0.015) was resulted in the same treatment for the two seasons, respectively. On the other hand, this treatment gave the lowest mean values of losses (53.89±0.36 and 50.66±0.61 kg/ton) due to fruit infestation thus resulted in the highest healthy fruit yield (10.18±0.02 and 10.5±0.02 ton/fed) in both seasons, respectively (Table 3). Harmony results were obtained by Cabello *et al.* (2009) who used the egg parasitoid, *T. achaeae* as a candidate against *T absoluta* and stated that this parasitoid played a good role to control the pest in greenhouses. Cabello *et al.* (2012) demonstrated that utilization of *T. achaeae* against the tomato leaf miner recorded better reduction in comparison to *T. urquijoi* in a greenhouse. However, heavy infestation with greatest mean by the pest for all foliage part (infested leaves, mines/leaf, surviving larvae, and infested stems) was (40.72±0.195, 27.16±0.027, 9.29±0.018 and 16.02±0.018) and (37.17±0.098, 26.51±0.027, 8.65±0.015 and 15.3±0.012) at seasons of 2013 and 2014, respectively. It was recorded associated with untreated plants, thus caused significant fruit losses due to infestation (514.16±0.36 and 503.7±0.61kg/ton) and resulted finally the lowest values of fruit yield (1.22±0.02 and 1.31±0.02 ton/fed.) throughout the experimental seasons, respectively (Tables 1 & 2 and 3). In this regard, Silva *et al.* (2011) studied the potential damage of this pest and reported that *T. absoluta* infestation may be reached about (100%) in the absence of control strategies.

Regarding the proper timing of the pest control, results demonstrated that early starts, at seedling stage (10 days post planting) achieved the highest potential, major foliage protection and played a vital role in suppression of *T. absolute* damage comparable to those at flowering or fruiting stages (Tables 1 & 2 and 3). This may be due to early

Table (1): Efficiency of different bio-control techniques against *T. absoluta* damage on certain vegetative parts of tomato plants during season of 2013

Treatments	Mean ± SE			
	Infested leaves	Mines/ leaf	Surviving larvae	Infested stems
T1	1.13±0.195^{a}	1.51±0.027^{a}	0.4±0.018^{a}	0.43±0.018^{a}
T 2	1.59±0.195^{b}	1.61±0.027^{b}	0.53±0.018^{b}	0.48±0.018^{b}
T 3	2.54±0.195^{c}	1.73±0.027^{c}	0.67±0.018^{c}	0.56±0.018^{c}
T 4	1.95±0.195^{b}	1.62±0.027^{b}	0.55±0.018^{b}	0.49±0.018^{b}
T 5	6.92±0.195^{g}	2.4 1±0.027^{g}	1.36±0.018^{g}	0.93±0.018^{g}
T 6	5.19±0.195^{e}	2.11±0.027^{e}	1.06±0.018^{e}	0.79±0.018^{e}
T 7	5.65±0.195^{f}	2.29±0.027^{f}	1.17±0.018^{f}	0.85±0.018^{f}
T 8	4.06±0.195^{d}	1.85±0.027^{d}	0.86±0.018^{d}	0.73±0.018^{d}
T 9	15.74±0.195^{k}	2.94±0.027^{k}	2.16±0.018^{k}	1.33±0.018^{k}
T 10	13.88±0.195^{i}	2.65±0.027^{i}	1.82±0.018^{i}	1.13±0.018^{i}
T 11	14.38±0.195^{j}	2.77±0.027^{j}	2.04±0.018^{j}	1.18±0.018^{j}
T 12	12.73±0.195^{h}	2.55±0.027^{h}	1.57±0.018^{h}	1.02±0.018^{h}
Control	40.72±0.195l	27.16±0.027l	9.29±0.018l	16.02±0.018l
$LSD_{0.05}$	0.37	0.051	0.053	0.051

Table (2): Efficiency of different bio-control techniques against *T. absoluta* damage on certain vegetative parts of tomato plants during season of 2014

Treatments	Mean ± SE			
	Infested leaves	Mines/ leaf	Surviving larvae	Infested stems
T1	1.06±0.098^{a}	1.39±0.027^{a}	0.37±0.015^{a}	0.31±0.012^{a}
T 2	1.37±0.098^{b}	1.51±0.027^{b}	0.49±0.015^{b}	0.37±0.012^{b}
T 3	2.32±0.098^{d}	1.63±0.027^{c}	0.58±0.015^{c}	0.47±0.012^{c}
T 4	1.59±0.098^{b}	1.53±0.027^{b}	0.52±0.015^{b}	0.39±0.012^{b}
T 5	5.65±0.098^{h}	2.22±0.027^{g}	1.13±0.015^{g}	0.81±0.012^{g}
T 6	4.45±0.098^{f}	1.88±0.027^{e}	086±0.015^{e}	0.65±0.012^{e}
T 7	4.90±0.098^{g}	2.04±0.027^{f}	0.98±0.015^{f}	0.70±0.012^{f}
T 8	3.44±0.098^{e}	1.72±0.027^{d}	0.79±0.015^{d}	0.59±0.012^{d}
T 9	14.05±0.098^{l}	2.68±0.027^{k}	1.95±0.015^{k}	1.16±0.012^{k}
T 10	12.26±0.195^{j}	2.52±0.027^{i}	1.52±0.015^{i}	1.00±0.012^{i}
T 11	13.11±0.098^{k}	2.59±0.027^{j}	1.63±0.0150j	1.09±0.012^{j}
T 12	11.84±0.098^{i}	2.35±0.027^{h}	1.30±0.015^{h}	0.93±0.012^{h}
Control	37.17±0.098^{m}	26.51±0.027^{l}	8.65±0.015^{l}	15.3±0.012^{l}
$LSD_{0.05}$	0.28	0.078	0.044	0.034

* Means with the same letter are not significantly different.

Table (3): Efficiency of different bio-control treatments against *T. absoluta* damage on tomato plants at harvest, during seasons of 2013 and 2014

Treatments	Mean No.± SE					
	Mean No. of infested fruits /10 fruits		Losses due to infestation Kg/ton		Mean value of yield Tone / fed.	
	2013	2014	2013	2014	2013	2014
T1	0.70±0.012^{a}	0.61±0.015^{a}	53.89±0.36^{a}	50.66±0.61^{a}	10.18±0.02^{a}	10.5±0.02^{a}
T 2	0.73±0.012^{b}	0.68±0.015^{b}	60.09±0.36^{b}	57.78±0.61^{b}	9.56±0.02^{b}	9.82±0.02^{b}
T 3	0.83±0.012^{d}	0.80±0.015^{d}	62.99±0.365^{c}	60.93±0.61^{c}	9.27±0.02^{d}	9.45±0.02^{d}
T 4	0.78±0.012^{c}	0.72±0.015^{c}	62.23±0.36^{c}	59.05±0.61^{bc}	9.47±0.02^{c}	9.66±0.02^{c}
T 5	1.32±0.012^{h}	1.27±0.015^{h}	102.13±0.36^{g}	97.84±0.61^{g}	7.42±0.02^{h}	7.73±0.02^{h}
T 6	1.12±0.012^{f}	1.03±0.015^{f}	93.24±0.36^{e}	90.19±0.61^{e}	7.68±0.02^{f}	7.98±0.02^{f}
T 7	1.25±0.012^{g}	1.14±0.015^{g}	95.27±0.36^{f}	93.57±0.61^{f}	7.60±0.02^{g}	7.86±0.02^{g}
T 8	1.003±0.012^{e}	0.93±0.015^{e}	88.05±0.36^{d}	84.9±0.61^{d}	7.95±0.02^{e}	8.23±0.02^{e}
T 9	1.76±0.012^{l}	1.65±0.015^{l}	175.2±0.36^{k}	167.3±61^{k}	5.07±0.02^{l}	5.32±0.02^{l}
T 10	1.66±0.012^{j}	1.51±0.015^{j}	163.18±0.36^{i}	159.17±0.61^{i}	5.48±0.02^{j}	5.63±0.02^{j}
T 11	1.69±0.012^{k}	1.57±0.015^{k}	166.57±0.36^{j}	163.14±0.61^{j}	5.35±0.02^{k}	5.53±0.02^{k}
T 12	1.50±0.012^{i}	1.36±0.015^{i}	159.85±0.36^{h}	148.4±0.61^{h}	5.60±0.02^{i}	5.92±0.02^{i}
Control	6.93±0.012^{m}	5.85±0.015^{m}	514.16±0.36^{l}	503.7±0.61^{l}	1.22±0.02^{m}	1.31±0.02^{m}
$LSD_{0.05}$	0.035	0.036	1.044	1.89	0.067	0.058

* Means with the same letter are not significantly different.

protection of different vegetative parts of the tomato plant, as well increasing the natural suppression by extensive releases of the *Trichogramma*, which may give their effect early and continue all over the growing season and/or the potential role of other combined bio-insecticides against larval stage of the pest, which may achieve a good foliage growth rate, cause significant and greatest reduction of fruit loss due to infestation and then finally record highest healthy fruit yield. Furthermore, significant reduction resulted in the pest population by these techniques showed harmony performance with the fact of, this pest attacks tomato crops during its whole growing cycle, causing a great economic loss in tomato fruit yield, thus it is necessary to start the control program at the early stage, tomato seedling stage (Picanco *et al.*, 2007). However, a relatively low efficiency of the pest control methods was recorded when they were applied at fruiting stage. Moreover, the results stated a moderate level of efficiency for minimizing the infestation on both foliage and fruits, when different tested technique was applied at the flowering stage. Statistical analysis showed significant positive correlations between losses of fruits due to infestation and each mean number of infested leaves, mines, surviving larvae and infested stems in both seasons, respectively recording (0.989 and 0.988), (0.952 and 0.953), (0.99 and 0.987) and (0.96 and 0.96) for the previous trials, respectively. On the other hand, obtained results showed significant negative correlations (0.95 and 0.96), (0.755 and 0.757), (0.86 and 0.84), (0.75 and 0.76) and (0.85 and 0.87) between tomato fruit yields and each of the infested leaves, mines, surviving larvae, stem and infested fruit in both 2013 and 2014, respectively. Among different bio-control treatment, that applied at flowering and fruiting stages, the technique of using 4 releases of *Trichogramma,* at the rate of (80.000 parasitoid/fed.), combined with 4 applications of Protecto, at the rate (300 gm/fed.) and spinosad bait, (5 ml/liter), as full traps between rows (one trap/10m) or 4 releases of *Trichogramma,* at the rate of (80.000 parasitoid/fed.) combined with 4 applications of Protecto, at the rate of (300 gm/fed.) and spinosad bait, performed best covering of tomato protection and decreased pest infestation comparing to other tested treatments at both flowering and fruiting growth stages (Tables 1 & 2 and 3). Thus, the significant reduction may be due to this technique that targeted different pest stages (egg, larvae and moths) by natural wasp suppression, additional positive effect of *B. thuringiensis* (*B.t.*) against the larvae and attractions of moths by bio-insecticide spinosad baits. The obtained results are in line with Rizk (2010) who evaluated the use of *T. evanescens* releases alone and in combination with *B. thuringiensis* application against *Phythoremia. operculella* (Zeller) in potato fields and demonstrated that the 3 releases of *T. evanescens* integrated with (*B.t.*) gave the second rank for protecting of the potato tuber moth and relatively high mean values of tuber yield. However, statistical analysis indicated that nine or six releases of the *Trichogramma* alone, at the rate of (80.000 parasitoids/ fed.), recorded relatively low efficacy of pest reduction at the same criteria comparing to the other studied treatments at the previous period, respectively. Regarding to different bio-control techniques during the experimental period, the obtained results recorded second degree of efficiency by 8 releases of *Trichogramma,* at the rate of (80.000 parasitoid/fed.) combined with 4 applications of radiant, at the rate of (60 ml/fed.), at seedling stage, related to the same criteria. In this regard, Hamdy and El-Sayed (2013) reported that the bio-insecticide, Spinetoram (Radiant) was more effective against *T. absoluta* infesting tomato leaves than the selected chemical insecticides. Moreover, analysis of the results showed a 3rd efficiency level by 6 releases of the parasitoid combined with both protecto and spinosad bait (as traps) for decreasing the damage in all tested traits based on the same criteria. Insignificant differences were detected between the two treatments that received 8 releases of *Trichogramma* combined with 4 applications of radiant and 6 releases of the parasitoid combined with both *B.t.* and spinosad bait, carried out for decreasing the pest damage on different foliage parts in both seasons (Tables 1 and 2). These findings are in harmony with those of (Siqueira *et al.*, 2000; Lietti *et al.*, 2005 and Silva *et al.*, 2011) who mentioned that *T. absoluta* has a short generation time, high biotic potential, internal living and endophytic behavior that increases the risk of developing resistance to traditional insecticides, that performed partially successful tactic to control this pest and becomes necessary to utilize different control methods targeting all *Tuta* stages (egg, larvae and moths). The obtained results agree with Khidr *et al.* (2013) who stated that the application of 3 different control strategies including (*B. t.* + *T. evanescens* + trapping) occupied second degree of efficacy for reducing the pest infestation and gave the highest protection of fruit yield. Present finding is also in line with the previous investigations who evidenced the important role of the timing of releasing the *Trichogramma* and demonstrated that early releases with the first appearance of different target lepidopteran moths achieved best efficacy for controlling these pests (El-Heneidy *et al.*, 2004; Rizk, 2010; El-Heneidy *et al.*, 2010 and Khidr *et al.*, 2013).

In conclusion; Early releases of *T. evanescens,* at the seedling stage of the tomato plant, can provide a vital role in reducing the pest damage along the season. A combination among *T. evanescens* + *B.t.* + spinosad bait was superior over other tested treatments when applied either at flowering and/or fruiting growth stages. Utilization of different control tactics, that target all *T. absoluta* stages, a moderate level of infestation, can achieve a potential healthy fruit yield when applied at the proper time.

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