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Original article (Orijinal araştırma)

Possibilities for biological control of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) in the western Mediterranean Region of Turkey

Batı Akdeniz Bölgesi'nde *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) ile biyolojik mücadele olanaklarının araştırılması

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Summary

After *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) was first detected in 2009, it quickly became the dominant pest in tomato-growing areas of Turkey. Both its feeding behavior and resistance to insecticides has forced growers to use alternative control measures. This study examined the effectiveness of individual and combined use of the predatory insect, *Nesidiocoris tenuis* (Reuter, 1895) (Hemiptera: Miridae) and the egg parasitoid, *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae) as biological control measures in the western Mediterranean Region of Turkey. For this purpose, greenhouse trials were conducted in a single-crop tomato cultivation period between 2011-2012 and 2013-2014. A reduction in infested fruit of 95.1 and 94.5% was achieved, respectively, in plots where *N. tenuis* and *T. evanescens* were released together. However, no significant difference was found between the plots with *N. tenuis* alone and the combination of *N. tenuis* and *T. evanescens*.

Keywords: Biological control, *Nesidiocoris tenuis,* tomato, *Trichogramma evanescens*, *Tuta absoluta*

Özet

Domates güvesi, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)'nın Türkiye'de ilk defa 2009 yılında tespit edilmesinden sonra domates yetiştiriciliğinin yapıldığı alanlarda ana zararlı konumuna gelmiştir. Zararlının gerek beslenme davranışı ve gerekse insektisitlere karşı dayanıklılık oluşturması üreticileri alternatif mücadele yöntemleri kullanmaya zorlamıştır. Bu çalışma ile Batı Akdeniz Bölgesinde zararlı ile biyolojik mücadelede avcı böcek *Nesidiocoris tenuis* (Reuter, 1895) (Hemiptera: Miridae) ve yumurta parazitoidi *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae)'in tek başına ve birlikte etkinliklerinin belirlenmesi hedeflenmiştir. Bu amaçla, 2011-2012 ve 2013-2014 tek ekim örtüaltı domates yetiştirme periyodunda sera denemeleri yapılmıştır. Kontrol parsellerindeki bulaşık meyve oranına göre *N. tenuis* ve *T. evanescens*' in birlikte salındığı parsellerde ilk yıl ve ikinci yıl çalışmasında sırasıyla, %95.1 ve 94.5 oranında azalma sağlamıştır. Bununla birlikte, *N. tenuis* ve *N. tenuis +T. evanescens* salınan parsellerde istatistiksel bir farklılık saptanmamıştır.

Anahtar sözcükler: Biyolojik mücadele, *Nesidiocoris tenuis*, domates, *Trichogramma evanescens*, *Tuta absoluta*

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Introduction

Historically, tomato cultivation in Turkey has been seriously impacted by whiteflies, *Bemisia tabaci* (Gennadius, 1889) and *Trialeurodes vaporariorum* (Westwood, 1856) (Hemiptera: Aleyrodidae), vegetable leafminers, *Liriomyza trifolii* (Burgess, 1880) (Diptera: Agromyzidae) and spider mites, *Tetranychus* spp. (Acarina: Tetranychidae) (Tunç & Göçmen, 1994; Bulut & Göçmen, 2000; Yaşarakıncı & Hıncal, 1997). Tomato leafminer, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), a native to South America, is one of the most devastating pests in Turkey and worldwide (Desneux et al., 2010, Kılıç, 2010); this species was introduced to Spain in 2006 (Urbaneja et al., 2007). Subsequently, it migrated and was soon discovered in France, Italy, Malta, the Netherlands, England, Hungary and Bulgaria. It was also found in North Africa, including Algeria, Morocco and Tunisia (Desneux et al., 2010). It was first detected in the İzmir Urla, Çanakkale and Balıkesir Provinces of Turkey in August 2009 (Kılıç, 2010) and quickly spread to all other regions in 2010, except for eastern Anatolia. This quick-proliferating pest caused significant product losses in the Mediterranean and Aegean Regions in greenhouse production.

The tomato leafminer is the main pest in open field and greenhouse tomato production. The larvae attack all growth stages of the tomato and feed on everything except for the root. When the larva hatches, it immediately begins to feed and enters the leaves and stems. The feeding process involves opening galleries between two epidermal layers in the leaves. The plant is at risk dehydration from the excess tunneling. Black excrement is left behind in the galleries of the leaf and fruit. The damaged fruit loses its market value and decomposition occurs with infection by secondary microorganisms on the opened fruit (EPPO, 2005). Chemical control of this pest is insufficient on its own and without effective control, the pest can damage up to 80-100% of greenhouse and field tomato crops (Apablaza, 1992; Lopez, 1991).

Controlling *T. absoluta* is very difficult for three reasons; 1) larvae feed in leaf tissue that is hard to access, 2) insecticide resistance has developed in some *T. absoluta* populations, and 3) its rapid proliferate rate. Yalçın et al. (2015) reported that *T. absoluta* populations obtained from the Aydın Province of Turkey had higher resistance; up to 8, 3.79 and 6.4 times for indoxacarb, metaflumizone and spinosad, respectively. Therefore, research on alternative methods for controlling this pest has become a necessity. Mass trapping with pheromone traps and a combination of light and water is one of the alternative control methods that has obtained satisfactory results, especially in low or moderate pest densities (Cocco et al., 2012; Aksoy & Kovanci, 2016). However, the prominent non-chemical control method for *T. absoluta* is biological control. Many studies have been conducted using predators, such as *Nabis pseudoferus* Remane, 1949 (Cabello et al., 2009b), *Nesidiocoris tenuis* (Reuter, 1895) (Calvo et al., 2012), and parasitoids, such as *Trichogramma achaeae* Nagaraja & Nagarkatti, 1969 (Cabello et al., 2009a; Chailleux et al., 2013), *Trichogramma euproctidis* (Girault, 1911) and *T. evanescens* (Chailleux et al., 2013). Most of these studies were conducted over a short-term period, and were laboratory or semi-field studies. However, in the western Mediterranean Region of Turkey, the vegetable-growing period lasts about 9 months from September to June, and pest and beneficial populations are greatly affected by the climatic conditions that include lower temperatures in the winter months. We examined the individual or combined use of *N. tenuis* and *T. evanescens* for biological control of *T. absoluta*. Also, we aimed to determine the performance of other potentially beneficial aspects, such as in the cold period in the winter months.

Material and Methods

Prey, predator and parasitoid rearing

Tuta absoluta rearing

The initial population of the prey, *T. absoluta,* was collected from commercial greenhouses in Antalya. Tomato plants with four to five leaves were placed in 30 x 30 x 50 cm cages with three sides covered in insect net for ventilation. Newly emerged adults were transferred to the cages. Tomato plants with *T. absoluta* eggs were kept in a climate chamber (25±1ºC, 65±5% RH and 16L:8D h photoperiod).

***Trichogramma evanescens* rearing**

Trichogramma evanescens was grown on *Ephestia kuehniella* Zell., 1879 eggs in a conditioning chamber (25±1ºC, 65±10% RH and 16L:8D h photoperiod). One-day old *E. kuehniella* eggs were kept for 30 min at -20ºC. These eggs were then scattered evenly on damp 1-cm wide paper. The egg cards were cut into strips and placed in 16 cm x 1.5 cm glass tubes, where *Trichogramma* individuals were released for 24 h (Bulut & Kılınçer, 1987; Öztemiz, 2001).

***Nesidiocoris tenuis* rearing**

Nesidiocoris tenuis adults were collected from tomato fields in Antalya Province and cultivated on tomato seedlings in cages covered with fine netting. The cages were placed in the conditioning chambers (25±1ºC, 65±10% RH and 16L:8D h photoperiod). *Ephestia kuehniella* eggs were placed in cages as prey.

First-year study (2011-2012)

Four treatments were examined; *N. tenuis* released alone, *T. evanescens* released alone, combined release of *N. tenuis* and *T. evanescens,* and control (no beneficial released).

The first-year greenhouse study was conducted over a single tomato-growing period. All plots were surrounded by fine nets before the seedlings were planted. Treatment were replicated three times in the randomized block design. Each plot was 10 m^2 with 20 plants. *Solanum lycopersicum* L. cv. Bestona was used in the trial and planted on 21 September 2011.

Adult tomato leafminers (1 female + 1 male per plant) taken from the stock culture were released into each cage in all plots on 17 October 2011. A total of 20 mated females were released in each treatment.

Trichogramma evanescens were released by hanging parasitoid cards. Parasitoids on the card were 6-7 days old after parasitization, and considered to be at pupal stage and about to emerge. Each release was 75 parasitoids/m^2, and this was repeated twice per week for a total of seven releases (Cabello et al., 2009a). Five additional releases were made, starting from 15 March in the spring of 2012 (Table 1).

Nesidiocoris tenuis was also released at 2 adults/m^2 following the pest release and *E. kuehniella* eggs were used as support food on the plant to establish *N. tenuis*. Beneficial insects were not released in control plots (Table 1), and no pesticides applied against any pest or diseases in all plots.

Second-year study (2013-2014)

The second-year greenhouse study was conducted as above in a single-crop tomato cultivation period. Tomato seedlings (cv. Bestona) were planted on 30 September 2013.

Additional data about releasing the predators and parasitoids for first and second years are given in Table 1.

Sampling of *Tuta absoluta*

Monitoring of the eggs and larvae of *T. absoluta* were performed weekly using five randomly selected plants in each plot. All parts of the plant were examined. The numbers of eggs and larvae of *T. absoluta* were recorded for each plant.

Sampling of the egg parasitoid, *Trichogramma evanescens*

Three leaves (from the upper, middle and bottom side of the plant) of five randomly selected plants were sampled weekly to determine the parasitism levels of *T. absoluta* eggs in each plot. Parasitism was determined by counting blackened eggs (i.e., parasitized) and transparent eggs (i.e., non-parasitized) (Ayvaz et al., 2008).

Sampling of the predator, *Nesidiocoris tenuis*

The nymph and adult stages of *N. tenuis* were separately counted at weekly intervals from five randomly selected plants in each plot. All parts of the plants were examined for the presence of these predators.

Table 1. Biological control of *Tuta absoluta* in the tomato-growing periods 2011-2012 and 2013-2014

Treatment	Release rates (m^2)	Total release (m^2)	Release frequency and dates
2011-2012 growing season			
Trichogramma evanescens	75	900	7 releases in autumn (17, 20, 24, 27 and 31 October, and 3 and 7 November 2011)
			5 releases in spring (15,18, 24 and 28 March, and 3 April 2012)
Nesidiocoris tenuis	2	2	1 release (17 October 2011)
Nesidiocoris tenuis + *Trichogramma evanescens*	2 +75	2 + 900	1 release (17 October 2011) + 7 releases in autumn (17, 20, 24, 27 and 31 October, and 3 and 7 November 2011)
			5 releases in spring (15,18, 24 and 28 March and 3 April 2012)
Control			No beneficial released
2013-2014 growing season			
Trichogramma evanescens	75	900	7 releases in autumn (23, 28 and 31 October, and 4, 7,11 and 14 November 2013)
			5 releases in spring (11,14, 18, 21 and 25 March 2014)
Nesidiocoris tenuis	2	2	1 release (23 October 2013)
Nesidiocoris tenuis + *Trichogramma evanescens*	2+ 75	2+ 900	1 release (23 October 2013) + 7 releases in autumn (23, 28 and 31 October, and 4, 7,11 and 14 November 2013)
			5 releases in spring (11,14, 18, 21 and 25 March 2014)
Control			No beneficial released

Sampling of damaged fruit

Fifty tomato fruits sampled from randomly selected plants in each plot were inspected to determine whether they were infected at harvest.

Data analyses

The number of *T. absoluta* larvae counted during the season for each plot and the number of damaged fruit were subjected to analysis of variance (ANOVA). The significance threshold for Tukey's HSD test was $P \leq 0.001$. Additionally, biological efficacy was calculated using Abbott's formula (Abbott, 1925).

Results

First-year study

The mean number of tomato leafminer eggs in the control and beneficial released plots were quite low until February, then strongly increased, especially with *T. evanescens* released alone and in the control plots. The control plots yielded the highest tomato leafminer counts at 133.8 eggs/plant on 7 May 2012. Comparable results were obtained for the larval stage of the pest. The larval population reached its highest density in the autumn on 21 November 2011 (8.5 larvae/plant in the control) and in the spring on 14 May (195.4 larvae/plant in the control) (Figures 1 & 2). *Tuta absoluta* larvae in plots with *N. tenuis* released alone, and combined *N. tenuis* and *T. evanescens* release were under 10 larvae/plant. However, in the plots with *T. evanescens* released alone, the pest population was greater than 100 larvae/plant (Figure 2).

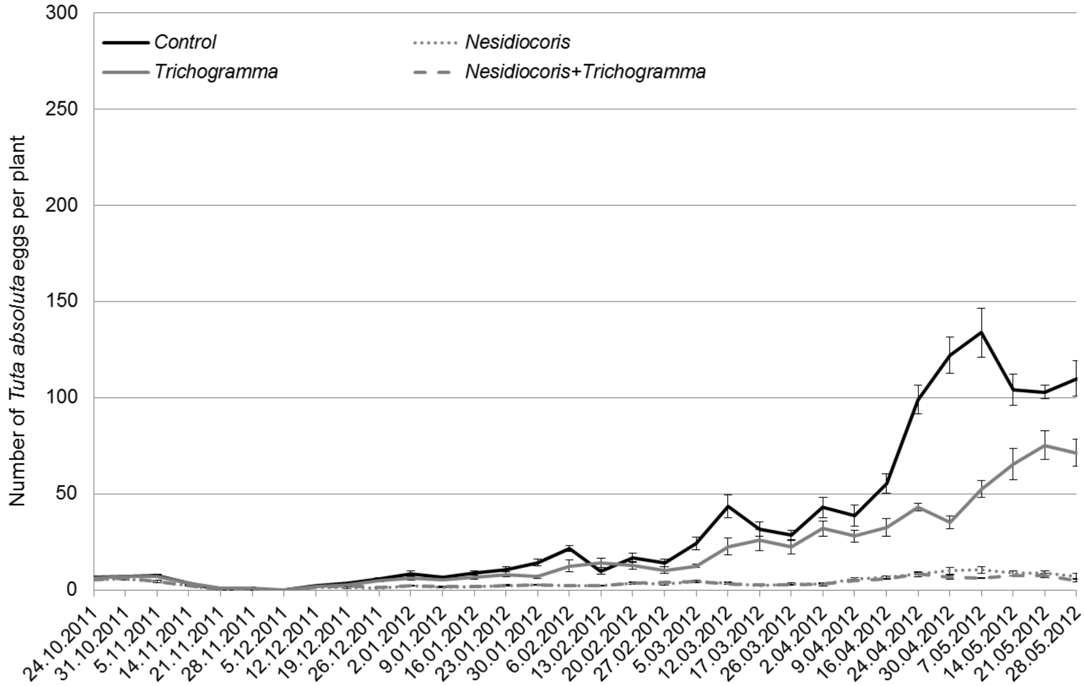

Figure 1. Egg density (mean±SE) of tomato leafminer at all treatments (control, *Nesidiocoris tenuis* and *Trichogramma evanescens* released alone, and in combination) in 2011-2012.

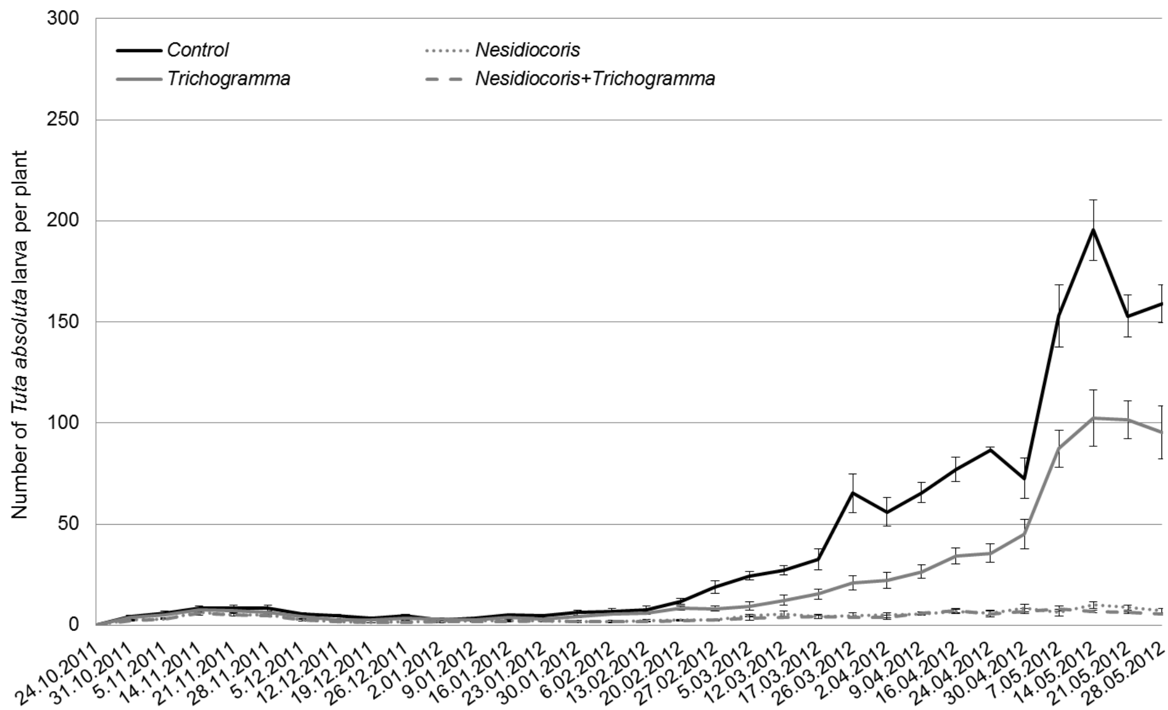

Figure 2. Tomato leafminer larvae (mean ±SE) at all treatments (control, *Nesidiocoris tenuis* and *Trichogramma evanescens* alone, and in combination) in 2011-2012.

Table 2. Total mean numbers of tomato leafminer larvae for all treatments (control, *Nesidiocoris tenuis* and *Trichogramma evanescens* alone, and in combination) in 2011-2012

Treatment	No. of tomato leafminer (mean±SE)
Nesidiocoris tenuis	133.7±21.4 c*
Trichoramma evanescens	696.6±86.0 b
Nesidiocoris tenuis + *Trichogramma evanescens*	118.7±13.7 c
Control	1288.7±115.8 a

*Means followed by a different letter differ significantly at P < 0.001.

The total number of tomato leafminer larva in the plots with *N. tenuis* (both alone and combined with *T. evanescens*) was significantly different from the control and individual *T. evanescens* released plots ($F_{3,11} = 121.2$; $P < 0.001$). However, the number of *T. absoluta* larvae in plots in which *N. tenuis* and *T. evanescens* were released did not show any significant difference compared to those with *N. tenuis* released alone (Table 2).

The predator, *N. tenuis,* established in plots from the date of release and there was no need for additional releases. The number of *N. tenuis* was higher than 10 adults and nymphs in plots where it was released alone and in combination with *Trichogramma* at 26 March 26 2012 (Figure 3). The pest population was 4.7 and 3.9 larvae/plant in plots with *N. tenuis* released alone and with *N. tenuis* and *T. evanescens* in combination, respectively, on that day. The numbers of *N. tenuis* alone and combined with *T. evanescens* released plots were almost at the same from the beginning to the end of the experiment (Figure 3).

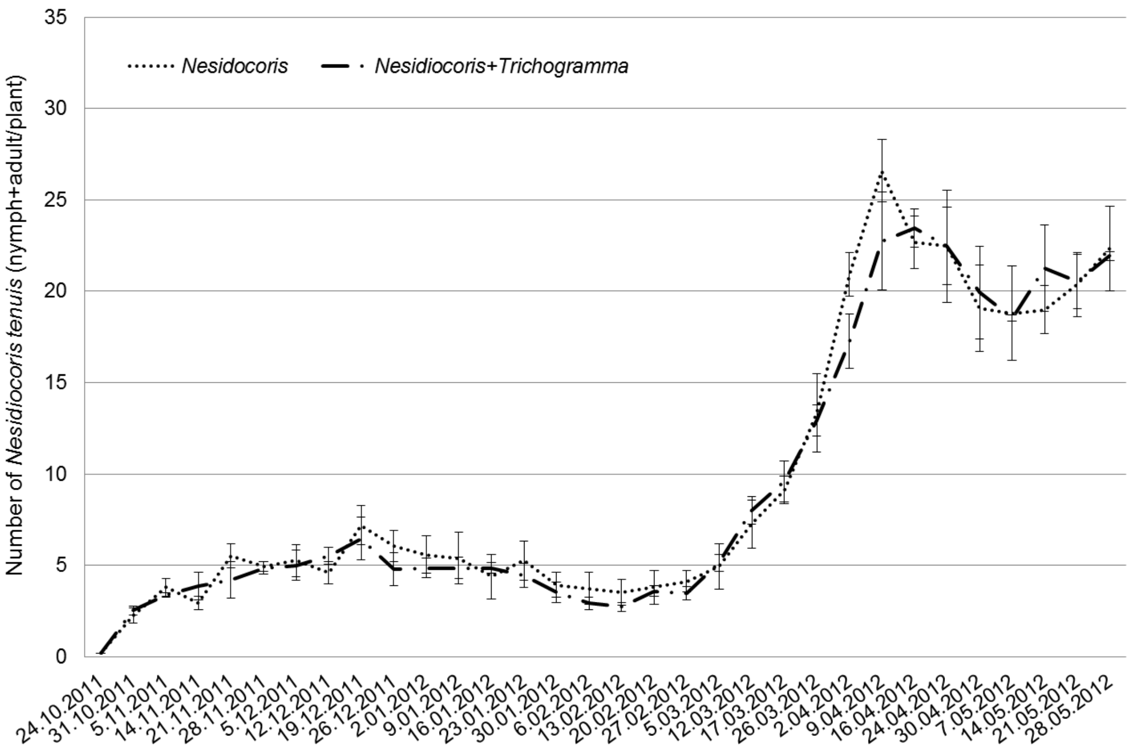

Figure 3. The population of *Nesidiocoris tenuis* (mean±SE) in two biological control treatments (*Nesidiocoris tenuis* released alone, and in combination with *Trichogramma evanescens*) in 2011-2012.

Because no parasitized eggs were detected in the plots where *T. evanescens* released at the beginning of March, five additional releases were performed. The maximum parasitization ratio was observed on 9 April 9 2012 (45.4%). When evaluating the greenhouse establishment of the beneficial insects after five releases *T. evanescens* in the spring, parasitoids could not be detected in the greenhouse after 7 May 2012 (Figure 4).

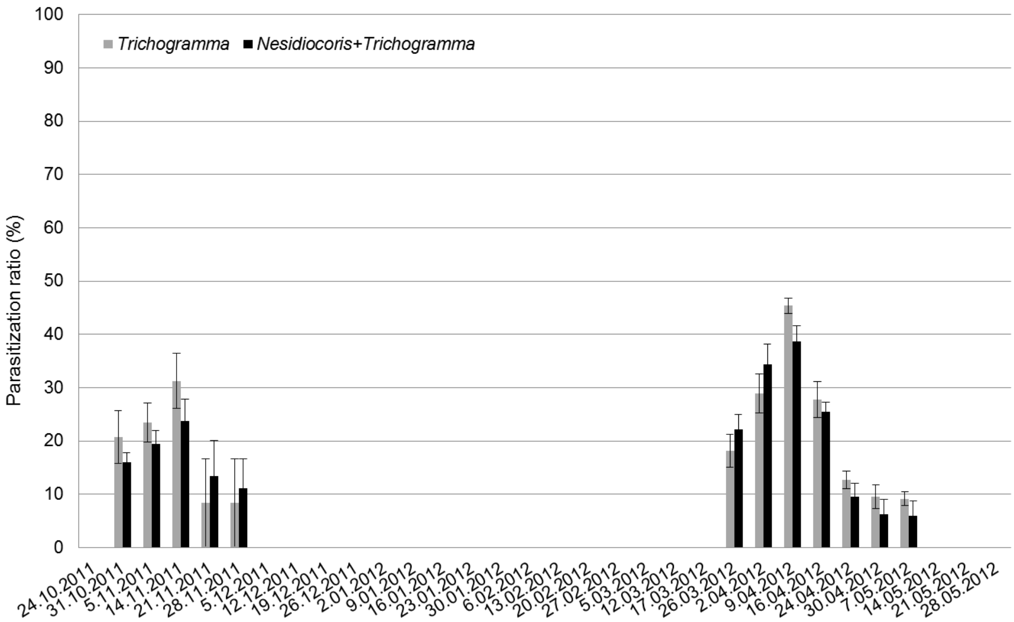

Figure 4. The parasitization ratio (mean±SE) of the tomato leafminer eggs in plots with *Trichogramma evanescens* released in 2011-2012.

The mean infested fruit ratios were 5.1, 36.6 and 2.5 in *N. tenuis, T. evanescens* and *N. tenuis plus T. evanescens* released plots, respectively and all showed a significant difference compared to the control plots ($F_{3,11}$ = 317.9; P< 0.001). Compared to the infected fruit ratio in the control, a reduction of 90.0 and 95.1%, respectively, was achieved in the plots with *N. tenuis* released alone and in combination with with *T. evanescens*, respectively. In plots with *T. evanescens* was released alone, the efficiency ratio was 28.4% (Table 3).

Table 3. Mean ratio of infested fruit (%) at harvest in the first-year plots and effects of application (corrected with Abbott's formula)

Treatment	Infested fruit ratio (%±SE)	Efficiency (%)
Nesidiocoris tenuis	5.1±0.9 c	90.0
Trichoramma evanescens	36.6±2.7 b	28.4
Nesidiocoris tenuis + *Trichogramma evanescens*	2.5±0.5 c	95.1
Control	51.1±4.1 a	-

*Means followed by a different letter differ significantly at P < 0.001.

Second-year study

The highest number of tomato leafminers in the control plots reached 169.9 eggs/plant on 18 March 2014 (Figure 5). From the beginning of February, the mean number of larvae population rapidly increased in the control plots. The tomato leafminer population reached its highest larval density on 1 April 2014 with 265.1 larvae/plant, again in the spring period and in control plots. On the same date, the pest population in the plots with *T. evanescens* released alone was 208.3 larvae/plant. However, in plots with *N. tenuis* released alone, the maximum pest population reached 24.7 larvae/plant (Figure 6).

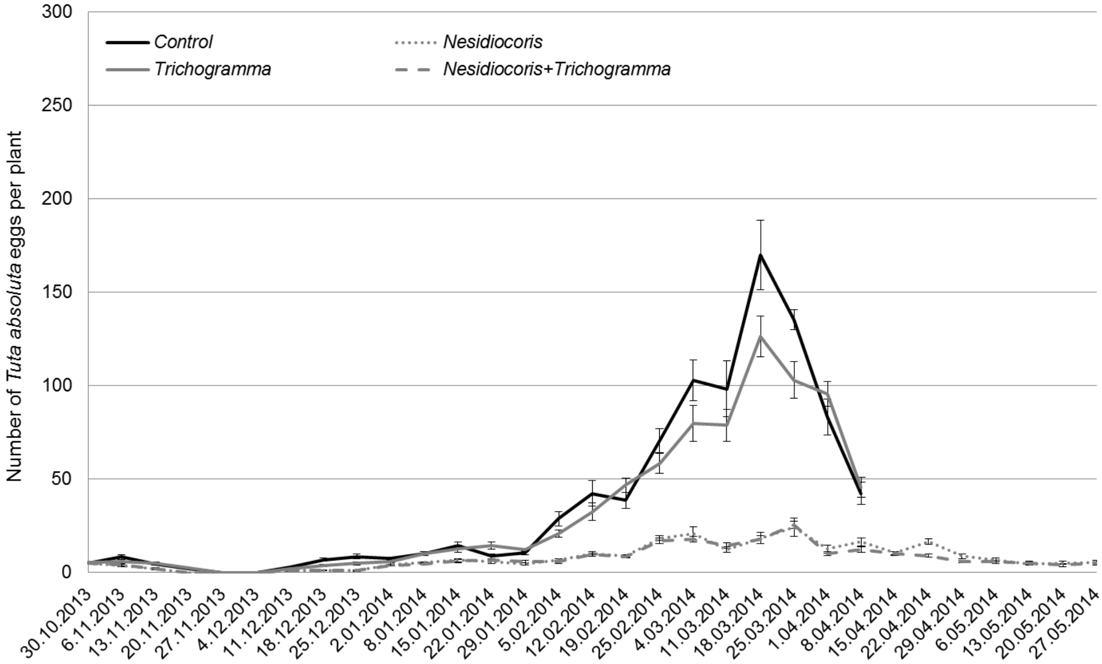

Figure 5. Egg density (mean±SE) of tomato leafminer for all treatments (control, *Nesidiocoris tenuis* and *Trichogramma evanescens* released alone, and in combination) in 2013-2014.

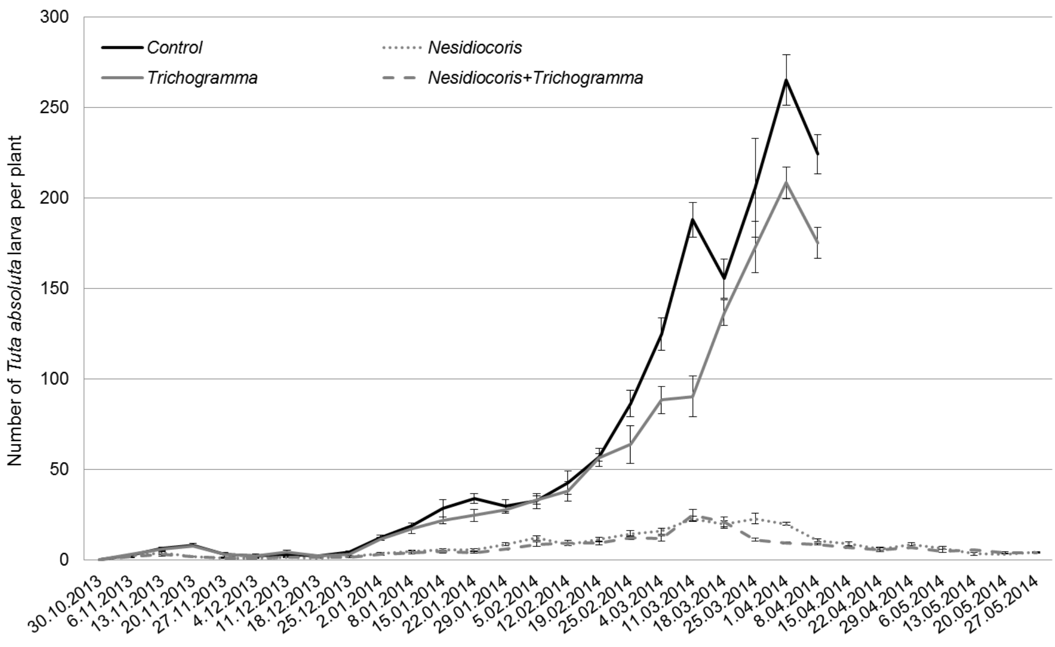

Figure 6. Tomato leafminer larvae (mean±SE) in all treatments (control, *Nesidiocoris tenuis* and *Trichogramma evanescens* released alone, and in combination) in 2013-2014.

Throughout the growing period, a mean of 1536.9 *T. absoluta* larvae per plant were counted in control plots. Although the number of tomato leafminer larvae per plant in plots with *T. evanescens* released alone was statistically different from control plots ($F_{3,11} = 232.9$; $P < 0.001$), it was still quite high and caused serious fruit loss. However, the pest population in the plots with both *N. tenuis* released alone and in combination with *T. evanescens* were 241.1 and 197.9 larvae/plant, respectively (Table 4).

Table 4. Total mean numbers of tomato leafminer larvae for all treatments (control, *Nesidiocoris tenuis* and *Trichogramma evanescens* alone, and in combination) in the second year

Treatment	No. of tomato leafminer (mean±SE)
Nesidiocoris tenuis	241.1±20.1 c*
Trichoramma evanescens	1197.6±88.9 b
Nesidiocoris tenuis + *Trichogramma evanescens*	197.9±17.3 c
Control	1536.9±94.6 a

*Means followed by a different letter differ significantly at P < 0.001.

The *N. tenuis* population was below 10 adults and nymphs until 18 March in plots with *N. tenuis* released alone and in combination with *T. evanescens*. After that date, the *N. tenuis* population remained above that level until the end of the experiment (Figure 7).

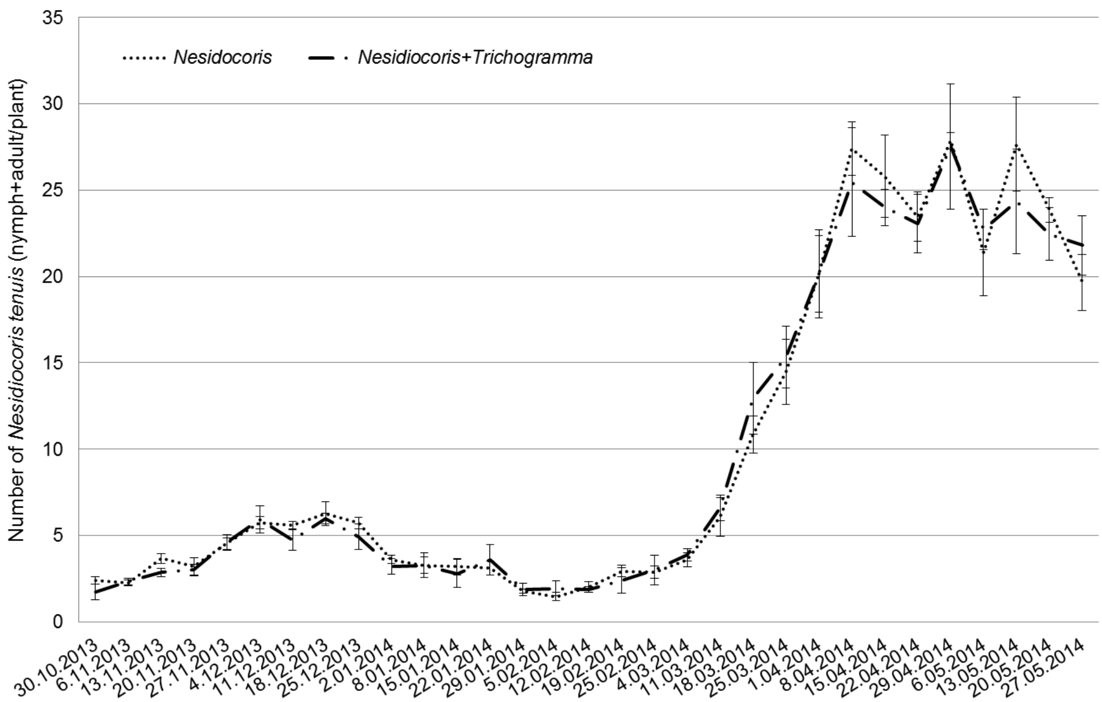

Figure 7. The population of *Nesidiocoris tenuis* (mean±SE) in two biological control treatments (*Nesidiocoris tenuis* alone, and in combination with *Trichogramma evanescens*) in 2013-2014.

In the second-year study, *T. evanescens* releases were made from March onwards. The maximum parasitization ratio was 30.5% on 1 April 2014 in the plot with *T. evanescens* released alone (Figure 8). In the plots with *T. evanescens* released alone, the intensity of tomato leafminers reached 208.3 larvae/plant on 8 April 2014. On that date, in both control plots and plots with *T. evanescens* released alone the damage ratio was 100%.

The damaged tomato fruit number in *N. tenuis* released plots (both alone and combined with *T. evanescens*) showed a significant difference compared to control plots and plots with *T. evanescens* released alone ($F_{3,11} = 179.8$; $P < 0.001$). When *N. tenuis* was released alone, the damage ratio in fruit was 5.1 and 4.3% in the first and second years, respectively. When *N. tenuis* was released in combination with *T. evanescens*, this ratio dropped to 2.5 and 2.9%, respectively. The two beneficial insects together or *N. tenuis* alone did not result in any statistically significant effect on the damage ratio (Tables 3 & 5).

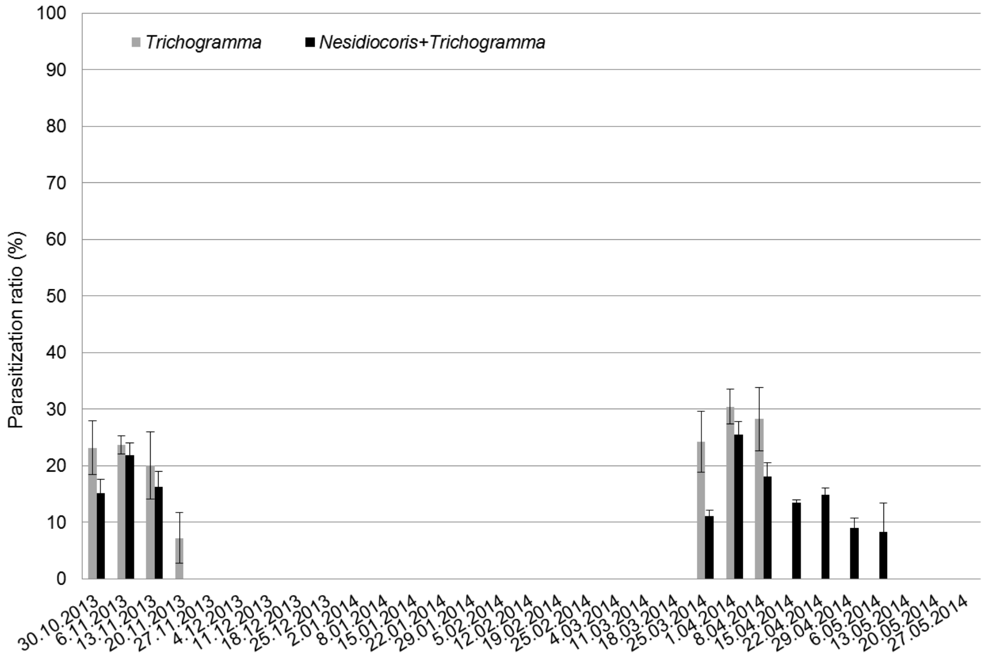

Figure 8. The parasitization ratio (mean±SE) of the tomato leafminer eggs in plots with *Trichogramma evanescens* released in 2013-2014.

Table 5. Mean ratio of infested fruit (%) at harvest in the second-year plots and effects of application (corrected with Abbott's formula)

Treatment	Infected fruit ratio (%±SE)	Efficiency (%)
Nesidiocoris tenuis	4.3±0.4 b*	91.8
Trichoramma evanescens	44.2±2.7 a	16.0
Nesidiocoris tenuis + *Trichogramma evanescens*	2.9±0.3 b	94.5
Control	52.6±4.7 a	-

*Means followed by a different letter differ significantly at P < 0.001.

Discussion

This study aimed to determine the individual and collective effectiveness of the predator insect, *N. tenuis,* and the egg parasitoid, *T. evanescens,* against tomato leafminers, an invasive pest that recently expanded into Turkey.

Given that *T. evanescens* was undetectable in both years at the beginning of March, five additional releases were performed. In the plots with *T. evanescens* released alone, 28.4 and 16.0% reductions in damaged fruit number were obtained compared to the control plots in first and second year study respectively. In the second-year study, the damage ratio in the control plot and the plots testing *T. evanescens* reached 100% in April towards to end of the growing season. Many studies have been conducted to determine the effectiveness of *Trichogramma* species against the tomato leafminer. Domingues et al. (2003) reported a damage ratio in fruit of 13% with *Trichogramma pretiosum* Riley, 1879 release. In a study with another species, *T. achaeae* reduced damage by 92% when used in rate of 75 adult/m^2 in 3-4 days intervals in August and September (Cabello et al., 2009a). This may explain the differences in egg parasitoid species.

Another key factor for this failure was the climatic conditions. The mean temperatures in the two years were 19.6 and 21.4ºC, respectively, during the additional release of *T. evanescens* in the spring. However, these temperatures were not appropriate for *T. evanescens*. The adaptation of *T. evanescens* to the climate, or tomato plant and its cultivars, may be a reason for the failure of its efficiency. Contrary to previous studies, *T. evanescens* was not able to increase its population (Chailleux et al., 2013).

In a study of the predatory insect, *N. tenuis*, the ratio of *T. absoluta* contamination was reduced by 97% in the leaves and 100% in the fruit (Mollá et al., 2009). Similar results were obtained for *Nabis pseudoferus* and the eggs of tomato leafminers were reduced by 92-96% (Cabello et al., 2009b). In the present study, *N. tenuis* reduced fruit damage by more than 90%.

During the study, the mean temperature between December and February was 12.3ºC in 2011-2012 and 14.2ºC in 2013-2014. The lower development threshold of tomato leafminers is 8°C (Barrientos et al., 1998), while this value is 10.3ºC for *N. tenuis* (Sanchez et al., 2009). Furthermore, the weather conditions were more moderate, especially in the second year of the study, which proved advantageous for the tomato leafminers that have a lower development threshold. Remarkable damage on fruit was observed in plots where *N. tenuis* was released in March. Therefore, it is believed that additional releases would be appropriate in the spring, depending on the pest intensity.

Calvo et al. (2012) recommended that applications of *N. tenuis* with *T. achaeae,* and with *N. tenuis, T. achaeae* and *Bacillus thuringiensis* have no additional positive effect on *T. absoluta* compared to releasing *N. tenuis* alone. In addition, offspring of *Trichogramma* developed on *T. absoluta* eggs displayed low parasitism and poor biological traits. For instance, some biological characteristics, such as healthy wing occurrence after emergence or adult longevity, were negatively affected compared to the controls reared on the cultured host. Therefore, the parasitoids that developed on *T. absoluta* had little success controlling it (Chailleux et al., 2013). In addition, Chailleux et al. (2013) stated that the combination of *Macrolophus pygmaeus* (Rambur, 1839) and egg parasitoids (*T. achaeae* or *T. euproctidis*) indirectly reduced their effectiveness against *T. absoluta* because the predator insect consumed the parasitoid eggs together with non-parasitoid eggs. However, Oztemiz et al. (2012) reported that the combination of *N. tenuis* and *T. evanescens* has the potential to control *T. absoluta*. This difference can be associated with the effect of host plant cultivar, climatic conditions and time of release.

In plots where *T. evanescens* and *N. tenuis* were released together to control tomato leafminers, no statistically significant benefit was observed with the addition of the egg parasitoid *T. evanescens*. Considering these findings, *N. tenuis* can be effectively used alone to control tomato leafminers.

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