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Orijinal araştırma (Original article)

Growth inhibitory effects of bio- and synthetic insecticides on *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)

Biyo- ve sentetik insektisitlerin *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)'ya gelişme engelleyici etkileri

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Summary

The study aimed to found out the potential efficacy of some bio- and synthetic insecticides used against *Tuta absoluta* (Lepidoptera: Gelechiidae) in tomato greenhouses. Chlorantraniliprole+abamectin, metaflumizone, azadirachtin, emamectin benzoate, spinosad, *Bacillus thuringiensis* and *B. thuringiensis*+azadirachtin mixture were applied by spraying method on tomato leaf, which 5 third-stage larvae existed on. Distilled water was used as a control to compare with insecticides. Each of the applications was performed in six replications. The assessments were done at the 1, 3, 5 and 7 DAA (Days After Application) according to the number of live individuals and larval weight.

Mean numbers of live metaflumizone-, azadirachtin-, *Bacillus thuringiensis*- and *B. thuringiensis*+azadirachtin-treated larvae at the 7 DAA were recorded as 0.33, 1.17, 0.67 and 0.33, respectively. Growth inhibition indexes of same larvae groups were 0.64, 0.17, 0.65 and 0.80, respectively. All individuals in chlorantraniliprole+abamectin-, emamectin benzoate- and spinosad-treated larvae were died at the 7 DAA. Growth inhibition indexes of these larvae groups were 0.97, 0.82 and 0.89, respectively. Pupations of metaflumizone-, azadirachtin-, *Bacillus thuringiensis*- and *B. thuringiensis*+azadirachtin-treated larvae were determined as 6.67, 20.0, 16.67 and 6.67 percent, respectively. Azadirachtin-treated larvae showed the success of adult emergence in the rate of 6.67%. However, none of the larvae treated with other insecticides could show the success of adult emergence. Mean weight of the control larvae group gradually decreased and also, the rate of pupation and adult emergence in this group were 56.67 and 50.00 percent. In addition, it was determined that *B. thuringiensis*+azadirachtin mixture constituted a synergistic effect on the pest.

Key words: Azadirachtin, *Bacillus thuringiensis*, emamectin benzoate, spinosad, *Tuta absoluta*

Özet

Bu çalışmada *Tuta absoluta* (Lepidoptera: Gelechiidae)'ya karşı seralarda yaygın olarak kullanılan sentetik insektisitlerin ve biyo-insektisitlerin potansiyel etkinliği ortaya çıkarılmaya çalışılmıştır. Denemede Chlorantraniliprole+abamectin, metaflumizone, azadirachtin, emamectin benzoate, spinosad, *Bacillus thuringiensis* ve *B. thuringiensis*+azadirachtin karışımı üzerinde 5 adet üçüncü dönem larva bulunan domates yapraklarına püskürtme yöntemiyle uygulanmıştır. Kontrol uygulaması olarak da saf su kullanılmıştır. Her bir uygulama altı tekerrürlü olarak gerçekleştirilmiştir. Değerlendirmeler uygulamalardan sonraki 1., 3., 5. ve 7. günlerde canlı birey sayıları ve larva ağırlıkları üzerinden yapılmıştır.

Metaflumizone, azadirachtin, *Bacillus thuringiensis* ve *B. thuringiensis*+azadirachtin ile muamele edilen larvaların canlılık oranları 7. gün sonunda sırasıyla 0,33, 1,17, 0,67 ve 0,33 olarak kaydedilmiştir. Aynı larvaların gelişme gerileme indeksi sırasıyla 0,64, 0,17, 0,65 ve 0,80'dir. Chlorantraniliprole+abamectin, emamectin benzoate ve spinosad ile muamele edilen larvaların tamamı 7. gün sonunda ölmüştür. Bu larvaların gelişme gerileme indeksi ise sırasıyla 0,97, 0,82 ve 0,89'dur. Metaflumizone, azadirachtin, *Bacillus thuringiensis* ve *B. thuringiensis*+azadirachtin ile muamele edilen larvaların pupa olma oranları sırasıyla %6,67, 20,0, 16,67 ve 6,67 olarak belirlenmiştir. Azadirachtin ile muamele edilen larvalar %6,67 oranında ergin olma başarısı gösterirken diğer insektisitler ile muamele edilen larvaların hiçbirisi ergin olma başarısı gösterememiştir. Kontrol grubundaki larvalarda ortalama ağırlık giderek azalmıştır. Ayrıca bu grupta ki larvaların pupa ve ergin olma oranları %56,67 ve %50,00 olarak bulunmuştur. Bunun yanı sıra *B. thuringiensis*+azadirachtin karışımının sinerjistik bir etki meydana getirdiği tespit edilmiştir.

Anahtar sözcükler: Azadirachtin, *Bacillus thuringiensis*, emamectin benzoate, spinosad, *Tuta absoluta*

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Introduction

The amount of vegetables produced in Turkey was approximately 28.5 million tons in 2013, 41.5% (11.8 million tons) of which include tomato (*Solanum lycopersicum* L.) (Solanaceae) (TÜİK, 2013).

Tomato is one of the major agriculture products due to high nutritional value and tasty as well as cheap. Tomato is a vegetable of which processed consumption is common as well as fresh consumption. Also, Turkey has an income of approximately US$400 million from tomato exports, which is 57% of total vegetable exports (Anonymous, 2014). One of the major insect pest of tomato both in the field and greenhouse conditions is the Tomato leafminer, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) that is originated from South America.

The tomato leafminer was described first as *Phthorimaea absoluta* by Meyrick (1917) in Peru and then as *Gnorimoschema absoluta* by Gates Clarke (1962). Afterwards, it is defined as *Scorbipalpula absoluta* by Povolny (1974) and finally as *Tuta absoluta* (Meyrick) by Povolny (1994) (EPPO, 2010; Muniappan, 2014).

Tuta absoluta was transported to Argentina from Chili in 1962 according to García-Marí & Espul (1982). A study conducted in Japan by Gates Clarke (1962) recorded some microlepidopters from Gelechiidae family on *Solanum lyratum*, and moreover there were many gelechiids fed on Solanaceae plants, and one of these gelechiids is the tomato leafminer. As for Turkey, the first record for *T. absoluta* was made in İzmir in 2009 (EPPO, 2010; Kılıç, 2010).

This pest lays eggs on leaves by 73%, on veins and stems by 21%, on sepals by 5% and on fruits by 1% (Muniappan, 2014). Larvae of the pest do not enter diapause as long as sufficient food and the pest may give 10-12 generations per a year (EPPO, 2005). This oligophagous pest cannot only feed on tomato but also other crops and weeds from Solanaceae family (EPPO, 2005; USDA, 2011a, b). In addition, the pest feeds on bean (*Phaseolus vulgaris* L.) was also reported (EPPO, 2009). This pest feeds on leaves, shoots, stems, flowers and fruits of tomato, but only on leaves and stems or tubers of other hosts (USDA, 2011a).

The tomato leafminer causes serious economic harms in tomato plants under greenhouse conditions in the province of Isparta is an important pest, the control of which is difficult for reasons such as wide host range and a potential of fast and high progeny. However, highly more synthetic insecticides are applied against the pest in this province. The intensive chemical applications also create residual problems and a possibility of pest resistance development together with environmental pollution (Braham et al., 2012). The pest has resistance to abamectin, cartap, methamidophos, permethrin and deltamethrin has been known (Siqueira et al., 2000, 2001; Lietti et al., 2005). Moreover, the methods that can be applied against the pest within the scope of integrated pest management such as biological control cannot be used because of the intensive chemical applications.

For the reasons discussed herein, studies on bio-insecticides which are an important component of integrated pest management have increased. In parallel with this development, their side effects on natural enemies and other non-target organisms have also became an important research subject.

The results of the study on the effect of lambda cyhalothrin and spinosad on the natural enemies *Coleomegilla maculata* (DeGeer) and *Hippodamia convergens* (Coleoptera: Coccinellidae), *Geocoris punctipes* (Hemiptera: Lygaeidae), *Bracon mellitor*, *Cardiochiles nigriceps*, and *Cotesia marginiventris* (Hymenoptera: Braconidae) in cotton by Tillman & Moonroney (2000) demonstrated that spinosad does not adversely affect *G. punctipes*, *H. convergens* and *C. maculata*; however, only *B. mellitor* among 6 natural enemy species used in the study was affected by spinosad. After different insecticide applications against aphids on each plant row in field experiments of the same study, the status of the pests and their natural enemies was examined. The results obtained from field experiments of the study indicated that the density of the natural enemies on the control and spinosad-treated plant-rows were higher than one on the lambda cyhalothrin-treated plant-rows. Also, it is declared in the study that lambda cyhalothrin has more negative effect on the natural enemies than spinosad. Based on the review by Biondi et al. (2012), it is possible to say that spinosad has slightly effect on the natural enemies, but shows more negative effect on parasitoids. In the review, acute toxicity of spinosad on 22 parasitoid species from the order of Hymenoptera was investigated according to several studies on the side effect of spinosad, and it is announced that these parasitoids can be affected at lethal levels unlike predators. Ghosh et al. (2010) studied the effect of spinosad on *Heliothis armigera* Hübner (Lepidoptara: Gelechiidae) and thereof natural enemies by comparing with effects of some synthetic insecticides. The results of the study showed that spinosad is effective against the pest and more safety than quinalphos, lambda cyhalothrin and cypermethrin for three major predators of the pest *Menochilus sexmaculatus* F. (Coleoptera: Coccinellidae), *Syrphus corollae* F. (Diptera: Syrphidae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). In addition to this, it is pointed out in the study that spinosad is safety for both of larvae and adult stages of the predators. Liu et al. (2012) stated that lambda cyhalothrin is more effective on the parasitoid *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) and the predator *C. maculata*, both natural enemies of the diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). However, spinosad has less toxicity on *C. maculata* adults and larvae, and slightly toxicity on *D. insulare*. A semi-field study by Lopez et al. (2011) demonstrated that the application of emamectin benzoate at the dose of 14.25 mg a.i./L shortly before the release of *Nesidiocoris tenuis* Reuter, *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) and *Diglyphus isaea* Walker (Hymenoptera: Eulophidae) in tomato greenhouse has not adverse effect on them. Also, the results of the study indicated that this bio-insecticide can be used within the scope of IPM programs.

Bio-insecticides derived from the trees *Azadirachta indica* A. Juss and *Melia azedarach* L. have no or less negative effects on natural enemies due to action mode and poor contact effect (Erdoğan, 2013). Tunca et al. (2012) investigated the side effects of azadirachtin, capsaicin, d-Limonene and pyrethrum on *Venturia canescens* (Grav.) (Hymenoptera: Ichneumonidae). According to the results of the study, azadirachtin caused the increase in the development time of the parasitoid, whereas the decrease in the longevity and the rate of parasitoid emergence. Besides that, pyrethrum has a greater negative effect on the progeny, development time and longevity of the parasitoid. Moreover, the study explained that bio-insecticides and plant extracts have a repellent effect on the parasitoid adults and therefore, azadirachtin, pyrethrum, capsaicin and d-Limonene are not suitable to use with the parasitoid *V. canescens*. Vinuela et al. (2000) explored the effects of Ingestion of azadirachtin by two pests, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) and *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and three natural enemies, *C. carnea*, *Opius concolor* (Szépligeti) (Hymenoptera: Braconidae) and *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae). The researchers, based on the results of the study, suggested that azadirachtin at the concentration of 1 mg a.i./L has a highly impact on *C. capitata* larvae. Also, it is noticed that last stage larvae of *S. exigua* were extremely sensitive, and fecundity of surviving adults exposed to 10 mg a.i./L concentration of azadirachtin and egg fertility were significantly reduced.

A study conducted by Schoenly et al. (2003) examined the effects of *Bacillus thuringiensis* on non-target herbivore and natural enemy assemblages in tropical irrigated rice. The results of the study demonstrated that *B. thuringiensis*, although a significantly impact on lepidopteran larvae, has no important effect on the natural enemies. Nevertheless, parasitoids compared to predators were reported as more susceptible to *B. thuringiensis* in the study.

For these reasons, some synthetic insecticides used in the province were determined and this study compared with growth inhibitory effects of some bio- and synthetic insecticides on larvae of *T. absoluta*. Thus, the potential use of these bio-insecticides was tried to be determined in order to contribute to the reduction of the adverse environmental impacts.

Material and Method

Productions of host plant and *Tuta absoluta*

Tomato plant (*Solanum lycopersicum* L.) (Solanaceae), which is the major host plant of *T. absoluta* were growth in a controlled climate room. For this goal, Tomato seedlings (var. Tayfun F1) were sown in 5 l plastic pots, containing a mixture of peat, soil and perlite in ratio of 1:1:1. Afterwards, tomato plants brought to an insect rearing room were infected with larvae of *T. absoluta* collected in tomato greenhouses in Isparta. The stock culture of the pest was generated in the insect rearing room. The third-stage larvae (ca. 7-9 day-old and 5.09-5.84 mm long) used in the experiment were provided from this stock culture of the pest. Daily maintenance and control for the plant and the pest were done regularly, and plant irrigation was made when considered necessary. The conditions of climate rooms performed productions of tomato plant and *T. absoluta* were set to temperature 26±1ºC, relative humidity 60±5% and photoperiod 16:8 h.

Insecticides used in the experiment

Active ingredients and ratios, commercial names, formulations and application doses of insecticides used in the bioassay were given in Table 1.

Table 1. Bio- and synthetic insecticides and application doses used in the experiment

	Active ingredients and ratios	Commercial names and formulations	Application doses
Synthetic insecticides	Chlorantraniliprole+abamectin (45+18 g L^{-1})	Voliam Targo 063 SC	80 ml/100 L
	Metaflumizone (240 g L^{-1})	Alverde SC	100 ml/da
Bio-insecticides	Azadirachtin A (10 g L^{-1})	NeemAzal-T/S EC	500 ml/100 L
	Emamectin benzoate (50 g L^{-1})*	Bekchi 5 SG	30 g/100 L
	Spinosad (480 g L^{-1})	Laser SC	25 ml/100 L
	Bacillus thuringiensis var. kurstaki (32000 IU mg^{-1})*	Delfin WG	100 g/100 L
	Bacillus thuringiensis+azadirachtin mixture	Delfin+NeemAzal-T/S	(50 g+250 ml)/100 L

*Because these insecticides are unregistered for *Tuta absoluta* in Turkey, recommended doses of them for *Heliothis armigera* were used in the study.

Insecticide applications

Tomato leaves were placed in the culture plates with a dimension of 6x9x13 cm, at the base of which located blotting paper. Then, 5 larvae at homogeneous weight were put in each of culture plates using a fine paintbrush after weighing individually. Medium-sized and sessile leaves of tomato were used to keep larvae easily when weighing of them during the assessment and fresh tomato leaves were added in the culture plates when considered necessary. Insecticides used in the experiment were applied by spray tower with 1 bar pressure, so that 2 mg solution per cm^2 on tomato leaf which 5 larvae existed on. Distilled water was applied as a control to compare with the insecticides and all applications were repeated 6 times to reduce the error degree of freedom in the experiment.

After the applications of the insecticides, the culture plates were kept in a climate chamber set to temperature 26ºC, relative humidity 60% and photoperiod 16:8 h and also, assessments were done at the 1, 3, 5 and 7 DAA (Days After Application). Larvae were checked if live or not, gently touching with the tip of the fine paintbrush. During the assessments, numbers of live individuals were recorded and larval weights were measured with electronic precision scales (Model: Kern ABJ 220-4m, max=220 mg, min=10 mg, e=1 mg, d=0.1 mg) in milligrams (mg). After surviving larvae became pupae, adult emergence was monitored up to 10 days under the same conditions with larvae.

Data analysis

To analyze the data obtained from the experiment, Tukey test applied after One-Way ANOVA for numbers of live individuals and larval weights (mg/larvae), using SPSS® (Version 15.00, November 2006, SPSS Inc., Chicago, IL, USA.).

Growth Inhibition Index (GII) was calculated with mean larval weights from the following equation: GII= (CL-TL)/CL where CL is the mean larval weight gained in the control after 7 days of applications and TL is the mean larval weight gained in the treatment after 7 days of applications (Abdelgaleil & El-Eswad, 2005; Gökçe et al., 2012; Turanlı et al., 2012). Also, the percentage effects of insecticides were calculated by Abbott's formula (Abbott, 1925; Karman, 1971). In addition, pupation and adult emergence percentage out of a total of 30 larvae were determined at the end of the experiment.

Results

Survival rate of *Tuta absoluta* larvae after insecticide applications

Mean numbers of live individuals at the 1, 3, 5 and 7 DAA were found as 2.50, 2.17, 0.50 and 0.33, respectively in metaflumizone-treated larvae of *T. absoluta*. As for chlorantraniliprole+abamectin-treated larvae, mean number of live individuals was 0.5 at the 1 DAA. In subsequent assessments, all individuals were died in chlorantraniliprole+abamectin-treated larvae. Mean numbers of live individuals at the 1, 3, 5 and 7 DAA of azadirachtin were 4.67, 3.83, 1.83 and 1.17, respectively. In emamectin benzoate-treated larvae, mean numbers of live individuals at the 1 and 3 DAA occurred as 1.33 and 0.67, respectively. Mean numbers of live individuals at the 1, 3, 5 and 7 DAA were realized as 2.67, 1.50, 0.17 and 0.00 respectively in spinosad-treated larvae, 4.17, 0.83, 0.67 and 0.67 respectively in *Bacillus thuringiensis*-treated larvae, 4.17, 0.33, 0.33 and 0.33 respectively in *B. thuringiensis*+azadirachtin mixture-treated larvae. In control larvae group, the mean numbers were 4.83, 4.17, 2.83 and 2.83, respectively (Figure 1).

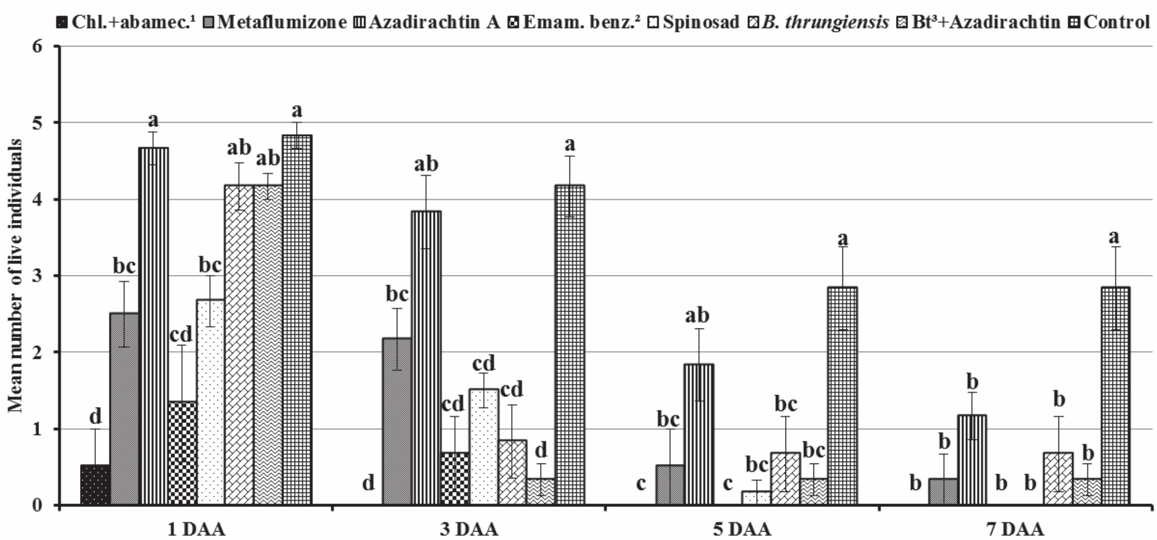

Figure 1. Mean numbers (±SE) of live larvae of *Tuta absoluta* at the 1, 3, 5 and 7 DAA. ¹Chl.+abamec.: Chlorantraniliprole+abamectin, ²Emam. benz.: Emamectin benzoate, ³Bt.: *Bacillus thuringiensis*. All data were analyzed using SPSS® (Version 15.00, November 2006, SPSS Inc., Chicago, IL., USA). Among vertical bars designated by the same letter at the same day do not differ significantly (p>0.05; n=6, total 30 larvae for each insecticide application) according to a Tukey test.

The differences between mean numbers of live individuals in azadirachtin-, *B. thuringiensis*- and *B. thuringiensis*+azadirachtin mixture-treated larvae at the 1 DAA were found statistically insignificant. Metaflumizone and spinosad active ingredients took place in the same statistical group. Chlorantraniliprole+abamectin was the most efficient at the 1 DAA and located in the different statistical group from the control. Azadirachtin and the control have no statistically differences at the 3 and 5 DAA. It is determined that chlorantraniliprole+abamectin and *B. thuringiensis*+azadirachtin mixture were in the same statistically group at the 3 DAA. As for emamectin benzoate, spinosad and *B. thuringiensis*, no statistical differences were found among them. All insecticides were involved in the same statistical group at the 7 DAA and they were statistically different from the control is observed in Figure 1.

Effects of synthetic insecticides on *T. absoluta* have begun from the 1 DAA and continued increasingly up to the 7 DAA. Effect of chlorantraniliprole+abamectin active ingredient was 89.66% at the 1 DAA and it rose to 100.00% at the assessments performed in subsequent days.

The percentage effects of metaflumizone at the 1, 3, 5 and 7 DAA were calculated by Abbott's formula as 48.28, 48.00, 82.35 and 88.24 percent, respectively (Figure 2).

As concerns bio-insecticides, they did not have an important effect on the pest at the 1 DAA, except emamectin benzoate. Effects of emamectin benzoate at the 1 and 3 DAA were 72.41% and 84.00%, respectively and it reached to 100.00% in subsequent assessments. The highest effective bio-insecticide was spinosad with 44.83%, followed by emamectine benzoate. Percentage effects of both *B. thuringiensis* and *B. thuringiensis*+azadirachtin mixture were 13.79% at the 1 DAA. It is observed that percentage effect of azadirachtin was 3.45% at the 1 DAA. Effects of spinosad, *B. thuringiensis* and *B. thuringiensis*+azadirachtin mixture from the 3 DAA and effect of azadirachtin from the 5 DAA were increased.

Percentage effects at the 3, 5 and 7 DAA occurred as 64.00, 94.12 and 100.00 percent respectively in spinosad-treated larvae, 80.00, 76.47 and 76.47 percent respectively in *B. thuringiensis*-treated larvae, 92.00, 88.24 and 88.24 percent respectively in *B. thuringiensis*+azadirachtin mixture-treated larvae and 8.00, 35.29 and 58.82 percent respectively in azadirachtin-treated larvae (Figure 2).

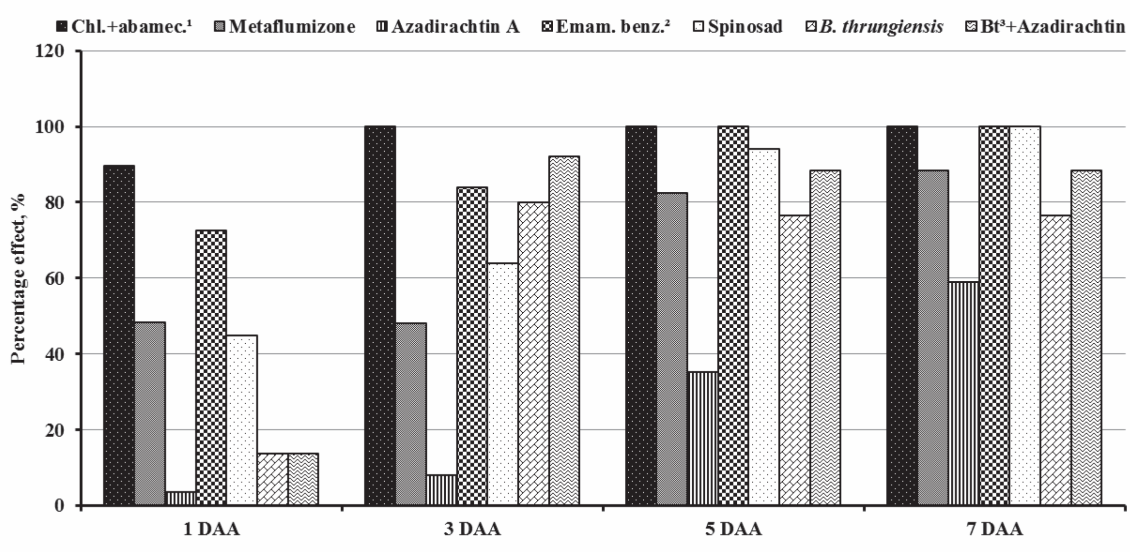

Figure 2. Efficacy of insecticides on larvae of *Tuta absoluta* at the 1, 3, 5 and 7 DAA by Abbott formula. [1]Chl.+abamec.: Chlorantraniliprole+abamectin, [2]Emam. benz.: Emamectin benzoate, [3]Bt.: *Bacillus thuringiensis*.

Growth inhibitory effects of insecticides on *Tuta absoluta* larvae

Mean weights at the 1, 3, 5 and 7 DAA of larvae selected homogeneously weight before applications were seen in Figure 3. Mean weights of the control larvae at the 1, 3, 5 and 7 DAA became 17.35, 13.88, 9.13 and 7.83 mg/larvae, respectively. Mean weight of chlorantraniliprole+abamectin-treated larvae at the 1 DAA was 1.67 mg/larvae. In subsequent assessments performed on chlorantraniliprole+abamectin-treated larvae, larval weight could not be measured due to absence of live larvae. As regards mean weights of metaflumizone-treated larvae were 8.53, 6.25, 1.50 and 1.02 mg/larvae at the 1, 3, 5 and 7 DAA, respectively (Figure 3).

Mean larval weights at the 1, 3, 5 and 7 DAA of bio-insecticides were 16.88, 13.25, 6.75 and 3.17 mg/larvae respectively in azadirachtin-treated larvae, 12.78, 1.75, 1.38 and 1.18 mg/larvae respectively in *B. thuringiensis*-treated larvae, 8.12, 0.67, 0.58 and 0.50 mg/larvae respectively in *B. thuringiensis*+azadirachtin-treated larvae. Mean weights of spinosad-treated larvae at the 1, 3 and 5 DAA were weighted as 2.70, 2.52 and 0.28 mg/larvae, respectively. As a result of weighing performed at the 1 and 3 DAA, mean weights of emamectin benzoate-treated larvae were calculated as 6.13 and 2.50 mg/larvae, respectively (Figure 3).

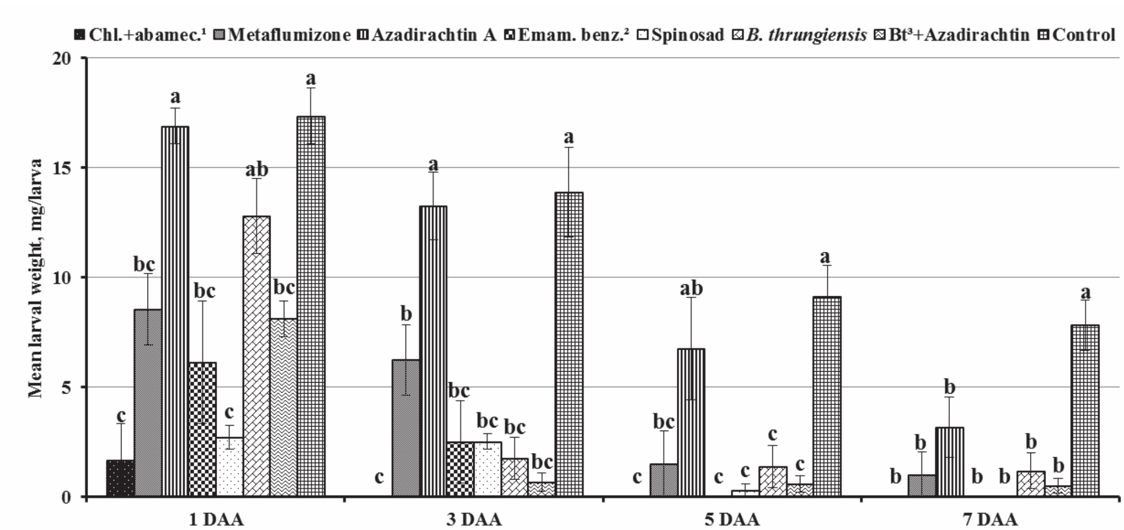

Figure 3. Mean larval weights (±SE) of *Tuta absoluta* at the 1, 3, 5 and 7 DAA. ¹Chl.+abamec.: Chlorantraniliprole+abamectin, ²Emam. benz.: Emamectin benzoate, ³Bt.: *Bacillus thuringiensis*. All data were analyzed using SPSS® (Version 15.00, November 2006, SPSS Inc., Chicago, IL., USA). Among vertical bars designated by the same letter at the same day do not differ significantly (p>0.05; n=6, total 30 larvae for each insecticide application) according to a Tukey test.

Effects of insecticides on larval growth index calculated after 7 days of applications confirmed previous results that mean weights of both bio-insecticide treated larvae and synthetic insecticide treated larvae decreased at a similar rate day by day after applications. As regards to synthetic insecticides, growth inhibition index was 0.97 in chlorantraniliprole+abamectin-treated larvae and 0.64 in metaflumizone-treated larvae. As for bio-insecticides, growth inhibition indexes after 7 days of applications were similar, except in azadirachtin-treated larvae (0.17). Growth inhibition indexes were determined as 0.82 in emamectin benzoate-treated larvae, 0.89 in spinosad-treated larvae, 0.65 in *B. thuringiensis*-treated larvae and 0.80 in *B. thuringiensis*+azadirachtin mixture-treated larvae (Figure 4).

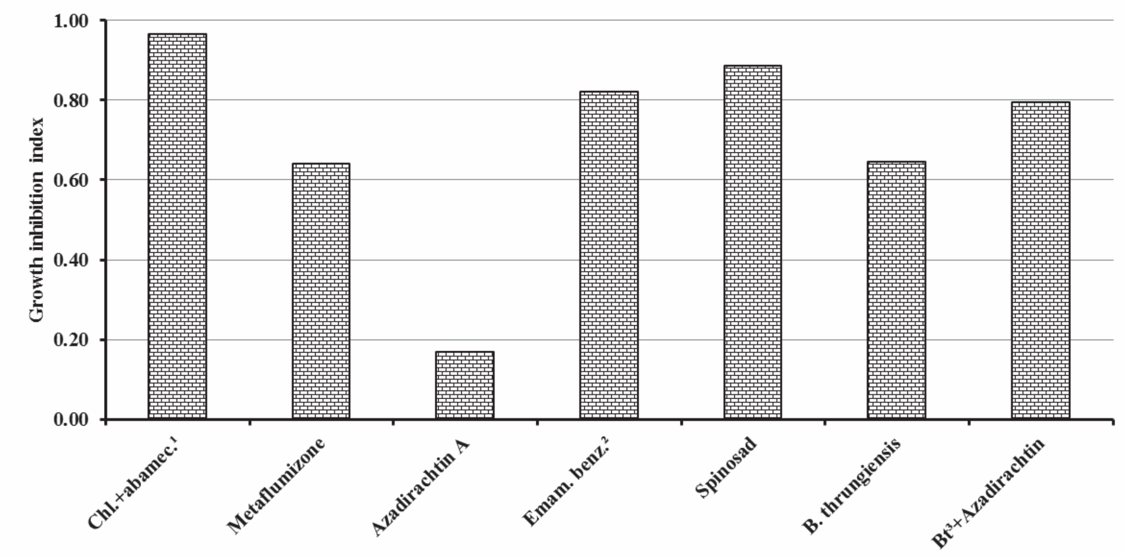

Figure 4. Growth inhibition indexes of insecticide-treated larvae at the 7 DAA. [1]Chl.+abamec.: Chlorantraniliprole+abamectin, [2]Emam. benz.: Emamectin benzoate, [3]Bt.: *Bacillus thuringiensis*.

The percent ratios of pupation and adult emergence of treated-larvae

The percentage of pupation and adult emergence out of a total of 30 larvae treated with insecticide or water were given in Table 2. The percentage of pupation and adult emergence in the control group were 56.67 and 50.00 percent, respectively. None of chlorantraniliprole+abamectin-, spinosad- and emamectin benzoat-treated larvae could be success to pupation. The data in table 2 demonstrated that both bio-insecticide treated larvae and synthetic insecticide treated larvae could not be adult, except azadirachtin-treated larvae (6.67%).

Table 2. The percentage of pupation and adult emergence of insecticide or water treated-larvae (%)*

Insecticides	Pupation	Adult emergence
Chlorantraniliprole+abamectin	0.00	0.00
Metaflumizone	6.67	0.00
Azadirachtin A	20.00	6.67
Emamectin benzoate	0.00	0.00
Spinosad	0.00	0.00
Bacillus thuringiensis	16.67	0.00
Bacillus thuringiensis+azadirachtin mixture	6.67	0.00
Control	56.67	50.00

*The percentage of pupation and adult emergence were calculated out of total 30 larvae for each insecticide or water application.

Discussion

The data analysis practiced at the end of the present study shows that chlorantraniliprole+abamectin synthetic insecticide had a high impact on the pest at the 1 DAA. Braham et al. (2012) reported that chlorantraniliprole+abamectin was extremely effective on *Tuta absoluta*, but on the other hand, this chemical has a high side impact on natural enemies and brings about the problem of resistance development. Effects of bio-insecticides had a low impact on the pest at the 1 DAA in the present study, except spinosad and emamectin benzoate. All bio-insecticides used in the experiment had similar percentage effects with synthetic insecticides from the 3 DAA, except azadirachtin. Also, effects of bio- and synthetic insecticides took place in the same statistical group at the 7 DAA. It is seen that azadirachtin had an impact on the pest too as compared with the control. Gacemi & Guenaoui (2012) conducted a study on the efficacy of emamectin benzoate on *T. absoluta* in tomato greenhouse, and based on the results of this study, suggested that emamectin benzoate was effective on larvae of *T. absoluta* and its percentage effect on the pest reached up to 87%. A study conducted by Isman (1993) indicated that azadirachtin had anti-feeding effect on larvae of six different noctuids (*Actebia fennica* (Tausch.), *Mamestra configurata* Walker, *Peridroma saucia* Hubner, *Melanchra picta* (Harr.), *Spodoptera litura* (Fab.) and *Trichoplusia ni* Hubner). Nannini et al. (2011) announced in a study about efficiency of 11 different larvicides on newly emerged and older larvae of *T. absoluta* under controlled laboratory conditions that spinosad had an extremely effect on the pest. As for metaflumizone and azadirachtin, their effects on the pest were lower than spinosad but higher than control. Santos et al. (2011) conducted a study between 2001 and 2005 to find out the effect of some insecticides on *T. absoluta* and other pests in pole tomato. According to the results of the field study, the researches denoted that spinosad was effective in controlling *T. absoluta*. Dağlı et al. (2012) determined in a study investigated the efficiency of different insecticides on *T. absoluta* that spinosad and chlorantraniliprole+abamectin showed a hundred percent effect on the pest, but effects of metaflumizone and azadirachtin on the pest were lower. A study on management of *T. absoluta* with insecticides on tomatoes in both laboratory and greenhouse performed by Braham & Hajji (2012) showed that spinosad was effective in the ratio of 91% on *T. absoluta*. Hafsi et al. (2012) explored larvicide and ovicide efficiencies of some insecticides on *T. absoluta* in semi-natural conditions. The study stated that spinosad had a 70% impact on the second-stage larvae of *T. absoluta*, and effect of azadirachtin was so low; however, mixture of azadirachtin and neem oil had a significant effect on eggs and larvae of *T. absoluta*. Also in this present study, effect of *Bacillus thuringiensis*+azadirachtin mixture was realized in a high proportion on the pest from the 3 DAA.

In the present study, mean larval weight in the control also decreased gradually according to the assessments made at the 1, 3, 5 and 7 DAA. The proportion of pupation and adult emergence of the control larvae group were determined as 56.67 and 50.00 percent. Therefore, it is thought that they lost weight because they experienced the water loss during pupation. Chlorantraniliprole+abamectin-treated larvae could show the success of pupation. The proportion of pupation was 6.67% in Metaflumizone-treated larvae and 20.00% in azadirachtin-treated larvae. *B. thuringiensis*-treated larvae and *B. thuringiensis*+azadirachtin mixture-treated larvae showed the achievement of pupation in the ratio of 16.67 and 6.67 percent, respectively. However, none of larvae in both two larvae group could manage to be adult. According to this, bio-insecticides used in the present study had an important effect on larvae of *T. absoluta* and were highly effective on the achievement of pupation and adult emergence of larvae. It is also observed that feeding ratio of both bio-insecticide treated larvae and synthetic insecticide treated larvae on tomato leaf was extremely decreased. Durmuşoğlu et al. (2011) reported in a study on effects of some plant origin insecticides on larvae of *T. absoluta* in laboratory conditions that the applications of these plant origin insecticides done by dipping method had an effect on the fourth-stage larvae of the pest. Hanafy & El-Sayed (2013) noted in a study on efficacy of some bio-insecticides on *T. absoluta* and *Heliothis armigera* that spinosad and emamectin benzoate were more successful in controlling *T. absoluta* rather than synthetic insecticides. In the present study, effects of *B. thuringiensis* and *B. thuringiensis*+azadirachtin mixture were very high from the 3 DAA. Hafsi et al. (2012) informed that *B. thuringiensis* had an impact on *T. absoluta* and could be used instead of synthetic insecticides. In a study examined effects of 17 different insecticides on *T. absoluta*, it is reported that effects of spinosad, emamectin benzoate, azadirachtin and *B. thuringiensis* were found as 96.0, 92.2, 78.8 and 75.9 percent, respectively (Moussa et al., 2013).

Based on the several studies on the effects of bio-insecticides and extracts derived from plants on the pest and thereof natural enemies (Tillman & Moonroney, 2000; Vinuela et al., 2000; Schoenly et al., 2003; Ghosh et al., 2010; Lopez et al., 2011; Biondi et al., 2012; Liu et al., 2012; Erdoğan, 2013), it is possible to say that the insecticides obtained naturally materials and organisms such as animals, plants, bacteria and minerals have less negative effects on natural enemies than synthetic insecticides.

Consequently, growth inhibitory effects of bio- and synthetic insecticides on *T. absoluta* and the decreases of mean larval weight in bio- and synthetic insecticide treated larvae groups after 7 days of applications showed similarity. Therefore, it is possible to say according to the results obtained from the present laboratory test that bio-insecticides may have a potential use instead of synthetic insecticides in controlling the pest. However, these results should certainly be supported by large-field experiments. In cases of necessarily chemical application, the usage of bio-insecticides instead of synthetic ones is more appropriate in terms of ensuring a successful controlling, environmental pollution and human health. In addition, anyway spinosad and azadirachtin are within the recommended chemicals against the pest in Turkey (Keçeci, 2010). Studies will be conducted on register of *B. thuringiensis* and emamectin benzoate against the pest may be useful. In addition, it is understood that mixtures of these active ingredients can be used in controlling the pest. Bio-insecticide usage in controlling pests may provide a significant advantage for integrated pest management applications. This last point should receive attention because it is important to have data in controlling *T. absoluta*. The data indicate that bio-insecticide usage in an agroecosystem will give an opportunity for biological control applications.

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