

Research Article

Efficacy of mixture of emamectin benzoate with some insecticides on the mortality and esterase activity of fourth instar larvae of *Tuta absoluta* (Lepidoptera: Gelechiidae)

Mohsen Taleh¹, Hooshang Rafiee Dastjerdi^{1*}, Bahram Naseri¹, Aziz Sheikhi Garjan² and Khalil Talebi Jahromi³

1. Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran.
2. Iranian Research Institute of Plant Protection, Tehran, Iran.
3. Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

Abstract: The tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is one of the most damaging tomato pests in the world and in Iran. The toxicity of acetamiprid, eforia (thiamethoxam + lambda-cyhalothrin) and hexaflumuron alone and in mixture with emamectin benzoate was studied against 4th-instars of *Tuta absoluta* (Meyrick) at 25 ± 2 °C, 65 ± 5% RH and 16:8 (L:D) h. Moreover, the mixtures of examined insecticides with emamectin benzoate at LC₁₅:LC₁₅ ratio were assessed on the general esterase enzyme activity and total protein content of 4th-instars. The highest toxicity was found for emamectin benzoate after 72 h (LC₅₀ = 0.48 mg a.i./l), followed by acetamiprid (LC₅₀ = 46.94 mg a.i./l), eforia (LC₅₀ = 156.24 mg a.i./l) and hexaflumuron (LC₅₀ = 670.32 mg a.i./l). Mixing emamectin benzoate with acetamiprid at the ratio of LC₅₀:LC₅₀ and LC₂₅:LC₂₅ resulted in synergistic impacts while mix of two other ratios of the same pesticides represented additive influences. The mixture of emamectin benzoate with either hexaflumuron or eforia at all ratios created antagonistic and additive effects, respectively. Mixing emamectin benzoate with either acetamiprid or eforia increased larval esterase activity, however, there was no significant difference between emamectin benzoate in mixture with hexaflumuron and using it alone. Mixing emamectin benzoate with the examined insecticides considerably decreased the larval total protein content. Based on the findings of this work, the mixtures of eforia and acetamiprid with emamectin benzoate represented greater negative effects against 4th-instars compared to emamectin benzoate alone and the control.

Keywords: mixture effects, emamectin benzoate, acetamiprid, eforia, *Tuta absoluta*

Introduction

Tuta absoluta Meyrick (Lepidoptera: Gelechiidae) is a native microlepidopteran pest in the Southern America which can affect all aerial

parts of the host plants (leaves, flowers, stems and fruits). This pest has a high potential to cause up to 100% economic losses (Torres *et al.*, 2001; EPPO, 2005; Desneux *et al.*, 2010). Chemical control of the leafminers such as *T. absoluta* is difficult due to their hidden behavior inside the leaf, high reproductive capacity, polyvoltinism and poor spraying technology, so it is necessary to apply various insecticides several times in a season to control these pests (Temerak, 2011).

Handling Editor: Saeid Moharramipour

*Corresponding author: Hooshangrafiee@gmail.com
Received: 16 December 2019, Accepted: 28 June 2020
Published online: 31 August 2020

However, *T. absoluta* has a great potential to develop resistance to conventional insecticides such as organophosphates (OPs), pyrethroids and avermectins (Siqueira *et al.*, 2000; Siqueira *et al.*, 2001; Roditakis *et al.*, 2013). Higher levels of *T. absoluta* resistance to abamectin, permethrin, Cartap and spinosad are related to the extensive application of these insecticides by farmers (Siqueira *et al.*, 2000; Campos *et al.*, 2014).

Using mixtures of pesticides is an effective way to postpone developing insecticide resistance or to overcome current resistance in a pest species. This method is generally utilized, in the field, to increase the control spectrum for aggressive multiple pests simultaneously or against a single pest (Ishaaya *et al.*, 1985). The mixture of insecticides with various modes of action could result in synergistic, antagonistic or additive effects against an insect species. If the mixtures would be synergistic, the costs of excessive use of insecticides might effectively be reduced (Wolfenbarger and Cantu, 1975).

Insecticides could influence the activity of metabolic enzymes such as glutathione S-transferases (GSTs), esterases, mixed function oxidases (MFOs) as well as total protein content. General esterases and GSTs have an important role in detoxifying of synthetic and non-synthetic insecticides (Vanhaelen *et al.*, 2001). Some studies demonstrated that the mixture of two different insecticides might result in inhibition of the detoxification enzymes in many pests and lead to their effective control (Martin *et al.*, 2003). Several researchers reported that OPs combined with pyrethroids had synergistic effect against several pests (All *et al.*, 1977; Asher *et al.*, 1986). For example, mixture of chlorpyrifos with either emamectin benzoate, indoxacarb or spinosad showed synergistic effect against *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) (Saddiq *et al.*, 2017). The mixtures of emamectin benzoate with deltamethrin significantly increased toxicity against adult of *Musca domestica* L. (Diptera: Muscidae) Also, emamectin benzoate mixed with bifenthrin showed an antagonistic effect against this species (Khan *et al.*, 2013). Findings of Ghoneim *et al.* (2012) indicated that chlorpyrifos produced high synergistic effect

when mixed with hexaflumuron and triflumuron and produced an additive effect when mixed with chlorfluazuron, chromafenozide and tebufenozide on *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae).

Emamectin benzoate is a new insecticide isolated from the avermectin group of natural products. These products have been utilized to control lepidopteran pests on vegetable crops worldwide, with a special efficacy against *T. absoluta* (Liguori *et al.*, 2008).

The present study was done to find out the toxicity of mixtures of emamectin benzoate with either eforia, acetamiprid, or hexaflumuron against larval *T. absoluta* as well as their effects on some biochemical aspects such as general detoxifying esterases and total protein content in the pest. It is hypothesized that the mixtures of tested insecticides could delay the development of insecticide resistance and reduce the costs and risks of overuse of them.

Materials and Methods

Insects

The specimens of *T. absoluta* were collected from tomato greenhouses around Ardabil city, Iran. The insects were maintained in the growth chamber on the commercial tomato (*Solanum lycopersicum* L. var. Super Strain B) at 25 ± 2 °C, $65 \pm 5\%$ RH and photoperiod of 16: 8 (L: D) h.

Insecticides

The insecticides had the commercial formulations as follows: emamectin benzoate (Proclaim 5% SG; Syngenta, Switzerland), acetamiprid (Acetamiprid 20% WP; Ariashimi, Iran), eforia (thiamethoxam + lambda-cyhalothrin) (Eforia 247% OD; Syngenta, Switzerland), hexaflumuron (Hexaflumuron 10% EC; Ariashimi, Iran).

Bioassays

The toxicity of tested insecticides was assessed against 4th-instar of *T. absoluta* using tomato leaves. Tomato leaves were dipped into five different concentrations of each insecticide for 15 s and left to dry for 1 h under room temperature. Twenty 4th-instar larvae of *T. absoluta* were

transferred on treated leaves placed in 9 cm diameter Petri dishes. Tween-80 was added to the insecticide preparations as a surfactant at a concentration of 0.05% (v/v). Leaves dipped in distilled water+Tween-80 were considered as the control. The mortality of larvae was recorded at 72 h after treatments. The tests were done in triplicates for each insecticide concentration (Galdino *et al.*, 2011). Toxicity index of insecticides was evaluated by Sun method (1950); (LC_{50} of the efficient compound/ LC_{50} of the other compound \times 100).

Mixtures

Newly molted 4th-instars of *T. absoluta* were exposed to binary mixtures of emamectin benzoate with either acetamiprid, eforia or hexaflumuron at LC_{10} , LC_{15} , LC_{25} and LC_{50} using the leaf-dip method. Twenty larvae were released in each Petri dish. Leaves dipped in distilled water were used as control. Mortality was recorded at 72 h after treatments. The experiments were conducted in triplicates for each mixture (Galdino *et al.*, 2011; Abbas *et al.*, 2015).

Determination of general esterases activity

General esterases activity of 4th-instars treated with emamectin benzoate mixed with either eforia, acetamiprid, or hexaflumuron at LC_{15} : LC_{15} ratio, was assayed according to the method of van Asperen (1962). The substrates of α -Naphthyl acetate (α -NA) and β -naphthyl acetate (β -NA) were used for α -esterase and β -esterase activities, respectively. Fifteen 4th-instars were homogenized in 70 μ l of 40 mM phosphate buffer (pH 7.0) inside microtube (2 ml) on ice. The solutions were then centrifuged at 10,000 g at 4 °C for 20 min and supernatant was used as enzyme solution. The quantity of 90 μ l of the substrate was added to the microplate, then preincubated at 30 °C for 10 min. The amount of 90 μ l of Fast Blue RR salt was added to the microplate, then the naphthol formation was assayed by measurement of absorbance at 450 and 540 nm for α -NA and β -NA, respectively through microplate reader (Anthos Labtec Instruments GmbH, Austria). All experiments were repeated three times.

Determination of total protein content

The effect of mixtures of emamectin benzoate with either acetamiprid, eforia or hexaflumuron at LC_{15} : LC_{15} ratio, on the total protein content of 4th-instars was determined based on Bradford (1976) method, using bovine serum albumin (Bio-Rad) (Sigma) as a standard protein. The measurement was carried out using a microplate reader at 595 nm. All experiments were repeated three times.

Data analysis

Probit analysis was used for calculating lethal concentrations (LC_{30} , LC_{50} , and LC_{90}) in SPSS software (ver.16). The mean mortality of 4th-instars treated with different concentrations of each insecticide, after checking for normality by the Shapiro-Wilk method, was analyzed by one-way analysis of variance (ANOVA) at a 5% level of significance by LSD test. Abbott's formula (Abbott, 1925) was used for correcting mortality data.

The expected mortality (M_E) for Mixing emamectin benzoate with different insecticides was calculated via equation $M_E = M_B + M_A (1 - M_B)$, where M_B is the observed mortality caused by different insecticides and M_A is the observed mortality caused by emamectin benzoate. Results from a chi-square test, $\chi^2 = (M_{AB} - M_E)^2 / M_E$, where M_{AB} shows the found mortality for mixing emamectin benzoate with different insecticides, were compared to the chi-square table value. When the determined chi-square value was overstepped the table value (df = 1), it would be a non-additive effect. The $M_{AB} - M_E > 0$ shows synergism; and the $M_{AB} - M_E < 0$ indicates antagonism (Koppenhofer and Kaya, 1996).

Data for general esterases activity and total protein content were exposed to ANOVA. The differences between means were isolated by LSD test in SPSS software ver. 16 at $P = 0.05$.

Results

Estimating LC_{50} values

Toxicity values and toxicity indices estimated for all insecticides against 4th-instars of the *T. absoluta* are shown in Table 1. The data

demonstrated that emamectin benzoate showed the highest toxicity ($LC_{50} = 0.48$ mg a.i/l), followed by acetamiprid ($LC_{50} = 46.94$ mg a.i/l), eforia ($LC_{50} = 156.24$ mg a.i/l) and hexaflumuron ($LC_{50} = 670.32$ mg a.i/l).

Mixtures

Binary mixtures of emamectin benzoate with eforia, acetamiprid, and hexaflumuron at different concentrations showed additive, synergistic and

antagonistic interactions (Table 2). The mixtures of emamectin benzoate with acetamiprid at LC_{10} : LC_{10} and LC_{15} : LC_{15} ratios showed additive effects. The emamectin benzoate mixed with acetamiprid at LC_{25} : LC_{25} and LC_{50} : LC_{50} ratio showed synergistic interaction and its mixtures with eforia at LC_{10} : LC_{10} , LC_{15} : LC_{15} , LC_{25} : LC_{25} and LC_{50} : LC_{50} showed additive effects. The mixture of hexaflumuron with emamectin benzoate showed an antagonistic interaction.

Table 1 Toxicity of tested insecticides against 4th-instars of *Tuta absoluta* under laboratory conditions.

Insecticide	LC_{10} (mg AI/L) (95% CL)	LC_{15} (mg AI/L) (95% CL)	LC_{25} (mg AI/L) (95% CL)	LC_{50} (mg AI/L) (95% CL)	Slope \pm SE	χ^2	Toxicity index at LC_{50}
Emamectin benzoate	0.14 (0.11-0.16)	0.18 (0.15-0.2)	0.25 (0.22-0.28)	0.48 (0.45-0.51)	2.4 ± 0.16	1.32	100
Acetamiprid	15.04 (10.68-18.88)	18.7 (14.04-22.68)	25.79 (20.95-29.85)	46.94 (42.17-52.11)	2.5 ± 0.28	2.08	1.02
Eforia	24.94 (13.53-36.92)	35.42 (21.24-49.4)	59.48 (41.07-76.37)	156.22 (130.93-184.41)	1.6 ± 0.19	1.86	0.3
Hexaflumuron	323.9 (254.93-377.18)	372.24 (305.12-423.17)	457.13 (396.79-502.86)	670.32 (626.56-716.59)	4 ± 0.49	0.67	0.07

Table 2. Mixture of emamectin benzoate with several insecticides and their interactive effects (at different doses) on 4th-instars of *Tuta absoluta* under laboratory conditions.

Mixture	Ratio	Observed mortality (%)	ME (%) ¹	χ^2	P	N ²	Interaction ³
Emamectin benzoate + Acetamiprid	$LC_{10} + LC_{10}$	28	20.8	2.49	0.05	180	additive
Emamectin benzoate + Eforia	$LC_{10} + LC_{10}$	23	22.56	0.008	0.05	180	additive
Emamectin benzoate + Hexaflumuron	$LC_{10} + LC_{10}$	8	19.04	6.40	0.05	180	antagonistic
Emamectin benzoate + Acetamiprid	$LC_{15} + LC_{15}$	35	26.05	3.07	0.05	180	additive
Emamectin benzoate + Eforia	$LC_{15} + LC_{15}$	32	29.45	0.22	0.05	180	additive
Emamectin benzoate + Hexaflumuron	$LC_{15} + LC_{15}$	10	26.05	9.88	0.05	180	antagonistic
Emamectin benzoate + Acetamiprid	$LC_{25} + LC_{25}$	60	45.25	3.59	0.05	180	synergistic
Emamectin benzoate + Eforia	$LC_{25} + LC_{25}$	53	46.71	0.84	0.05	180	additive
Emamectin benzoate + Hexaflumuron	$LC_{25} + LC_{25}$	12	32.84	13.22	0.05	180	antagonistic
Emamectin benzoate + Acetamiprid	$LC_{50} + LC_{50}$	95	75.56	5.00	0.05	180	synergistic
Emamectin benzoate + Eforia	$LC_{50} + LC_{50}$	78	76.60	0.02	0.05	180	additive
Emamectin benzoate + Hexaflumuron	$LC_{50} + LC_{50}$	8	52.16	37.38	0.05	180	antagonistic

¹ The expected mortality of mixture.

² Total number of insects exposed.

³ The type of interaction (synergistic, additive or antagonistic) was determined by comparing the expected and observed mortalities as described by Koppenhofer and Kaya (1996).

General esterases activity

α -esterase activity was higher in mixture of emamectin benzoate with either acetamiprid (0.616 μ mol/min/mg protein) or eforia (0.562 μ mol/min/mg protein) at LC_{15} : LC_{15} ratio as compared with emamectin benzoate alone (0.354

μ mol/min/mg protein) or control (0.31 μ mol/min/mg protein) ($F = 24.262$; $df_{te} = 4, 10$; $P < 0.05$). The activities obtained for β -esterase ($F = 0.862$; $df_{te} = 4, 10$; $P > 0.05$) showed no significant differences between the mixtures, emamectin benzoate alone and the control (Fig. 1).

Total protein content

The data in Fig. 2 indicate that protein content in larvae treated with emamectin benzoate mixed with either acetamiprid (1.025 µg/mg), eforia (0.924 µg/mg) or

hexaflumuron (1.105 µg/mg) at LC₁₅:LC₁₅ ratio was significantly reduced as compared with emamectin benzoate alone (1.84 µg/mg) or the control (1.912 µg/mg) (F = 19.607; df_{e,t} = 4,10; P < 0.05).

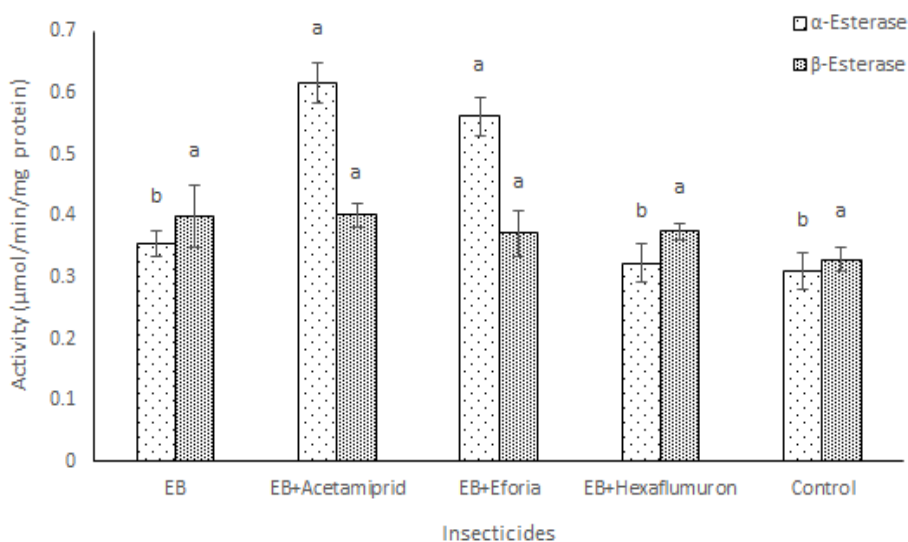


Figure 1 The effects of insecticides mixture on α- and β- esterase activity (mean ± SE) in 4th-instars of *Tuta absoluta*. Various letters represent that the enzymes' specific activities vary significantly from each other by LSD test (P < 0.05). EB: Emamectin benzoate.

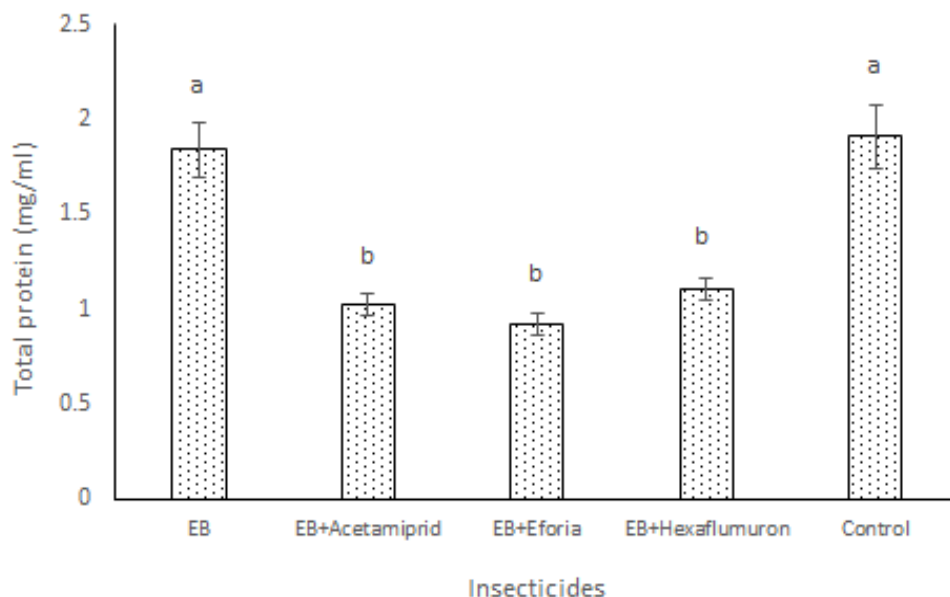


Figure 2 The effects of interaction of emamectin benzoate with other insecticides on the total protein content (mean ± SE) of 4th-instars of *Tuta absoluta*. Different letters represent the significant difference between the protein contents by LSD test (P < 0.05). EB: Emamectin benzoate.

Discussion

Based on the results of this study, the emamectin benzoate showed the best efficacy against 4th-instars of *T. absoluta*. Many researchers have reported the efficacy of emamectin benzoate on different insect pests like *T. absoluta* (Gacemi and Guenaoui, 2012) and *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) (López *et al.*, 2010). Moreover, Mahmoud *et al.* (2013) showed a remarkable reduction in the population of *T. absoluta* and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) treated with emamectin benzoate.

Acetamiprid was the second most effective insecticide against larval *T. absoluta*, in this study, similar to Nozad *et al.* (2017) findings. However, Yankova and Ganeva (2013) reported that acetamiprid had inadequate effectiveness towards larval *T. absoluta*. Wafaa (2011) noted that acetamiprid could be considered as a promising candidate in controlling *Bemisia tabaci* (Homoptera: Aleyrodidae) with a lower value of harmful effect on beneficial insect species. eforia was another effective insecticide in our study. The results of Duchovskienė (2016) indicated that eforia was a suitable insecticide to reduce abundance of the most harmful lepidopteran pests in white cabbages.

Mixture of emamectin benzoate with acetamiprid showed synergistic effects against 4th-instars of *T. absoluta*. Mahmoud *et al.* (2013) noted that emamectin benzoate combined with either imidacloprid, indoxacarb, profenofos, chlorfenapyr or methomyl caused a considerable population reduction in 3rd-instars of *T. absoluta*. Mahmoud *et al.* (2014) demonstrated that chlorantraniliprole insecticide was the most toxic against *T. absoluta* followed by 20% emamectin benzoate + 60% bifenthrin and lambda-cyhalothrin. In our study, the mixture of hexaflumuron with emamectin benzoate showed antagonistic interactions. It can be suggested that emamectin benzoate caused cessation of feeding in larvae, so hexaflumuron could not enter larval body through the digestive tract resulting in antagonistic interaction. Metayi *et al.* (2015)

reported that all mixtures of emamectin benzoate (at LC₁₀ or LC₂₅) with novaluron or diflubenzuron (at LC₁₀ or LC₂₅) resulted in antagonistic effects.

The emamectin benzoate can influence the arthropods' nervous system by incrementing chloride ion flux at the neuromuscular junction leading to termination of irreversible paralysis and feeding (Ishaaya, 2001). Neonicotinoids such as acetamiprid can interact with nicotinic acetylcholine receptors and disturb the nervous system of the insect resulting in the insects' death (Belzunces *et al.*, 2012). Accordingly, the mixtures of these insecticides with various modes of action supplement the action of one another for control of *T. absoluta*. Also, Corbett (1974) has a general theory to explain the synergistic interactions among insecticides. According to his theory, one toxicant in the mixture synergizes the toxicity of the other one by participating in its metabolic detoxification. Moreover, in our study, when the mixture of emamectin benzoate with other insecticides was applied, they might bind to detoxifying enzymes and then prevent the binding and subsequent degradation of emamectin benzoate by these enzymes, thereby enhancing the toxicity of this insecticide. Formerly, it had been supposed that OPs in mixture with pyrethroids inhibit the detoxifying enzymes such as monooxygenases and esterases in different insect pests (Bryne and Devonshire, 1991; Martin *et al.*, 2003).

The results regarding esterase enzyme activity and protein content of 4th-instar larvae, support the observed toxicity ratios of the mixtures. Esterases, as important detoxifying enzymes, hydrolyze the esteric bond in synthetic chemicals (Hemingway and Karunatne, 1998). In the present study, the activity of α -esterase increased when larvae were treated with a mixture of emamectin benzoate with either acetamiprid or eforia. This shows that α -esterase enzyme has an important role in detoxification of these mixtures, suggesting a high toxicity of the mixtures of tested insecticides to *T. absoluta*. In the study of Zhu *et al.* (2017), acephate-only treatment reduced esterase activity by 40%, and

the mixture of imidacloprid+ acephate suppressed esterase activity by 45% in *Apis mellifera* L. (Hymenoptera: Apidae). Probably, in our study, mixtures have the ability to suppress the activity of other detoxification enzymes such as GST and MFOs. The biochemical analysis of GST and arboxylesterase by Badawy *et al.* (2015) indicated the detoxification of the low doses of pymetrozine and acetamiprid in *A. mellifera* by these enzymes. According to Abdel-Mageed and Shalaby (2011), thiamethoxam + lambda-cyhalothrin (Engeo) resulted in a considerable increment in acetylcholinesterase activity of *S. littoralis*. The role of esterases in detoxification of abamectin and resistance of Brazilian populations of *T. absoluta* against this insecticide is demonstrated by Siqueira *et al.* (2001). Zibae *et al.* (2016) indicated that exposing 4th-instars of *T. absoluta* to chlorpyrifos increased the activity of esterases after 24 and 48 hrs. Esterases and MFOs are the enzymes responsible for reduced toxicity of spinosad to *T. absoluta* (Reyes *et al.*, 2012).

In this study, the amount of total protein of larvae decreased by mixture of emamectin benzoate with either acetamiprid, eforia or hexaflumuron, suggesting that these mixtures have high toxicity against *T. absoluta* (although the mixture of hexaflumuron with emamectin benzoate showed an antagonism interaction). This might be caused by the degradation of proteins into amino acids, that with the entry of these amino acids to TCA cycle, they will aid store energy for the insect (Nath *et al.*, 1997). Furthermore, Nath *et al.* (1997) noted that sublethal concentrations of ethion and fenitrothion resulted in depleting the protein content of 5th-instars of *Bombyx mori* (L.) (Lepidoptera: Bombycidae) achieved by an increase in free amino acids. The reduction in protein content induced by emamectin benzoate + hexaflumuron in our study was similar to the finding of Assar *et al.* (2016), who stated that total protein and lipid contents of *S. littoralis* larvae were decreased by emamectin, hexaflumuron and teflubenzuron. Wilde *et al.* (2016) reported that the level of protein in

tissue extracts of *A. mellifera* was significantly reduced from the 1st to the 2nd-instars treated with imidacloprid. According to Manal and Abdel-Mageed (2018), the total protein content of *M. domestica* adults treated with tested mixtures such as deltamethrin + abamectin, lambda-cyhalothrin + khaya extract, indoxacarb + pomegranate, chlorantraniliprole + jojoba oil and methomyl + jojoba oil was reduced compared with control.

Several studies showed that populations of *T. absoluta* might have multiple insecticide resistance (Siqueira *et al.*, 2000; Siqueira *et al.*, 2001; Guedes and Siqueira, 2012). Pesticide mixtures with various modes of action might postpone the resistance development of pest population (Bielza *et al.*, 2009). Therefore, pesticide mixtures can overcome or hinder pest resistance and control a broad spectrum of pest species. Moreover, the expenses of excessive use of insecticides and the risks of environmental pollution could be decreased. Therefore, it is possible to use it as a component of integrated pest management programs in case of synergistic and additive interactions. According to the results of this work, the mixtures of emamectin benzoate with eforia and acetamiprid are good options for managing *T. absoluta*.

Acknowledgments

The University of Mohaghegh Ardabili (Ardabil, Iran) has financially supported this study, which is appreciated.

References

- Abbas, N., Crickmore, N. and Shad, S. A. 2015. Efficacy of insecticide mixtures against a resistant strain of housefly (Diptera: Muscidae) collected from a poultry farm. International Journal of Tropical Insect Science, 35: 48-53.
- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.
- Abdel-Mageed, A. E. M. and Shalaby, Sh. E. M. 2011. Toxicity and biochemical impacts

- of some new insecticide mixtures on cotton leafworm *Spodoptera littoralis* (Boisd.). *Plant Protection Science*, 47(4): 166-175.
- All, J. N., Ali, M., Hornyak, E. P. and Weaver, J. B. 1977. Joint action of two pyrethroids with methyl-parathion, methomyl, and chlorpyrifos on *Heliothis zea* and *H. virescens* in the laboratory and in cotton and sweetcorn. *Journal of Economic Entomology*, 70: 813-817.
- Asher, K. R. S., Eliyahu, M., Ishaaya, I., Zur, M. and Ben-Moshe, E. 1986. Synergism of pyrethroid-organophosphorus insecticide mixtures in insects and their toxicity against *Spodoptera littoralis* larvae. *Phytoparasitica*, 14: 101-110.
- Assar, A. A., Abo El-Mahasen, M. M., Dahi, H. F. and Amin, H. S. 2016. Biochemical effects of some insect growth regulators and bioinsecticides against cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Journal of Bioscience and Applied Research*, 2(8): 587-594.
- Badawy, M. E. I., Nasr, H. M. and Rabea, E. I. 2015. Toxicity and biochemical changes in the honey bee *Apis mellifera* exposed to four insecticides under laboratory conditions. *Apidologie*, 46: 177-193.
- Belzunces, L., Tchamitchian, S. and Brunet, J. L. 2012. Neural effects of insecticides in the honey bee. *Apidologie*, 43: 348-370.
- Bielza, P., Fernández, E., Graválos, C. and Albellán, J. 2009. Carbamates synergize the toxicity of acrinathrin in resistant western flower thrips (Thysanoptera: Thripidae). *Journal of Economic Entomology*, 102: 393-397.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*, 72: 248-254.
- Bryne, F. J. and Devonshire, A. L. 1991. In vivo inhibition of esterase and acetylcholinesterase activities by profenofos treatment in the tobacco whitefly *Bemisia tabaci* (Genn) implications for routine biochemical monitoring of these enzymes. *Pesticide Biochemistry and Physiology*, 40: 198-204.
- Campos, M. R., Rodrigues, A. R. S., Silva, W. M., Silva, T. B. M., Silva, V. R. F., Guedes, R. N. C. and Siqueira, H. A. A. 2014. Spinosad and the tomato borer *Tuta absoluta*: A bioinsecticide, an invasive pest threat, and high insecticide resistance. *PLOS ONE*, 9: e103235.
- Corbett, J. R. 1974. *The Biochemical Mode of Action of Pesticides*. New York: Academic Press.
- Desneux, N., Wajnberg, E., Wyskuys, K.A.G., Burgio, G., Arpaia, S., NarváezVazquez, C.A. and *et al.* 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of Pest Science*, 83: 197-215.
- Duchovskienė, L. 2016. The efficacy of different insecticides for control of Lepidopteran pests on cabbage in Lithuania. *Sodininkystė ir Daržininkystė*, 35(3/4): 65-75.
- EPPO, 2005. Data sheets on quarantine pests: *Tuta absoluta*: 2005 OEPP/EPPO, European and Mediterranean Plant Protection Organization, Bulletin OEPP/EPPO, 35: 434-435.
- Gacemi, A. and Guenaoui, Y. 2012. Efficacy of emamectin benzoate on *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) infesting a protected tomato crop in Algeria. *Academic Journal of Entomology*, 5 (1): 37-40.
- Galdino, T. V. S., Picanço, M. C., Morais, E. G. F., Silva, N. R., Silva, G. A. R. and Lopes, M. C. 2011. Bioassay method for toxicity studies of insecticide formulations to *Tuta absoluta* (MEYRICK, 1917). *Science and Agrotechnology*, 35(5): 869-877.
- Ghoneim, Y. F., Singab, M., Abou-Yousef, H. M. and Abd-El-Hai, N. S. 2012. Efficacy of certain insecticides and their mixtures with the tested IGRs against a field strain of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) under laboratory conditions. *Australian Journal of Basic and Applied Sciences*, 6(6): 300-304.

- Guedes, R. N. C. and Siqueira, H. A. A. 2012. The tomato borer *Tuta absoluta*: insecticide resistance and control failure. CAB Reviews, 7(055): 1-7.
- Hemingway, J. and Karunatne, S. H. P. 1998. Mosquito carboxylesterases: A review of the molecular biology and biochemistry of a major insecticide resistance mechanism. Medical and Veterinary Entomology, 12: 1-12.
- Ishaaya, I. 2001. Biochemical processes related to insecticide action: An overview. In: Ishaaya, I., (Ed.), Biochemical sites of insecticides action and resistance. Berlin. Springer. pp. 1-16.
- Ishaaya, I., Mendelson, Z., Ascher, K. R. S. and Casida, J. E. 1985. Mixtures of synthetic pyrethroids and organophosphorus compounds for controlling the whitefly, *Bemisia tabaci*, Phytoparasitica, 13: 76-77.
- Khan, H. A. A., Akram, W., Shad, S. A. and Lee, J. J. 2013. Insecticide mixtures could enhance the toxicity of insecticides in a resistant dairy population of *Musca domestica* L. PLOS ONE, 8(4): e60929.
- Koppenhofer, A. M. and Kaya, H. K. 1996. Additive and synergistic interaction between entomopathogenic nematodes and *Bacillus thuringiensis* for scarab grub control. Biological Control, 8: 131-137.
- Liguori, R., Cestari, P., Serrati, L. and Fusarini, L. 2008. Emamectina benzoate (AFFIRM®): innovative insetticida par la difesa contro I lepidopteri fitofagi. Atti Giornate Fitopatologiche, 23-28.
- López, J. D., Latheef, M. A. and Hoffman, W. C. 2010. Effect of emamectin benzoate on mortality, proboscis extension, gustation and reproduction of the corn earworm *Helicoverpa zea*. Journal of Insect Science, 10(89). Available online: insectscience.org/10.89.
- Mahmoud, M. M., Soliman, A. S. H. Abdel-Moniem, B. and Abdel-Raheem, M. A. 2013. Impact of some insecticides and their mixtures on the population of tomato borers, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in tomato crop at Upper Egypt. Archives of Phytopathology and Plant Protection. <http://dx.doi.org/10.1080/03235408.2013.857226>
- Mahmoud, M. M., Soliman, A. S. H., Abdel-Moniem, B. and Abdel-Raheem, M. A. 2014. Effect of certain low toxicity insecticides against tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) with reference to their residues in harvested tomato fruits. International Journal of Agricultural Research, 9(4): 210-218.
- Manal, A. R. and Abdel-Mageed, A. R. 2018. Toxicity of traditional, novel and bio-insecticides and their mixtures against house fly *Musca domestica* in relation to some biochemical activities. Research Journal of Environmental Toxicology, 12 (1): 1-10.
- Martin, T., Ochou, O. G., Vaissayre, M. and Fournier, D., 2003. Organophosphorus insecticides synergise pyrethroids in the resistant strain of cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) from West Africa. Journal of Economic Entomology, 92: 468-474.
- Metayi, M. H. A., Ibrahiem, M. A. M. and El-Deeb, D. A. 2015. Toxicity and Some Biological Effects of Emamectin Benzoate, Novaluron and Diflubenzuron against Cotton Leafworm. Alexandria Science Exchange Journal, 36(4): 350-357.
- Nath, B. S., Suresh, A., Varma, B. M. and Kumar, R. P. S. 1997. *Bombyx mori* (Lepidoptera: Bombycidae) in response to organophosphorus insecticides toxicity. Ecotoxicology and Environmental Safety, 36(2): 169-173.
- Nozad-Bonab, Z., Hejazi, M. J., Iranipour, Sh. and Arzanlou, M. 2017. Lethal and sublethal effects of some chemical and biological insecticides on *Tuta absoluta* (Lepidoptera: Gelechiidae) eggs and neonates. Journal of Economic Entomology, 1-7.
- Reyes, M., Rocha, K., Alarcón, L., Siegwart, M. and Sauphanor, B. 2012. Metabolic mechanisms involved in the resistance of field populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) to spinosad.

- Pesticide Biochemistry and Physiology, 102: 45-50.
- Roditakis, E., Skarmoutsou, C. and Staurakaki, M. 2013. Toxicity of insecticides to populations of tomato borer *Tuta absoluta* (Meyrick) from Greece. *Pest Management Science*, 69: 834-840.
- Saddiq, B., Ejaz, M., Shad, S. A. and Aslam, M. 2017. Assessing the combined toxicity of conventional and newer insecticides on the cotton mealybug *Phenacoccus solenopsis*. *Ecotoxicology*. 26: 1240-1249.
- Siqueira, H. A. A., Guedes, R. N. C. and Picanco, M. C. 2000. Cartap resistance and synergism in populations of *Tuta absoluta* (Lep., Gelechiidae). *Journal of Applied Entomology*, 124: 233-238.
- Siqueira, H. A. A., Guedes, R. N. C., Fragoso, D. B. and Magalhaes, L. C. 2001. Abamectin resistance and synergism in Brazilian populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *International Journal of Pest Management*, 47: 247-251.
- Sun, Y. P. 1950. Toxicity index-An improved method of comparing the relative toxicity of insecticides. *Journal of Economic Entomology*, 43: 45-53.
- Temerak, S. A. 2011. The status of *Tuta absoluta* in Egypt. EPPO/IOPC/FAO/NEPP Joint, International Symposium on management of *Tuta absoluta* (tomato borer) Conference, Agadri, Morocco.
- Torres, J. B., Faria, C., Evangelista, W. S. J. and Pratisoli, D. 2001. Within-plant distribution of the leaf miner *Tuta absoluta* (Meyrick) immatures in processing tomatoes, with notes on plant phenology. *International Journal of Pest Management*, 47(3): 173-178.
- van Asperen, K. 1962. Study of housefly esterases by means of sensitive colorimetric method. *Journal of Insect Physiology*, 8: 401-416.
- Vanhaelen, N., Haubruge, E., Lognay, G. and Francis, F. 2001. Housefly glutathione S-transferase and effect of Brassicaceae secondary metabolites. *Pesticide Biochemistry and Physiology*, 71: 170-177.
- Wafaa, A. AL-Kh. 2011. Field efficacy of some neonicotinoid Insecticides on whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) and its natural enemies in cucumber and tomato plants in Al-qassim region, KSA. *Journal of Entomology*, 8 (5): 429-439.
- Wilde, J., Fraczek, R. J., Siudaa, M., Bak, B., Hatjina, F. and Miszczak, A. 2016. The influence of sublethal doses of imidacloprid on protein content and proteolytic activity in honey bees (*Apis mellifera* L.). *Journal of Apicultural Research*, 55(2): 212-220.
- Wolfenbarger, D. A. and Cantu, E. 1975. Enhanced toxicity of carbaryl when combined with synergists against larvae of the bollworm, *Heliothis zea* and the tobacco budworm, *Heliothis virescens*. *Florida Entomologist*, 58: 103-104.
- Yankova, V. and Ganeva, D. 2013. Possibilities for control of tomato leaf miner *Tuta absoluta* (Meyrick) by application of insecticides in tomato greenhouse growing. *Bulgarian Journal of Agricultural Science*, 19(4): 728-731.
- Zhu, Y. C., Yao, J., Adamczyk, J. and Luttrell, R. 2017. Synergistic toxicity and physiological impact of imidacloprid alone and binary mixtures with seven representative pesticides on honey bee (*Apis mellifera*). *PLOS ONE*, 12(5): e0176837.
- Zibae, I., Bandani, A. R. and Sabahi, G. H. 2016. The expression profile of detoxifying enzyme of tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) to chlorpyrifos. *Arthropods*, 5(2): 77-86.

تأثیر اختلاط امامکتین بنزوات با برخی حشره‌کش‌ها روی مرگ‌ومیر و فعالیت آنزیم استراز لاروهای سن چهارم مینوز برگ گوجه‌فرنگی، (*Tuta absoluta* (Lepidoptera: Gelechiidae))

محسن طلعه^۱، هوشنگ رفیعی دستجردی^{۱*}، بهرام ناصری^۱، عزیز شیخی گرجان^۲ و خلیل طالبی جهرمی^۳

۱- گروه گیاه‌پزشکی، دانشکده کشاورزی و منابع طبیعی، دانشگاه محقق اردبیلی، اردبیل، ایران.

۲- مؤسسه تحقیقات گیاه‌پزشکی کشور، تهران، ایران.

۳- گروه گیاه‌پزشکی، دانشکده کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران.

پست الکترونیکی نویسنده مسئول مکاتبه: Hooshangrafiee@gmail.com

دریافت: ۲۵ آذر ۱۳۹۸؛ پذیرش: ۸ تیر ۱۳۹۹

چکیده: مینوز برگ گوجه‌فرنگی (*Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae)) یکی از مضرترین آفات گوجه‌فرنگی در جهان و ایران به‌شمار می‌رود. در این بررسی، سمیت حشره‌کش‌های استامی‌پرید، افوریا (تیامتوکسام + لامبدا سای‌هالوترین) و هگزافلومورون به‌تنهایی و در ترکیب با امامکتین بنزوات علیه لاروهای سن چهارم این آفت در شرایط آزمایشگاهی (دمای 25 ± 2 درجه سلسیوس، رطوبت نسبی 5 ± 65 درصد و دوره نوری ۸:۱۶ ساعت روشنایی: تاریکی) تعیین شد. علاوه بر این، اثر اختلاط حشره‌کش‌های مورد بررسی با امامکتین بنزوات با نسبت $LC_{15} : LC_{15}$ روی فعالیت استرازهای عمومی و محتوای پروتئین کل لاروهای سن چهارم مورد بررسی قرار گرفت. بالاترین میزان سمیت پس از ۷۲ ساعت برای امامکتین بنزوات ($LC_{50} = 0/48$ میلی‌گرم ماده مؤثر بر لیتر) به‌دست آمد، بعد از آن استامی‌پرید ($LC_{50} = 46/94$ میلی‌گرم ماده مؤثر بر لیتر)، افوریا ($LC_{50} = 156/24$ میلی‌گرم ماده مؤثر بر لیتر) و هگزافلومورون ($LC_{50} = 670/32$ میلی‌گرم ماده مؤثر بر لیتر) قرار داشتند. اختلاط امامکتین بنزوات با استامی‌پرید با نسبت $LC_{50} : LC_{25}$ و $LC_{50} : LC_{25}$ منجر به اثر سینرژیستی شد. اختلاط امامکتین بنزوات با هگزافلومورون و افوریا در همه نسبت‌ها به ترتیب اثر آنتاگونیستی و تجمیعی نشان داد. اختلاط امامکتین بنزوات با استامی‌پرید و افوریا باعث افزایش فعالیت استراز شد، اما بین اختلاط امامکتین بنزوات با هگزافلومورون و تیمار امامکتین بنزوات تفاوت معنی‌داری وجود نداشت. اختلاط امامکتین بنزوات با حشره‌کش‌های مورد بررسی محتوای پروتئین کل لاروهای سن چهارم را به‌طور معنی‌داری کاهش داد. براساس نتایج این تحقیق، اختلاط افوریا و استامی‌پرید با امامکتین بنزوات در مقایسه با تیمار امامکتین بنزوات و شاهد، روی لاروهای سن چهارم مینوز گوجه‌فرنگی سمیت بالایی داشت.

واژگان کلیدی: اختلاط، امامکتین بنزوات، استامی‌پرید، افوریا، مینوز گوجه‌فرنگی