International Journal of Advanced Biological and Biomedical Research
Available online at http:www.ijabbr.com
Volume 8, Issue 2 (2020) pp. 180-192 DOI: 10.33945/SAMI/IJABBR.2020.2.8

Original Article

Effects of Emamectin Benzoate Combined with Acetamiprid, Eforia and Hexaflumuron Against *Tuta Absoluta* (Lep.: Gelechiidae)

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Received: 2 September 2019, Revised: 05 November 2019, Accepted: 10 November 2019

ABSTRACT

Tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is one of the most destructive pests of tomato in many parts of the world including Iran. The toxicity of emamectin benzoate alone and combined with acetamiprid, eforia (thiamethoxam +lambda-cyhalotrin) and hexaflumuron were evaluated against 2nd instar larvae of *Tuta absoluta* (Meyrick) under 25 ± 1 °C, the relative humidity of 75 ± 5% and a photoperiod of 16:8 (L:D). Also, the mixtures of tested insecticides with emamectin benzoate at LC_{15}:LC_{15} ratio were evaluated on the activity of general esterase enzyme and total protein content of 2nd instar larvae. After 96 hours, emamectin benzoate had the highest toxicity (LC_{50} = 0.52 mg A.I./L), followed by acetamiprid (LC_{50} = 56.39 mg A.I./L) and eforia (LC_{50} = 312.01 mg A.I./L). Hexaflumuron showed no toxicity against larvae. The combination of emamectin benzoate with acetamiprid at LC_{50}:LC_{50} ratioproduced synergistic effects and all of the other ratios showed additive effects. The emamectin benzoate combined with either eforia or hexaflumuron at all of the ratios produced additive and antagonistic effects, respectively. Esterase activity of larvae increased when emamectin benzoate was mixed with either acetamiprid or eforia, but no significant differences were seen between emamectin benzoate alone and its mixture with hexaflumuron. The combination of emamectin benzoate with tested insecticides significantly reduced the total protein content of larvae. According to the results of this study, the mixtures of acetamiprid and eforia with emamectin benzoate showed higher negative impacts against 2nd instar larvae than emamectin benzoate alone.

Key words: Mixture, Esterase, Total protein, Emamectin benzoate, *Tuta absoluta*

Introduction

Tuta absoluta Meyrick (Lepidoptera: Gelechiidae) is a native micro lepidopteran pest of South America which can affect all aerial parts of the host plants (leaves, flowers, stems and fruit). 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 is difficult due to its hidden behavior inside the leaf, high reproductive capacity, polyvoltine nature and poor spraying technology, so it is necessary to apply various insecticides several times in a season to control the pest (Braham and Hajji, 2011). 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, Cartap and permethrin (Siqueira *et al*., 2000) and spinosad (Campos *et al*., 2014) are related to the extreme application of these insecticides by farmers.

Using the mixtures of pesticides is one of the effective ways to postpone the development of insecticide resistance or to struggle current resistance in a pest species. This method is generally used, in the field, to increase the spectrum of control when multiple pests are aggressive simultaneously or against a single pest (Ishaaya *et al*., 1985). The combination of insecticides with various modes of action could either be synergistic, additive or antagonistic 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), esterase, mixed function oxidase (MFO) as well as total protein content. General esterases and GSTs have an important role in detoxifying synthetic and non-synthetic insecticides (Vanhaelen *et al*., 2001). Research demonstrated that the combination of two different insecticides might result in inhibition of the detoxification enzymes in many pests and hence can lead to effective control of them (Martin *et al*., 2003). Several researchers reported that OPscombined with pyrethroids had synergistic effect against several pests (All *et al*., 1977; Asher *et al*., 1986). For example, combination of chlorpyrifos with either emamectin benzoate, indoxacarbor spinosad showed a synergism against *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) (Saddiq *et al*., 2017). The mixtures of emamectin benzoate with deltamethrin significantly increased toxicity against *Musca domestica* L. (Diptera: Muscidae) Also, emamectin benzoate mixed with bifenthrin showed an antagonistic effect against this housefly (Khan *et al*., 2013). Findings of (Ghoneim *et al*., 2012) indicated that chlorpyrifos produced high synergism when mixed with hexaflumuron and triflumuron and produced an additive effect when mixed with chlorfluazuron, chromafenozide and tobufenozite on *Spodopteralittoralis* (Boisd.) (Lepidoptera: Noctuidae).

Emamectin benzoate is a new insecticide isolated from the avermectin family of natural products. These products have been used for the control of Lepidoptera pests on vegetable crops worldwide, with a special efficacy against *T. absoluta* (Gacemi and Guenaoui, 2012)

The present study was done to find out the toxicity of mixtures of emamectin benzoate with either acetamiprid, eforia or hexaflumuron against *T. absoluta* larvae as well as their effects on the biochemical traits 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 extreme use of them.

Experimental

Insects

The specimens of *T. absoluta* were collected from tomato farms located in Moghan region, Ardabil, Iran. The insects were maintained in the growth chamber on the commercial tomato (*Solanum lycopersicon* L. var. Super Strain B) under controlled conditions at 25±1 C°, the relative humidity of 75 ± 5% and a photoperiod of 16:8 (L:D).

Insecticides

Commercial formulations of the following insecticides were used: emamectin benzoate (Proclaim® 5% SG; Syngenta, Switzerland), acetamiprid (Mospilan 20% WP; Ariashimi, Iran), eforia (thiamethoxam+lambda-cyhalotrin) (Eforia 240% OD; Syngenta, Switzerland), hexaflumuron (Consalt 10% EC; Ariashimi, Iran)

Bioassays

The toxicity of tested insecticides was assessed against 2nd instar larvae of *T. absoluta* using the tomato leaves. The tomato leaves were dipped into five different concentrations of each insecticide for 15 s and left to be dried for 1 h under room temperature. Twenty 2nd instar larvae of *T. absoluta* were transferred on treated leaves located in a 10 cm diameter Petri dish. Tween-80 was added to the concentrations as a surfactant at a concentration of 0.05% (v/v). Leaves dipped in distilled water+Tween-80 as control. The mortality of larvae was recorded at time intervals of 24, 48, 72 and 96 h after treatments. The experiments were done in triplicates for each insecticide concentration, and each bioassay was repeated three times (Galdino *et al*., 2011).

Combinations

Newly molted 2nd instar of *T.absoluta* was exposed to binary mixtures of emamectin benzoate with acetamipridor eforia at LC_{10}, LC_{15}, LC_{25} and LC_{50}, and hexaflumuron at 100 mg/L^{-1} using the leaf-dip method. Twenty larvae were released in each Petri dish. Leaves dipped in distilled water were used as control. Mortality was scored at time intervals of 24, 48, 72 and 96 h after treatments. The experiments were conducted in triplicates for each insecticide concentration, and each bioassay was repeated three times (Galdino *et al*., 2011; Abbas, 2015).

Determination of general esterase activity

General esterase activity of 2nd instar larvae treated with emamectin benzoate combined with either acetamiprid or eforia at LC_{15}:LC_{15} ratio, and hexaflumuron at LC_{15}:100 mg L^{-1} ratio, was assayed according to the method of van Asperen (1962). *α*-Naphthyl acetate (*α*-NA) and *β*-naphthyl acetate (*β*-NA) were used as substrates for *α*-esterase and *β*-esterase activity, respectively. Fifteen 2nd instar larvae were homogenized in 70 µl of 40 mM phosphate buffer (pH 7.0) on ice. They were then centrifuged at 10,000 g at 4 °C for 20 min. The quantity of 90 µl of the substrate was added to the microplate, thenpreincubated at 30 °C for 10 min. The amount of 90 µl of Fast Blue RR salt was added to the microplate, then the naphthol production

was monitored by measuring absorbance at 450 and 540 nm for *α*-NA and *β*-NA, respectively, using microplate reader (Anthos 2020, Austria). All experiments were replicated three times.

Determination of total protein content

The effect of mixtures of emamectin benzoate with either acetamiprid or eforia at LC_{15}:LC_{15} ratio and hexaflumuron at LC_{15}:100 mg L^{-1} ratio, on the total protein content of 2nd instar larvae was determined according to Bradford (1976), using bovine serum albumin (Bio-Rad) (Sigma) as a standard protein. The measurement was performed using a microplate reader at 595 nm. All experiments were replicated three times.

Data analysis

The data were analyzed using PROBIT in order to determine lethal concentrations (LC_{30}, LC_{50}, and LC_{90}). The mean mortality of 2nd instar larvae treated with different concentrations of each insecticide, after checking for normality, 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 the combination of emamectin benzoate with different insecticides was accounted through formula $M_E=M_B+M_A$ $(1-M_B)$, M_B signifying observed mortality caused by different insecticides; M_A signifying observed mortality caused by emamectin benzoate. Results from a chi-square test, $\chi^2=(M_{AB}- M_E)^2/M_E$, where M_{AB} is the observed mortality for the combination of emamectin benzoate with different insecticides, were compared to the chi-square table value. If the calculated chi-square was overstepped the table value (df=1), it would be a nonadditive effect. The difference $M_{AB}- M_E>0$ indicated synergism; and the difference $M_{AB}- M_E<0$ indicated antagonism (Koppenhofer and Kaya, 1996).

General esterase activity and total protein content data were subjected to ANOVA. Means were compared by LSD test and significant differences were recorded with SPSS software ver. 16 at P=0.05.

Results and discussion

LC values

Toxicity values for all insecticide treatments on 2nd instar larvae of *T. absoluta* are shown in Table 1. The data demonstrated that emamectin benzoate showed the highest toxicity (LC_{50}=0.52 mg A.I./L), followed by acetamiprid (LC_{50}=56.39 mg A.I./L) and eforia (LC_{50}=312.01 mg A.I./L). Hexaflumuron had no toxicity against *T. absoluta*.

Table 1. Toxicity of three tested insecticides against 2nd instar larvae of *Tuta absoluta* under laboratory conditions

Insecticide	LC_{10} (mg AI /L)	LC_{15} (mg AI /L)	LC_{25} (mg AI /L)	LC_{50} (mg AI /L)	Slope ±SE	χ^2	Toxicity* index at LC_{50}
Emamectin benzoate	0.14	0.18	0.26	0.52	2.3±.27	3.63	100
Acetamiprid	18.07	22.47	30.98	56.39	2.5±.28	2.08	0.92
Eforia	49.79	70.74	118.78	312.01	1.6±.19	1.88	0.16

* LC_{50} of the efficient compound/LC_{50} of the other compound × 100 (Sun, 1950)

Combinations

Binary mixtures of emamectin benzoate with acetamiprid, eforia 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}, LC_{15}:LC_{15} and LC_{25}:LC_{25} ratios showed additive effects. The emamectin benzoate mixed with acetamiprid at 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 combination of hexaflumuron with emamectin benzoate showed an antagonism interaction.

Table 2. Combination of emamectin benzoate with several insecticides on 2nd instar larvae of *Tuta absoluta* under laboratory conditions

Combination	Ratio	Observed mortality (%)	ME (%)[a]	X^2	P	N[b]	Interaction[c]
Emamectin benzoate+Acetamiprid	LC_{10}+ LC_{10}	27	20.8	1.84	0.05	180	additive
Emamectin benzoate+Eforia	LC_{10}+ LC_{10}	25	22.56	0.26	0.05	180	additive
Emamectin benzoate+Hexaflumuron	LC_{10}+100 mg l^{-1}	8	19.04	6.4	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}+100 mg l^{-1}	10	26.05	9.88	0.05	180	antagonistic
Emamectin benzoate+Acetamiprid	LC_{25}+ LC_{25}	58	45.25	3.59	0.05	180	additive
Emamectin benzoate+Eforia	LC_{25}+ LC_{25}	53	46.71	0.84	0.05	180	additive
Emamectin benzoate+Hexaflumuron	LC_{25}+100 mg l^{-1}	12	32.84	13.22	0.05	180	antagonistic
Emamectin benzoate+Acetamiprid	LC_{50}+ LC_{50}	95	75.56	5	0.05	180	synergistic
Emamectin benzoate+Eforia	LC_{50}+ LC_{50}	78	76.6	0.02	0.05	180	additive
Emamectin benzoate+Hexaflumuron	LC_{50}+100 mg l^{-1}	10	52.16	34.07	0.05	180	antagonistic

[a] The expected mortality of combination

[b] Total number of insects exposed

[c] 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 esterase activity

The data obtained for α-eterase assay ($F=24.207$; $df_{t,e}=4, 10$; $P<0.05$) showed the highest activity when emamectin benzoate mixed with either acetamiprid (0.496 µmol/min/mg protein) or eforia (0.442 µmol/min/mg protein) at LC_{15}:LC_{15} ratio as compared with emamectin benzoate alone (0.234 µmol/min/mg protein) or control (0.19 µmol/min/mg protein). The activities obtained for *β*-

esterase (F=0.87; $df_{t,e}$=4, 10; P>0.05) showed no significant differences among the combinations, emamectin benzoate alone and control (Figure 1).

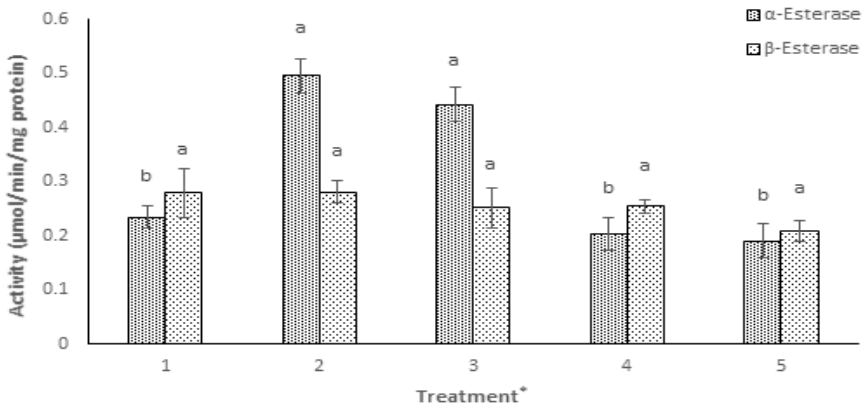

Figure 1. The effects of insecticides mixture on α- and β- esterase activity (mean ± SE) in 2nd instar larvae of *Tuta absoluta*
Different letters indicate that the specifc activities of the enzymes are signifcantly different from each other by LSD test (P<0.05)
* Emamectin benzoate alone (1), mixtures of emamectin benzoate with: acetamiprid (2), eforia (3), hexaflumuron (4) and control (5)

Total protein content

The data in Figure 2 shows that protein content in larvae treated with emamectin benzoate mixed with either acetamiprid (0.845 µg/mg) or eforia (0.744 µg/mg) at LC_{15}:LC_{15} ratio and hexaflumuron (0.925 µg/mg) at LC_{15}:100 mg L^{-1} ratio significantly reduced as compared with emamectin benzoate alone (1.66 µg/mg) or control (1.732 µg/mg) (F=19.607; $df_{t,e}$=4, 10; P<0.05).

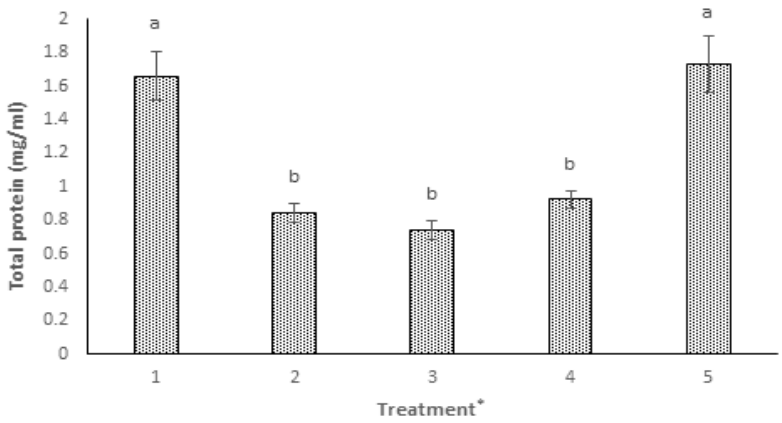

Figure 2. The effects of interaction of emamectin benzoate with other insecticides on the total protein content (mean ± SE) of 2nd instar larvae of *Tuta absoluta*
Different letters indicate that the protein contents are signifcantly different from each other by LSD test (P<0.05)
* Emamectin benzoate alone (1), mixtures of emamectin benzoate with: acetamiprid (2), eforia (3), hexaflumuron (4) and control (5)

Discussion

Based on the results of this study, the emamectin benzoate showed the best efficacy against 2nd instar larvae of *T. absoluta*. Many researchers 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 by emamectin benzoate.

Acetamiprid was the second most effective insecticide against *T. absoluta*, in this study, similar to (Nozad *et al*., 2017) findings. However, (Yankova and Ganeva, 2013) reported that acetamiprid had inadequate effectiveness towards *T. absoluta*. (AL-Kherb, 2011) noted that acetamiprid could be considered promising candidates in controlling *Bemicia 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 cabbage.

Combination of emamectin benzoate with acetamiprid showed synergistic effects against 2nd instar larvae of *T. absoluta*. (Mahmoud *et al*., 2013) noted that emamectin benzoate combined with either imidacloprid, indoxacarb, profenofos, chlorfenapyr or methomyl achieved a considerable reduction population in third instar larvae of *T. absoluta*. (Mahmoud *et al*., 2014) demonstrated that chlorantraniliprole insecticide was the most toxic against *T. absoluta* followed by 20% emamectinbenzoate+60% bifenithrin and lambda-cyhalothrin. In our study, the combination of hexaflumuron with emamectin benzoate showed antagonistic interactions. It can be suggested that emamectin benzoate caused larvae to face the cessation of feeding, so hexaflumuron could not enter larvae body through the digestive tract resulting in antagonistic interaction. (Metayi *et al*., 2015) presented that all mixtures of emamectin benzoate (at LC_{10} or LC_{25}) with novaluron or diflubenzuron (at LC_{10} or LC_{25}) resulted in antagonistic effects.

The emamectin benzoate can affect the nervous system of arthropods by increasing chloride ion flux at the neuromuscular junction, resulting in cessation of feeding and irreversible paralysis (Ishaaya, 2001). By inhibiting the nicotinic acetylcholine receptor (nAChR), neonicotinoids such as acetamiprid disrupt the insect's nervous system by prolonged activation of the receptors as they are not hydrolyzed by acetylcholinesterase, leading to the death of the insects (Belzunces *et al*., 2012). Accordingly, the mixture of these insecticides with different modes of action supplements the action of anotherone for *T. absoluta* control. 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 the metabolic detoxification of it. Moreover, in our study, when the combination 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, so enhanced the toxicity of this insecticide. Formerly, it has been supposed that OPs in combination 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 2nd instar larvae, support the observed toxicity ratios of the combinations. 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*. The biochemical analysis of carboxylesterase and GST by (Badawy *et al*., 2014) showed that these enzymes detoxified the low doses of acetamiprid and pymetrozine in *Apis mellifera* L. (Hymenoptera:Apidae). According to (Abdel-Mageed and Shalaby, 2011), thiamethoxam + lambda-cyhalothrin (Engeo^{R}) caused a significant increase in acetylcholinesterase activity of *S. littoralis*. The role of esterases in resistance of Brazilian populations of *T. absoluta* against abamectin is reported by (Siqueira *et al*., 2001). (Zibaee *et al*., 2016) showed that the exposure of 4th instar larvae of *T. absoluta* to chlorpyrifos increased the esterases activity after 24 and 48 h. Esterase and MFO are two enzymes responsible for reducing toxicity of spinosad in *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*. This might be due to the dissociation of protein into amino acids, that with the entry of these amino acids to TCA cycle, they will aid to store energy for the insect (Nath *et al*., 1997). Furthermore, (Nath *et al*., 1997) noted that sublethal concentrations of fenitrothion and ethion caused depletion in total protein content of 5th instar *Bombyx mori* (L.) (Lepidoptera: Bombycidae) larvae that was followed by an addition 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 decreased with emamectin, hexaflumuron and teflubenzuron. (Wilde *et al*., 2016) reported that the level of protein in tissue extracts of *A. mellifera* 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, lambada-cyhalothrin+khaya extract, indoxacarb+pomegranate, chlorantraniliprole+jojoba oil and methomyl+jojoba oil 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). Pesticides mixtures with different modes of action might postpone the resistance development of pest population (Bielza *et al*., 2009). Therefore, it can manage pesticide 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 reduced. Therefore, it can be used as a component of integrated pest management programs in case of synergistic and additive interactions. In this study, the mixtures of emamectin benzoatewith acetamiprid and eforia are good options for managing *T. absoluta*.

Conclusions

Generally, the mixtures of acetamiprid and eforia with emamectin benzoate showed higher negative impacts against 2nd instar larvae than emamectin benzoate alone. Therefore, it can manage insecticide resistance and reduce damage of *T. absoluta*.

Acknowledgements

This work was fnancially supported by the University of Mohaghegh Ardabili (Ardabil, Iran), which is appreciated.

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How to cite this article: Mohsen Taleh, Hooshang Rafiee Dastjerdi, Bahram Naseri, Aziz Sheikhi Garjan, Khalil Talebi Jahromi, Effects of Emamectin Benzoate Combined with Acetamiprid, Eforia and Hexaflumuron Against *Tuta Absoluta* (Lep.: Gelechiidae). *International Journal of Advanced Biological and Biomedical Research*, 2020, 8(2), 180-192. Link: http://www.ijabbr.com/article_37168.html