

Efficacy of *Bacillus thuringiensis* Compared with Some Chemical Insecticides in Controlling Tomato Leafminer, *Tuta absoluta* (Meyrick)

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ABSTRACT

Tuta absoluta (Meyrick) (Lep.: Gelechiidae) is a major pest of tomato crops that causes high yield losses. Currently, in most countries, *T. absoluta* management is mainly based on chemical control. Nonetheless, special emphasis is being placed on implementing environmentally safe strategies. Commercial formulations based on *Bacillus thuringiensis* may be a good alternative, as they have been used to control other insect pests successfully. The laboratory and greenhouse data presented in this work are evidence that *B. thuringiensis* is highly efficient in controlling *T. absoluta*. Moreover, the toxicity effect of three pesticides (Biolep, Abamectin, and Indoxacarb) was evaluated against the first and last instar larvae of *T. absoluta*. Results indicate that the Bt formulation has the highest efficacy compared to the chemical pesticides. Abamectin and Indoxacarb had low mortality effect on the first and last instars of *T. absoluta*. In conclusion, the Bt formulations are recommended as an effective, environmentally friendly, and safe biopesticide for controlling tomato leafminer.

Keywords: Biopesticide, Environmentally friendly pesticide, Integrated Pest Management.

INTRODUCTION

Tomato, *Lycopersicon esculentum* Mill, is one of the most widely grown and consumed crop worldwide (Ramkumar *et al.*, 2020). It is grown with high levels of production in the European Union (EU). Italy and Spain combined produce nearly two-thirds (62.9%) of the EU total production (Eurostat, 2019). Yield losses in tomato cultivations are caused by several insect pests, especially the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in the Mediterranean Basin countries (EPPO, 2008; EPPO, 2009a; EPPO, 2009b; Desneux *et al.*, 2010; Abd El-Ghany *et al.*, 2018). In addition to boundary small markets between Iran, Iraq, and Turkey, the ferry services of passengers and goods across the Persian Gulf present a potential bridge for this pest

to cross from Arabian countries to the southern ports of Iran. In Iran, this destructive pest could damage 165 thousand ha of tomato farming and also pose a threat to the new biocontrol program in tomato greenhouses (Farrokhi *et al.*, 2011). Tomato leafminer attack tomato plants and cause losses that vary from 40 to 100%. At the beginning of the attack, small mines are observed, which increase in size until almost all the leaflets are taken (Giustolin *et al.*, 2001).

Infestation of tomato plants occurs throughout the entire crop cycle. Feeding damage is caused by all larval instars and throughout the whole plant. The larval instars of tomato leafminer have chewing mouthparts with various sensory structures on their olfactory and gustatory organs that provide essential role in the larval feeding

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behavior (Abd El-Ghany and Faucheux, 2021). The larvae feed on the mesophyll tissue of the leaves, forming irregular leaf mines that may later become necrotic (Younes *et al.*, 2018). They attack leaves and flowers, mine stalks, apical buds, and green or ripe fruits, causing quality and yield losses of up to 100%, if no control methods are applied (Apablaza, 1998). Moreover, larvae can form extensive galleries in the stems, which affect the development of the plants. The indirect damage can often be manifested as a result of bacterial or fungal infections. Fruit is attacked by the larvae, and the entry-ways are used by secondary pathogens, leading to fruit rot. The extent of the infestation is partly dependent on the tomato variety, which have different susceptibility levels depending on the variety (Abdel-Razek *et al.*, 2021). Potential yield loss in tomatoes (quantity and quality) is significant and can reach up to 100% if the insect pest infestations are not managed. If the food and climatic conditions are favorable, the larvae feed almost continuously, and generally do not enter diapause leading to 10–12 generations per year (Tropea-Garzia *et al.*, 2012).

Owing to the increasing environmental hazards and developing resistance towards excessive use of chemical insecticides, a newer safe biopesticide became a demand (Valizadeh *et al.*, 2020, 2021). Biological control strategies including semiochemicals, plant extracts, and microbial control agents are becoming alternative sources that are increasingly important in agriculture as components of integrated pest management and resistance management strategies (Medeiros, 2006; Abd El-Ghany *et al.*, 2016; Abdel-Razek *et al.*, 2017; Abd El-Ghany, 2019).

The use of biopesticides is an effective way of coping with insect pests (Valizadeh *et al.*, 2013a; 2021; Oftadeh *et al.*, 2014). Of the total production of biopesticides, entomopathogenic bacteria, *Bacillus thuringiensis* (Bt) occupies the first place (90%) of the total biopesticides market. Bt has been commercially used in the

biological control of insect pests for the last 4 decades. Bt strains can produce toxic compounds of numerous chemical structures and properties. Selectivity of Bt δ -endotoxins against the larvae of target insects was documented (Merdan *et al.*, 2010, Salama *et al.*, 2015, Jalapathi *et al.*, 2020). The potential activity of *B. thuringiensis* isolates against some economically important lepidopteran insect pests has been studied under laboratory, greenhouse, and open-field conditions (Ramirez *et al.*, 2005; Salama *et al.*, 2012, Abd El-Ghany *et al.*, 2015).

Keeping in sight the promise that these biological control agents show, we designed the present study to investigate the various factors affecting the performance of *B. thuringiensis* and to control the tomato leafminer moth, *T. absoluta*. The aim of this study was to evaluate the efficacy of *Bacillus thuringiensis* compared to some commercially available chemical insecticides to control *T. absoluta* under laboratory conditions.

MATERIALS AND METHODS

Tomato Varieties and Planting

Falcato cultivar of tomato was planted in pots with a diameter of 15 cm in the plant protection clinic of Sirjan, Kerman, Iran (3261086 North, 370151 East). Tomato plants were kept in laboratory conditions (25±2°C, 60-70% RH) that were used for rearing the insect pest, and their leaves were used in bioassay tests.

Insects Rearing

Since the first instar larvae of tomato leafminer are more sensitive than the other ages and the fourth instar larvae is a critical stage of life (the most damaging instar), the two larval instars were used to conduct the experiments, which were developed under laboratory conditions (25±2°C, 60-70% RH

and 16 hours photoperiod). To obtain the same larvae age for use in the experiments, tomato leaves containing osprey eggs were isolated and placed in a growth chamber at $25\pm 2^\circ\text{C}$, $60\pm 10\%$ RH with a photoperiod of 16:8 hour (L:D). The leaves were inspected daily and used for experiments as soon as the first instar larvae hatched. To obtain the fourth instar larvae, hatched larvae were reared until the emergence of the fourth instar larvae.

Initially, an adults of tomato leafminer from Pardis Greenhouse Complex in Sirjan, Kerman, Iran, (3261086 North, 370151 East) on tomato plants were collected. After identification, moths were released on tomato plants in cages with dimensions $30\times 100\times 100$ cm. After a few days, when the spots of leafminer larvae from entering into parenchyma tissue were visible, the larvae were collected from the cage and transferred to another cage on healthy plants. For the experiments, the leaves were transferred into special rearing containers (diameter of 4 cm and a height of 2.5 cm) containing a layer of 0.7% agar gel and covered with netting cover to exchange air. Then, the rearing containers were kept inside the growth chamber with temperature conditions of $25\pm 2^\circ\text{C}$, RH of $60\pm 10\%$, and a photoperiod of 16:8 hour (L:D). To more accurately control the environmental conditions inside the growth chamber, a hygrometer or hydrometer and a maximum-minimum thermometer were used.

Laboratory Bioassay

One Biological insecticide (Biolep) and two chemical pesticides were evaluated against *T. absoluta* larvae. Biolep contains the active ingredient spores and crystals of endotoxin *Bacillus thuringiensis* variety kurstaki (109 spore g^{-1}) Water soluble Powders (WP), which is used specifically for the control of Lepidopterous insect pests and was prepared by the Biorun Company, Iran. Chemical pesticides including Abamectin EC 1/8% and Indoxacarb SC

15% (Ariashimi Company) were used in bioassay tests. Tests at the recommended concentration of each compound (Biolep 5,000 ppm (mg L^{-1}), Abamectin 500 ppm, and Indoxacarb 50 ppm), half of the recommended concentration, and twice the recommended concentration were evaluated against the first and fourth instar larvae of tomato leafminer (Table 1).

For each concentration, 10 replicates, and for each treatment 10 larvae per replicate were exposed to these pesticides. Then, tomato leaves were placed in a plastic container with a diameter of 4 cm and a height of 2.5 cm on agar gel 0.7 % and larvae were put on the leaves by a fine brush. One day after the larvae entered between the leaves, the concentrations of pesticides with sampler was calculated, measured, and sprayed on each container, while for the control, distilled water was used. After 48 and 72 hours, the larvae mortality percentages were determined by counting the number of dead larvae per container. Larvae were considered dead if they did not respond to brush stimulation when viewed under the binocular apparatus. Finally, the mortality rate was calculated and the percentage of casualties was determined.

All statistical analyses were done using software Statplus (version 4.9, 2007). The first step was to calculate the mean, then, find out the difference between the means, in this case, the differences between indicated, and secondly the significant differences between the values at 5% probability. To compare data obtained from studies, using ANOVA-One-Way ANOVA was performed using the Fisher LSD test.

RESULTS

Evaluation of Pesticides against 1st Instar Larvae of Tomato Leafminer after 48 Hours

The outcome of various concentrations of the Biolep, Abamectin, and Indoxacarb mortality rate on the first instar larvae of tomato leafminer after 2 days showed that the highest mortality rate was in the

**Table 1.** The abbreviation and concentration of the compounds used in the study.

Compounds	Abbreviation	Concentration
Indoxacarb	RR/2 IN	25 ppm
Abamectin	RR/2 A	250 ppm
Biolep	RR/2 Biolep	2500 ppm
Indoxacarb	RR IN	50 ppm
Abamectin	RR A	500 ppm
Biolep	RR Biolep	5000 ppm
Biolep	2RR Biolep	10000 ppm
Biolep and Indoxacarb	RR Bio + RR/2 IN	5000 ppm Biolep +25 ppm Indoxacarb
Biolep and Abamectin	RR Bio + RR/2 A	5000 ppm Biolep +250 ppm Abamectin
Biolep and Indoxacarb	RR/2 Bio + RR IN	2500 ppm Biolep +50 ppm Indoxacarb
Biolep and Abamectin	RR/2 Bio + RR A	2500 ppm Biolep +500 ppm Abamectin
Biolep and Indoxacarb	RR/2 Bio + RR/2 IN	2500 ppm Biolep +25 ppm Indoxacarb
Biolep and Abamectin	RR/2 Bio + RR/2 A	2500 ppm Biolep +250 ppm Abamectin
Biolep, Abamectin and Indoxacarb	RR Bio + RR/3 A + RR/3 IN	5000 ppm Biolep +166 ppm Abamectin + 16 ppm Indoxacarb
Biolep, Indoxacarb and Abamectin	RR/3 Bio + RR/3 IN + RR/3 A	1666 ppm Biolep +166 ppm Abamectin + 16 ppm Indoxacarb

treatment twice the recommended dose of the commercial formulation of Biolep (Biolep 10,000 ppm), an average of $99/99 \pm 0/001$. The least percentage of mortality rate was in the treatment using one-half the concentration of Abamectin (Abamectin 250 ppm), an average of $16.00 \pm 4/00$ (Figure 1) ($P=0$, $df=15, 16$, $F=219.5347$).

Evaluation of Pesticides against 1st Instar Larvae of Tomato Leafminer after 72 Hours

The mortality rate of the first instar larvae of tomato leafminer after 3 days was recorded. The highest mortality percentages (an average of $99/99 \pm 0/001$) were achieved in the treatment with Biolep 10,000 ppm, Biolep 5,000 ppm, Biolep 5,000 ppm+Abamectin 250 ppm, Biolep 5,000 ppm+Indoxacarb 25 ppm, Biolep 2500 ppm+Indoxacarb 50 ppm, Biolep 2500 ppm+Abamectin 500 ppm, Biolep 25,000 ppm+Abamectin 250 ppm, Biolep 2,500 ppm+Indoxacarb 25 ppm, Biolep 1,666 ppm+Indoxacarb 16 ppm+Abamectin 166 ppm, and Biolep 5,000 ppm+Indoxacarb 16 ppm+Abamectin 166 ppm. The lowest mortality percentages were achieved by Abamectin 250 ppm ($22.00 \pm 2/50$) and Indoxacarb 25 ppm ($22/00 \pm 2/90$) (Figure 2) ($P=0$, $df=15, 16$, $F=504.213$).

Evaluation of Pesticides against 4th Instar Larvae of Tomato Leafminer after 48 Hours

The results of Biolep, Abamectin, and Indoxacarb mortality percentage on the last instar larvae of tomato leafminer after 2 days were recorded. The highest mortality percentage ($97.00 \pm 1/52$) was obtained in Biolep 2,500 ppm+Abamectin 500 ppm, whereas the lowest mortality percentage was obtained in Indoxacarb 25 ppm ($8.00 \pm 2/4$) (Figure 3) ($P=0$, $df=15, 16$, $F=201.6285$).

Evaluation of Pesticides against 4th Instar Larvae of Tomato Leafminer after 72 Hours

The results of mortality percentage on the last instar larvae of tomato leafminer after 3 days indicates that Biolep was the most potent concentration that causes the highest mortality for the 4th instar larvae of *T. absoluta*, and the lowest mortality percentage ($19/00 \pm 5/46$) was obtained by Abamectin 250 ppm (Figure 4) ($P=0$, $df=15, 16$, $F=220.4041$).

DISCUSSION

The use of various bio-rational insecticides has shown to provide a promising strategy

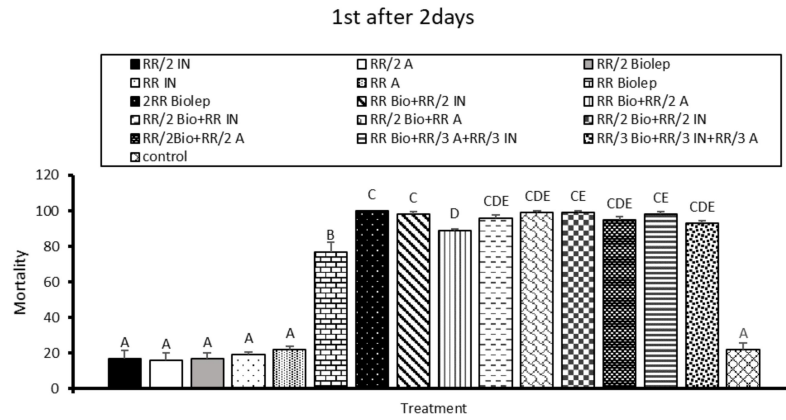


Figure 1. Mortality percentage of 1st instar larvae of tomato leafminer moth treated with tested insecticides after 48 hours.

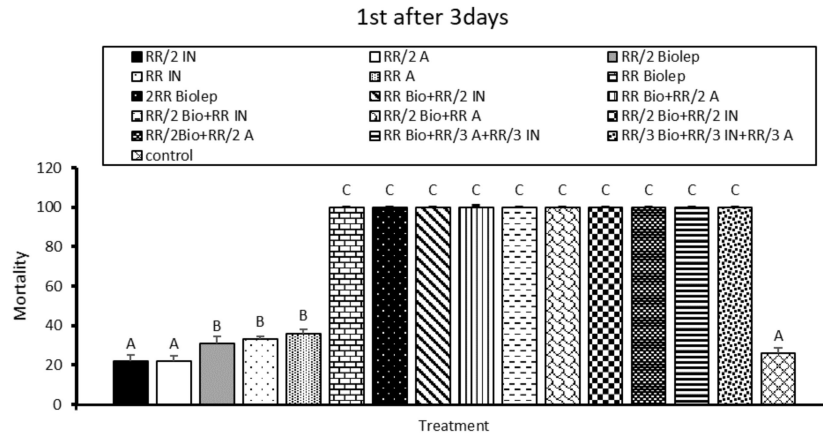


Figure 2. Mortality percentage of 1st instar larvae of tomato leafminer moth treated with tested insecticides after 72 hours.

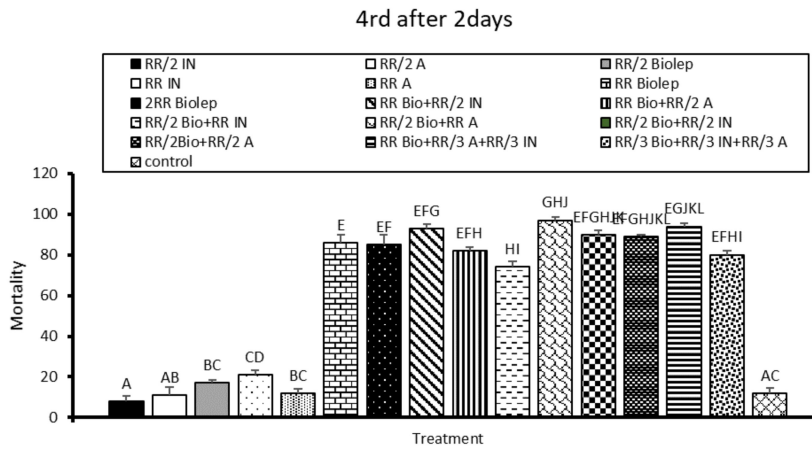


Figure 3. Mortality percentage of 4th instar larvae of tomato leafminer moth treated with tested insecticides after 48 hours.



4rd after 3days

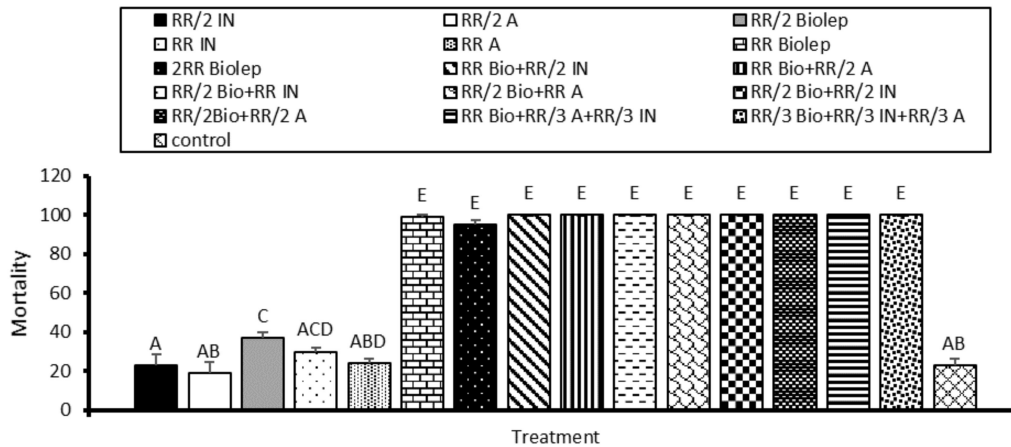


Figure 4. Mortality percentage of 4th instar larvae of tomato leafminer moth treated with tested insecticides after 72 hours.

for insect pests control; providing safe alternatives, being harmless to vertebrates and other non-target fauna and because of the specificity of most of the entomopathogens, which affect a small range of insect hosts (Abd El- Ghany *et al.*, 2018). Among various bio-rationale insecticides, Bt has been used for controlling different insect pests (Merdan *et al.*, 2010; Salama *et al.*, 2012; Abd El-Ghany *et al.*, 2015; Alsaedi *et al.*, 2017; Hosseinzadeh *et al.*, 2019). The present study aimed to compare the efficacy of bio-rationale insecticide (Bt) with chemical insecticides for controlling tomato leafminer, *T. absoluta*. The mortality percentage of tomato leafminer larvae (1st, 4th instars) were evaluated after 48 hours and 72 hours with different concentrations of the insecticides (Biolep, Abamectin, and Indoxacarb). The results indicate that different concentrations of Abamectin and Indoxacarb have low toxicity effect, and show no significant difference with the control against the tested larval instars. Indoxacarb and Bt are both included in the lists of protocols approved for *T. absoluta* control (SEWG, 2008 in Spain; IRAC, 2009 in Argentina; Mallia, 2009 in Malta; FREDON, 2009 in France;

USDA-APHIS, 2011). Reports from various authors present contradictory results regarding the effectiveness of Indoxacarb and Bt, while Indoxacarb had higher efficacy than Bt (Derbalah *et al.*, 2012). Similarly, Indoxacarb was more effective than Bt at reducing the infestation in tomato leaves/fruits (Nazarpour *et al.*, 2016). Although Indoxacarb has good efficacy, it is an insecticide; therefore, its application is recommended for high population densities (Shahini *et al.*, 2021). In contrast, the present study shows that the potency of Bt pesticide is higher than Indoxacarb.

The results obtained by Alsaedi *et al.* (2017) revealed high mortality for different larval instars (1st-4th) of *T. absoluta* after fed with leaves treated with *B. thuringiensis* suspensions. They recommended the suspension of *B. thuringiensis* var. Kurstaki as a potential safe tool for using in integrated management of *T. absoluta* in the agricultural ecosystems. Gonza'lez-Cabrera *et al.* (2011) confirmed that impact of the bacterial pesticides against tomato leafminer showed the greatest sensitivity against the 1st instar, however, the sensitivity of the second and third larval instars showed lower impact. The mortality

rate of the first instar larvae was 90 times that of the control. Similar findings were reported by Hosseinzadeh *et al.* (2019) who confirmed that the first larval instar of this pest was the most sensitive to all biological insecticides.

Based on the previous studies, it is evident that different larval instars of *T. absoluta* were susceptible to various Bt strains. Also, they indicated that the susceptibility of larvae decreased with the larval developmental stage (Roh *et al.*, 2007; Gonza'lez *et al.*, 2011).

On the other hand, Youssef and Hassan (2013) reported that the mortality rate of the first instar larvae of this pest was lower than the last instar larvae because Bt compounds are digestive and first instar larvae have less feeding, therefore, fewer toxins enter the body.

Chemical control has been the most important tool in suppressing *T. absoluta* since its dispersion in South America in the 1970s. However, reports of control failure have demonstrated the high resistance of this to multiple classes of insecticides (Siqueira *et al.*, 2000; Haddi *et al.*, 2012; Roditakis *et al.*, 2018; Peng Han *et al.*, 2018). Reports from Chile, Argentina, and particularly Brazil suggest that insecticide resistance evolves quickly in this species as an intrinsic response to insecticide use or overuse (Siqueira *et al.*, 2000; Lietti *et al.*, 2005; Silva *et al.*, 2011; Guedes and Picanço, 2012; Peng Han *et al.*, 2018).

Intensive use of synthetic insecticides for *T. absoluta* management coupled with insect biological traits, such as a high reproductive potential and multivoltine, endophytic larval feeding behavior and mining habit as well as polyphagy have increased *T. absoluta* selection pressure for insecticide resistance (Siqueira *et al.*, 2000; Brévault *et al.*, 2008). Synthetic insecticides are employed as the primary method of control against insect pest's infestation (Valizadeh *et al.*, 2013b, 2014; Tarusikirwa *et al.*, 2020). For example, Tunisia registered 18 new insecticides during 2009–2011 following the *T. absoluta* invasion,

although they all turned out fine active (Abbes *et al.*, 2012; Zekeya *et al.*, 2017; Tarusikirwa *et al.*, 2020). In South America and Europe, resistance has been reported against conventional insecticides such as Organophosphates (OPs), pyrethroids, cartap, diamides, and avermectins (Biondi *et al.*, 2018; Silva *et al.*, 2011; Tarusikirwa *et al.*, 2020). The main resistance mechanisms evolved through altered target-site sensitivity and/or enhanced detoxification, depending on the chemical class (Guedes *et al.*, 2019; Tarusikirwa *et al.*, 2020). In northern Nigeria, resistance was reported in cyhalothrin (a Type II pyrethroid), propoxur, and chlorpyrifos-methyl via enzyme mutation, underlying the challenges in managing this invasive pest using pesticides (Bala *et al.*, 2019). Given the prohibitive costs of synthetic pesticides for African farmers, the evolution of pesticide resistance will further compound losses on already resource-constrained farmers (Tarusikirwa *et al.*, 2020).

Environmental friendly and controlling strategies have been developed for controlling *T. absoluta*. This includes cultural control measures (crop rotation, selective removal, and destruction of infected plant material) (Korycinska and Moran, 2009), microbial control (Molla *et al.*, 2011; Abdel El-Ghany *et al.*, 2016 and 2018), using natural enemies (parasitoids, predators) (Desneux *et al.*, 2010; Chhetri, 2018), botanical extracts (Abd El-Ghany *et al.*, 2018), and production of resistant tomato cultivar varieties (Abdel-Razek *et al.*, 2021). The integration of these methods with each other and minimum use of less environmentally hazardous insecticides are important to control *T. absoluta* without disturbing the ecological and biological world.

However, repeated and intensive use of Bt formulations may lead to resistance in the case of diamondback moth, *Plutella xylostella* L. (Tabashnik and Carriere, 2017; Jalapathi *et al.*, 2020). The high reproductive rates of *T. absoluta*, along with previous reports on resistance



developed by other insect species to *B. thuringiensis*-based formulations indicate that it is highly desirable to alternate *B. thuringiensis* treatments with the use of other chemical pesticides (Gassmann *et al.*, 2009). This strategy may also include the alternative use of different *B. thuringiensis* formulations based on subspecies commonly bearing different toxin profiles (i.e. sub sp. kurstaki or aizawai) (Schnepf *et al.*, 1998). These formulations can be more than 95% effective when used at the proper concentration (González-Cabrera *et al.*, 2011). Several studies are published in this point using bacterial, fungus, and virus against *T. absoluta* (Desneux *et al.*, 2010; Abd El-Ghany *et al.*, 2018).

In this study, one-half concentration (1/2 RR) Biolep combined with the dose Recommended Rate (RR) Abamectin and one-half concentration (1/2 RR) Biolep combined with one-half concentration (1/2 RR) Abamectin showed the highest mortality rates against the tomato leafminer larvae. This agreed with Liu and Sengonca (2003) who reported that half of the Biolep combined with half the recommended concentration of Abamectin can be most effective with the least amount of toxin to control the larvae. Therefore, Biolep is a good combination that with the lowest has the greatest effect to control this insect pest (Balzan and Moonen, 2012). The obtained results are in harmony with some previous studies. For instance, in South America, especially Chile, Brazil, and Argentina, tomato leafminer resistance to various insecticides (organophosphates, Pyrethroids, Abamectin, Permethrin, and Cartap) has been recorded (Lietti *et al.*, 2005). The authors suggest that once *T. absoluta* appears in pheromone traps, preventive measures such as *B. thuringiensis* should be initiated and could even be integrated with predator and/or parasitoid releases.

In this study, the overall conclusion is that the combination of Bt-based formulation with chemical insecticides (Abamectin) have high efficiency against *T. absoluta* larvae.

Utilization of chemical insecticides alone does not have good effect for controlling the first and last instar larvae of this insect pest. However, Bt formulation (Biolep) causes high mortality percentage after 72 hours against the first and last instar larvae and persists longer than chemical pesticides, thus it could replace the chemicals used for controlling *T. absoluta*. Recommendation for combination of Bt- formulation with abamectin in IPM strategies pests a highly effective method to control tomato leafminer and reduces development of resistance to the chemicals insecticides.

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اثربخشی باسیلوس تورنجینسیس در مقایسه با برخی از حشره کش شیمیایی بر روی پروانه مینوز *T. absoluta* (Meyrick)، گوجه فرنگی،

س. ابوالقاسمی، ک. احمدی، و ح. م. تکلوزاده

چکیده

شب پره مینوز برگ گوجه فرنگی با نام علمی (*Tuta absoluta* (Meyrick) (Lep.: Gelechiidae) یکی از آفت‌های اصلی محصولات گوجه‌فرنگی است که سبب کاهش شدید عملکرد می‌شود. در حال حاضر، مدیریت کنترل *T. absoluta* در اکثر کشورها عمدتاً مبتنی بر کنترل شیمیایی است. با این وجود، تأکید ویژه ای بر اجرای روش های ایمن از نظر زیست محیطی است. فرمولاسیون های تجاری با منشا *Bacillus thuringiensis* ممکن است جایگزین خوبی باشند، زیرا از آنها برای کنترل موفقیت آمیز سایر آفات حشرات استفاده شده است. بررسی های آزمایشگاهی، گلخانه‌ای و میدانی انجام شده نشان می‌دهند که *B. thuringiensis* در کنترل *T. absoluta* بسیار کارآمد است. در این مطالعه اثر غلظت‌های مختلف سه آفت کش بیولپ، آبامکتین و ایندوکساکارب بر مرگ و میر لاروهای سن اول و آخر بر روی برگ‌های گوجه‌فرنگی در اتاقک رشد برای کنترل مؤثر شب پره برگ گوجه‌فرنگی با استفاده از کمترین مقدار مورد بررسی قرار گرفت. آزمایش سموم با غلظت توصیه شده هر ترکیب، نصف دوز توصیه شده، دو برابر غلظت توصیه شده و کاربرد دو و سه سموم با هم انجام شد. در این مطالعه، نتیجه کلی این بود که آبامکتین و ایندوکساکارب تأثیر کمی در کنترل لاروهای سن اول و آخر این آفت دارند. اما تلفیق کاربرد ترکیب باکتریایی مورد استفاده با ترکیبات شیمیایی را می‌توان برای کنترل این آفت جایگزین کرد.