Journal of Agricultural Science and Technology (JAST)
In Press, Pre-Proof Version

Effectiveness of *Bacillus thuringiensis* (Shigetane) Commercial Products against the tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)

Burçin Çiçek[1], Mahmut Mete Karaca[1], and Kamil Karut[1*]

ABSTRACT

The tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), is one of the most important pests causing significant economic losses in plant species belongingthe Solanaceae family. The preferred management method for *T. absoluta* currently involves insecticide application. However, beside the undesired effects of insecticides, chemical treatments can also negatively impact the efficiency of integrated pest management programs (IPM). *Bacillus thuringiensis* (Shigetane 1902) (Bacillales: Bacillaceae) (Bt) is a pathogen with formulations used as host-specific bioinsecticides. These formulations decompose quickly in the environment, thereby reducing non-target effects and residue problems compared to chemical pesticides. In this study, the effectiveness of six commercial *Bt* products, belonging to *aizawai* and *kurstaki* strains, against *T. absoluta* was assessed under laboratory conditions, using manufacturer-recommended doses. The efficacy of the *Bt* products varied between 70 and 97.5%. The lowest and highest mortalities were recorded in *B. thuringiensis* var. *aizawai* and *B. thuringiensis* var. *kurstaki* products, respectively. Mortality reached 100% within three days following insecticide treatments, whereas peak mortality in *Bt* applications was noted after a post-treatment period of fifteen days. These findings highlight the potential of certain *Bt* products as effective components of IPM programs for *T. absoluta*, suggesting the need for further field studies to optimize their use in agricultural practices.

Keywords: *Bacillus thuringiensis*, development time, mortality, tomato, *Tuta absoluta*

INTRODUCTION

The tomato laf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), originating from South America, stands as one of the most economically detrimental pests affecting a range of plant species within the Solanaceae family (Miranda et al., 1998; Garzia, 2009). Initially reported in Spain in 2006, the pest subsequently spread throughout Europe and the Mediterranean countries (Urbaneja et al., 2007; Arno et al., 2009). In Turkey, following its

[1] Department of Plant Protection, Faculty of Agriculture, Cukurova University, 01330, Adana, Turkiye.
*Corresponding author; e-mail: karuti@cu.edu.tr

first appearance in 2009, it rapidly proliferated and emerged as a prominent pest in both greenhouse and field tomato cultivation (Kılıç, 2010; Karut et al., 2011*)*. *T. absoluta* larvae feed between the two epidermal layers of tomato leaves, creating irregular transparent galleries that eventually turn brown, causing complete leaf desiccation. Furthermore, the larvae also feed on tomato fruits, and their excrement fosters an environment conducive to decay and the development of secondary microorganisms. Collectively, these damages result in significant losses in fruit quality and yield (Korycinska and Moran 2009; Desneux et al., 2010).

The predominant method for controlling *T. absoluta* involves insecticide application among existing practices (Tropea et al., 2012; Roditakis et al., 2018). However, due to the limited penetration of insecticides into plant tissues and the rapid development of resistance attributed to *T. absoluta*'s high reproductive capacity, chemical control alone often fails to yield the desired results (Biondi et al., 2018; Buragohain et al., 2021). Moreover, the indiscriminate and intensive use of insecticides poses adverse effects on human and environmental health. Consequently, alternative control methods, such as biological and biotechnical control, have gained preference for the better management of the pest (Lietti et al., 2005; Gonzales-Cabrera et al., 2011; Desneux et al., 2022).

Numerous natural enemies of *T. absoluta* from Hymenoptera and Hemiptera group of insects have been identified (Miranda et al., 1998; Marchiori et al., 2004; Luna et al., 2007; Bajonero 2008; Cabello et al., 2009; Kabiri et al., 2010; Doğanlar and Yiğit 2011). In addition to predators and parasitoids, microorganisms are also employed for pest control (Buragohain et al., 2021). *Bacillus thuringiensis* (Shigetane 1902) (Bacillales: Bacillaceae) (*Bt*) is a unique soil-dwelling bacterium utilized in the biological control of *T. absoluta* (Palma et al., 2014; Dammak et al., 2016; Biondi et al., 2018). Commercial products derived from various subspecies of *Bt* are deployed in managing insect species across different families. While *B. t* var. *kurstaki* is effective against lepidopteran larvae, *B. t* var. *israelensis* and *B. t* var. *tenebrionis* are used to control mosquitoes and coleopteran pest species, respectively (Gelernter, 2004; Palma et al., 2014; Dammak et al., 2016).

Studies investigating the efficacy of *Bt* products against on *T. absoluta* commenced with *B. t* var. *kurstaki* (*Btk*), sourced from South America in the early 2000s. Giustolin et al. (2001) demonstrated *Btk* induced mortality across all developmental stages of *T. absoluta* larvae. Subsequently, there has been a notable increase in research assessing the efficacy of *Bt* products in managing the pest (Niedmann and Meza-Basso, 2006; Gonzalez-Cabrera, 2011; Sarr et al., 2021). Niedmann and Meza-Basso (2006) revealed that two indigenous strains of Bt exhibited lethal effects against *T. absoluta* in Chile. Gonzalez-Cabrera (2011) reported that the impact of *T. absoluta* could be significantly diminished by exclusively applying *B. t*-based formulations, obviating the need for chemical insecticides. Sarr et al. (2021) demonstrated a reduction in the proportion of damaged fruits and an improvement in tomato yield, particularly with the application of *Bt* products. Furthermore, it has been drevealed that more favorable outcomes in pest management could be achieved by combining *Bt* with various biocontrol agents (Gonzalez-Cabrera et al., 2011; Alsaedi et al., 2017; Jamshidnia et al., 2018; Asma et al., 2018).

Environmentally friendly agents such as *Bt* strains are essential for a sustainable Integrated Pest Management (IPM) program against tomato pests. Therefore, this study aims to evaluate the effects of specific *Bt* commercial products with the potential to be used in biological control programs against *T. absoluta*.

MATERIAL AND METHODS

Host Plant Rearing

Tomato (*Lycopersicon esculentum* L.) cultivar Soray was used as a host plant in this study. The production of tomato plants was carried out in the a specialized rearing room adjusted at 25±2 °C temperature and 70±5% humidity with long day lighting (16 Light: 8 Dark)h. The plants were grown in pots (15x15 cm) containing potting soil.

Tomato Leaf Miner Rearing

The initial population of *T. absoluta* was obtained from tomato fields of Adana, and bioassay studies were completed at Cukurova University, Faculty of Agriculture, Department of Plant Protection, Laboratory of Insect Molecular Genetics and Biotechnology. The production was carried out in three fully grown tomato plants in net cages. The cages, each measuring 70x70x150 cm, were placed in the rearing room adjusted to 25±2 °C temperature of and 70±5% humidity, with long-day lighting (16 Light: 8 Dark)h. To maintain the *T. absoluta* production, dead tomato plants were replaced with new healthy plants during mass rearing period.

Bacillus thuringiensis Products

In this study, six registered *Bt* products in Turkey were tested. In addition to those products, two commercial insecticides, spinetoram 120 g L−1 (Radiant™, Dow AgroSciences, Istanbul, Turkey), and spinosad 480 g L−1 (Laser™, Dow AgroSciences, Istanbul,Turkey), widely preferred in pest control by growers, were used as positive controls. The features and recommended doses of the products are given in Table 1. Except for Dacron, all products were registered against *T. absoluta*.

Bioassay Experiment

Leaves obtained from the upper half of 40 cm tall tomato plants (40 cm in height) were used in the experiments. The recommended doses of the products, given in Table 1, were prepared using distilled water, and were applied to the tomato leaves by leaf dipping method. In the process, the leaves were dipped in the prepared solution for three seconds and then, allowed to dry on a paper for 30 minutes under laboratory conditions. The petiole of the tomato leaves were wrapped in wet cotton to provide moisture and keep the leaves alive during the experiments. The leaves were placed in rectangular transparent plastic containers of 12x6x6 cm, where the lids were covered with nets for ventilation. One newly hatched first instar of *T. absoluta* larvae was transferred to each leaf with the help of a fine-tipped paint brush. The first instar larvae were obtained from *T. absoluta* eggs kept in cabinet adjusted to 25±1°C temperature and 70±5% humidity. The larvae released on leaves treated with distilled water were considered as controls. The prepared units were placed in a cabinet adjusted to 25±1°C temperature, 70±5% humidity, and long-day lighting (16 hour Dark:8 hour Light). Experimental units were checked daily, and the number of live/dead larvae and the development of the larvae that remained alive were recorded. The stages of the larvae were determined depending on the head capsules they left after each molting. A total of ten individuals were used per replicate and each treatment was set up with 10 replicates (100 individuals) in bioassay experiments. The mean development time of larval instars was determined from individuals that remained alive and completed the immature development (Kandil et al., 2020). To determine adult longevity, individuals reaching the adult stage were carefully transferred to separate containers, and provided with honey as a regular consistent food source. These containers were kept under controlled environmental conditions, including a temperature of 25±1°C, relative humidity of 70±5%, and a photoperiod of 16 hours light/8 hours dark. Each adult was observed daily, and their survival was recorded until death.

Table 1. Characteristis of *Bacillus thuringiensis* products and insecticides used in the experiments.

		Bt products			
Name	Formulation	Strain	Isolate	Bacteria density	Recommended rate
Agree 50	WG	*B. thuringiensis spp. aizawai+*	GC-91	%50	100 g /100 L
Dacron	WP	*B. thuringiensis berliner var kurstaki*	Serotype 3a 3b, SA- 11 5300	32000 IU/mg	100 g /100 L
Delfin	WG	*B. thuringiensis berliner var kurstaki*	Serotype 3a 3b, SA- 11	32000 IU/mg	100 g /100 L
Dipel DF	WG	*B. thuringiensis subsp kurstaki*	ABTS-351	32000 CLU/mg	100 g /100 L
Florbac	WG	*B. thuringiensis var. aizawai*	ABTS-1857	35000 DBM/mg	150 g /100 L
Rebound	WP	*B. thuringiensis var. kurstaki*	-	16000 IU/mg	200 g /100 L
		Insecticides			
			Active ingredient		
Laser	SC		480 g/l Spinosad		25 ml/100 l
Radiant	SC		120 g/l Spinetoram		50 ml/ da

Statistical Analyses

Corrected mortality rates of 3, 7, 10, and 15 days after application and cumulative mortality were calculated using the Abbott formula (Abbott, 1925). Before conducting the analysis, we assessed the normality using the Shapiro–Wilk test and checked for homogeneity of variances using Levene’s test. In case of violation of assumptions, the data were transformed using Log10(X+1) and arcsin for homogeneity of variances. The original data were presented in the results. Data were analyzed using the One-Way ANOVA, followed by separation of means using the Tukey test. All analyses were conducted using SPSS 25.0 (Chicago, IL, USA).

RESULTS

Effects of *B. thuringiensis* Products on Mortality of *Tuta absoluta*

The insecticides, spinosad and spinetoram, exhibited the highest cumulative mortality rates, both reaching 100%, indicative of their potent lethal effects. Delfin and Dacron, belonging to *Bt* category, followed with mortality rates of 97.5 and 92.5%, respectively, and were statistically fell within the same group [F(7,79)=9.74, P=0.0001].The remaining *Bt* products demonstrated mortality rates ranging from 86.2 to 70.0%, showcasing variability in their effectiveness (Figure 1).

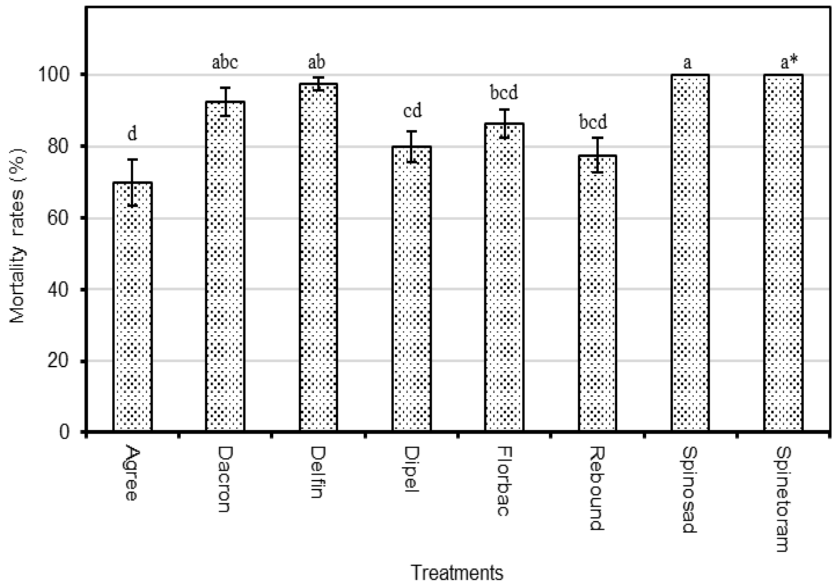

Fig. 1. Cumulative mortality rates (±SE) of *Tuta absoluta* caused by different *Bacillus thuringiensis* products and two insecticides (Spinosad and Spinetoram). * Values with different letters denote statistically significant difference (Tukey; $p< 0.05$).

On the third day after application, a 100% mortality rate was observed for spinosad and spinetoram, while Dipel exhibited a low rate of 1%. Mortality rates increased for all products by day 7, ranging from 9 for Agree to 50% for Delfin, signifying varied responses to the treatments. On day 10, except for Delfin (67%), all products exhibited mortality rates below 50%, indicating sustained but varied efficacy across treatments. On the 15th day, the highest and lowest mortality rates were observed in Delfin (94.2) and Agree (55.2%) treatment units, respectively, highlighting the durability and variability of the treatments (Table 2).

In the first instar larvae, the highest mean number of dead individuals was detected for Delfin (3.6), followed by Rebound (1.9) and Dipel (1.6). The three products differed statistically from the control experimental unit[F(6, 69)=10.1, P=0.0001]. No mortality was observed in Agree, which belonged to the same category as the control group. In the second instar larvae, the highest average numbers of dead individuals were found in Dacron (4.6), followed by Florbac (4.5). Other products showed varied mean mortality values between 0 and 2.8; the differences were statistically significant [F(6, 69)=16.08, P=0.0001]. In the third instar larvae, the mean numbers of dead individuals were close to each other, with the highest in Agree and Dacron (2.8). All the products were statistically different from the control, but showed no difference between each other [F(6, 69)=8.27, P=0.0001]. In the fourth instar larvae, the highest mean mortality values was determined as two for both Agree and Florbac. The values varied between 0.6 and 1.5 for the other products. In the pupal stage, the highest and lowest mean numbers of dead individuals were recorded respectively for Rebound (0.8), and Delfin (0.1); the statistical difference, however was not significant [F(6,69)=1.40, P=0.226]. In the sum of the first and second instars, the mean number of dead individuals exceeded approximately 50% for the three products (Dacron, Delphin and Florbac) (Table 3).

Table 2. Corrected mortality rates (±SE) of commercial *Bacillus thuringiensis* (Bt)-based products and two insecticides (Spinetoram and Spinosad) after post-treatment period of 3, 7, 10 and 15 days.

Products	Days			
	3	7	10	15
Agree	0.00±0.00^{b*}	9.00±3.48^{c}	13.00±3.34^{b}	55.25±4.38^{c}
Dacron	0.00±0.00^{b}	16.00±4.00^{bc}	44.00±6.86^{a}	64.50±7.00^{bc}
Delfin	0.00±0.00^{b}	50.00±7.60^{a}	67.00±9.07^{a}	94.25±3.07^{a}
Dipel	1.00±1.00^{b}	32.00±4.16^{ab}	46.00±7.18^{a}	78.50±4.47^{abc}
Florbac	0.00±0.00^{b}	21.00±4.33^{bc}	45.00±7.49^{a}	76.25±6.57^{abc}
Rebound	0.00±0.00^{b}	26.00±6.15^{abc}	37.00±6.15^{ab}	82.25±6.17^{ab}
Spinetoram	100±0.00^{a}	-	-	-
Spinosad	100±0.00^{a}	-	-	-

* Means within the same column with different letters denote statistically significant difference (Tukey; $p < 0.05$).

Table 3. Mean (±SE) numbers of mortality *Tuta absoluta* individuals at different larval instars treated with commercial *Bacillus thuringiensis* (Bt)-based products

Products	Larval instars and pupa				
	I	II	III	IV	Pupa
Agree	0.0±0.00^{c*}	2.0±0.36^{bc}	2.8±0.44^{a}	2.0±0.61^{a}	0.7±0.30^{a}
Dacron	0.5±0.30^{bc}	4.6±0.76^{ab}	2.8±0.46^{a}	1.2±0.38^{a}	0.3±0.30^{a}
Delphin	3.6±0.61^{a}	2.8±0.44^{abc}	2.0±0.55^{a}	1.4±0.26^{a}	0.1±0.10^{a}
Dipel	1.6±0.54^{ab}	2.5±0.54^{abc}	2.6±0.30^{a}	1.5±0.37^{a}	0.2±0.13^{a}
Florbac	1.0±0.29^{bc}	4.5±0.63^{a}	2.4±0.26^{a}	0.6±0.26^{a}	0.3±0.15^{a}
Rebound	1.9±0.62^{ab}	1.5±0.16^{c}	2.0±0.59^{a}	2.0±0.53^{a}	0.8±0.29^{a}
Control	0.0±0.00^{c}	0.0±0.00^{d}	0.1±0.10^{b}	0.6±0.22^{a}	0.4±0.22^{a}

* Means within the same column with different letters denote statistically significant difference (Tukey; $p < 0.05$).

Effects of *B. thuringiensis* Products on Development and Longevity of *Tuta absoluta*

In the first instar, statistically significant differences were observed in mean development times. The longest and shortest times were recorded for Dipel (4.37 days) and the control (3.33 days), respectively [$F(5, 161)=2.72$, $P=0.02$] (Table 4). In the second instar, the mean development times varied between 2.77 and 5.00 days. In the third instar, a statistically significant difference in mean development times was observed, exceeding those of the control [$F(5, 161)=9.62$, $P=0.0001$]. In the pupal stage, mean development times were close to each other and did not show statistically significant differences [$F(5, 161)=1.83$, $P=0.10$]. Total mean development times ranged between 24.5 and 20.27 days, with statistically significant differences observed [$F(5, 161)=12.7$, $P=0.0001$]. Except for Florbac, adult longevities in all treatments were longer and statistically different from control [$F(5, 161)=6.42$, $P=0.0001$] (Table 4).

Table 4. Mean (±SE) development time (day) of different larval stages, and adult longevity of *Tuta absoluta* calculated from the larvae do not dead and complated development after *Bacillus thuringiensis* treatment.

Products		Larval instars and pupa						Longevity
	n	I	II	III	IV	Pupa	Total	
Agree	24	3.45±0.20^{ab*}	4.16±0.48^{ab}	4.54±0.37^{a}	3.54±0.24^{ab}	8.12±0.06^{a}	23.83±0.63^{ab}	12.33±1.13^{ab}
Dacron	6	4.33±0.42^{ab}	4.83±0.30^{a}	3.50±0.22^{ab}	3.66±0.33^{ab}	8.16±0.18^{a}	24.50±0.42^{a}	15.16±0.60^{a}
Dipel	16	4.37±0.32^{a}	3.62±0.32^{ab}	3.37±0.28^{ab}	3.56±0.47^{ab}	8.12±0.17^{a}	23.06±0.50^{ab}	13.68±0.76^{a}
Florbac	12	3.91±0.28^{ab}	5.00±0.68^{a}	3.33±0.28^{ab}	2.83±0.40^{ab}	7.58±0.19^{a}	22.66±0.93^{abc}	8.66±1.00^{b}
Rebound	18	3.66±0.19^{ab}	2.77±0.26^{c}	2.88±0.25^{b}	4.22±0.40^{a}	7.94±0.17^{a}	21.50±0.49^{bc}	14.66±0.94^{a}
Control	86	3.33±0.13^{b}	3.50±0.14^{bc}	2.74±0.09^{b}	2.72±0.13^{b}	7.88±0.07^{a}	20.27±0.26^{c}	11.04±0.32^{ab}

* Means within the same column with different letters denote statistically significant difference (Tukey; $p < 0.05$).

DISCUSSION

Although there were statistical differences, the effectiveness of *B. thuringiensis* (*Bt*) products, manifest by mortality rates exceeding 70.0%, was confirmed in this study. Similarly the effectiveness of *Bt* products on larval mortality of *T. absoluta* was confirmed under laboratory and greenhouse conditions (Hafsi et al., 2012; Birgücü et al., 2014; Jallow et al., 2019; Kandil et al., 2020; Sandeep Kumar et al., 2020a, b; Buragohain et al., 2021; Sarr et al., 2021). Although the application method is different, Hafsi et al. (2012) also found an average of 72.5% larval mortality at seven days after the treatment of the *Bt* product (Bt 32000) under laboratory conditions. Jallow et al. (2019), reported 55%–65% mortality when second- instar *T. absoluta* larvae were exposed to tomato leaves treated with *Bt* (Dipel).

It can be suggested that the high mortality rate in the first two larval stages, with over 50% mortality in three *Bt* products (Delfin, Florbac, and Dacron), could increase the success in the biological control of tomato leaf miner. Similar results were reported in different studies, and mortality in the first and second larval stages was found to be higher than other larval stages in comparison (Giustolin et al., 2001; Gonzalez-Cabrera et al., 2011; Hashemitassuji et al., 2014). Coelho and França (1987) argued that this was because the new larva that emerged from the egg was feeding by chewing the leaf surface to reach the mesophyll layer. This behavior increases the chance of getting bacterial toxins into the digestive system of the larvae.

B. thuringiensis products prolonged the larval development period in infected individuals that survived and completed their development. These results were aligned with other researchers who demonstrated the effect of Bt products on *T. absoluta* larvae, reporting a significant increase in larval and pupal development periods (Kandil et al., 2020). Similar results were also reported for other lepidopteran pests. Yang et al. (2008) determined that the *Bt* YL17 isolate distrupted the development of the 3rd larval stage of *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae), and prolonged the total immature development. Erb et al. (2001) reported that *Bt* had a sub-lethal effect on *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Lymantridae) fourth instar larvae and prolonged the development period. Barker (1998) reported 12.4 days longer total immature development time for *Bt* treated *Cochylis hospes* (Walsingham, 1884) (Lepidoptera: Tortricidae) larvae compared to the control group. Similarly, Huarong et al. (2005) found that after *Bt* applications, larval development of *Ostrinia nubilalis* (Hübner, 1796) (Lepidoptera: Pyralidae) was prolonged when compared to the control.

The mortality rates of the *Bt* products were found to be close to the that of the insecticides. However, the highest mortality rate (100%) was reached on the 3rd day in insecticide treatments, while it was reached on the 15th day in *Bt* treatments. This could be due to the different modes of action of the insecticide and *Bt*. While insecticides lead to immediate death after application, mortality in *Bt* applications may occur after a few hours or weeks (Perez et al., 2015). *B. thuringiensis* strains generate toxins during both the initial sporulation phase and the growth stage, resulting in the formation of parasporal crystalline inclusions. Upon ingestion by insects, these toxins dissolve within the midgut. Subsequently, midgut proteases trigger the toxins through proteolysis, binding them to precise receptors on the insect cell membrane. This binding results in cell disruption, ultimately leading to the death of the insect (Schnepf et al., 1998; Palma et al., 2014).

In this laboratory study, we demonstrated that *Bt* products are at least as effective as insecticides but require more time to achieve the maximum mortality rate. Therefore, for a successful IPM program in greenhouses, these products should be applied repeatedly at specific time window (one week) supported with supplemental application of other natural enemies, such as predators or parasitoids.

ACKNOWLEDGEMENTS

The authors thank Dr. Arif Arslan (Cargill, Alberta, Canada) and Mohammed A. Mohammed for language editing the manuscript; Cukurova University Scientific Project Foundation Units (CU-BAP) (grant numbers FYL-2019-12065).

REFERENCES

1. Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*., 18: 265–267.
2. Alsaedi, G., Ashouri A. and Talaei-Hassanloui, R. 2017. Evaluation of *Bacillus thuringiensis* to control *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under laboratory conditions. *Agric. Sci.*, 8: 91–599.
3. Arno, J., Sorribas, R., Prat, M., Matas, M., Pozo, C., Rodriguez, D., Garreta, A., Gomez, A. and Gabarra, R. 2009. *Tuta absoluta*, a new pest in IPM tomatoes in the northeast of Spain. *IOBC/WPRS Bull.*, 49: 203–208.
4. Asma, C., Ons, I., Sabrine, B. and Kaouthar, L. 2018. Life-stage-dependent side effects of selected insecticides on *Trichogramma cacoeciae* (Marchal) (Hymenoptera: Trichogrammatidae) under laboratory conditions. *Phytoparasitica*, 46: 105–113.
5. Aynalem, B. 2022. Empirical review of *Tuta absoluta* Meyrick effect on the tomato production and their protection attempts. *Adv. Agric.*, 2595470. https://doi.org/10.1155/2022/2595470.
6. Bajonero, J. 2008. Biology and life cycle of *Apanteles gelechiidivoris* (Hymenoptera: Braconidae) parasitoid of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agron. Colomb.*, 26(3): 417–426.
7. Barker, J. F. 1998. Effect of *Bacillus thuringiensis* subsp. *kurstaki* toxin on the mortality and development of the larval stages of the banded sunflower moth (Lepidoptera: Cochylidae). *J. Econ. Entomol.*, 91 (5): 1084–1088.

8. Birgücü, A. K., Çelikpençe, Y., Karaca, İ. and Bayındır A. 2014. Growth inhibitory effects of bio- and synthetic insecticides on *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae). *Turk. J. Entomol.*, 38 (4): 389–400.
9. Biondi, A., Guedes R. N. C., Wan F. H. and Desneux N. 2018. Ecology, worldwide spread, and management of the invasive South American Tomato Pinworm, *Tuta absoluta*: Past, present, and future. *Annu. Rev. Entomol.*, 63: 239-258.
10. Buragohain P., Kumar Saikia, D., Sotelo-Cardona, P. and Srinivasan, R. 2021. Evaluation of Bio-Pesticides against the South American Tomato Leaf Miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in India. *Horticulturae*, 7(9): 325.
11. Cabello, T., Gallego, J. R., Vila, E., Soler, A., Del Pino, M., Carnero, A., Hernandez, E. and Polaszek, A. 2009. Biological control of the South American Tomato Pinkworn, *Tuta absoluta* with releases of *Trichogramma achaeae* in tomato greenhouses of Spain. *IOBC/ WPRS Bull.*, 49: 225–230.
12. Coelho, M. C. F. and França, F. H. 1987. Biologia e quemotaxia da larva e descriçao da pupa e adulto da traça-do-tomateiro. *Pesqui Agropecu Brasil*, 22: 129–135.
13. Dammak, M., Ben Khedher, S., Boukedi, H., Ikbel, C., Laarif, A. and Tounsi, S. 2016. Involvement of the processing step in the susceptibility/tolerance of two lepidopteran larvae to *Bacillus thuringiensis* Cry1Aa toxin. *Pestic. Biochem. Physiol.*, 127: 46–50.
14. Desneux, N., Han, P., Mansour, R., Arno, J., Brévault, T., Campos, M. R., Chailleux, A., Guedes, R. N. C., Karimi, J., Konan, K. A. J., Lavoir, A. V., Luna, M. G., Perez‐Hedo, M., Urbaneja, A., Verheggen, F. J., Zappala, L., Abbes, K., Ali, A., Bayram, Y., Cantor, F., Andrew, G. S. Cuthbertson, A. G. S., Vis, R. D., Erler, F., Firake, D. M., Haddi, K., Hajjar, M. J., Ismoilov, K., Jaworski, C. C., Kenis, M., Liu, H. T., Madadi, H., Martin, T., Mazih, A., Messelink, G. J., Mohamed, S. A., Nofemela, R. S., Oke, A., Ramos, C., Ricupero, M., Roditakis, E., Shashank, P. R., Wan, F. H., Wang, M. H., Wang, S., Zhang, Y. B. and Biondi, A. 2022. Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. *J. Pest Sci.*, 95:17–39.
15. Desneux, N., Wajnberg, E., Burgio G., Arpaia, S., Wyckhuys-Kris A. G., Narvaez-Vasquez, C.A., Gonzalez-Cabrera, J., Tabone, E., Frandon J., Pizzol, J., Poncet C. and Urbaneja A. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *J. Pest Sci.*, 83: 197–215.

16. Doğanlar, M. and Yiğit, A. 2011. Parasitoid complex of the Tomato Leaf Miner, *Tuta absoluta* (Meyrick 1917), (Lepidoptera: Gelechiidae) in Hatay, Turkey. *KSU J. Nat. Sci.*, 14(4): 28–37.
17. Erb S., Bourchier, R., van Frankenhuyzen, K. and Smith, S. 2001. Sublethal effects of *Bacillus thuringiensis* Berliner subsp. *kurstaki* on *Lymantria dispar* (Lepidoptera: Lymantriidae) and the Tachinid parasitoid *Compsilura concinnata* (Diptera: Tachinidae). *Environ. Entomol.*, 30(6): 1174–1181.
18. Garzia, G.T. 2009. *Physalis peruviana* L. (Solanaceae), a host plant of *Tuta absoluta* in Italy. *IOBC/WPRS Bull.*, 49: 231–232.
19. Gelernter W. 2004. The rise and fall of *Bacillus thuringiensis* tenebrionis. *Phytoparasitica*, 32: 321–324.
20. Giustolin, T. A., Vendramim, J. D., Alves, S. B., Vieira S. A. and Pereira, R.M. 2001. Susceptibility of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae) reared on two species of lycopersicon to *Bacillus thuringiensis* var. *kurstaki*. *J. Appl. Entomol.*, 125: 551–556.
21. Gonzalez-Cabrera, J., Molla, O., Monton H. and Urbaneja, A. 2011. Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *BioControl*, 56: 71–80.
22. Hafsi, K. A., Chermiti, B. and Nasraoui, B. 2012. Response of the tomato miner *Tuta absoluta* (Lepidoptera: Gelechiidae) to thirteen insecticides in semi-natural conditions in Tunisia. *Bull. OEPP/EPPO Bull.*, 42 (2): 312–316
23. Hashemitassuji, A., Safaralizadeh, M. H., Aramideh, S. and Hashemitassuji, Z. 2014. Effects of *Bacillus thuringiensis* var. *kurstaki* and spinosad on three larval stages 1st, 2nd and 3rd of tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in laboratory conditions. *Arch. Phytopathol. Plant Prot.*, 48(5): 377–384.
24. Huarong, L., Oppert, B., Higgins, R. A., Huang, F., Buschman, L. L. and Zhu, K. Y. 2005. Susceptibility of Dipel resistant and susceptible *Ostrinia nubilalis* (Lepidoptera: Crambidae) to individual *Bacillus thuringiensis* protoxins. *J. Econ. Entomol.*, 98(4): 1333–1340.
25. Jallow, M. F. A., Dahab A. A., Albaho, M. S. and Devi, V. Y. 2019. Efficacy of some biorational insecticides against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under laboratory and greenhouse conditions in Kuwait. *J. Appl. Entomol.*, 143: 187–195.

26. Jamshidnia, A., Abdoli, S., Farrokhi, S. and Sadeghi, R. 2018. Efficiency of spinosad, *Bacillus thuringiensis* and *Trichogramma brassicae* against the Tomato Leafminer in greenhouse. *BioControl*, 63: 619–627.
27. Kabiri, F., Vila, E. and Cabello T. 2010. *Trichogramma achaeae*: An excellent biocontrol agent against *Tuta absoluta*. *Sting Newsletter Biol. Control*, 33: 5–6.
28. Kandil, M. A., Abdel-Kerim R. N. and Moustafa M. A. 2020. Lethal and sub-lethal effects of bio-and chemical insecticides on the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egyption J. Biol. Pest Control*, 30:1–7.
29. Karut, K., Kazak C.. Döker I. and Ulusoy M. R. 2011. Mersin ili domates seralarında Domates yaprak galeri güvesi *Tuta absoluta* (Meyrick. 1917) (Lepidoptera: Gelechiidae)'nın yaygınlığı ve zarar durumu. *Turk. Entomoloji Derg.*, 35 (2): 339–347. (in Turkish with abstract in English)
30. Kılıç, T. 2010. First record of *Tuta absoluta* in Turkey. Phytoparasitica, 38: 243–244.
31. Korycinska, A. and Moran, H. 2009. Plant Pest Notice: South American tomato moth, *Tuta absoluta*. *Dep. Environ. Food Rural Aff. Food Environ. Res. Agency* (Fera), 56: 1–4.
32. Lietti, M. M. M., Botto, E. and Alzogaray, R.A. 2005. Insecticide resistance in Argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotrop. Entomol.*, 34 (1): 113–119.
33. Luna, M. A. G., Sanchez, N. and Pereyra, P.C. 2007. Parasitism of *Tuta absoluta* (Lepidoptera: Gelechiidae) by *Pseudapanteles dignus* (Hymenoptera, Braconidae) under laboratory conditions. *Environ. Entomol.*, 36(4): 887–893.
34. Marchiori, C. H., Silva, C. G. and Lobo, A. P. 2004. Parasitóides de *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) coletados em plantas de tomate em lavras, estado de Minas gerais, Brasil. *Braz. Arch. Biol. Technol.*, 50(6): 434–437.
35. Miranda, M. M. M., Picanço, M., Zanuncio, J. C. and Guedes, R.N.C. 1998. Ecological life table of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Biocontrol Sci. Technol.*, 8: 597–606.
36. Niedmann L. L. and Meza-Basso, L. 2006. Evaluation of native strains of *Bacillus thuringiensis* as an alternative of integrated management of the tomato leaf miner (*Tuta absoluta* Meyrick; Lepidoptera: Gelechiidae) in Chile. *Agricultura Técnica*, 66(3): 235–246.

37. Palma L., Muñoz, D., Berry, C. Murillo, J. and Caballero, P. 2014. *Bacillus thuringiensis* toxins: An overview of their biocidal activity. *Toxins*, 6(12): 3296–3325.
38. Perez, J., Bond, C., Buhl, K. and Stone, D. 2015. *Bacillus thuringiensis* (Bt) General Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services. http://npic.orst.edu/factsheets/btgen.html.
39. Roditakis, E., Vasakis, E., Garcia-Vidal, L., Del Rosario, M. A. M., Rison, J. L., Haxaire-Lutun, M. O., Nauen, R., Tsagkarakou, A. and Bielza, P. (2018). A four-year survey on insecticide resistance and likelihood of chemical control failure for tomato leaf miner *Tuta absoluta* in the European/Asian region. *J Pest Sci., 91*(1), 421–435.
40. Sandeep, Kumar, J., Jayaraj, J., Shanthi, M., Theradimani, M., Venkatasamy, B., Irulandi, S. and Prabhu, S. 2020 (a). Potential of Cry1Ac from *Bacillus thuringiensis* against the tomato pinworm, *Tuta absoluta* (Meyrick) (Gelechiidae: Lepidoptera). *Egyption J. Biol. Pest Control*, 30: 81
41. Sandeep Kumar, J., Jayaraj, J., Shanthi, M., Theradimani, M., Venkatasamy, B., Irulandi, S. and Prabhu, S. 2020 (b). Potential of standard strains of *Bacillus thuringiensis* against the tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egyption J. Biol. Pest Control*, 30: 123.
42. Sarr, O. M., Bal, A. B., Fossati-Gaschignard O. and Gauthier, N. 2021. Effectiveness of two biopesticides against the invasive tomato pest *Tuta absoluta*. *Entomologia Exp. et Appl.*, 169: 674–685.
43. Schnepf, E., Crickmore, N., van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D. R. and Dean, D. H. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.*, 62: 775–806.
44. Tropea Garzia, G. G., Siscaro, G., Biondi, A. and Zappalà, L. (2012). *Tuta absoluta*, a South American pest of tomato now in the EPPO region: Biology, distribution, and damage. EPPO Bulletin, 42(2): 205-210.
45. Urbaneja, A., Vercher, R., Navarro, V., García Marí, F. and Porcuna, J. 2007. La polilla del tomate, *Tuta absoluta*. *Phytoma España*, 194: 16–23.
46. Yang, X., Oluwafemi, A. R. and Zhang, H. 2008. Screening of highly toxic *Bacillus thuringiensis* and its effects on the growth and development of *Spodoptera exigua* (Lepidoptera: Noctuidae). *Entomol. Gen.*, 31(1): 95–104.