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The effect of sublethal doses of *Bacillus thuringiensis* Berliner on *Tuta absoluta* (Meyrick) on resistant and susceptible tomato cultivars

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Abstract

The effect of *Bacillus thuringiensis* (*Bt*) Berliner on the *Tuta absoluta* (Meyrick) on two resistant ('King Stone') and susceptible ('Rio Grande') tomato (*Solanum lycopersicum* L.) cultivars was studied under laboratory conditions. In the bioassay tests, the lethal concentration (LC_{50}) values of *Bt* were determined for the 2nd- and 3rd-instar larvae of *T. absoluta* on two cultivars. In another experiment, the effect of sublethal doses of *Bt* on 2nd- and 3rd-instar larvae on two susceptible and resistant cultivars was determined. Also, the nutritional indices and the enzyme content of larvae on two susceptible and resistant cultivars under the sublethal doses of *Bt* were investigated. All nutritional indices and the enzyme content of the treated larvae were significantly different compared to those of the control group. The lowest efficiency of the conversion of ingested food (7.82%), the efficiency of the conversion of digested food (4.34%), the relative consumption rate (22.29 mg mg^{-1} day^{-1}), and the relative growth rate (1.16 mg mg^{-1} day^{-1}) was determined for the resistant cultivar 'King Stone' at the LC_{25} concentration of *Bt*. The lowest amount of α-amylase (0.98 nM min^{-1} mg^{-1} protein), protease (12.61 µM min^{-1} mg^{-1} protein), and lipase (0.182 nM min^{-1} mg^{-1} protein) and the highest amount of phenoloxidase (0.337 µM min^{-1} mg^{-1} protein) were determined for the resistant cultivar 'King Stone' at the LC_{25} concentration of *Bt*. The results showed that the integration of *Bt* application and host plant resistance significantly increased the efficiency of *Bt*.

Keywords: biocontrol, digestive enzymes, nutritional indices, *Solanum lycopersicum*.

Introduction

The tomato leaf miner, *Tuta absoluta* (Meyrick), is a serious pest of tomatoes throughout the world (Nouri-Ganbalani et al., 2016). The use of pesticides as an important part of integrated pest management (IPM) programmes is of great importance in the control of tomato leafminer (Khani et al., 2020). Control of *T. absoluta* is very difficult with chemicals alone due to the rapid capability to develop insecticide-resistant strains (Roditakis et al., 2015). For this reason, there is an urgent need to develop environmentally friendly and effective IPM strategies for the control of this pest. Other pest control methods, such as the potential use of biological control agents (Sylla et al., 2016), botanical insecticides (Ghanim, Abdel Ghani, 2014), mating disruption techniques (Cocco et al., 2013), and the use of insecticide-treated nets (Biondi et al., 2015), have been investigated and documented.

Host plant resistance is an environmentally friendly control method that is an important part of IPM programmes. The development of insect-resistant cultivars has a stable and cumulative effect on the pest population without harmful effects on the environment (Kumari et al., 2022).

Bacillus thuringiensis (*Bt*) strains are highly effective against all larval stages of *T. absoluta* (Mollá et al., 2011; Kumar et al., 2020). A combination of resistant cultivars and biological control is one of the methods that has received attention in recent years to control some pests. Saeidi and Raeesi (2021) revealed that the integration of host plant resistance and biological control was effective in the sustainably management of *T. absoluta* under greenhouse conditions. A combination of host plant resistance and biological control using *Trichogramma brassicae* significantly reduced the

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number of infested leaves, the number of larvae per plant, the number of mines per leaf, and the number of infested fruits per plant. Buragohain et al. (2021) also revealed that the implementation of an IPM package including microbial pesticides (*B. thuringiensis* var. *kurstaki* and *Beauveria bassiana*), neem products, and chlorantraniliprole was in line with usual farmer practices (i.e., calendar-based application of chemical pesticides), significantly reduced *T. absoluta* infection without any reduction of the marketable yield (about 25 and 22 t ha^{-1}), and even reduced crop protection costs (50%).

Some studies have been conducted on the sublethal effects of *Bt* on various pests (Ghassemi-Kahrizeh, Aramideh, 2015; Fathipour et al., 2019; Fernandes et al., 2021; Poulin et al., 2022), Several studies have also been conducted on the effects of *Bt* on *T. absoluta* (Nozad-Bonab et al., 2017; Jamshidnia et al., 2018; Jalapathi et al., 2020). To our knowledge, there is not much information on the sublethal effects of *Bt* on *T. absoluta*, therefore, this study may provide more information on this issue.

Based on the results of the present study, in order to reduce the use of pesticides against this important and major pest, protect the environment and reduce the damage caused by this pest, the necessary recommendations for the agricultural sector are provided.

The present study was conducted to investigate the effects of sublethal doses of *Bt* on *T. absoluta* on two resistant and susceptible tomato cultivars.

Material and methods

The present study was conducted at the Department of Plant Protection, Mahabad Branch, Islamic Azad University in West Azarbaijan Province, Iran, from 2020 to 2021.

Preparation and planting of tomatoes. Seeds of two tomato (*Solanum lycopersicum* L.) cultivars 'King Stone' as a resistant cultivar and 'Rio Grande' as a susceptible cultivar were obtained from the Karaj Seed and Seedling Breeding Institute, Iran. The seeds of the tested cultivars were soaked in water for 24 h and then grown in a greenhouse in seedling trays with a peat moss grow medium. After six leaves of the plant, the seedlings were grown in plastic pots (diameter 30 cm and height 42 cm) in a mixture of soil, sand, and manure in the ratio of 1:1:2 in greenhouse conditions at 25 ± 2°C, 65 ± 5% RH, and 16/8 (light/dark) h. To prevent the plants from infesting by other pests, the pots were placed under a 50-mesh net.

Rearing of Tuta absoluta. Leaves infested with the larvae of *T. absoluta* were collected from tomato plants in the greenhouse and transported to the Insectology Laboratory of the Department of Plant Protection, Mahabad Branch, Islamic Azad University in West Azarbaijan Province. Then, the larvae developed and matured on the leaves until they reached the adult stage. After the identification of the species in the Entomology Department of Urmia University in West Azarbaijan Province, Iran, using adult morphology (Bloem, Spaltenstein, 2011), insects were reared by two methods. One of the methods was to release the adult insects on the pots of different tomato cultivars under the net in the greenhouse and rear two generations on each cultivar at 25 ± 2°C, 65 ± 5% RH, and 16/8 (light/dark) h. In another method, to study the life table of insects, leaves infested with pest larvae were collected from the greenhouse and kept in plastic Petri dishes with lids (diameter 8 cm and height 1.5 cm) until the pupae stage. The larvae were fed with fresh tomato leaves every two days. To provide ventilation in the lids of the larvae rearing containers, small holes with a diameter of one centimeter were made and covered with a 50-mesh net. To prevent the leaves from drying out, the petioles of the leaves were kept with moist cotton pieces wrapped around the petiole. After emerging, the pupae were kept in plastic containers (diameter 3 cm and height 5 cm) in the laboratory until adult insects emerged.

To feed adult insects, a 10% water and honey solution was used. Adult males and females (15 pairs) from each cultivar for mating in equal proportions were placed in special cylindrical containers (diameter 11.5 cm and height 9.5 cm with net cover). To lay eggs, a leaf with 5 leaflets was placed in an egg-laying chamber, and a new leaf with 5 leaflets was replaced every two days until the 1st-instar larvae were observed on the leaves. Then the infested leaves were transferred to containers where larvae turned into pupae. The colony was maintained in a growth chamber at 25 ± 2°C, 65 ± 5% RH, and 16/8 (light/dark) h. Two generations of those insects were reared on different tomato cultivars, and the eggs of the 2nd generation were used to begin the experiment.

Bioassay experiments of Bacillus thuringiensis (*Bt*). *Bacillus thuringiensis* var. *kurstaki* was prepared in the form of wettable powder manufactured in Murcia, Spain. Due to the insecticidal nature of this agent, which is effective in digestion, a bioassay was performed by immersing tomato leaves in a toxic solution. Preliminary experiments were conducted to determine and estimate the lethal concentration (LC_{50}) of *Bt*, including the exposure of five concentrations of bacteria along with the control treatment, and to determine the lowest and highest concentrations (80–20%) for the 2nd- and 3rd-instar larvae. After determining the concentrations that caused the lowest and highest mortality, three other concentrations were calculated using a logarithmic method. Different concentrations were prepared and mixed, and tomato leaves of two tested tomato cultivars (Khatami et al., 2022) were immersed in toxic solutions. To spread the solutions evenly on the surface of the leaves, 1% of wetting agent Tween 20, which has the ability to spread and stick, was used. After contamination, the leaves were dried at the laboratory temperature. Distilled water with Tween 20 was used in the control treatment. After the evaporation of water from the surface of the leaves, 15 larvae of the 2nd and 3rd stages were placed on the leaves separately with a soft brush. To exchange the air inside the Petri dishes, their lids were closed with nets. Larval mortality was calculated after 24, 48, and 72 h. Dead larvae were recorded by touching their head with a hot needle. In that way, the moving larvae were considered alive, and the larvae not reacting to the impact and change in the colour of the body were considered dead. The bioassay experiments were performed on both susceptible and resistant tomato cultivars using the 2nd- and 3rd-instar larvae. Three replicates were performed

for each cultivar and larval stage. Subsequently, the LC_{90}, LC_{50}, LC_{25}, and LC_{10} values of *Bt* were calculated for both cultivars.

Interaction of the sublethal doses of Bt with cultivar resistance. Based on the experiments related to the study of the pest life table on different cultivars (Khatami et al., 2022), two susceptible and resistant tomato cultivars were selected, and the LC_{10} and LC_{25} concentrations of *Bt* were sprayed on the plants of the tested cultivars. In this experiment, the 2nd- and 3rd-instar larvae of the pest were evaluated. The experiment was conducted in a completely randomised block design with three replications. The first factor was two (susceptible and resistant) tomato cultivars, and the second factor was two (LC_{10} and LC_{25}) concentrations of *Bt* and the control treatment (distilled water).

Effect of the sublethal doses of Bt on nutritional indices. To investigate the effects of *Bt* on nutritional indices, LC_{10} and LC_{25} concentrations were evaluated. To determine the weight of larvae, consumed food, and produced waste, a sensitive scale with an accuracy of 0.01 mg was used. The experiment was done in four replications. In each repetition, 15 newly emerged 4th-instar larvae were used (less than 24 h), which were kept hungry for 12 h until their stomach contents were emptied. For each cultivar, the control group was treated with water only. Leaf discs (a diameter of 8 cm) were immersed in a given concentration of *Bt* for 20 s, and their initial weight was recorded. Then the larvae were weighed and allowed to feed on the leaves. After 24 h, the remaining leaves were removed and replaced with freshly treated leaves. At the end of each day, the remaining leaves were weighed and dried in an oven at 60°C for 48 h and weighed again to calculate the dry weight of the consumed food. At the end of each day, the produced waste was collected, dried in an oven, and weighed. At the end of the experiment, the larvae were weighed and their mortality was recorded. To determine the dry weight of the larvae used, they were dried in an oven and weighed again. The experiment continued for three days, and the observations were recorded at the end of each day.

The nutritional indices were determined according to the formulas provided by Waldbauer (1968):

$$\text{Approximate digestibility (AD)} = [(W_i - W_f) / W_i] \times 100, \quad (1)$$

where W_i is the dry weight of food consumed per larva during the feeding time, mg; W_f is the dry weight of produced waste, mg;

$$\text{Efficiency of the conversion of ingested food (ECI)} = [(W_t - W_0) / W_i] \times 100, \quad (2)$$

where W_t is the dry weight of the larva after feeding during T_t, mg; W_0 is the initial dry weight of the larva before feeding, mg;

$$\text{Efficiency of the conversion of digested food (ECD)} = [(W_t - W_0) / (W_i - W_f)] \times 100 \quad (3)$$

$$\text{Relative consumption rate (RCR)} = W_i / (T_t \times W_0), \quad (4)$$

where T_t is the feeding time of the larva (days).

$$\text{Relative growth rate (RGR)} = (W_t - W_0) / (T_t \times W_0) \quad (5)$$

Effect of the sublethal doses of Bt on the biochemical compounds of larvae. Measurement of biochemical compounds activity. Anatomy and separation of digestive organs. To dissect and isolate the digestive tube, the Esmaeily and Bandani (2015) method was used. For this purpose, 3rd-instar larvae were used. The larvae were first anesthetised on ice. In 1% NaCl (sodium chloride) solution, the beginning and end of the larval body were taken with two tweezers and pulled in two opposite directions to separate the digestive tube. In this experiment, 90 digestive tubes were transferred to 1.5 mL microtubes containing 550 µL of distilled water and stored at a temperature of 4°C. Finally, the upper part of the mixture was removed and stored at −20°C to be used as a source of enzymes in next experiments.

Measurement of phenoloxidase enzyme activity was performed by the method of Terra and Ferreira (1994). 20 µL of hemolymph were dissolved with 100 µL of the anticoagulant. For the substrate, 10 mM of L-de hydroxy phenylalanine (L-DOPA) was used. The samples were centrifuged at 4°C for 10 min at 13,000 rpm. The activity was read at a wavelength of 490 nm for 5 min using an ELISA BioTek ELx800 microplate reader (USA). The activity was presented in the units of phenoloxidase per milligram of hemolymph protein, where each unit represents the amount of enzyme required to increase absorption by 0.001 units per minute. The experiment was done with three replications and with 10 larvae in each repetition.

Measurement of α-amylase enzyme activity was performed by the method of Bernfeld (1955). The α-amylase activity was assayed by the dinitrosalicylic acid (DNS) procedure using 1% soluble starch (Merck) at pH = 3 as a substrate described by Bandani et al. (2009). 20 µL of enzyme supernatant solution was incubated for 30 min at 35°C with 50 µl of universal buffer and 30 µL of soluble starch. The reaction was stopped by addition 100 µL of DNS and heated in boiling water for 10 min. The absorbance was then read at 545 nm after cooling in ice for 5 min.

Measurement of lipase enzyme activity was performed by the method of Tsujita et al. (1989). 20 µL of the samples and 30 µL of P-nitrophenyl butyrate as a substrate were mixed with 50 µL of the phosphate buffer. The resulting mixture was kept at 37°C to stop the enzyme activity. Then the samples were cooled, and the absorbance was read at a wavelength of 450 nm. Using P-nitrophenol butyrate, one enzyme unit releases one nanomole of P-nitrophenol per minute at pH = 7 and 37°C.

Measurement of protease enzyme activity was performed by the method of Ranjbar et al. (2011) with some modifications. To measure the protease activity, the 0.01% azocasein substrate was used. 100 µL of the phosphate buffer, 40 µL of azocasein, and 20 µL of the sample were mixed, and the resulting mixture was kept in an oven at 30°C for 60 min. Then, 150 µL of 30% trichloroacetic acid was added to the samples to stop the reaction. The samples were refrigerated at 4°C for 15 min and then centrifuged at 10,000 rpm for 5 min. 100 µL of the resulting mixture was mixed with 100 µL of one molar NaOH and poured into the microplate wells, and the absorbance of the samples was measured at a wavelength of 400 nm.

Statistical analysis. To calculate the LC_{10}, LC_{25}, LC_{50}, and LC_{90} values of *Bt*, the data obtained from the 2nd- and 3rd-instar larvae mortality were analysed. The analysis of the data on the effect of sublethal doses of *Bt* was done using the two-way analysis of variance (ANOVA) and the software SPSS, version 21 (IBM Corp., USA). The normality of the data was determined by the Levene's test, and the assumption of homogeneity of variances was fulfilled ($P > 0.05$). When evaluating the use of resistant cultivar with sublethal doses of *Bt*, the data of mortality from different treatments were transformed by the arcsin(x) method, and then the two-way ANOVA was performed. A comparison of means was done using the Tukey's HSD test at a 5% level.

Results

Bacillus thuringiensis (Bt) bioassay. The *Bt* mortality rates on 2nd- and 3rd-instar larvae of *T. absoluta* on the resistant and susceptible tomato cultivars after 24, 48 and 72 h is presented in Table 1. The LC_{50} values obtained from the effect of *Bt* on the larvae of *T. absoluta* showed that the LC_{50} of *Bt* decreased with time, that is, the mortality increased. The 2nd-instar larvae were most susceptible to *Bt*, and the mortality decreased with increasing larval age.

Table 1. Effect of the LC_{10}, LC_{25}, LC_{50}, and LC_{90} concentrations of *Bacillus thuringiensis* on the 2nd- and 3rd-instar larvae of *Tuta absoluta* on the tested tomato cultivars

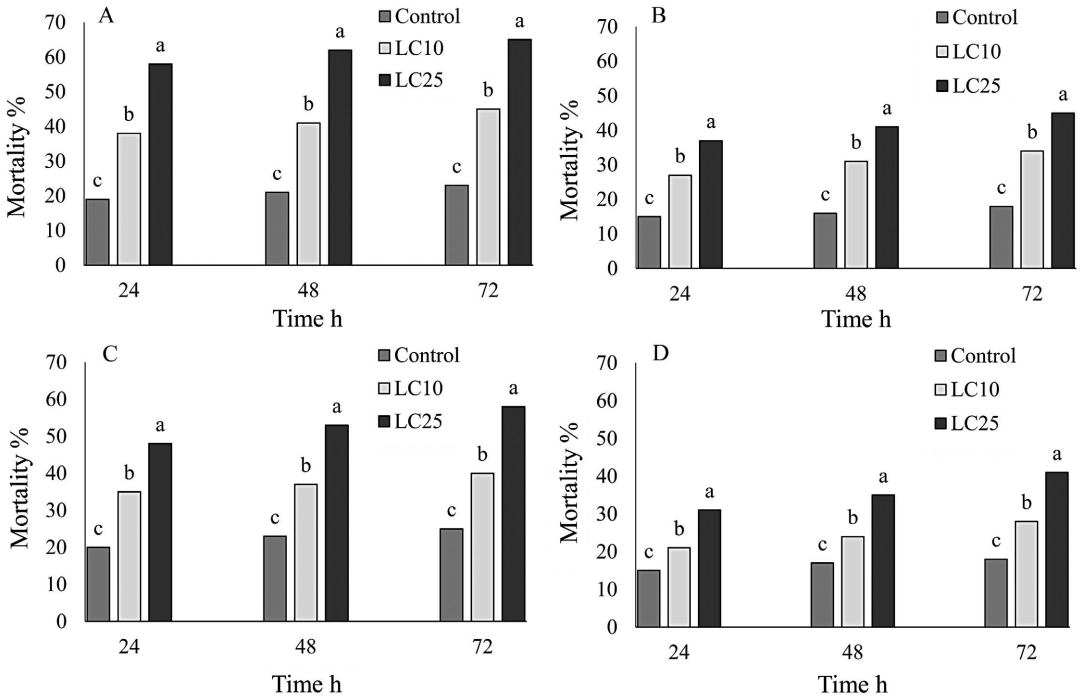
Cultivar	Larval stage	Time h	Slop ± SE	Chi-square	Lethal concentration (LC) ppm				
					LC_{10}	LC_{25}	LC_{50}	LC_{90}	lower–upper limit
'King Stone' (resistant)	2nd	24	3.33 ± 0.53	1.84	694.26	1432.74	2145.49	3781.53	2953.85–4105.32
		48	4.82 ± 0.73	1.53	546.48	1284.10	2013.85	3691.43	2841.19–3971.41
		72	3.90 ± 0.57	0.48	462.43	1122.60	1783.59	3470.93	2438.82–3745.67
	3rd	24	4.43 ± 0.73	2.53	748.52	1958.73	2655.53	4535.84	3773.61–5409.84
		48	4.67 ± 0.88	0.25	632.28	1830.75	2761.81	4321.43	3652.39–5376.51
		72	3.94 ± 0.16	0.64	569.82	1464.64	2157.74	3843.93	3370.27–4934.95
'Rio Grande' (susceptible)	2nd	24	5.30 ± 0.62	2.43	1520.71	2572.84	3259.48	4895.74	4419.29–5565.74
		48	5.23 ± 0.64	3.21	1439.47	2491.38	3195.28	4873.27	4143.48–5282.37
		72	4.93 ± 0.53	2.64	1118.75	2235.84	2939.81	4626.29	3975.72–4973.63
	3rd	24	4.45 ± 0.43	2.73	1889.73	3778.38	3809.48	5689.18	4913.83–6534.73
		48	4.73 ± 0.82	3.34	1710.18	3619.73	3719.53	5279.72	4732.26–6198.72
		72	4.48 ± 0.83	3.73	1636.62	3472.63	3506.83	5192.86	4534.94–5834.18

The results of the analysis of the insecticidal properties of *Bt* on the 2nd-instar larvae showed that the LC_{50} after 24 h was 2145.49 ppm for the resistant 'King Stone' and 3259.48 ppm for the susceptible 'Rio Grande', while the value of LC_{50} for the 3rd-instar larvae was 2655.53 and 3809.48 ppm for 'King Stone' and 'Rio Grande', respectively (Table 1). In both tomato cultivars tested, the *Bt* mortality on the larvae of *T. absoluta* increased with time.

Effect of the sublethal doses of Bt on cultivar resistance. The mortality of the 2nd- and 3rd-instar larvae of *T. absoluta* at the sublethal doses of *Bt* on the resistant and susceptible tomato cultivars at different times is presented in Figure. Significant differences were found between the larval mortaliy at different times in different cultivars at the sublethal doses of *Bt*.

The results of ANOVA for the mortality data of the 2nd-instar larvae of *T. absoluta* due to *Bt* in the tested tomato cultivars at 24 h ($F_{3,11} = 28.43$; $P = 0.002$), 48 h ($F_{3,11} = 35.13$; $P = 0.001$), and 72 h ($F_{3,11} = 32.61$; $P = 0.005$), and for the mortality data of the 3rd-instar larvae of *T. absoluta* due to *Bt* in the tested tomato cultivars at 24 h ($F_{3,11} = 18.45$; $P = 0.001$), 48 h ($F_{3,11} = 15.74$; $P = 0.001$), and 72 h ($F_{3,11} = 28.43$; $P = 0.001$) showed that there was a significant difference in mortality between the treatments at the 95% confidence level. The highest mortality of 2nd-instar larvae (65%) was found in the resistant 'King Stone' at LC_{25} of *Bt* after 72 h, and the lowest mortality of 2nd-instar larvae (15%) was found in the susceptible 'Rio Grande' after 24 h in the control treatment (Figure A and B). Also, the highest mortality of 3rd-instar larvae (58%) was found in the resistant 'King Stone' at LC_{25} of *Bt* after 72 h, and the lowest mortality of 3rd-instar larvae (15%) was found in the susceptible 'Rio Grande' after 24 h in the control treatment (Figure C and D). The results showed that in the resistant 'King Stone', the low concentrations of *Bt* caused a higher larval mortality, while in the susceptible 'Rio Grande', higher concentrations were required for a higher larval mortality. In other words, the host plant resistance increases the susceptibility of larvae to *Bt*. The concentration of *Bt* had an inverse relationship with the resistance of the cultivar, so the resistant 'King Stone' required a lower concentration of *Bt* to control the pests.

Effect of Bt on the nutritional indices of larvae. The results of the effect of the sublethal doses of *Bt* on the nutritional indices of the 4th-instar larvae in two resistant and susceptible tomato cultivars are presented in Table 2. All nutritional indices of the pest were affected by the sublethal doses of *Bt*, and there were significant differences between the two tested cultivars. The efficiency of the conversion of ingested food (ECI), the efficiency of the conversion of digested food (ECD), the approximate digestibility (AD), the relative consumption rate (RCR) and the relative growth rate (RGR) of treated larvae showed significant differences compared to those of the control group. The lowest of ECI (7.82%), ECD (4.34%), RCR (22.29 mg mg^{-1} day^{-1}), and RGR (1.16 mg mg^{-1} day^{-1}) were found in the resistant 'King Stone' at the LC_{25} of *Bt*.



'King Stone' and *B. thuringiensis* on the 2nd- (A) and 3rd-instar larvae (C) of *T. absoluta*, 'Rio Grande' and *B. thuringiensis* on the 2nd- (B) and 3rd-instar larvae (D) of *T. absoluta*

Figure. Mortality of the two-stage *Tuta absoluta* larvae in the tested tomato cultivars under the sublethal doses of *Bacillus thuringiensis* at different times

Table 2. The mean (± SE) of nutritional indices of the 4th-instar larvae of *Tuta absoluta* on two tested tomato cultivars under the sublethal doses of *Bacillus thuringiensis* (*Bt*)

Cultivar	*Bt* concentration	AD	ECI	ECD	RCR	RGR
		%			mg mg^{-1} day^{-1}	
'King Stone' (resistant)	control	49.75 ± 4.43 c	19.46 ± 2.20 b	7.45 ± 0.28 b	34.81 ± 0.13 a	2.85 ± 0.29 b
	LC_{10}	53.24 ± 3.82 b	12.31 ± 1.23 b	5.43 ± 0.52 c	28.63 ± 0.09 c	1.84 ± 0.21 d
	LC_{25}	64.32 ± 3.34 a	7.82 ± 1.19 c	4.34 ± 0.73 d	22.29 ± 0.15 e	1.16 ± 0.19 e
'Rio Grande' (susceptible)	control	47.82 ± 3.23 c	24.12 ± 0.93 a	9.42 ± 0.34 a	35.84 ± 0.01 a	3.15 ± 0.20 a
	LC_{10}	49.19 ± 2.72 c	17.43 ± 1.43 b	6.14 ± 0.15 b	30.74 ± 0.01 b	2.60 ± 0.18 c
	LC_{25}	53.42 ± 3.29 b	12.18 ± 1.63 c	5.36 ± 0.71 c	26.96 ± 0.01 d	1.93 ± 0.16 d

Note. AD – the approximate digestibility, ECI – the efficiency of the conversion of ingested food, ECD – the efficiency of the conversion of digested food, RCR – the relative consumption rate, RGR – the relative growth rate; means in a column followed by different letters are significantly different (Tukey HSD; $P < 0.05$).

Effects of Bt on the biochemical characteristics of 3rd-instar larvae. The results of the sublethal doses of *Bt* on the biochemical characteristics of the 3rd-instar larvae in two tested tomato cultivars are presented in Table 3. All biochemical characteristics of the pest were affected by the sublethal doses of *Bt*, and significant differences were found between the two tomato cultivars tested. The lowest amount of α-amylase (0.98 nM min^{-1} mg^{-1} protein), protease (12.61 μM min^{-1} mg^{-1} protein), and lipase (0.182 nM min^{-1} mg^{-1} protein) and the highest amount of phenoloxidase (0.337 μM min^{-1} mg^{-1} protein) were determined in the resistant 'King Stone' at the LC_{25} of *Bt*.

Table 3. Mean (± SE) of the biochemical characteristics of the 3rd-instar larvae of *Tuta absoluta* on two tested tomato cultivars at the sublethal doses of *Bacillus thuringiensis* (*Bt*)

Cultivar	*Bt* concentration	α-Amylase nM min^{-1} mg^{-1} protein	Protease μM min^{-1} mg^{-1} protein	Lipase nM min^{-1} mg^{-1} protein	Phenoloxidase μM min^{-1} mg^{-1} protein
'King Stone' (resistant)	control	2.28 ± 0.06 a	34.52 ± 2.20 ab	0.298 ± 0.01 ab	0.197 ± 0.01 d
	LC_{10}	1.73 ± 0.05 c	21.84 ± 1.23 c	0.215 ± 0.01 c	0.284 ± 0.01 b
	LC_{25}	0.98 ± 0.01 e	12.61 ± 1.19 e	0.182 ± 0.01 d	0.337 ± 0.01 a
'Rio Grande' (susceptible)	control	2.45 ± 0.04 a	36.17 ± 0.93 a	0.353 ± 0.01 a	0.157 ± 0.01 d
	LC_{10}	2.03 ± 0.05 b	27.82 ± 1.43 b	0.269 ± 0.01 b	0.264 ± 0.01 c
	LC_{25}	2.54 ± 0.02 d	18.19 ± 1.63 d	0.210 ± 0.01 cd	0.321 ± 0.01 a

Note. Means in a column followed by different letters are significantly different at $P < 0.05$ according to Tukey HSD.

Discussion

It is very important to develop alternative pest management strategies aiming for the minimum use of pesticides and conservation of natural enemies for maintaining the ecological balance of the environment. Host plant resistance is of vital importance in the integrated pest management. To implement this, a detailed knowledge of the pest biology on different host plant cultivars is required (Kumari et al., 2022). In the study conducted by Khatami et al. (2022), the demographic characteristics of *T. absoluta* on different tomato cultivars were compared using the age and bisexual stage life table method. To determine the resistance and susceptibility of tomato cultivars to this pest, two resistant ('King Stone') and susceptible ('Rio Grande') cultivars were selected for *Bt* bioassay tests and to study the effect of sublethal doses of that bacterium on those cultivars. The results of the present study showed different effects of the resistance and susceptibility of tomato cultivars on the biological indices of *T. absoluta*. The efficiency and mortality of *Bt* were different in the 2nd- and 3rd-instar larvae and the resistant and susceptible tomato cultivars.

Bacillus thuringiensis (Bt) bioassay. The susceptibility of the 2nd-instar larvae of *T. absoluta* to *Bt* was higher than that of the 3rd-instar larvae, and the mortality of the *Bt* increased with time. These results are consistent with those of other researchers (Giustolin et al., 2001; Kumar et al., 2020). Mollá et al. (2011) reported that the younger instar larvae of *T. absoluta* were highly susceptible to *Bt* compared to the older instar larvae, which is consistent with the results of the present study.

Use of the sublethal doses of Bt combined with cultivar resistance. According to the calculated LC_{50} values, the mortality of the *Bt* on the 2nd- and 3rd-instar larvae was higher in the resistant cultivar than in the susceptible one. Therefore, if the biological control is combined with host plant resistance, the efficiency of the biological agent is increased. The mortality of *Bt* on the pest is increased probably due to the fact that on the resistant cultivar, pests are affected by the chemical or mechanical defense of the host plant. The results of the present study are in agreement with Giustolin et al. (2001), who reported that the application of *Bt kurstaki* on tomato (PI 134417, resistant cultivar) leaves had a synergistic or additive effect with the resistant genotype on larval survival.

Effects of Bt on the nutritional indices of larvae. In this study, food treated with the sublethal doses of LC_{10} and LC_{25} of *Bt* was provided to the 4th-instar larvae of *T. absoluta*, and the nutritional indices including the efficiency of the conversion of ingested food (ECI), the efficiency of the conversion of digested food (ECD), the approximate digestibility (AD), the relative consumption rate (RCR), and the relative growth rate (RGR) were measured. The results showed that all the nutritional indices (ECI, ECD, RCR, and RGR) had a significant decrease in the larvae feeding on the food treated with *Bt* compared to the control, which was strongly correlated to the larval mass, the amount of food consumed, and the waste mass produced. These data suggest that the *Bt* treated leaves showed a decreased amount of α-amylase, protease, and lipase compared to the larvae fed on control leaves. Similar findings were reported by Walker et al. (1998) suggesting that the inhibition of these digestive enzymes with *Bt* results in the reduction of CI, ECI, and ECD.

The highest RCR value of the control larvae demonstrated that the weight of food consumed compared to the average weight gain of the larvae during the feeding period was the highest for those larvae. Also, the RGR value was the highest for the larvae reared on the susceptible cultivar control leaves and the lowest for those reared on the resistant cultivar leaves containing LC_{25} of *Bt*. This demonstrated that the larvae fed on the control leaves of susceptible cultivars had a higher efficiency than the larvae fed on the leaves containing the LC_{10} and LC_{25} concentrations of *Bt* in both resistant and susceptible cultivars depending on the conversion of ingested feed to body mass. The ability of *Bt* to reduce RCR, ECI, and RGR has been observed previously (Shannag et al., 2015). The results of this study were in agreement with those of Nouri-Ganbalani et al. (2016), who reported that the nutritional indices (CI, ECI, ECD, RCR, and RGR) of the 3rd-instar larvae of *Plodia interpunctella* fed diets containing the LC_{30} concentration of *Bt* were significantly lower than those of control larvae.

In the present study, the highest AD value was determined for the larvae that reared on the leaves treated with LC_{25} of *Bt* of the resistant cultivar, while the lowest value of this index was observed for the larvae that reared in the control group of susceptible cultivar. These results are in contrast to Nouri-Ganbalani et al. (2016), who reported that the AD index of the 3rd-instar larvae of *P. interpunctella* diets containing the LC_{30} of *Bt* was significantly lower than those of control larvae. The reason for this difference is possibly related to the different types of pests investigated in the two studies. They did the study on the storage pests, and this study was done on agricultural pests, so the nature of the nutrients of these two pests is different. The results of the study are in agreement with Nathan et al. (2005), who reported that the nutritional indices of the 4th-instar larvae of *Canphalocrocis medinalis* after the treatment with *Bt kurstaki* were decreased compared to the control.

The effect of Bt on the biochemical characteristics of larvae. Alpha-amylases are the main digestive enzymes that act in the first stage of malto-polysaccharides digestion (Da Lage, 2018). The results of present study showed that there was a significant difference in the amount of α-amylase in different concentrations of *Bt*. A few alkalophilic bacteria isolated from the Lonar crater have been introduced as inhibitors of the α-amylase enzyme. The inhibitory property of the enzyme by *Bt serovar finitimus* has been reported by Dhote et al. (2014). One of the most effective conditions for the interaction between insect amylase and amylase inhibitors is the condition of the insect's gut. The activity of the amylase inhibitor was almost constant over the range of pH, and the optimum pH for the amylase inhibitor was pH 9 (Dhote et al., 2014). The pH level of the digestive tube of *T. absoluta* is alkaline, therefore, the inhibitor that has the most inhibitory properties at this pH can control amylase with the appropriate concentration in the interior of the insect's gut (Majidiani et al., 2015).

The hydrolysis of peptide bonds in the insects is carried out by a group of enzymes called proteases. These enzymes are very important in digesting food and breaking down food proteins into amino acids needed by the insects (Terra, Ferreira, 1994). However, inhibiting these enzymes and preventing the formation of essential amino acids leads to the death of insects and microorganisms (Budatha et al., 2008).

The results of the present study showed that the protease enzyme activity of the larvae treated with different concentrations of *Bt* decreased and had a significant difference compared to the control. With the increasing *Bt* concentration, the amount of protease enzyme decreased. The presence of delta-endotoxin of *Bt* in the midgut of the insect causes it to bind to the protein receptors of midgut epithelial cells resulting in the destruction of the cell wall (Jurat-Fuentes, Jackson, 2012), which leads to a decrease in the production of the protease enzyme and a decrease in the activity of this enzyme.

The lipase enzyme breaks carboxyl ester bonds in triacylglycerols, phospholipids, and galactolipids. This group of enzymes plays an important role in the storage, using, and transport of the lipids in insects. Lipases also are the basis of many physiological processes of insects such as growth and development, reproduction, and defense against pathogens (Grillo et al., 2007). The results of this study showed a decrease in the lipase enzyme activity in the larvae treated with different concentrations of *Bt*.

The results showed that the amount of phenoloxidase enzyme increased compared to the control. The phenoloxidase activity plays an important role in the innate immune response of insects (Taleh et al., 2014). The insect prophenoloxidase (PPO) is an important innate immunity protein involved in cellular and humoral defense (Lu et al., 2014). Phenoloxidase is one of the defence components of the insect that playing an important role in the process of blood coagulation, nodule (capsule) melanisation, and wound healing, and it is mainly synthesised by onocytoids (Cerenius et al., 2008). In insects, the phenoloxidase activity leads to the formation of melanin, which is deposited around the capsule and helps to eliminate the external agent. The amount of this enzyme is very low in the hemolymph of healthy insects but increases rapidly in the insects infested with fungi or other pathogens (Cerenius et al., 2008). Similar results have been reported for some pests such as *Eurygaster integriceps* (Zibaee et al., 2011) and *Andrallus spinidens* (Firouzbakht et al., 2016).

Conclusions

1. The mortality of *Bacillus thuringiensis* (*Bt*) on the *Tuta absoluta* larvae was higher in the resistant tomato cultivar 'King Stone' compared to the susceptible cultivar 'Rio Grande'.
2. The use of sublethal doses of *Bt* on the resistant cultivar significantly increased the mortality of the 2nd- and 3rd-instar larvae compared to the susceptible and control cultivars.
3. The nutritional indices and digestive enzyme activity of the larvae treated with the sublethal doses of *Bt* differed significantly between the resistant 'King Stone' and susceptible 'Rio Grande'.
4. The activity of phenoloxidase enzyme increased in the *T. absoluta* larvae treated with the sublethal doses of *Bt* compared to the control.
5. The effect of sublethal doses of *Bt* on all tested traits and indices was significantly higher in the resistant 'King Stone' than in the susceptible 'Rio Grande'.
6. The results showed that combining the *Bt* application with the resistance of host plant, the efficiency of *Bt* increased significantly.

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