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MICROBIOLOGY

Effects of *Bacillus thuringiensis* var. *israelensis* Strains and Toxins on the Pine Processionary Moth *Thaumetopoea wilkinsoni* (Lepidoptera: Notodontidae)

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Abstract—The pine processionary moth *Thaumetopoea wilkinsoni* (Tams, 1926) (Lepidoptera: Notodontidae) is one of the most harmful species that causes destruction in pine ecosystems and also causes critical skin reactions in humans and animals due to its urticating hairs. This study aimed to evaluate the efficacy of different strains [*Bti* ATCC 35646 (wild-type strain), *Bti* pHT315, and *Bti* pHT*ppk* (mutant strain)] of Diptera-targeted *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) and toxins (spore/crystal mixtures) obtained from these strains against the 4th larval stage of *T. wilkinsoni* under laboratory conditions. *T. wilkinsoni* eggs were collected from pine trees at Ondokuz Mayıs University in Samsun, Turkey, in 2022. Pine needles were contaminated with bacterial strains of different concentrations (1×10^7, 1×10^8, 1×10^9 cfu/mL) in 5 mL and toxins in different concentrations (0.15, 0.3, 0.6, 1.25, 2.5, 5 mg/mL) in 2 mL. Larvae were placed in containers with 30 larvae in each group and were observed for 25 days. As a result of the study, *Bti* ATCC 35646 with 63.4% mortality and the lowest LC_{50} value of 1.2×10^9 cfu/mL among the three strains of *Bti* and *Bti* pHT*ppk* with 76.7% mortality and the lowest LC_{50} value of 1.2 mL among the toxins from the *Bti* strains had found to be virulent for *T. wilkinsoni*. The results obtained from our study suggest that Diptera-targeted *Bti* is virulent to *T. wilkinsoni* and that this strain and spore/crystal mixture can be used in the biological control of this species.

Keywords: *Bacillus thuringiensis israelensis*, biological control, pine processionary moth, survival, toxin

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INTRODUCTION

Pinaceae is the largest family of conifers including more than 200 species common throughout the Northern Hemisphere (Farjon, 2017). One of the most important genera belonging to this family is *Pinus*. Distinct parts of trees belonging to the *Pinus* genus are rich in Mg, P, Na, Fe, Cu, and Zn minerals as well as high oil content (Dziedziński et al., 2021). Additionally, *Pinus* species have essential oils that exhibit antioxidant (Abbou et al., 2019; Bouyahya et al., 2019), anticoagulant (Abbou et al., 2019), antibacterial (Demirtaş, 2021), anticancer (Khatamian et al., 2022), antifungal (Karličić et al., 2021), anti-inflammatory (Abbou et al., 2019), anti-hemolytic (Meziti et al., 2019), larvicide (Koutsaviti et al., 2015; Mitić et al., 2019), and herbicide (Hamrouni et al., 2015) properties. Essential oils from these species and the leaves of the trees are used in traditional practices for treating various diseases, including diarrhea, wounds, rheumatism, cough, gastrointestinal diseases, hypertension, and hemorrhoids (El Omari et al., 2021). Besides, the bark, needle, and other parts of these species have been used as excellent raw materials for many years (Li et al., 2015).

The pine processionary moth *Thaumetopoea wilkinsoni* (Tams, 1926) (Lepidoptera: Notodontidae) is a pest invading the *Pinus* species, which is important medically, economically and ecologically. This insect is an important defoliator of pine forests. Pine processionary moth larvae can cause deformations in saplings, stunting, and loss of yield in pine nuts (Aydın et al., 2018), and even indirectly cause tree death by facilitating the attacks of other pests, mostly bark beetles (Barta et al., 2020). Additionally, the larvae of this species also cause health problems by causing painful skin irritation, rashes, and allergic reactions in humans and animals because their hair contains the urticating protein thaumetopoein (Barta et al., 2020).

In addition to being a major forest pest, it has extremely harmful effects in terms of public health, so it is inevitable to control this insect. Chemical and mechanical-physical methods are generally used in

controlling this pest (Cebeci et al., 2010). Considering both the difficulties in mechanical-physical control and the adverse effects of insecticides used in chemical control on human health and other organisms, biological control methods should be preferred in the control of this species (Onaran and Kati, 2010). Various studies have been conducted in this context on nematodes (Karabörklü et al., 2015) and fungi (Güven et al., 2021; Topkara et al., 2022; Yanar et al., 2023), as well as essential oils and leaf extracts (Semiz, 2017; Faria, 2021). Bacteria are the most commonly used species in the control of this species within the scope of biological control (Gindin et al., 2007a, 2008; Yılmaz et al., 2013). *Bacillus thuringiensis* (*Bt*), an entomopathogenic microorganism, is a ubiquitous Gram-positive bacterium (Rabha et al., 2023). *Bt* produces various proteins toxic to different invertebrates (Santos et al., 2022). Protoxins of *Bt* produced in an inactive form are proteolytically activated by the midgut protease of the target insect. Activated toxins interact with midgut receptors in target insects, and then migrate to the cell membrane to form pores, destroying the midgut epithelium and insect death (Bravo et al., 2007; Vachon et al., 2012; Pardo-López et al., 2013). *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) is the first subspecies of *Bt* used as an effective biological control agent against the larvae of many Diptera species in the world (Ben-Dov, 2014). The effectiveness of *Bti* in other insects (Federici and Bauer, 1998; Porcar et al., 2009; Bordalo et al., 2020; Tudoran et al., 2021; Yanar et al., 2022) as well as in flies and mosquitoes (Abuldahab et al., 2019; Nasser et al., 2021; Poulin et al., 2022) has also been examined.

Many studies have been conducted to obtain a hypertoxic mutant strain and increase the efficacy of *Bti* (Doruk et al., 2013; Bahareth et al., 2018; Zorzetti et al., 2018; Valtierra-de-Luis et al., 2020; Ioannou et al., 2021). In one of these studies, Doruk et al. (2013) found that increased polyphosphate (polyP) levels in the cells affect bioinsecticide biosynthesis by *Bti*. They overexpressed the polyphosphate kinase gene in *Bti*, which is responsible for polyP synthesis, and demonstrated that this recombinant strain (*Bti* pHT*ppk*) is approximately eight times more toxic than the wild type against late the 2nd instar laboratory-reared *Culex quinquefasciatus* (Say, 1823) (Diptera: Culicidae) larvae.

This study aimed to determine the effectiveness of different strains of *Bti* [*Bti* ATCC 35646 (wild-type strain), *Bti* pHT315, and *Bti* pHT*ppk* (mutant strain)] and the toxins (spore/crystal mixtures) obtained from these strains against the 4th instar larvae of *T. wilkinsoni*. Most of the studies have shown the effects of bacterial strains on the early stages of insects (Gindin et al., 2007b; Cebeci et al., 2010). In this study, our purpose in using the 4th instar larvae was to determine the effects of the bacterial strains used in the study on the late instar of *T. wilkinsoni* larvae.

MATERIALS AND METHODS

Obtaining Larvae

Thaumetopoea wilkinsoni eggs were collected from the needles of *Pinus sylvestris* L. at Ondokuz Mayıs University Kurupelit Campus in Samsun, Turkey (41°22′26.5116″ N 36°13′17.6340″ E) in 2022 and brought to the laboratory. The eggs were disinfected with 10% sodium hypochlorite (Code: 105614, Merck, Darmstadt, Germany) for about 7 min, and then washed with distilled water for about 7 min and rinsed. The disinfected eggs were taken to the air-conditioning room at a relative humidity of 70% and 24°C (12 h dark/12 h light). The hatched larvae were given pine needles to feed on.

Strains

Bti ATCC 35646 was kindly provided by Gwo-Chyuan Shaw (National Yang-Ming University, Taiwan), control strain harboring pHT315 (*Bti* pHT315) and *ppk* overexpressing strain (*Bti* pHT*ppk*) were from our previous study (Doruk et al., 2013). Briefly, the polyphosphate kinase gene (*ppk*) of *Bti* (GenBank: EAO55476.1) with its own promoter was amplified from the genomic DNA of *Bti* using forward (5' GTGAACAGTCTGCATTAGCAG 3') and reverse (5' GCCGCCAGCACCTTATCCTTG 3') primers and was cloned into pHT315 plasmid (Arantes and Lereclus, 1991). The resultant plasmid (pHT*ppk*) was introduced into *Bti* via electroporation. *Bti* cells carrying empty pHT315 plasmid were used as control.

Protein Extraction for Toxicity Measurements

Proteins were extracted according to the procedure of Donovan et al. (1988). 10 mL of cells (spore/crystal mixture) from 72 h grown cultures were harvested, and washed once with 5 mL of 1 M NaCl (Code: 106404, Merck, Darmstadt, Germany) and twice with 5 mL of deionized water. Then, the cells were suspended in deionized water at 100 mg wet cell weight per mL. Equal amounts of lysis buffer [TE buffer (100 mM Tris-HCl, 20 mM EDTA pH 7.4) containing 10 mg/mL lysozyme] were added to the cell suspension and incubated at 37°C for 30 min. After incubation, 10 μL of 2% SDS was added and the suspension was vortexed for 30 s, centrifuged for 5 min in a microfuge and then the pellet (spore/crystal mixture) was suspended in 50 μL of 0.2% SDS. Protein concentrations were determined by the BCA Protein Assay Method with bovine serum albumin as the standard (Smith et al., 1985).

Larvicidal Activity

Larvae were exposed to spore/crystal mixtures isolated from equal volumes of each bacterial strain. 2 mL of serially diluted spore/crystal mixtures ranging from

5 to 0.15 mg/mL were applied to *P. sylvestris* needles on the boxes. Thirty of the fourth instar larvae were then placed in the boxes and the viability of the larvae was checked daily. On the first day of the experiment, the needles given to the bacteria/toxin applied groups were consumed by the larvae in a short time. Thus, it was ensured that the larvae received the bacteria/toxins into their bodies. After the first needles were consumed by the larvae, fresh needles were given every day to all experimental groups, including the control groups. LC_{50} values were determined at the end of the 25th day by taking the average results of three independent experiments.

The Experimental Setup

The hatched larvae were fed with the needles of the *P. sylvestris* (disinfected with 50% ethyl alcohol, and then washed with distilled water), and the fourth instar larvae were used for the experiment. The experiment was carried out in two stages. In the first stage, different concentrations (1×10^7, 1×10^8, and 1×10^9 cfu/mL) of *Bti* ATCC 35646, *Bti* pHT315, and *Bti* pHT*ppk* strains were poured into separate containers with 5 mL for each dose and spread all over the plant needles. After plant needles infested with bacteria were placed in containers, the 4th instar larvae were placed in containers with 30 larvae per container and three replicates per group. A total of 900 larvae, including the control group, were used at this stage.

In the second stage, different concentrations (0.15, 0.3, 0.6, 1.25, 2.5, and 5 mg/mL) of toxin (spore/crystal mixture) of *Bti* ATCC 35646, *Bti* pHT315, and *Bti* pHT*ppk* strains of 2 mL were applied to *P. sylvestris* needles for each group, and then the 4th instar larvae were placed in containers with 30 larvae per container and three replicates per group. The experiment was carried out in three repetitions and a total of 1710 larvae, including the control group, were used for the experiment. In both stages of the experiment, disinfected *P. sylvestris* needles were given to the larvae in the control group. In both stages, the larvae were observed for 25 days.

Statistical Analyses

The Kaplan–Meier test was used to calculate the survival rates of *T. wilkinsoni* larvae. The survival rates of the *T. wilkinsoni* larvae infected with different concentrations of three different *Bti* strains and toxins from these strains were compared with the control group with the Log-Rank test. The Cox-Regression analysis was used to compare the mortality risk of larvae exposed to three different *Bti* strains and toxins from these strains. The lethal dose (LC_{50}) was calculated using Probit analysis. SPSS version 22.0 was used for these tests.

RESULTS

The survival rates of *T. wilkinsoni* larvae infected with *Bti* ATCC 35646, *Bti* pHT315, and *Bti* pHT*ppk* strains and toxins from these strains are shown in Table 1. Among the groups infected with different *Bti* strains, the lowest survival rate was found in the group infected with the highest concentration (1×10^9 cfu/mL) of *Bti* ATCC 35646 (36.6%). The survival rates in larvae exposed to different concentrations of *Bti* ATCC 35646 were between 36.6–61.1%, in those exposed to *Bti* pHT315 were between 43.3–63.3%, and in those exposed to *Bti* pHT*ppk* were 47.7–64.4. The highest survival rate was in the control group (95.5%). Among the survival rates of *T. wilkinsoni* larvae exposed to toxins from *Bti* strains, the lowest survival rate was in larvae exposed to the highest concentration (5 mg/mL) of *Bti* pHT*ppk* (23.3%). The survival rates in larvae exposed to different concentrations of *Bti* ATCC 35646 toxin were between 44.4–94.4%, in those exposed to *Bti* pHT315 toxin were between 46.7–93.3%, and in those exposed to *Bti* pHT*ppk* toxin were between 23.3–83.3%. It was determined that the highest survival rate was in the control group (97.8%) (Fig. 1).

According to the Log-Rank test results, it was found that the control group was statistically different from the infected larvae exposed to *Bti* strains, but there was no statistical difference between the infected larvae (Table 2). In the groups infected with toxins, it was determined that the control group was statistically different from the larvae of the groups infected with toxins. It was noted that there was no difference between the groups in which the *Bti* ATCC 35646 and *Bti* pHT315 toxins were applied, but the group in which *Bti* pHT*ppk* was applied was statistically different from the other two groups.

According to the results of Cox regression analysis, it was determined that the *Bti* ATCC 35646 strain increased the risk of death 14 times, and the *Bti* pHT*ppk* strain increased the risk of death 11 times. While the *Bti* ATCC 35646 toxin increased the risk of death 14 times, the *Bti* pHT*ppk* toxin increased the risk of death 25 times (Table 3).

LC_{50} values for the 4th instar *T. wilkinsoni* larvae fed with *P. slyvestris* needles exposed to different *Bti* strains and toxins from these strains were shown in Table 4. Accordingly, the *Bti* ATCC 35646 strain had the lowest LC_{50} value (1.2×10^9 cfu/mL), while the *Bti* pHT315 strain had the highest LC_{50} value (1.8×10^9 cfu/mL). Among the larvae exposed to different *Bti* toxins, the lowest LC_{50} value was in the *Bti* pHT*ppk* group (1.2 mL), while the highest LC_{50} value was in the *Bti* pHT315 group (5.3 mL).

Mean survival times for different *Bti* strains and toxins from these strains applied to *T. wilkinsoni* larvae were shown in Table 5. Among the groups exposed to *Bti* strains, the shortest survival time (12.9 days) was found in the group exposed to the highest concentra-

Table 1. Survival rates of *Thaumetopoea wilkinsoni* larvae infected with different *Bti* strains and toxins

				Censored	
Groups (Strains)	Concentrations	Total *N*	*N* of Events	*N*	%
Control	–	90	4	86	95.5
Bti ATCC 35646	1×10^7	90	35	55	61.1
	1×10^8	90	41	49	54.4
	1×10^9	90	57	33	36.6
Bti pHT315	1×10^7	90	33	57	63.3
	1×10^8	90	39	51	56.6
	1×10^9	90	51	39	43.3
Bti pHT*ppk*	1×10^7	90	32	58	64.4
	1×10^8	90	39	51	56.6
	1×10^9	90	47	43	47.7
Groups (Toxins)					
Control		90	2	88	97.8
Bti ATCC 35646	0.15	90	5	85	94.4
	0.30	90	7	83	92.2
	0.60	90	18	72	80
	1.25	90	32	58	64.4
	2.5	90	40	50	55.6
	5.0	90	50	40	44.4
Bti pHT315	0.15	90	6	84	93.3
	0.30	90	9	81	90
	0.60	90	17	73	81.1
	1.25	90	28	62	68.9
	2.5	90	33	57	63.3
	5.0	90	48	42	46.7
Bti pHT*ppk*	0.15	90	15	75	83.3
	0.30	90	25	65	72.2
	0.60	90	35	55	61.1
	1.25	90	45	45	50
	2.5	90	54	36	40
	5.0	90	69	21	23.3

tion of *Bti* ATCC 35646. The survival times of larvae exposed to different concentrations of *Bti* ATCC 35646 were between 12.9–17.7 days, those exposed to *Bti* pHT315 were between 14.7–18.0 days, and those exposed to *Bti* pHT*ppk* were between 16.1–18.0 days. The longest survival time was found in the control group (24.2 days). In the groups exposed to different *Bti* toxins, it was noted that the shortest survival time (10.8 days) was in the group exposed to the highest concentration of *Bti* pHT*ppk*. The survival times of larvae exposed to different concentrations of *Bti* ATCC 35646 toxin were between 15.7–23.9 days, those exposed to *Bti* pHT315 toxin between 16.2–23.8 days, whereas those exposed to *Bti* pHT*ppk* were between 10.8–22.0 days. The longest survival time was in the control group (24.6 days).

DISCUSSION

Bt strains are thought to have insecticidal activity for target pests belonging to one or more orders, but the contrary has been proven in studies (Redmond et al., 2020). In this study, the effectiveness of different strains of Diptera-targeted *Bti* (*Bti* ATCC 35646, *Bti*

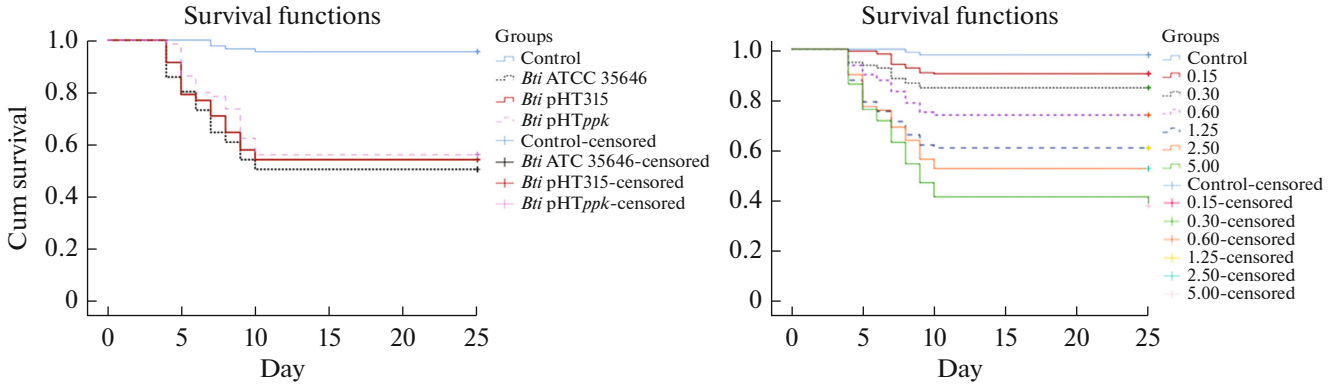

Fig. 1. Cumulative survival rates of *Thaumetopoea wilkinsoni* larvae infected with different *Bti* strains and toxins.

pHT315, and *Bti* pHT*ppk*) and the toxins (spore/crystal mixtures) from these strains against the 4th instar larvae of *T. wilkinsoni* were determined.

It was determined that the highest survival rates of *T. wilkinsoni* larvae infected with different *Bti* strains and toxins from these strains were in the control groups. The lowest survival rate of larvae infected with different strains of *Bti* was found in the group exposed to the highest concentration (1×10^9 cfu/mL) of *Bti* ATCC 35646. The lowest survival rate of larvae infected with toxins from *Bti* strains was noted in the group exposed to the highest concentration (5 mg/mL) of *Bti* pHT*ppk*. There are two interesting results here. The first is that the survival rate of larvae exposed to toxins from strain *Bti* pHT*ppk* is about half that of those exposed to this strain. In fact, the lowest survival rate among the groups exposed to both the *Bti* strains and the toxins from these strains was in the group exposed to the toxin obtained from the *Bti* pHT*ppk* strain. Crystal toxins or δ-endotoxins are considered the main factor conferring insecticidal properties to *Bt* (Bouslama et al., 2020). This idea is in line the result obtained from our study.

The second interesting result is that the *Bti* ATCC 35646 strain at the highest concentration had the lowest survival rate, while the larvae exposed to the spore/crystal mixture from this strain increased in survival rate. This did not meet our expectations because we expected that those exposed to *Bti* pHT*ppk* toxin would have the lowest survival rate in larvae infected with the *Bti* pHT*ppk* strain. The mutant strain we used in our study was obtained from the study conducted by Doruk et al. (2013). The related authors determined that the mutation created a stress situation in the bacteria and the mutant strain produced more sigma factor (σ^E), which enables the adaptation of bacteria to stress conditions, in all samples studied. We think that the toxicity of the mutant strain, which is currently under stress, may have decreased because it has a disadvantage compared to the wild strain in adapting to field conditions and maintaining its viability.

In addition, the survival rates of the groups exposed to both *Bti* strains and toxins from these strains decreased with concentration increases. Guo et al. (2020) reported that the larval mortality rates increased with the concentration increase of the *Bt*

Table 2. Log-Rank (Mantel-Cox) test results of *Thaumetopoea wilkinsoni* larvae infected with different *Bti* strains and toxins

	Control		*Bti* ATCC 35646		*Bti* pHT315		*Bti* pHT*ppk*	
Groups (Strains)	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*
Control			37.551	.000	35.840	.000	51.709	.000
Bti ATCC 35646	37.551	.000			.118	.731	1.674	.196
Bti pHT315	35.840	.000	.118	.731			2.445	.118
Bti pHT*ppk*	51.709	.000	1.674	.196	2.445	.118		
Groups (Toxins)								
Control			25.803	.000	23.273	.000	50.457	.000
Bti ATCC 35646	25.803	.000			.536	.464	30.801	.000
Bti pHT315	23.273	.000	.536	.464			39.326	.000
Bti pHT*ppk*	50.457	.000	30.801	.000	39.326	.000		

Table 3. Cox regression analysis results of *Thaumetopoea wilkinsoni* larvae infected with different *Bti* strains and toxins

Groups (Strains)	B	SE	Wald	df	*p*	Exp(B)
Control			29.781	3	.000	
Bti ATCC 35646	2.592	.508	28.172	1	.000	14.790
Bti pHT315	2.440	.509	25.611	1	.000	13.086
Bti pHT*ppk*	2.592	.509	23.390	1	.000	11.694
Groups (Toxins)						
Control			62.888	3	.000	
Bti ATCC 35646	2.679	.712	14.165	1	.000	14.567
Bti pHT315	2.594	.712	13.274	1	.000	13.389
Bti pHT*ppk*	3.225	.710	20.623	1	.000	25.141

B: Coefficient of regression, SE: Standard error, Wald: Significance of the regression coefficients, df: Degree of freedom, *p*: Significant, Exp(B): Hazard proportion.

Table 4. LC_{50} values of *Thaumetopoea wilkinsoni* larvae infected with different *Bti* strains and toxins

Groups (Strains)	LC_{50}, cfu/mL (*FL*, 95%)	Intercept	Slope ± *SE*	X^2	df^b
Bti ATCC 35646	1.2×10^9	−2.1 ± 0.1	0.3 ± 0.1	1.9	1
Bti pHT315	1.8×10^9	−3 ± 0.1	0.35 ± 0.1	0.3	1
Bti pHT*ppk*	1.7×10^9	−2 ± 0.1	0.3 ± 0.1	1.1	1
Groups (Toxins)	LC_{50}, mL (*FL*, 95%)				
Bti ATCC 35646	3.9	−0.7 ± 0.1	1.0 ± 0.1	2.9	4
Bti pHT315	5.3	−0.7 ± 0.1	1.0 ± 0.0	1.0	4
Bti pHT*ppk*	1.2	−0.8 ± 0.0	1.0 ± 0.0	0.8	4

Cry1Ac toxin applied to *Plutella xylostella* larvae. Ramasubramanian et al. (2022), on the other hand, noted that the larval mortality rates increased with the concentration increases of *Bacillus* applied to the 3rd and the 4th instar *Cnaphalocrocis medinalis*. These findings coincide with our results.

In previous studies, the effectiveness of commercial *Bt* products was evaluated against the larvae of pine processionary moths *T. wilkinsoni* and *T. pityocampa*. Cebeci et al. (2010) applied *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) bioinsecticides Foray 76B and VBC 60074, which were based on a mixture of spores and parasporal crystals of *Btk* strain ABTS-351, to *T. pityocampa* and determined that these insecticides cause 97–99% mortality. Zamoum et al. (2016) in their study, in which they applied Foray 48B (which was based on a mixture of spores and parasporal crystals of *Btk* strain ABTS-351) containing *Btk* 3a and 3b serotypes to pine processionary moth, noted that the mortality rate seven days after the application varied between 41–75% and 14 days after this rate ranged between 73–93%. The effects of *Bt* applied to pine processionary moth on mortality in the EU and some Mediterranean countries have been shown in various studies. Osuna et al. (1994) recorded a mortality rate of 100% for serotypes of H7 (*B. thuringiensis* subsp. *aizawai*), H27 (*B. thuringiensis* subsp. *mexicanensis*), and 3a3b3c (*Btk*), while Ghent (2003) 95–98% for *Btk* strains and Martin et al. (2003) 75–98% for Foray 96 B, Foray 76 SI, and Foray 48 SI (which were based on a mixture of spores and parasporal crystals of *Btk* strain ABTS-351). In Turkey, 100% mortality for *Bt* strain (Besceli, 1969) and 94% mortality for MVP, which was Cry 1AC from *Btk*, (Ozcankaya and Can, 2004) were noted in various studies conducted within the scope of combating pine processionary moth. Gindin et al. (2007b) determined that the larvae were sensitive to *Bt* application in the study they applied Delfin WG (*Btk,* serotype 3a 3b, strain SA-11), Dipel DF, and Foray 48B (both were based on a mixture of spores and parasporal crystals of *Btk* strain ABTS-351) to *T. wilkinsoni*. The results of our study are parallel to the results of all these studies in terms of lethality.

In various studies, the effectiveness of different *Bt* strains against pine processionary moths was also evaluated. Yılmaz et al. (2013) in their study, in which they

Table 5. Means for survival time of *Thaumetopoea wilkinsoni* larvae infected with different *Bti* strains and toxins

Groups (Strains)	Mean[a]				
	Concentrations	estimate, days	std. error	95% confidence interval	
				lower bound	upper bound
Control	–	24.244	.370	23.519	24.970
Bti ATCC 35646	1×10^7	17.756	.965	15.864	19.647
	1×10^8	16.689	.969	14.789	18.588
	1×10^9	12.989	.977	11.073	14.905
Bti pHT315	1×10^7	18.022	.971	16.119	19.925
	1×10^8	16.967	.980	15.047	18.887
	1×10^9	14.767	.958	12.888	16.645
Bti pHT*ppk*	1×10^7	18.011	.992	16.066	19.956
	1×10^8	17.533	.908	15.753	19.314
	1×10^9	16.178	.902	14.411	17.945
Groups (Toxins)					
Control		24.633	.256	24.131	25.136
Bti ATCC 35646	0.15	23.967	.450	23.084	24.849
	0.30	23.367	.593	22.205	24.529
	0.6	21.289	.787	19.746	22.832
	1.25	18.256	.964	16.365	20.146
	2.5	16.711	.987	14.777	18.645
	5	15.744	.999	13.787	17.702
Bti pHT315	0.15	23.867	.448	22.988	24.745
	0.30	23.267	.549	22.191	24.342
	0.6	21.378	.795	19.819	22.936
	1.25	18.722	.987	16.788	20.656
	2.5	18.100	.963	16.212	19.988
	5	16.222	.991	14.281	18.164
Bti pHT*ppk*	0.15	22.056	.697	20.690	23.421
	0.30	19.767	.895	18.013	21.520
	0.6	17.933	.943	16.086	19.781
	1.25	15.800	.980	13.878	17.722
	2.5	14.022	.961	12.139	15.905
	5	10.867	.840	9.220	12.514

applied different *Bt* isolates against *T. wilkinsoni*, determined that the isolates could be used as environmentally safe insecticides to control this pest species. Rausell et al. (1999) applied Cry1B, Cry1C, and Cry1E toxins and *B. thuringiensis*-based bioinsecticides (Cordalene, Dipel, Foray 48B, and Foray 76B) to *T. pityocampa* and determined that Cry1B was toxic to larvae. The results of these studies are consistent with the findings obtained from our study in terms of lethality of *Bt*.

When LC_{50} values were compared, the highest LC_{50} values among all groups were in the groups exposed to the *Bti* pHT315 strain and toxin obtained from this strain. The lowest LC_{50} value for larvae exposed to different *Bti* strains was found in the group exposed to the *Bti* ATCC 35646, and the lowest value for toxins was

determined in the group exposed to the *Bti* pHT*ppk*. These findings are in parallel with the survival data. It can be said that the *Bti* ATCC 35646 strain and the *Bti* pHT*ppk* toxin are the most effective because the low LC_{50} value means that the applied strain is effective even in small amounts. In our previous study (Yanar et al., 2022), where we applied different *Bti* pHT*ppk* concentrations to the 2nd instar *T. wilkinsoni* larvae, we recorded the LC_{50} value as 3.4×10^7 cfu/mL for the group exposed to the highest concentration of *Bti* pHT*ppk*. Although we used the same strain, the reason why we found the LC_{50} value higher compared to our previous study is that we used the 4th instar larvae in our study. As the larval age increases, the LC_{50} value also increases.

CONCLUSIONS

Bti ATCC 35646 with 63.4% mortality and the lowest LC_{50} value of 1.2×10^9 cfu/mL among the three strains of *Bti* and *Bti* pHT*ppk* with 76.7% mortality and the lowest LC_{50} value of 1.2 mL among the toxins from the *Bti* strains had found to be virulent for *T. wilkinsoni*. Results from our study suggest that Diptera-targeted *Bti* is also virulent against one insect from a different order, *T. wilkinsoni*, and that testing different strains of *Bt* with toxins can be developed prospectively as a management strategy for pests. In addition, it emphasizes that the effectiveness of mutant strains in living organisms may be more ineffective than wild-type strains.

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AUTHOR CONTRIBUTION

EFT, OY, and TD designed the experiment; EFT, OY and TD performed bioassays; OY and YT performed the statistical analysis. EFT and OY wrote the manuscript; EFT, OY, TD and YT revised the manuscript. All the authors read and approved the manuscript.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and vertebrate animal subjects. No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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