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Effectiveness of *Bacillus thuringiensis* var. *kurstaki* on *Thaumetopoea solitaria* Frey. (Lepidoptera: Thaumetopoeidae) Larvae in Laboratory Conditions

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Abstract: This study was conducted to determine the effect of *Bacillus thuringiensis* var. *kurstaki* on the larvae of *Thaumetopoea solitaria* in the search for an alternative control method with minimal undesirable side effects. Four larval stages were tested with various concentrations of the bacterium under controlled conditions by dipping pistachio saplings in relevant suspensions and feeding larvae on their leaves. The effect of *B. thuringiensis* var. *kurstaki* was significantly higher on the 1st instar larvae than on the 2nd and the 3rd instar larvae, and the effect was significantly higher on the 2nd instar larvae than on the 3rd instar larvae. LC_{50} for the 4th instar larvae was also greater than that for all the other larval stages and its confidence limits did not overlap with those of the other stages. High larval mortality (78%-100%) was attained in the first week after the treatment especially for the first 3 larval stages with the application of the highest 3 concentrations (10^4, 10^5, and 10^6 µg l^{-1}). The results show that *B. thuringiensis* var. *kurstaki* is a good candidate for suppressing *T. solitaria* populations in pistachio orchards and could be used as a biological control agent against the pest.

Key Words: Biological control, microbial control, biopesticide, entomopathogenic bacteria, dose-mortality test

Bacillus thuringiensis var. *kurstaki*'nin *Thaumetopoea solitaria* Frey. (Lepidoptera: Thaumetopoeidae) Larvalarına Karşı Laboratuar Koşullarında Etkinliği

Özet: Bu çalışma *Thaumetopoea solitaria* mücadelesinde negatif yan etkisi minimum olan bir alternatif metot arayışı içerisinde *Bacillus thuringiensis* var. *kurstaki*'nin zararlı larvalarına etkisini belirlemek amacıyla yürütülmüştür. Bakterinin bir seri konsantrasyonu, antepfıstığı fidanlarının uygun süspansiyonlara daldırılması ve yapraklarının larvalara besin olarak sunulması yöntemi ile kontrollü koşullarda, zararlının dört farklı dönemine karşı test edilmiştir. *B. thuringiensis* var. *kurstaki*'nin etkisinin birinci larva dönemine ikinci ve üçüncü larva döneminden, ve ikinci larva dönemine ise üçüncü larva döneminden önemli derecede daha fazla olduğu tespit edilmiştir. Dördüncü larva dönemine ait LC_{50} değeri diğer dönemlerden daha yüksek çıkmış olup güvenirlik aralığı diğer dönemlerin LC_{50} güvenirlik aralıkları ile çakışmamaktadır. En yüksek üç konsantrasyonun (10^4, 10^5 ve 10^6 µg l^{-1}) uygulandığı ilk üç larva döneminde bir hafta sonunda yüksek larva ölüm oranları (% 78-100) elde edilmiştir. Sonuçlar *B. thuringiensis* var. *kurstaki*'nin antepfıstığı bahçelerinde *T. solitaria* popülasyonlarını baskı altında tutmak için ümit vaat eden bir etmen olduğunu ve zararlıya karşı bir biyolojik mücadele etmeni olarak kullanım olanağını ortaya koymuştur.

Anahtar Sözcükler: Biyolojik mücadele, mikrobiyal mücadele, biyopestisit, entomopatojen bakteri, doz-ölüm testi

Introduction

Turkey is the third biggest producer of pistachio in the world after Iran and the USA, with an average of 51,428.57 tons of pistachio production between 1999 and 2005. Annually, an average of 1438.86 tons of pistachio was exported between 1998 and 2004 (FAO, 2006). Pest organisms are important factors affecting the quality and quantity of pistachio production. As a result of several studies conducted previously, over 40 pest insects and mites were recovered on *Pistachia* species and 20 of

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these were found to have great importance in Turkey (Özer, 1958; Ulu et al., 1972; Çelik, 1975; Günaydın, 1978). An insect pest, *Thaumetopoea solitari* Frey. (Lepidoptera: Thaumetopoeidae), was found to be quite common in pistachio orchards in Turkey (Mart and Karaat, 1990; Mart et al., 1995), and it also exists in Greece (Mourikis et al., 1998), Iran (Davatchi, 1958), and Israel (Haperin, 1983).

Although *T. solitaria* damage is economically important on *Pistachia vera* L., it is also reported to feed on some other *Pistachia* species, *Schinus terebinthifolius* Raddi (Haperin, 1983), *Cupressus sempervirens* L. (Rougeot and Viette, 1978; De Freina and Witt, 1987), and *Fraxinus* L. (De Freina and Witt, 1987). The larvae of the pest first impair pistachio buds in early season, reducing the number of shoots emerging, and then voraciously consume the leaves until pupation. A high pest population, especially on young trees, can leave trees without leaves partially or completely, hampering the development of the trees. As a result, the pest causes a reduction in yield (Mart and Eskalen, 2000; Uygun et al., 2002).

In order to control the pest populations, eggs and larvae in early stages are mechanically killed and/or the larvae are sprayed with chemical insecticides, preferably at early stages. Since the eggs are covered with scales by females, their colour is almost the same as that of branches and thus they are difficult to detect on trees for mechanical pest control. Because of hazardous features of chemical insecticides to both the environment and non-target organisms, alternative means of *T. solitaria* control with minimal negative side effects need to be considered. Mart et al. (1995) suggested the use of *Bacillus thuringiensis* as an environmentally friendly alternative method against *T. solitaria* larvae. Insecticides based on this bacterium are known to be effective, with various successes, against *T. processionea* L. (Martin and Bonneaux, 2006), a pest of oak trees, *T. wilkinsoni* Tams (Gindin et al., 2007a, 2007b) and *T. pityocampa* (Schiff.) (Battisti et al., 1998), pests of pine trees; however, they have not been tested against *T. solitaria*. In this study, we tested various concentrations of *B. thuringiensis* var. *kurstaki* on 4 larval instars of the pest to explore its potential as an alternative biological control agent.

Materials and Methods

The insect:

The insect cultures were started with their eggs collected from pistachio orchards in Gaziantep during the overwintering period. The eggs were kept in paper bags in a refrigerator at +4 °C until the pistachio trees in the region had leaves. The eggs were transferred into controlled room conditions (21 ± 5 °C, 50 ± 5 relative humidity, and 16:8 photoperiod) on different dates to provide larvae at different stages for biological tests. Initially the larvae were kept in 500 ml glass jars incorporated with nylon mesh for aeration. Once the larvae passed the second stage, they were kept in large plastic containers (3-5 l) having large openings covered with nylon mesh on both sides for sufficient aeration. The larvae were provided with fresh pistachio leaves on shoots or small stems daily and the containers were cleaned when required.

Testing *B. thuringiensis* var. *kurstaki* on *T. solitaria*:

The effect of *B. thuringiensis* var. *kurstaki* was tested by providing the larvae with treated 2-year-old pistachio saplings or leaves. A larvicide based on *B. thuringiensis* var. *kurstaki* (Delfin WG, consisting of 32,000 IU mg^{-1} spores) was employed for the biotests. Homogeneous bacterial suspensions were prepared by blending the required amount of the agent in 1 l of distilled water on a magnetic stirrer, and then mixing it into 5 l of distilled water, providing a final amount of 6 l of required bacterial suspension. Considering the preliminary test results (the data are not presented), selected bacterial concentrations were 10^2, 10^3, 10^4, 10^5, and 10^6 µg l^{-1} for the 1st instar larvae, and 10^1, 10^2, 10^3, 10^4, 10^5, and 10^6 µg l^{-1} for the 2nd, 3rd, and 4th instar larvae. The suspensions were prepared separately for each larval stage prior to the onset of the experiment. Control saplings were treated with distilled water alone. The foliage of the experimental saplings was dipped into appropriate bacterial suspensions by tilting and bending the plants so that the whole foliage was in the suspension. After treatment for 10 s, the saplings were left to dry under room conditions (McGuire et al., 1997). Thereafter, 20 larvae were placed on each sapling for the biotests on the 1st and the 2nd instar larvae. Twenty larvae were placed in jars and they were provided with the leaves of the treated saplings daily for the biotests on the 3rd and the 4th instar larvae, since they are very

active. If the larvae are placed on a sapling they leave it and cannot return. The experiment was checked daily to monitor mortality and the dead larvae were removed from the experiment. The whole experiment was repeated twice and conducted under the conditions of 20 ± 5 °C, 50 ± 5 relative humidity, and 16:8 photoperiod. The experiments were terminated either when mortality reached 100%, especially for high concentrations, or 10 days after the onset of the experiment.

Statistical analyses:

Sixth day larval mortality obtained from tests of various concentrations of *B. thuringiensis* var. *kurstaki* suspensions was subjected to probit analyses using Polo-Pc (LeOra, 1987) to estimate the LC_{50} values (the concentration at which 50% of the larvae died) and the slopes of the regression lines. Furthermore, the hypotheses that the lines are the same and that the lines are parallel are tested for larval stages using the same statistics software.

Results

Testing of various concentrations of *B. thuringiensis* var. *kurstaki* on *T. solitaria* resulted in time depended increasing mortality for all the tested larval stages (Figures 1-4). The data indicated that noticeable mortality started on the 2nd day for the 2nd and 3rd instar larvae (Figures 2 and 3) and on the 3rd day for the 1st and 4th instar larvae (Figures 1 and 4). When 10^6 µg l^{-1} was applied, 100% mortality occurred on the 6th day for the 1st instars and on the 7th day for the 2nd and 3rd instars. For the 4th instars the mortality reached 96.67% on the 10th day of the experiment.

Lower concentration treatments were more effective against younger larvae. Treatment with 100 µg l^{-1} killed over 40% of the 1st instar larvae in 1 week (Figure 1) but caused less than 10% mortality on the 3rd and 4th larval stages (Figures 3 and 4). However, the highest concentration was still quite effective on the 4th larval stage (Figure 4). The probit analysis results are presented in the Table. According to the probit analyses, LC_{50} value and slope of regression line increased while intercept decreased as older larvae were subjected to the test. Nonetheless, the results also revealed that the slopes for the 1st, 2nd, and 3rd instar larvae are not significantly different from each other (X^2 = 4.10, d.f. = 2, P = 0.128). Therefore, it can be concluded that the effect of *B. thuringiensis* var. *kurstaki* was significantly higher on the 1st instar larvae than on the 2nd and the 3rd instar larvae, and the effect was significantly higher on the 2nd instar larvae than on the 3rd instar larvae. LC_{50} for the 4th instar larvae was also greater than all the other larval stages and its confidence limits did not overlap with those of the other stages.

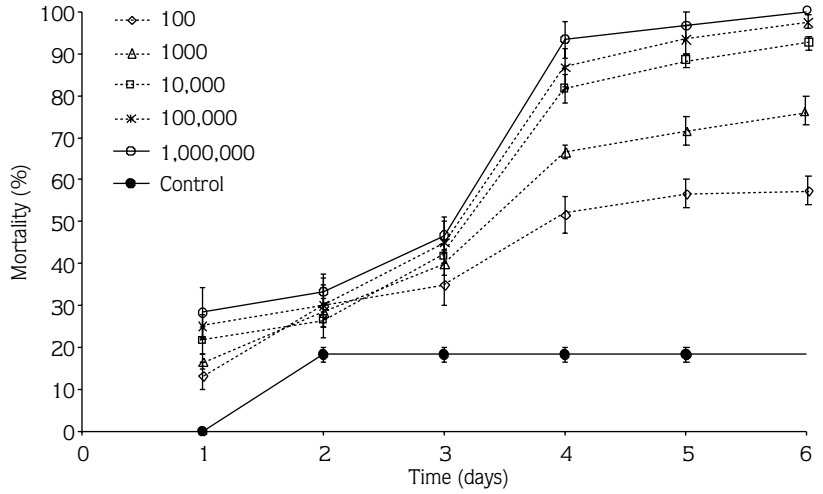

Figure 1. Mortality of 1st instar *Thaumetopoea solitaria* larvae fed on pistachio saplings treated with *Bacillus thuringiensis* var. *kurstaki*. Bars represent standard errors. (concentration unit = µg l^{-1})

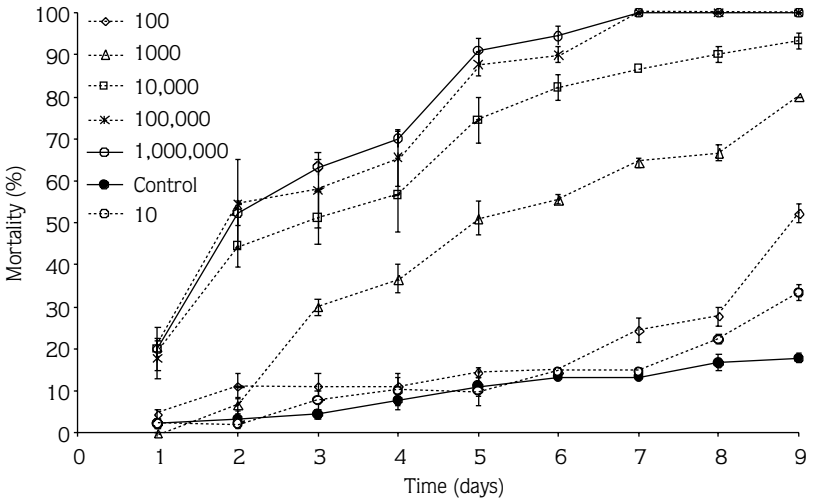

Figure 2. Mortality of 2nd instar *Thaumetopoea solitaria* larvae fed on pistachio saplings treated with *Bacillus thuringiensis* var. *kurstaki*. Bars represent standard errors. (concentration unit = µg l^{-1})

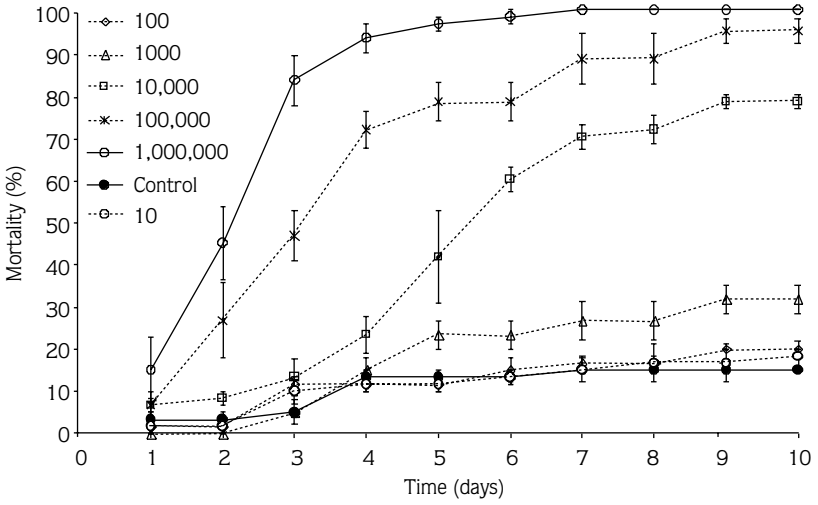

Figure 3. Mortality of 3rd instar *Thaumetopoea solitaria* larvae fed on leaves of pistachio saplings treated with *Bacillus thuringiensis* var. *kurstaki*. Bars represent standard errors. (concentration unit = µg l^{-1})

A positive effect of *B. thuringiensis* var. *kurstaki* application was also evident from the appearances of pistachio saplings during and after the experiments. While the saplings in the control group were left with no leaves, those that received *B. thuringiensis* var. *kurstaki*, especially high concentrations, were almost undamaged by the pest.

Discussion

In order to demonstrate the effect of *B. thuringiensis* var. *kurstaki* to *T. solitaria*, various concentrations of the bacterium were tested against 4 larval instars. Larval mortality increased as the concentration was increased or the exposure period was prolonged. The beginning of noticeable mortality of 1st and 4th instar larvae was 1 day

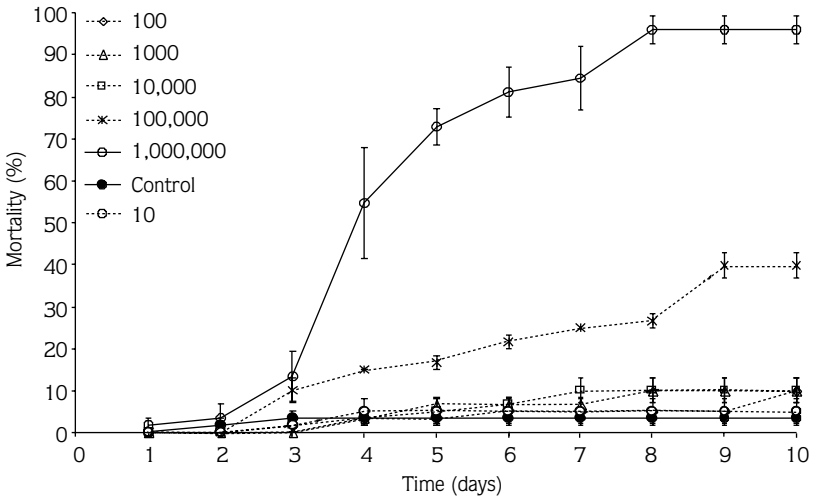

Figure 4. Mortality of 4th instar *Thaumetopoea solitaria* larvae fed on leaves of pistachio saplings treated with *Bacillus thuringiensis* var. *kurstaki*. Bars represent standard errors. (concentration unit = µg l^{-1})

Table. Results of probit analyses of *Bacillus thuringiensis* var. *kurstaki* against *Thaumetopoea solitaria* larvae.

Larval Stage	LC_{50} (µg l^{-1}) (95% confidence limits)	't' for slope	Slope (± s.e.)	intercept	g (95%)	X^2 (d.f.)
I	146.17 (30.79-392.50)	6.19	0.66 ± 0.11	-1.44	0.100	3.48 (13)
II	1891.83 (787.30-3954.39)	11.33	0.73 ± 0.06	-2.41	0.054	24.80 (16)
III	12117.00 (5789.50-21,915.00)	7.31	0.95 ± 0.13	-3.90	0.072	7.67 (16)
IV	0.33×10^6 (0.22×10^6-0.48×10^6)	5.92	1.79 ± 0.30	-9.88	0.110	7.24 (16)

later than that of the 2nd and 3rd instars (Figures 1-4). In the case of 1st instar larvae, this could be due to substantially lower larval intake of the bacterium and/or longer time required for reorganisation of the colony and for initiation of feeding (personal observation). The 4th instar larvae, on the other hand, probably responded later because of basically its larger body size. As environmental impact on the bacterium is possible in field conditions, quick action of the pathogen is desirable. Demolin and Martin (1998) reported that 2 commercial products remained active for 6-8 days in a forest environment. In the present study, high larval mortality was attained in the first week after the treatment especially for the first 3 larval stages with the application of the highest 3 concentrations.

The effect of *B. thuringiensis* var. *kurstaki* was higher on earlier larval stages (Table); therefore, it is likely that the bacterium would be more successful when applied to the larvae as early as possible in field conditions. Early application would also be required to prevent further damage to pistachio trees. The same phenomenon was also reported for the effect of *B. thuringiensis* on the

larvae of *T. pityocampa* (Battisti et al., 1998). A similar age dependent dosage requirement also commonly applies to other insects (Frankenhuyzen et al., 1997; Evans, 1999; Tripathi and Singh, 2003).

B. thuringiensis var. *kurstaki* was found to be highly effective especially against earlier larval stages when the whole foliage was covered by the method employed in this study. However, because it would be difficult to achieve the same coverage of foliage in field applications, and due to probable effects of environmental factors on the bacterium, the concentration required to reach the same mortality level would possibly be higher in field applications. Conditions at the time of application were found to be important for the effectiveness of *B. thuringiensis* var. *kustaki* for the control of *T. processionea* (Martin and Bonneau, 2006). Therefore, further studies are needed, especially on its use in field conditions. Insecticides based on *B. thuringiensis* were already recommended against pine processionary caterpillars in Turkey (Aydınoğlu et al., 2002). Moreover, further studies investigating the natural enemies of *T. solitaria* and their effectiveness could reveal other potential biological control agents, as demonstrated for pine processionary caterpillars (Avcı and Oğurlu, 2002; Doğanlar et al., 2002).

The results of the present study show that *B. thuringiensis* var. *kurstaki* is a good candidate for suppressing *T. solitaria* populations in pistachio orchards and could be used as a biological control agent against the pest once its efficiency is further supported by field trials.

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