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Control of pine processionary moth, *Thaumetopoea pityocampa* with *Bacillus thuringiensis* in Antalya, Turkey

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Abstract: *Taumetopoea pityocampa (Den. and Schiff.) is one of the most common defoliator insects found in Turkey. Although several methods have been used in attempting to control this major forest pest up to now but the problem still remains largely unsolved in Turkey. There is an urgent need to control and minimize the damages caused by these defoliating caterpillars. Therefore, we planned and applied field treatments using by Foray 76B and VBC 60074 to put forward to the efficiency of these bioinsecticides against PPM. The bioinsecticides included in Bacillus thuringiensis subsp. kurstaki (Btk). The vulnerable performance of a single application has been observed in the field trial of these bioinsecticides with mortality rates ranging from 97 to 99% in Turkey's pine forests.*

Key words: *Bacillus thuringiensis sub sp. kurstaki, Pine processionary moth, Pine, Biological control, Mortality percentage*
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Introduction

The pine processionary moth (PPM), *Thaumetopoea pityocampa* (Den. and Schiff) is one of the most important forest pest in Turkey. The larvae of this pest feed on coniferous species, *Pinus brutia*, *P. nigra*, *P. pinaster*, *P. pinea* and *Cedrus libani* into an area of over 1.5 million hectare (Atakan, 1991). Larval defoliation results in decreasing the annual diameter increment of host trees. The diameter decreases have been reported to be from 12% to 65% (Babur, 2002; Carus, 2004; Kanat *et al*., 2005). Defoliated trees can become highly prone to the incidence of secondary insects. These insects can cause to tree mortality (Akkuzu and Selmi, 2002; Avci and Ogurlu, 2002). Therefore, the protection of coniferous forests requires regular application of various control methods against PPM. The control methods involve in mechanical-physical, chemical, bio-technical and biological measures for PPM management. As far as control concerned, the measures mainly based on destroying egg-batches (Ozkazanc, 1987) and winter nests (Acatay, 1953; Besceli, 1969), using by various chemicals (Besceli, 1969; Ozkazanc, 1986 and 1987; Kanat and Sivrikaya, 2004), pheromone traps (Kucukosmanoglu and Arslangundogdu, 2002), vegetal oils (Kanat and Alma, 2004), entomopathogenic fungi (Er *et al*., 2007), parasitoids-predators (Besceli, 1969; Tosun, 1975; Eroglu and Ogurlu, 1993; Avci, 2000; Kanat and Mol, 2008) and microbial pathogens (Besceli, 1969; Ozkazanc, 1986; Ozcankaya and Can, 2004) in Turkey up to now. There are considerable limitations to the control methods widely used in Turkey. The use of mechanical-physical control methods in nature stands put forward to some difficulties due to prove costly over large and the height of the trees. In addition, the health risks associated with PPM's urticant hairs (Turkmen and Oner, 2004) which cause pustules and wounds on the oral and nasal mucosa of humans seriously limit the extent to which labour-intensive mechanical removal of nests can be used as a control method. Since cutting out winter nests on terminal buds deform to host trees and the death of parasitoids-predators into the burned nests, the mechanical-physical methods are insufficient and inappropriate measures. The applications based on chemical preparations effect negatively on environment and the natural enemies of pest are susceptible to these treatments. Furthermore, Kanat and Sivrikaya (2004) reported the need for scientists to explore and focus on alternative biological methods due to population levels of PPM increased rapidly in the year following the chemical application. Pheromone traps which are used for monitoring of PPM populations considered as an insufficient control measure when these are applied lonely against the pest in infected areas. Kucukosmanoglu and Arslangundogdu (2002) tested different types of pheromone trap during 1993 in Turkey. The mean number of PPM captured by one trap was 100 during two weeks. If the egg-batches are supposed to contain about 270 eggs, the collecting of one egg-batch equals to almost activity of three traps. Therefore, this control method is combined with the others for the aim of assistance. Although some of biological methods such as vegetal oils, entomopathogenic fungi, parasitoids-predators resulted in noteworthy outputs according to activations against pest in laboratory, there is no detailed literature about field experiments in Turkey. Since the use of *Btk* preparations for 40 years, they have the acceptance for field and laboratory treatments by international organizations (WHO, 1999).

Some studies and experiments have been conducted to evaluate the effectiveness of *Btk* preparations against PPM by ground spraying in Turkey. Ozcankaya and Can (2004) obtained various results from treatments with different commercial preparations, including *Btk,* in Mugla. Ozkazanc (1986) tested different pesticides at different rates, including *Btk* formulations by spraying pine plantations in Kilis-Hazaltepe. Despite of its efficacy and environmentally friendly profile (Kumar *et al*., 2008), *Btk* usage has not been one of the control agents of PPM in Turkey while it is

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the most widely used method in control of PPM in the EU and in some Mediterranean countries (Kallidis, 1966; Demolin and Milet, 1981; Masutti and Battisti, 1990; Saphir *et al.*, 1997; Navon, 2000; Shevelev *et al.*, 2001; Battisti, 2004; Ben Jamaa, 2005; Niccoli, 2005; Panzavolta, 2005; Pilarska, 2005; Gindin *et al.*, 2007). Also, Btk preparations used against mosquito species show significant results for control (Senthil Kumar *et al.*, 2009)

Our extensive literature search confirmed that there had not been yet any comprehensive study on *Bacillus thuringiensis* subsp. kurstaki *Btk* aerial application in field conditions in Turkey. In this study, our aim was to investigate the efficacy of the improved *Btk* bioinsecticides Foray 76B and VBC 60074 on PPM populations by aerial application.

Materials and Methods

This study was conducted between September 2005 and March 2006 on 8 year-old *Pinus brutia* stands in Antalya-Doyran at 95 m altitude. The previous winter nests remaining from 2005 and egg batches on shoots were observed the pine forests of Antalya at the beginning of the first week of September 2005 to have an idea about population density and infestation levels of PPM. Therefore, the region having four to more winter nests and four to seven egg batches on the young pine trees was considered as an appropriate place to establish the experiment. After that, hatching of eggs were observed to find out the preliminary larval stages, especially L_1 and L_2 instars of PPM with two or three day intervals during the following days till December 2005. The timetable of the field studies and trials can be seen at the Table 1. The treatment was realized when almost all egg batches hatched and nearly 30% of L_1 instars turned to and L_2 stage.

Two different *Btk* formulations were used for checking the effectiveness on PPM in this study; (1) Foray 76B having a potency of 16.700 I.U per mg is a water based formulation, (2) VBC 60074 having a potency of 64 B.I.U per kg is a wettable granule formulation. Both bioinsecticides contain the spores and crystals of *Btk*. We arranged the field trial by means of two-factor randomised block design within three blocks having a size of 15 ha. Each block was subdivided into three plots having a size of 5 ha. The buffer zones were then established to avoid cross contamination from treatments between the blocks and plots (Fig. 1). Foray 76B was applied at the rate of 2.5 litres 50 BIU^{-1} ha^{-1} on three plots. VBC 60074 was applied at the rate of 0.6625 kg + 2.5 litres water 50 $BIUha^{-1}$ on three plots. These bioinsecticides were sprayed at 16 °C, 3 km h^{-1} wind speed and in partly cloudy weather conditions on December 21, 2005 by Pawnee Brave (PA-36) aircraft. The aircraft equipped with 4 Micronair AU 4000 rotary atomizers. During the spraying operation, the aircraft flew 5-7 m above the forest canopy.

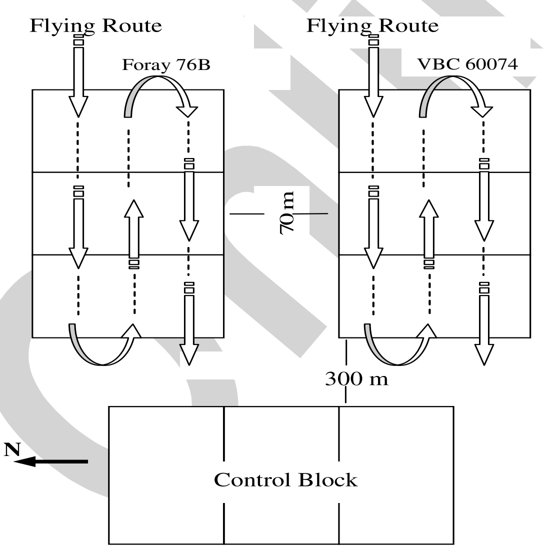

Fig. 1: Flying route of aircraft

After the application on December 21, 2005, twenty-five nests were collected from each plot on January 19, 2006 (P_1), February 09, 2006 (P_2) and March 03, 2006 (P_3). Determination of the numbers of dead and live larvae into the collected nests was counted. There were totally collected 675 nests on shoots during P_1, P_2 and P_3 (Fig. 2). To calculate the percentage of larval mortality, the dead larvae numbers were divided by the dead and live larvae numbers for each nest.

Data were analysed using ANOVA. Before performing ANOVA, data transformation was done with Arcsin. Means were compared with Duncan multiple range test at alpha level of 0.05.

Results and Discussion

The mortality rates obtained in the treatment plots were significantly greater than the mortality rate in the control plots. In

Table - 1: The timetable of field studies and trials

Studies	Time intervals
Determining and establishment of experimental blocks	From end of September, 2005 to Mid-October, 2005
Observation of egg-hatching	From end of September, 2005 to end of October, 2005
Development of L_1 and L_2 stages	From Mid-October, 2005 to Mid-December, 2005
Spraying operation	December 21, 2005
First sampling (P_1)	January 19, 2006
Second sampling (P_2)	February 09, 2006
Third sampling (P_3)	March 03, 2006

Table - 2: Percentages of larvae mortality

Bioinsecticide	Sampling periods		
	P_1	P_2	P_3
Control	$0.97^{a*} \pm 0.34$ n = 75	$0.44^{a} \pm 0.06$ n = 75	$3.15^{b} \pm 0.46$ n = 75
Foray 76B	$97.54^{c} \pm 0.81$ n = 75	$99.83^{d} \pm 0.18$ n = 75	$99.27^{e} \pm 0.46$ n = 75
VBC 60074	$97.30^{c} \pm 0.13$ n = 75	$99.66^{df} \pm 0.48$ n = 75	$99.39^{ef} \pm 0.29$ n = 75

* Means ± SD with different superscript letters are statistically significant for bioinsecticides within the same column and for nest collection periods within the same row as determined by Duncan multiple range test (p<0.05)

Table - 3: Larvae mortality (%) for each plot

Application	Periods	Control plots			Foray 76B plots			VBC 60074 plots		
		I	II	III	I	II	III	I	II	III
1st	P_1	1.33	0.90	0.66	98.23	96.65	97.71	97.43	97.17	97.29
1st	P_2	0.48	0.45	0.37	99.88	99.62	99.96	99.11	99.94	99.93
1st	P_3	2.95	3.66	2.81	99.18	98.85	99.77	99.06	99.64	99.45

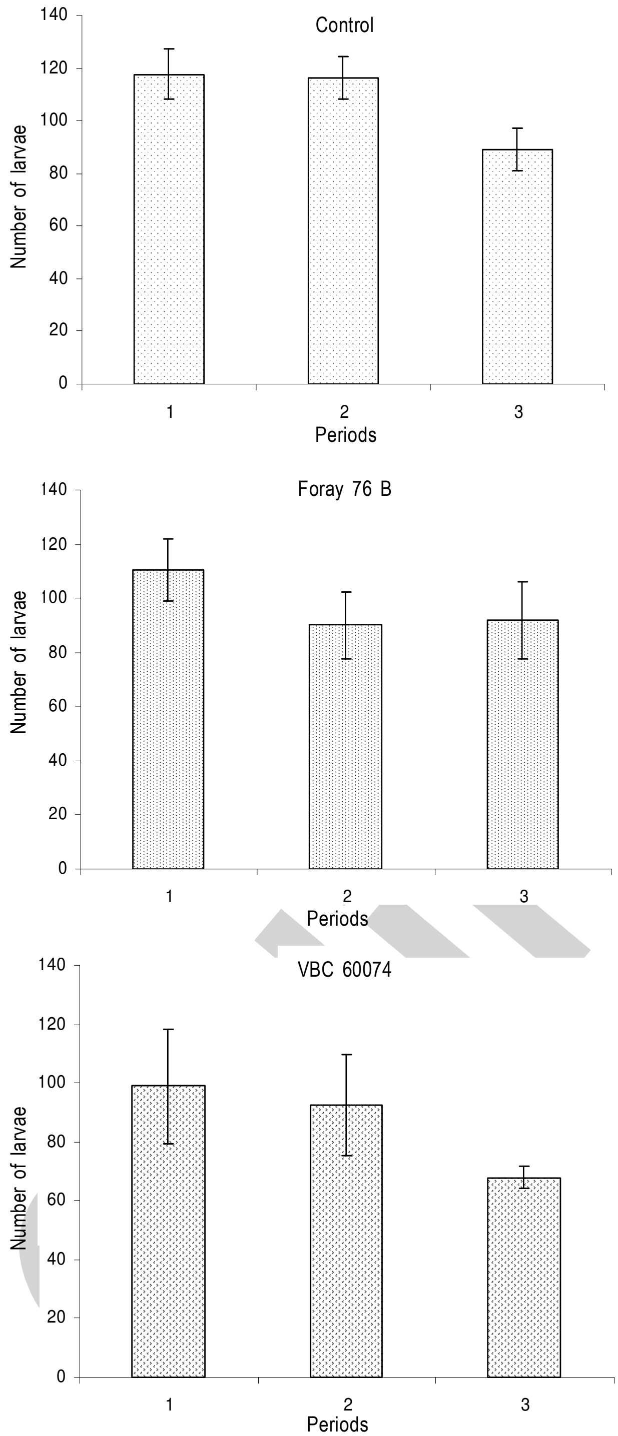

Fig. 2: Number of average larvae (dead and live) in each nest

addition, there was no significant difference between mortality rates of bioinsecticides in both treatment plots (Table 2). The statistical analysis showed a minor difference between Foray 76B and VBC 60074. The plots treated with VBC 60074 had not statistically significant while Foray 76B had a slight statistical contrast according to their effects on the percentage of larval mortality between P_2 and P_3 periods. Although this contrast was not statistically significant at $p<0.05$ level stated that VBC 60074 had a slightly long lasting residual dose than Foray 76B preparation on the needles.

Both the bioinsecticides provided acceptable results, as far as larval mortality was concerned, in 28 days after spraying (Table 3). These results were similar to the results of other authors studying on the Bacillus thrungiensis based products (Besceli, 1969; Ozkazanc, 1986; Avtzis, 1998; Battisti *et al*., 1998; Ozcankaya and Can, 2004).

Various *Btk* commercial preparations were used against PPM by several researchers. According to their studies, the larval mortality rates were 71-80% (Niccoli and Pelagatti, 1986), 96% (El Yousfi, 1990), 93-99% (Sanchis *et al*., 1990), 100% (Osuna *et al*., 1994), 89-100% (Avtzis, 1998), 95-98% (Ghent, 2003), 75-98% (Martin *et al*., 2003), 85% (Orphanides *et al*., 2003) and 100% (Vandenbrouck, 2007) in EU and in some Mediterranean countries. Ground applications of the *Btk* resulted in larval mortality 100% (Besceli, 1969), 40-99% (Ozkazanc, 1986) and 94% (Ozcankaya and Can, 2004) in Turkey. The results of our analysis coincide with those mentioned authors.

In the control plots, average of percentage mortality values showed a slight increase throughout the survey periods although remaining significantly lower than the mortality rate in the treatment plots. The mortality rate differences among P_1, P_2 and P_3 within the control plot can be attributed to the effect of parasitical, predatorial, pathogenetic and other environmental factors. However, the low mortality results obtained in the control plot revealed that the biotical and abiotical factors were not favourable to the natural control of the PPM caterpillar in the area. The percentage mortality values in the control plot were 0.44% after 22 days and 3.15% after 45 days (Table 2). However, the results were not consistent with the study conducted in Greece by Avtzis (1998). The author obtained 40.5 and 52.3% of larval mortality in control plots at almost similar time intervals.

These results suggest that the application of Foray 76B and VBC 60074 gave satisfied results against PPM when used in proper conditions in Turkey's pine forests.

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