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Efficacy of *Bacillus thuringiensis* and Mineral Oil against *Phyllocnistis citrella* Stainton (Lepidptera: Gracillariidae)

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ABSTRACT

A laboratory experiments was conducted to examine the efficacy of *Bacillus thuringiensis* for control of citrus leafminer, *Phylocnistis citrella* Stainton, in isolated citrus leaves in Mazandaran province, Iran. Several preparations of *Bt* tested at the concentrations of 0.5, 1, 3 and 6 g per liter of water with or without mineral oil in these bioassays. In all tests, the bacterial suspensions or mineral oils were applied on the leaves of citrus with second or third instars of leafminer larvae in a leaf-dip bioassay. For all concentrations of *Bt* treatments, with the exception of 0.5% the number of larval mortality was significantly ($P < 0.01$) decreased compared to un-treated control group. The larval mortality positively correlated with *Bt* concentrations. The number of larvae per leaf, were significantly ($P < 0.01$) reduced by treatment with *Bt* plus mineral oil. Treatment with *Bt* plus mineral oil reduced the CLM larvae numbers to a level not significantly different from treatment with *Bt* alone. These data suggest that the activity of *Bt* is enhanced, probably due to increased penetration of *Bt* into leaf cuticles by treatment of mineral oil.

Key Words: *Phyllocnistis citrella; Bacillus thuringiensis;* Mineral oil; Bioassay

INTRODUCTION

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton originated in Asia but now is distributed throughout the five continents where citrus is grown. The CLM is an important pest of citrus and related species of Rutaceae family and some related ornamental plants. The CLM mines leaves, surface tissue of young shoots, stems and less frequently the fruit. The lamina of mined leaves dries and rolls, reducing leaf area and photosynthetic activity of the plant. Although citrus leaf-miner causes indirect damage to young leaves, which predisposes them to infection by canker, controlling citrus leaf-miner is also a vital component of canker management (Belasque *et al.,* 2005). Several insecticides are used against this pest, but these may involve un-desirable effects on the environment, including interference in control of the pest by natural enemies. Biological control is the best option for controlling this pest. The effective control of CLM is very complicated, because of its high migration ability from outside of orchards and the high fertility of CLM. It has been proven that citrus leaf epidermis provides substantial protection for CLM and the difficulty of direct contact of chemical to the larval body (Pena, 1997).

It has been shown that the CLM has also a long history of resistance to many insecticides and development of resistance against the chemicals sometimes makes it difficult to obtain enough control (Mafi & Ohbayashi, 2006). *Bacillus thuringiencis* (*Bt*) subsp *Krustaki* is the bacterial insecticide most widely used for controlling Lepidoptera larvae population (Broderick *et al.,* 2000). It is safe for many non-target insects and has a minimal impact on the environment (Jyoti & Brewer, 1999). Since CLM is protected inside the mine it is suggested that the mineral oils used as a surfactant would reduce the surface tension and increase the penetration of the *Bt* suspension through the leaf epidermis. Petroleum oil reduced infestation by preventing oviposition and there is a negative effect between the number of mines/leaf and concentration of oil (Dias *et al.,* 2005).

The first record of CLM from southern part of Iran dates back to 1961, but in the northern of Iran its presence was noted for the first time in September 1994. Since then, it has shown a dramatic increase and widespread dispersal. Almost all commercial varieties are affected but data on economic losses are not available. The pest has about 5 - 9 generations during the year, with peak periods in early during summer and autumn. Preliminary field trials with selected insecticides indicate the superiority of Dimilin (diflubenzuron) over diazinon, Zolone (phosalone) and Ekamet (etrimfos) in controlling of CLM in the northern Iran, but it was not totally effective (Jafari, 1996). No information on other methods of control or on indigenous parasitoids is available. The aim of the present study was to evaluate the toxicity of the commercial formulation of *Bt* with and without mineral oil on second and third in star larvae of the CLM in laboratory conditions.

MATERIALS AND METHODS

Laboratory bioassays. The toxicity of insecticides against CLM was conducted under laboratory condition using commercial *Bt* pesticides with or without mineral oil at research station of Sari Agricultural and Natural Resources

Table I. The effect of different concentrations of *Bt* (0.5, 1, 3 & 6 gram per liter of water) on percentage of CLM larvae mortality (Mean ± sd)

Treatment	Mean comparison		
	c	b	a
Control	8.49±1.5		
0.5	35.40±7.5	35.40±7.5	
1		40.07±5.3	40.07±5.3
3		46.96±5.7	46.96±5.7
6			61.16±7.5

a, b, c, Means did not followed by the same letters in rows are significantly different ($P<0.01$).

Table II. The effect of different post treatment time of *Bt* on percentage of CLM larvae mortality (Mean ± sd)

Post treatment time (h)	Mean comparison	
	b	a
24	16.81±3.8	
48	35.37±4.64	35.37±4.64
72		47.45±6.70
96		54.10±7.15

a, b, Means did not followed by the same letters in rows are significantly different ($P<0.01$).

University in 2006. The insecticides and respectively concentrations used were *Bt* (0.5, 1, 3 & 6 g L^{-1} of water) in experiment 1, different percentage (0.1, 0.2, 0.3, 0.5) of mineral oil (MO) in experiment 2 and BT (0.5, 1, 3 & 6 g L^{-1}) + 0.5% MO in experiment 3. In each experiment a control group was run using sterile water. The leaf-dip bioassays were devised to test the toxicities of *Bt* pesticide. In assay, only leaves with actively feeding second or third in star leaf-miner larvae were completely excised with petioles from citrus Thomson trees and used for bioassays. To keep the leaves turgescent during the bioassay, each petiole was covered by wet cotton. Leaves were dipped separately for approximately 10 seconds into each treatment. Air-dried for approximately 2 h and placed at the bottom of the plastic petridishes (9 cm diameter × 2.5 cm high). These dishes were lined with a wet filter paper and covered with a plastic lid. The experiment for each treatment was replicated four times along with distilled water treated as a control group. After 24, 48, 72 and 96 h of post-treatment the numbers of live and dead larvae for each replicate were counted under a stereo-micro-scope. Variable measured per replicate of each treatment were the average number of mines per leaf larval mortality (the proportion of larvae that were dead).

Statistical analysis. The experiment was conducted in a completely randomized design using factorial arrangements of treatments (four replications for each treatment). Normality of the data was assessed using probability plots. The normal distributed was approximated for the number of dead larvae per leaf when these data were reciprocally transformed using:

$$ArcSin\ \sqrt{\frac{y}{100}}\ .$$

Mortality data were corrected using Abbott's formula (Abbott, 1925). The analysis of data was performed on each dependent variable using the ANOVA. If a significance effect of variables was calculated, the means were contrasted by Duncan's multiple range tests.

RESULTS

Analyses of variance indicated significant differences among *Bt* treatments ($P < 0.01$). The results clearly demonstrated that the efficacy of *Bt* against CLM increased with increasing *Bt* concentration (Table I). The comparison between different post treatment times showed significant differences ($P < 0.05$) among the periods of the post treatments (Table II). Post-treatment for 96, 72 and 48 h were more effective than 24 h on pest mortality. There was no significant difference between interaction of *Bt* and post treatment time on CLM larvae mortality. Results for different concentrations of *Bt* plus MO indicated significant differences among treatments. The results showed that the treatment of *Bt* plus MO increased the mortality of CLM larvae at higher concentration of *Bt* (Table III). The comparison between different post treatment times showed significant differences ($P < 0.05$) among the periods of the post treatments on CLM larvae mortality (Table IV). No statistically differences were observed in CLM larvae mortality between *Bt* treated groups in comparison with their counterparts *Bt* plus MO groups (Table V).

DISCUSSION

The effect of insecticides in citrus orchards against the CLM is difficult to achieve the maximum CLM larval mortality and it is not very sufficient, because several generations of CLM are usually overlapping and the CLM larvae are protected by a cuticular layer of the leaves in the serpintine mine and the pupal stage is also protected by the rolled leaf margins (Raga *et al.,* 2001). The results of present study clearly demonstrated that the efficacy of *Bt* and *Bt* plus MO against CLM increased with the increasing *Bt* concentration. It is known that the larval mortality varies with spray volume suggesting that oil reduced infestation by acting as an oviposition deterrent (Liu *et al.,* 2001).

Pesticides may be applied to protect new flushes of growth when the leaves are most vulnerable to CLM damage. However, the best foliar insecticides confer only 2 weeks of leaf miner infestations (Michaud & Grant, 2003). These data showed that the *Bt* and *Bt* plus MO are active against the leaf-miner demonstrating that these bio-pesticiedes penetrate into leaf mines, thereby killing the larvae. Recently, the toxicity of different insecticides to the citrus leaf-miner and its parasitoids was evaluated under laboratory conditions in Japan (Mafi & Ohbayashi, 2006). They found that the percentage corrected mortality of eggs of the citrus leaf-miner exposed to insecticides (dipping method bioassay) ranged from 3 to 44%, but all the insecticides tested showed almost over 90% mortality to the first in star larvae of citrus leaf-miner.

It has been demonstrated that using petroleum oil spray residues reduced infestations of CLM by preventing

Table III. The effect of different concentrations of *Bt* (0.5, 1, 3 & 6 gram per liter of water) plus MO (0.5%) on percentage of CLM larvae mortality (Mean ± sd)

Treatments	Mean comparison		
	c	b	a
control	8.48±1.5		
0.5		53.38±3.4	
1		54.72±5.6	54.72±5.6
3		56.25±4.8	56.25±4.8
6			63.13±5.5

a, b, c, Means did not followed by the same letters in rows are significantly different (P<0.01)

Table IV. The effect of different post treatment time of *Bt* plus MO (0.5%) on percentage of CLM larvae mortality (Mean ± sd)

Post treatment time (h)	Mean comparison	
	b	a
24	42.6±10	
48	59.27±9.6	59.27±9.6
72	64.82±8.3	64.82±8.3
96		85.82±5.9

a, b, Means did not followed by the same letters in rows are significantly different (P<0.05)

Table V. Mean comparison between the effect of *Bt* and *Bt* plus MO (0.5%) on percentage of CLM larvae mortality (Mean ± sd)

Treatments	BT	BT+MO
0	8.49±1.51	8.49±1.51
0.5	35.40±7.60	56.04±3.46
1	38.22±5.62	53.73±5.65
3	46.96±5.72	54.24±4.91
6	61.16±7.56	63.21±5.61

oviposition is related to the concentration of oil in sprays and timing of spray (Beattie *et al.*, 1995). Both of Abamectin and Lufenuron pesticides along with petroleum oil provided a significant increase in CLM larval activity (Raga *et al.,* 2001). However, the efficacy of petroleum-derived spray oils used as oviposition deterrents to control citrus leaf-miner is related to timing of spray application the amount of oil deposited on sprayed surfaces (dose) and the persistence of oil molecules on sprayed surfaces or efficacy is also related to increasing molecular weight of oil molecules as reflected by *nCy* values and therefore persistence of oil molecules on sprayed surfaces (Liu *et al.,* 2001). Therefore the petroleum oils alone or combine with microbial agent as emulsifier, which has synergist and less harmful effect for the environment recommended for using in IPM program (Khyami & Ateyyat, 2002). In our experiment, by comparing the activity of the commercial formulation between *Bt* and *Bt* plus MO against the CLM, we observed that the CLM larval mortality was higher (not statistically) in *Bt* plus MO treated groups than the *Bt* alone. Several research groups have shown that, the application of Abamectin in combination with petroleum oil provides the most synergistic effect to control of the *Helicoverpa armigera* and CLM (Wang *et al.,* 2005).

Studies show that neonicotinoid, pyrethroid and growth regulator insecticides have a significant, negative impact on some predators, which are appearing to be the most important biological control agents of leaf-miners. Depending on the rate of insecticide used the number and timing of applications and the level of coverage of the tree. Thus, it is necessary to be aware of the effect of these pesticides on beneficial insects including parasitoid and predators for these reasons it is better to use biopesticides such as *Bt* and/or *Bt* plus MO (Villanueva-Jiménez *et al.,* 2000; Grafton & Gu, 2003). Sometimes the indirect damage of CLM is very important. Mining of immature foliage by the larvae can lead to reduced growth rates, yield and mined surfaces serve as foci for the establishment of diseases such as citrus canker, *Xanthomonas citri*. In the absence of citrus canker, citrus leaf-miner is a serious pest of rapidly growing immature or pruned trees. But in presence of citrus canker, it is a major pest of both immature and mature trees (Liu *et al.,* 2001). Therefore it is important to select less toxic chemicals against the natural enemies in order to expect both the activity of natural enemies and control effect of insecticides for suppressing the infestation of CLM. The higher activity of *Bt* in *Bt* plus MO treated groups at the present study may be due to increased penetration of *Bt* through the mine by helping of MO.

For better understanding it is necessary to investigate the third generation pesticides such as growth regulators in combination with mineral oil, microbial and fungi insecticides to get much more suitable results in the field conditions. However, more field studies will need to be performed to understand the effect of *Bt* and *Bt* plus MO against *P. citrella* and to determine the optimum timing of the multiple application.

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