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Insecticidal Effect of *Bacillus thuringiensis* var Kurstaki on the Various Instars Larvae of *Plutellax ylostella* L. (Lep.: plutellidae) Under Laboratory Condition

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ABSTRACT

Due to economic importance of diamondback moth pest and resistance to conventional insecticides, it is necessary to use novel and suitable compounds in control programs. Therefore, we evaluated the toxicity of *Bacillus thuringiensis* to four instars larvae of diamondback moth, *Plutella xylostella* (L.). In this study 1st, 2nd, 3rd and 4th instars larvae were exposed to different concentrations of *Bacillus thuringiensis*. The exposure times were 24, 48 and 72 h for oral trials. Experiments were performed in complete randomized block design with four replications. After treatment the samples were held under constant conditions in laboratory rearing room (25±2°C, 50±5% RH and 14 and 10 hrs. L: D photoperiod). The maximum mortality rate for 1st, 2nd, 3rd and 4th instars larvae in 90, 140, 200 and 250 ppm of *Bacillus thuringiensis* was achieved 98.33, 97.67, 96.67 and 90% after 72 h, respectively. Our results suggest *Bacillus thuringiensis* could be an important agent in control of larval instars of *Plutella xylostella*.

Key words: *Bacillus thuringiensis*, Bioassay, cabbage, *Plutella xylostella*

INTRODUCTION

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: plutellidae) is the most destructive insect pest of cruciferous crops throughout the world (Talekar and Shelton, 1993).The pest was controlled easily with insecticides until 1980 when severe failures of pesticides began to occur and progressively grocers reported resistance problems (Dunhawoor *et al.*, 1998). New insecticides are continuously being developed as existing insecticides become useless, but *Plutella xylostella* has developed resistance very quickly to many of these (Nisin, *et al*. 2000); (Shelton, *et al.* 2000). Microbial insecticides are a promising alternative, the most widely used microbial insecticide. *Bacillus thuringiensis* Berliner, is highly toxic to certain pests, yet it has little or no adverse effect on most non target organisms, including humans (Flexner *et al*. 1986); (Wilcox, *et al.* 1986).

Bacillus thuringiensis is a rod-shaped, gram-positive, soil bacterium that produces crystalline inclusions during sporulation. *Bacillus thuringiensis* is not a single entity, but a highly diverse one with about 50 serotypes that are further subdivided into 63 serovars based on H-flagellar antigen technique (Thiery and Frachon, 1997). *Bacillus thuringiensis* is especially useful for control of diamondback moth, a worldwide pest of cruciferous vegetables. *Bacillus thuringiensis* does not harm the hymenopterous parasitoids of diamondback moth (Brunner and Stevens, 1986), but it is highly effective against diamondback moth that are resistant to conventional insecticides (Sun, *et al.* 1986). The objective of the

present study was to investigate the different doses effects of *Bacillus thuringiensis* on the various instars larvae of *P. xylostella* under laboratory conditions and determination of concentration at which maximum mortality occur.

MATERIALS AND METHODS

Insects

Plutella xylostella larvae and pupae were collected from cabbage (*Brassica oleracea* var. *capitata*) in an experimental field of the college of Natural Resource and Environment, during February 2010 in the Urmia area (Iran) agricultural university. Pest were reared in a colony of 2-3 generations in an automatic climate apparatus at (25±2) ^{o}C, under a 14: 10 L: D photoperiod, and fed on cabbage seedlings. The 1st , 2nd, 3rd and 4th instars larvae were used in bioassay.

Bioassay and determination of different doses concentrations

A leaf dip bioassay method was followed as described by Tabashnik, *et al.* (1991) using fully opened cabbage leaves. The leaves without the inside of the main plant from separate pots, were first washed with distilled water containing 0.1% Triton X-100 thoroughly and dried. Leaf disc of 5±0.5 cm diameter were cut from cabbage leaves and dipped in solutions of different concentrations prepared with *B. thuringiensis*. The tests of the Biturin *B. turingiensis* var *kurstaki* toxin product of Biotechnology Companies, mehr Asian (Mabko) in Iran (Semnan) in solution 3.6 percent of the materials were used effectively. Each disc was dipped for 10-15 s. and allowed to air dry for a period of 1 h. Then the discs were placed individually into small Petri dishes (7 cm diameter). There were five concentrations ranging from 30 to 90 ppm for the1st instars, 50 to 140 ppm for the 2nd instars, 90 to 200 ppm for the 3rd instars and 150 to 250 ppm for the 4th instars. Larvae were allowed to feed for 72 h at 25±2^{o}C and more than 50% R. H. For toxicity bioassay experiment, 1st, 2nd, 3rd and 4th instars larvae were treated by oral application through cabbage leaf discs. Larval mortality was recorded up to 72 h of treatment. Because diamondback moth is such a sensitive insect to *B. thuringiensis* the 72 h mortality data observed were more efficient, and were strongly associated with results from longer bioassays.

Data analysis

Abbott's correction (Abbott, 1925) was applied to all data in the dose-response experiments. Larvicidal activities of *B. thuringiensis* of the different crud extracts and fractions were statistically analyzed by general linear model (univariate) analysis of variance. The data were transformed by Arcsin$\sqrt{\%}$. Probit analyses were done to calculate median lethal concentration (LC_{50}) using SPSS 19 version software package.

RESULTS

In all four instars of the diamondback moth, effects of concentration and time were significant as was the interaction between concentrations and time. Mortality increased with increasing concentration and time in all instars. The susceptibility of the 1st instar to *B. thuringiensis* were analyzed by leaf dip bioassay and mortality at 30, 39, 52, 68 and 90 ppm doses determined for 1st instar larvae at 24, 48 and 72 h after application is shown in Table 1. In the 1st instar the LC_{50} values at 48 and 72 h after treatment were very similar, at 0.994 and 0.822 ppm, respectively. Therefore, the LC_{50} (0.822 ppm) applied for 72 h were highest mortality concentration in our study. Using the formulaω^2, the results for 1st instar, ω^2 =0.90 for concentration, ω^2=0.064 for time.

Table 1.Toxicity of *Bacillus thuringiensis* to 1st instar *Plutella xylostella*

LC_{50} (ppm)	**95% Confidence limit**		Intercept	**Slope ± SE**	*p*	X^2	Treated time (h)
	Lower	**Upper**					
0.587	1.393	2.372	0.814	3.513±0.372	0.175	1.360	24
0.455	0.994	1.413	1.397	4.080±0.387	0.749	1.216	48
0.385	0.822	1.116	1.774	4.283±0.416	0.452	2.632	72

The susceptibility of the 2nd instar to *B. thuringiensis* was analyzed by leaf dip bioassay and mortality at 50, 66, 85, 110 and 140 ppm doses determined for 2nd instar larvae at 24, 48 and 72 h after application is shown in Table 2. In the 2nd instar the LC_{50} values at 48 and 72 h after treatment were very similar, at 0.688 and 0.593 ppm, respectively. Therefore, the LC_{50} (0.593 ppm) applied for 72 h were highest mortality concentration in this study. Using the formula ω^2, the results for 2nd instar, ω^2 =0.88 for concentration, ω^2=0.077 for time.

Table 2: Toxicity of *Bacillus thuringiensis* to 2nd instar *Plutella xylostella*

LC_{50} (ppm)	**% Confidence limit**		Intercept	Slope ± SE	*p*	X^2	Treated time (h)
	Lower	**Upper**					
1.003	2.129	3.385	-0.005	4.027±0.411	0.719	1.345	24
0.688	1.306	2.793	0.601	3.756±0.403	0.014	2.982	48
0.593	1.018	2.085	0.838	3.692±0.419	0.964	0.278	72

Also sensitivity of 3rd instar to *B. thuringiensis* at 90, 110, 132, 162 and 200 ppm doses determined for 3rd instar larvae at 24, 48 and 72 h after application is shown in Table 3. In the 3rd instar the LC_{50} values at 48 and 72 h after treatment were different, at 1.219 and 0.933 ppm, respectively. Therefore, the LC_{50} (0.933 ppm) applied for 72 h were highest mortality concentration in our study. Using the formula ω^2, the results for 3rd instar, ω^2 =0.90 for concentration, ω^2=0.06 for time.

Table 3: Toxicity of *Bacillus thuringiensis* to3rd instar *Plutella xylostella*

LC_{50}(ppm)	**% Confidence limit**		Intercept	Slope±SE	*p*	X^2	Treated time(h)
	Lower	**Upper**					
1.371	1.284	1.467	-0.562	4.106±0.498	0.802	0.998	24
1.219	1.144	1.290	-0.409	4.765±.517	0.870	0.714	48
0.933	0.812	1.021	0.118	3.884±0.534	0.767	1.142	72

The susceptibility of the 4th instar to *B. thuringiensis* was analyzed by leaf dip bioassay and mortality at 150, 170, 190, 220 and 250 ppm doses determined for 4th instar larvae at 24, 48 and 72 h after application is shown in Table 4. In the 4th instar the LC_{50} values at 48 and 72 h after treatment were similar, at 1.952 and 1.711 ppm, respectively. Therefore, the LC_{50} (1.711 ppm) applied for 72 h were highest mortality concentration in this study. Using the formula ω^2, the results for 4th instar, ω^2 =0.89 for concentration, ω^2 =0.064 for time.

Table 4: Toxicity of *Bacillus thuringiensis* to 4th instar *Plutella xylostella*

LC_{50}(ppm)	95% Confidence limit		Intercept	Slope±SE	*p*	$\mathbf{X^2}$	Treated time (h)
	Lower	Upper					
2.204	3.195	4.123	-2.745	7.998±0.838	0.322	3.488	24
1.952	3.015	3.926	-2.040	7.023±0.783	0.975	0.215	48
1.711	2.646	3.270	-1.697	7.278±0.813	0.378	3.078	72

The result shows that the concentration factor was more important than time in all four larval instars. Comparison of mortality on the various instars larvae of *Plutella xylostella* to *B. thuringiensis* by leaf dip bioassay in different concentrations and times (Table 5).

Table 5: Comparison of mortality on the various instars larvae of *Plutella xylostella* to *Bacillus thuringiensis* by leaf dip bioassay in different concentrations and times.

	Comparing the average concentrations					Times are compared(h)		
Instar	**1**	**2**	**3**	**4**	**5**	**24**	**48**	**72**
1st	29.432 ± 0.567^{e}	36.432 ± 0.634^{d}	50.623 ± 0.430^{c}	58.545 ± 0.467^{b}	66.572 ± 0.726^{a}	34.343 ± 0.241^{c}	41.952 ± 0.715^{b}	47.932 ± 0.820^{a}
2nd	28.889 ± 0.572^{e}	38.163 ± 0.402^{d}	49.961 ± 0.325^{c}	59.514 ± 0.952^{b}	65.986 ± 0.235^{a}	32.491 ± 0.625^{c}	41.767 ± 0.474^{b}	48.762 ± 0.775^{a}
3rd	33.789 ± 0.522^{e}	42.737 ± 0.525^{d}	50.624 ± 0.464^{c}	58.545 ± 0.148^{b}	66.572 ± 0.490^{a}	36.887 ± 0.628^{c}	41.440 ± 0.439^{b}	48.761 ± 0.525^{a}
4th	25.309 ± 0.314^{c}	34.231 ± 0.48258^{d}	44.75 4± 0.699^{c}	55.177 ± 0.835^{b}	60.614 ± 0.601^{a}	29.278 ± 0.314^{c}	37.095 ± 0.489^{b}	44.624 ± 0.350^{a}

*Dissimilar letters indicate significant differences statistically significant at the 5 percent level by the Tukey test.

DISCUSSION

A recent survey of biopesticide researchers working in developing countries indicated that formulation was the most important issue in the development of biological insecticides (Harris and Dent, 2000). Microbial pesticides, particularly *B. thuringiensis*, are likely to become increasingly important as pest resistance and environmental concerns reduce the usefulness of conventional insecticides. Although laboratory selection has increased resistance to *B.thuringiensis* in several species of insects McGaughey, (1985); mcGaughey and Beeman, (1988); Stone, *et al.* (1989); Miller, *et al.* (1990). The results of experiments in this study show that concentrations of 90, 140, 200 and 250 ppm on 1st, 2nd , 3rd and 4th instars larvae provides the highest mortality base on intestinal activity of *B. thuringiensis*, respectively. The greatest casualty is being treated after 72 hours. The results of this study, in various instar larvae of this pest with Talekar and Griggs, (1986); Krieg and Langenbruch, (1981); Johnson *et al*., (1990) and Van Rie *et al*., (1990) is somewhat similar, test results, but different Mohan and Ggjar, (2000) and Tang *et al.*, (1997), that reason, not using this toxin is against this pest in Iran, Protection of crucifer crops from damage often requires application of insecticide to plant foliage, sometimes as frequently as twice per week. However, resistance to insecticides is widespread, and includes most classes of insecticides including some *Bacillus thuringiensis* products. Rotation of insecticide classes is recommended, and the use of *B. thuringiensis* is considered especially important because it favors survival of parasitoids. Even *B. thuringiensis* products should be rotated, and current recommendations generally suggest alternating the kurstaki and aizawa strains because resistance to these microbial insecticides occurs in some locations.

In summary, as in most cases, the best opportunity to manage resistance to *B. thuringiensis* in diamondback moth is to take action before resistance occurs. *B. thuringiensis* should be used judiciously to conserve its efficacy against diamondback moth. Management programs that emphasize biological and cultural controls can integrate *B. thuringiensis* and other insecticides

sparingly, thereby prolonging their usefulness.

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