Effects of *Helicoverpa armigera* Nucleopolyhedrosis Virus (HaNPV) on the Larvae of the Diamondback Moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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Abstract

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Laboratory studies were performed to determine the insecticidal activity of baculovirus against diamondback moth, *Plutella xylostella*. The nucleopolyhedrosis (HaNPV) was tested against 2nd instar larvae fed on cabbage leaf disks treated with aqueous suspensions of occlusion bodies (OB). Lethal concentrations values (LC_{25}, LC_{50}, and LC_{75}) were 2.2×10^3, 3.8×10^4, and 6.6×10^5 PIB/ml for 2nd larval instars, respectively. Median lethal time (LT_{50}) to similar response levels (mortality rates 50–75%) decreased with decreasing larval age (from 114.23 to 106.05 h). Larval development time and pupal weight were not affected by different concentrations (LC_{25}, LC_{50}, and LC_{75}) of HaNPV. Significant differences were found in the pupal rate and adult emergence in larvae treated by different concentrations. In conclusion, HaNPV treatment failed to cause high mortality rates in *P. xylostella* larvae, but it had prompt deleterious effects on survivor's development and emergence.

Keywords: DBM; HaNPV; LC_{50}; LT_{50}; biological parameters

Diamondback moth, *Plutella xylostella* (Linnaeus, 1753) (Lepidoptera: Plutellidae) is a major pest of cruciferous plants around the world and in Iran (Talekar & Shelton 1993; Mahmoudvand *et al*. 2011). It is an oligophagous pest and feeds on plants that contain mustard oil and glycosides (Ooi 1986). *P. xylostella* is known to be resistant to main groups of insecticides (Perez *et al.* 1995), ranking among the twenty most resistant pest species reported up to now (Mota-Sanchez *et al*. 2002; Mohan & Gujar 2003; Shelton 2004).

According to many researchers, to eliminate the problem of pest resistance to chemical pesticides, an integrated pest management program (IPM) should be used. In line with this, the uses of microbial control agents (MCAs) are discussed. Baculovirus-based insecticides are firm candidates for effective and environmentally-safe control agents (Moscardi 1999). The taxonomy of the family *Baculoviridae* genera has recently been changed on the basis of the hosts. Now there are four genera: the *Alphabaculovirus* (lepidopteron-specific nucleopolyhedro virus), *Betabaculovirus* (lepidopteron specific granulovirus), *Gammabaculovirus* (hymenopteron-specific nucleopolyhedrovirus), and *Deltabaculovirus* (dipteron-specific baculovirus) (Jehle *et al*. 2006). The nucleopolyhedrosis (NPV) is a natural microbial pathogen of lepidopteron pests and it infects insect midgut cells and causes chronic infections (Kumar *et al*. 2008). NPV products are commercially available for heliothine pests in the USA and Australia (Mensah 2002; Raymond *et al*. 2006). NPVs have limitations as insecticides, especially due to their production costs. Through the build-up of infectious particles and secondary cycles of infection in pest populations, they can be an effective means of long-term

population control (Young 1998; Bonsall 2004). This in turn can lead to increased spray intervals and reduced costs for growers (Moscardi 1999). Pathogenicity and virulence of the baculovirus are the most frequently evaluated parameters. According to Tanada and Fuxa (1987) and Shapiro *et al*. (2005), pathogenicity can be defined as the ability of an organism to cause disease and virulence refers to the degree of pathology caused by the organism. The screening of highly-virulent isolates is the first step in the biopesticide development, but other factors need to be taken into consideration, including persistence, host range, and impact on non-target insects (Shapiro *et al*. 2005). The purpose of the present study is to explore further effects of HaNPV on larval mortality and debilitating effects on *P. xylostella*.

MATERIAL AND METHODS

Insect rearing. The initial *P. xylostella* colony was collected in August of 2010 from cauliflower fields of Shahre-Rey, south of Tehran, Iran. For egg laying, about 500 adults of *P. xylostella* were placed in a plastic cage (60 × 40 × 40 cm) and eggs were transferred to leaves of cauliflower, *Brassica oleracea* var. *botrytis* cv. White Cloud as food material to continue their development. Insect stock was maintained at 25 ± 1°C and 65 ± 5% relative humidity under a 16 h light : 8 h darkness photoperiod in a growth chamber.

Nuclear polyhedrosis virus preparation. *Helicoverpa armigera* nuclear polyhedrosis virus (HaNPV) was provided by the Plant Protection Research Institute of Iran. 10-day old larvae of *H. armigera* were infected with a viral suspension of 1×10^7 PIB/ml on the artificial diet. After 4 days, the infected cadavers were collected and homogenised by a homogeniser in Tris-HCl 50mM (pH = 7.2). The viral suspension was filtered through a two-layer filter cloth to remove large debris and the filtrate was centrifuged at 700 *g* for 1 min and the supernatant was centrifuged at 3800 *g* for 10 minutes. Finally, pure polyhedral inclusion bodies were separated from the supernatant and resuspended in 50mM Tris-HCl (pH 7) and stored in a deep freezer (Navon & Ascher 2000). The number of PIBs/ml was determined by using Neubaur Hemocytometer (tiefe depth profondeur 0.100 mm and 0.0025 mm^2 area; HBG GmbH, Frankuft am Main, Germany). For experiments, viral suspensions ranging 10^2–10^5 PIBs/ml was prepared by diluting with 50mM Tris-HCl.

Bioassay procedures. Seven concentrations of HaNPV from 10^2 to 10^5 PIB/ml were used for the experiment and each was replicated three times. For each replicate ten 2nd instar (6-day old) larvae of *P. xylostella* were placed individually in paper cups (6 × 3 cm) containing two cabbage leaf discs (1 cm diameter). Fresh leaves used in the experiment were previously surface sterilised in 0.5% sodium hypochlorite solution for 10 min and washed three times in sterile distilled water. The leaf discs were contaminated by 100 µl of the appropriate concentration of HaNPV. Larvae fed on leaf discs treated with only 50mM Tris-HCl served as control. The larvae were allowed to feed on the HaNPV treated leaf discs for 48 hours. Surviving *P. xylostella* larvae were then transferred to individual cups. Fresh untreated leaves (surface sterilized in 0.5% sodium hypochlorite solution for 10 min and washed three times in sterile distilled water) were fed to all the larvae from day 3 of the experiment.

Bioassay was conducted at 25 ± 1°C and 65 ± 5% humidity under 16 h light : 8 h darkness photoperiod and larval mortality was recorded every 24 h until the larvae either died or pupated.

Data analysis. One-way ANOVA was performed using SPSS software (1998). The larvae that were unable to move and feed were proclaimed dead. Because infected larvae were dead on days 7 or 8, mortality on the eighth day and percentage pupation, larval period, pupal period, and pupal weight were calculated on the basis of the initial number of larvae used in each treatment. Pupae were weighed using an electronic balance. Percentage mortality was corrected by the equation: $M\ [\%] = [(t - c)/(100 - c)] \times 100$, where: M – corrected mortality; c – percentage mortality in controls; t – percentage mortality in treatments (Abbott 1925; Duddield & Jordan 2000). Corrected percentage mortality, pupation rate, larval period, pupal period, and pupal weight were separated and compared using a one-way Duncan test among treatments. All tests were conducted with $\alpha = 0.05$.

RESULTS

Infectivity. For 2nd larval instars, mortality increased with HaNPV concentration rate (Figure 1). Probit analysis of mortality data enabled calculation of the dose–response relationships for larval instar, obtaining the following equations and LC_{50} with 95% fiducial limits: $y = 0.544x - 2.49$, $\chi^2(5) = 1.48$, and 3.8×10^4 PIB/ml (1.6×10^4–9.8×10^4). Also, calculated LC_{25} and LC_{75} were equal to 2.2×10^3 and 6.6×10^5 PIB/ml for 2nd larval instars, respectively.

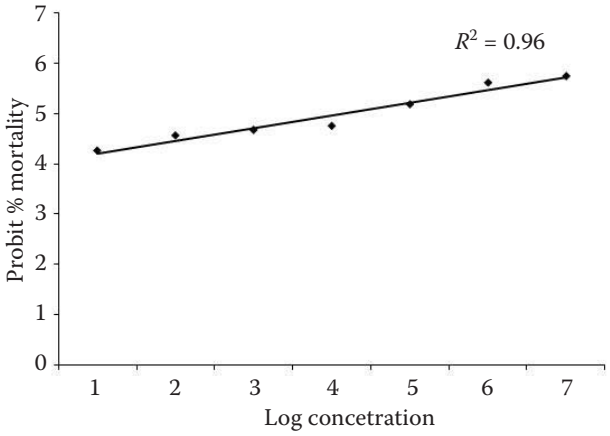

Figure 1. Probit of percentage of 2nd larval instars mortality of *P. xylostella* at different concentrations of HaNPV

The fit of the transformed data was acceptable using the chi-squared test (Table 1). The results indicated a positive relationship between larval mortality and concentration values which differed statistically, based on the non-overlap of fiducial limits. Most larvae died in the last instar. Median lethal time (LT_{50}) to similar response levels (mortality rates 50–75%) decreased with decreasing larval age (from 114.23 h to 106.05 h) (Table 2).

Effects on development. When 2nd instars larvae fed on the leaf discs treated with HaNPV, mortality, pupation rate, larval period, and adult emergence showed significant variation in different concentrations of HaNPV (mortality: $F_{(3)}$= 191.918, P < 0.00; pupation rate: $F_{(3)}$= 265.326, P < 0.000; larval period: $F_{(3)}$= 1.537, P < 0.278; adult emergence: $F_{(3)}$= 265.326, P < 0.001), but in regard to pupal weight it was not significant (pupal weight: $F_{(3)}$= 4.579, P < 0.038) (Table 3).

The mortality in concentration of 6.6×10^5 PIB/ml HaNPV was the highest (72%). The pupal weight and the pupation rate decreased significantly with increasing HaNPV concentration in compare with control.

Larval period did not change statistically with different concentrations of HaNPV in compare with control (Table 3). Adult emergence in all concentrations of HaNPV was significantly decreased compared with control.

DISCUSSION

Bioassay using 2nd instar larvae of *P. xylostella* with HaNPV isolate with different lethal concentrations was evaluated. LC_{25}, LC_{50}, and LC_{75} values were 2.2×10^3, 3.8×10^4, and 6.6×10^5 PIB/ml for 2nd larval instars, respectively. The HaNPV from Iran proved effective at 3.8×10^4 PIB/ml based on the LT_{50} value (114.23 h) for 2nd instar larvae of *P. xylostella*. Median lethal time (LT_{50}) to similar response levels (mortality rates 50–75%) decreased with decreasing larval age (from 114.23 h to 106.05 h).

In previous studies Padmavathamma and Veeresh (1991) investigated the effect of PxMPV on the diamondback moth, *P. xylostella* and found the highest percentage of larval mortality (67.93%) for the highest concentration (1.7×10^9 PIB/ml) and the lowest percentage of mortality (20.67%) for the lowest concentration (1.7×10^1 PIB/ml). The highest mortality on the 1st to 3rd larval instars occurred at the highest concentrations (1.7×10^7, 1.7×10^8, and 1.7×10^9 PIB/ml, respectively). Farrar *et al*. (2007) stated that LD_{50} value of NPV virus on the diamondback moth, *P. xylostella,* was 167 occlusion bodies (OB) per each larva. Also Biever and Andrew (1984) estimated the LC_{50} value of NPV virus on 3rd instar larvae of the diamondback moth, *P. xylostella,* equal to 1.5×10^4 PIB/ml. In addition, the LC_{50} value of the PxGV Indian virus on 2nd larval instar of *P. xylostella* was estimated at

Table 1. Calculated LC_{25}, LC_{50}, and LC_{75} values of HaNPV treated 2nd instar larvae of the diamondback moth, *P. xylostella*

LC_{25}	LC_{50}	LC_{75}	Slope	Intercept	χ^2	*df*	Pr > χ^2
(PIB/ml) (lower–upper limit)							
2.2×10^3 (3.5×10^2–6×10^3)	3.8×10^4 (1.6×10^4–9.8×10^4)	6.6×10^5 (2.2×10^5–5.8×10^6)	0.544	–2.49	1.48	5	0.915

Table 2. LT_{50} values of different concentrations of HaNPV on 2nd larval instars of *P. xylostella*

Treatments	NPV dose (PIB/ml)	LT_{50} (h)	95% limit		Slope	Intercept	χ^2
			lower	upper			
LC_{50}	3.8×10^4	114.23	105.45	130.57	9.39	–19.32	5.876
LC_{75}	6.6×10^5	106.05	99.58	113.70	12.89	–26.12	0.854

Table 3. Mortality, growth, and development of *P. xylostella* 2nd instar larvae when exposed to HaNPV simultaneously[1]

HaNPV (PIB/ml)	Larval period[2] (days)	Pupation rate (%)	Pupal weight (mg)	Adult appearnce (%)	Total mortality (%)
2.3×10^3	10.33 ± 0.88[a]	73.3 ± 3.33[c]	3.90 ± 0.06[ab]	73.26 ± 3.33[c]	24.60 ± 3.67[b]
3.8×10^4	10.33 ± 0.88[a]	46.63 ± 1.93[b]	3.60 ± 0.23[a]	46.63 ± 1.93[b]	52.00 ± 2.30[c]
6.6×10^5	9.00 ± 0.58[a]	23.30 ± 1.90[a]	3.30 ± 0.12[a]	23.30 ± 0.00[a]	72.00 ± 0.00[d]
0	8.67 ± 0.33[a]	98.87 ± 1.13[d]	4.80 ± 0.55[b]	98.87 ± 1.13[d]	1.13 ± 1.13[a]
df	3	3	3	3	3
F	1.537	265.326	4.579	265.326	191.918
P	0.278	0.001	0.038	0.001	0.001

[1]2nd instar larvae of *P. xylostella* were fed with leaf discs infected by HaNPV; [2]data are means (± SE); [3]died out pupae were applied in the mortality rate and percentage of mortality was corrected by Abbot's formula; [a–d]means within the same column followed by a different letter are significant at $P < 0.05$, Duncan test

5.89 OB/mm^2 (Rabindra *et al*. 1997). Dezianian *et al*. (2010) announced the LC_{50} of PxGV virus against 2nd larval instars of the diamondback moth, *P. xylostella* was 1.39×10^6 granules/ml. Fahimi *et al*. (2008) stated that LC_{50} of Taiwanii-PxGV virus on 2nd instar larvae of *P. xylostella* was 1.55×10^7 OB/ml. These studies showed that *P. xylostella* have been susceptible to baculoviruses. Differences between these studies and the present study could be due to differences in the response of species differences in the strength of pathogen virus or both, tested virus type, larval instars and methods to be used.

In the present study, larvae of *P. xylostella* started to die from the 5th day after infection with HaNPV. Trang and Chaudhari (2002) showed that the LT_{50} values for NPV against the 2-day old larvae of *Spodoptera litura* (Fab.) was 4.4 to 5.4 days at the lowest and highest concentration.

NPV infection is limited to particular tissues in midgut, and causes some infected columnar epithelial cells to rupture. LT_{50} value of PxGV virus in the concentration of 10^6 OB/ml on 2nd larval instars of diamondback moth, *P. xylostella* (at 27°C) and on 3rd larval instars (at 27°C) was reported as equal to 4.98 and 5.51 days, respectively (Kadir *et al*. 1999). Dezianian *et al*. (2010), stated that LT_{50} values of PxGV virus on 1st to 3rd larval instars of the diamondback moth, *P. xylostella* in the concentrations of 3.11×10^9, 3.11×10^7, and 3.11×10^5 OB/ml were 3.81–6.95, 4.97–9.74, and 5.15–9.41 days, respectively. Also the value of LT_{50} of PxGV-Taiwanii on 2nd larval instars of the diamondback moth, *P. xylostella* in the concentrations of 749.89 g/mm^2 (with 57.78% mortality) and of 1883.65 g/mm^2 (with 75.56% mortality) was 6.04 and 6.85 days (Fahimi *et al*. 2008).

Exposure of *P. xylostella* larvae to HaNPV showed that pupation rate and pupal weight were reduced. These results agree with the finding of Liu *et al*. (2006) and Marzban *et al*. (2009). When evaluating the use of pathogens as microbial control agents, time to death (speed of killing) should be considered (Marzban *et al*. 2009). Developmental time is an important aspect of the biology of an insect. Prolonged developmental time at any leaf stage would mean greater exposure to natural enemies and environmental stresses, which could reduce the rate of population buildup of the insect. Furthermore, a longer generation time could result in fewer generations per season. In this study, HaNPV adversely affected the growth and development of diamondback moth.

Based on experimental data obtained in this study, HaNPV virus could be considered as an effective pathogenic agent in the control programs of the diamondback moth. Therefore, it seems further experiments in the field and laboratory results generalisation are needed. The study also suggested further studies on the possibility of transmission of HaNPV virus to the next generation and application of synergists factors in increasing its virulence.

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