Saudi Journal of Biological Sciences 27 (2020) 1419–1422

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Manifold passages in an assorted infection in a host could improve virulence of *Helicoverpa armigera* Nucleopolyhedrovirus (HaNPV)

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ARTICLE INFO

Article history:
Received 15 December 2019
Revised 15 February 2020
Accepted 29 February 2020
Available online 10 March 2020

Keywords:
American bollworm
NPV
Lethal time
Toxicity
Vertical transmission

ABSTRACT

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) is serious pests of cotton and several other crops. *Helicoverpa armigera Nucleopolyhedrovirus* (HaNPV) can be important alternative to synthetic insecticides for the management of *H. armigera*. However, the efficacy of HaNPV can vary in horizontal and vertical transmission. In the current study, we evaluated the efficacy of HaNPV of a virulent strain (vertically transmitted up to six generations) and wild strains (used after isolation from the field infected larvae). Both strains were applied to the 2nd instar larvae of *H. armigera* @ 1×10^9 polyhedral inclusion bodies (PIB)/ml. There were six replications of each strain (strains). The results indicated higher mortalities in larvae exposed to virulent strains (68.33 ± 6.07%) as compared to wild strain (45 ± 2.24%). Virulent strains killed the larvae quite faster than wild strain. The lethal time (LT_{50}) to kill 50% of the larvae by virulent strain was 7.15 days and for wild strain it was 19.47 days. The results showed that multiple passage of HaNPV through several generations enhances its efficacy to kill *H. armigera* larvae faster. The results of this study will be helpful to manage *H. armigera* and other related lepidopoterous pests.

1. Introduction

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae), is a devastating insect pest which feeds on many host plants (Marzban et al., 2009). It has a wide geographical range, high survival rate and a great propensity to become resistant against many chemical insecticides (Wakil et al., 2009a, 2009b, 2012). Injudicious use of insecticides to control this pest has posed serious threat to human health and the environment. Therefore, there is a need to find an alternative pest control tactics that are environmentally benign (Cherry et al., 1997). The entomopathogens particularly Nucleopolyherosis viruses (NPVs) are not only safer to environment but also effective against lepidopterous insect pests (Nawaz et al. 2019). Nucleocapsid Nucleopolyhedrosis virus (HearNPV) also called as HaSNPV was first discovered in China during 1975 and has been used for more than 25 years for the management of *H. armigera* (Zhang, 1994). Since that more than 600 viruses of family Baculoviridae (NPV and *Granuloviruses*) have been tested against *H. armigera* (Jayaraj, 1985; Nathan and Kalaivani, 2006; David, 2008).

Lot of research has been conducted on the genetics of Baculoviruses (Herniou et al., 2003) but limited work was done on their ecology, epidemiology and transmission. The vertical transmission (from parents to off-springs) as well as horizontal transmission (from diseased individuals to healthy ones) of the baculoviruses occurs (Fine, 1984; Andreadis, 1987). Principally

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.02.023
1319-562X/

the horizontal transmission takes place when an infected larva with NPV dies and disruption of the dead body occur and numerous occlusion bodies (OBs) are released onto foliage and soil. The OBs infect the susceptible hosts after being ingested. In addition to that horizontal transmission also occurs by contamination of host plant through defecation and regurgitation of the infected larvae (Ali et al., 1987; Vasconcelos et al., 1996; Young, 1998; Keddie et al. 1989). Cannibalism and predation were also advocated routes of horizontal transmission of virus by some experts (Dhandapani et al., 1993; Evans, 1986). The ecological factors such as rainfall, wind storm (Fuxa and Richter, 1991), and contaminated ovipositors of parasitic hymenopterans also assist horizontal transmission (Hamm et al., 1988).

Vertical transmission embraces embryonic infection, transovarian (in the egg) transmission and transovum (on the egg) transmission (Cory and Myers, 2003; Fuxa, 2004). Vertical transmission of NPV in insects was reviewed in detail by Kukan (1999). The vertical transmission rate of NPVs varied from 0.5 to 57.1% in different species of Lepidoptera. Whereas, in the field, decontaminated egg masses produced only 1–9% infected larvae and contaminated egg masses resulted in 2–80% infected larvae. It proved transovum transmission (which can be nullified by decontamination) as the key factor for vertical transmission whereas transovarian transmission as the minor but statistically significant one.

It has been described that manifold passage of virus infection in host could be related to virulence efficiency of NPV in *H. armigera.* The increase in virulence of NPV leads to increase in survival of viruses to control the *H. armigera* population under economic threshold level by biological control rather than chemical control. In the current study, we aim to produce a virulent strain of HaNPV for the management of *H. armigera*. We hypothesized that multiple passages of HaNPV will increase its virulence.

2. Materials and methods

2.1. Collection and rearing of Helicoverpa armigera

The larvae of *H. armigera* were collected from wheat field located at Multan during April 2014 and shifted to laboratory of Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan. They were reared by following the methodology of Zhang et al. (2015) with little modification. Briefly, larvae were placed in Petri dishes containg artificial diet. The Petri dishes were placed at 25 ± 2 °C temperature, 70 ± 5% relative humidity, and 16:8 h L:D photoperiod. After pupation, male and female pupae (one pair) were placed inside glass jar. On moth emergence, they were fed on 2% honey solution. Napiliner was hanged inside jar for egg laying. The eggs were carefully removed from napiliner and placed in separate jars for hatching. The newly hatched larvae were placed singly in petri dishes.

Table 1
Daily mortality (%) of *Helicoverpa armigera* larvae after application of virulent and wild NPV.

Exposure time	Mortality of larvae (%)	
	Virulent NPV	Wild NPV
After 4 day exposure	46.67 ± 2.11 a	11.67 ± 3.07 b
After 6 day exposure	53.33 ± 3.33 a	25.00 ± 4.28 b
After 8 day exposure	65.00 ± 2.24 a	26.67 ± 3.33 b
After 10 day exposure	65.00 ± 2.24 a	40.00 ± 2.58 b
After 12 day exposure	65.00 ± 2.24 a	40.00 ± 2.58 b
After 14 day exposure	65.00 ± 2.24 a	40.00 ± 2.58 b
After 16 day exposure	65.00 ± 2.24 a	40.00 ± 2.58 b
After 18 day exposure	65.00 ± 2.24 a	41.67 ± 1.67b
After 20 day exposure	66.67 ± 3.33 a	43.33 ± 2.11 b
After 22 day exposure	68.33 ± 3.07 a	46.57 ± 3.33 b

Means with different letters in rows are significantly different (*t*-test, P < 0.05).

2.2. NPV virulent and wild strains

NPV virulent strain was obtained after constant vertical transmission in *H. armigera*. This host-level selection process reduced the genetic variability in the viral population to a degree sufficient to notably increase vertical transmission without resort to viral cloning. The selection was carried out for up to six generations and then the NPVs produced in selection was purified through homogenization and density gradient centrifugation (31–54% CsCl), counted with a Petroff-Hauser counting chamber under phase microscopy, and stored at −4°C until use. While, wild strain was collected from the wheat and gram fields from Salarwahin Kohna, Khanewal Punjab, Pakistan and Taunsa Sharif, Dera Ghazi Khan, Punjab, Pakistan by collecting *H. armigera* larvae hanging with the plants with blackish colour and visually seemed NPV infected. The NPV were isolated by homogenization and density gradient centrifugation (31–54% CsCl), and stored at −4°C till use.

2.3. Bioassay

The toxicity of NPV was tested @ 1×10^9 PIB/ml for both wild and virulent strains against 2nd instar larvae of *H. armigera*. The above given dose was mixed in the artificial diet for each treatment separately. The larvae were placed singly in 24 well plates along with small piece of diet (1 cm^3). There were a total of six replications for each treatment. The plates were placed in laboratory under controlled temperature (26 ± 1 °C), relative humidity (70 ± 5%) and a photoperiod (16:8 h L:D). Similar numbers of plates were placed as control with untreated diet. The mortality was reorded on alternate days for a total of 22 days.

2.4. Data analysis

Median lethal time (LT_{50} and LT_{90}) was calculated by probit analysis using SPSS software (Version 23.0). LT_{50} values were considered to be significantly different based on non-overlapping of 95% confidence limits. Mortality data was corrected by Abbot' formula if there was more than 10% mortality in control. Daily mean percent mortality in each treatment was compared by applying Student's t. test using Statistix 8.1 (Statistix, Tallahassee, FL, USA).

3. Results

The percent mortality of *H. armigera* larvae in the virulent NPV treatment was significantly higher after 4 days' exposure (t = 9.39, P < 0.001), 6 days' exposure (t = 5.22, P < 0.001), 8 days' exposure (t = 9.55, P < 0.001), 10–16 days' exposure (t = 7.32, P < 0.001), 18 days' exposure (t = 8.37, P < 0.001), 20 days' exposure (t = 5.92, P < 0.001), and 22 days' exposure (t = 4.78, P < 0.001) compared to the wild NPV treatment. After four days of exposure, virulent strain caused higher mortality (46.67 ± 2.11%) as compared to wild type strain which caused same mortality after 22 days (Table 1).

The overall mortality of *H. armigera* was also higher by virulent strain (68.33 ± 6.07%) as compared to wild strain of NPV (45 ± 2.24%) (Fig. 1). The estimated lethal time was significantly lower for virulent strain (LT_{50} = 7.15 days, LT_{90} = 38.86 days) as compared to that of wild strain (LT_{50} = 7.15 days, LT_{90} = 38.86 - days) (Table 2). The data indicated that virulent NPV is good option for the control of *H. armigera* and can be included in management strategies.

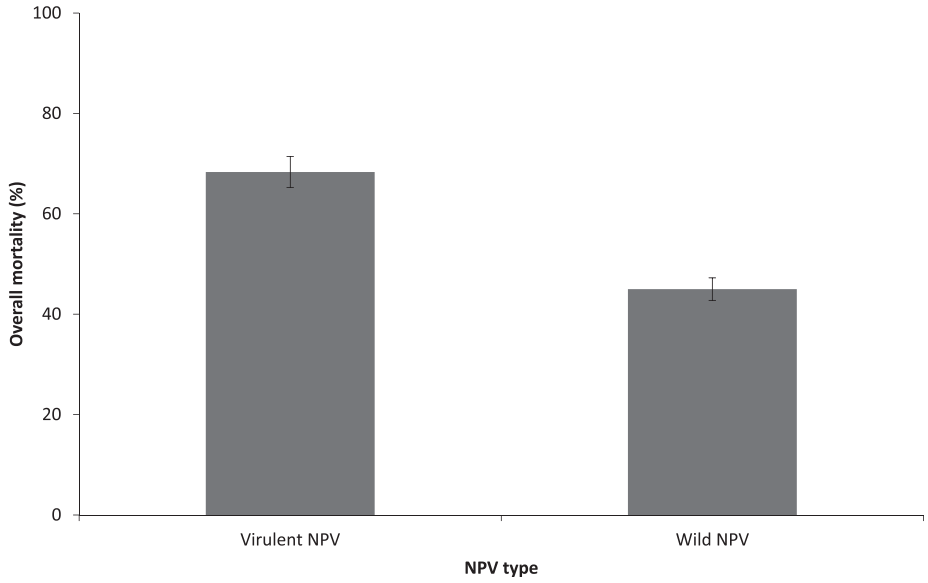

Fig. 1. Overall mortality (%) of larvae of *Helicoverpa armigera* larvae after application of virulent and wild NPV.

Table 2
Time mortality response (LT_{50}) of *Helicoverpa armigera* to NPV strains.

NPV Strain	LT_{50}[a] (95% FL) (Days)	LT_{90} [b] (95% FL[c]) (Days)	d.f.	$\chi 2$[d]	Intercept	P	N[e]
Virulent	7.15 (5.93–8.33) a	38.86 (29.32–59.39) a	9	12	−1.49 ± 0.21	0.213	50
Wild	19.47 (16.19–25.37) b	141.94 (82.31–355.98) b	9	5.51	−1.91- ±0.22	0.78	50

Confidence limits followed by the same letter are overlapping so the LT_{50} are not statistically different.
[a] LT_{50}, time for 50% of the population to be killed.
[b] LT_{90}, time for 90% of the population to be killed.
[c] FL, fiducial limits.
[d] Chi-square.
[e] Number of workers exposed.

4. Discussion

The insect viruses have been used successfully and the selection of virulent strain of NPV is necessary for the development of effective viral insecticides (Gupta et al., 2007). In the present study, the virulent NPV caused significantly higher mortality of *H. armigera* after 22 days' exposure than wild NPV. Previously, Milks et al. (2001) reported that the recombinant NPV killed 30% more larvae of *Trichoplusia ni* (Lepidoptera: Noctuidae) than wild type virus. Similarly, the LD_{50} of recombinant virus was lower (4.4 times) compared to the wild type virus against second instar larvae of *T. ni*. However, at LD_{90}, the survival of recombinant and wild type viruses were not significantly different (Li et al., 2003).

Yu et al. (2017) has been reported that novel recombinant RjAa17f-HearNPV could improve the insecticidal effect against *H. armigera*. Murillo et al. (2003) conducted experiments on three noctuid moths (*Spodoptera exigua, S. littoralis* and *S. frugiperda*) which were hosts of three distinct NPVs. They found that each virus killed its respective host more effectively (*S. exigua* NPV most effective against *S. exigua* and so on) as compared to others however, *S. littoralis* variant (*S. littoralis* NPV) was almost equally effective against all three noctuid species. Bhutia et al. (2012) reported that Sl-NPV application caused larvae mortality more than chemical insecticide (indoxacarb) in *S. litura*. It has been reported that application of *S. exigua*-NPV (Se-NPV) was effective enough in controlling *S. exigua* both in the laboratory and greenhouse experiment. In laboratory experiment, Se-NPV caused 77.5% larval mortality within 5 days of exposure. Furthermore, Se-NPV also decreased feeding capacity and pupal weights. While in green house experiment, the application of Se-NPV has caused 100% of larval mortality (Supyani et al., 2014).

5. Conclusion

Virulent NPV could be more effective than wild NPV for the control of *H. armigera* larvae. However, assessment of the ecological risks linked with the release of virulent NPV must be tested before the commercialization. Mono-specific NPVs are extremely unlikely to present a risk to any non-target species and highly suitable pest control agent, particularly when used in urban areas and nature reserves.

Acknowledgements

Authors are thankful to the Institute of Pure and Applied Biology for providing lab facilities to conduct this study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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