Interaction of HaNPVs with two novel insecticides against Helicoverpa armigera Hubner (Noctuidae: Lepidoptera)

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A B S T R A C T

Nucleopolyhedrosis viruses can be utilized for effective management of agriculture pests. Their efficacy can be increased if they are mixed with certain insecticides. In the current study, HaNPV was mixed with two insecticides: spinetoram and emamectin benzoate in various combinations and applied to larvae of H. armigera in laboratory conditions. There were a total of 15 combinations of HaNPV with each of the two insecticides in addition to five doses of HaNPV and three doses of insecticides alone. The synergistic and antagonistic effects of combinations were explored. The results revealed that there was synergistic effect of HaNPV @ 0.5 × 10⁶ PIB/ml × Spinetoram @ 40, 20, 10 ml/100 L of water. In case of emamectin benzoate, synergistic effects were recorded at 1 × 10⁶ PIB/ml HaNPV × emamectin benzoate @ 100 ml/100 L of water. However, 0.5 × 10⁶ PIB/ml HaNPV has synergistic effects with all three doses of emamectin benzoate. The results suggested that HaNPV can be used in combination with spinetoram and emamectin benzoate for the management of resistant population of H. armigera.

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1. Introduction

The cotton bollworm, Helicoverpa armigera (Lepidoptera: Noctuidae), is a serious pest of agriculture in Asia, Europe, Africa, USA and Oceania (Guo, 1997; Czepak et al., 2013). It has been reported to damage about 200 plant species including some important agriculture crops like cotton, maize, beans and tomato (Pogue, 2004; Moral-Garcia, 2006; Baker et al., 2008, 2010) and is mainly controlled by insecticides (Brevault and Achaleke, 2005). However, due to the over-reliance on insecticides, this pest has shown resistance against many insecticides that is major cause of sporadic outbreaks of this pest (Ahmad et al., 2001, Torres-Vila et al., 2002, Ahmad et al., 2003, Rajagopal et al., 2009, Alvi et al., 2012, Qayyum et al., 2015, Ahmad et al., 2019).

The entomopathogens can be very effective alternatives of synthetic insecticides to manage lepidopterous insect pests. The efficiency of the entomopathogens can be increased by adding small quantities of synergistic substance like optical brighteners, inorganic acids or sub-lethal concentrations of synthetic insecticides (Peters and Coaker, 1993; Shapiro and Dougherty, 1994; Cisneros et al., 2002). However, the interaction between pathogen and other compounds could be either antagonistic or additive (Pingel and Lewis, 1999; Koppenhofer and Kaya, 2000). Such interactions have been studied between spinetoram insecticide and nucleopolyhedrovirus for various Spodoptera species (Lepidoptera: Noctuidae) (El-Helaly and El-Bendary, 2013; Mendez et al., 2002). But
extensive studies involving the interaction between NPVs and insecticides with novel mode of actions (e.g. spinetoram, emamectin benzoate etc.) are lacking for *H. armigera*.

Spinetoram is primarily a stomach poison with some contact toxicity. It is a mixture of two spinosyns A and D and is obtained from soil actinomycete *Saccharopolyspora spinosa* Mertz and Yao (*Actinomycetales: Pseudonocardiaceae*) after fermentation (Sparks et al., 1998). Spinetoram targets the binding sites on nico- tinic acetylcholine receptors (nAChRs) and GABA receptors of insect nervous system (Salgado, 1998). After exposure to spinetoram, the insect stops feeding followed by paralysis and death. It is usually used against Lepidoptera and Diptera but its novel mode of action makes it relatively safer for non-target organisms and environment (Bret et al., 1997; Saunders and Bret, 1997).

Emamectin benzoate is a mixture of avermectins containing about 80% avermectin B1a and 20% avermectin B1b and is produced after fermentation of soil bacterium *Streptomyces avermitilis* (Lankas and Gordon 1989; Hayes and Laws, 2013). Emamectin benzoate is a selective insecticide, acaricide and nematicide which kills the target organisms by disrupting γ-aminobutyric acid (GABA) gated chloride channels, glutamate-gated chloride channel and other chloride channels in nervous system (Xu et al., 2016). This insecticide is classified as an environment friendly insecticide and is less toxic to beneficial insects (MacConnell et al., 1989; Jansson and Dybas, 1998).

Based on the need for designing effective and sustainable management strategy for *H. armigera*, it is very important to evaluate the toxicity of two insecticides spinetoram and emamectin, and NPV as alone and in combination with NPV. From this, we will be able to conclude whether or not these two insecticides play a role in protecting NPV. The aim of this work was to enhance the efficacy of NPVs by combining it with sub-lethal concentrations of spinetoram and emamectin in order to have complete and economical control of *H. armigera*.

## 2. Materials and methods

### 2.1. Collection and rearing of Helicoverpa armigera

The larvae of *H. armigera* were collected from gram field and shifted to glass jars containing artificial diet (Table 1). The jars were placed in laboratory under controlled temperature (25 ± 2 °C) and relative humidity (60 ± 5%). They were reared until pupation. After that the pupae were identified to male and female and shifted to glass jar containing napiliner for egg laying. In each glass jar, one pair of male and female was released with 2% honey solution as diet. The eggs were collected from napiliner and shifted to their natural diet as describe above. After hatching, 2nd instar larvae were used in the experiment.

### 2.2. Treatment of *H. armigera* larvae with insecticides and HaNPV

Second instar *H. armigera* larvae were inoculated with HaNPV by incorporating HaNPV @ in the diet with following treatments at five doses of NPV: 4 × 10⁹ PIB/ml, 2 × 10⁹ PIB/ml, 1 × 10⁹ PIB/ml, 0.5 × 10⁹ PIB/ml, 0.25 × 10⁹ PIB/ml. About 100 larvae were treated at each HaNPV concentration for 24 h. The HaNPV used in our previous experiments was also used in this study (Abid et al., 2020). After 24 h of exposure to HaNPV, the larvae were transferred to a diet containing either spinetoram @ 40, 20, 10 ml/100 L of water or emamectin @ 400, 200, 100 ml/100 L of water. There were a total 42 combinations as given in Table 2. Each combination was replicated 12 times (each replication contained two larvae). After 72 hrs mortality was recorded.

### 2.3. Data analysis

The data of mean mortality was subjected to Analysis of Variance and means were separated by Tukey’s HSD test using Statistix 8.1v (Analytical software, 2005). The mortality data were corrected using Abbott’s formula (Abbott, 1925), if the mortality rate in the control was more than 5%. Median lethal concentrations (LC₅₀) were determined by probit analysis using SPSS software (Version 23.0 for windows, SPSS Inc., Chicago, USA).

## 3. Results

### 3.1. Effect of sole and combination of HaNPV and insecticides

The results of various insecticides alone and in combination with HaNPV are given in Table 2. Higher doses of HaNPV showed antagonistic effects with both of the insecticides. However, synergistic effect was recorded of HaNPV @ 0.5 × 10⁹ PIB/ml × Spinetoram @ 40, 20, 10 ml/100 L of water. In case of emamectin benzoate, synergistic effects were recorded at 1 × 10⁹ PIB/ml HaNPV × emamectin benzoate @ 100 ml/100 L of water. However, 0.5 × 10⁹ PIB/ml HaNPV has synergistic effects with all three doses of emamectin benzoate.

### 3.2. Lethal concentration

The Table 3 showed the LC₅₀ values of HaNPV, spinetoram, emamectin benzoate and their combinations. It was observed that LC₅₀ values of spinetoram, emamectin benzoate were decreased with the increase in concentration of HaNPV. However, LC₅₀ values were lower for HaNPV + Spinetoram as compared to HaNPV + Emamectin combinations. The lowest LC₅₀ value were observed in 4 × 10⁹ HaNPV + Spinetoram combination (61.12 mg/l) followed by 2 × 10⁹ HaNPV + Spinetoram (67.53 mg/l), 1 × 10⁹ HaNPV + Spinetoram (75.34 mg/l) and 0.5 × 10⁹ HaNPV + Spinetoram (91.47 mg/l) and 0.25 × 10⁹ HaNPV + Spinetoram (241.19 mg/l). These LC₅₀ values were lower than spinetoram alone (332.37 mg/l). Similarly, in case of HaNPV + Emamectin combinations, the lowest LC₅₀ value was recorded by 4 × 10⁹ HaNPV + Emamectin benzoate (372.13 mg/l), 2 × 10⁹ HaNPV + Emamectin benzoate (418.87 mg/l), 1 × 10⁹ HaNPV + Emamectin benzoate (527.42 mg/l), 0.5 × 10⁹ HaNPV + Emamectin benzoate (641.72 mg/l), 0.25 × 10⁹ HaNPV + Emamectin benzoate (1709.91 mg/l).

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### Table 1

| Component         | Quantity |
|-------------------|----------|
| Chickpea flour    | 100 g*   |
| Yeast             | 30 g     |
| Wesson's salt mix | 7 g      |
| Methyl Para ben   | 2 g      |
| Sorbic acid       | 1 g      |
| Ascorbic acid     | 3 g      |
| Agar              | 13 g     |
| Vanderzant vitamin solution | 8 ml    |
| Streptomycin sulphate | 40 mg   |
| Carbendazim       | 675 mg   |
| Formalin          | 2 ml     |
| Water             | 720 ml   |

*Whole chickpea seeds could also be used (soak in distilled water overnight).  
**28% solution in distilled water.

* not included in diets used for inoculation of larvae with virus and post-inoculation rearing.
Table 2
Antagonistic and synergistic effect of HaNPV with spinetoram and emamectin benzoate.

| Treatment | Average Mortality (%) ± SEM | Synergistic/Antagonistic Effect |
|-----------|-----------------------------|--------------------------------|
| HaNPV @ 4 x 10^9 PIB/ml | 71 ± 9.64 A-E |                                  |
| HaNPV @ 2 x 10^9 PIB/ml | 75 ± 7.53 A-D |                                  |
| HaNPV @ 1 x 10^9 PIB/ml | 62.5 ± 8.97 A-G |                                |
| HaNPV @ 0.5 x 10^9 PIB/ml | 29 ± 11.44F-I |                                |
| HaNPV @ 0.25 x 10^9 PIB/ml | 165 ± 7.11 I |                                |
| HaNPV @ 4 x 10^9 PIB/ml + Spinetoram @40 ml/100 l of water | 100 ± 0.00 A |                                |
| HaNPV @ 2 x 10^9 PIB/ml + Spinetoram @20 ml/100 l of water | 91.5 ± 5.61 AB |                                  |
| HaNPV @ 2 x 10^9 PIB/ml + Spinetoram @10 ml/100 l of water | 75 ± 7.53 A-D |                                  |
| HaNPV @ 2 x 10^9 PIB/ml + Spinetoram @40 ml/100 l of water | 100 ± 0.00 A |                                  |
| HaNPV @ 2 x 10^9 PIB/ml + Spinetoram @20 ml/100 l of water | 87.5 ± 6.52 AB |                                  |
| HaNPV @ 2 x 10^9 PIB/ml + Spinetoram @10 ml/100 l of water | 71 ± 7.43 A-E |                                  |
| HaNPV @ 1 x 10^9 PIB/ml + Spinetoram 480 SC @40 ml/100 l of water | 100 ± 0.00 A |                                  |
| HaNPV @ 1 x 10^9 PIB/ml + Spinetoram @20 ml/100 l of water | 83.5 ± 7.11 ABC |                                  |
| HaNPV @ 1 x 10^9 PIB/ml + Spinetoram @10 ml/100 l of water | 67 ± 7.11 A-F |                                  |
| HaNPV @ 0.5 x 10^9 PIB/ml + Spinetoram @40 ml/100 l of water | 100 ± 0.00 A |                                  |
| HaNPV @ 0.5 x 10^9 PIB/ml + Spinetoram @20 ml/100 l of water | 79 ± 9.64 A-D |                                  |
| HaNPV @ 0.5 x 10^9 PIB/ml + Spinetoram @10 ml/100 l of water | 58.5 ± 12.05B-H |                                  |
| HaNPV @ 0.25 x 10^9 PIB/ml + Spinetoram 480 SC @40 ml/100 l of water | 75 ± 9.73 A-D |                                  |
| HaNPV @ 0.25 x 10^9 PIB/ml + Spinetoram @20 ml/100 l of water | 33.5 ± 9.40 E-I |                                  |
| HaNPV @ 0.25 x 10^9 PIB/ml + Spinetoram @10 ml/100 l of water | 21 ± 9.65 HI |                                  |
| Spinetoram @40 ml/100 l of water | 46 ± 7.43C-I |                                  |
| Spinetoram @20 ml/100 l of water | 25 ± 7.54 GHI |                                  |

Table 3
Lethal concentration estimation of HaNPV, spinetoram, emamectin benzoate and their combinations against H. armigera.

| Treatment | LC25 (mg/l) (95% CL) | LC50 (mg/l) (95% CL) | LC50 (mg/l) (95% CL) | Slope | \( \chi^2 \) | df | P | N |
|-----------|----------------------|----------------------|----------------------|-------|--------|-----|---|---|
| HaNPV | 0.32 (0.12–0.52) x 10^9 | 0.97 (0.63–1.48) x 10^9 | 7.94 (3.98–37.48) x 10^9 | 0.02 (±0.12) | 3.97 | 0 | 0.264 | 144 |
| 4 x 10^9 HaNPV + Spinetoram | 36.25 (0.22–68.34) | 61.12 (2.66–94.53) | 164.87 (115.73–644.80) | 5.31 (±2.57) | 0.37 | 1 | 0.541 | 96 |
| 2 x 10^9 HaNPV + Spinetoram | 39.19 (1.19–71.10) | 67.53 (8.52–101.19) | 189.89 (137.40–509.61) | 5.22 (±2.78) | 0.76 | 1 | 0.382 | 96 |
| 1 x 10^9 HaNPV + Spinetoram | 43.54 (3.28–75.15) | 75.34 (17.09–108.84) | 213.52 (156.18–553.08) | 5.31 (±2.12) | 1.22 | 1 | 0.269 | 96 |
| 0.5 x 10^9 HaNPV + Spinetoram | 55.41 (12.51–84.96) | 91.47 (40.49–122.75) | 237.12 (177.18–525.42) | 5.67 (±2.09) | 1.44 | 1 | 0.239 | 96 |
| 0.25 x 10^9 HaNPV + Spinetoram | 129.51 (66.79–175.01) | 241.19 (179.11–364.01) | 786.14 (469.35–3638.22) | 5.95 (±1.56) | 1.18 | 1 | 0.276 | 96 |
| Spinetoram | 192.39 (62.49–519.49) | 332.37 (231.29–904.07) | 1462.49 (653.29–57150.77) | 5.02 (±0.56) | 0.25 | 1 | 0.613 | 96 |
| 4 x 10^9 HaNPV + Emamectin benzoate | 254.77 (16.89–388.69) | 372.13 (78.10–499.53) | 764.46 (592.23–1805.04) | 10.54 (±4.09) | 0.04 | 1 | 0.834 | 96 |
| 2 x 10^9 HaNPV + Emamectin benzoate | 280.73 (59.60–411.32) | 418.87 (173.87–547.91) | 895.97 (697.99–1799.58) | 10.17 (±5.57) | 0.16 | 1 | 0.689 | 96 |
| 1 x 10^9 HaNPV + Emamectin benzoate | 323.29 (116.01–467.90) | 527.42 (302.45–687.41) | 1336.74 (1002.38–2660.45) | 8.64 (±2.44) | 0.15 | 1 | 0.701 | 96 |
| 0.5 x 10^9 HaNPV + Emamectin benzoate | 393.26 (183.50–542.75) | 641.72 (428.54–820.07) | 1627.08 (1208.53–3191.75) | 8.90 (±2.24) | 0.84 | 1 | 0.359 | 96 |
| 0.25 x 10^9 HaNPV + Emamectin benzoate | 890.09 (501.88–1217.92) | 1709.91 (1247.19–3569.89) | 5911.49 (3066.06–63172.92) | 7.69 (±2.17) | 0.36 | 1 | 0.547 | 96 |
| Emamectin benzoate | 834.32 (480.16–1116.25) | 1541.06 (1151.37–2691.30) | 4944.94 (2785.88–11224.05) | 8.06 (±2.16) | 0.61 | 1 | 0.435 | 96 |

a LC25 = Lethal concentration to kill 25% population.
b CL = Confidence limits.
c LC50 = Lethal concentration to kill 50% population.
d LC50 = Lethal concentration to kill 90% population.
e \( \chi^2 \) = Chi-square.
f = Total numbers exposed.
4. Discussion

In Pakistan, farmers usually rely on synthetic insecticides to manage lepidopterous pests which have caused insecticide resistance and very harmful effects on non-target organisms and the environment (Ferré and van Rie, 2002; Sayyed and Wright, 2006). Therefore, sole reliance on synthetic chemicals should be avoided to prevent such negative effects. On the other hand, use of microbial organisms for management of insect pests is safer but it requires long time to reduce their population as their action is very slow. The findings of the current study revealed that mixing of NPV with synthetic chemicals could be very effective, quicker in action and safer to manage insect pests. However, this mixture is not suitable for use at every ratio of both ingredients: some ratio will cause antagonistic effects while some synergistic effect. Our study revealed that there is synergistic effect between HaNPV and spinetoram at higher doses while antagonistic effect at higher doses.

The combination of HaNPV with spinetoram was caused higher mortalities of H. armigera as compared to spinetoram, emamectin benzoate, HaNPV alone and combination of HaNPV with emamectin benzoate. In the current study, both additive and antagonistic effects were observed between HaNPV and two insecticides. There was antagonistic interaction between HaNPV and two insecticides. There was antagonistic interaction between spinetoram with 0.5 mg/C2 and emamectin benzoate. In the current study, both additive and antagonistic effects were observed between HaNPV and two insecticides. There was antagonistic interaction between HaNPV and two insecticides. There was antagonistic interaction between spinetoram with 0.5 mg/C2 and emamectin benzoate. In the current study, both additive and antagonistic effects were observed between HaNPV and two insecticides.

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