Saudi Journal of Biological Sciences 27 (2020) 2124–2128

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

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

Allah Ditta Abid [a,b,*], Shafqat Saeed [a,c,*], Syed Muhammad Zaka [a], Muhammad Ali [d,e], Muhammad Sohail Shahzad [b], Muhammad Iqbal [f], Umbreen Shahzad [g], Naeem Iqbal [c], Suliman M. Alghanem [h]

[a] Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan 60800, Pakistan
[b] Department of Plant Protection, Karachi Ministry of National Food Security, Pakistan
[c] Institute of Plant Protection, MNS-University of Agriculture, Multan 60000, Pakistan
[d] Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan 60800, Pakistan
[e] Qauid-i-Azam University, Islamabad, Pakistan
[f] Cotton Research Institute, Multan 60000, Pakistan
[g] College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus, Layyah, Pakistan
[h] Biology Department, Faculty of Science, Tabuk University, Tabuk 71491, Saudi Arabia

ARTICLE INFO

Article history:
Received 11 May 2020
Revised 12 June 2020
Accepted 12 June 2020
Available online 20 June 2020

Keywords:
Synergistic effect
Combination
Spinetoram
Emamectin benzoate
HaNPV

ABSTRACT

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 @ 0.5×10^9 PIB/ml × Spinetoram @ 40, 20, 10 ml/100 L of water. In case of emamectin benzoate, synergistic effects were recorded at 1×10^9 PIB/ml HaNPV × emamectin benzoate @ 100 ml/100 L of water. However, 0.5×10^9 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*.

1. Introduction

The cotton bollworm, *Helicoverpa armigera* (Lepidopetra: 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

* Corresponding authors at: Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan 60800, Pakistan.
E-mail addresses: adabid5@gmail.com (A.D. Abid), shafqat.saeed@mnsuam.edu.pk (S. Saeed).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.06.023
1319-562X/

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 nicotinic 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 chlorine 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.

Table 1
Artificial diet for rearing of *H. armigera* larvae.

Component	Quantity
Chikpea flour	100 g*
Yeast	30 g
Wesson's salt mix	7 g
Methyl Paraben	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 checkpea 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.

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: 4×10^9 PIB/ml, 2×10^9 PIB/ml, 1×10^9 PIB/ml, 0.5×10^9 PIB/ml, 0.25×10^9 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 (LC_{50}) 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 antagositic effects with both of the insecticides. However, synergistic effect was recorded of HaNPV @ 0.5×10^9 PIB/ml × Spinetoram @ 40, 20, 10 ml/100 L of water. In case of emamectin benzoate, synergistic effects were recorded at 1×10^9 PIB/ml HaNPV × emamectin benzoate @ 100 ml/100 L of water. However, 0.5×10^9 PIB/ml HaNPV has synergistic effects with all three doses of emamectin benzoate.

3.2. Lethal concentration

The Table 3 showed the LC_{50} values of HaNPV, spinetoram, emamectin benzoate and their combinations. It was observed that LC_{50} values of spinetoram, emamectin benzoate were decreased with the increase in concentration of HaNPV. However, LC_{50} values were lower for HaNPV + Spinetoram as compared to HaNPV + Emamectin combinations. The lowest LC_{50} value were observed in 4×10^9 HaNPV + Spinetoram combination (61.12 mg/l) followed by 2×10^9 HaNPV + Spinetoram (67.53 mg/l), 1×10^9 HaNPV + Spinetoram (75.34 mg/l) and 0.5×10^9 HaNPV + Spinetoram (91.47 mg/l) and 0.25×10^9 HaNPV + Spinetoram (241.19 mg/l). These LC_{50} values were lower than spinetoram alone (332.37 mg/l). Similarly, in case of HaNPV + Emamectin combinations, the lowest LC50 value was recorded by 4×10^9 HaNPV + Emamectin benzoate (372.13 mg/l), 2×10^9 HaNPV + Emamectin benzoate (418.87 mg/l), 1×10^9 HaNPV + Emamectin benzoate (527.42 mg/l), 0.5×10^9 HaNPV + Emamectin benzoate (641.72 mg/l), 0.25×10^9 HaNPV + Emamectin benzoate (1709.91 mg/l).

Table 2
Antagonistic and synergistic effect of HaNPV with spinetoram and emamectin benzoate.

Treatment	Average Mortality (%) ± SEM	Synergistic/Antagonistic Effect
HaNPV @4 × 10^9 PIB/ml	71 ± 9.64 A-E	
HaNPV @2 × 10^9 PIB/ml	75 ± 7.53 A-D	
HaNPV @1 × 10^9 PIB/ml	62.5 ± 8.97 A-G	
HaNPV @ 0.5 × 10^9 PIB/ml	29 ± 11.44F-I	
HaNPV @ 0.25 × 10^9 PIB/ml	16.5 ± 7.11 I	
HaNPV @4 × 10^9 PIB/ml × Spinetoram @40 ml/100 l of water	100 ± 0.00 A	Antagonistic
HaNPV @4 × 10^9 PIB/ml × Spinetoram @20 ml/100 l of water	91.5 ± 5.61 AB	Antagonistic
HaNPV @4 × 10^9 PIB/ml × Spinetoram @10 ml/100 l of water	75 ± 7.53 A-D	Antagonistic
HaNPV @2 × 10^9 PIB/ml × Spinetoram @40 ml/100 l of water	100 ± 0.00 A	Antagonistic
HaNPV @2 × 10^9 PIB/ml × Spinetoram @20 ml/100 l of water	87.5 ± 6.52 AB	Antagonistic
HaNPV @2 × 10^9 PIB/ml × Spinetoram @10 ml/100 l of water	71 ± 7.43 A-E	Antagonistic
HaNPV @1 × 10^9 PIB/ml × Spinetoram 480 SC @40 ml/100 l of water	100 ± 0.00 A	Antagonistic
HaNPV @1 × 10^9 PIB/ml × Spinetoram @20 ml/100 l of water	83.5 ± 7.11 ABC	Antagonistic
HaNPV @1 × 10^9 PIB/ml × Spinetoram @10 ml/100 l of water	67 ± 7.11 A-F	Antagonistic
HaNPV @ 0.5 × 10^9 PIB/ml × Spinetoram @40 ml/100 l of water	100 ± 0.00 A	Synergistic
HaNPV @ 0.5 × 10^9 PIB/ml × Spinetoram @20 ml/100 l of water	79 ± 9.64 A-D	Synergistic
HaNPV @ 0.5 × 10^9 PIB/ml × Spinetoram @10 ml/100 l of water	58.5 ± 12.05B-H	Synergistic
HaNPV @ 0.25 × 10^9 PIB/ml × Spinetoram 480 SC @40 ml/100 l of water	75 ± 9.73 A-D	Synergistic
HaNPV @ 0.25 × 10^9 PIB/ml × Spinetoram @20 ml/100 l of water	33.5 ± 9.40 E-I	Antagonistic
HaNPV @ 0.25 × 10^9 PIB/ml × Spinetoram' @10 ml/100 l of water	21 ± 9.65 HI	Antagonistic
Spinetoram @40 ml/100 l of water	46 ± 7.43C-I	
Spinetoram @20 ml/100 l of water	25 ± 7.54 GHI	
Spinetoram @10 ml/100 l of water	16.5 ± 7.11 I	
HaNPV @4 × 10^9 PIB/ml × Emamectin benzoate @400 ml/100 l of water	100 ± 0.00 A	Antagonistic
HaNPV @4 × 10^9 PIB/mlx Emamectin benzoate @200 ml/100 l of water	100 ± 0.00 A	Antagonistic
HaNPV @4 × 10^9 PIB/ml × Emamectin benzoate @100 ml/100 l of water	83.5 ± 7.11 ABC	Antagonistic
HaNPV @2 × 10^9 PIB/ml × Emamectin benzoate @400 ml/100 l of water	100 ± 0.00 A	Antagonistic
HaNPV @2*×10^9 PIB/ml × Emamectin benzoate @200 ml/100 l of water	100 ± 0.00 A	Antagonistic
HaNPV @2 × 10^9 PIB/ml × Emamectin benzoate @100 ml/100 l of water	83.5 ± 7.11 ABC	Antagonistic
HaNPV @1 × 10^9 PIB/ml × Emamectin benzoate @400 ml/100 l of water	100 ± 0.00 A	Antagonistic
HaNPV @1 × 10^9 PIB/ml × Emamectin benzoate @200 ml/100 l of water	100 ± 0.00 A	Antagonistic
HaNPV @1 × 10^9 PIB/ml × Emamectin benzoate @100 ml/100 l of water	87.5 ± 6.52 AB	Synergistic
HaNPV @ 0.5 × 10^9 PIB/ml × Emamectin benzoate @400 ml/100 l of water	100 ± 0.00 A	Synergistic
HaNPV @ 0.5 × 10^9 PIB/ml × Emamectin benzoate @200 ml/100 l of water	75 ± 7.54 A-D	Synergistic
HaNPV @ 0.5 × 10^9 PIB/ml × Emamectin benzoate @100 ml/100 l of water	62.5 ± 8.97 A-G	Synergistic
HaNPV @ 0.25 × 10^9 PIB/ml × Emamectin benzoate @400 ml/100 l of water	33.5 ± 7.11 E-I	Antagonistic
HaNPV @ 0.25 × 10^9 PIB/ml × Emamectin benzoate @200 ml/100 l of water	21 ± 7.43 HI	Antagonistic
HaNPV @ 0.25 × 10^9 PIB/ml × Emamectin benzoate @100 ml/100 l of water	29 ± 9.65F-I	Antagonistic
Emamectin benzoate 25 WG @40 ml/100 l of water	58.5 ± 10.36B-H	
Emamectin benzoate @20 ml/100 l of water	41.5 ± 8.34 D-I	
Emamectin benzoate @10 ml/100 l of water	16.5 ± 7.11 I	
Control	8.5 ± 5.62 I	

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

Treatment	LC_{25}[a] (mg/l) (95% CL[b])	LC_{50}[c] (mg/l) (95% CL[b])	LC_{90}[d] (mg/l) (95% CL[b])	Slope	χ^2[e]	df	P	N[f]
HaNPV	0.32 (0.12–0.52) × 10^9	0.97 (0.63–1.48) × 10^9	7.94 (3.98–37.48) × 10^9	0.02 (±0.12)	3.97	3	0.264	144
4 × 10^9 HaNPV + Spinetoram	36.25 (0.22–68.34)	61.12 (2.66–94.53)	164.87 (115.73–464.80)	−5.31 (±2.57)	0.37	1	0.541	96
2 × 10^9 HaNPV + Spinetoram	39.19 (1.19–71.10)	67.53 (8.52–101.19)	189.89 (137.40–509.61)	−5.22 (±2.28)	0.76	1	0.382	96
1 × 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 × 10^9 HaNPV + Spinetoram	55.41 (12.51–84.96)	91.47 (40.49–122.75)	237.12 (177.18–525.42)	−6.07 (±2.09)	1.44	1	0.23	96
0.25 × 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 (62.49–218.49)	332.37 (231.29–904.07)	1462.49 (653.29–57150.77)	−5.02 (±1.56)	0.25	1	0.613	96
4 × 10^9 HaNPV + Emamectin benzoate	254.77 (16.89–388.69)	372.13 (76.10–499.53)	764.46 (592.23–1805.04)	−10.54 (±4.39)	0.04	1	0.834	96
2 × 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 (±3.57)	0.16	1	0.689	96
1 × 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 × 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 × 10^9 HaNPV + Emamectin benzoate	890.09 (501.881–1217.92)	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)	4944.9 (2785.88–31224.05)	−8.06 (±2.16)	0.61	1	0.435	96

[a] LC_{25} = Lethal concentration to kill 25% population.
[b] CL = Confidence limits.
[c] LC_{50} = Lethal concentration to kill 50% population.
[d] LC_{90} = Lethal concentration to kill 90% population.
[e] = 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 low 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 spinetoram at higher doses of HaNPV (Table 3). However, synergistic interaction was observed between spinetoram with 0.5×10^9 PIB/ml dose of HaNPV. Similar results were recorded for emamectin benzoate where there was synergistic interaction of HaNPV and emamectin benzoate at 0.5×10^9 PIB/ml dose of HaNPV. Our results are in agreement to those who reported synergistic action between synthetic insecticides and NPV (Senthil et al., 2005; Singh et al., 2009; Shaurub et al., 2014; Nasution et al., 2015) against *S. litura* larvae. The interaction between microbial agent and insecticides depends upon the type of insecticide and insect pest under study. For example, there was synergistic interaction between NPV and Azadiractum (Wakil et al. (2012) and NPV and Bacillus thuringiensis (Qayyum et al., 2015) against *H. armigera*, NPV and Imidacloprid (Trang et al., 2002) against *S. litura*. The antagonistic interaction between NPV and insecticide might be due to the decrease in feeding potential or change in pH of insect gut (El-Helaly and El-Bendary, 2013).

5. Conclusion

The HaNPV can be mixed with spinetoram and emamectin benzoate for the management of *H. armigera* however for better results their mixture should be made at lower doses of HaNPV. Better results are obtained by combining HaNPV with spinetoram instead of emamectin benzoate.

Conflict of Interest

The authors declare no potential conflicts of interest.

Acknowledgement

The authors are thankful to Institute of Pure and Applied Biology for providing laboratory facilities to conduct this study. The authors are also thankful to Dr. Husnain Ali Sayyed (Late) for his help and guidance in designing and performing this experiment.

References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Ahmad, M., Rasool, B., Ahmad, M., Russell, D.A., 2019. Resistance and synergism of novel insecticides in field populations of cotton bollworm Helicoverpa armigera (Lepidoptera: Noctuidae) in Pakistan. J. Econ. Entomol. 112, 859–871.

Ahmad, M., Arif, M.I., Ahmad, Z., 2001. Resistance to carbamate insecticides in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. Crop Prot. 20, 427–432.

Ahmad, M., Arif, M.I., Ahmad, Z., 2003. Susceptibility of Helicoverpa armigera (Lepidoptera: Noctuidae) to new chemistries in Pakistan. Crop Prot. 22, 539–544.

Alvi, A.H.K., Sayyed, A.H., Naee, M., Ali, M., 2012. Field Evolved Resistance in Helicoverpa armigera (Lepidoptera: Noctuidae) to bacillus thuringiensis toxin Cry1Ac in Pakistan. PLoS One 7, 1–9. https://doi.org/10.1371/journal.pone.0047309.

Analytical software, 2005. Statistix 8.1 for Windows. Analytical Software, Tallahassee.

Baker, G.H., Tann, C.R., Fitt, G.P., 2008. Production of Helicoverpa spp. (Lepidoptera, Noctuidae) from different refuge crops to accompany transgenic cotton plantings in eastern Australia. Aust. J. Agric. Res. 59, 723–732.

Baker, G.H., Tann, C.R., Fitt, G.P., 2010. A tale of two trapping methods: Helicoverpa spp. (Lepidoptera, Noctuidae) in pheromone and light traps in Australian cotton production systems. Bull. Entomol. Res. 101 (1), 9–23.

Brevault, T., Achaleke, J., 2005. Status of pyrethroid resistance in the cotton bollworm, *Helicoverpa armigera*, in Cameroon. Resistant Pest Manage. Newsletter 15, 4–7.

Bret, B.L., Larson, L.L., Schoonover, J.R., Sparks, T.C., Thompson, G.D., 1997. Biological properties of spinosad. Down Earth 52, 6–13.

Cisneros, J., Perez, J.A., Penagos, D.I., Ruız, V.J., Goulson, D., Caballero, P., Cave, R.D., Williams, T., 2002. Formulation of a baculovirus with boric acid for control of Spodoptera frugiperda (Lepidoptera: Noctuidae) in maize. Biol. Control 23, 87–95.

Czepak, C., Albernaz, K.C., Vivan, L.M., Guimaraes, H.O., Carvalhais, T., 2013. First reported occurrence of Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) in Brazil. Pesq. Agropec. Trop. 43 (1), 110–113.

El-Helaly, A.A., El-bendary, H.M., 2013. Impact of Spinosad and nucleeopolyderosis virus alone and in combination against the cotton leaf worm Spodeptera littralis under laboratory. App. Sci. Rep. 2 (1), 17–21.

Ferré, J., van Rie, J., 2002. Biochemistry and genetics of insect resistance to Bacillus thuringiensis. Annu. Rev. Entomol. 47, 501–533.

Guo, Y.Y., 1997. Progress in the researches on migration regularity of cotton bollworm and relationships between the pest and its host plants. Acta Entomol. Sin. 40, 1–6.

Hayes, W.J., Laws, E.R., 2013. Handbook of pesticide toxicology, classes of pesticides. Jr. Elsevier 3, 1451–1453.

Jansson, R.K., Dybas, R.A., 1998. Avermectins: Biochemical mode of action, biological activity and agricultural importance. In: Ishaaya, I., Degheele, D., Insecticides with Novel Modes of Action—Mechanisms and Application. Springer, Berlin, Heidelberg, New York, pp. 153–170.

Koppenhofer, A.M., Kaya, H.K., 2000. Interactions of a nucleopoly- hedrovirus with azadirachtin and imidacloprid. J. Invertebr. Pathol. 75, 84–86.

R.G. Lankas, L.R. Gordon, 1989. Toxicology. In: Campbell. W.C. (Ed.), Ivermectin and Abamectin, Springer Verlag, New York. NY, pp. 10–142.

MacConnell, J.G., Demchak, R.J., Preiser, F.A., Dybas, R.A., 1989. Relative stability, toxicity, and penetrability of abamectin and its 8,9-oxide. J. Agric. Food Chem. 37, 1498–1501.

Mendez, W.A., Valle, J., Ibarra, J.E., Cisneros, J., Penagosand, D.I., Williams, T., 2002. Spinosad and nucleopolyhedrovirus mixtures for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize. Biol Control 25, 195–206.

Moral-Garcia, F.J., 2006. Analysis of the spatiotemporal distribution of Helicoverpa armigera (Hübner) in a tomato field using a stochastic approach. Biosyst. Eng. 93, 253–259.

Nasution, D.E.A., Miranti, M., Melanie, M., 2015. Biological Test of Formulation of Subculture *Helicovepa armigera* Nuclear Polyhedrosis Virus (HaNPV) on Mortality of *Spodoptera litura* Larvae infested to cabbage (*Brassica oleracea* Var. capitata Linn.) Plantation. Knowl. Publ. Serv. 2, 646–648.

Peters, S.E.O., Coaker, T.H., 1993. The enhancement of Pieris brassicae (L.) (Lep., Pieridae) granulosis virus infection by microbial and synthetic insecticides. J. Appl. Entomol. 116, 72–79.

Pingel, R.L., Lewis, L.C., 1999. Effect of Bacillus thuringiensis, Anagrapha falcifera multiple nucleopolyhedrovirus, and their mixture on three lepidopteran corn ear pests. J. Econ. Entomol. 92, 91–96.

Pogue, M.G., 2004. A new synonym of Helicoverpa zea (Boddie) and differentiation of adult males of H. zea and H. armigera (Hübner) (Lepidoptera: Noctuidae: Heliothinae). Ann. Entomol. Soc. Am. 97, 1222–1226.

Qayyum, M.A., Wakil, W., Arif, M.J., Sahi, S.T., 2015. Bacillus thuringiensis and nuclear polyhedrosis virus for the enhanced biocontrol of Helicoverpa armigera. Int. J. Agric. Biol. 17, 1043–1048.

Rajagopal, R., Arora, N., Sivakumar, S., Rao, N.G.V., Nimbalkar, S.A., Bhatnagar, R.K., 2009. Resistance of Helicoverpa armigera to Cry1Ac toxin from Bacillus thuringiensis is due to improper processing of the protoxin. Biochem. J. 419, 309–316.

Salgado, V.L., 1998. Studies on the mode of action of spinosad: insect symptoms and physiological correlates. Pestic. Biochem Phys. 60, 91–102.

Saunders, D.G., Bret, B.L., 1997. Fate of spinosad in the environment. Down Earth 52, 14–20.

Sayyed, A.H., Wright, D.J., 2006. Genetics and evidence for an esterase-associated mechanism of resistance to indoxacarb in a field population of diamondback moth (Lepidoptera: Plutellidae). Pest Manage. Sci., New York 62 (11), 1045–1051.

Senthil, S.N., Kalaivani, K., Chung, P.G., 2005. The effects of azadirachtin and nucleopolyhedro virus on midgut enzymatic proWle of Spodoptera litura Fab.

(Lepidoptera: Noctuidae). Pestic. Biochem. Physiol. 83, 46–57. https://doi.org/10.1016/j.pestbp.2005.03.009.
Shapiro, M., Dougherty, E.M., 1994. Enhancement in activity of homologous and heterologous viruses against the gypsy moth (Lepidoptera: Lymantriidae) by an optical brightener. J. Econ. Entomol. 87, 361–365.
Shaurub, E.H., Meguid, A.A., Aziz, N.M.A., 2014. Effect of individual and combined treatment with Azadirachtin and *Spodoptera littoralis* multicapsid nucleopolyhedro virus (SpliMNPV, Baculoviridae) on the Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Ecol. Balkanica 6, 93–100.
Singh, H., Singh, H.R., Yadav, R.N., Yadav, K.G., Yadav, A., 2009. Efficacy and economics of some bio-pesticide in management of Helicoverpa armigera (HUB) on chickpea. Pestology 33, 36–37.
Sparks, T.C., Thompson, G.D., Kirst, H.A., Hertlein, M.B., Larson, L.L., Worden, T.V., Thibault, S.T., 1998. Biological activity of the spinosyns, new fermentation derived insect control agents, on tobacco budworm (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 91, 1277–1283.
Torres-Vila, L.M., Rodrıguez Molina, M.C., Lacasa Plasencia, A., Bielza-Lino, P., Rodríguez-del-Rincón, Á., 2002. Pyrethroid resistance of Helicoverpa armigera in Spain: current status and agroecological perspective. Agric. Ecosyst. Environ. 93, 55–66.
Trang, T., Kieu, T., Chaudhari, S., 2002. Bioassay of nuclear polyhydrosis virus (NPV) and in combination with insecticide on Spodeptera litura (Fab). Omerica 10, 45–53.
Wakil, W., Ghazanfar, M.U., Nasir, F., Qayyum, M.A., Tahir, M., 2012. Insecticidal efficacy of Azadirachta indica, nucleopolyhedrovirus and chlorantraniliprole singly or combined against field populations of Helicoverpa armigera Hübner (Lepidoptera: noctuidae). Chilean J. Agric. Res. 72 (1), 53.
Xu, X., Sepich, C., Lukas, R.J., Zhu, G., Chang, Y., 2016. Emamectin is a non-selective allosteric activator of nicotinic acetylcholine receptors and GABAA/C receptors. Biochem. Biophys. Res. Commun. 473 (4), 795–800.