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Original article

Field evaluation of nucleopolyhedrosis virus and some biorational insecticides against *Helicoverpa armigera* Hubner (Noctuidae: Lepidoptera)

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ABSTRACT

American bolloworm, *Helicoverpa armigera* Hubner (Noctuidae: Lepidoptera) is considered as a major pest of various crops all over the world. It is mainly controlled by indiscriminate use of synthetic insecticides in the world due to which this pest developed resistance to most of the available insecticides. Therefore, in the current study, the efficacy of virulent strain of HaNPV (0.5×10^9 PIB/ml) alone and in combination with recommended doses of spintoram (20 g/100 L of water) and emamectin benzoate (200 ml/100 L of water) was tested in field. The combination of HaNPV with spintoram and emamectin benzoate 100% reduced the larval population as compared to emamectin benzoate and HaNPV alone. This suggested that the combination of spintoram and emamectin benzoate with HaNPV could be used in field to manage the infestation of *H. armigera*.

1. Introduction

American Bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) is a major pest of several field crops and vegetables causing losses of several billions dollars annually. It has wide hosts range including approximately all the commercially grown plant families. This could be one of the reason of evolution of resistance in this pest against different insecticides (Wang and Qin, 2007). The other reasons for quick resistance development in *H. armigera* could be its short life cycle, high fecundity and favorable environmental conditions prevailing in the area (Denholm et al. 1998).

In some countries, the problem of insecticide resistance in *H. armigera* has worsened causing 20–30% crop loss (Bhargava et al., 2008). The indiscriminate use of chemical insecticides to control. this pest led to development of resistance .to several insecticides groups including pyrethroids (>800 folds), organophosphates (>700 folds), carbamates (>300 folds) and a low level of resistance (8 folds) (Ahmad et al. 1995; Ahmad et al., 2002; Faheem et al. 2013 Yang et al. 2013) and serious harmful effects on the environment (Sabir et al. 2011). Thus usage of synthetic insecticide should be minimized and replaced with environmentally benign methods to reduce insecticide resistance and delay development of resistance in insect pests (Bielza 2008).

Therefore, there is a dire need to find some alternative methods and compounds for the sustainable management of *H. armigera* without damaging the non-target organisms and the environment. The use of entomopathogenic micro-organisms could be an effective option for *H. armigera* but a few products are available in the

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market for its management (Qayyum et al. 2020). Although, *Bacillus thuringiensis* (*Bt*) crops significantly reduced the problem of cotton bollworms but some pests like *H. armigera* have developed resistance to these Bt crops (Tabashnik et al. 2008, 2009; Alvi et al., 2012). At the same time, heavy usage of non-selective chemical insecticides for sucking pests has led to decline in population of biological control agents, allowing *H. armigera* to resurge.

Another option for the management of *H. armigera* is the use of nuclear polyhedrosis virus (NPV) which species-specific and have almost no harm to the beneficial natural enemies of pests and the environment (Caballero et al., 1992; Cory et al., 1997). These NPV have been successfully used against some lepidopterous pest species (Liu et al. 2006; Inceoglu et al. 2006; Kumar et al. 2011; Ahmad et al. 2018; Ayyub et al. 2019), but there are few studies in which authors have evaluated field efficacy NPV against *H. armigera.* Some authors have reported increased in the efficacy of NPV when they are mixed with sub-lethal doses of synthetic insecticides both in laboratory and field conditions (Charati et al. 1999; Bret et al. 1997; Ayyub et al. 2019). To the best of our knowledge, none of any studies reported the impact of NPV along with insecticides against *H. armigera*. In the current study, efficacy of HaNPV along with two insecticides (spintoram and emamectin benzoate) was assessed against *H. armigera* under field conditions. We hypothesized that the combination of NPV with insecticide will be more effective as compared to sole insecticide or NPV against *H. armigera.*

2. Materials and methods

2.1. Study site and layout of experiment

The cotton field comprised of 594 × 480 sq.ft (6 Acres) was selected and divided into four similar blocks each of 594 × 110 sq.ft (1.5 acres). There were six treatments: Spintoram, Emamectin benzoate; lab selected HaNPV; Spintoram + HaNPV; Emamectin benzoate + HaNPV and Control and each treatment was replicated four times. The plot size per treatment was 90 × 100 sq.ft and plot to plot distance was 9 feet whereas block to block distance was 10 feet. However, on boundaries of North south 4.5ft was untreated area and from east west boundaries 5 ft were untreated area. The experiment was laid out in randomized complete block design.

2.2. Cultivation of cotton

The field was lazer leveled before seed bed preparation. The seed bed preparation was made by two ploughings followed by one deep ploughing, 1 rotavater and again two ploughings to make the soil good for cotton seed germination. The sowing was made on April 29, 2014 by planting 8 kg seed/Acre of MNH-886 by planter with row to row distance 2.5feet and plant to plant distance 0.75ft. Soon after sowing, irrigation was made and till germination irrigation was made after every 3 days and after that at every 7 days interval. However, the irrigation was skipped if rainfall occurred at the time of irrigation. The gap flailing was made after 3rd irrigation. The thinning was done at 6 leaf stage. 1 bag of DAP and 1 bag of CAN per acre was added at the time of seed bed preparation and then half bag of urea at every alternate irrigation through fertigation was used till 10th of October 2014. Pest scouting was started on 30th may 2014 and continued till October 31st, 2014 after 7 days interval. Sucking pests were controlled by applying relevant insecticides.

2.3. Field spraying and data recording

Field doses of each treatment, HaNPV @ 0.5×10^9 PIB/ml alone and in combination with recommended doses of spintoram (20 g/100 L of water) and emamectin benzoate (200 ml/100 L of water) were prepared and sprayed in cotton field at dusk so that larvae have enough time to pick up NPV from foliage. The most virulent strain of HaNPV was used in field experiments (Abid et al. 2020). The data of *H. armigera* population was recorded 24 h before spraying each field and then at 24 h interval for a total of eight days. During data recording, plants were carefully observed for dead larvae that were collected and brought to laboratory for confirmation of the NPV infection.

2.4. Data analysis

The data of reduction (%) of *H. armigera* population in different treatments was subjected to randomized complete block design (RCBD) analysis of variance (ANOVA) and means were separated by Tukey's HSD test. All tests were performed using Statistix 8.1v (Analytical software, 2005).

3. Results

The number of larvae observed per 25 plants at several intervals after application are presented in Table 1. There was significant.difference among the treatments after 1 day exposure ($F = 2.85$, $DF = 5$, $P = 0.05$). After 2 days post application, there were no significant differences in the treatments compared with the control ($F = 1.59$, $df = 5$, $P = 0.22$). After 3 days post application, the number of larvae varied from 1.75 to 2.25 in spintoram, emamectin benzoate, NPV and their mixture treatments which were significantly less than the control treatment where 5.00 larvae/25 plants were observed ($F = 4.89$, $df = 5$, $P = 0.01$).

Similarly, the number of larvae in all the treatments (except control) were significantly lower viz. 0.25–2.00 after 4 days exposure which were significantly higher than the control containing 6.50 larvae ($F = 25.52$, $df = 5$, $P < 0.01$). After 5 days of exposure, no population was observed in Spintoram + NPV, while higher population was observed in control viz. 8.50 larvae/25 plants ($F = 51.31$, $df = 5$, $P < 0.01$). After 6 days of treatment, larval population in control increased to 9.75 larvae/25 plants while larval

Table 1
Average number of *Helicoverpa armigera* larvae after application of spintoram, emamectin benzoate, NPV and their mixtures.

Treatment	Number of larvae / 25 plants							
	After 1 day exposure	After 2 day exposure	After 3 day exposure	After 4 day exposure	After 5 day exposure	After 6 day exposure	After 7 day exposure	After 8 day exposure
Control	2.50 ± 1.04b	2.75 ± 0.48ab	5.00 ± 0.91a	6.50 ± 0.64a	8.50 ± 1.04a	9.75 ± 0.48a	9.75 ± 0.85a	12.25 ± 0.48a
Spintoram	3.25 ± 0.48b	2.75 ± 0.25ab	2.25 ± 0.25b	1.00 ± 0.41bc	0.50 ± 0.29bc	0.50 ± 0.29b	0.00 ± 0.00b	0.00 ± 0.00c
Emamectin benzoate	2.25 ± 0.25b	3.00 ± 0.41a	2.00 ± 0.41b	2.00 ± 0.41b	1.50 ± 0.29b	0.75 ± 0.48b	0.50 ± 0.29b	1.50 ± 0.29b
NPV	3.00 ± 0.41b	1.75 ± 0.25b	1.75 ± 0.63b	1.25 ± 0.48bc	0.75 ± 0.48bc	0.50 ± 0.29b	0.25 ± 0.25b	0.50 ± 0.29c
Spintoram + NPV	2.75 ± 0.25b	2.00 ± 0.71ab	1.75 ± 0.25b	0.25 ± 0.25c	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c
Emamectin benzoate + NPV	5.50 ± 1.04a	2.00 ± 0.41ab	2.00 ± 0.41b	1.00 ± 0.41bc	0.50 ± 0.29bc	0.50 ± 0.29b	0.50 ± 0.29b	0.00 ± 0.00c

Values within columns followed by the same letter do not differ significantly at the $P > 0.05$.

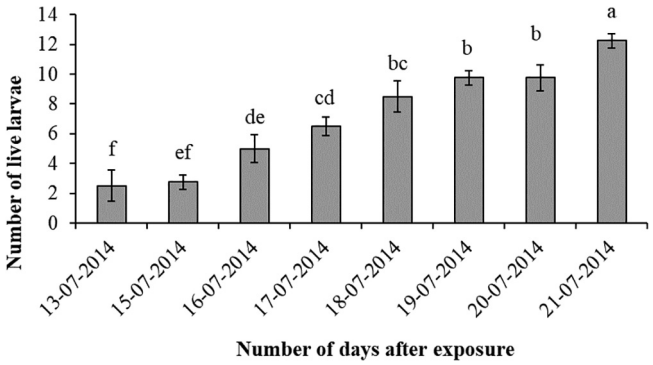

Fig. 1. Number of larvae recorded in the control treatment at different intervals. Bars with different letters show significant differences at $p < 0.05$.

population was significantly lower in all other treatments varying from 0.00 to 0.75 larvae/25 plants ($F = 133.62$, $df = 5$, $P < 0.01$). After 7 days post-treatment, larval population in control increased to 9.75 larvae/25 plants while larval population was significantly lower in all other treatments varying from 0.00 to 0.50 larvae/25 plants ($F = 92.08$, $df = 5$, $P < 0.01$). Finally, larval population reduced to 0.00 in spintoram, spintoram + NPV and emamectin benzoate + NPV while in control population increased to 12.25 larvae.25 plants after 8 days exposure ($F = 359.91$, $df = 5$, $P < 0.01$).

3.1. Number of larvae in control treatment

The live number of larvae in the control treatment were significantly increased with the passage of time ($F = 19.33$, $df = 7$, $P < 0.001$, Fig. 1).

3.2. Number of larvae in spintoram treatment

The live number of larvae of *H. armigera* in the spintoram treatment were significantly reduced after eight days exposure to spintoram ($F = 25.65$, $df = 7$, $P < 0.001$). There were no larvae found after 7 and 8 days exposure to spintoram (Fig. 2).

3.3. Number of larvae in emamectin benzoate treatment

The live number of larvae of *H. armigera* after 6 and 7 days exposure to emamectin benzoate were significantly lower compared with the exposure to emamectin benzoate after 1st, 2nd, 3rd, 4th, 5th and 8th days ($F = 4.96$, $df = 7$, $P = 0.002$). However,

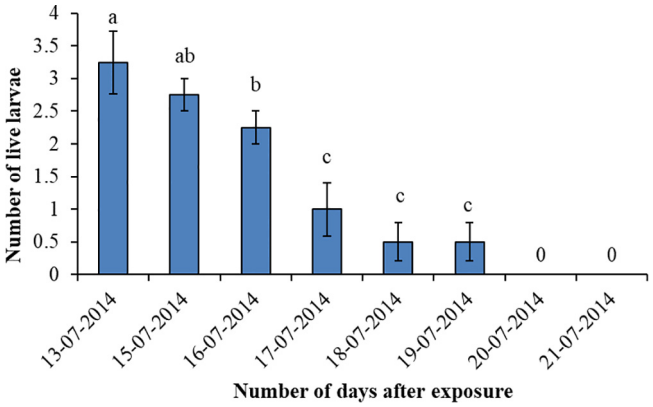

Fig. 2. Number of larvae recorded in the spintoram treatment at different intervals. Bars with different letters show significant differences at $p < 0.05$.

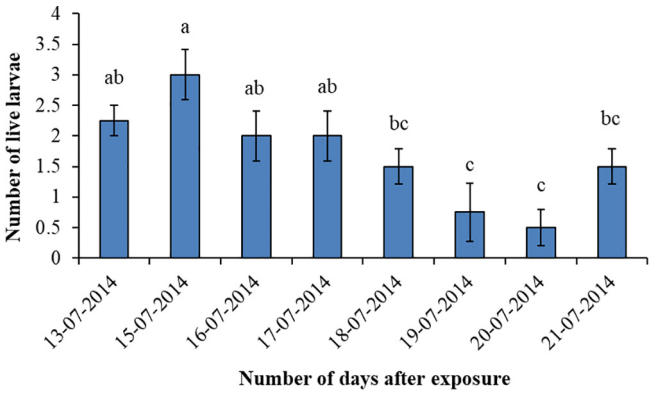

Fig. 3. Number of larvae recorded in the emamectin benzoate treatment at different intervals. Bars with different letters show significant differences at $p < 0.05$.

there were no significant differences in larvae after first 5th and 8th day exposure to emamectin benzoate (Fig. 3).

3.4. Number of larvae in NPV treatment

The live number of larvae of *H. armigera* in the NPV treatment were significantly decreased ($F = 4.62$, $df = 7$, $P = 0.003$) with the passage of time to exposure (Fig. 4).

3.5. Number of larvae in spintoram + NPV treatment

live number of larvae of *H. armigera* in the spintoram + NPV treatment were highly significantly decreased ($F = 14.09$, $df = 7$, $P < 0.001$) with the passage of time to exposure (Fig. 5). There were no larvae found 4 days after exposure to spintoram + NPV mixture.

3.6. Number of larvae in emamectin benzoate + NPV treatment

The live number of larvae of *H. armigera* in the emamectin benzoate + NPV treatment were highly significantly decreased ($F = 13.89$, $df = 7$, $P < 0.001$) with the passage of time to exposure (Fig. 6). There were no larvae found 8 days after exposure to emamectin benzoate + NPV mixture.

4. Discussion

In Pakistan, farmers use different insecticides as a major tool for the management of various lepidopteran pests. However, extensive

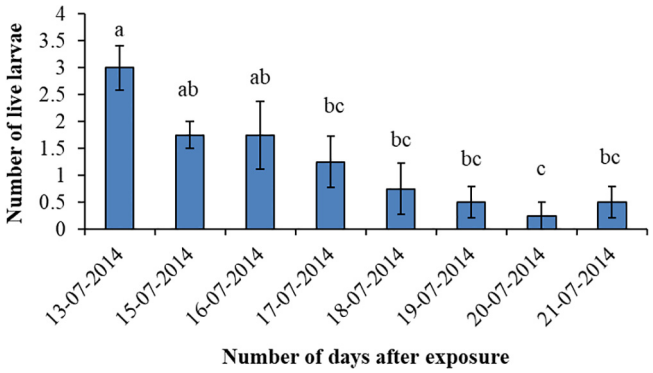

Fig. 4. Number of larvae recorded in the NPV treatment at different intervals. Bars with different letters show significant differences at $p < 0.05$.

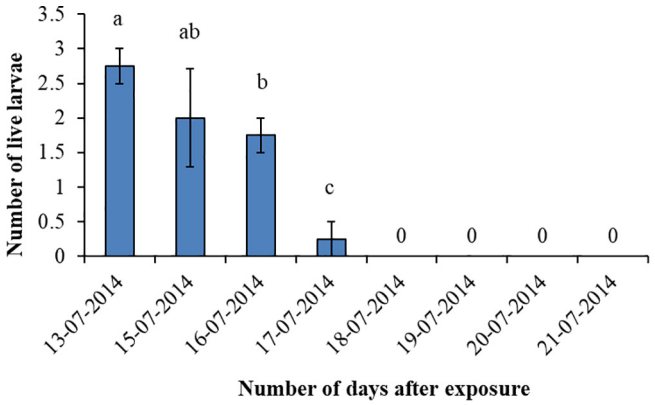

Fig. 5. Number of larvae recorded in the spintoram + NPV treatment at different intervals. Bars with different letters show significant differences at $p < 0.05$.

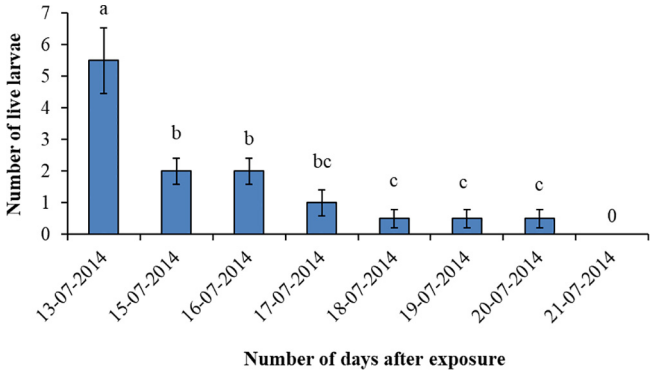

Fig. 6. Number of larvae recorded in the emamectin benzoate + NPV treatment at different intervals. Bars with different letters show significant differences at $p < 0.05$.

use of insecticides has created the resistance in lepidopterous insect pests (Kaur and Dilawari 2011, Shad et al. 2012, Faheem et al. 2013, Abbas et al. 2014, Jan et al. 2015, Qayyum et al., 2015). Authors have reported resistance in *H. armigera* to a wide range of insecticides and might be the main reason for sporadic out breaks of this pest (Alvi et al. 2012, Faheem et al. 2013, Qayyum et al., 2015). In the current study, field efficacy of NPV alone and its combination with two new chemistry insecticides (spintoram and emamectin benzoate) was evaluated. The results indicated that the combination of NPV + spintoram caused 100% reduction of *H. armigera* from cotton field followed by the combination of NPV + emamectin benzoate and NPV alone. However, larval population was increased in control plots.

The higher reduction of larval population in plots where NPV + spintoram was sprayed due to additive effect of spintoram when mixed with NPV. Spintoram insecticide is extracted from *Saccharopolyspora spinosa,* a soil bacterium is an effective bio-pesticide with very low mammalian and beneficial insect toxicity. The spintoram has a dual mode. of action, the nicotinic acetylcholine receptor, and GABA receptor allosteric modulators. Spintoram has been used effectively for the .management of different insect pests especially lepidopterans (Abbas et al. 2015; Jan et al. 2015; Ullah et al. 2016). However, emamectin benzoate has been derived from *Streptomyces avermitilis* and is a glutamate-gated. chloride .channel allosteric modulator (Sparks et al. 2012; Sparks and Nauen 2015). In the current study, spintoram, as well as emamectin benzoate significantly lower the number of ABW larvae under field trials to 100% after one week of application.

In the current study, NPV significantly reduced the number of H. armigera larvae under field applications, when applied alone. Previously, NPV as an effective microbial insecticide has been reported in *H. armigera* (Gupta et al. 2007, Marzban et al. 2009, Pugalenthi et al. 2013), *Spodoptera littoralis* (Sutanto et al. 2014) and *Spodoptera litura* (Maqsood et al. 2017). Several hurdles have been faced due to low persistent, slow action and needed repeated applications when the microbial agents are used individually in integrated pest management. Combined application of microbes with biopesticides may help to control *H. armigera*, when these mixtures may enhance their virulence than expected in a single application. In the present study, the mixtures of NPV and spintoram/emamectin benzoate significantly reduced the number of H. armigera larvae under field conditions. These results are in agreement with the results of previous studies where a combination of NPV and flubendiamide insecticide significantly reduced the lepidopteran larvae (Shaurub et al. 2014, Maqsood et al. 2017). Similar results were obtained by Nawaz et al. (2019) who reported NPV as effective tool for the management of H. armigera under laboratory conditions.

5. Conclusion

In conclusion, NPV, spintoram and emamectin benzoate are biorational insecticides and have the potential to be effectively used in integrated pest management strategies as a key component to minimize the insecticides resistance developed in this pest. The present results suggest that NPV with a combination of insecticides should be used for suppression of the ABW larvae under field conditions.

Declaration of Competing Interest

The authors declare no potential conflicts of interest.

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