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Biological Control and Crop Protection

Comparative bio-efficacy of nuclear polyhedrosis virus (NPV) and Spinosad against American bollwormm, *Helicoverpa armigera* (Hubner)

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ABSTRACT

American bollworm (ABW), *Helicoverpa armigera* (Hubner), is considered as a major pest of cotton, *Gossypium hirsutum*, all over the globe. Due to its destructive feeding nature and continuous consumption of the same chemicals, it devolved resistant against many insecticides. Therefore, a combined application of bio- and synthetic-pesticide need to evaluate against this pest. The entomopathogenic viruses like nuclear polyhedrosis virus (NPV), a member of baculoviruses, can be the potential candidates for better control against ABW. The present study was conducted to assess the comparative efficacy of NPV and Spinosad 240SC (with the concentration of 250 mL · ha^{-1}) against ABW in the controlled environment. The ABW was treated with different concentrations of NPV and Spinosad separately and in a combination of NPV with 0.1% Spinosad. The results revealed that highest concentrations showed highest mortality (95%) followed by 95%, 92%, 84%, 82% and 78% mortality at 1×10^9, 1×10^8, 1×10^7, 1×10^6 and 1×10^5 POBs, respectively. Spinosad when mixed in diet give 100% mortality at 0.8% followed by 50.87%, 42.10%, 29.82%, 26.31% and 22.80% mortality at 0.4%, 0.2%, 0.1%, 0.5% and 0.025% respectively. The results of this study revealed that microbial control of ABW through NPV is an effective tool. The repeated use of synthetic pesticides caused the resurgence of many insect pests, and this study results would provide useful insight to build a framework for future investigations for the management of many major insect pests.

Introduction

American bollworm (ABW), *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), has been observed most dangerous pest and yield decliner of the field crops and vegetables in Pakistan since the 1990s and early 21st century. It has become the most hazardous pest due to its polyphagous nature, migratory behavior, high fecundity rate, multiple generations and can develop the resistance against insecticides. It is estimated that annually, 2.5 million bales losses were caused by this insect pest to the cotton crop (Karim, 2000; Muhammad et al., 2009).

Different levels of resistance (moderate to high) to a wide range of insecticides have been reported worldwide, including Pakistan, among the field population of ABW (Sayyed and Wright, 2006; Damalas and Eleftherohorinos, 2011; Ishtiaq and Saleem, 2011). Therefore, the management of this pest for an extended period with reduced risk of insecticides is the dire need of time and a pragmatically suggested option. Among the various biological control agents used as an alternative to synthetic insecticides (Ali et al.,

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Table 1
Effect of different concentrations of Spinosad on larvae of American bollworm, *Helicoverpa armigera* after different time intervals in diet mixed method.

Concentrations %	24 h	48 h	72 h
0.8	$16.81^{A} \pm 0.39$	$33.81^{A} \pm 0.54$	$50.87^{A} \pm 0.81$
0.4	$15.30^{A} \pm 0.25$	$26.02^{B} \pm 0.49$	$42.10^{AB} \pm 0.66$
0.2	$10.15^{AB} \pm 0.43$	$20.31^{C} \pm 0.26$	$29.82^{BC} \pm 0.36$
0.1	$8.39^{AB} \pm 0.39$	$15.23^{CD} \pm 0.31$	$26.31^{C} \pm 0.31$
0.05	$3.51^{B} \pm 0.26$	$11.86^{D} \pm 0.51$	$22.80^{C} \pm 0.44$
0.025	$3.79^{B} \pm 0.33$	$11.61^{D} \pm 0.47$	$22.80^{C} \pm 0.37$
CONTROL	$2.07^{B} \pm 0.22$	$2.38^{E} \pm 0.37$	$3.71^{D} \pm 0.28$

2015, 2018a,b,c,d,e), the preference for entomopathogenic Nuclear Polyhedrosis Virus (NPV) has been increased in the recent years. Furthermore, new chemistry insecticides have been established from natural sources, disturb the normal physiological functions of the targeted species in pest management programs (Qasim et al., 2018a; Schneider et al., 2003; Thompson et al., 2000; Dhadialla et al., 1998) by targeting insect development mechanism like moulting as well as chloride channel activator. Another advantage of new chemistry insecticide over the broad-spectrum insecticides is that they are selective to target pest species and less dangerous to natural enemies (Grafton-Cardwell et al., 2005).

Among microbial insecticides, the insect pathogenic viruses such as baculoviruses were used successfully for biological control under integrated pest management (IPM) program. It has been used for more than 20 years with great success (Zhang, 1989) in different parts of the world for managing different insect pest species based on their several advantages. These microbial agents are highly host-specific and are known to be entirely safe for humans, animals and non-target beneficial organisms such as bees, predatory insects and parasitoids (Monobrullah and Nagata, 1999; Nakai et al., 2003; Islam et al., 2018a, b). Keeping in view the facts as mentioned above, the present study was carried out to evaluate the effectiveness of NPV against ABW under controlled conditions.

Materials and methods

The study was conducted in Integrated Pest Management (IPM) Lab, Department of Entomology, University of Agriculture, Faisalabad, Pakistan during 2016–2018.

Preparation of artificial diet

To prepare the artificial diet for lepidopterans, the product F9772 (Lot# 110216-01) was used according to the manufacturer's recommendations. Moreover, following ingredients were used; agar (19.0 g), distilled water (875 mL) (stirred the agar until the agar was dissolved completely) and 144.0 g dry mix ingredients (Sucrose, Soy Flour, 50%, Wheat Germ, stabilized, Salt Mix, Wesson, USDA Vitamin Premix, Fiber, Sorbic Acid, Methyl Paraben, Ascorbic Acid). Agar solution was boiled and stir until a smooth, and even consistency was achieved. Pour the diet into small cups approximately one-third of a cup, and left to cool and solidify ready for the addition of larvae.

Rearing of insects

The larvae of ABW were collected from the host field and reared in the laboratory under controlled conditions (temperature $25 \pm 2\,^{\circ}C$ and relative humidity (RH) 75%). The cubes of the artificial diet were prepared and shifted to plastic vials. A single larva was allowed to feed on the diet in each plastic vial. For adults, a 10% sugar solution was provided until adult dies, for egg laying on muslin cloth. The laid eggs were collected after 24 h in a petri dish in a growth chamber for eclosion. After eclosion, larvae were shifted to an artificial diet for further experiments.

Isolation of NPV from infected ABW from filed

Infected larvae of ABW were collected from the field. For isolation of the virus, a standard procedure was adopted in which homogenization, filtration and centrifugation were done. The occlusion bodies were purified (Tompkins, 1991). The Polyhedral occlusion bodies (POB) per millilitre were counted using hemocytometer under the light microscope.

Bioassay

Accessing the efficacy of NPV on ABW

The homogenized population of ABW was reared, and selected second and third instar (20 larvae) were allowed to feed on the artificial diet mix with NPV formulation. Six different formulations of NPV (1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9 and 1×10^{10} POBs) were used to assess the efficacy of NPV. The experiment was repeated thrice, and control was kept untreated. Mortality data was taken after the interval of every 24 h subjected to different dose formulation until all the larvae died.

Assessing the efficacy of Spinosad

Spinosad 240SC (Arysta Life Science, Pakistan) was assessed against ABW at the rate of 80–100 $mL \cdot acre^{-1}$. Six concentrations of spinosad (0.8%, 0.4%. 0.2%, 0.1%, 0.05% and 0.025%) were made and tested against the ABW larvae through diet mix method. The experiment was repeated thrice, along with the control treatment. Mortality data for all treatments were taken at the interval of 24 h, 48 h and 72 h.

Assessing the efficacy of combine toxicity of NPV and Spinosad

Various concentrations of NPV were also mixed with 0.1% spinosad to check the combined toxicity of NPV and Spinosad. Twenty larvae were allowed to feed on a diet mixed with NPV and Spinosad in combination. Mortality data were taken after 24 h subjected to concentrations of NPV and Spinosad.

Statistical analysis

Mortality data were subjected to Abbott's formula (Fleming and Retnakaran, 1985). Data were analyzed using Statistica software and means were separated by using Tukey's HSD test. The Co-Toxicity test was calculated by the following formula.

$$CTF = OC\text{-}OE/OE \times 100$$

CTF means co toxicity factor, OC means observed percentage mortality and OE is the result of the combined application. The OE is the expected percentage of mortality and represents the sum of percentage mortality produced by each of the treatment used in combination. The value CTF varies between 20 and −20. Value of CTF above 20 revealed that there was a synergetic effect between two mixtures. The value of CTF in −20 showed that there was an antagonistic relationship between two mixtures and the value of CTF between 20 and −20 showed that there was an additive effect on comparative efficacy.

Results

ANOVA parameters indicated that all NPV concentrations were significantly effective against the second and third instar larvae of ABW ($F = 592$, $P = 0.000$). Results showed that higher concentrations had given the highest mortality when treated with NPV alone in diet mixed method. It was also observed that the effectiveness

Table 2
Mortality percentage of American bollworm, *Helicoverpa armigera* against different concentrations of NPV alone and in a combination of 0.1% Spinosad.

Concentrations	DAT 1 ± S.E.	DAT 2 ± S.E.	DAT 3 ± S.E.	DAT 4 ± S.E.	DAT 5 ± S.E.	DAT 6 ± S.E.	DAT 7 ± S.E.	DAT 8 ± S.E.	DAT 9 ± S.E.	DAT 10 ± S.E.
1×10^{10} alone	$20.07^{A} \pm 0.25$	$28.39^{A} \pm 0.39$	$36.14^{A} \pm 0.80$	$45.61^{A} \pm 0.82$	$57.89^{A} \pm 0.88$	$66.67^{A} \pm 0.90$	$79.63^{A} \pm 1.11$	$92.15^{A} \pm 2.11$	$95.00^{A} \pm 2.11$	$95.00^{A} \pm 2.05$
1×10^{9} alone	$15.39^{B} \pm 0.35$	$20.05^{AB} \pm 0.42$	$32.71^{AB} \pm 0.77$	$42.10^{A} \pm 0.87$	$49.12^{B} \pm 0.79$	$50.08^{B} \pm 0.85$	$74.07^{A} \pm 1.95$	$84.31^{A} \pm 2.01$	$98.03^{A} \pm 2.02$	$100.00^{A} \pm 1.99$
1×10^{8} alone	$10.06^{C} \pm 0.40$	$16.67^{BC} \pm 0.25$	$27.54^{BC} \pm 0.31$	$33.42^{B} \pm 0.60$	$42.10^{C} \pm 0.65$	$50.81^{B} \pm 0.79$	$59.25^{B} \pm 0.81$	$66.59^{B} \pm 0.80$	$78.43^{B} \pm 1.25$	$92.15^{B} \pm 2.07$
1×10^{7} alone	$10.55^{C} \pm 0.30$	$13.45^{BC} \pm 0.22$	$24.12^{CD} \pm 0.49$	$31.57^{B} \pm 0.59$	$38.59^{CD} \pm 0.59$	$44.49^{B} \pm 0.91$	$53.70^{B} \pm 0.86$	$62.74^{B} \pm 0.91$	$68.62^{C} \pm 0.97$	$84.31^{C} \pm 1.75$
1×10^{6} alone	$6.45^{C} \pm 0.45$	$11.62^{BC} \pm 0.49$	$20.70^{CD} \pm 0.25$	$28.07^{BC} \pm 0.36$	$36.84^{CD} \pm 0.25$	$44.58^{B} \pm 0.83$	$50.01^{B} \pm 0.79$	$58.82^{B} \pm 0.88$	$62.74^{C} \pm 0.92$	$82.35^{C} \pm 1.90$
1×10^{5} alone	$6.38^{C} \pm 0.54$	$10.08^{CD} \pm 0.50$	$17.28^{D} \pm 0.51$	$24.56^{C} \pm 0.22$	$35.08^{D} \pm 0.51$	$42.53^{B} \pm 0.76$	$50.82^{B} \pm 0.90$	$56.86^{B} \pm 0.79$	$60.78^{C} \pm 0.88$	$78.43^{C} \pm 1.20$
Control	$2.09^{D} \pm 0.39$	$2.04^{D} \pm 0.30$	$3.06^{E} \pm 0.33$	$4.44^{D} \pm 0.29$	$5.31^{E} \pm 0.42$	$5.60^{C} \pm 0.29$	$7.29^{C} \pm 0.45$	$8.61^{C} \pm 0.25$	$10.02^{D} \pm 0.49$	$10.05^{D} \pm 0.39$
1×10^{10} + Spin.	$16.81^{A} \pm 0.39$	$33.81^{A} \pm 0.54$	$50.87^{A} \pm 0.81$	$71.93^{A} \pm 1.11$	$94.73^{A} \pm 1.97$	$95.00^{A} \pm 2.09$	$95.00^{A} \pm 2.01$	$95.00^{A} \pm 2.11$	$95.00^{A} \pm 2.02$	$95.00^{A} \pm 2.11$
1×10^{9} + Spin.	$15.30^{A} \pm 0.25$	$26.02^{B} \pm 0.49$	$42.10^{AB} \pm 0.66$	$64.91^{AB} \pm 0.95$	$83.82^{A} \pm 1.50$	$90.74^{A} \pm 1.91$	$95.00^{A} \pm 2.11$	$95.00^{A} \pm 2.04$	$95.00^{A} \pm 1.97$	$95.00^{A} \pm 1.91$
1×10^{8} + Spin.	$10.15^{AB} \pm 0.43$	$20.31^{C} \pm 0.26$	$29.82^{BC} \pm 0.36$	$42.10^{BC} \pm 0.88$	$51.85^{B} \pm 0.89$	$64.81^{B} \pm 0.89$	$73.52^{B} \pm 1.89$	$84.31^{B} \pm 1.93$	$98.03^{A} \pm 2.11$	$100.00^{A} \pm 2.08$
1×10^{7} + Spin.	$8.39^{AB} \pm 0.39$	$15.23^{CD} \pm 0.31$	$26.31^{C} \pm 0.31$	$40.35^{BC} \pm 0.76$	$48.24^{B} \pm 0.66$	$53.70^{B} \pm 0.66$	$50.87^{C} \pm 0.91$	$62.74^{C} \pm 0.58$	$65.85^{B} \pm 0.88$	$74.51^{B} \pm 1.39$
1×10^{6} + Spin.	$3.51^{B} \pm 0.26$	$11.86^{D} \pm 0.51$	$22.80^{C} \pm 0.44$	$35.08^{C} \pm 0.57$	$46.49^{B} \pm 0.91$	$51.85^{B} \pm 0.81$	$45.50^{C} \pm 0.88$	$57.86^{C} \pm 0.81$	$62.74^{B} \pm 0.79$	$70.58^{B} \pm 1.85$
1×10^{5} + Spin.	$3.79^{B} \pm 0.33$	$11.61^{D} \pm 0.47$	$22.80^{C} \pm 0.37$	$35.18^{C} \pm 0.61$	$46.49^{B} \pm 0.88$	$50.16^{B} \pm 0.77$	$45.20^{C} \pm 0.81$	$56.65^{C} \pm 0.88$	$60.78^{B} \pm 0.91$	$68.62^{B} \pm 0.91$
Control	$2.07^{B} \pm 0.22$	$2.38^{E} \pm 0.37$	$3.71^{D} \pm 0.28$	$5.09^{D} \pm 0.37$	$7.70^{C} \pm 0.21$	$9.08^{C} \pm 0.44$	$9.76^{D} \pm 0.30$	$10.09^{D} \pm 0.33$	$10.05^{C} \pm 0.27$	$10.06^{C} \pm 0.21$

Table 3
Effect of Spinosad on mean larval mortality of American bollworm, *Helicoverpa armigera* larvae treated with different concentration of NPV in a combination of 0.1% Spinosad from day 1–3.

Concentrations	Day-1				Day-2				Day-3			
	Actual mortality	Expected mortality	CTF	Effect	Actual mortality	Expected mortality	CTF	Effect	Actual mortality	Expected mortality	CTF	Effect
NPV6	$20.07^{A} \pm 0.25$	28.39	−9.94	Additive	$25.35^{A} \pm 0.39$	43.62	−18.27	Additive	$44.63^{A} \pm 0.61$	62.45	−17.82	Additive
NPV5	$15.39^{B} \pm 0.35$	23.78	−8.67	Additive	$20.26^{AB} \pm 0.26$	35.28	−15.02	Additive	$42.98^{A} \pm 0.69$	59.02	−16.04	Additive
NPV4	$10.06^{C} \pm 0.40$	18.45	−10.06	Additive	$15.17^{AB} \pm 0.49$	31.9	−16.73	Additive	$32.16^{B} \pm 0.55$	53.85	−21.69	Antagonistic
NPV3	$10.55^{C} \pm 0.30$	18.94	−10.51	Additive	$15.17^{AB} \pm 0.33$	28.68	−13.51	Additive	$30.40^{B} \pm 0.49$	50.43	−20.03	Antagonistic
NPV2	$6.45^{C} \pm 0.45$	14.84	−9.03	Additive	$10.08^{BC} \pm 0.51$	26.85	−16.77	Additive	$17.83^{C} \pm 0.33$	47.01	−29.18	Antagonistic
NPV1	$6.38^{C} \pm 0.54$	14.77	−11.54	Additive	$10.08^{BC} \pm 0.41$	25.31	−15.23	Additive	$17.83^{C} \pm 0.41$	43.59	−25.76	Antagonistic
Spinosad	8.39				15.23				26.31			

of NPV increased with time. Mean percent mortality comparison showed that maximum mortality (95.00 ± 2.05%) was seen at 1×10^{10} POB which was 9.95 times higher than control, followed by 95.00 ± 1.99% at 1×10^{9} POB which was 9.95 times higher than control. However, most diluted NPV dose (1×10^{5} POB) caused enough larval mortality, reaching up to 78.43 ± 1.20% which was 7.80 times higher than control (10.05 ± 0.39%) (Table 2).

Overall, analysed data indicated that all spinosad concentrations were significantly lethal to the second and third instar larvae of ABW (F = 27.4, P = 0.000). Mean percent mortality comparison showed that maximum mortality (50.87 ± 0.81%) was observed at (0.8%) which was 13.71 times higher than control followed by 42.10 ± 0.66% at (0.4%) which was 11.34 times higher than control. Though, most diluted dose (0.025%) caused only 22.80 ± 0.37% mortality of ABW larvae, which was 6.14 times higher than control (3.71 ± 0.28%) (Table 1).

ANOVA parameters indicated that the combined effect of all NPV concentrations and spinosad proved to be significant against the second or third instar larvae of ABW (F = 680, P = 0.000). Mean percent mortality comparison showed that maximum mortality (95.00 ± 2.06%) was observed at NPV6 + Spinosad (1×10^{10} POB + 0.1%) which was 9.91 times higher than control followed by (95.00 ± 1.98%) at NPV5 + Spinosad (1×10^{9} POB + 0.1%) which was 9.91 times higher than control. Even though the combination of NPV1 + Spinosad (1×10^{5} POB + 0.1%) was also too much lethal to ABW larvae, causing 80.71 ± 1.91% mortality, which was 7.99 times higher than control (10.09 ± 0.31%) (Table 2).

Combined application of different concentrations of NPV with 0.1% Spinosad

When different concentrations of NPV (1×10^{5}, 1×10^{6}, 1×10^{7}, 1×10^{8}, 1×10^{9} and 1×10^{10} POBs) were mixed with 0.1% of Spinosad, the mortality of larvae increased. It was observed that the mortality of larvae was higher in the combined application as compared to the individual application of both NPV and Spinosad. It was also observed that higher concentrations of NPV with a low concentration of Spinosad give an additive effect (CTF in between −20 and +20). While in the case of low NPV and Spinosad concentration gives an antagonistic impact (CTF ≥ −20). According to results of day one and two, all NPV concentrations showed the additive effect with Spinosad (CTF in between −20 and +20). After three days of application, two highest concentrations of NPV gave an additive impact while the rest of the concentrations gave antagonistic effects (CTF ≥ −20) (Table 3).

Discussion

While using the bio-agent in IPM, there are some limitations such as these agents are slow in action, low persistence in environment and need of a repeated application to infect the host population. The combined use of microbial agents may help in resolving these problems and increasing the pathogenicity, persistence and rate of infection (Ali et al., 2015, 2016). The issue of insecticide resistance can also be minimized by using these agents as they used different tactics to kill or control host, as compared to other conventional insecticides (Bala et al., 2018). ABW is the pest of great importance that attacks many valuable crops.

The resistance of ABW against many insecticides has been noted, and it was the issue in focus for many years. Entomopathogens can control the pest having resistance against insecticides (Cloyd, 2014). The rotation of crops and pesticides can also be helpful to minimize the problem of resistance (Zahn and Morse, 2013). When two or more bio-agents are used in combination, they increase the effectiveness, and host spectrum of each other and reduce the mortality time (Kalantari et al., 2014). It can be imagined that the mutual action of microbes may increase the virulence as expected alone. The polyhedral bodies of NPV attach to the midgut of host and multiply, thereby destroying the gut cells. The midgut is the first binding site of POBs, where they multiply and then infection transfer from cell to cell, causing the death of the host (Liu et al., 2006; Arif et al., 2018; Qasim et al., 2018b; Shakeel et al., 2018).

Among these concentrations, after the exposure to treated diet, mortality was recorded. High concentrations of both NPV and Spinosad gives the highest mortality. Such as NPV gave highest mortality (95%, 95% and 92.15%) at 1×10^{10}, 1×10^{9} and 1×10^{8} POBs concentrations and these results were parallel to those results of Arrizubieta et al. (2016), who reported that a mixture of NPV and insecticides against *H. armigera* was effective under laboratory condition. These results showed that effectiveness and tenacity of mixture caused significant mortality of larvae. Spinosad gave the highest mortality (95%, 95% and 95%) and 0.8%, 0.4% and 0.2% concentrations and these results were supported by Jat and Ameta (2013). The study results concluded that Flubendiamide 480SC and Spinosad 45SC (at 200 mL · ha^{-1}) were found to be effective against the larvae of ABW, and these insecticides caused 89.94% and 74.67% mortality, respectively.

The viruses multiply within the body of the host and destroy the host cell tissues. The pathogenicity and rate of response depend upon the dose, temperature and larval body size, and it varies from 4 to 14 days (Jones et al., 1994). Thus the pathogenicity varies with treatment and time, as the dose increase the control efficacy also increases.

Combination of two or more bio-agent can be an option to delay the resistance and increase the effectiveness (Kalantari et al., 2014). In the current study, both additive and antagonistic interactions were observed, and these results were supported with the findings of Wang et al. (2009) who found that lethal and sub-lethal doses of spinosad increase the mortality and delay the developmental process. The additive impact was supported by the results of Wakil et al. (2012), who found that there was an additive effect between NPV and *Azadiractum* against ABW. Moreover, Qayyum et al. (2015) also reported the additive impact of NPV and *Bacillus thuringiensis* against ABW. In our study, the antagonistic effect was supported by the outcomes of Trang et al. (2002) as they observed the antagonistic effect of NPV and Imidacloprid. The antagonistic effect might be due to the decrease in feeding potential or pH change of gut (El-Helaly and El-bendary, 2013).

Conclusion

We speculated that microbial control through NPV and Spinosad is an effective approach against ABW. The repeated use of synthetic pesticides against major pests could be the main reason of pest resurgence, and this study results would provide useful insight to build a framework for future investigations and to reduce the use of toxic chemicals for the control of insect pests of major crops.

Conflict of interest

All authors declare no conflict of interests.

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