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Evaluation of Nuclear Polyhedrosis Virus (NPV) and Emamectin Benzoate against *Spodoptera litura* (F.) (Lepidoptera: Noctuidae)

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Abstract

The Oriental leaf worm moth, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), is among the most voracious insect pests in a variety of economically important crops, particularly cotton. It has developed resistance against conventional chemical insecticides. Therefore, it is vital to evaluate an integrated application of bio- and synthetic pesticide against this pest. Nuclear polyhedrosis virus (NPV) can be a potent alternative to control this pest. The present study was conducted to evaluate the efficacy of NPV and emamectin benzoate (Proclaim® 19EC) against three geographically distinct populations of *S. litura* in vitro conditions. Second and fourth larval instars were treated by three different concentrations of NPV (NPV-1 2×10^9, NPV-2 3×10^9, and NPV-3 4×10^9 POB ml^{-1}) and emamectin benzoate (EB 0.1 ppm) alone and in combination. The results showed that the highest mortality rate (83.28%) was recorded for NPV-3 + EB, followed by NPV-2 + EB, NPV-1 + EB, EB, NPV-3, NPV-2, and NPV-3 at all the tests. Moreover, Faisalabad (FSD) population was found more susceptible, followed by Layyah (LY) and Multan (ML) populations. Reduction in pupation, adult emergence and egg eclosion was found directly related to the pathogenicity of the applied pathogens. The results of this study revealed that biorational control of *S. litura* with combined application of NPV + emamectin benzoate was an effective tool.

Keywords: Nucleopolyhedrosis virus, Emamectin benzoate, *Spodoptera litura*, Efficacy

Background

The Oriental leaf worm moth, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) is a serious polyphagous and cosmopolitan insect pest of cash crops, vegetable and ornamentals (Senthil-Nathan and Kalaivani, 2005). In Pakistan, *S. litura* causes heavy losses in various regions such as the northern and southern districts of Punjab (Ahmad et al. 2007). The increase in area under cultivation of succulent crops like soybean, cotton, mung bean, cabbage, and vegetables provide ideal conditions for its vigorous reproduction, resulting in a rapid increase of generations and population size (Gao et al. 2004). Mainstay of the farming community to combat this pest is the use of synthetic chemical insecticides which not only cause serious harm to environment and human health but also develop resistance against these agents (Aydin and Gurkan, 2006). Indiscriminate use of these chemical has also led to resistance in *S. litura* population in different geographical areas of the Punjab (Pakistan) (Shad et al. 2012; Ahmad and Mehmood, 2015). This situation demands to evaluate safer eco-friendly alternatives.

Nucleopolyhedrosis viruses (NPVs) can be potent alternatives to the synthetic insecticides against *S. litura* (Ahmad et al. 2018). NPVs belong to family baculoviruses (BV), which is specific against a variety of insect pests of forests and economically important crops (Tang

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et al. 2011). This family comprises 600 viruses, including two genera, NPV and Granuloviruses (GV) (Hu et al. 2003). NPVs have great potentials against many lepidopterous pests (Tang et al. 2011 and Zhang et al. 2015).

Emamectin benzoate is a semi-synthetic derivative of abamectin which has been developed for the purpose of controlling variety of lepidopterous pests worldwide (Jansson et al. 1997). Moreover, new chemical insecticides have been established from natural sources; disturb the normal physiological functions of the targeted species (Thompson et al. 2000). Environmental Protection Agency (EPA) has classified emamectin benzoate as a reduced-risk compound due to environmentally benign characteristics (Saunders and Bret, 1997). It has both stomach and contact mode of actions, primarily acting on nicotinic acetylcholine receptor and subsequently on butyric acid receptors. Emamectin benzoate in contrast to synthetic insecticides, has low mammalian toxicity and exerts no toxic effect on non-target organisms. Due to strong mode of action, it is widely used against lepidopterous and dipterous insect pest (Stanley et al. 2006; Wang et al. 2013). In Pakistan, emamectin benzoate has been registered for the control of several species of Lepidoptera in field crops and vegetables.

Integration of synthetic insecticides with baculovirus enhances the efficacy of baculovirus, especially against *S. litura* (Arti and Yogita, 2014; Shaurub et al. 2014; Ayyub et al. 2019 Nawaz *et al.,* 2019). Keeping in view the importance of low input based crop production and reduction of pesticides' load on the vegetable crops, the present study aimed to assess the efficacy of NPV alone and in combination with emamectin benzoate against 2nd and 4th larval instars of *S. litura* from various localities of Punjab (Pakistan) under laboratory conditions.

Material and methods

Insect culture

Larvae of *S. litura* were collected from three different localities viz. Faisalabad (FSD), Multan (ML), and Layyah (LY), Pakistan and shifted to the Microbial Control Laboratory, Department of Entomology, University of Agriculture, Faisalabad. *S. litura* was mass cultured under the laboratory conditions at 26 ± 2 °C, 70 ± 5% relative humidity (R.H.) and a photoperiod of 12:12 (L:D) h on a semi artificial diet, following the method of Saljoqi et al. (2015). Adults of *S. litura* were fed on 10.0% honey solution and provided with a coarse surface of tissue as nappy liner to lay eggs.

Insecticide used

The commercial liquid formulation of Proclaim® (Emamectin benzoate, 19 g l^{-1}, 19EC; Syngenta Pakistan, Ltd., Karachi, Pakistan) was used in the experiment. The insecticide was applied at 0.1 ppm.

Viral isolation and suspension preparation

NPV isolates were obtained from NPV-infected larvae stored in the Microbial Control Laboratory. The presence of NPV was confirmed by inverted microscope (× 40) with Giemsa staining (Yaman et al. 2001). Virus isolation and propagation were carried out in vivo as described by Monobrullah and Nagata (2000). Purified occlusion bodies (POBs ml^{-1}) were counted five times, using a hemocytometer under inverted microscope. A dilution of various concentrations (2×10^9; 3×10^9; and 4×10^9 POBs ml^{-1}) was prepared in distilled water from stock suspension (Cory and Myers, 2003).

Bioassay

Efficiency of emamectin benzoate and NPV was evaluated against 2nd and 4th larval instars. Both pathogens were applied, using a diet incorporation method. A diet piece of 0.5 g was offered to the larvae placed in glass vials (7 cm height, 3 cm in diameter) (Qayyum et al. 2015). The artificial diet, mixed with the three concentrations of NPV (2×10^9, 3×10^9, and 4×10^9 POB ml^{-1}) and emamectin benzoate (0.1 ppm) or a combined suspension of these was used. Each treatment contained 15 larvae (as a replicate), and was repeated thrice. Larvae were fed on the treated artificial diets for 48 h. After 48 h, survivors were transferred into new vials containing non-treated fresh diet. Bioassay were conducted at 25 ± 2 °C, 65 ± 5% R.H. and a photoperiod of 14:10 (D:L) h in an incubator. Mortality rate was recorded every 24 h and the last count was recorded till pupation of both larval instars. The larvae were poked with a blunt needle and those unable to move in a coordinated manner were considered as dead (Ma et al. 2008; Qayyum et al. 2015). Pupation and adult emergence data were also recorded hereafter. The emerged adults were allowed to mate freely at each treatment and egg hatching percentage was calculated.

Statistical analysis

Mortality means were corrected using Abbott's formula (Abbott, 1925) and the data was subjected to one-way analysis of variance (ANOVA), using Minitab software (Minitab, 2002) and significance of means were separated by Tukey's HSD test at 1.0% significance level (Sokal and Rohlf, 1995).

Results and discussion

Efficacy of NPV and emamectin benzoate was challenged against 2nd and 4th larval instars of *S. litura* on the artificial diet and all treatments were found significant. Highest mortality was recorded for NPV-3 + EB,

Table 1 Mean mortality (%±SE) of second and fourth larval instars of *Spodoptera litura* from three different field populations treated with nucleopolyhedrosis virus and emamectin benzoate (NPV-1 2 × 10^9 POBs ml^{-1}, NPV-2 3 × 10^9 POBs ml^{-1}, NPV-3 3 × 10^9 POBs ml^{-1}, EM 0.1 ppm)

Treatments	Second instar			Fourth instar		
	FSD	LY	ML	FSD	LY	ML
NPV-1	22.27 ± 1.13e	15.76 ± 0.87e	13.38 ± 0.92ef	17.46 ± 1.09f	12.80 ± 0.86f	8.99 ± 0.55f
NPV-2	35.66 ± 1.55d	27.35 ± 1.11d	20.31 ± 1.14e	28.21 ± 1.61ef	23.38 ± 1.24ef	16.45 ± 1.03e
NPV-3	44.92 ± 1.73cd	38.25 ± 1.38c	31.79 ± 1.50de	39.79 ± 1.59de	32.43 ± 1.43de	25.50 ± 1.20d
EM	49.52 ± 1.67c	42.64 ± 1.57c	37.09 ± 1.39d	44.42 ± 1.70d	38.62 ± 1.54d	30.74 ± 1.37d
NPV-1 + EM	68.83 ± 2.12b	63.43 ± 1.83bc	56.13 ± 2.10c	62.27 ± 2.67c	57.51 ± 2.29c	50.31 ± 2.06c
NPV-2 + EM	89.84 ± 2.70ab	81.42 ± 2.61b	73.28 ± 2.74b	80.72 ± 3.80b	73.49 ± 2.84b	66.82 ± 2.24b
NPV-3 + EM	100.00 ± 0.00a	96.13 ± 2.62a	87.93 ± 2.82a	96.19 ± 3.20a	88.41 ± 3.20a	83.28 ± 2.95a

Mean sharing the same letters within each column are not significantly different at 1.0% level

followed by NPV-2 + EB, NPV-1 + EB, EB, NPV-3, NPV-2, and NPV-3 at all the tests. For 2nd instar larvae, the highest larval mortality rate (49.52%) was recorded for emamectin benzoate in FSD population ($F_{7,71}$ = 118.0, $p \leq 0.01$), followed by LY ($F_{7,71}$ = 97.7, $p \leq 0.01$) and then ML ($F_{7,71}$ = 112, $p \leq 0.01$) populations (Table 1). In the combined treatments, maximum larval mortality (100.0%) was recorded at the highest concentration of NPV (4 × 10^9 POB ml^{-1}) and emamectin benzoate (0.1 ppm) (Table 1). In case of 4th instar larvae, significant differences were found among all the treatments. In individual applications, the highest larval mortality (44.42%) was recorded for emamectin benzoate in FSD population ($F_{7,71}$ = 128.0; $p \leq 0.01$), followed by LY ($F_{7,71}$ = 87.6; $p \leq 0.01$) and then ML ($F_{7,71}$ = 121; $p \leq 0.01$). Similarly, the highest mortality (96.19%) was recorded in FSD population, followed by LY and ML populations for a high concentration of NPV (4 × 10^9 POB ml^{-1}) with emamectin benzoate (0.01 ppm) (Table 1). With regard to proportion of insects succeeded to pupate (Table 2), the interaction of NPV and emamectin benzoate varied greatly and significantly.

The combined applications of microbial pathogens may enhance their pathogenicity, persistence and infection rate (Ali et al. 2016). Moreover, the issue of insecticide resistance can also be minimized as they exhibit novel modes of actions, which is lacking in conventional chemical insecticides (Qayyum et al. 2015 and Bala et al. 2018).

Combined action of both the two pathogens was proved to be more fatal than their sole application and lower proportion of pupation was observed for all populations (2nd instar: FSD 0.00 ± 0.00, LY 2.96 ± 0.27, ML 7.40 ± 0.73; 4th instar: FSD 3.70 ± 0.37, LY 8.88 ± 0.71, ML 13.33 ± 0.96) than the control treatment (˃ 94.0%). Larval mortality increased as the numbers of polyhedral occlusion bodies (POBs) were increased in individual or combined applications. Similar findings were reported by Nawaz et al. (2019) who reported a high mortality of *Helicoverpa armigera* (H.) larvae with the increase of POBs number. Obtained results are also parallel to the findings of Arrizubieta et al. (2016), who reported the highest effectiveness of NPV and insecticides mixture against *H. armigera.* This might be attributed to the fact that the polyhedral bodies of NPV attach to the midget of host and multiply, thereby destroying the gut cells.

Table 2 Mean pupation (%±SE) of second and fourth larval instars of *Spodoptera litura* from three different field populations treated with nucleopolyhedrosis virus and emamectin benzoate. (NPV-1 2 × 10^9 POBs ml^{-1}, NPV-2 3 × 10^9 POBs ml^{-1}, NPV-3 4 × 10^9 POBs ml^{-1}, EM 0.1 ppm)

Treatments	Second instar			Fourth instar		
	FSD	LY	ML	FSD	LY	ML
NPV-1	71.85 ± 2.89b	79.25 ± 2.59b	85.18 ± 2.82b	78.51 ± 2.85b`	85.18 ± 2.67b	91.11 ± 3.22ab
NPV-2	63.70 ± 2.53bc	68.14 ± 2.27bc	76.29 ± 2.56bc	69.63 ± 1.95bc	74.07 ± 2.50bc	80.74 ± 2.41b
NPV-3	51.85 ± 2.34cd	59.25 ± 2.19cd	65.18 ± 1.88cd	58.51 ± 1.67c	65.18 ± 1.95cd	72.59 ± 2.16bc
EM	47.40 ± 2.14d	54.81 ± 1.35d	61.48 ± 1.73cd	53.33 ± 1.42c	60.74 ± 1.71d	66.66 ± 2.57c
NPV-1 + EM	28.88 ± 1.18e	37.77 ± 1.12be	43.70 ± 1.42d	34.81 ± 1.25d	41.48 ± 1.42be	48.14 ± 1.67d
NPV-2 + EM	9.62 ± 0.25f	16.29 ± 0.96f	20.74 ± 1.05e	17.77 ± 0.98e	25.18 ± 1.15f	32.59 ± 1.23e
NPV-3 + EM	0.00 ± 0.00g	2.96 ± 0.27g	7.40 ± 0.73f	3.70 ± 0.37f	8.88 ± 0.71g	13.33 ± 0.96f
Control	94.81 ± 2.97a	96.29 ± 3.17a	97.03 ± 3.04a	95.55 ± 3.26a	97.03 ± 2.98a	98.51 ± 2.97a

Mean sharing the same letters within each column are not significantly different at 1.0% level

Table 3 Mean adult emergence (%±SE) of second and fourth larval instars of *Spodoptera litura* from three different field populations treated with nucleopolyhedrosis virus and emamectin benzoate. (NPV-1 2 × 10^9 POBs ml^{-1}, NPV-2 3 × 10^9 POBs ml^{-1}, NPV-3 4 × 10^9 POBs ml^{-1}, EM 0.1 ppm)

Treatments	Second instar			Fourth instar		
	FSD	LY	ML	FSD	LY	ML
NPV-1	68.14 ± 2.34b	75.55 ± 1.92b	80.74 ± 2.54b	74.81 ± 2.48b	82.96 ± 2.74b	87.40 ± 2.73ab
NPV-2	57.77 ± 2.48bc	63.70 ± 1.45c	71.85 ± 2.19bc	63.70 ± 2.74c	71.85 ± 2.94bc	76.29 ± 1.96b
NPV-3	48.88 ± 1.53cd	54.81 ± 2.09cd	62.96 ± 1.95cd	55.5 ± 1.92cd	62.22 ± 1.92c	67.46 ± 1.52bc
EM	42.96 ± 1.66d	51.85 ± 1.48cd	58.51 ± 1.67cd	47.40 ± 1.82d	54.81 ± 1.47de	62.96 ± 1.74c
NPV-1 + EM	25.18 ± 1.17e	32.59 ± 1.25d	37.77 ± 1.22d	31.85 ± 1.15e	36.29 ± 1.24e	45.28 ± 1.25d
NPV-2 + EM	6.66 ± 0.22 f	14.81 ± 1.17e	19.25 ± 1.07e	12.59 ± 0.54f	23.70 ± 1.05f	27.40 ± 1.13e
NPV-3 + EM	0.00 ± 0.00 f	1.48 ± 0.17f	4.44 ± 0.21f	0.00 ± 0.00g	6.66 ± 0.13g	9.62 ± 0.39f
Control	94.07 ± 3.33a	95.55 ± 3.11a	96.29 ± 3.17a	94.81 ± 3.85a	96.29 ± 3.61a	97.77 ± 3.11a

Mean sharing the same letters within each column are not significantly different at 1.0% level

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 (Arif et al. 2018). In the present study, a high mortality rate was recorded in the combined treatments than in the sole application of NPV or emamectin benzoate. Additive or synergistic effects can be resulted by dual action of both pathogens, which may broaden the action spectrum. Similar findings were also reported (Qayyum et al. 2015; Ayyub et al. 2019 and Nawaz et al. 2019; Maqsood et al. 2019). Contrarily, Trang et al. (2002) reported antagonistic effects of NPV and imidacloprid. Antagonism may be caused due to reducing feeding or changing in pH of gut (El-Helaly and El-bendary 2013).

Apart from untreated larvae, the highest level of pupation (2nd instar: FSD 71.85 ± 2.89, LY 79.25 ± 2.59, ML 85.18 ± 2.42; 4th instar: FSD 78.51 ± 1.85, LY 85.18 ± 2.67, ML 91.11 ± 3.22) was observed in NPV1 treatment for both 2nd and 4th larval instars respectively (Table 2). Adult emergence and egg eclosion of both instars were found inversely related to pathogenicity of NPV and emamectin benzoate. Significant differences were observed at individual and combined treatments for all populations. Lowest adult emergence (2nd instar: FSD 0.00 ± 0.00, LY 1.48 ± 0.17, ML 4.44 ± 0.21; 4th instar: FSD 0.00 ± 0.00, LY 6.66 ± 0.13, ML 9.62 ± 039) and egg hatchability (2nd instar: FSD 0.00 ± 0.00, LY 0.00 ± 0.00, ML 2.93 ± 0.09; 4th instar: FSD 4.85 ± 0.08, LY 10.42 ± 0.47, ML 16.19 ± 0.92) was recorded in larvae treated with NPV-3 and EMB in 2nd and 4th instars, respectively, whereas the highest level was recorded in larvae treated with NPV-1. Overall, combined application exerted more hazardous effect on adult emergence and egg eclosion than the individual applications and was inversely proportional to the pathogenicity dependent manners (Tables 3 and 4). Similar findings were also observed by Qayyum et al. (2015) with *Bacillus thuringiensis* and NPV against *H. armigera*. Different susceptibility levels were observed in various populations. This might show the level of resistance in insects towards insecticides to which they have been exposed frequently and successively. Resistant populations (ML), frequently exposed to the insecticides, attained cross resistance against emamectin benzoate and vice versa.

Table 4 Mean egg eclosion (%±SE) of second and fourth larval instars of *Spodoptera litura* from three different field populations treated with nucleopolyhedrosis virus and emamectin benzoate. (NPV-1 2 × 10^9 POBs ml^{-1}, NPV-2 3 × 10^9 POBs ml^{-1}, NPV-3 4 × 10^9 POBs ml^{-1}, EM 0.1 ppm)

Treatments	Second instar			Fourth instar		
	FSD	LY	ML	FSD	LY	ML
NPV-1	63.39 ± 2.41ab	70.61 ± 2.47b	76.46 ± 2.89b	74.45 ± 1.35a	83.14 ± 2.23ab	89.48 ± 2.40ab
NPV-2	51.91 ± 2.17bc	62.58 ± 2.09c	68.79 ± 2.73bc	60.44 ± 2.27c	72.39 ± 2.15b	77.07 ± 1.99bc
NPV-3	39.68 ± 1.34c	47.10 ± 1.58d	51.02 ± 1.93c	45.08 ± 1.58d	54.91 ± 1.42c	63.20 ± 2.29c
EM	27.28 ± 1.21cd	38.50 ± 1.31e	42.25 ± 1.25cd	32.59 ± 1.25e	43.95 ± 1.30cd	52.58 ± 1.60cd
NPV-1 + EM	18.49 ± 1.03d	27.47 ± 1.12f	35.96 ± 1.39d	25.15 ± 1.09ef	36.41 ± 1.23d	41.25 ± 1.42d
NPV-2 + EM	11.45 ± 0.22e	18.40 ± 1.01g	21.18 ± 1.14e	18.22 ± 0.48f	24.05 ± 1.11e	33.28 ± 1.19de
NPV-3 + EM	0.00 ± 0.00f	0.00 ± 0.00h	2.93 ± 0.09f	4.85 ± 0.08g	10.42 ± 0.47f	16.19 ± 0.92f
Control	83.37 ± 2.63a	90.63 ± 3.61a	92.39 ± 2.82a	92.74 ± 3.39a	93.89 ± 2.92a	95.38 ± 3.23a

Mean sharing the same letters within each column are not significantly different at 1.0% level

Conclusion

Integration of NPV and emamectin benzoate could be effectively used against the notorious pest (*S. litura*). Both of the two pathogens represented better tools to combat resistance related issues. Further evaluations under field conditions is needed for further validation.

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Authors' contributions
All authors equally participated in this study. WW conceived and designed the study. MSQ and MY conducted the research and prepared first draft. MAQ performed statistical analysis and prepared graphs. WW supervised, reviewed and edited MS for final submission. The author(s) read and approved the final manuscript.

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Not applicable

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Competing interests
The authors declare that they have no competing interests.

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