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Evaluation of indigenous the nucleopolyhedrovirus (NPV) of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in combination with chlorantraniliprole against *Spodoptera* species

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Abstract

Background: The armyworms, *Spodoptera exigua* (Hübner), and *S. litura* (Fabricius) (Lepidoptera: Noctuidae) are polyphagous pests of many cash crops. Heavy crop losses have been reported for the fruit and vegetable crops each year owing to the diverse impact on global economies. The present study was aimed to sort out a novel method of pest control using the insect's own nucleopolyhedrosis virus (NPV) alone and in combination with a new chemistry insecticide chlorantraniliprole.

Results: In the study, the effect of indigenous isolated nucleopolyhedrovirus (NPV) and the chemical insecticide (chlorantraniliprole) formulations against the 2nd and 4th larval instars of *S. litura* and *S. exigua*, collected from the different geographical region of Punjab (Pakistan) province, was evaluated. Three concentrations of the NPV isolate, sub-lethal (1×10^4, 6×10^4 POB ml^{-1}), lethal (3×10^5 POB ml^{-1}), and chlorantraniliprole 0.01 $\mu l\ l^{-1}$, were applied alone and in combination against the 2nd and 4th larval instars of both pest species. The lethal concentration of NPV + chlorantraniliprole exhibited synergistic interaction and caused high larval mortality against both instars, while in all other combinations, additive effect was observed. Moreover, NPV + chlorantraniliprole at lethal concentration exhibited decreased pupation, adult emergence, and egg eclosion.

Conclusion: The implications of using NPV alone and in combination with an insecticide are discussed briefly in this study.

Keywords: Nucleopolyhedrovirus, Chlorantraniliprole, *Spodoptera exigua*, *S. litura*, Combined effect

Background

Spodoptera exigua (Hübner) and *S. litura* (Fabricius) (Lepidoptera: Noctuidae) are native to Asia (Farahani et al. 2012). Both attack more than 90 plant species including major crops such as sugar beet, cotton, soybean, and potatoes (Fu et al. 2017; Luna-Espino et al. 2018). The mechanism of resistance in *S. exigua* has been reported against different classes of insecticides (Ishtiaq et al. 2012). Furthermore, extensive use of synthetic insecticides has resulted in enormous environmental pollution and a negative impact on non-target insect species (Ahmad et al. 2008). This situation forced the researchers to develop eco-friendly alternatives

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(Sayyed et al. 2012). Baculoviruses consist of rod-shaped, dsDNA-occluded viruses that infect insect pests belonging to the orders Lepidoptera, Hymenoptera, and Diptera (Williams et al. 2017). Due to their fast-killing nature and host specificity, some of these viruses have been used as commercial bioinsecticides (Sosa-Gómez 2017). Baculoviruses comprise two genera, granuloviruses (GVs) and nucleopolyhedroviruses (NPVs). The *S. litura* multiple nucleopolyhedroviruses (SlM-NPV) have been molecularly characterized, and their biological activity has been tested against *S. litura* populations in Pakistan (Ahmad et al. 2018; Ali et al. 2018; Ayyub et al. 2019) and all over the world (Laarif et al. 2011 and Kumar et al. 2012a, 2012b).

Chlorantraniliprole is a Ryanoid class insecticide (anthranilic diamide), which demonstrates maximum mortality against the target insect pests even at a low application rate (Cordova et al. 2007). Furthermore, this insecticide is compatible to use with NPV to enhance the pathogenic efficacy. Ryanodine receptors are ion channels that maintain calcium levels in the sarcoplasmic reticulum of muscle cells. Chlorantraniliprole opens the ryanodine receptor for an uncontrolled release of calcium from muscle cells, which reduces feeding, paralysis, and ultimately death of target species (Lahm et al. 2007). Previous studies represent the efficacy of NPVs of specific regions, but until now, no published data is available on the effect of a local isolate of NPV against *Spodoptera* species when applied in combination with chlorantraniliprole. Therefore, the present study was conducted to evaluate the effect of the local isolate of *S. litura* NPV alone and in combination with chlorantraniliprole against *Spodoptera* spp.

Methods

Rearing of insects

About 150 larvae (3rd and 4th instars) each of *S. litura* and *S. exigua* were collected by plucking the infested leaves from the field during the winter cropping season of 2018–2019 to establish insect cultures. The larvae along with the leaves were carried in plastic jars and shifted to the rearing cages in the laboratory with the help of a camel hairbrush. Pest species were identified based on the morphological characters traced with the help of a taxonomic key. The larvae were mass-reared on artificial diets at 25 ± 2 °C, 70 ± 5% RH and a photoperiod of 14:10 h (L:D). The standard established laboratory protocol was followed (Ahmad et al. 2020) The larvae were pupated in vials containing the artificial diet. The emerged adults (approximately 120 males and females) were shifted from vials to plastic cages/boxes for mating and egg-laying purposes. The adults were provided with 10% honey solution in cages. After hatching, the neonate larvae were shifted to vials containing the artificial diet until pupation. The F_1 generation of reared test insects was mass cultured and used in bioassay experiments.

Chlorantraniliprole is a new insecticide with a novel mode of action. The semi-viscous formulation of it is provided byDuPont™ Operations Private Limited, Pakistan, containing 20% W/V active ingredient Rynaxpyr (200 ml/l), and 80% other ingredients (800 ml/l) was used for bioassays. The formulation was applied by dissolving in distilled water at a concentration of 0.01 μl l^{-1} mixed with the diet, thoroughly mixed in an electric shaker for 30 s.

Viral isolation and suspension preparation

NPV-infected larvae were macerated, and obtained polyhedral (occlusion bodies) were passed through the cheesecloth to remove large debris. Semi purified occlusion bodies were centrifuged at 3000 rpm for 45 min. For further purification of the occlusion bodies, the resulting suspension was centrifuged at a high speed (16, 000 rpm) for 10 min. The suspension was transferred to glass vials and stored at 4 °C. The concentrations of polyhedral occlusion bodies (POBs) as a stock solution (2×10^8 POB l^{-1}, 3×10^8 POB l^{-1}, and 4×10^8 POB l^{-1}) were prepared from indigenous NPV using Neubauer hemocytometer (Shapiro et al. 2005). From the stock solution, 1 ml suspension from each concentration was prepared as NPV-1 (1×10^6 POB ml^{-1}), NPV-2 (1×10^7 POB ml^{-1}), and NPV3 (4×10^8 POB ml^{-1}). For bioassay, 2 sub-lethal concentrations of NPV (1×10^4; 6×10^4) and one lethal concentration of NPV (3×10^5 POB ml^{-1}) were used to study the synergistic, additive, or antagonism effect against *Spodoptera* species (these sub-lethal concentrations were selected from a preliminary bioassay).

Insect bioassay

The 2nd and 4th larval instars of *S. litura* and *S. exigua* were tested by NPV and chlorantraniliprole alone and in combination. The desired concentration of NPV from the stock solution was mixed in the artificial diet for even distribution. All the bioassays were conducted under Biosafety Level 2 to avoid any source of contamination or leakage of the pathogens. The experiments were conducted in a plastic vial (base radius of 2.4 cm × height 6 cm). Sub-lethal concentrations of NPV-1 (1×10^4 POB/larva) and NPV-2 (6×10^4 POB/larva) and lethal concentration of NPV-3 (3×10^5 POB/larva) were used to calculate the percentage mortality, pupation, adult emergence, and egg eclosion from the 2nd and 4th larval instars of *S. litura* and *S. exigua*. Each plastic vial contained a 2-mm cube of diet previously soaked in respective NPV and chlorantraniliprole concentration. Twenty pre-starved (24 h) larvae of both instars of *S.*

litura and *S. exigua* were allowed to feed the artificial diet until complete consumption in the individual container. After being fed, the larvae were removed and then released in plastic vials containing an artificial diet until the larvae died or pupated. The bioassay was conducted at 25 ± 2 °C, 70 ± 5% RH and L16:D8 h photoperiod. Each treatment was replicated 3 times, and each bioassay was repeated thrice independently. The number of dead insects was recorded daily, and surviving insects were monitored for % pupation and adult emergence. Larvae were considered dead when they were unable to move (Ma et al. 2008). Emerged adults from each treatment were shifted from vials to plastic cages/boxes for mating and egg-laying purposes.

Statistical analysis

The mortality rate of targeted insects was corrected using Abbott's formula (Abbott 1925). The co-toxicity factor (CTF), observed mortality (Oc), and expected mortality (Oe) were calculated using the equation (Mansour et al. 1966) CTF = (Oc − Oe)/Oe × 100 to study the synergistic (cytotoxicity factor above 20), additive (cytotoxicity factor between 20 and − 20), and antagonistic (cytotoxicity factor − 20 or above) interaction between the treatments. The means were compared by using the Tukey-Kramer HSD test at $P = 0.05$ (Sokal and Rohlf 1995).

Results

Mortality of *S. litura*

The 2nd instar larvae showed a 38.10% mortality rate when exposed to a sub-lethal concentration of chlorantraniliprole (0.01 ppm), while the larvae treated with NPV (sub-lethal 1 × 10^4, sub-lethal 6 × 10^4 POB ml^{-1}, and lethal 3 × 10^5 POB ml^{-1}) showed 34.02, 48.41, and 60.48% mortality, respectively. A combination of the lethal concentration of NPV (3 × 10^5 POB ml^{-1}) with a sub-lethal concentration of chlorantraniliprole (0.01 ppm) showed a synergistic action (CTF ≥ 20) with a mortality of 89.35%, while the rest of the combination exhibited an independent effect (CTF ≤ 20) (Table 1). Similarly, integration of a sub-lethal + lethal combination of NPV on the 4th instar larvae showed the highest level of mortality in the 4th instar larvae than the chlorantraniliprole alone treatment. The combined treatment of NPV + chlorantraniliprole interaction produced an antagonistic or additive effect while combing chlorantraniliprole with NPV at 3 × 10^5 POB ml^{-1} showed a mortality rate of 75.70 ± 1.76% (Table 1).

Mortality of *S. exigua*

Sub-lethal effect of chlorantraniliprole (0.01 ppm) against the 2nd instar larvae showed 33.66% mortality rate, whereas three concentrations of NPV (sub-lethal 1 × 10^4, sub-lethal 6 × 10^2 POB ml^{-1}, and lethal 3 × 10^5 POB ml^{-1}) produced 30.48, 42.70, and 54.92% mortality, respectively. Simultaneous action of NPV (3 × 10^5 POB ml^{-1}) with a sub-lethal concentration of chlorantraniliprole (0.01 ppm) showed a synergistic action (CTF ≥ 20) with a mortality of 84.02%, while the rest of the combination exhibited an independent effect of each other (CTF ≤ 20) (Table 2). Similarly, the integration of a sub-lethal + lethal combination of NPV on the 4th instar larvae showed the highest level of mortality in the 4th instar larvae than the chlorantraniliprole alone treatment. In the combined treatment of NPV + chlorantraniliprole, 2 interactions produced an additive effect, while combining chlorantraniliprole with NPV at 3 × 10^5 POB ml^{-1} caused enhanced mortality of 57.30% (Table 2).

Table 1 Mortality percentage of the 2nd and 4th instars of *Spodoptera litura* larvae treated with nucleopolyhedrovirus alone and in combination with chlorantraniliprole

Treatments (POB ml^{-1})	2nd instar larvae (n = 20)				4th instar larvae (n = 20)			
	Mortality%	Expected mortality	CTF	Interaction	Mortality %	Expected mortality	CTF	Interaction
NPV-1 (1 × 10^4)	34.02				20.43			
NPV-2 (6 × 10^4)	48.41				32.69			
NPV-3 (3 × 10^5)	60.48				42.29			
Chl	38.10				24.43			
NPV-1+Chl	74.13	75.58	− 6.63	Additive	42.52	43.61	− 0.85	Additive
NPV-2+Chl	85.56	75.67	14.54	Additive	55.53	54.17	12.14	Additive
Npv-3+Chl	89.35	79.42	20.16	Synergistic	75.70	65.91	19.13	Synergistic
Control	2.38				1.59			
F	251				275			
DF	7,71				7,62			
P	< 0.01				< 0.01			

CFT co-toxicity factor

Table 2 Mortality percentage of the 2nd and 4th instars of *Spodoptera exigua* larvae treated with nucleopolyhedrovirus alone and in combination with chlorantraniliprole

Treatments (POB ml^{-1})	2nd instar larvae (n = 20)				4th instar larvae (n = 20)			
	Mortality%	Expected mortality	CTF	Interaction	Mortality %	Expected mortality	CTF	Interaction
NPV-1 (1×10^4)	30.48				15.89			
NPV-2 (6×10^4)	42.70				25.51			
NPV-3 (3×10^5)	54.92				38.68			
Chl	33.66				20.56			
NPV-1+Chl	65.90	60.25	1.78	Additive	37.59	31.12	10.14	Additive
NPV-2+Chl	71.53	68.24	3.74	Additive	46.67	38.31	17.21	Additive
Npv-3+Chl	84.02	70.13	26.22	Synergistic	57.30	45.12	21.57	Synergistic
Control	3.17				3.18			
F	271				117			
DF	7,71				7,62			
P	< 0.01				< 0.01			

CFT co-toxicity factor

Pupation percentage of *S. litura*

A significant interaction was found between NPV and chlorantraniliprole in terms of the number of larvae that enter the pupation stage (2nd instar $F7,71 = 257$, $p \leq 0.01$, 4th instar $F7,71 = 319$, $p \leq 0.01$). The integration effect of both agents was lethal as compared to their sole applications. The combined lethal concentration of NPV with chlorantraniliprole against the 2nd and 4th larval instars of *S. litura* produced synergistic interaction with a decreasing percentage of pupation (3.11 and 4.15%, respectively). In contrast, the highest percentage of pupation (40.67 and 36.26%, respectively) was observed at a low concentration of chlorantraniliprole (Table 3).

Pupation percentage of *S. exigua*

Among treatment of NPV and chlorantraniliprole, the interaction was significant for pupation percentage (2nd instar $F7,71 = 221$, $p \leq 0.01$, 4th instar $F7,71 = 342$, $p \leq 0.01$). The lethal combination effect of NPV with chlorantraniliprole was synergistic with a high rate of mortality in the 2nd and 4th larval instars of *S. exigua* and a low percentage of pupation, while the rest of the combinations of lethal concentration of NPV with chlorantraniliprole produced synergistic interaction with decreasing percentages of pupation (5.33 and 12.307%, respectively). On the other hand, a low concentration of chlorantraniliprole produced the highest percentage of pupation (51.89 and 41.22%, respectively) (Table 4).

Adult emergence and egg eclosion percentage of *S. litura*

Adult emergence rate was inversely proportional to all the treatments of NPV and chlorantraniliprole (Table 3). The lowest adult emergence (8.22 and 1.00%) and egg eclosion (2.94 and 4.32%) were observed when a high

Table 3 The mean pupation, adult emergence, and egg eclosion (% ± SE) of the 2nd and 4th instars (n = 20) of *Spodoptera litura* larvae treated with nucleopolyhedrovirus alone and in combination with chlorantraniliprole

Treatments (POB ml^{-1})	Pupation		Adult emergence		Egg eclosion	
	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar
NPV-1 (1×10^4)	71.81 ± 2.22	74.63 ± 2.23	71.11 ± 2.73	64.44 ± 1.93	67.21 ± 2.70	76.63 ± 3.12
NPV-2 (6×10^4)	61.01 ± 2.57	65.81 ± 1.93	60.15 ± 2.74	52.96 ± 2.11	54.99 ± 3.84	65.65 ± 3.10
NPV-3 (3×10^5)	54.67 ± 1.47	50.52 ± 2.18	50.00 ± 1.96	48.52 ± 1.97	42.38 ± 2.69	49.33 ± 2.61
Chl	40.67 ± 2.05	36.26 ± 2.14	47.78 ± 2.51	31.85 ± 2.83	35.00 ± 3.45	38.04 ± 3.91
NPV-1+Chl	25.15 ± 2.41	24.44 ± 1.27	47.41 ± 1.77	22.16 ± 1.19	24.93 ± 2.58	29.29 ± 2.56
NPV-2+Chl	2.63 ± 2.18	13.67 ± 2.11	27.96 ± 2.55	6.30 ± 2.63	11.37 ± 3.62	18.54 ± 3.76
NPV-3+Chl	03.11 ± 0.00	4.15 ± 1.87	8.22 ± 1.58	01.00 ± 0.10	2.94 ± 2.35	4.32 ± 2.38
Control	96.41 ± 2.98	97.5 ± 2.37	94.85 ± 2.15	95.07 ± 1.34	87.37 ± 4.63	92.74 ± 4.02
F	257	319	291	366	44	53
Df	7,71	7,71	7,71	7,71	7,71	7,71
P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Table 4 The mean pupation, adult emergence, and egg eclosion (% ± SE) of the 2nd and 4th instars (n = 20) of *Spodoptera exigua* larvae treated with nucleopolyhedrovirus alone and in combination with chlorantraniliprole

Treatments (POB ml^{-1})	Pupation		Adult emergence		Egg eclosion	
	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar
NPV-1 (1×10^4)	82.52 ± 1.43	78.59 ± 2.58	69.89 ± 1.75	79.26 ± 1.88	74.94 ± 3.40	87.23 ± 5.55
NPV-2 (6×10^4)	72.70 ± 1.14	71.30 ± 2.54	64.44 ± 2.65	75.56 ± 2.12	62.72 ± 2.60	77.54 ± 4.25
NPV-3 (3×10^5)	60.44 ± 1.59	65.52 ± 2.14	52.22 ± 3.14	65.93 ± 2.17	49.53 ± 3.17	58.08 ± 3.99
Chl	51.89 ± 2.45	41.22 ± 1.93	44.07 ± 1.17	51.74 ± 2.38	40.68 ± 5.26	49.29 ± 5.07
NPV-1+ Chl	30.37 ± 0.00	34.07 ± 2.24	36.67 ± 3.06	50.37 ± 1.91	33.38 ± 2.17	42.14 ± 2.98
NPV-2+Chl	21.59 ± 1.85	20.56 ± 1.48	12.00 ± 2.66	32.44 ± 1.82	20.99 ± 2.10	25.07 ± 4.88
NPV-3+Chl	5.33 ± 1.37	12.30 ± 3.17	4.15 ± 2.74	17.59 ± 1.54	5.50 ± 1.23	9.55 ± 2.26
Control	97.29 ± 2.58	96.63 ± 2.65	95.56 ± 1.12	96.69 ± 2.65	90.63 ± 2.71	93.89 ± 8.56
F	221	342	168	219	36	26
Df	7,71	7,71	7,70	7,71	7,71	7,71
P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

concentration of NPV (3×10^5) was combined with chlorantraniliprole against the 2nd and 4th larval instars, respectively. Adult emergence and egg eclosion rates were high at the sole application of chlorantraniliprole. The combined application of microbes produced fatal effects on adult emergence and egg eclosion as compared to their sole application.

Adult emergence and egg eclosion percentage of *S. exigua*

The relationship between adult emergence rate and bioassay treatment was inversely proportion (Table 4). The percentage of adult emergence (4.15 and 17.59%) and egg eclosion (5.50 and 9.55%) of the 2nd and 4th larval instars, respectively, were the lowest when a lethal concentration of NPV (3×10^5 POB ml^{-1}) was combined with chlorantraniliprole. Sole application of both agents produced a high percentage of adult emergence and egg eclosion. The integration effect of biorational insecticides was toxic for adult emergence and egg eclosion than that of their sole application.

Discussion

The practical use of microbial insecticides is limited due to host specificity and slow in action. Combing biorational insecticides with microbes is a premixing strategy to manage insecticide overuse (Kumar et al. 2012a, 2012b). Biological insecticides have been used to control lepidopteran insect pests of agricultural, horticultural, and forest importance (Nathan and Kalaivani 2006).

A molecularly identified isolate of NPVs has been used to control *Spodoptera* spp. in Pakistan (Ahmad et al. 2018; Ali et al. 2018) and worldwide (Luna-Espino et al. 2018). In the present study, after the viral infection, *Spodoptera* spp. showed stunted growth and prolonged larval and pupal duration. Insect pests of vegetable, ornamental, and field crops have been shown more vulnerability to the pathogenicity of NPV infection (Rios-Velasco et al. 2011). Early instar larvae were more succumb to pathogenic infection than older larvae because early ones usually consumed more surface of viral treated leaves (Gothama et al. 1995). It is likely due to more deposition of cuticular melanism in the oldest larvae which prevents the entrance of pathogens (Wilson et al. 2001).

Chlorantraniliprole is a novel insecticide (Cordova et al. 2006). In the present study, it showed a good control against both larval instars of *Spodoptera* spp., but the 2nd instar larvae showed high susceptibility. Low concentrations of chlorantraniliprole gave fair control against lepidopteron insect pests (Cordova et al. 2006; Wakil et al. 2013; Wang et al. 2013). Synergistic interaction was produced in the present study when a high concentration of NPV was combined with chlorantraniliprole. Similar synergistic interaction was reported by Wakil et al. (2012) for *Bacillus thuringiensis* + chlorantraniliprole. On the other hand, additive or synergistic effects have been observed at high concentrations of NPV + biorational insecticides (Wakil et al. 2013). A possible reason for NPV-Ch synergism is that the selection pressure of insecticides makes them more vulnerable to viral occlusion bodies (OB). Exposure to chemical insecticide increases the developmental period, and this allows the viral infection to develop in the insect (Kumar et al. 2008). The antagonistic or additive interaction in the present study might be due to a decrease in normal feeding on treatment or a change of gut pH (El-Helaly and El-Bendary 2013). The results of the present study showed that the sub-lethal concentration of chlorantraniliprole + high concentration of NPV could be a better strategy to manage insecticidal resistance.

Conclusion

The obtained data showed that the lethal concentration of NPV in combination with chlorantraniliprole caused high larval mortality rates of *Spodoptera* spp. under laboratory conditions and could be suggested as an effective strategy to control the population *Spodoptera* spp., but further experiments under field conditions would be very helpful for the integration of NPV biopesticides with insecticides in the IPM program.

Abbreviations

NPV: Nucleopolyhedroviruses; dsDNA: Double-stranded deoxyribonucleic acid; GVs: Granuloviruses; SIM-NPV: *Spodoptera litura* multicapsid nuclear polyhedrosis virus; RH: Relative humidity; L:D: Light:dark; W/V: Weight by volume; POB: Polyhedral occlusion body; CTF: Co-toxicity factor; Oc: Observed mortality; Oe: Expected mortality; HSD: Honestly significant difference

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Authors' contributions

GS designed and supervised the overall research. NAM and MAA conducted the experiments. MRS contributed to the statistical analysis. MAM helped in the extraction of NPV. MF contributed to the data analysis and manuscript write-up. All authors have read and approved the final manuscript.

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Availability of data and materials

Data will be accessible on request from the corresponding authors.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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