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MANAGEMENT OF AMERICAN BOLLWORM (*HELICOVERPA ARMIGERA*) USING NATIVE ISOLATED *SPODOPTERA LITURA* ASSOCIATED NUCLEOPOLYHEDRO VIRUSES (SlitNPV)

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ABSTRACT

Helicoverpa armigera (Lepidoptera, Noctuidae) is polyphagous insect pests of many economically important crops. In present research, native Nucleopolyhedroses virus (SlitNPV) were isolated from infected *Spodoptera litura* present in cotton field and observed under microscope through Geimsa Stain. Viral suspensions were prepared for bioassays. To observe the effect of Nucleopolyhedroses virus (NPV) and Spinosad, various doses of sub-lethal (< LC50) and lethal (>LC50) were applied alone and in combination with Spinosad (0.01 ppm). The biological activities of native NPV isolate and its interaction were studied against larvae of *H. armigera* (2nd and 4th instars). The biological attributes were severely affected and highest mortality of *H. armigera* was observed at early instars as compared to last larval instars. The mortality and means of *H. armigera* was done using Abbott's formula and Tukey-Kramer (HSD) test. The additive and synergistic interaction was found among different instars against various doses of NPVs and spinosad. A synergistic interaction of lethal dose of NPV (lethal (>LC50) was observed with spinosad against 2nd as well as 4th instar larvae of *H. armigera*. The current study suggests that environment friendly biological insecticides should be developed that can effectively be used to manage different insect pests of major crops by reducing and the use of synthetic chemicals and encourages developing. The results from this study indicated that *S. litNPV* was effective against *H. armigera* and can easily be used to manage these pests successfully confirming an alternative strategy than commercial based insecticide.

Key words: Nucleopolyhydroviruses, Insect pest management, Microbial insecticides, PCR

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INTRODUCTION

The *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) also known as tomato fruit worm is a noctuid polyphagia pest in nature which occurred worldwide (Queiroz-Santos *et al*., 2018). *H. armigera* is the host of more than 200 plant species belonging to 68 different families including economical important cereals (maize, sorghum), vegetables (chick pea, pigeon pea, soybean, groundnut) and oilseed and fiber crops (sunflower, cotton) (Rasool, 2015). It feed on nitrogenous part of the plant like flower and fruit (Fitt, 1989). Farmers use mainly chemicals that cause resistance against this pest. Further, these chemicals are hazardous to user, damaging to non-target insects and cause environmental pollution (Alvi *et al*., 2018). In Pakistan, *H. armigera* has developed moderate to high levels of resistance against pyrethroid, chlorinated hydrocarbons, organophosphates, carbamates, neonicotinoids, and insect growth regulators (IGRs) (Ahmad *et al*., 1995; Nauen and Bretschneider, 2002; Shad *et al.,* 2012; Hussain *et al*., 2015).

To avoid the use of extensive insecticide, there is need to develop microbial based pesticides from insect viruses, protozoa, fungi and bacteria which are attractive, easily biodegradable and environmentally safe (Çakici *et al*., 2014; Ahmad *et al.,* 2018). Baculoviruses belonging to family baculoviridae have been classified into two genera (Granuloviruses, GVs) and (Nucleopolyhedroviruses, NPVs) which are double stranded DNA circular viruses that mainly infect lepidopterous pests (Clem and Passarelli, 2013). NPVs are host specific natural microbial insecticide against lepidopterous insect that are beneficial to natural enemies and develop low level of insecticide resistance (Nguyen *et al*., 2013; Kumari and Singh, 2009; Rios-Velasco *et al*., 2011; Zhang *et al*., 2015; Ahmad *et al*., 2018, 2020b). *H. armigera* associated NPVs have been identified and characterized (Mehrvar *et al*., 2008; Kumar *et al*., 2012; Ferrelli *et al*., 2016) and their pathogenicity in the field and laboratory have been proved effective (Figueiredo *et al*., 1999; Ogembo *et al*., 2007). Spinosad is the microbial insecticide derived from fermentation of soil bacteria *Saccharopolyspora spinosa and* effectively used against lepidopterous and dipterous insect pests in integrated pest management programme due to low mammalian toxicity and no non-target effect (Bret *et al*., 1997; Sparks *et al*., 1997; Wang *et al*., 2013). The baculovirus-based insecticides in combination with insect natural enemies such as parasitoids, predators or other

pathogens have been used against many lepidopterous insect pests (Elvira *et al*., 2013; Ahmad *et al.,* 2018). Recently, different major insect pests (Ahmad *et al.,* 2018, 2020ab), phytopathogens (Ahmad *et al.,* 2017) as well as entomopathogens (Ahmad *et al.,* 2018) have characterized by several researchers from Pakistan (Ahmad *et al.,* 2017, 2018). Mostly nucleopolyhedroviruses are species specific and recommended to control particular targeted pest. We have isolated and identified indigenous isolates of such entomopathogens. The present study was conducted to observe the cross pathogenicity of recently identified slitNPV against *H. armigera* alone and in combination of spinosad.

MATERIALS AND METHODS

Insect Collection and Rearing on Artificial diets: The 5th and 6th instar larvae of army worm, *S. litura* and bollworm, *H. armigera* were collected from vegetable fields and shifted to Dr. Jam Laboratory, Department of Entomology, University of Agriculture Faisalabad for laboratory rearing on artificial diets following standard established protocol (Ahmad *et al.,* 2018; 2020b). Briefly, in 300 ml of distilled water (H_2O), 150g chickpea flour, 24g brewer's yeast 1.5g Methy-4-hydroxybenzoate 0.75g Sorbic acid 2.35g L-Ascorbic acid 0.75g Streptomycin sulfate were mixed for 25 min by adding 0.75g vitamin B12 and 6 ml brassica oil in mixture. In another beaker having 400 ml of distilled H20, 9g Agar Technical was dissolved for 5 min and after cooling at room temperature, the mixture was poured into a diet tray for solidification and stored at 4ºC until use. The pieces of diet were cut and placed into vials and collected larvae were fed on artificial diet. The larvae were allowed to pupate into same vials after food consumption until adult emergence. The adults emerged from vials were provided with 10% honey solution in plastic cages/box during mating and egg laying period. The hatching was done in same adult cages and then neonates were shifted to vials on artificial diet until further pupation. The F1 generation (2nd and 4th instar) larvae of reared targeted insects were used in experiments under laboratory conditions (25±2ºC, 70±5ºC % RH) and a photoperiod (14:10 h, L: D).

Detection and Microscopic Examination: The larvae of *Spodoptera litura* with viral symptoms were collected from farmers' fields. The larval bodies were examined under light microscope for the presence of viral occluded bodies by spreading small part of tissue on a glass slide. In order to stain the cells, 1ml Giemsa's stain was added on thin film of glass line and incubated for 10 min. The excess amount of stain was washed with tap water and allowed for air dry for 5-10 sec (Wigley, 1976). One drop of immersion oil was placed on thin side of film and then the slide was observed under microscope (Micros, Austria) at 40X for the detection the viral bodies of nuclear polyhedrosis viruses (NPV).

Insecticide and Viral suspension preparation: The liquid formulation of Spinosad containing spinosyn A to spinosad D (85:15) from Dow Agro Sciences, Limited, Pakistan was used in the Bioassay. The insecticides and viral suspension were prepared according to recommended protocol (Ahmad *et al*., 2020b). Briefly, the NPV infected 5th or 6th larval instar were collected and brought to Dr. Jam Laboratory, UAF. The infected insects were homogenized in 50ml tube containing 0.5% SDS. Homogenate was then filtered through double layers of cheese cloth and transferred to 50 mL round-bottom polycarbonate tubes. After centrifugation at 2700 rpm, supernatant was decanted and pellets were suspended in 20 ml 0.1% SDS. This step was repeated and then pellets were re-suspended in 20 ml 0.5 M NaCl. Finally, supernatant was poured out and obtained pellets were re-dissolved in 0.5 -1 ml of ddH20 containing sodium azide. Suspension was transferred to glass vials and stored at 4°C. Using Neubauer haemocytometer, the concentration of polyhedral occlusion bodies (POBs) as stock solution NPV1 (1 x 10^6 POB mL^{-1}) and NPV2 (1 x 10^7 POB mL^{-1}) were prepared from indigenous NPV isolate.

Efficacy evaluation against *H. armigera*: One single sub-lethal concentration (0.01ppm) of spinosad and three different concentrations (1 x 10^4; 5 x 10^4; and 3 x 10^5 POB mL^{-1}) of isolated SlitNPV were implemented against *H. armigera*. The required concentration of NPVs and Spinosad was mixed with artificial diets during diet preparation. Sub-lethal concentration 10µl NPV1 (1 x 10^4 POB/larva), 50µl NPV1 (5 x 10^4 POB/larva) and lethal concentration 30µl NPV2 (3 x 10^5 POB/larva) were used against (2nd and 4th instar) larvae of *H. armigera*. The NPV and Spinosad incorporated diet pieces (cubes of 2mm^2) were fed to twenty pre-starved (24 h) larvae of both instars of *H. armigera* in the plastic vials (base radius 2.4 cm × height 6 cm) individually (Fig.5). Then larvae were shifted to new artificial diet (without insecticides and virus) until death or pupated and adult emergence. All the experiments were repeated 3 times each with 3 replicates under 25 ± 2°C, 70± 5 % R.H. and L16: D8 h photoperiod. The data of mortality and other biological features were recorded regularly as described (Ma *et al.,* 2008).The Co-toxicity factor (CTF), and Observed and expected mortality was determined with equation (CTF = (Oc-Oe)/Oe ×100). The synergistic, additive and antagonistic interaction of NPV with Spinosad against *H. armigera* were calculated as synergistic (cytotoxicity factor above 20), additive (cytotoxicity factor between 20 and -20) and antagonistic (cytotoxicity factor -20 or above) interaction between the treatments (Mansour *et al*., 1966). The mortality (LC50) was determined by using Abbott's formula (Abbott, 1925) and means were separated using Tukey-Kramer (HSD) test (Sokal and Rohlf, 1995) at confidence interval 95%. The analysis was conducted through statistical software one-way ANOVA using Minitab 13.2 (Minitab, 2003).

RESULTS

Viral infection and Symptomatology: The naturally infected army worm (*Spodoptera litura)* with nucleopolyhydrovirus (NPV) was collected from fields showing viral symptoms (Fig. 1). The symptoms associated with NPV infection confirmed the presence of Occlusion bodies (Obs) from infected specimens which were isolated successfully from *Spodoptera litura* body and effect of this isolate was tested against *Helicoverpa armigera*. The symptoms of viral infection in *H. armigera* were found as ruptured larval body and reddish cuticle of swollen larvae after treatment. For microscopic examination, a drop of thin smear of infected larvae stained with Giemsa stain indicated polyhedral particles under light and inverted microscope (Fig. 2). Then isolated virus collected from field was also cultured in a *S. litura* laboratory colony and isolation and purification were kept at -80°C for further studies.

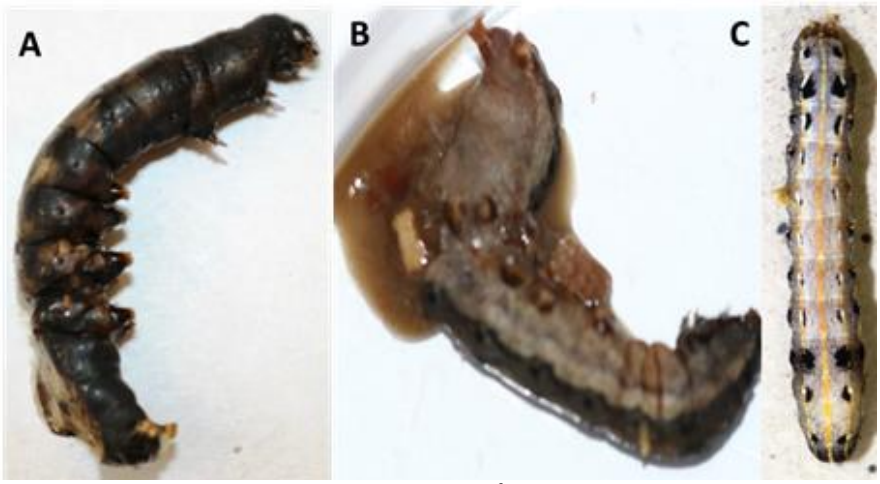

Figure 1. Healthy (C) and NPV infected (A-B) dead last Instar 6th larvae with swollen malformed ruptured larva body *Spodoptera litura* releasing a virus-laden fluid.

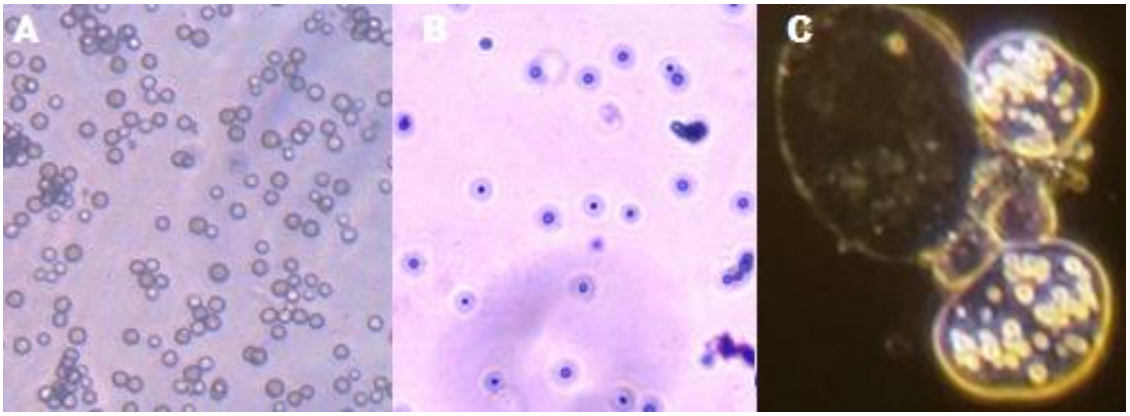

Figure 2. Microscopic examination of NPV; (A) Occlusion bodies on thin slide (B) Giemsa staining of viral particles (C) accumulation of occlusion bodies in insect cells and released as cell ruptured. (40X)

Mortality of *H. armigera*: The nucleopolyhedrovirus (NPVs) are natural biological control agents used against majority of lepidopterous pests. Here, identified NPVs showed effectiveness against polyphagus pest, *Helicoverpa armigera*. The 2nd instar larvae of *H. armigera* treated with sub-lethal dose of Spinosad (0.01ppm) showed mortality of 25.04±3.25 % while larvae exposed to two sub-lethal and one lethal dose of NPV (Sublethal: 1×10^4, Sublethal: 5×10^4POB mL^{-1} and Lethal: 3×10^5 POB mL^{-1}) resulted 28.57±3.51, 35.56±3.74 and 67.20±1.25 % mortality, respectively. The lethal dose of NPV (3×10^5 POB mL^{-1}) in combination with spinosad (0.01ppm) exhibited synergistic action (CTF≥20) with mortality of 95.45±1.59 % while additive effect in rest of combination was observed (CTF≤20) (Table 1). Similarly, sub-lethal and lethal combination of NPV with spinosad caused higher larval mortality in 4th instar larvae of *H. armigera*, however, at alone application of NPV mortality was higher as compared to spinosad alone treatment. In combined application of NPV and Spinosad, sublethal doses of NPV with spinosad produced antagonistic or additive effect while combining Spinosad at 0.01 ppm with NPV at 3×10^5 POB mL^{-1}

resulted synergistic interaction with mortality 60.16±5.18% (Table 1). Larval mortality with time course of second and 4th instar *of* targeted insects (*H. armigera)* populations was observed 25%-58% for 4th and 80 -95% for 2nd larval instars in 196h (Fig 5-6).

Figure 5. Healthy and NPV infected *H. armigera* (A-B) healthy and NPV infected 2nd instar and (C-D) healthy and infected 4th instar larvae respectively. The infected samples with swollen malformed ruptured larva body,

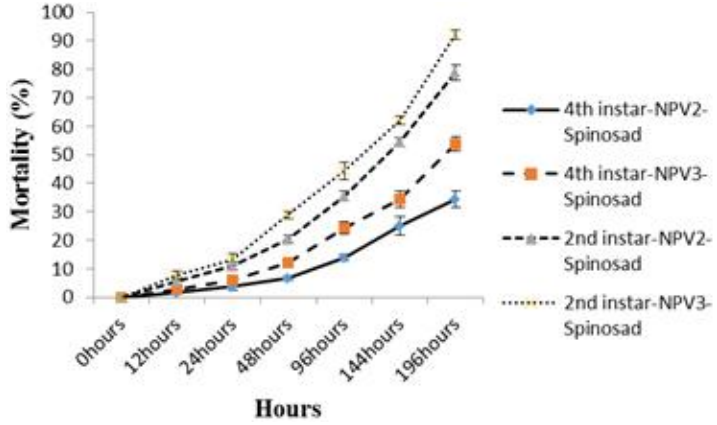

Figure 6. Larval mortality (%±SE) time course of second and 4th instar *of* targeted insect (*H. armigera)* populations treated with dose rate of NPV2-3 with their respective combination of one dose of Spinosad (Sp: 0.01ppm).

Table 1. The larval mortality (%±SE) of second and fourth instar of targeted insect (*H. armigera)* populations treated with three dose rate of NPV1 ($1^{*}10^{4}$), NPV2 ($6^{*}10^{4}$), NPV3 ($3^{*}10^{5}$) and one dose of Spinosad (Sp: 0.01ppm and their respective combination.

Treatments	Mortality (%)	Expected Mortality	CTF	Interaction	Mortality %	Expected Mortality	CTF	Interaction
	2nd Instar larvae				4th instar larvae			
NPV-1 ($1^{*}10^{4}$)	28.57±3.51a				12.72±2.45a			
NPV-2 ($6^{*}10^{4}$)	35.56±3.74b				21.84±2.15b			
NPV-3 ($3^{*}10^{5}$)	67.20±1.25c				32.27±1.90c			
Spinosad	25.04±3.25a				22.45±2.01b			
NPV-1+Spinosad	50.80±3.14d	56.5a	-23.122	Antagonistic	27.41±2.14d	36.75a	-22.21	Antagonistic
NPV-2+Spinosad	60.44±1.40e	55 a	13.523	Additive	40.66±1.68e	45.45b	16.86	Additive
Npv-3+Spinosad	95.52±1.61f	75.8b	21.345	Synergistic	58.81±2.86f	60.16c	26.17	Synergistic
Control	4.60±1.19g				3.17±1.04g			
F	170				193			
DF	7,51				7,62			
P	< 0.01				< 0.01			

Pupation percentage of *H. armigera:* The interaction of NPVs and Spinosad was significant with respect to number of larvae enter in to pupation stage (second instar F771= 243, P≤0.01, fourth instar F771= 312, P≤0.01). The sublethal and lethal effect of NPV in combination with spinosad was more fatal than that of sole applications. Whereas combined lethal effect of NPV with Spinosad produced synergistic effect in 2nd and 4th instar larvae of *H. armigera* with lower percentage of pupation (7.30±2.73 and 15.52±2.37%). In all treatment of bioassay, highest level of pupation (53.67±2.61 and 60.37±2.13%) was observed in sublethal concentration of Spinosad (Table 2).

Adult emergence and egg eclosion percentage of *H. armigera*: The sublethal and lethal doses of NPV and spinosad were inversely proportion to adult emergence and egg eclosion (Table 2). Lethal dose of NPV (3×10^5) with spinosad exerted hazardous effect with lowest adult emergence (5.06±1.29 and 18.26±2.07) and egg eclosion (11.16±2.15 and 17.11±5.97%) in second and fourth instar, respectively whereas highest percentage of adult emergence and egg eclosion was recorded in larvae treated with Spinosad. Combined application of microbes was proved to fetal effect on adult emergence and egg eclosion as compared to their sole application.

Table 2. The pupation, adult emergence and egg ecclusion (%±SE) of second and fourth instar of targeted insects (*H. armigera)* populations treated with three dose rate of NPV1 (1^*10^4), NPV2 (6^*10^4), NPV3 (3^*10^5) and one dose of Spinosad (Sp: 0.01ppm and their respective combination.

Treatments	*Pupation*	*Adult emergence*	*Egg ecclusion*	*Pupation*	*Adult emergence*	*Egg ecclusion*
	2nd Instar Larvae			**4th Instar Larvae**		
NPV-1	81.52±1.61a	74.37±1.99a	78.24±3.18a	87.48±2.13a	82.22±1.98a	94.75±2.92a
NPV-2	66.22±2.47b	66.67±1.96b	71.89±2.45b	75.44±2.42b	77.78±3.06b	82.78±3.90b
NPV-3	60.70±1.58c	56.70±2.41c	55.35±3.64c	67.41±1.19c	68.15±1.86c	69.35±2.55c
Sp	53.67±2.61d	48.52±2.51d	46.63±4.16d	60.37±2.13d	54.44±2.54d	54.53±3.46d
NPV-1+ SP	38.33±2.66e	42.59±1.57e	38.39±2.54e	45.56±3.17e	56.30±2.16d	45.57±3.45e
NPV-2 + SP	27.81±2.34f	17.19±2.61f	24.23±3.60f	36.30±2.74f	37.15±2.18e	33.48±2.94f
NPV-3+ SP	7.30±2.73g	5.06±1.29g	11.16±2.15g	15.52±2.37g	18.26±2.07f	17.11±5.97g
Control	97.73±2.17h	96.29±1.17h	92.39±4.82h	97.51±1.87h	96.58±2.51h	87.91±5.19h
F	243	283	29	312	213	34
Df	7,61	7,61	7,61	7,61	7,61	7,61
P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

DISCUSSION

The *Helicoverpa armigera* (Lepidoptera: Noctuidae) is very destructive pest (Cloyd, 2014; Queiroz-Santos *et al*., 2018) which feed on leaves, flowers and fruits (Fitt, 1989) of more than 200 plant species (Rasool, 2015). In Pakistan, several researchers have documented insecticide resistance of *H. armigera* for pyrethroid, chlorinated hydrocarbons, organophosphates, carbamates, neonicotinoids, insect growth regulators (IGRs) as well as Bt (Hussain *et al*., 2015; Shad *et al.,* 2012; Ahmad *et al*., 2019). The non-judicial use of insecticide has created pest resurgence, insecticide resistance and environment problems (Cherry *et al*., 1997; Ahmad *et al.,* 2007; Ahmad *et al.,* 2008; Shad *et al*., 2012; Ahmad *et al.,* 2018, 2020) motivating researchers to find out some environmentally friendly tactics to control the agriculture pests (Ahmad *et al*., 2018). The combined application of microbial and conventional insecticides as well as nanoparticles with suitable concentration are the need of time to overcome the limits of costly, slow acting and non-environment friendly insecticides (Ahmad *et al.,* 2020; Jafir *et al*., 2021; Qamar *et al.,* 2021). Bio- rational insecticides in combination with microbes is valuable strategy to overcome resistance related issues in insect pests (Ahmad *et al.,* 2018, 2020). Worldwide, NPVs and Nematodes have been utilized alone and in combination against various insect pests of major crops by different scientists (Shapiro *et al.,* 2005; Mendez *et al.,* 2002; Figueroa *et al.,* 2015; Malik *et al.,* 2020). Recently, nucleopolyhedroviruses (SlitNPV) have been isolated, characterized and used to control *Spodoptera litura* in Pakistan (Ali *et al.,* 2017; Ahmad *et al.,* 2018; Ayyub *et al.,* 2019). The result of the present study confirmed first time that *S. litura* associated NPV not only kills *S. litura* but also *H. armigera* by retarding its growth and increasing larval and pupal duration after infection. Enhanced pathogenicity of NPV has been observed against different vegetable, ornamental and field crops (Rios-Velasco *et al*., 2011; Arrizubieta *et al.,* 2013; Arrizubieta *et al.*, 2015). The neonate larvae were more susceptible to pathogenic infection than older larvae because neonate larvae consumed more area of viral treated leave (Kumar *et al.*, 2008). In the present study, the higher dose rate of SplitNPV in combination with Spinosad caused higher mortality in early instar of *H. armigera* as compared to later instar. Similar result was obtained showing that 2nd instar

larvae of *H. armigera* were died more rapidly and susceptible to viral infection (Nawaz *et al.,* 2020) at combined treatment of NPV and chlorantraniliprole (Wakil *et al.,* 2012). It might be due to more deposition of cuticular melanism in older larvae which prevent the entrance of pathogen (Wilson *et al.,* 2001). The current study showed that sublethal dose rate of spinosad is effective against both larval instar of *H. armigera*. Similarly, sub lethal application of spinosad reduced survival and reproduction rate of *H. armigera* and *Spodoptera exigua* (Lepidoptera: Noctuidae) (Wang *et al.,* 2009; Wang *et al.,* 2013). Spinosad is natural class of insecticide targeting nicotinic acetylcholine and butyric acid receptors (Capinera and Froeba, 2007) and enhanced the survived larval developmental and pupal duration while, decreasing larval and pupal weight, and adult emergence ratio, longevity and fecundity of adult fertility. These unique characteristic of spinosad makes the insect pest of vegetable and field crops more vulnerable to microbial insecticide (Wang *et al.,* 2014). In china, spinosad has been used to manage the resistance in diamond back moth against conventional insecticides and *Bacillus thuringiensis* (Bt) (Zhao *et al.,* 2006; Wang and Wu, 2012). The mechanism of insecticide resistance in insect pest can be reduced by combing microbial biorational insecticide including nanoparticles (Jafir *et al.,* 2021; Qamar and Ahmad, 2021; Qamar *et al.,* 2021). Nathan and Kalaivani (2005) observed maximum larval mortality of *S. litura* Fabricius (Lepidoptera: Noctuidae at higher dose rate of NPV and AZA as compared to their sole application. Similarly, Jafir *et al.,* (2021) applied nanoparticles and insecticides against *S. litura* and found high efficacy of nanoparticles to control this notorious pest. In current bioassay higher dose rate of NPV in combination with spinosad caused higher larval mortality and produced synergism against *H. armigera* (2nd instar larvae). Pineda *et al.* (2014) observed that mixture of *Spodoptera frugiperda* multicapsid nucleopolyhedrovirus (SfMNPV) and AZA resulted in synergistic interaction. Qayyum *et al.* (2015) also reported similar additive effect for NPV and *Bacillus thuringinsis*. The reason behind bio rational insecticide (Spinosad) with NPV synergism is creation of physiological or chemical pressures in insects and making them more susceptible to occlusion bodies (OB). NPV infected larvae exhibited oozing of body content and become pale in color. The body of insects become transparent and larvae move towards the tip of plants and then ultimately die (Nathan *et al.,* 2006; Kumar *et al.,* 2008). Bio rational insecticide prolong the larval duration and this allows the viral infection to develop in the insect (Kumar *et al.,* 2008). The antagonistic interaction of NPV (high dose) with imidacloprid has been observed (Trang *et al.,* 2002). The present study has shown that the higher dose rate of NPV in combination with spinosad exhibited synergistic additive effect. The antagonistic or additive interaction in present study could be due to decreased in normal feeding on treatment or a change of gut pH (El- Helalyand and El-bendary, 2013). The present study further indicated that combined application of sub lethal dose of spinosad with medium sub lethal or lethal doses of NPV could be better management strategy to reduce insecticide resistance and to better control *H. armigera*.

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REFERENCES

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

Ahmad, J.N., M. Manzoor, Z. Aslam and S.J.N Ahmad (2020a). Molecular and Enzymatic Study on Field evolved Resistance of Red Palm Weevil (RPW) (*Rhynchophorus ferruginous*) and its management through RNAi in Pakistan. Pakistan J. Zool. 52(2):477-486.

Ahmad, J.N., M. Rashid, S.J.N. Ahmad, S. Maqsood, I. Ahuja and A.M. Bones (2018). Molecular Identification and Pathological characteristics of native isolated NPV against *Spodoptera litura* (Fabricius) in Pakistan. Pakistan J. Zool. 50:2229-2237.

Ahmad, J.N., S.J.N. Ahmad, M.A. Ahmad, N. Contaldo, S. Paltrinieri and A. Bertaccini (2017). Molecular and Biologic Characterization of a phytoplasma associated with *Brassica campestris* phyllody disease in Punjab province. Eur. J. Pl. Pathol.149:117-125.

Ahmad, M., M.I. Arif and M. Ahmad (2007). Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. Crop Prot. 26(6): 809-817.

Ahmad, M., I.M. Arif and Z. Ahmad (1995). Monitoring insecticide resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. *J. Econ. Entomol. 88*:4771-776.

Ahmad, M., SA.H. Sayyed, M.A. Saleem and M. Ahmad (2008). Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. Crop Prot. 27(10): 1367-1372.

Ahmad, J.N., R. Mushtaq, S.J.N. Ahmad., M.A. Malik., M. Manzoor, Tahir, Z. Aslam S. Maqsood., I. Ahuja and A.M. Bones (2020b). Sub-lethal Dose Reponses of Native Polyhydroviruses and Spinosad for Economical and Sustainable

Management of *Spodoptera litura* in Pakistan. Pakistan J. Zool. 52(3): 289-299.

Ahmad. S., H.M.N. Cheema, A.A. Khan, S.A. Khan and J.N. Ahmad (2019). Resistance Status of *Helicoverpa armigera* against Bt Cotton in Pakistan. Transg. Res. 28:199-212.

Alvi, A. M., N. Iqbal, M.A. Bashir, M.I.A. Rehmani, Z. Ullah, A. Latif and Q. Saeed (2018). Efficacy of Rhazya stricta leaf and seed extracts against *Rhyzopertha dominica* and *Trogoderma granarium*. Kuwait J. Sci. 45(3).

Arrizubieta, M., O. Simón, L.M. Torres-Vila, E. Figueiredo, J. Mendiola, A. Mexia, P. Caballero and T. Williams (2015). Insecticidal efficacy and persistence of a co-occluded binary mixture of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) variants in protected and field-grown tomato crops on the Iberian Peninsula. Pest Manag. Sci. 72(4): 660-70.

Arrizubieta, M., T. Williams, P. Caballeroa and O. Simon (2013). Selection of a nucleopolyhedrovirus isolate from *Helicoverpaarmigera* as the basis for a biological insecticide. Pest Manag. Sci. 70(6): 967-974.

Ayyub, M. B., A. Nawaz, M.J. Arif and L. Amrao (2019). Individual and combined impact of nuclear polyhedrosis virus and spinosad to control the tropical armyworm, *Spodoptera litura* (Fabricius)(Lepidoptera: Noctuidae), in cotton in Pakistan. Egypt. J. Biol. Pest Cont. 29(1): 1-6.

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

Çakici, F.Ö., A. Sevim., Z. Demirbag and I. Demir (2014). Investigating internal bacteria of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) larvae and some Bacillus strains as biocontrol agents. Turk. J. Agric. For. 38(1):99-110.

Capinera, J. L. and J.G. Froeba (2007). Behavioral responses of *Schistocercaamericana* (Orthoptera: Acrididae) to azadirex (neem)-treated plants. J. Econ. Entomol. 100: 117-122.

Cherry, A. J., M. Parnell, D. Grzywacz, M. Brown and K.A. Jones (1997). The optimization of in vivo nuclearpolyhedrosis virus production of *Spodopteraexempta* (Walker) and *Spodoptera exigua* (Hubner). J. Invert. Pathol. 70:50-58.

Clem, R.J. and A. L. Passarelli (2013). Baculoviruses: sophisticated pathogens of insects. PLoS pathogens, *9*(11): p.e1003729.

Cloyd,R.A. 2014. How to minimize insecticide/ miticide resistance. Available at: http://extension.psu.edu/plants/greenindustry/landscaping/integrated-pestmanagement/fact-sheets/howtominimize-insecticide-miticide-resistance. (Accessed: May 15, 2014)

El- Helaly, A A. and H.M. El-bendary (2013). Impact of Spinosad and Nucleopolyhedrovirus alone and in combination against the Cotton Leaf worm *Spodopteralittoralis* under laboratory. App. Sci. Rep. 2 (1): 17-21.

Elvira, S., M.A. Ibargutxi., N. Gorria., D. Muñoz., P. Caballero and T. Williams (2013). Insecticidal characteristics of two commercial *Spodoptera exigua* nucleopolyhedrovirus strains produced on different host colonies. J. econ. Entomol. 106(1):50-56.

Ferrelli, M.L., C. Taibo., P. Fichetti., A. Sciocco-Cap and J.D Arneodo (2016). Characterization of a new Helicoverpa armigera nucleopolyhedrovirus variant causing epizootic on a previously unreported host, Helicoverpa gelotopoeon (Lepidoptera: Noctuidae). J. Invert. Path. 138-89-93.

Figueiredo, E., D. Muñoz., A. Escribano, A. Mexia., J.M. Vlak and P. Caballero (1999). Biochemical identification and comparative insecticidal activity of nucleopolyhedrovirus isolates pathogenic for Heliothis armigera (Lepidoptera., Noctuidae) larvae. J. App Entomol. *123*(3):165-169.

Figueroa, J.I., R.E. Coronado, S. Pineda, J.M. Chavarrieta, and A.M. Martínez-Castillo (2015). Mortality and food consumption in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae treated with spinosad alone or in mixtures with a nucleopolyhedrovirus. Flo. Entomol. 98(3):1009-1011.

Fitt, G.P. (1989). The ecology of Heliothis species in relation to agroecosystems. Ann. rev. Entomol. 34(1):17-53

Hussain, D., A. Hussain., M. Qasim and J. Khan (2015). Insecticidal susceptibility and effectiveness of *Trichogramma chilonis* as parasitoids of tomato fruit borer, Helicoverpa armigera. *Pakistan J. Zool. 47*(5).

Jafir, M., J. N. Ahmad., M. J. Arif., S. Ali and S. J. N. Ahmad (2021). Characterization of *Ocimum basilicum* synthesized silver nanoparticles and its relative toxicity to some insecticides against tobacco cutworm, *Spodoptera litura* Feb. (Lepidoptera; Noctuidae). Ecotoxicol. Environ. saf. 218(2021): 112278 https://doi.org/10.1016/j.ecoenv.2021.112278

Kumar, M., P. K. Sharma and A. K. Sharma (2012). Studies on pheromone catches of Helicoverpaarmigera hubner and relation of moth activity with larval infestation on tomato in Baghpat Uttar Pradesh. Int. J. Microb. Res. Technol. *2*:1-3.

Kumar, S. N., K. Murugan and W. Zhang (2008). Additive interaction of HelicoverpaarmigeraNucleopolyhedrovirus and AzadirachtinNachimuthu. BioControl. 53:869-880

Kumari, V. and N. P. Singh (2009). *Spodoptera litura* nuclear polyhedrosis virus (NPV-S) as a component in Integrated Pest Management (IPM) of *Spodoptera litura* (Fab.) on cabbage. J. Biopest. 2:84-86.

Ma, X., X. Liu, X. Ning, B. Zhang, F. Han, X. Guan, Y. Tan and Q. Zhang (2008). Effects of *Bacillus thuringiensis* toxin Cry1Ac and *Beauveria bassiana* on Asiatic corn borer (Lepidoptera: Crambidae). J. Invert. Pathol. 99: 123-128.

Malik, M.A., S.J.N. Ahmad., M. J. Arif and J. N. Ahmad (2020). Management of Diamond Back Moth (*Plutella xylostella*) using Indigenous Isolated Granulovirus and *Azadirachta indica*. Pakistan J. Zool. 52(2) 573-583 DOI: http://dx.doi.org/10.17582/journal.pjz/2020.52

Mansour, N. A., M.E. Eldefrawi, A. Toppozada and M. Zeid (1966). Toxicological studies on the Egyptian cotton leafworm, *Prodenialitura* VI potentiation and antagonism of carbamate insecticide. J. Econ. Entomol. 59: 307-311.

Mehrvar, A., R.J. Rabindra, K. Veenakumari and G.B. Narabenchi (2008). Molecular and biological characteristics of some geographic isolates of nucleopolyhedrovirus of *Helicoverpa armigera* (Lep.: Noctuidae). J. Entomol. Soci. Iran. 28(1): 39-60.

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

Minitab, (2002). Software Inc., Northampton, MA, USA

Nathan, S.S. and K. Kalaivani (2005). Efficacy of nucleopolyhedrovirus (NPV) and azadirachtin on *Spodopteralitura*Fabricius (Lepidoptera: Noctuidae). Biol. Cont. (34): 93-98.

Nathan, S.S. and K. Kalaivani (2006). Combined effects of azadirachtin and nucleopolyhedrovirus (SpltNPV) on*Spodoptera litura*Fabricius (Lepidoptera: Noctuidae) larvae. Biol. Cont. 39:96-104.

Nauen, R. and T. Bretschneider. (2002). New modes of action of insecticides. Pesticide Outlook. 13(6):241-245.

Nawaz, A., H. Ali, M. Sufyan, M.D. Gogi, M.J. Arif, A. Ali, and H.A. Ghramh (2020). In-vitro assessment of food consumption, utilization indices and losses promise of leafworm, *Spodoptera litura* (Fab.), on okra crop. J. Asia-Paci. Entomol. 23(1): 60-66.

Nguyen, Q., L. Nielsen and S. Reid (2013). Genome scale transcriptomics of baculovirus-insect interactions. Viruses. *5*(11):2721-2747.

Ogembo, J.G., S. Chaeychomsri., K. Kamiya., H. Ishikawa., Y. Katou., M. Ikeda and M. Kobayashi (2007). Cloning and comparative characterization of nucleopolyhedroviruses isolated from African bollworm, *Helicoverpa armigera,* (Lepidoptera: Noctudiae) in different geographic regions. J. Insect biotech. Sericol. 76(1):139-149.

Pineda, S., C.A. Pérez-Robledo, R.E. Hernández, J.I. Figueroa de la Rosa, J.M. Chavarrieta and A.M. Martínez (2014). Combined and individual effects of a nucleopolyhedrovirus and azadirachtin on the mortality and maize-leaf consumption of *Spodoptera frugiperda*. Phytoparasitica. 42:571-578.

Qamar, S. R and J. N. Ahmad. 2021. Nanoparticles: Mechanism of biosynthesis using plant extracts, bacteria, fungi, and their applications. J. Mol. Liq. 334(2021): 116040.

Qamar, S.U.R., S. Tanwir., W. A. Khan, J. Altaf and J. N. Ahmad (2021). Biosynthesis of silver nanoparticles using *Ocimum tenuiflorum* extract and its efficacy assessment against *Helicoverpa armigera.* Int. J. Pest Manag. https://doi.org/10.1080/09670874.2021.1980244

Qayyum, M.A., W. Wakil1, M.J. Arif and S.T. Sahi (2015).*Bacillus thuringiensis* and Nuclear Polyhedrosis Virus for the Enhanced Bio-control of *Helicoverpa armigera*. Int. J. Agric. Biol., 17(5):1043-1048.

Queiroz-Santos, L., M.M. Casagrande and A. Specht (2018). Morphological Characterization of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae: Heliothinae). Neot. Entomol. 47(4):517-542

Rasool, A. (2015). Molecular evidence of insecticide resistance in *Helicoverpa armigera* and expression profiles of genes involved in metabolic resistance (Doctoral dissertation, Pakistan Institute of Engineering and Applied Sciencel Nilore, Islamabad).

Rios-Velasco, C., G. Gallegos-Morales, M.C.D. Rincón-Castro, E. Cerna-Chávez, S.R. Sánchez-Peña and M.C. Siller (2011). Insecticidal activity of native isolates of *Spodoptera frugiperda* multiple Nucleopolyhedrovirus from soil samples in Mexico, Flo. Entomol. 94(3):716-718.

Shad, S.A., A.H. Sayyed, S. Fazal, M.A. Saleem, S.M. Zaka and M. Ali (2012). Field evolved resistance to carbamates, organophosphates, pyrethroids, and new chemistry insecticides in *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). J Pest Sci., 85:153-162.

Shapiro, A., T.B. Green, S. Rao, S. White, G. Carner, P.P.C, Mertens, J.J. Becnel (2005). Morphological and molecular characterization of a Cypovirus (Reoviridae) from the Mosquito Uranotaenia sapphirina (Diptera: Culicidae). J. Virol.. 79:9430-9438.

Sokal, R. and F.J Rohlf, 1995.Biometry, (3rd Edition). Freeman and Company, New York, USA.

Sparks, T.C., G.D. Thompson, H.A. Kirst, M.B. Hertlein, L.L. Larson, T.V. Worden (1997) Biological activity of the spinosyns, new fermentation derived insect control agents, on tobacco budworm (Lepidotera). J. Econ. Entomol. 96 (6): 1277-1287.

Trang, T., T. Kieu and S. Chaudhari (2002). Bioassay of nuclear polyhedrosis virus (npv) and in Combination with insecticide on *Spodoptera litura* (Fab).Omerica. 10: 45-53.

Wakil, W., M.U. Ghazanfar, F. Nasir, M.A. Qayyum, and M. Tahir (2012). Insecticidal efficacy of *Azadirachta indica*, nucleopolyhedrovirus and chlorantraniliprole singly or combined against field populations of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). Chilean J. Agri. Res. 72(1): 53.

Wang, D., P. Gong, M. Li, X. Qiua and K. Wang (2009). Sublethal effects of spinosad on survival,growth and reproduction of *Helicoverpa armigera*(Lepidoptera: Noctuidae). Pest Manag Sci., 65(2): 223-227.

Wang, D., Y. M. Wang, H, Y. Liu, Z. Xin and M. Xue (2013). Lethal and Sublethal Effects of Spinosad on *Spodoptera exigua* (Lepidoptera: Noctuidae). J. Econ. Entomol. 106(4): 1825-1831.

Wang, X. and Y. Wu. 2012. High levels of resistance to chlorantraniliprole evolved in field populations of Plutella xylostella. J. Econo. Entomol. *105*(3):1019-1023.

Wang, X., C. Zhang., M. Wang and W. Lu (2014). Genome-scale metabolic network reconstruction of Saccharo polyspora spinosa for spinosad production improvement. Microb. cell fact. *13*(1):.41-48.

Wigley, T. M. L. and L.N. Plummer (1976). Mixing of carbonate waters. Geochimica et Cosmo. Acta. 40(9): 989-995.

Wilson, K., S.C. Cotter. A.F. Reeson and J.K Pell (2001). Melanism and disease resistance in insects. Ecol. let. *4*(6):637-649.

Zhang, S., F. Wu, Z. Li, Z. Lu, X. Zhang, Q. Zhang, and X. Liu (2015). Effects of nucleopolyhedrovirus infection on the development of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and expression of its 20-hydroxyecdysone– and juvenile hormone–related genes. Fl. Entomol. 98(2):682-689.

Zhao, J. Z., H.L. Colins, Y.X. Li, R. F. L. Mau, G.D.Thompson, M. Hertlein, J.T. Andaloro,R. Boykin, and A. M. Shelton (2006). Monitoring of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad, mindoxacarb and emamectin benzoate. J. Econ. Entomol. 99: 176-181.