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COMPARATIVE TOXICITY ASSESSMENT OF NUCLEAR POLYHEDROSIS VIRUS AND SPINOSAD AGAINST *Spodoptera litura* (FABRICIOUS) IN SEMI FIELD CONDITIONS

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Spodoptera litura Fabricius (Noctuidae; Lepidoptera) is most damaging insect pest of various crops. Entomopathogens have potential to manage insect pests by minimizing resistance and reducing health hazards associated with synthetic toxic chemicals. The Nuclear polyhedrosis virus isolate of *S. litura* (V-*Splt*NPV) was isolated from infected larvae and presence of viral occlusion bodies were confirmed using an inverted microscope. Laboratory as well as greenhouse experiment was performed to evaluate the pathogenicity of V-*Splt*NPV. For laboratory studies,2nd, 3rd and 4th instar larvae of *S. litura* were exposed to various concentrations (1×10^8, 1×10^7, 1×10^6, 1×10^5 and 1×10^4 OBs/ml) of V-*Splt*NPV. The results revealed that highest V-*Splt*NPV concentration (1×10^8 OBs/ml) caused 88.08% mean mortality while lower concentration (1×10^4 OBs/ml) of V-*Splt*NPV resulted in lower mean mortality (58.75%) of 2nd instar larvae of *S. litura*. Larval mortality was enhanced with increase in concentration of V-*Splt*NPV and mortality reduced with increase in age of *S. litura* larvae. Around 65.52% mean mortality of 4thinstar was recorded against 1×10^8 OBs/ml which was reduced to 36.72% against 1×10^4 OBs/ml of V-*Splt*NPV. In greenhouse experiment a combined application of spinosad with V-*Splt*NPV (1×10^8 OBs/ml) caused 92.98% mortality of *S. litura* while individual application of V-*Splt*NPV (1×10^8 OBs/ml) resulted 39.65% mean mortality of 3rd instar larvae. Similarly, individual exposure of spinosad caused around 42% mean mortality of 3rd instar larvae under semi-field conditions. The native isolate V-*Splt*NPV have potential to be used in integrated manner with other IPM tactics to significantly reduce the use of the insecticides.

Keywords: *Spodoptera litura*, nuclearpolyhedrosis virus, spinosad, occlusion bodies. Entomopathogens.

INTRODUCTION

The *Spodoptera litura (*Fabricius) causes severe damage to important crops like cotton, tobacco, groundnut, soybean, and vegetables (Qin *et al*., 2004). It is commonly known as tobacco caterpillar and found in South Asian countries especially Pakistan and India (Kranthi *et al*., 2002; Ghaffar *et al*., 2002). It is also known as armyworm and can cause 26–100% yield reduction (Dhir *et al*., 1992). In Pakistan, *S. litura* can is found on the cotton crop at the beginning of the cotton season (Saleem *et al*., 2016). Chemical insecticides are the most commonly used tool for the management of *S. litura*. It is estimated that about thirty insecticides from various groups are applied to manage *S. litura* in Pakistan (Saleem *et al*., 2008). This indiscriminate and unselective use of insecticides along with the poor practice of insecticide application without the pest scouting are the important factors for the resistance development in *S. litura* (Ahmad and Arif, 2010). The *S. litura* has gained resistance against various insecticide groups such as synthetic pyrethroids, carbamates and organophosphates (Ahmad *et al*., 2007).

The negative effect of chemical insecticides on environment, human health (Nawaz *et al*., 2014) and resistance development in insects are key reasons to adopt alternative methods of pest management (O'Callaghan and Brown, 2009). Different microbial pesticides are being used all over the world to manage various insect pests without affecting environment (Islam and Omar, 2012).

Nuclear polyhedrosis viruses (NPVs) belong to the Baculoviridae family and are the pathogens which infest many insect pests and other arthropods. They are used as biopesticides against targeted insect pests (Deng *et al*., 2007). They have been deployed as insecticides since more than 75 years. NPVs are rod-shaped double-stranded DNA viruses which infect arthropods (Jehle *et al*., 2006). Many lepidopteran pests including *S. Litura* (Fabricius) has shown susceptibility to NPV isolates (Kumar *et al*., 2011; Laarif *et al*., 2011; Khattab, 2013; Ahmad *et al*., 2018). NPVs can persist in the environment especially in soil for long time.(Berling *et al*., 2009).Spinosad is a biobased insecticide which is very effective against target insect pests. It is known as low risk insecticide and considered safe for non-target

organisms. NPVs and spinosad when used in combination, gives effective results against the cotton leaf worm (El-Helaly and El-bendary, 2013; Ayyub *et al*., 2019). Efficacy of *Spil*NPV increased by mixing with spinosad against *S. littoralis* larvae (Khattab, 2007). About 30% increase in control of *S. frugiperda* was observed by using mixtures of spinosad and *Sf*MNPV (Mendez *et al*., 2002). The current research work also indicated the efficacy of indigenous isolate of NPV with spinosad under laboratory and semi-field conditions and could be the base of microbial insecticide development in Pakistan.

MATERIALS AND METHODS

***Insect population and insect virus*:** Larvae of *Spodoptera litura* were collected from various cotton fields of district Faisalabad, Pakistan and brought into the laboratory for rearing in controlled conditions. Larvae of *S. litura* were fed on artificial diet (ul Haq *et al*., 2015) in controlled environment (26 ± 2°C, 70 ± 5 RH, 12:12 h light: dark photoperiod) in IPM laboratory, Department of Entomology, University of Agriculture, Faisalabad, Pakistan. Newly hatched larvae were transferred indivuallyto plastic vials (3.2cm height, 3cm diameter) containing a small piece of artificial diet. The NPV infected isolates (38) of *S. litura* larvae were collected from different districts (Faisalabad, Multan, Vehari and Bahawalpur) of Punjab province (Pakistan) (Fig.1).

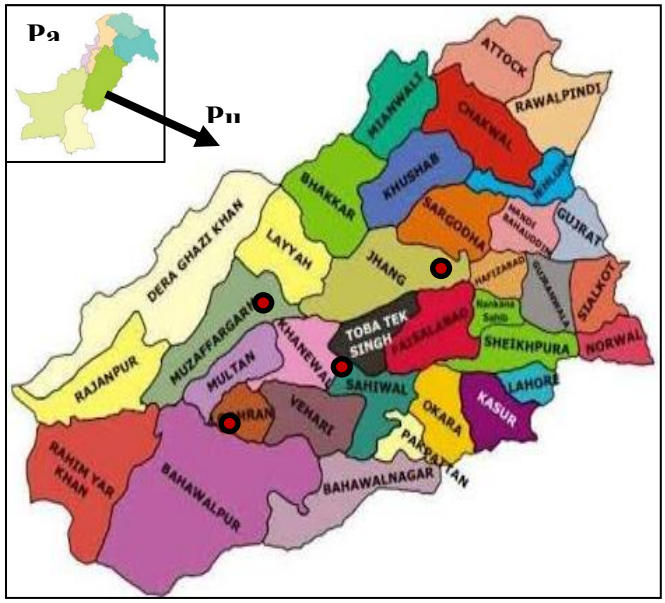


Figure 1. Map showing sampling districts of Punjab, Pakistan (Vehari, Multan, Faisalabad and Bahawalpur).

Collected isolates showing symptoms brought to the laboratory and observed to confirm the presence of NPV by inverted microscope with Giemsa staining (Yaman *et al*., 2001). Two screening tests were conducted to evaluate the pathogenicity of these isolates and most virulent strain isolate was selected for further experimentation (Ayyub *et al*., 2019).The name was given to the isolate with reference to the location from which it was collected (V= Vehari, *Splt*NPV= *S. litura* NPV).The virus propagation was carried out *in vivo* as described(Hunter-Fujita *et al*., 1998 ; Monobrullah and Nagata 2000). Purified occlusion bodies (OBs/ml) were counted five times using hemocytometer under inverted microscope. A dilution of various concentrations (1x 10^4-1x10^8 OBs/ml) of V-*Splt*NPV were prepared in distilled water from stock suspension (Cory and Myers, 2003). The selected isolate V-*Splt*NPV was used for mass culturing and greenhouse experiments.

***Leaf disc bioassay*:** The *S. litura* larvae were obtained from laboratory rearing colony. Cotton leaves of 3 cm diameter were cut and placed in the plastic container (7cm height and 3cm in diameter). Various concentrations (1x 10^{4-8} OBs/ml) were prepared and 5-10 µl viral concentration was applied on leaf discs with a micropipette. Control treatment were applied using only distilled water. Newly molted 30 larvae of 2nd, 3rdand4th instar were placed in a treated container and allowed to feed on contaminated leaf discs. After 24h, larvae were shifted on fresh leaves. Fresh leaves were provided daily until pupation. All plastic containers were placed in a growth chamber with controlled conditions (25±2°C, 70±5% R.H and 14:10 (D:L) photoperiod). Mortality data was recoded after every 48h. All experiments were replicated three times.

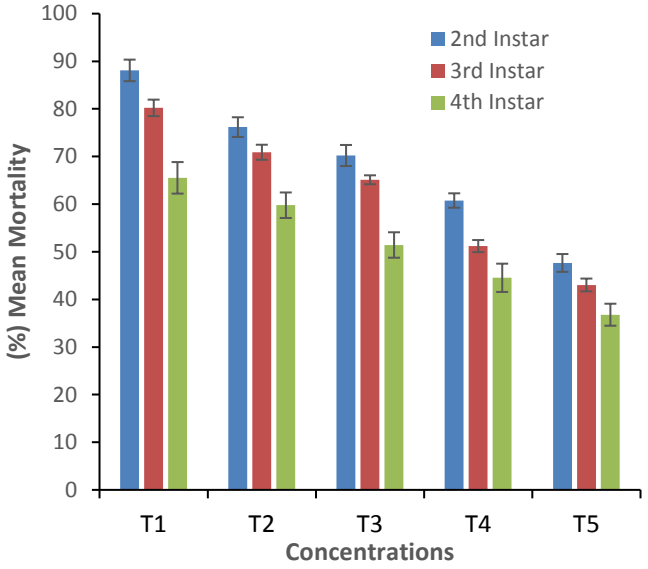
***Greenhouse experiment with NPV and spinosad*:** The experimentwas performed in green house by using potted cotton plants of the same age (50 days). Three different concentrations (1×10^{6-8} OBs/ml) of V-*Splt*NPV were used for bioassay. Spinosad (Tracer 240 SC, Dow AgroSciences) was used with three V-*Splt*NPV concentrations for combined treatment and also evaluated individually as well. For the greenhouse experiment, 1 ml of virus concentration was thoroughly mixed with 19 ml distilled water to make 20 ml (calibrated) final volume. The recommended concentration of spinosad (1%) was sprayed on potted cotton plants for individual toxicity assessment. The combined application of spinosad with various concentrations (1×10^{6-8} OBs/ml) of NPV comprised of 20ml solution which was sprayed along with 0.1% Tween-80 as an adjuvant. Twenty larvae of 3rdinstar of *S. litura* were released separately on each potted cotton plant with the camel hair brush. Cotton plants were covered with 0.5 mm^2 meshed mosquito net to avoid larval escape. The concentrations were sprayed on cotton plants with a hand sprayer. Mortality was observed daily until pupation.

***Statistical analysis*:** Corrected mortalities were calculated by Abbott's formula (1925) and data were analyzed by using

Statistica 8.1 software and means were separated by Tukey's HSD test at α = 5% (Sokal and Rohlf, 1995).

RESULTS

The bioassay was conducted against 2nd, 3rdand 4th instar larvae of *S. litura*. The comparison of mean mortalities of tested larval instars (Fig.2) showed that *S. litura* larvae were adversely effected when exposed to various concentrations of V-*Splt*NPV (1×10^8, 1×10^7, 1×10^6, 1×10^5 and 1×10^4 OBs/ml). The mean mortality ranging from 88.08%-36.78% of tested larval instars were recorded at the end of experiment. The mortality of tested larvae was dose depended as higher mean mortalities were observed for 2nd instar larvae when compared to 3rd and 4th instar larvae.

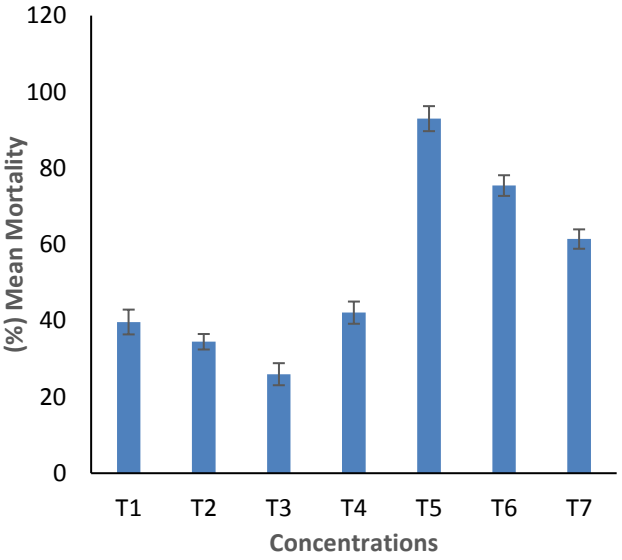


T1, 1×10^8 OBs/ml; T2, 1×10^7 OBs/ml; T3, 1×10^6 OBs/ml; T4, 1×10^5 OBs/ml, T5, 1×10^4 OBs/ml

Figure 2. Percentage mean mortality of different larval instars of *S. litura* against various concentrations applied on cotton leaves in laboratory.

The concentration 1×10^8 OBs/ml (T1) caused 88.08% mean mortality of 2nd instar larvae which was reduced to 80.21% for 3rd instar larvae while 65.52% mean mortality of 4th instar larvae was noticed on same concentration. After application of 1×10^7 OBs/ml concentration (T2) 76.32% mean mortality of 2nd instar larvae was recorded whereas 70.89% and 59.77 % mean mortality of 3rd and 4th instar larvae was observed, respectively. Reduced mortality was noticed after application of lower concentrations. When the concentrations of 1×10^6 OBs/ml (T3) was applied 70.21%, 65.11% and 51.42% mean mortalities were observed against 2nd, 3rd and 4th instar larvae of *S. litura*, respectively. Mean mortalities of 60.76 % of 2nd instar, 51.19% of 3rd instar and 44.53% of 4th instar larvae were recorded against the concentration of 1×10^5 OBs/ml (T4). The 1×10^4 OBs/ml concentration (T5) resulted 47.65% mean mortality which was reduced to 36.78% for 4th instar larvae of *S. litura*.

Greenhouse experiment results showed that combination of spinosad with V-*Splt*NPV concentrations was more effective as compared to alone application of V-*Splt*NPV (Fig.3). Alone application of V-*Splt*NPV with highest concentration (1×10^8 OBs/ml) resulted 39.65% mean mortality of 3rd instar larvae of *S. litura* when released on cotton plant. Whereas combined application of same concentration (1×10^8 OBs/ml) of V-*Splt*NPV caused 92.98% mean mortality which was much higher than the mortality caused by individual applications of V-*Splt*NPV (39.65%) and spinosad (42.10%). The minimum concentration of V-*Splt*NPV (1×10^6 OBs/ml) when combined with spinosad was even effective by causing 61.4% followed by 75.43% (by1×10^7 OBs/ml) mean mortality of 3rd instar larvae of *S. litura* The individual application of both concentrations showed less than 50% mean mortality than in combination with spinosad.



T1, 1×10^8 OBs/ml; T2, 1×10^7 OBs/ml; T3, 1×10^6 OBs/ml; T4, Spinosad; T5, Spinosad + 1×10^8 OBs/ml; T6, Spinosad +1×10^7OBs/ml; T7, Spinosad + 1×10^6 OBs/ml.

Figure 3. Percentage mean mortality of 3rd instar larvae against various concentrations sprayed on cotton plants in greenhouse

DISCUSSION

V-*Splt*NPV isolate was effective to control the larval population of *S. litura*. Older larvae were more resistant to the concentration of V-*Splt*NPV due to physiological changes related to pupation, and resistance against the infection process on the later larval stage. The increase in larval age actually decreases the efficacy of the V-*Splt*NPV. The

mortality of 2nd instar larvae was 88.08% while that of 4th instar was 65.52%. Such observations were agreed with the results of Kumar *et al.* (2011) who revealed that decreasing trend of mortality was observed for second to third instar larvae of *S. litura* due to *Splt*NPV. The physiological changes associated with pupation might not allow infection at the lateral developmental stage as the older larvae (10 days) which were found to be more resistant to *Sl*NPV. The possibility of biovirus not getting sufficient time to replicate or kill the larvae may not be ruled out. Such suggestion gets support from the findings of Teakle*et al*. (1986). In addition, Tuan *et al*. (1998) and Kamala (1992) also observed significant differences in LC_{50} values among different larval instars of *S. litura* and *Trichoplusia ni* (Milks *et aI*., 1998).

The results may differ with previous findings because it has been also reported that the variation in the lethal activity of NPV isolates may depend on the insect population (Erlandson, 2009). In addition, the dose dpendedt mean mortalities of tested *S. litura* are in agreement to the results of Pritha *et al*. (2018) who reported 30.55% to 86.11% mean mortalities of 2nd instar larvae of *S. litura* when exposed to different concentrations of *Sl*NPV. There are number of studies to support the stated results (Tuan *et al*., 1998; Kamala, 1992; Milks *et aI*., 1998; Trang and chaudhari, 2002; Kumari and Singh, 2002).

The combination of spinosad with entomopathogens proved to be suitable because spinosad has no antiviral, antifungal or antibacterial activity (Bret *et al*., 1997). Spinosad has been distinguished as a biopesticide, as spinosyns are produced by fermentation of soil bacterium *actinomycete* (Copping and Menn, 2000). Spinosad has insecticidal properties that differentiate it from other entomopathogenic biopesticides (Salgado, 1998). Bret *et al*. (1997) noted that the combination of spinosad with *Sl*NPV resulted in better control for the management of *S. furgiperda* population. The individual application of spinosad showed 42.1% mean mortality and 39.65% mean mortality was recoded at highest concentration of V-*Splt*NPV. On the other hand, 92% mean mortality was recorded in combined application of Spinosad and V-*Splt*NPV. Such findings are in agreement with findings of El-Helaly and El-bendary (2013) who observed maximum larval mortality (55%) of *S. littoralis* against the combined treatment of *Sl*NPV and spinosad. While alone treatment of *Sl*NPV caused 20.11% mortality and spinosad gave 26.66% mortality of *S. littoralis*. The highest mortality rate may be due to the additive/synergistic impact or the compatibility of Spinosad and V-*Splt*NPV. The additive effects of spinosad and *Sf*MNPV combination was also reported in previous findings of Mendez *et al*. (2002) against larvae of *S. furgiperda* as combined treatment caused higher mortality when compared with the alone application. Similarly, 40% more control of *S. litura* was reported when exposed to the combined formulation of *Splt*NPV and azadirachtin (Cook *et al*., 1996). The combined application of *Ag*MNPV and spinosad also increased the mortality of pickleworms larvae up to 78% (Jackson *et al*., 2014). While significantly lower mortality of 32% and 24% was observed against *Ag*MNPV and spinosad, respectively when applied alone. Overall, it is evident that spinosad is compatible with NPV based products and can be effective if applied carefully.

NPV also proved to be compatible with other important pest management compounds. Nathan and Kalaivani (2005) reported maximum mortality (92.7%) with azadirachtin (AZA) and NPV against *S. litura* larvae but less mortality was recorded when treated alone with NPV (28.5%) and AZA (36.3%). Similarly, Shaurub *et al*. (2014) suggested that the mixture of NPV with AZA enhance larval mortality of *S. littoralis* significantly as compared to individual treatments.

***Conclusion*:** The results exhibited dose and stage dependent mean mortality of target pest. The native isolate V-*Splt*NPV showed 88.08% mean mortality of 2nd instar *S. litura* in laboratory. V-*Splt*NPV is also compatible with spinosad causing 92.98% mortality of 3rd instar *S. litura* in semi-natural conditions. Overall, the integration of NPV with low risk insecticides can be very effective for the management of insect pests of economic importance.

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