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Research Article

Invitro assessment of native nuclear polyhedrosis virus efficacy for the management of *Spodoptera litura* reared on artificial diet

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Abstract

Microbial based insecticides have great potential for the management of various agricultural insect pests. Present research was conducted to assess the efficacy of indigenous nuclear polyhedrosis virus (NPV) for the management of *Spodoptera litura* (Fabricius). A field survey was conducted for collection of NPV infected larvae of *S. litura* with specific symptoms. Out of 38 collected NPV infected isolates (larvae) the best isolate showed maximum mortality in *S. litura* larvae in two screening experiments. The selected isolate was given the name V-*Splt*NPV and was multiplied for further experimentation. The V-*Splt*NPV identified by Giemsa stain under an inverted microscope. Concentrations of V-*Splt*NPV were prepared by dilution with distilled water and counted by hemocytometer. Effectiveness of native isolate V-*Splt*NPV was assessed against 2nd, 3rd and 4th and 5th instar *S. litura* larvae on artificial diet in laboratory. Artificial diet pieces were contaminated with various concentrations (1×10^8, 1×10^7, 1×10^6, 1×10^5 and 1×10^4 OBs/ml) of V-*Splt*NPV and offered to *S. litura* larvae. The highest concentration (1×10^8 OBs/ml) of V-*Splt*NPV caused maximum (68.52%) mean mortality of 2nd instar larvae while minimum mean mortality (51.11%) of 5th instar larvae was occurred by tested concentrations. Conclusively, local isolate if isolated and multiplied can be proved best for the management of several insect pest species.

Keywords: Artificial diet; Biological control; Nuclear polyhedrosis virus; NPV; *Spodoptera litura*

Introduction

Spodoptera species (Lepidoptera: Noctuidae) contain the insect pests of economically important crops and widely distributed throughout sub-tropical, tropical and the temperate zones **[1]**. About 30 species of *Spodoptera* have been described till now with their presence in six continents around the globe **[2]**. Their larvae attack on more than 120 different plant species including various vegetables, flowers and weeds etc. **[3, 4]** The main insect pests of genus *Spodoptera* are

Spodoptera litura (Fabricius), *Spodoptera exigua* (Hübner), *Spodoptera littoralis* (Biosduval) and *Spodoptera frugiperda* (Federici). The *S. litura* (Lepidoptera: Noctuidae) is the most important species of *Spodoptera genus.* It has main economic importance due to higher reproductive potential and attack on verity of agricultural crops. **[5, 6]** The *S. litura* is also known to infest broad range of succulent crops **[7]**. Its larvae attack on more than 112 plant species from 40 different plant families **[8]**.

Larvae of *S. litura* have wide host range, which can be important factor for their survival **[9, 10]**. Selection of host plants mainly relay on the nutritional differences which are linked with the primary as well as secondary metabolites which are present in those plants **[9]**. The *S. litura* is mainly a leaf eater as it chew the leaves but also attack on buds, flowers, immature bolls and old leaves. Its larvae prefer to attack on the midrib veins of leaves. In case of severe attack larvae can defoliate the complete plant. Due to severe attack fields of infected crop look like standing sticks **[11]**. The female of *S. litura* normally lay eggs (1000-2000) in cluster of 200-300 at lower side of the leaf and cover them with abdominal hairs to protect from natural enemies **[12]**. Incubation of *S. litura* is completed in 4 days in warm weather **[5]**. The *S. litura* larvae has six instars and complete its period in 16-24 days depending upon host plant and environmental conditions **[13]**. Pupation occurs inside the soil debris and pupation occur for 8-12 days. The adult remain alive for mostly 4-10 days. Its total life cycle is about 5-6 weeks with 3-4 generations in a year **[14]**. The *S. litura* also known as cotton leafworm, tropical armyworm, cluster caterpillar, tobacco caterpillar and common cutworm **[15]**. In ideal conditions, its population increased and move across fields as large masses in search of food, so called as armyworm. Its larvae have ability to cause upto 100% crop loss. **[16]** It is distributed worldwide especially in subcontinant **[17]**. Various artificial diets have been developed for rearing of Lepidopterous insect pests like *S. litura* **[18]**. Mostly artificial diets proved themselves effective for successful rearing of those insects but in some cases there is loss of reproductive potential which ultimately enhance the developmental period and reduced the rate of fecundity **[19]**.

The *S. litura* is now severe insect pest of Pakistan which attack in various areas of Pakistan **[20]**. The increase in productions of various succulent crops such as vegetables, cotton, mung bean, soya bean and cabbage provide ideal circumstances to *S. litura* for vigorous reproduction that results in its rapid population increase **[21]**. Despite the modern technology, few control strategies have been recommended to manage the *S. litura* population **[22]**.

Repeated and irrational chemical insecticide use is causing resistance in various insect pests against those chemical insecticides in many countries **[23]** including Pakistan. The increase of pesticide use has led to a heavy cost regarding environmental and human health issues **[20]**.

Microbial biopesticides especially virus based pesticides are a strong biological agent of major insect pest due to their safety to non-targeted organism **[24]**. Among different families of viruses, the Baculoviridae family has been most extensively used as a biopesticide to control the world's most damaging insect pests **[25]**. Baculoviruses considered as the most intensively studied the insect pathogenic virus **[26]**. Baculoviruses have about 600 different host insect species to infect, which includes members of different orders such as Lepidoptera, Hymenoptera, and Diptera. Putative members of baculoviruses have been found from Coleoptera, Orthoptera, Neuroptera, Trichoptera, and Thysanura **[27]**. The pathogenicity level of these viruses against

targeted pest and their presence varies among different geographic isolates [28].

Propagation or multiplication of nuclearpoly hedrosis virus is mainly done by *in vivo* and *in vitro* [29, 30]. Commonly used technique is *in vivo*, inn which simply infecting the healthy targeted larvae by NPVs mixed diet and harvest the infected larval population for the virus propagation [31].

Present research was conducted to evaluate the efficacy of native nuclearpolyhedrosis virus isolate against the larvae of *S. litura* which was reared on artificial diet.

Materials and methods

Rearing of insects

The larvae of *Spodoptera litura* (Fabricius) were collected from agricultural fields of university of Agriculture, Faisalabad and brought to the laboratory for rearing under controlled environment (70 ± 5% RH, 26 ± 2°C Temp, 12:12h light: dark photoperiod) in IPM laboratory, Entomology department, University of Agriculture, Faisalabad. Artificial diet was prepared as previously described [32]. Newly hatched larval population was transferred indivually to plastic vials (3 cm diameter, 3.2 cm height) containing artificial diet piece.

Pupae were shifted in plastic jar and kept until adult emergence. The mouth of the jar was tightly covered with muslin cloth. The 10% honey solution (cotton plug was soaked in honey and placed in jar) was given to adults. Paper strips hanged in the jar for egg laying. Eggs were collected from the paper strip and shifted to a petri dish for hatching. Freshly hatched larvae then again shifted to artificial diet for rearing as described above. More than ten generations of *S. litura* population were reared in the laboratory before the experimentation.

Collection, isolation and identification of NPV infected larvae

NPV infected larvae with specific symptoms were collected from agriculture fields. After isolation and identification of V-*Splt*NPV described by [32] V-*Splt*NPV was propagated in 4th larval instar of *S. litura* by offering them V-*Splt*NPV contaminated diet and allowed to feed on contaminated diet for 8 days.

Concentrations were prepared after 6 counts under Neubauer hemocytometer [**33**]. These concentrations were used in the experiments. Various concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 OBs/ml) of V-*Splt*NPV were prepared by diluting them in distilled water.

Potential assessment of NPV isolates at different dose rates in laboratory conditions

Efficacy of five concentrations (1×10^4 to 1×10^8 OBs/ml) of V-*Splt*NPV were evaluated under laboratory conditions. Artificial diet pieces of 10g were cut and prepared concentrations were applied on that pieces with the help of micropipette. The contaminated diet pieces were placed in plastic vials.

Newly molted 30 larvae of 2nd, 3rd, 4th and 5th in stars were individually placed in plastic vials containing diet pieces and allowed to feed. For control treatment, diet pieces treated with distilled water were used. All plastic vials were placed in a growth chamber with controlled environment (70 ± 5% RH, 26 ± 2°C Temp, 12:12h light: dark photoperiod). Mortality was recorded after every 48 h until pupation. (Concentrations repeated 3 times). All experiments/ each treatment was were replicated three times using

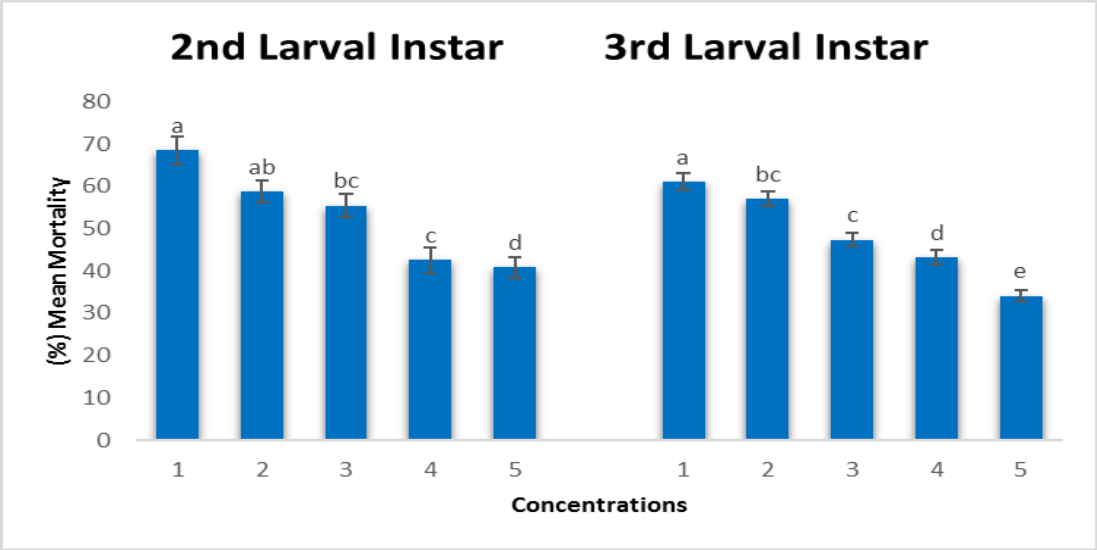
Data analysis

Completely Randomized Design. After the completion of the experiments, corrected mortalities were calculated by Abbott's [34] formula and mortality data was analyzed by using Statistic 8.1 software and means were separated by Tukey's HSD test at $\alpha = 5\%$.

Results and Discussion

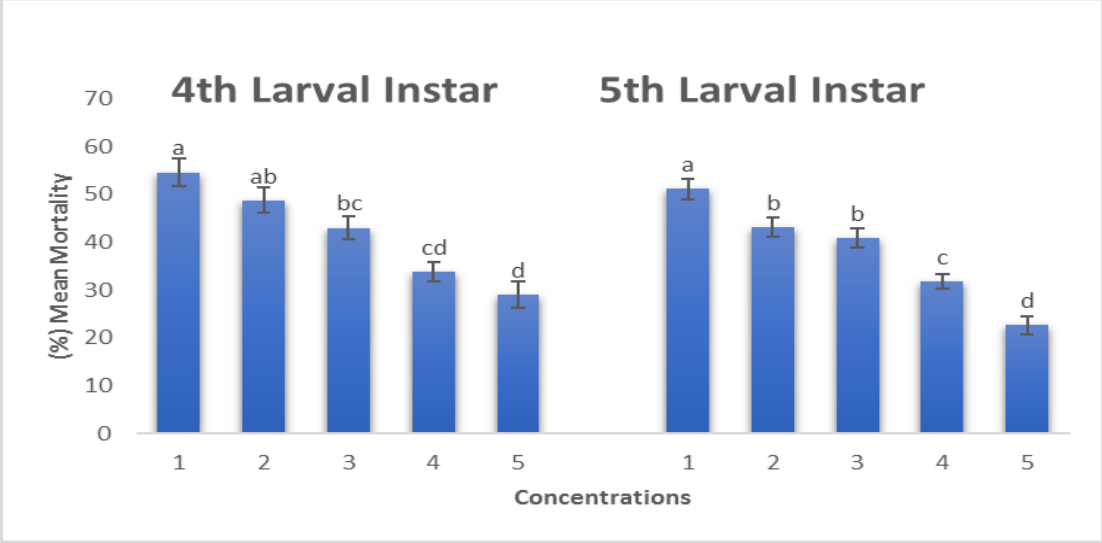
The V-*Splt*NPV bioassays performed against 2nd, 3rd ,4th and 5th instar of *S. litura* larvae

showed different mean mortalities ranges against various concentrations as 22.68% to 68.52% for tested larvae (Fig 1 & 2). According to the results 2nd instar larvae were more susceptible than the other tested larvae of *S. litura*. Similarly, 5th instar larvae were most resistant than the other larval instars. Higher mean mortalities (68.52%) of 2nd instar larvae of *S. litura* were observed in the bioassay (Fig 1).



1; 1×10^8 OBs/ml, 2; 1×10^7 OBs/ml, 3; 1×10^6 OBs/ml,
4; 1×10^5 OBs/ml, 5; 1×10^4 OBs/ml

Figure 1. Assessment of NPV isolates at various concentrations against 2nd and 3rd instar *S. litura* larvae



1; 1×10^8 OBs/ml, 2; 1×10^7 OBs/ml, 3; 1×10^6 OBs/ml,
4; 1×10^5 OBs/ml, 5; 1×10^4 OBs/ml

Figure 2. Assessment of NPV isolates at various concentrations against 4th and 5th instar *S. litura* larvae

Mean mortalities were decreasing with increasing the larval age as 3rd instar larvae showed (61.12%), 4th instar larvae resulted (54.59%) mean mortality and the 5th instar showed least mean mortality (51.11%) (Fig 2). Mean mortalities were increased with increasing the concentrations.

The current outcomes are in line with **[35]** who recorded 30.55% to 86.11% mean mortalities of 2nd instar *S. litura* larvae when exposed to different formulations of *Sl*NPV. Furthermore, the recent results are also supported by Monobrullah and Nagata **[36]** as they suggested increase in the value of LD_{50} for second to fifth larval instar of *S. litura* showing an increase in resistance against NPV with an increasing larval age. There are number of studies to support current findings **[37-40]**.

Older instars showing resistant to the formulations of V-*Splt*NPV may be due to the physiological variations which are related to the pupation, resulted resistance development against the process of infection on the older larval instars. Such results were agreed with the findings of **[41]**.

The results are in accordance with **[42]** that the inverse correlation among susceptibility of insect larvae, associated with an increase in larval biomass with the increase in larval age. Our research in line with the findings of **[43]** who revealed that the increasing trend in the LC_{50} values of *Splt*NPV was observed for second to third larval instar of *S. litura*. Similar results were discussed by **[44]** who observed the increasing LC_{50} values of NPV formulation with increasing the larval age of *Heliothis punctigera* (Wallengren). Similarly, **[42]** noticed the difference in the values of LC_{50} among 1st and 5th larval instar of *S. litura*. The value of LC_{50} was 34 000 times higher for the 5th larval instar when compared with 1st larval instar of *Mamestra brassicae* (Linnaeus), while the 5th and the 6th larval instars were almost at par for the resistant to viral infectivity.

The results may differ with previous findings because it has been also reported that the change in the lethal activity of NPV isolates may depend on the insect population [**45**] Our findings supported by **[47]** as they observed considerable differences in the LD_{50} values between *S. exigua* NPV isolates from Thailand, United States, and Spain. While **[47]** reported that there was no such difference between the LD_{50} values of two geographically different NPV isolates of *M. brassicae*, collected from the Germany and Netherlands.

The physiological variations linked with the pupation process might not allowed infection at the lateral developing stage as the old age larvae (10 days) were more resistant to *Sl*NPV formulations. The opportunity of biovirus not getting enough time to multiply the virus or to kill the larvae may not be ruled out. Such observations get support from the results of instars **[42, 44]**. In line to our research Milks [**39**] also noticed a decreasing trend in LC_{50} values with the decrease in larval age. According to them, for eight day old larvae LC_{50} value was 1.4×10^{8} PIB/ml while for two day old larvae of *S. litura* LC_{50} value was 1×10^{3} PIB/ml. Over all, the results were in accordance with previous studies which indicated the efficacy of experiments and the procedure to execute them in different conditions.

Conclusion

Conclusively native isolate V-*Splt*NPV have the ability to manage the *S. litura* population presented in agricultural fields of Pakistan. The V-*Splt*NPV can be used in integrated manner with other integrated pest management tactics to significantly reduce the use the toxic chemical pesticides.

Authors' contributions

Conceived and designed the experiments: MB Ayyub & A Nawaz, Performed the experiments: MB Ayyub, S Zaman & T Anwar, Analyzed the data: G Ullah & M Iqbal, Contributed materials/ analysis/ tools:

MB Ayyub, A Rasul & F Amjad, Wrote the paper: MB Ayyub.

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