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Bioassay, cross-infectivity and shelf life studies of *Spodoptera litura* nuclear Polyhedrosis Virus

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Abstract
An investigation was carried out to determine the lethal dose and lethal time of *SL*NPV against *S. litura*, besides cross infectivity and shelf life under laboratory condition in Department of Entomology, Dr. PDKV Akola, Maharashtra. Bioassays conducted against late second instar larvae showed LC_{50} value of 6.02 x 10^5 POBs/ml and LC_{90} of 5.08 x 10^9 POBs/ml. The data on the lethal time required revealed that the LT_{50} value was 98 hrs (4.08 days) and LT_{90} was 167 hrs (6.95 days). The cross infectivity studies confirmed the species specificity of *Sl*NPV as no cross-infectivity was recorded against other tested insects, *viz*, gram pod borer, diamond back moth, castor semilooper and soybean semilooper. The laboratory analysis for shelf-life of *Sl*NPV showed reduction in POB count/ml over a period of 6 months. The POB count indicated reduction of 20.71%, 14.28% and 9.48% in case of sole NPV, NPV with UV protection agent and NPV with antimicrobial agent, respectively. Addition of UV protectant and antimicrobial agents in the crude *Sl*NPV proved to confer prolonged viability of POBs/ml in NPV suspension. Thus, the present investigation confirmed the efficacy of *Sl*NPV as biocontrol agents against *S. litura* that can be exploited under field conditions while planning for pest control strategies in an ecofriendly manner.

Keywords: Nuclear polyhedrosis virus, Species specificity, Shelf life, LC_{50} value, LT_{50}, *Spodoptera litura*,

Introduction
Pesticides are considered to be the most effective tool for combating the pest problems inimical to the interest of man. They have dominated the field of pest control for long. Even today they are used on a large scale by most of the cultivators in their spray schedules, because of quick knock-down effects and noticeable results of these chemicals. Pesticides have been used extensively to control different pests, both in agriculture, forestry, and public health sectors. However, extensive reliance on pesticides and their indiscriminate and intensive use has led to serious consequences like pest became resistance to pesticides, pest resurgence due to the destruction of their natural enemies, soil and water pollution due to presence of pesticide residues etc. Also use of toxic chemicals has caused ill effects to non-target organisms including human being [3].

The *Spodoptera litura*, commonly known as tobacco leaf eating caterpillar, is a polyphagous insect pest of national importance causing economic damage to a number of economically important agricultural crops viz. tobacco, cole crops, castor, cotton, sunflower, soybean, chilli [9] reported to feed on more than 290 plant species belonging 80 to 90 families [10].This pest has the high reproductive capacity and ability to migrate over large distance in the adult stage. The damage is caused by the larvae feeding mainly on the foliage resulting in significant reduction in foliage and yield, if it is not properly managed. [14] *Spodoptera* is reported to cause enormous losses in yields of different crops as reported from different parts of the country. Yield losses in groundnut up to 71% have been reported in the irrigated tracts of Andhra Pradesh, Karnataka and Tamil Nadu [4] reported a 26-100 % yield loss in the field of groundnut due to *S. litura*. In Vidarbha region of Maharashtra state, outbreak of *S. litura* on soybean crop was reported in eastern districts of Vidarbha during 2008-09 which caused heavy losses to soybean growers. In North India, [8] carried out a field survey in 14 villages of Sirsa district (Haryana), and an increasing trend in population of *S. litura* (Fab.) larvae per plant of *Bt* cotton and concluded that *S. litura* (Fab.) is an emerging threat on *Bt* cotton (*Cry 1Ac*) under north Indian conditions.

Several synthetic insecticides are recommended for the management of this pest.

However, indiscriminate and frequent application of these chemicals resulted in problems like development of resistance, secondary pest resurgence and environmental pollution. In the southern parts of India, it is reported to have developed resistance virtually to almost all conventional insecticides such as cypermethrin, fenvalerate, endosulfan, quinalphos and monocrotophos [2]. With these bitter experiences of chemical insecticides, there has always been a need of an effective alternate pest control strategy. Accordingly, efforts have been made towards the development of alternative management strategies and biological control provides one such alternative as an important component of integrated pest management.

Among the various biological control agents used for pest suppression, insect viruses have been the most exciting and promising group of pathogens being considered for use in insect pest management. Nuclear Polyhedrosis Viruses (NPVs) are the best known for their effects and account for 41% of described arthropod viruses showing great promise for practical use. Due to the merits like naturally occurring entities, self-perpetuation, safety, host specificity and environmental stability, NPV have been considered as a potential substitute to chemical insecticides for control of *Spodoptera litura*. Amongst various insect viruses, Nuclear Polyhedrosis Viruses (NPV) is more successful in pest management. [11] Fortunately, the pest is highly susceptible to its NPV and studies have shown that the virus can be used effectively as biopesticide in the field. Considering the reliability, suitability and effectiveness of *Sl*NPV in terms of economic and ecological reasons, its utilization in pest management has received a great deal of significance. Keeping these things under consideration, the investigation focuses towards the efficacy, cross infectivity and shelf life studies under laboratory condition.

Materials and Methods

The present investigation was undertaken during 2015-2016 in the Department of Agricultural Entomology, PGI, PDKV AKOLA. The experimental site is situated in Vidarbha region of Maharashtra and located at an altitude of 282 m above MSL with latitude of 20° 42' N and 77° 02' E. For this experiment, the late instar larvae of *Spodoptera litura* were collected from pigeon pea, castor, soybean fields in and around Dr. PDKV Campus, which served as a starter culture for its mass rearing under laboratory conditions. They were brought into the laboratory and reared.

After pupation, the pupae were surface sterilized by dipping in 0.25% Sodium hypochlorite solution. After washing in water the pupae were dried in the air and kept in 10 inch diameter petri plate for emergence in emergence cage. The emerged moths were collected and released in to separate chambers in a proportion of 1:1(male: female).Honey (10%) solution dipped in cotton swab was provided as adult food. After hatching the first instar larvae were transferred to fresh castor leaves till they reach late second instar larvae. Rearing was carried out at room temperature aseptically with periodical disinfection of the rearing chamber and rearing material with Formaldehyde.

Determination of Lethal dose and lethal time

The *Sl*NPV procured from Dr. PDKV experimental fields were used for bioassay studies. The polyhedral occlusion bodies in NPV suspension were counted by using Neubaur haemocytometer and the number of POB was calculated by using the formula given in below.

Number of Polyhedra per ml = D X X/N X K

Where,
D = dilution factor, X = total number of squares counted,
N = Number of squares counted, K = Volume of above one small square in cm^3

Bioassay was conducted by leaf dip method. The late second instar larvae (5 days old) were used as test insects for bioassay studies. Around six concentrations in geometrical proportion were selected for the preliminary trials. The mortality data in preliminary trials were further used for determining the actual concentration for the detailed bioassay. A solution for higher concentration was prepared initially and then serially diluted to get required lower concentration and count also was taken for knowing the accurate POB concentration in them. Each concentration had three replications of 10 larvae each. The concentration used for bioassay were $4x10^4$, $7.5x10^5$,$1.3x10^6$, $1.2x10^7$, $3.2x10^8$, $1.6x10^9$ POBs/ml.The late second instar larvae were released on *Sl*NPV treated castor leaves and allowed to feed for 72 hrs. Then they were replaced with fresh castor leaves for up to 8th day. Observations on larval mortality were recorded from 2nd to 8th day. The mortality data recorded were corrected depending upon the mortality in control following Abbots formula and subjected to probit analysis (Finney, 1964) for determining the median lethal concentration and also time wise mortality was recorded to know the median lethal time. The LC_{50} and LT_{50} values were utilized for evaluation of efficacy of *Sl*NPV formulation.

Determination of cross infectivity of *Sl*NPV against different insect pests

Further, *Sl*NPV having $1x10^9$ POBs/ml was used for cross infectivity studies. Cabbage leaves were treated with *Sl*NPV and 2nd instar DBM larvae were allowed to feed on it. Three sets of 10 DBM larvae were taken for the same. Mortality or any deformities were assessed daily from 2nd day to 8th day of treatment. Likewise castor leaves for castor semilooper and soybeans leaves for soybean semilooper were used for bioassay. Similarly for *Helicoverpa armigera*, gram seeds treated with *Sl*NPV were used for bioassay as above. Observations were recorded on larval mortality and /or deformity if any, to determine the possible cross infectivity.

Determination of effect of different adjuvants on the shelf life of *Sl*NPV

The *Sl*NPV having $1x10^9$ POBs/ml was kept in the laboratory at room temperature. 20 ml distilled water was taken in the small borosil beaker and 20 ml *Sl*NPV was added. Three eppendorf tubes were taken and filled with 900 micro litre of distilled water and 100 microlitre of *Sl*NPV was taken for making three time dilution. One drop of the suspension was poured in haemocytometer. The count was taken for checking the presence of viable amount of POBs under microscope with the help of Haemocytometer during each month upto a period of 6 months to assess the shelf life of *Sl*NPV under test. Three different types solutions by adding adjuvant like Ranipal as UV protectant, antibiotic like streptomycin also mixed to check their additive action in the shelf life of NPV. The containers were kept undisturbed by wrapping them with black velvet paper to avoid sunlight.

Results and Discussion

Traditionally, the enormous amount of chemical pesticides

including persistent organic compounds have been used for the pest control. But recently, there is increasing concern about over reliance on pesticide and their ill effects on man, wild life and the environment as a whole. Thus, the interest on the search for biotic agents has emerged that can control important pests of crops.

Lethal dose and lethal time of *Sl*NPV against *Spodoptera litura*

The *Sl*NPV bioassays conducted against late 2nd instar larvae of *Spodoptera litura* showed mortality in various concentrations in the range of 30.55 to 86.11 % after 8 days of treatment when exposed to five different concentrations used for determination of LC_{50}. The data after subjecting to probit analysis revealed that LC_{50} was $6.02x10^5$ POBs/ml and LC_{90} value was $5.82x10^8$ POBs/ml with fiducial limits at 95% in the range of $1.44x10^5$ – $1.72x10^6$ POBs/ml. The chi-square value obtained was 0.750 indicating a good fit and the value of slope was 0.43 (Table 1). In consistence to the present findings, it is reported the LC_{50} values against 2 days old *Spodoptera* larvae to be 1 x 10^3 POBs/ml, and against 8 days old larvae was 1.5 x 10^9 POBs/ml. [13] LT_{50} values were reported to be increased from 4.4 days against 2 days old larvae to 9.4 days against 8 days old larvae. Similarly, [6] reported LT_{50} value of 4.35 days and LT_{90} value of 6.58 days against *Helicoverpa armigera* fed with Ar1b-HearNP, HZ8-HearNPV and wt-HearNPV, which supports the present findings regarding the lethal time to kill 50 % population.

Fig 1: NPV infected virosed larvae after bioassay.

Table 1: Median Lethal Concentration of *Sl*NPV against *S. litura*

Probit Analysis				
LC_{50} (POBs/ml)	**LC_{90} (POBs/ml)**	**Fiducial Limits at 95 %**	**X^2**	**Slope ± SE**
$6.02x10^5$	$5.8x10^8$	($1.44x10^5$ – $1.72x10^6$)	0.750	0.43±0.08

(Chi square for heterogeneity tabular value at 0.05 level – 7.815)

Similarly, a laboratory bioassay was conducted with the LC_{90} value obtained in first bioassay study to know the lethal time required to kill 50 % and 90 % population of *Spodoptera*. The data obtained from mortality after subjecting to Probit Analysis [5] revealed that the LT_{50} value was 98 hours indicating that the lethal time required for killing 50 % *Spodoptera* population was 4.08 days after the application of *Sl*NPV. The LT_{90} obtained was 167 hrs requiring 6.95 days killing 90% population of *S. litura* after the application of *Sl*NPV. The values of fiducial limits at 95% were in the range of 90.53 – 106.85 hrs and chi-square value of 8.310 with a slope of 5.57 (Table 2).

Table 2: Lethal time (LT_{50} & LT_{90}) of *Sl*NPV against *S. litura*

Probit Analysis				
LT_{50} (hr)	**LT_{90} (hr)**	**Fiducial Limits at 95 %**	**X^2**	**Slope ± SE**
98 hr (4.08 days)	167 hr (6.95 days)	(90.532 – 106.855 hr)	8.310	5.57±0.593

(Chi square for heterogeneity tabular value at 0.05 level – 12.592)

Cross infectivity of *Sl*NPV against different insect pests

The cross infectivity studies may widen the scope of controlling multiple insect species occurring in same cropping system although NPVs are highly host specific. Accordingly, cross infectivity studies of *Sl*NPV were conducted against diamond backmoth, gram podborer, soybean semilooper and castor semilooper and the results revealed that no-cross infectivity was observed in any of the test insects as the inoculated larvae of all the four insect pests did not show any symptoms of viral infection rather all the larvae entered into pupal stage and emerged into normal adults which confirms the species specificity of viral biopesticides in general and *Sl*NPV in particular as observed in present investigation. The experiment on safety of *Sl*NPV formulation revealed that the virus formulation tested at a concentration of $1x10^9$ POBs/ml was not harmful to soybean semilooper, castor semilooper, *Spodoptera litura*, *Helicoverpa armigera*. Therefore it is highly host specific in action (Table 3). In a study specifically on *S. litura* NPV, [7]also recorded that there was no cross infectivity of *Sl*NPV observed to other groundnut pests tested, viz., *Heliothis armigera* (Hub.), *Aproaerema modicella (*Deventer), *Empoasca kerri* Pruthi and *Aphis craccivora* Koch, and was also safer to their predators, viz., *Coccinella septumpunctata* Linn., *Menochilus sexmaculatus*. In consistence to our present findings, [1] another research on host specificity did not show any larval mortality in nine lepidopteran insects viz *Helicoverpa armigera, Spodoptera litura, Agrotis ipsilon, Pieris brasssicae, Plutella xylostella, Galleria melonella, Corcyra cephalonica, Bombyx mori and Spilosoma obliqua* when treated with *Sl*NPV.

Table 3: Cross infectivity of *Sl*NPV against different insect pests

Treatment	Insect Pest	No. of insects treated	No. of virosed larvae
*Sl*NPV ($1x10^9$POBs/ml)	*Helicoverpa armigera*	30	Nil (on 7th day)
Control (distill water)	*Helicoverpa armigera*	30	Nil (on 7th day)
*Sl*NPV ($1x10^9$POBs/ml)	*Diamond back moth*	30	Nil (on 7th day)
Control (distilled water)	*Diamond back moth*	30	Nil (on 7th day)
*Sl*NPV ($1x10^9$ POBs/ml)	*Castor semilooper*	30	Nil (on 7th day)
Control (distilled water)	*Castor semilooper*	30	Nil (on 7th day)
*Sl*NPV ($1x10^9$ POBs/ml)	*Soybean semilooper*	30	Nil (on 7th day)
Control (distilled water)	*Soybean semilooper*	30	Nil (on 7th day)
*Sl*NPV ($1x10^9$POBs/ml)	*Spodoptera litura*	30	30 (on 6th day)
Control (distilled water)	*Spodoptera litura*	30	Nil (on 6th day)

Effect of different adjuvants on the shelf life of *Sl*NPV under storage conditions

The present studies on shelf life of *Sl*NPV formulation under

storage condition by adding different adjuvants at room temperature revealed that the retention of POBs/ml in a higher number in addition to antimicrobial agents for a period of 6 months. In the third sample of *Sl*NPV having an antibacterial agent, the shelf life could be maintained more effectively with less reduction of POBs as compared to other two samples under the study. The initial count was reduced down to 9.48 % after 6 months period. Thus, the comparative performance between the three samples showed *Sl*NPV+Antibacterial agent to be a better one to maintain shelf life as compared to sole *Sl*NPV or *Sl*NPV + UV protectant (Table 4, 5). The effect of different storage conditions on the virulence of NPV samples stored in earthen pots and at room temperature maintained efficacy upto four months and after that virulence started decreasing [12] are in consistence with our study. The decreased efficacy of samples stored under room temperature may be due to increased bacterial activity. When the NPV samples were tested for the bacterial load, it was 3.7 times more in the samples at room temperature after six months of storage which are in line to the present findings where the *Sl*NPV + antibacterial agent has maintained relatively better POB count as compared to other two samples under study.

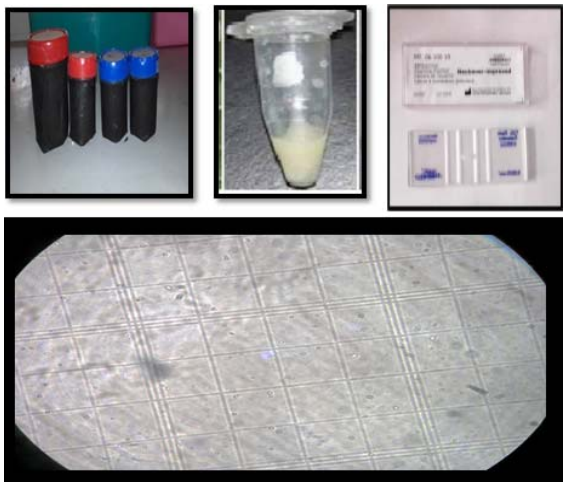

Fig 2: POBs under compound microscope

Table 4: Reduction of POBs/ml over a period of six months

Duration of shelf life	POBs/ml in *Sl*NPV (sole)	POBs/ml in *Sl*NPV + UV Protectant	POBs/ml in *Sl*NPV + Antibacterial agent
1st month	$1.4x10^9$	$1.4x10^9$	$1.4x10^9$
2nd month	$1.3x10^9$	$1.31x10^9$	$1.4x10^9$
3rd month	$1.22x10^9$	$1.25x10^9$	$1.38x10^9$
4th month	$1.20x10^9$	$1.23x10^9$	$1.33x10^9$
5th month	$1.11x10^9$	$1.22x10^9$	$1.30x10^9$
6th month	$1.11x10^9$	$1.20x10^9$	$1.27x10^9$
Per cent Reduction after 6 months	20.71%	14.28%	9.48%

Table 5: Effect of adjuvants on the shelf life of *Sl*NPV under storage condition

Source	D.F	SS	MSS	CAL. F	CD (5%)	S.Em
Treatment	2	.091	0.046	78.352	.027	.009
Replication	6	.006	0.001	1.596		
Error	18	0.010	0.001			
Total	20					

Tabular value at 0.05 level – 9.48

Conclusion

In conclusion, the studies revealed that the median lethal dose (LC_{50}) to kill 50 per cent of the population was $6.02x10^5$ POBs/ml, whereas the lethal time to kill 50 % population of *S. litura* by *Sl*NPV was 4.08 days and to kill 90 % population was 6.95 days. It has been found that the *Sl*NPV is a species specific viral biopesticide as no cross infectivity was observed against four lepidopteran pests tested. Antibacterial agent may be used for conserving POBs under storage condition for better shelf life of *Sl*NPV.

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