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Research Article

Pathogenicity of Nucleopolyhedrovirus (NPV) against *Spodoptera litura* (Fabricius)

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ABSTRACT: The management of *Spodoptera litura* by entomopathogenic Nucleopolyhedrosis Viruses (NPVs) is one of the eco-friendly methods. The present study was aimed to evaluate Nucleopolyhedrosis Virus (NPV) against *S. litura* and its potential as a bio-pesticide. The Occlusion Bodies (OBs) of *S. litura* nucleopolyhedrosis virus was isolated from infected larvae collected from cabbage field. Pathogenicity studies evaluated three NPV suspensions *viz.* SpltNPV-native, SpltNPV-commercial and SpltNPV-NIPHM against second and fourth instar larvae of *S. litura* recorded maximum mortality at 1×10^9 OBs/ml. The LC_{50} values of the SpltNPV-native, SpltNPV-commercial and SpltNPV-NIPHM suspensions against second instar larvae were 0.584, 0.540, 0.625 OBs/mm^2, respectively, which increased to 0.696, 0.620, 0.756 OBs/mm^2 against the fourth instar larvae. The LT_{50} at 1×10^9 OBs/ml was found to increase from 146.33, 137.51 and 155.88 h for SpltNPV-native, SpltNPV-commercial and SpltNPV-NIPHM suspension, respectively, against the second instar larvae to 178.51, 162.07 and 187.67 h, respectively, against the fourth instar larvae. The cumulative per cent mortality, LC_{50} and LT_{50} suggested that the second instar larvae were more susceptible and easier to kill than the fourth instar larvae.

KEY WORDS: LC_{50}, LT_{50}, mortality, Nucleopolyhedrosis virus, OBs, *Spodoptera litura*

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INTRODUCTION

Spodoptera litura (Fabricius) commonly known as tobacco cutworm is a serious polyphagous pest and is distributed throughout tropical as well temperate regions of world (Tenywa *et al*., 2018) It is a serious crop pest of important cash crops, vegetables, fodder, ornamental and horticulture (Ahmad *et al*., 2013).The larvae feeds on leaves, fresh emerging seedlings, brackets and soft shoots (Yinghua *et al*., 2017) and causes severe damage with yield loss ranging from 26-100% (Tuan *et al*., 2014). Farming community largely depends on conventional synthetic insecticides for the management of this pest. Negative effect of these synthetic insecticides on human health, environment (Xiao *et al*., 2019) and development of insecticide resistance are reasons to adopt and evaluate safer eco-friendly alternatives (Ahmad *et al*., 2018). Microbial bio-pesticides are environment-friendly, having specific activity towards their target pests and causes less exposure resulting in no persistent residues and can be employed for the eco-friendly management of pest populations (Singh and Joshi, 2020).

Among the entomopathogens, virus-based bio-control agents are gaining popularity because they are safe to non-target organism, have high specificity and are, thus, gaining popularity. Nucleopolyhedrosis belonging to family Baculoviridae are the most potential candidates. They are enveloped viruses with typical occlusion bodies and are promising candidates against a number of lepidopteron pests (El Sayed *et al*., 2022; Zhang *et al*., 2015) *viz. S. litura* (Fabricius), *S. littoralis* (Boisduval), *S. exigua* (Hubner) and *Helicoverpa armigera* (Hubner). The occlusions bodies (OBs) are an important structural feature of these viruses. The occurrence of these occlusion bodies inside the nucleus of infected cells is a characteristic feature of NPV's infection. Other remarkable features of the NPVs are their high epizootic levels, self-propagation, environment safety, etc. NPV infection can be recognized symptomatically as the larvae become flaccid and fragile. The outer body wall of the larvae often changes colour and appears shiny. The dead larvae hang upside down which is another typical feature of viral infection. At death, the body wall ruptures and releases the Occlusion Bodies (OBs).

The efficiency of baculoviruses is related to their biological activity that takes place in the target pests. The biological activity depends on the effectiveness of starting off an infection within the host and replicating, which ultimately causes death of the insect. The success of baculoviruses depends on their biological parameters, i.e., median Lethal Concentration (LC_{50}) of the virus suspension and median Lethal Time (LT_{50}) of the infected larvae which is calculated through bioassays (Susurluk *et al*., 2013). Thus, focusing on the benefits of entomopathogens and the success of viruses as bio-control agents, more attention need to be diverted towards exploitation of viruses as insect bio-control agents. Therefore, the present study was undertaken with the objective to evaluate the efficacy of nuclear poyhedrosis viruses against larvae of *S. litura.*

MATERIAL AND METHODS

Collection and rearing of *Spodoptera litura*

Larvae of *S. litura* were collected from field crops and brought to laboratory for rearing under controlled conditions at 25±2°C, 70±5 RH, 12:12h light: dark photoperiod. The healthy insect culture for use in bioassay studies was maintained on natural diet which consisted of leaves of host plants like cabbage or cauliflower. The insect larvae that were to be used for virus propagation and evaluation were reared on artificial diet (Chickpea flour (105 g),ascorbic acid (3.25 g), methyl-p-hydroxybenzoate (2 g), multivitamin (2 capsules), sorbic acid (1 g), 10% formaldehyde solution (2 ml), agar (12.75 g), streptomycin sulphate (0.25 g), vitamin e (2 g), yeast tablets (10 g), distilled water (780 ml).

Virus isolates

One isolate of *Spodoptera litura* Nucleopolyhedrosis Virus (SpltNPV-NIPHM) collected from National Institute of Plant Health Management (NIPHM), Hyderabad, and one native isolate obtained from diseased *Spodoptera litura* larvae collected from cabbage field were used in the present study.

Virus Bio-Pesticide

One commercial *Spodoptera litura* nucleopolyhedrosis virus formulation, Spodomar (Splt NPV), manufactured by Multiplex Bio-Tech Pvt. Ltd., Bangalore, India, was used for the present study

Virus Propagation

The virus was propagated by feeding fourth instar larvae of *S. litura* on artificial diet inoculated with virus suspension. The viral-infected larval cadavers were collected and stored for further process.

Extraction of Polyhedral Occlusion Bodies (POBs)

The extraction of POBs was done from NPV-infected larval cadavers with little modification to the methodology of Bhutia *et al.,* 2012. The collected larval cadavers were treated overnight with 0.1% SDS (@1ml/dead larvae) at 4°C, after which the cadavers were macerated to release all the POBs from insect cells. The resulting suspension was filtered through double layer of muslin cloth to remove insect debris. The filtrate was subjected to three rounds of differential centrifugations (at 500 rpm for 90 sec, 5000 rpm for 10 min and 5000 rpm for 15 min). After final centrifugation, the pellet obtained was collected, washed with distilled water and then re-suspended in distilled water and stored at -4°C for future use. The number of OBs in the stock suspension of SpltNPV was set at 1×109 OBs/ml after counting on Neubaur haemocytometer.

Microscopy

Staining of *S. litura* viral suspension was performed as described by Wigley (1976). A wet smear of virus suspension was spread on a glass slide and air dried. The slide was then flooded with 10% Giemsa's stain for about 10 min. The extra stain was washed with slow running water (Ahmad *et al*., 2018). This slide was viewed under light microscope and phase contrast microscope at 400x magnifications

Transmission Electron Microscopy

Transmission electron microscopy of the viral suspension was done using uranyl acetate staining method. After bath sonication of the viral suspension thoroughly for even distribution of the POBs in the liquid, an adequate volume of the NPV suspension was placed on carbon coated copper grid (200 mesh size, Tedpella, USA). The liquid was allowed to rest on the copper grid for 5 minutes. After that, aqueous uranyl acetate stain (2.0% w/v) was added and allowed to react for 2 to 3 minutes. The sample loaded on the grid was then viewed under Transmission Electron Microscope (model Hitachi H-7650, Japan) operated at 80 kV accelerating voltage (Bussola and Russel, 1999). The images were captured through a CCD camera at 4.0k - 6.0kx magnification.

Bioassay

Bioassay studies of SpltNPV against second and fourth instar larvae of *S. litura* was done according to the methodology of Lucien *et al* (2009) with slight modifications. The bioassay against *S. litura* larvae was performed according to diet incorporation method. Three different doses, viz.

1×10^7, 1×10^8 and 1×10^9 OBs/ml along with control (diet without virus inoculation) were evaluated against larvae of *S. litura*. The larvae were starved and then fed on the virus treated artificial diet which was inoculated with 10 µl of the respective concentrations of virus suspension. After 24h of feeding the larvae, the treated diet was replaced with fresh diet. Ten treatments with three replications per treatment were used. Each replication had 20 larvae of S. *litura* which were released on treated diet under laboratory conditions at 25±2°C and mortality was recorded upto ten days post treatment.

Determination of LC_{50} and LT_{50}

The median lethal concentration (LC_{50}), the concentration of the virus suspension that kills fifty per cent insect population, and the median lethal time (LT_{50}), the time required to kill fifty per cent insect population, was recorded at 95% confidence level. A record of mortality with respect to concentration and time was maintained and was subjected to Probit analysis using SPSS software.

Statistical analysis

The data was subjected to one-way Analysis of Variance (ANOVA) in SPSS 16.0 statistical software and means were compared at 5% level of significance by Tukey's post-hoc test. Means with $P < 0.05$ were considered to be significantly different from each other. The transformed mean values were obtained by applying one-way ANOVA under CRD in CPCS1 software.

RESULTS AND DISCUSSION

Microscopic structure of the virus

The Occlusion Bodies (OBs) obtained from virus infected cadavers appeared as bright, round structures at 400x magnification when viewed under light microscope and phase contrast microscope (Leica DM 5000B, Germany) (Plate 1, Plate 2). The Geimsa's staining recorded POBs as negatively stained particles (Plate 3). TEM indicated that a majority of the POBs had a roughly polyhedral shape (Plate 4). The size of the POBs ranged from 0.9-2.5 µm in diameter.

Pathogenicity of SpltNPV against *S. litura* larvae

Second instar larvae of *S. litura*

Cumulative per cent mortality recorded till fourth day after treatment was low. After six days post treatment, the mortality ranged from 3.33 to 46.67 per cent (Table 1). On eighth day after treatment, the maximum mortality (66.67%) was recorded in SpltNPV-commercial (1×10^9 OBs/ml) while the minimum mortality (28.33%) was recorded in SpltNPV-NIPHM (1×10^7 OBs/ml). After ten days of treatment, cumulative per cent mortality in second instar larvae ranged from 43.33 to 81.67 per cent. Cumulative percent mortality in SpltNPV-native was 78.33 per cent (1×10^9 OBs/ml) which was not significantly different from mortality recorded due to SpltNPV-commercial (81.67%) and the mortality (76.67%) due to SpltNPV-NIPHM suspension at the same concentration. These treatments were significantly better than all other treatments.

Fourth instar larvae of *S. litura*

Spodoptera litura larval mortality was low till fourth day post treatment. Maximum cumulative per cent mortality (38.33%) was recorded for SpltNPV-commercial at 1.0 ml/L concentration, which was at par with mortality (35.00%) in SpltNPV-native followed by mortality (31.67%) in SpltNPV-NIPHM, at the same concentration six days post treatment (Table 4). After eight days of treatment, mortality ranged from 3.33 to 58.33 per cent. All three Splt NPVs recorded maximum mortality at higher concentration (1×10^9 OBs/ml) and were at par with each other. On tenth day post inoculation, maximum larval mortality (78.33%) was recorded for SpltNPV-commercial virus suspension (1×10^9 OBs/ml). This was followed by 71.67 per cent mortality in SpltNPV-native and 68.33 per cent mortality in SpltNPV-NIPHM which were statistically at par with each other and significantly better than all other treatments and untreated control. At 0.8 ml/L, the cumulative per cent mortalities recorded were 56.67, 61.67 and 51.67 per cent for SpltNPV-native, SpltNPV-commercial and SpltNPV-NIPHM suspensions, respectively. Whereas, the minimum mortality (36.67%) was recorded in SpltNPV-NIPHM suspension (1×10^7 OBs/ml).

Dosage-mortality and Time-mortality response of *S. litura* larvae towards NPV

The LC_{50} values of the SpltNPV-native, SpltNPV-commercial and SpltNPV-NIPHM against second instar larvae were 0.584, 0.540, 0.625 OBs/mm^2, respectively (Table 2), which increased to 0.696, 0.620, 0.756 OBs/mm^2 against the fourth instar *S. litura* larvae (Table 5). The median Lethal Time (LT_{50}) at the highest concentration, 1×10^9 OBs/ml was found to increase from 146.33, 137.51 and 155.88 h for SpltNPV-native, SpltNPV-commercial and SpltNPV-NIPHM, respectively, against second instar larvae (Table 3) to 178.51, 162.07 and 187.67 h respectively in case of the fourth instar larvae of *S. litura* (Table 6). This also indicates that the second instar larvae of *S. litura* are more susceptible to SpltNPV under laboratory conditions.

Bright polyhedral structured occlusion bodies were recorded under microscope. Occlusion bodies from discharged

body fluid of virus infected insect larvae was also recorded by Kumar *et al*. (2011) and Khattab (2013). Negatively stained OBs were observed by Geimsa's staining and this was comparable with findings of Ahmad *et al.* (2018) who also recorded viral Occlusion Bodies (OBs) as polyhedral and negatively stained particles after Giemsa's staining. El Sayed *et al.* (2022) characterized nucleopolyhedrovirus isolated from the cotton leaf worm and recorded the presence of typical occlusion bodies with average size of (1.06 × 1.19 µm) under Transmission electron microscopy.

The results from the TEM studies of the native SpltNPV were consistent with those reported by Khattab (2013) who recorded size of the POBs to be 1.52 ± 0.11 µm in diameter. Similarly, Kumar *et al.* (2011) also carried electron microscopic studies of the polyhedral occlusion bodies of nucleopolyhedrosis viruses from different lepidopteran insects and their study revealed that polyhedral particle size (diameter) to be approximately 1.0 to 2.0 µm for *Amsacta albistriga* nucleopolyhedrovirus (Amal NPV), 0.9 to 2.9 µm for *Spodoptera litura* nucleopolyhedrovirus (SpltNPV) and 0.5 to 2.5 µm in case of *Helicoverpa armigera* nucleopolyhedro virus (HearNPV).

The higher concentrations of SpltNPV suspensions recorded maximum mortality of *S. litura* larvae. Therefore, we can infer that the larval mortality is directly proportional to the concentration of the virus suspension used against the larvae of *S. litura* as there is more number of POBs in the higher concentrations (1.0 ml/L) of the suspension than in lower concentrations. The larval mortality increases with an increase in the number of polyhedral occlusion bodies (POBs) (Yasin *et al*., 2020). Cumulative per cent mortality of the fourth instar larvae of *S. litura* was less as compared to that of the second instar larvae, thus, indicating the inverse relation of the pathogenicity of SpltNPV suspensions with the larval stage.

The present results can also be supported with the findings of Ayyub *et al*. (2020) from a bioassay conducted against second, third and fourth instar larvae of *S. litura*. They did a comparison of mean mortalities of tested larval instars and found that the mean mortality ranged from 36.78-88.08 per cent at the end of experiment. The mortality of tested larvae was found to be dose dependent. A similar trend was recorded in the present study also where the mortality of the larvae was directly related to the concentration of the viral suspension used against the insect larvae. Our results were also in corroboration with the results obtained by other Ali *et al*. (2018) who observed that the mortality of the *S. litura* larvae increased with dose. The findings of the present study were in line with the findings of Sarwar *et al*. (2021) who carried out an evaluation of NPV against different larval instars. The second instar larvae of *Spodoptera* species were found to be more susceptible to nucleopolyhedrosis virus in comparison to fourth instar larvae. The early instar larvae were found to be more sensitive to pathogenic infection than older larvae. This was probably because the younger larvae consumed a larger surface of viral treated food (Gothama *et al*., 1995). The reason for less death in case of later instar larvae could be higher deposition of cuticular melanism in the older larvae which hinders the entry of pathogens (Wilson *et al.* 2001). In the present study as well, the fourth instar larvae of *S. litura* showed less susceptibility towards SpltNPV treatment and mortality was less among the fourth instar larvae compared to the younger, second instar larvae.

Similarly, bioassay of an isolated strain of SpltNPV was conducted by Ahmad *et al*. (2018) against second, third, fourth and fifth instars larvae of *S. litura* under laboratory condition. They found that different instars of the insect showed a wide range of variation in their biological activity. They reported that the LC_{50} values of SpltNPV were inversely correlated with the age of the larvae, i.e., the LC_{50} values were the highest for fifth instar. They discussed that the reduction in susceptibility of the larvae towards SpltNPV was due to dilution effect as the larval weight increased with growth of the insect. In the present study also it was observed that the LC_{50} values were higher for fourth instar larvae as compared to the second instar larvae of *S. litura* and the LT_{50} was also directly related to the larval age which is again consistent with the findings of Ahmad *et al*. (2018).

The mortality caused by SpltNPV under the laboratory conditions, largely depends upon the concentrations of the viral suspension used and the size and age of the larvae of *S. litura*. The maximum mortality was recorded at the highest concentration (1.0 ml/Lor 1×10^9 POBs/ml) in all three SpltNPV on tenth day after treatment. Further, higher mortality was recorded in the second instar larvae of *S. litura* than the fourth instar larvae, thus showing inverse relationship with the larval stage of *S. litura*. A similar trend was suggested by the dosage-mortality and time-mortality response of the insect larvae towards SpltNPV. The LC_{50} and LT_{50} values of SpltNPV were higher against the fourth instar as compared to the second instar larvae of *S. litura*.

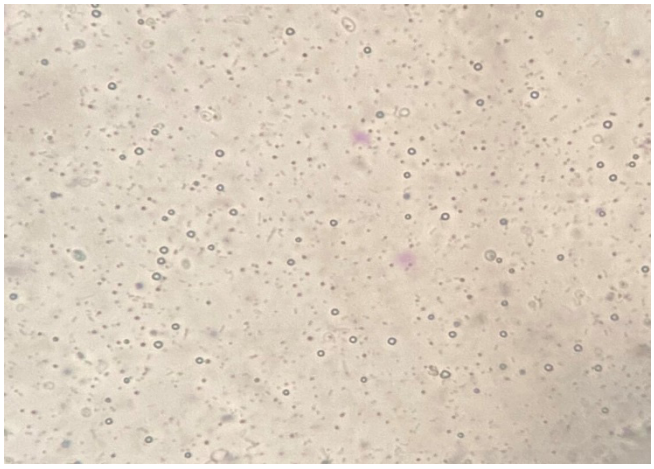


Plate 1. Occlusion bodies of SpltNPV-native under light microscope (400x magnification).

Plate 2. Occlusion bodies of SpltNPV-native under phase contrast microscope (400x magnification)**.**

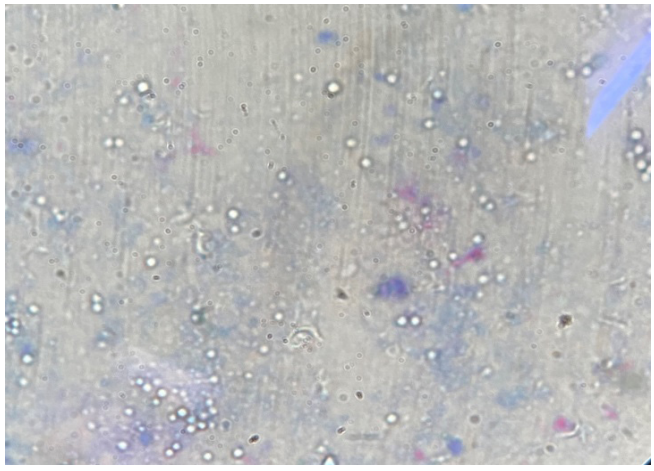
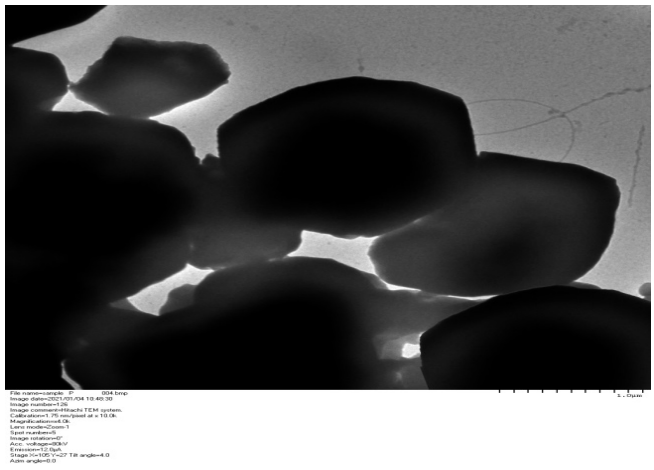


Plate 3. Negatively stained occlusion bodies of SpltNPV-nativeafter Geimsa's staining.



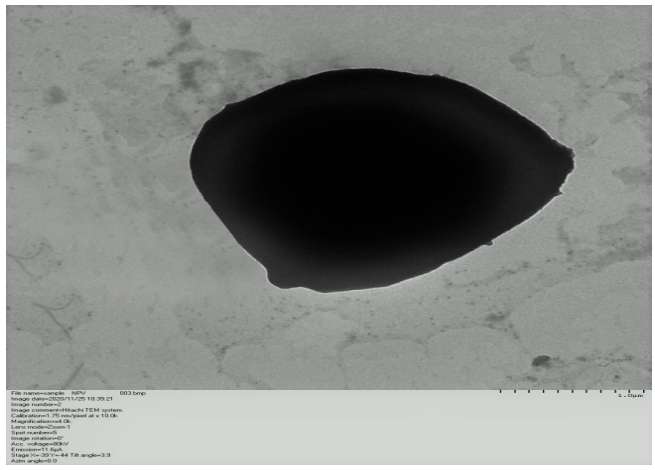


Plate 4. Transmission electron micrograph of OBs of SpltNPV (native).

Table 1. Evaluation of SpltNPV against second instar larvae of *S. litura* under laboratory conditions

Treatments	Concentrations (OBs/ml)	Per cent Mortality				
		Days after inoculation				
		2DAI	4DAI	6DAI	8DAI	10DAI
SpltNPV (Native)	1×10^7	3.33^{dc} (8.61)	8.33^{ed} (16.59)	20.00^{ed} (26.55)	33.33^{d} (35.23)	46.67^{d} (43.06)
	1×10^8	6.67^{dcb} (14.75)	20.00^{cb} (26.55)	35.00^{cb} (36.22)	51.67^{cb} (45.93)	65.00^{c} (53.70)
	1×10^9	13.33^{ba} (21.32)	21.67^{ba} (27.69)	45.00^{a} (42.10)	65.00^{a} (53.74)	78.33^{a} (62.26)
SpltNPV (Commercial)	1×10^7	3.33^{dc} (8.61)	10.00^{d} (18.42)	21.67^{ed} (27.69)	35.00^{d} (36.22)	50.00^{d} (44.98)
	1×10^8	8.33^{cb} (16.59)	21.67^{ba} (27.69)	38.33^{ba} (38.22)	51.67^{cb} (45.93)	68.33^{cb} (55.74)
	1×10^9	16.67^{a} (24.03)	28.33^{a} (32.12)	46.67^{a} (43.06)	66.67^{a} (54.72)	81.67^{a} (64.66)
SpltNPV (NIPHM)	1×10^7	1.67^{dc} (4.30)	6.67^{ed} (14.75)	13.33^{e} (21.32)	28.33^{d} (32.12)	43.33^{d} (41.14)
	1×10^8	3.33^{dc} (8.61)	13.33^{dc} (21.32)	28.33^{dc} (32.12)	48.33^{c} (44.02)	61.67^{c} (51.73)
	1×10^9	11.67^{ba} (19.87)	23.33^{ba} (28.84)	41.67^{ba} (40.18)	60.00^{ba} (50.76)	76.67^{ba} (61.12)
Control		0.00^{d} (0.00)	1.67^{e} (4.30)	3.33^{f} (8.61)	5.00^{e} (10.44)	6.67^{e} (14.75)
*(CD 5%)		8.68	5.31	5.25	6.23	3.51

Note: Values in parenthesis are arcsine transformation

*Critical difference at 5 percent

Mean value followed by same letter (a, b, c, d, e) in vertical column are not significantly different at 0.05% level of probability using Tukey's post hoc test

Table 2. Dose-mortality response of second instar larvae of *S. litura* towards SpltNPV

Treatment	LC_{50}			Slope ± S.E.	Heterogeneity	
	Concentrations (OBs/mm^2)	Fiducial Limits (ml/L)			χ^2	df
		Lower	Upper			
SpltNPV-Native	0.632	0.475	0.713	3.895±1.08	0.009	1
SpltNPV-Commercial	0.603	0.44	0.684	4.042 ± 1.10	0.022	1
SpltNPV-NIPHM	0.665	0.531	0.743	4.011 ± 1.07	0.038	1

*All values are non-significant at $p<0.05$; S.E.: standard error; df: degrees of freedom

Table 3. Time-mortality response of second instar of *S. litura* larvae towards SpltNPV

Treatment	Concentrat (OBs/ml)	LT_{50} (Hrs)	Fiducial Limits (Hrs)		Slope ± S.E.	Heterogeneity	
			Lower	Upper		$\chi 2$	df
SpltNPV-Native	1×10^7	267.18	231.77	332.14	2.889 ± 0.379	2.024	7
	1×10^8	189.49	171.11	215.7	2.797 ± 0.315	1.269	7
	1×10^9	146.33	133.68	161.04	2.894 ± 0.293	5.172	7
SpltNPV-Commercial	1×10^7	260.66	226.35	322.86	2.801 ± 0.365	1.511	7
	1×10^8	177.64	161.27	199.74	2.837 ± 0.309	2.166	7
	1×10^9	137.51	124.87	151.79	2.714 ± 0.283	7.039	7
SpltNPV-NIPHM	1×10^7	287.97	248.26	364.74	3.163 ± 0.433	2.027	7
	1×10^8	202.84	184.05	230.09	3.150 ± 0.347	1.59	7
	1×10^9	155.88	142.27	172.49	2.870 ± 0.297	4.605	7

*All values are non-significant at $p<0.05$; S.E.: standard error; df: degrees of freedom

Table 4. Evaluation of SpltNPV against fourth instar larvae of *S. litura* under laboratory conditions

Treatments	Concentrations (OBs/ml)	Per cent Mortality				
		Days after inoculation				
		2DAI	4DAI	6DAI	8DAI	10DAI
SpltNPV (Native)	1×10^7	1.67^{ba} (4.30)	3.33^{ed} (8.61)	11.67^{d} (19.87)	23.33^{d} (28.84)	38.33^{f} (38.22)
	1×10^8	3.33^{ba} (8.61)	10.00^{dcb} (18.43)	23.33^{cb} (28.84)	41.67^{b} (40.18)	56.67^{ed} (48.81)
	1×10^9	6.67^{ba} (14.75)	16.67^{ba} (24.03)	35.00^{a} (36.22)	53.33^{a} (46.89)	71.67^{ba} (57.83)
SpltNPV (Commercial)	1×10^7	3.33^{ba} (8.61)	8.33^{edc} (16.59)	16.67^{dc} (24.03)	28.33^{d} (32.12)	41.67^{f} (40.18)
	1×10^8	3.33^{ba} (8.61)	11.67^{cba} (19.87)	23.33^{cb} (28.84)	41.67^{b} (40.18)	61.67^{dc} (51.73)
	1×10^9	8.33^{a} (16.59)	18.33^{a} (25.29)	38.33^{a} (38.22)	58.33^{a} (49.78)	78.33^{a} (62.26)
SpltNPV (NIPHM)	1×10^7	1.67^{ba} (4.30)	3.33^{ed} (8.61)	11.67^{d} (19.87)	23.33^{d} (28.84)	36.67^{f} (37.24)
	1×10^8	1.67^{ba} (4.30)	6.67^{edc} (14.75)	18.33^{dc} (25.29)	36.67^{cb} (37.24)	51.67^{e} (45.94)
	1×10^9	3.33^{ba} (8.61)	13.33^{bca} (21.32)	31.67^{ba} (34.21)	51.67^{a} (45.94)	68.33^{cb} (55.74)
Control		0.00^{b} (0.00)	1.67^{e} (4.30)	1.67^{e} (4.30)	3.33^{e} (8.61)	5.00^{g} (10.44)
***(CD 5%)**		NS	7.78	5.4	4.62	5.82

Note: Values in parenthesis are arcsine transformation

*Critical difference at 5 percent

Mean value followed by same letter (a, b, c, d, e) in vertical column are not significantly different at 0.05% level of probability using Tukey's post hoc test

Table 5. Dose-mortality response of fourth instar larvae of *S. litura* towards SpltNPV

Treatment	LC_{50}			Slope ± S.E.	Heterogeneity	
	Concentration (OBs/ mm^2)	Fiducial Limits (ml/L)			χ^2	df
		Lower	Upper			
SpltNPV-Native	0.718	0.602	0.803	3.907 ± 1.06	0.015	1
SpltNPV-Commercial	0.674	0.563	0.745	4.442 ± 1.08	0.065	1
SpltNPV-NIPHM	0.755	0.641	0.859	3.651 ± 1.06	0.151	1

*All values are non-significant at p<0.05; S.E.: standard error; df: degrees of freedom

Table 6. Time-mortality response of fourth instar of *S. litura* larvae towards SpltNPV

Treatment	Concentration (ml/L)	LT_{50} (Hrs)	Fiducial Limits (Hrs)		Slope ± S.E.	Heterogeneity	
			Lower	Upper		2	df
SpltNPV-Native	1×10^7	323.08	270.78	436.31	3.085 ± 0.459	3.32	7
	1×10^8	225.59	203.01	260.81	3.258 ± 0.376	3.32	7
	1×10^9	178.51	163.58	198.07	3.193 ± 0.327	3.65	7
SpltNPV-Commercial	1×10^7	311.18	258.90	422.87	2.601 ± 0.380	1.819	7
	1×10^8	219.41	198.49	251.14	3.339 ± 0.374	5.093	7
	1×10^9	162.07	149.62	177.1	3.380 ± 0.323	7.673	7
SpltNPV-NIPHM	1×10^7	319.15	269.360	425.97	3.252 ± 0.484	3.38	7
	1×10^8	246.07	220.32	288.72	3.536 ± 0.429	1.937	7
	1×10^9	187.67	172.84	207.35	3.535 ± 0.359	1.99	7

*All values are non-significant at p<0.05; S.E.: standard error; df: degrees of freedom

REFERENCES

Ahmad JN, Mushtaq R, Ahmad SJN, Maqsood S, Ahuja I, Bones AM. 2018. Molecular identification and pathological characteristics of NPV isolated from *Spodoptera litura* (Fabricius) in Pakistan. *Pak J Zool,* **50:** 2229-2237. https://doi.org/10.17582/journal.pjz/2018.50.6.2229.2237

Ahmad M, Ghaffar A, Rafiq M. 2013. Host plants of leaf worm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in Pakistan. *Asian J Agric Biol*, **1:** 23-28.

Ahmed MAI, Sobhy AST, Farouk AA, Samir HMM. 2015. The effects of selected host plants on the efficacy of spinosad pesticide on cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) under laboratory conditions. *Adv Environ Bio,* **19:** 372-375.

Ali G, van der Werf W, Vlak JM. 2018. Biological and genetic characterization of a Pakistani isolate of *Spodoptera litura* nucleopolyhedrovirus. *Biocontrol Sci Tech,* **28**(1): 20-33. https://doi.org/10.1080/09583157.2017.1409339

Ayyub MB, Nawaz A, Gogi MD, Arif MJ, Amrao L, Ahmad JN. 2020. Comparitive toxicity assessment of nuclear polyhedrosis virus and spinosad against *Spodoptera litura* (Fabricius) in semi field conditions. *Pak J Agric Sci,* **57**(2): 445-450.

Bhutia KC, Chakravarthy AK, Doddabasappa B, Narabenchi GB, Lingaraj VK. 2012. Evaluation and production of improved formulation of nucleopolyhedrosis virus of *Spodoptera litura. Bull Insectology*, **65**(2): 247-256.

Bussola JJ, Russel LD. 1999. Electron microscopy: principles and techniques for biologists. Frankfurt, GE: Jones and Bertotlett.

El Sayed YA, Sayed S, Magdy A and Elmenofy W. 2022. Detection, characterization and virulence analysis of nucleopolyhedrovirus isolated from the cotton

leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Egyptian J Biol Pest Ctrl,* **32:** 74 https://doi.org/10.1186/s41938-022-00570-2

Evans H, Shapiro M. 1997. Viruses. In: Lacey L A (Ed.). Manual of Techniques in Insect Pathology. London, UK. https://doi.org/10.1016/B978-012432555-5/50005-1

Gothama AA, Sikorowski PP, Lawrence GW. 1995. Interactive effects of *Steinernema carpocapsae* and *Spodoptera exigua* nuclear polyhedrosis virus on *Spodoptera exigua* larvae. *J Invertebr Pathol*, **66**(3): 270-276. https://doi.org/10.1006/jipa.1995.1100

Khattab M. 2013. Isolation of Nucleopolyhedrovirus (NPV) from the beet armyworm *Spodopter aexigua* (Hubner) (SpexNPV). *Int J Environ Sci Engg,* **4:** 75-83.

Kumar CS, Rao GR, Sireesha K, Kumar PL. 2011. Isolation and characterization of baculoviruses from three major lepidopteran pests in the semi-arid tropics of India. *Indian J Virol*, **22**(1): 29-36. https://doi.org/10.1007/s13337-011-0029-0 PMid:23637499 PMCid:PMC3550719

Lucein KNG, Katuso T, Yositiskaand S, Masayuki N. 2009. Further Studies on biological activity and Identification of Nucleopolyhedroviruses Isolated from *Spodoptera litura* in Japan. *South Specific Studies*, **30:**1.

Sarwar G, Maan NA, Ayub MA, Shahid MR, Malik MA, Farooq M. 2021. Evaluation of indigenous the nucleopolyhedrovirus (NPV) of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in combination with chlorantraniliprole against *Spodoptera* species. *Egypt J Biol Pest Ctrl,* **31**(1): 1-7. https://doi.org/10.1186/s41938-021-00403-8

Singh H, Joshi N. 2020. Management of the aphid, *Myzus persicae* (Sulzer) and the whitefly, *Bemisia tabaci* (Gennadius), using biorational on capsicum under protected cultivation in India. *Egypt J Biol Pest Ctrl,* **30:** 1-9. https://doi.org/10.1186/s41938-020-00266-5

Susurluk H, Toprak U, Gurkan MO. 2013. Concentration of sodium dodecyl sulfate used in occlusion body extraction affects *Spodoptera littoralis* nucleopolyhedrovirus biological activity. *Turk J Biol*, **37**(2): 171-175 https://doi.org/10.3906/biy-1204-50

Tenywa MM, Nyamwaro SO, Kalibwani R, Mogabo J, Buruchara R, Fatunbi AO. 2018. Innovation opportunities in sorghum production in Uganda. *FARA Res Rep,* **18**(2): 20.

Tuan SJ, Lee CC, Chi H. 2014. Population and damage projection of *Spodoptera litura* (F.) on peanuts (Arachis *hypogaea* L.) under different conditions using the age-stage, two-sex life table. *Pest Manag Sci*, **70**(5): 805-813. https://doi.org/10.1002/ps.3618 PMid:23893943

Wigley PJ (1976) The epizootiology of a nuclear polyhedrosis virus disease of the winter moth, *Operophtera burmata* L. at Wistman's Wood, Dartmoor. Dissertation, University of Oxford.

Wilson K, Cotter SC, Reeson AF, Pell JK. 2001. Melanism and disease resistance in insects. *Ecology letters*, **4**(6): 637-649. https://doi.org/10.1046/j.1461-0248.2001.00279.x

Xiao JJ, Fu YY, Ye Z, Liu YY, Shi YH, Liao M, Cao HQ. 2019. Analysis of the pesticide behaviour in *Chaenomelis speciosa* and the role of digestive enzyme in vitro oral bio accessibility. *Chemosphere,* **231:** 538-545. https://doi.org/10.1016/j.chemosphere.2019.05.172 PMid:31151014

Yasin M, Qazi MS, Wakil W, Qayyum MA. 2020. Evaluation of Nuclear Polyhedrosis Virus (NPV) and Emamectin Benzoate against *Spodoptera litura* (F.)(Lepidoptera: Noctuidae). *Egypt J Biol Pest Ctrl,* **30**(1): 1-6. https://doi.org/10.1186/s41938-020-00271-8

Yinghua, S, Yan D, Jin C, Jiaxi W, Jianwu W. 2017. Responses of the cutworm *Spodoptera litura* (Lepidoptera: Noctuidae) to two Bt corn hybrids expressing Cry1Ab. *Scientific Rep,* **7:** 1-13. https://doi.org/10.1038/srep41577 PMid:28186125 PMCid:PMC5301207

Zhang S, Wu F, Li Z, Lu Z, Zhang X, Zhang Q, Liu X. 2015. Effects of Nucleopolyhedrovirus infection on the development of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and expression of its 20-hydroxyecdysone- and juvenile hormone-related genes. *Fla Entomol,* **98**(2): 682-689. https://doi.org/10.1653/024.098.0243