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ORIGINAL ARTICLE

Isolation and Characterization of Baculoviruses from Three Major Lepidopteran Pests in the Semi-Arid Tropics of India

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Abstract Baculoviruses were isolated from three major lepidopteran pests, *Helicoverpa armigera*, *Spodoptera litura* and *Amsacta albistriga* in the semi-arid tropics during natural epizootic conditions at ICRISAT fields, Patancheru, Andhra Pradesh, India. Biological, morphological and biochemical analysis identified these isolates as Nucleopolyhedroviruses (NPVs). Scanning electron microscopy of the occlusion bodies (OBs) purified from diseased larvae revealed polyhedral particles of size approximately 0.5–2.5 μm [*Helicoverpa armigera Nucleopolyhedrovirus* (HearNPV)], 0.9–2.92 μm [*Spodoptera litura Nucleopolyhedrovirus* (SpltNPV)] and 1.0–2.0 μm [*Amsacta albistriga Nucleopolyhedrovirus* (AmalNPV)] in diameter. Transmission electron microscopy of thin sections of OBs of the three isolates revealed up to 5–8 multiple bacilliform shaped particles packaged within a single viral envelope. The dimensions of these particles were 277.7 × 41.6 nm for HearNPV, 285.7 × 34.2 nm for SpltNPV and 228.5 × 22.8 nm for AmalNPV. Each of HearNPV and AmalNPV contained up to 6 nucleocapsids and SpltNPV contained up to 7 nucleocapsids per envelope. The estimated molecular weights of the purified OB (polyhedrin) protein of the three NPVs were 31.29–31.67 kDa. Virus yield (OBs/larva) was $5.18 \pm 0.45 \times 10^9$ for HearNPV, $5.73 \pm 0.17 \times 10^9$ for SpltNPV and $7.90 \pm 0.54 \times 10^9$ for AmalNPV. The LC_{50} values of various NPVs against 2nd and 3rd instar larvae indicated 2.30×10^4 and 1.5×10^5 OBs/ml for HearNPV, 3.5×10^4 and 2.4×10^5 OBs/ml for SpltNPV and 5.6×10^4 and 3.96×10^5 OBs/ml for AmalNPV. The lethal time required to cause 50% mortality (LT_{50}) for these three species were also defined. This study has shown that the NPVs infecting three major lepidopteran pests in India are multiple NPVs, and they have good potential to use as biocontrol agents against these important pests.

Keywords Insect virus · Baculoviruses · Lepidopteran pests · NPV · Electron microscopy · Bioassays

Introduction

Baculoviruses, pathogenic to arthropods, particularly of the order Lepidoptera, Hymenoptera and Diptera have enveloped rod shaped nucleocapsids with circular, double stranded DNA genome and characterized by the presence of occlusion bodies (OBs) [3]. The infectious virus particles are randomly embedded in proteinaceous OBs specially designed to protect infectivity outside their hosts in the environment for several years until the availability of susceptible host [14]. The nature and significance of these OBs remained a mystery for a long time until the electron microscope (EM) was available that the virus particle could be isolated and identified as the infectious viral agent. Based on the size, shape and occluded virion phenotype the baculoviruses are classified into two genera, Nucleopolyhedroviruses (NPVs) and Granuloviruses (GVs)

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[4, 8, 23, 34]. The OBs in subgroup NPVs are large (0.13–15 μm) and polyhedral shape called polyhedral occlusion bodies (POBs). The virions in this subgroup contain either a single nucleocapsid (SNPV) or multiple nucleocapsids (MNPV) per envelope. The size and shape of OBs varies considerably not only between the OBs from different insects, but often also within the same species. The most important characteristic feature of occluded insect viruses is their ability to produce virions sequestered (occluded) within the crystalline matrix protein of OB called polyhedrin/granulin.

Like other viruses, entomopathogenic viruses are obligate pathogens, can be replicate only in its host larvae [12]. The inoculation dose is expressed in units of OBs/ml, and the optimal dose varies with the virulence of the strain and age of the host [13]. Surface treatment is an efficient system that is easily automated and requires much less virus than diet incorporation [25]. However, soaked chickpea seeds treated with Helicoverpa armigera nucleopolyhedrovirus (HearNPV) to feed *Helicoverpa armigera* (Hubner) larvae was found to be more effective for mass production of virus [19]. It was defined as "one larval equivalent" when the virus produced at least 6×10^9 OBs/larva in late instars [11]. The virus yield increase exponentially with certain age of larva [29]. Generally earlier instars are highly susceptible to the virus with 100% mortality with shorter LT_{50} (lethal time required to cause 50% mortality) values [18, 22]. Whereas, late instars particularly from fifth instars are less susceptible, pupate and gives to malformed adults with short and ruffled wings which indicates that the effect of NPV is directly related to the age of the larvae at the time of infection [2].

Though there were several reports on the occurrence and their potential importance of baculoviruses still there are several gaps in the identification and morphological characterization of these viruses for their understanding and their exploitation in the present day plant protection with reference to India. Hence the present investigation was under taken to provide better clarification of baculoviruses isolated from three key lepidopteran insect pests important in semi-arid tropics (SAT) namely *H. armigera* (legume pod borer) Noctuidae, *Spodoptea litura* (Fabricius) (tobacco caterpillar) Noctuidae and *Amsacta albistriga* (Walker) (red hairy caterpillar) Arctiidae.

Materials and Methods

Collection of Virus Isolates

Surveys were conducted at ICRISAT campus in Patancheru (Andhra Pradesh, India) during natural epizootic conditions for identification of baculoviruses from major lepidopteran pest populations of legume crops. Typical symptoms of baculovirus infection were observed in *H. armigera* on pigeon pea and chickpea crops, *S. litura* and *A. albistriga* on groundnut. The diseased larvae were collected into 2 ml tubes and brought to the laboratory for further studies.

Extraction of OBs

The OBs were extracted from individual diseased larvae with slight modifications to the previously described method [6]. To each cadaver 1 ml sterile distilled water was added, disrupted by vortexing for about 2 min, extract was filtered through glass wool and the glass wool was washed with 500 μl of sterile distilled water and the filtrate centrifuged at $15{,}000 \times g$ for 5 min. The supernatant was removed carefully, pellet was washed with 2 ml distilled water and centrifuged as described above. The pellet was resuspended in 1 ml of sterile distilled water and stored at 4°C. The OBs were enumerated using Neubauer's haemocytometer mounted on a phase-contrast light microscope at 10×40 magnification.

Electron Microscopy

Morphology of the OBs extracted from individual larvae was studied under transmission electron microscope (TEM) and scanning electron microscope (SEM). For SEM, the OB suspensions were transferred into vials and fixed in 2.5% (v/v) glutaraldehyde in 0.05 M phosphate buffer, pH 7.2, for 24 h at 4°C and post fixed in 2% (v/v) aqueous osmium tetroxide in the same buffer for 2 h. Subsequently, samples were dehydrated by washing in a series of graded alcohol and dried to a critical drying point. Dried samples were mounted over the stubs with double-sided conductivity tape and sputter coated with gold for 3 min using an automated sputter coater (Model: JEOL-JFC 1600). The coated samples were mounted in SEM (JOEL-JSM 5600, JAPAN) and visualized and photographed at various magnifications. The sizes of the OBs were measured directly from the amplified photograph using a scale and dividing the value by the magnification of the photograph.

Transmission Electron Microscopic (TEM) Studies

Pellets of OBs were fixed in 2.5% (v/v) glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 24 h at 4°C and post fixed in 0.5% (v/v) aqueous osmium tetroxide in the same buffer for 2 h. After the post fixation samples were dehydrated in a series of graded alcohol, infiltrated and embedded in Araldite 6005 resin. Ultrathin sections (50–70 nm thickness) were cut with a glass knife on a Leica Ultra cut UCT-GA-D/E-1/00 ultramicrotome and

mounted on grids. Then sections were stained with saturated aqueous uranyl acetate and counter stained with 4% lead citrate and observed under TEM (Hitachi H-7500, JAPAN) at various magnifications at 80 kV current. The sizes of the OBs and nucleocapsids were measured directly from the amplified photographs using a precision ruler and dividing the value by the magnification of the photograph.

Determination of Molecular Weight of Occlusion Body Protein

The OB protein was purified as per the previously described method [21]. Purified protein concentration was estimated by Bradford method. The purity of polyhedrin preparations were analyzed in 12% SDS-PAGE gels [16]. The protein samples ($\sim$100 to 200 µg/10 µl) were mixed with equal volume of Laemmli buffer and denatured by heat treatment in boiling water bath for 3 min. Samples (10 µl) were loaded into wells of 12% SDS-PAGE and electrophoresed at 100 volts for approximately 2 h in Broviga apparatus. The gel was silver stained to visualize proteins [16]. The molecular weights of the protein bands were estimated by comparing with the protein molecular weight standards (MBI Fermentas Cat# SM0441). Standard graph was prepared by plotting the distance migrated by protein standards on X-axis and molecular weights on Y-axis.

Propagation of Baculoviruses and OB Purification

Baculoviruses were mass multipled at ICRISAT-NPV production laboratory according to the previously described procedures for HearNPV [9], for SpltNPV [15] and for AmalNPV [32]. The HearNPV was multiplied both on field collected and laboratory reared fourth instar larvae and the yield obtained was compared. Where as for mass multiplication of SpltNPV and AmalNPV, laboratory reared 5th instar larvae emerged from field collected egg masses were used for virus inoculation. After larval death, the cadavers were collected in jars containing distilled water and stored at 4°C for further processing. The larvae were ground in a blender and suspension was filtered through the double layered muslin cloth or plastic strainer to remove larval debris. Then the filtrate was centrifuged at 5,000 rpm for 10–15 min (Remi R8C). OBs sediment at the bottom of the tube were dissolved in distilled water and stored at 4°C for further studies.

Enumeration, Dilution and Counting of OBs

Enumeration of OBs in the viral suspension was done with the help of Neubauer's haemocytometer as per the standard procedure [7].

Bioassays

To test the virulence of NPVs against different age group larvae, bioassays were conducted on 2nd and 3rd instar larvae by providing 1.5–2.5 g diet per larva through surface contamination method [7]. Six concentrations were prepared by serial dilution. Concentrations from 1.8×10^7 to 1.8×10^2 OBs/ml against 2nd instar and 1.8×10^8 to 1.8×10^3 OBs/ml against 3rd instar larvae were tested. For each treatment three replications were maintained with ten larvae. For HearNPV and SpltNPV bioassay 50 µl of virus suspension was dispensed over the surface of artificial diet in the cell wells and spread uniformly over the surface using blunt glass rod and allowed it to dry for 10–20 min and then the larvae were released carefully over the surface of the diet. Whereas for AmalNPV bioassay, groundnut foliage was sprayed with virus for couple of times in plastic trays and allowed it to dry for 10–20 min and then the inoculated leaves were transferred to plastic cages and then the larvae were released carefully on to the leaf material. The larvae were reared under controlled conditions with 16 h light and 8 h dark photoperiod, 25 $\pm$ 2°C temperature and 70% relative humidity. Observations on mortality were recorded daily from fourth day after inoculation. The data were analyzed using Probit analysis software to arrive at lethal concentration of virus required to cause 50% mortality (LC_{50}) and lethal time required to cause 50% mortality (LT_{50}) [5].

Results

Identification and Collection of Baculoviruses

During natural epizootic conditions baculovirus infection was observed in *H. armigera*, *S. litura* and *A. albistriga* pest populations at ICRISAT fields. The diseased larvae were swollen, glossy and moribund. The larvae of *H. armigera* and *A. albistriga* were crawled to the top of the twigs (negative geotropism) on which they were fed. The diseased larvae were died and the body tissues were liquefied, in some larvae the cuticle was ruptured and discharging of white body fluid on to plant parts was observed. Observation of discharged body fluid under phase contrast microscope revealed that large number of round particles resembling as baculoviral OBs (Fig. 1).

Electron Microscopic (EM) Studies

Electron microscopic (EM) studies revealed typical baculovirus OBs of type *Nucleopolyhedrovirus* (NPV) with polyhedral structures and rod shaped nucleocapsids (NCs). The details of the EM study results were presented in

Table 1. Under SEM the OBs of three viruses appeared as crystalline structures of variable shapes of size 0.5–2.5 μm (HearNPV), 0.9–2.92 μm (SpltNPV) and 1.0–2.0 μm (AmalNPV) in diameter (Fig. 2). TEM of the cross-sectioned OBs revealed multiple nucleocapsids in each envelop, which were bacilliform shaped of dimensions 277.7 × 41.6 nm for HearNPV, 285.7 × 34.2 nm for SpltNPV and 228.5 × 22.8 nm for AmalNPV. The OBs of HearNPV and AmalNPV contained 2–6, and SpltNPV contained 5–7 nucleocapsids per envelope (Fig. 3).

SDS-PAGE Analysis of Major Occlusion Body Protein

In 12% SDS-PAGE analysis, the denatured purified protein preparations of three viruses resolved as single band (Fig. 4) of estimated molecular weights of 31.65 kDa (±0.00) (HearNPV), 31.29 kDa (±0.00) (SpltNPV) and 31.67 kDa (±0.295) (AmalNPV).

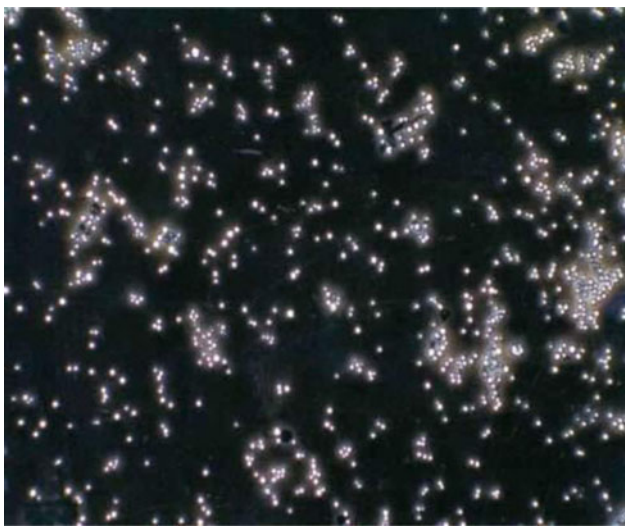

Fig. 1 Phase-contrast micrograph (magnification at 10 × 40) of white discharge body fluid obtained from diseased larvae showing baculovirus OBs

Propagation of Baculoviruses

Propagation of NPVs on respective larvae resulted in virus infection. HearNPV propagated on field collected larvae resulted in significantly higher yield ($5.35 \pm 0.31 \times 10^9$ OBs/larva) compared to laboratory reared larvae ($5.18 \pm 0.45 \times 10^9$ OBs/larva). The OB yield of SpltNPV and AmalNPV was recorded as $5.73 \pm 0.17 \times 10^9$ and $7.90 \pm 0.54 \times 10^9$ OBs/larva. The source and age of larvae used for multiplication, concentration of virus used for inoculation and yield obtained were presented in Table 2.

Bioassays

To test the virulence of NPVs, bioassays were conducted on second and third instar larvae and mortality data were recorded. The LC_{50} values observed for second and third instar larvae were 2.3×10^4 and 1.5×10^5 OBs/ml for HearNPV, 3.5×10^4 and 2.4×10^5 OBs/ml for SpltNPV and 5.6×10^4 and 3.96×10^5 OBs/ml for AmalNPV, respectively (Table 3). The LT_{50} values for second instar larvae, at highest virus concentration (1.8×10^7 OB/ml) was observed for three viruses as 122.64, 138.58 and 132.52 h, respectively, and the LT_{50} at lowest virus concentration (1.8×10^2 OB/ml) was observed as 230.68, 244.6 and 238.06 h, respectively. Similarly the LT_{50} for third instar larvae, at highest virus concentration (1.8×10^8 OB/ml) was observed as 123.6, 132.72 and 128.64 h, respectively, and at lowest virus concentration (1.8×10^3 OB/ml) the LT_{50} values were 216.07, 228.96 and 236.16 h, respectively (Table 4).

Discussion

The infected larvae showed pale swollen bodies and moribund. The larvae of *H. armigera* and *A. albistriga* were crawled to the top of the twigs (negative geotropism) on which they were fed. But the larva of *S. litura* had not shown this feature due to its burrowing and nocturnal

Table 1 Electron microscopy of baculoviruses isolated from three major insect pests of legume crops

Host insect	Crop	SEM		TEM		
		Shape of OBs	Size of OBs (μm)	Type of virion	Number of NCs/envelope	Dimensions of NC (nm)
Helicoverpa armigera	Pigeon pea and Chickpea	Irregular	0.5–2.5	Multiple enveloped	2–6	277.7 × 41.6
Spodoptera litura	Groundnut	Irregular	0.92–2.92	Multiple enveloped	5–7	285.7 × 34.2
Amsacta albistriga	Groundnut	Irregular	1.0–2.0	Multiple enveloped	2–6	228.5 × 22.8

SEM scanning electron micrograph; *TEM* transmission electron micrograph; *OBs* occlusion bodies; *NCs* nucleocapsids

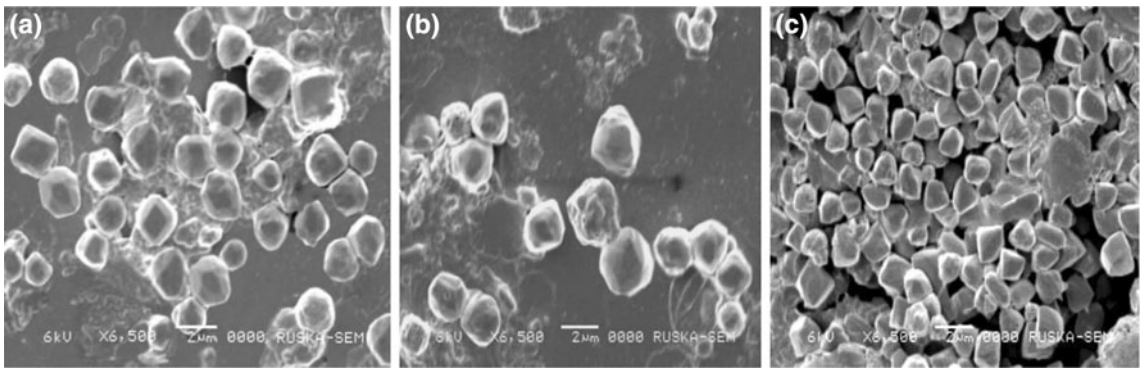

Fig. 2 Scanning electron micrographs of polyhedral OBs extracted from baculovirus infected larvae of **a** *Helicoverpa armigera*, **b** *Spodoptera litura* and **c** *Amsacta albistriga* (EM. Magnification = 6500×)

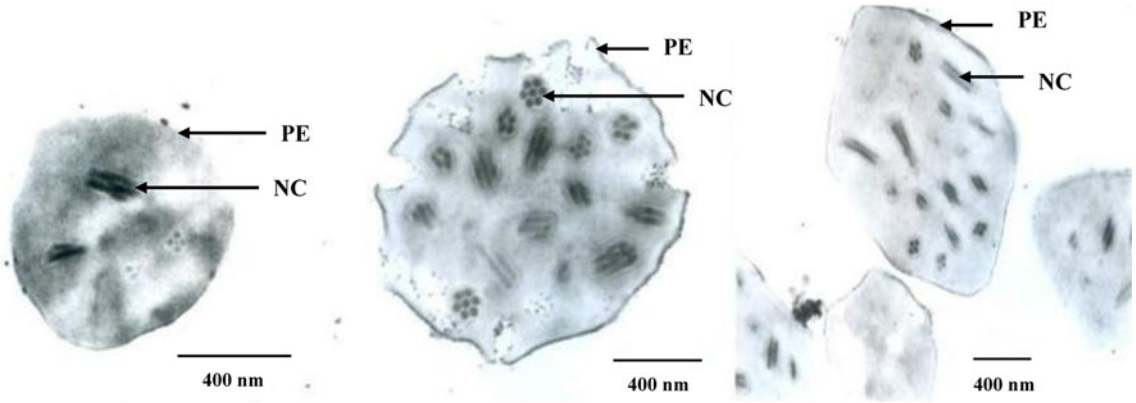

Fig. 3 Transmission electron micrographs of cross section of OBs obtained from baculovirus infected larvae of **a** *Helicoverpa armigera*, **b** *Spodoptera litura* and **c** *Amsacta albistriga* (magnification = 25000×); the size of the OB shown to original scale; details of OBs of three viruses showing multiple nucleocapsids surrounded by a single membrane, the polyhedral envelope (PE) and the bacilliform shaped nucleocapsids

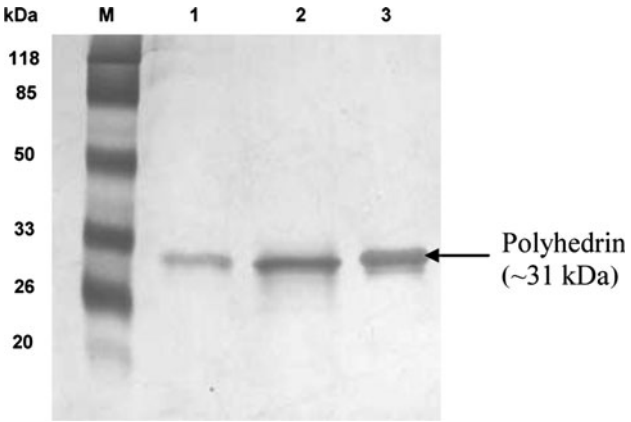

Fig. 4 SDS-PAGE (12%) profile of major occlusion body (OB) protein preparations of baculoviruses isolated from *Helicoverpa armigera* (*lane 1*); *Spodoptera litura* (*lane 2*); *Amsacta albistriga* (*lane 3*). The gel was silver stained and the OB protein of is indicated

habitat. The initial signs of baculoviral infection are gradual changes in the colour and luster of the integument. Infection of the epidermis caused the host to appear soft and in some larvae the cuticle was ruptured and discharging of body fluid on to plant parts was also observed. Observation of discharged body fluid under phase contrast microscope revealed the presence of OBs as shown in Fig. 1. In the present study, the morphological and

Table 2 Propagation of NPVs from field collected and laboratory reared larvae under controlled environment conditions at ICRISAT-NPV production laboratory

NPV type	Age of the larvae used for virus inoculation[a]	Virus yield (OBs/larva)	
		Field collected larvae	Laboratory reared larvae
HearNPV	4th instar	$5.35 \pm 0.31 \times 10^9$	$5.18 \pm 0.45 \times 10^9$
SpltNPV	5th instar	NT	$5.73 \pm 0.17 \times 10^9$
AmalNPV	5th instar	NT	$7.90 \pm 0.54 \times 10^9$

HearNPV—Helicoverpa armigera Nucleopolyhedrovirus; *SpltNPV—Spodoptera litura Nulceopolyhedrovirus*; *AmalNPV—Amsacta albistriga Nucleopolyhedrovirus*; *NT*—not tested

[a] 10^8 OBs/ml virus concentration was used for inoculating the larvae

Table 3 LC_{50} values of NPV isolates against second and third instar larvae

NPV isolate	LC_{50} values	
	Second instar larvae	Third instar larvae
HearNPV	2.3×10^4	1.5×10^5
SpltNPV	3.5×10^4	2.4×10^5
AmalNPV	5.6×10^4	3.9×10^5

HearNPV—Helicoverpa armigera Nucleopolyhedrovirus; *SpltNPV—Spodoptera litura Nulceopolyhedrovirus*; *AmalNPV—Amsacta albistriga Nucleopolyhedrovirus*. LC_{50} lethal concentration of virus required to cause 50% mortality

biological characteristics of baculoviruses isolated from the larvae of *H. armigera*, *S. litura* and *A. albistriga* were compared by conducting electron microscopic and bioassay studies. SEM showed that the OBs of three baculoviruses were appeared as irregular shape structures indicated that viruses isolated in this present investigation were NPVs rather than GVs. TEM studies on cross-sections of purified OBs of these viruses showed that each occlusion body contains 2–7 (multiple) nucleocapsids packaged within a single viral envelope. The nucleocapsids are elongated with parallel sides and two straight ends, measuring the sizes of 277.7 × 41.6 nm (HearNPV), 285.7 × 34.2 nm (SpltNPV) and 228.5 × 22.8 nm (AmalNPV). Similar studies, [31] on OBs of HearNPV and SpltNPV isolated in Taiwan also reported irregular shape with sizes raged from 0.79 ± 0.22 µm (HearNPV) and 1.61 ± 0.32 µm (SpltNPV), both the viruses were MNPVs. Recently, [10] demonstrated the MNPV nature of a NPV infecting *Trichoplusia ni* during isolation and characterization of a baculovirus associated with that insect parasitoid wasp, *Cotesia marginiventris*, on its host. In another study the polyhedra of the *Lymantria dispar* MNPV-NM isolate were observed as irregularly shaped, the average diameter of the polyhedra was 1.62 ± 0.33 µm; TEM revealed that LdMNPV-NM had bundles of virions in the nucleocapsid, which belonged to MNPV [27]. Similarly, [35] reported the morphology of a MNPV isolated from *Lonomia obliqua* (Lepidoptera: Saturniidae) with size ranged from 1 to 1.4 µm and the nucleocapsid dimensions of 270 × 36 nm. The OBs in the mid gut tissues of the tea looper (*Ectrophis obliqua*) were observed under TEM, the micrograph showed that the EcobSNPV were irregular shape and ranged in size from 0.7 to 1.7 µm in diameter and multiple rod-shaped virions measuring about 250 nm in length and 40 nm in width, were embedded in each OB with a single nucleocapsid packaged within the envelope of the virion [17]. Thus several studies in this group of viruses support the preset investigations.

In 12% SDS-PAGE analysis the molecular weight of the purified polyhedrin of three NPVs was determined as 31.65 kDa (±0.00), 31.29 kDa (±0.00) and 31.67 kDa (±0.295) for HearNPV, SpltNPV and AmalNPV, respectively. The present observations were close to that of [31] who worked on NPVs such as HearNPV, SpltNPV and *Spodoptera exigua* NPV (SeMNPV). Recently, [1] reported the molecular weight of 32 kDa for recombinant and wild type *A. californica* nucleopolyhedrovirus (AcAaIT and AcMNPV). Thus all of the baculovirus matrix proteins examined to date have molecular weights in the range from 25 to 32 kDa.

HearNPV multiplied on field collected larvae ($5.35 \pm 0.31 \times 10^9$ OBs/larva) recorded significantly higher yield compared to laboratory reared larvae ($5.18 \pm 0.45 \times 10^9$ OBs/larva). Similarly, the field collected larvae of *A. albistriga* were yielded 5.05 times more virus/larva [33], the NPV of *Hyblaea puera* (Cramer) (Lepidoptera: Hyblaeidae), the teak defoliator, when mass produced in situ reportedly yielded 2.56 times more HpNPV than when mass produced in the laboratory [28]. Fourth instar *H. armigera* larvae were inoculated with 10^8

Table 4 LT_{50} (h) values of NPV isolates against second and third instar larvae

Virus concentration (OB/ml)	LT_{50} (h) values					
	Second instar larvae			Third instar larvae		
	HearNPV	SpltNPV	AmalNPV	HearNPV	SpltNPV	AmalNPV
1.8×10^8	NT	NT	NT	123.60	132.72	128.64
1.8×10^7	122.64	128.58	132.52	134.25	140.4	144.0
1.8×10^6	131.28	133.62	136.64	136.42	143.0	148.64
1.8×10^5	142.32	146.76	149.72	150.0	156.12	162.42
1.8×10^4	153.30	158.60	162.72	161.22	176.08	182.06
1.8×10^3	191.18.	195.60	199.20	216.07	228.96	236.16
1.8×10^2	230.68	234.60	238.06	NT	NT	NT

HearNPV—Helicoverpa armigera Nucleopolyhedrovirus; *SpltNPV—Spodoptera litura Nulceopolyhedrovirus*; *AmalNPV—Amsacta albistriga Nucleopolyhedrovirus*; LT_{50} lethal time required to cause 50% mortality; *NT* not tested

OBs/ml and recorded higher larval mortality and higher quantity of viral yield per larva (2.81×10^8 OBs) and suggested 10^8 OBs/ml as optimum viral dose required for mass production of virus both on third and fourth instar larvae [9]. In the present study the OB yield obtained per larva was slightly higher than the earlier studies conducted by [9] and [20], which may be due to variation in the size of the larvae inoculated with the virus and the diet provided to the larvae. The host, diet, age and virus dosage, incubation, environment and preservation of virus infectivity are some of the major factors, which influence the production of NPVs. The optimum dose of viral inoculum also varies with the virulent strain and age of the host. The variation of virus yield between filed collected and laboratory reared larvae may be due to assorted sizes or stages in the field collected larvae. However mass production of HearNPV on field collected larvae is more feasible for large scale production. Where as for propagation of SpltNPV and AmalNPV laboratory reared 5th instar larvae found to be more efficient. Subsequently, due to heavy body weight of these insects the yield of NPV was also recorded as higher than HearNPV. In the present investigation the OB yield of SpltNPV was similar to the reports of [24], found higher than some studies [30] and lower than some other studies [15]. Whereas, the yield of AmalNPV ($7.90 \pm 0.54 \times 10^9$ OBs/larva) was noticed to be higher than HearNPV and SpltNPV which could be due to higher larval body weight.

Based on the LC_{50} and LT_{50} values the NPVs isolated in the present study were found as virulent. Similarly, bioassays among NPV isolates have been established in previous studies conducted by [26] on the variations in virulence of 34 isolates of HearNPV and concluded 56 fold difference in the activity of the isolates however they could not exclude the influence of factors such as solubility of OBs and the availability of virions which might have played critical role.

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References

1. Ashour MB, Ragheb DA, EI-Sheikh E-SA, Gomaa E-AA, Kamita SG, Hammock BD. Biosafety of recombinant and wild type *Nucleopolyhedrovirus* as bioinsecticides. Int J Environ Res Public Health. 2007;4(2):111–25.
2. Battu GS. Yield levels of the occlusion bodies obtained from the Baculovirus infected *Hellula Undalis* (Fabricius). Indian J Entomol. 1990;51:317–25.
3. Blissard GW, Rohrmann GF. *Baculovirus* diversity and molecular biology. Annu Rev Entomol. 1990;35:127–55.
4. Blissard GW, Black B, Crook N, Keddie BA, Posse R, Rohrmann GF, Theilmann DA, Volkman L. Family *Baculovirdae*. In: Van Regenmortel MHV, et al., editors, Virus taxonomy: seventh report of the international committee on taxonomy of viruses. San Diego: Academic Press; 2000. p. 195–202.
5. Chi H. Computer programme for the probit analysis. Taichung, Taiwan: National Chung Hsing University; 1997.
6. Christian PD, Gibb N, Kasprzak AB, Richards A. A rapid method for the identification and differentiation of *Helicoverpa Nucleopolyhedroviruses* (NPV *Baculoviridae*) isolated from the environment. J Virol Methods. 2001;96:51–65.
7. Evans HF, Shapiro M. Viruses. In: Lacy LA, editor. Manual of techniques in insect pathology. San Diego: Academic Press; 1997. p. 17–54.
8. Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. Virus taxonomy—seventh report of the international committee on taxonomy of viruses. San Diego: Academic Press; 2004. p. 195–202.
9. Gopali JB, Lingappa S. Refinement of technique for mass production of virus from *Helicoverpa armigera* (Hubner). Karnataka J Agric Sci. 2001;14(4):947–54.
10. Grasela JJ, Mc Intosh AH, Shelby KS, Long S. Isolation and characterization of a *Baculovirus* associated with the insect parasitoid wasp, *Cotesia marginiventris*, on its host, *Trichoplusia ni*. J Insect Sci. 2008;8(42):19.
11. Ignoffo CM. Standardization of products containing insect viruses. J Invertebr Pathol. 1966;48:547–8.
12. Ignoffo CM, Anderson R. Bio-insecticide. In: Peppler HJ, Parlman D, editors. Microbial technology. New York: Macmillan; 1979. p. 1–28.
13. Ignoffo M, Couch TL. The nuclear polyhedrosis virus of *Heliothis* species as a microbial insecticide. In: Burges HD, editor. Microbial control of pests and plant diseases 1970–1980. London: Academic Press; 1981. p. 329–62.
14. Jacques RP. Persistence, accumulation and denaturation of *Nuclear polyhedrosis* and *granulosis viruses*. In: Summers MD, Engler R, Falcon LA, Vail PV, editors. Baculoviruses for insect pest control: safety considerations. Washington, DC: American Society of Microbiology; 1975. p. 90–9.
15. Jun T, Shohei O, Takayoshi I, Madoka N, Yasuhisa K. Productivity and quality of polyhedral occlusion bodies of a *Nucleopolyhedrovirus* harvested from *Spodoptera litura* (Lepidoptera: Noctuidae) larvae. Appl Entomol Zool. 2007;42(1):21–6.
16. Kumar PL, Waliyar F. Diagnosis and detection of viruses infecting ICRISAT mandate crops: methods manual. Patancheru: International Crops Research Institute for the Semi-Arid Tropics; 2007.
17. Ma XC, Xu HJ, Tang MJ, Xiao Q, Hong Q, Zhang CX. Morphological, phylogenetic and biological characteristics of *Ectropis oblique* single-nucleocapsid *Nucleopolyhedrovirus*. J Microbiol. 2006;44(1):77–82.
18. Narayanan K. Studies on the *Nuclear polyhedrosis virus* of gram pod borer, *Heliothis armigera* (Hubner) (Noctuidae: Lepidoptera). Dissertation, Tamil Nadu Agriculture University; 1979.
19. Odak SK, Srivastava DK, Misra VK, Nema KK. Preliminary studies on the pathogenicity of *Bacillus thuringiensis* and *Nuclear polyhedrosis virus* on *Heliothis armigera* host in the laboratory and in pot experiments. Legume Res. 1984;5:13–7.
20. Pawar VM, Thombre UT. Prospects of *Baculovirus* in integrated pest management of pulses. In: Ananthkrishnan TN, editor. Emerging trends in biocontrol of phytophagous insects. New Delhi: Oxford and IBH publishing Company Private Limited; 1992. p. 253–8.
21. Quant RL, Pearson MN, Rohrman GF, Beaudreau GS. Production of polyhedrin monoclonal antibodies for distinguishing two *Orgyia pesudotsugata Baculoviruses*. Appl Environ Microbiol. 1984;48:732–6.

22. Rabindra RJ, Subramanian TR. Studies on the *Nuclear polyhedrosis* of *Heliothis armigera* (Hubner). Madras Agric J. 1974; 60:217–20.
23. Rohrmann GF. Nuclear polyhedrosis viruses. In: Webster RG, Granoff A, editors. Encyclopedia of virology. 2nd ed. London: Academic Press; 1999. p. 146–52.
24. Senthil Kumar CM, Sathiah N, Rabindra RJ. Optimizing the time of harvest of *Nucleopolyhedrovirus* infected *Spodoptera litura* (Fabricius) larvae under in vivo production systems. Curr Sci 2005;88(10):1682–4.
25. Shapiro M, Bell RA. Biological activity of *Lymantria dispar* nucleopolyhedrosis virus from living and virus killed larvae. Ann Entomol Soci Am. 1981;74:27–8.
26. Shapiro M, Ignoffo CM. *Nucleopolyhedrosis* of *Heliothis*: activity of isolates from *Heliothis zea*. J Invertebr Pathol. 1970;16:107–11.
27. Shim HJ, Roh JY, Choi JY, Li MS, Woo SD, Oh HW, Boo KS, Je Y. Isolation and Characterization of a *Lymantria dispar* multinucleocapsid *Nucleopolyhedrovirus* isolate in Korea. J Microbiol. 2003;41(4):306–11.
28. Sudheendrakumar VV, Sajeev TV, Varma RV. Mass production of *Nuclear polyhedron virus* of the teak defoliator, *Hyblaea puera* Cramer using host population in teak plantations. J Biol Control. 2004;18:81–4.
29. Teakle RE, Byrne VS. *Nuclear polyhedrosis virus* production in *H. armigera* infected at different J Invertebr Pathol. 1989;53: 21–4.
30. Tuan SJ, Chen WL, Kao SS. In vivo mass production and control efficacy of *Spodoptera litura* (Lepidoptera: Noctuidae) *Nucleopolyhedrovirus*. Chin J Entomol. 1998;18:101–16.
31. Tuan SJ, Kao SS, Cheng DJ, Hou RF, Chao YC. Comparison of the characterization and pathogenesis of three lepidoperan *Nucleopolyhedroviruses* (HearNPV, SpeiNPV and SpltNPV) isolated from Taiwan. Chin J Entomol. 1999;19(2):167–86.
32. Veenakumari K, Rabindra RJ, Srinivasa Naik CD, Shubha MR. Standardization of laboratory mass production of *Amsacta albistriga Nucleopolyhedrovirus*. J Biol Control. 2006;20:183–90.
33. Veenakumari K, Rabindra RJ, Srinivasa Naik CD, Shubha MR. In situ field level mass production of *Amsacta albistriga* (Lepidoptera: Arctiidae) *Nucleopolyhedrovirus* in a groundnut ecosystem in South India. Int J Trop Insect Sci. 2007;27(1):48–52.
34. Winstanley D, Reilly DO. *Granuloviruses*. In: Webster RG, Granoff A, editors. Encyclopedia of virology. 2nd ed. London: Academic Press; 1999. p. 69–84.
35. Wolff JLC, Moraes RHP, Kitajima E, Leal EDS, Zanotto PMA. Identification and characterization of a *Baculovirus* from *Lonomia obliqua* (Lepidoptera: Saturniidae). J Invertebr Pathol. 2002; 79:137–45.