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Characterization and field evaluation of *Spilosoma obliqua* nucleopolyhedrosis virus (*Spob*NPV) CRIJAF1 strain against jute hairy caterpillar, *Spilosoma obliqua* (Walker) infesting jute, *Corchorus olitorius* Linn.

Veegala Ramesh Babu[1,2*], G. Sivakumar[2] and S. Satpathy[1]

Abstract

Background *Spilosoma* (= *Spilarctia*) *obliqua* (Walker) (Lepidoptera: Arctiidae), once considered as a sporadic pest, is now considered as a major and polyphagous pest widely distributed throughout India. Recent outbreaks of this pest were manifested in both jute and sunnhemp. During a survey in and around adjoining villages of I.C.A.R-C.R.I.J.A.F, Barrackpore, an epizootic was observed in the field population of *S. obliqua* caused by a nucleopolyhedrosis virus. Hence, an attempt was made to isolate, characterize using electron microscopy and assess the efficacy of *S. obliqua* nucleopolyhedrosis virus under laboratory and field conditions.

Results Electron microscopic studies revealed typical baculovirus occlusion bodies of type nucleopolyhedrosis virus with tetrahedral and triangular in shape with the size ranging from 2.55 to 2.90 µm, with an average size of about 2.72 µm. Bioassay studies showed the larval mortality at low concentration of *Spob*NPV, i.e., 2.42×10^4 POBs/ml. The virus-infected *S. obliqua* was identified through the amplification of polyhedrin, *polh* gene sequences with the available sequences in public database, GenBank, NCBI. The sequence data generated for the polyhedrin gene were deposited in GenBank, and accession number was obtained, MN648213. Field experiment revealed 50.52, 63.25 and 82.91% larval reductions, respectively, at 2, 4 and 7 days after spray of *S. obliqua* nucleopolyhedrosis virus.

Conclusion The present study revealed that foliar application of *S. obliqua* nucleopolyhedrosis virus was very effective in causing mortality against *S. obliqua* larvae and thus can be used as an effective microbial bioagent in the integrated pest management of the caterpillar, *S. obliqua*.

Keywords *Spilosoma obliqua*, Nucleopolyhedrovirus, Characterization, Polyhedrin gene, Jute

Background

Jute (*Corchorus olitorius* Linn., *C. capsularis* Linn.; Family: Malvaceae) is an important commercial bast fiber crop next to cotton. In Indian perspective the cultivation of jute is mostly confined to Indo-Gangetic plains (Raja 2012) constituting the states of West Bengal, Bihar, Odisha and Assam, all of which contribute to more than 80% of total jute production (Chapke et al. 2006). However, the current average productivity of jute in India is

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2.5 t/ha (AINPJAF 2020). Both biotic and abiotic factors in duo are responsible for curtailing the productivity vis-a-vis fiber quality. Across all jute-growing regions of India the pest infestation inevitably causes approximately 20–25% yield loss coupled with deterioration in fiber quality (Satpathy et al. 2014). The crop phenology of jute is succumbed to various insect pests of different insect orders, commencing from the sowing of the crop in mid-March till harvesting of fiber in mid-August. The overwhelming pest numbers manifest the complete failure of the crop causing huge monitory loss to jute cultivators. It is no astonishing to say that off the various insect pests encompassing different insect orders, lepidopteran pests are of utmost importance.

Spilosoma (=*Spilarctia*) *obliqua* (Walker) (Lepidoptera: Arctiidae), vernacularly known as jute hairy caterpillar once regarded as sporadic pest, has accomplished the status of regular, polyphagous pest and ubiquitous throughout India (CPC 2004). The pest is accredited to infest myriad of plant species belonging to 25 families, including pulses, oilseeds, cereals, vegetables, mulberry, turmeric, bast fiber crops, viz., jute, mesta and ramie; medicinal and aromatic plants, causing heavy economic loss (Senthil Kumar et al. 2011). Recent outbreak of this pest is evident in jute and sunnhemp during 2011 and 2012 (Satpathy et al. 2014) causing great loss to fiber yield. The newly hatched neonate larvae from the single egg cluster laid by the female moth feed gregariously by scraping chlorophyll content of the leaves, while that of late larval instars are mostly confined solitarily causing severe defoliation (Selvaraj et al. 2015).

Many conventional chemical pesticides have been pulled back from usage in agricultural scenario per se environmental pollution, human health hazards (Damalas and Eleftherohorinos 2011) insecticide resistance and resurgence issues (Mishra et al. 2015) affirming and asserting the development and adoption of alternative pest management strategies. Biological control, an intrinsic component of integrated pest management relies on living microorganisms or their derivatives, plant extracts, predators and parasitoids and other natural compounds in management of insect pests in various agro-ecosystems in a sustainable way stands as a proxy strategy (Kumar et al. 2019). Microbial control encompassing entomopathogenic bacteria, fungi, viruses and nematodes or their derivatives caters the need of pest management in a sustainable way as they are less environmental toxic, target specificity and safety to non-target organisms (Kumar and Singh 2015) and hence are gaining popularity. Entomopathogenic viruses which are generally categorized under baculoviruses are efficient biocontrol agents due to their limited host range, least toxic to non-target organisms, well adapted to avoid the insect's defense mechanisms and rarely develop resistance (Gramkow et al. 2010), thus rifting and gaining momentum in pest control programs globally. Nucleopolyhedrosis viruses (NPV) which are obligate parasite and one among the two types of baculoviruses known for causing naturally high epizootics, self-perpetuating, safe to natural enemies and eco-friendly are a great success in agriculture and forestry (Ikeda et al. 2015). Therefore, it is quite evident that, in comparison with chemical control of insect pests, the usage of baculoviruses in the present pest management scenario is considerably cheap, practicable; eco-friendly and potential tool for management of insect pests of agricultural importance of farm crops (Gramkow et al. 2010). Hence, the present study aimed to isolate, characterize and evaluate the efficacy of *S. obliqua* nucleopolyhedrosis virus (*Spob*NPV) for the management of *S. obliqua* infesting jute crop under field conditions.

Methods

Insect culture

Neonate larvae of *S. obliqua* were collected from the fields of Research Farm, I.C.A.R-Central Research Institute for Jute and Allied Fibres, Barrackpore, and reared in the laboratory to adult moths on jute leaves in plastic containers having nylon mesh. The eggs laid by the adult female moths were used to maintain the healthy populations of the insect in the laboratory to carry out further experiments.

Nucleopolyhedrosis virus isolation

The NPV was isolated from the dead fourth instar larvae of *S. obliqua*. The larvae were showed typical "Wipel Krankheit or Tree Top Disease" with characteristic viral infection symptoms and found hanging from jute plants at farmer's field located in Berabaria, North 24 Parganas, West Bengal (22.75° N, 88.42°), India, while surveying during an epizootic in June 2020 (Fig. 1). The viral-infected larvae were individually transferred to small-sterile Eppendorf tubes (3 ml) and brought to Biocontrol Lab, Crop Protection Division, ICAR-CRIJAF, Barrackpore. The larval cadaver was homogenized with sterile distilled water for 3 min, and the crude homogenate obtained then was filtered thrice by muslin cloth to remove larval debris. The filtrate then was centrifuged for 1 min at 20 *g* to remove large particles. The resultant supernatant was once more resuspended in sterile distilled water and then centrifuged for 20 min at 1100 *g* and the pellet was collected. Sterile distilled water (50 ml) was used to resuspend the pellet, and this was regarded as the stock solution/suspension. The number of occlusion bodies (OBs) in the stock suspension was determined and deduced to 3.54×10^8 POB's/ml, using a Neubauer

Fig. 1 Larva of *Spilosoma obliqua* infected with NPV and showing "Wipel Krankheit or Tree Top Disease" symptom

hemocytometer in aqueous solution of 0.05% Triton X-100 (v/v).

Mass production of *Spob*NPV

Fifth instar larvae of *S. obliqua* were used for carrying out the in vivo mass production of *Spob*NPV in the plastic trays. About hundred 5th instar larvae of *S. obliqua* were reared on the natural diet viz., insecticide-free jute leaves. Prior to smearing with NPV the jute leaves were washed thoroughly by distilled water and air-dried. Using the polished blunt end of glass rod jute leaves were smeared with *Spob*NPV at 3.54×10^8 OBs/ml. The virus-contaminated leaves were then placed in plastic trays (50 cm L × 25 cm B × 6 cm H). Fifth instar larvae of *S. obliqua* were allowed to feed on these leaves. Post-consumption of the virus-treated leaves the larvae were fed fresh insecticidal-free jute leaves. All the dead larval cadavers were collected and processed post 6–8 days of inoculation. The OBs were quantified using a double-ruled improved Neubauer hemocytometer, and the virus suspension was standardized and stored at − 20 °C for further studies.

Larval bioassays studies

Estimation of occlusion bodies (OBs) in the *Spob*NPV viral suspension was carried out by an improved Neubauer hemocytometer. From the primary stock solution (3.54×10^8 OBs/ml), seven concentrations (3.54×10^8 POBs/ml to 354 POBs/ml) were prepared with 0.05% Triton X-100 through serial dilution. The surface-sterilized jute leaves were dipped in the respective NPV dilutions for 20–30 s and then air-dried, while that of the control treatment jute leaves dipped in sterile distilled water. The treated leaves were then placed in plastic Petri dishes (12 cm diameter). Larval bioassays were carried out for second, third and fourth larval instars of the test insect individually. Each bioassay study comprised of 20 corresponding larval instar per viral concentration and was replicated thrice along with a control treatment. Larval mortality was documented five days after treatment. The bioassay was repeated wherever control mortality exceeded 20%. The median lethal concentration (LC_{50}) was calculated for second, third and fourth larval instars in tandem.

Molecular characterization of SpobNPV

The occlusion bodies (polyhedra) from the larval cadavers were purified by centrifugation at 280 *g* for 1 min in microtubes (2 ml). The pellet was discarded retaining the supernatant having OB's. The supernatant was subjected to centrifugation once more, and OB's thus obtained were suspended in sterile distilled water and stored at -20 °C for further use. DNA extraction was performed using the traditional 0.3 M sodium carbonate solution. The POB's were dissolved 500 µl sodium carbonate buffer (0.3 M sodium carbonate, 300 mM EDTA and 5 M sodium chloride) and incubated at 37 °C for 60 min and centrifuged at 1100 *g* for 5 min. The supernatant was collected and again centrifuged at 4472 *g* for 30 min to pellet the virus particles. The pellet was dissolved in 400 µl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5) and incubated overnight at room temperature. The suspension having OB's was treated with equal volume of 10% SDS and 8 µL of proteinase K (10 mg/ml) and incubated at 65 °C for 3 h. DNA was extracted by using phenol: chloroform (1:1), followed by chloroform:isoamylalcohol (24:1). The clear aqueous phase was transferred and added with 1/10th volume of 3 M sodium acetate and 2.5 times volume of ethanol (100%) to precipitate DNA. The precipitate was centrifuged at 4472 *g* for 15 min. The pellet was washed with 70% ethanol and air-dried. DNA was resuspended in TE buffer, and extracted DNA was visualized in 0.8% agarose gel. Species-specific amplification of *polh* gene was carried out by designing primer sequences based on the *Spob*NPV sequences submitted to GenBank database of National Centre for Biotechnology Information. Accordingly, the primer sequence consisted of forward primer 5′-ATGCCAGACTTCTCG TACCG-3′ and reverse primer 5′-TAATACGCGGGA CCGGTGAAT-3′. Each 50 µl PCR mixture consisted of 50 ng template DNA, 0.25U of *Taq* DNA polymerase, 10X *Taq* buffer, 2.5 mM $MgCl_2$, 2.5 mM of each of four dNTPs and 1 µl each forward and reverse primer. The PCR consisted of an initial denaturation step of 95 °C

for 3 min and 35 cycles of denaturation at 95 °C for 30 s, annealing of primers at 54 °C for 1 min, extension at 72 °C for 1 min, followed by final extension step 72 °C for 10 min. The nucleotide sequence of the PCR product was submitted to GenBank, and accession numbers were obtained.

Electron microscopy

Scanning electron microscopy (SEM)

The SEM was conducted by fixing the samples containing OB's using 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4 °C overnight. Then the OB's were washed thrice with 0.1 M phosphate buffer (pH 7.2) with a time interval of 30 min between each wash. Post-fixation of the OB's was done using 1% aqueous osmium tetroxide (OsO_4) prepared in 0.1 M phosphate buffer (pH 7.2) for 4 h. Dehydration of the samples was done in ascending series of graded ethanol and dried to critical point drying with CPD (EMS 850) unit/vacuum desiccation for 35–45 min for complete drying of specimens. The dried samples were mounted over the stubs with double-sided carbon conductivity tape, and a thin layer of heavy metal (gold) was coated over the samples by using an automated sputter coater (Model—JEOL JFC-1600) for 3 min and scanned under scanning electron microscope (SEM—Model: JOEL-JSM 5600). The structure of the OB's was viewed at 3–5 µm size (10 kV) and captured at required magnifications (× 3000–× 5000) as per the standard procedures at RUSKA Lab's College of Veterinary Science, PVNRTVU, Rajendranagar, Hyderabad, India (Bozzola and Russell 1998a).

Transmission electron microscopy (TEM)

Samples containing OB pellets were fixed with 2.5% (v/v) glutaraldehyde prepared in 0.05 M phosphate buffer (pH 7.2) for 24 h at 4 °C and were washed twice with PBS (phosphate buffer saline) with a gap of 45 min between subsequent washes. Post-fixation of OB's was done in 0.1% (v/v) aqueous osmium peroxide (OsO_4) prepared in 0.1 M phosphate buffer (pH 7.2) for 2 h. Subsequently, the samples were washed thrice with deionized distilled water with time gap of 45 min between each wash. Later on dehydration of the samples containing OB's was done with ascending series of graded ethanol, infiltrated and embedded in Araldite 6005 resin or spur resin. Dehydration of the samples was carried out at 80 °C for 48 h to ensure complete polymerization. Ultrathin sections (60 nm thickness) were made using a glass knife on ultra-microtome (Leica Ultra cut UCT-GAD/ E-1/00), and the sections were mounted on three hundred mesh carbon-coated copper grids for staining. The samples were stained first with aqueous uranyl acetate (UA) (saturated in water) and later on with 4% Reynolds lead citrate (LC), respectively. The samples at various magnifications were viewed at 80 kV with transmission electron microscope (TEM) (Hitachi H-7500, Japan) as per the standard procedures at RUSKA Lab's College of Veterinary Science, PVNRTVU, Rajendranagar, Hyderabad, India. The morphology of the OBs and nucleocapsids were measured directly from the amplified photographs using a precision metallic ruler and dividing the value by the magnification of the photograph (Bozzola and Russell 1998b).

Field experiment

To evaluate the efficacy of *Spob*NPV field experiments were conducted at Central Research Farm, ICAR-Central Research Institute for Jute and Allied Fibers, Barrackpore, Kolkata, India, during 2020 and 2021 at the peak infestation of *S. obliqua*. The experimental plots of size (4 × 5 m) were laid out in randomized block design with 4 replications. Field efficacy of *Spob*NPV (3.54×10^8 POB's/ml) @ 2 ml/l was compared to *Bacillus thuringiensis* var. *kurstaki* (*Btk*) NCIM2514 at 2 ml/l (Dipel®), Margo Private Limited, Bengaluru, India, and conventional insecticide; chlorpyrifos 20EC (Dursban®), Crystal Crop Protection Limited, Ahmadabad, India, at 2 ml/l. Jute seed (variety JRO 204) was sown during late summer season (mid-May) with spacing of (30 × 15 cm) and raised, following the recommended package of practices. During the peak period of insect infestation pre-treatment observation on the number of larvae from 50 randomly selected plants was recorded at 1 day before spraying. Treatments were imposed with knapsack sprayer coinciding with the infestation third instar larvae. *Spob*NPV at 3.54×10^8 OBs/ml with molasses 5%, glycerol 10% and Stikker plus 0.1% was sprayed at 2 ml/l during evening hours. *Btk* NCIM2514 was sprayed at 2 ml/l. Post-treatment observations on the number of *S. obliqua* larvae were recorded at 2, 4 and 7 days after spray applications from 50 randomly selected plants.

Statistical analysis

The bioassay data were analyzed using SAS 9.2 with Probit for estimation of LC_{50} (lethal concentration to kill 50% population). The data on percent infestation were subjected to ANOVA using statistical software SAS 9.2. The percent reduction of insect infestation over control was calculated by modifying Abbott's formula (Abbott 1925) and (Fleming and Retnakaran 1985).

$$\text{Population reduction (\%)} = 1 - \frac{\text{Post-treatment population in treatment}}{\text{Pre-treatment population in treatment}} \times \frac{\text{Post-treatment population in control}}{\text{Pre-treatment population in control}}$$

Results

The natural epizootic by baculovirus caused massive infection in pest population of hairy caterpillar, *S. obliqua* in jute crop fields of villages adjoining to I.C.A.R-C.R.I.J.A.F, Barrackpore, Kolkata, India. The diseased larvae appeared swollen, glossy, spongy and moribund with typical nucleopolyhedrosis viral (NPV) characteristic feature of hanging down in inverted 'ʌ' on to the top of the plants. The cadavers were seen with liquefied body tissues and white body fluid oozing out from the ruptured cuticle, which when observed under phase contrast microscope revealed presence of baculoviral occlusion bodies (OBs). Typical characteristic baculoviral OBs of nucleopolyhedrovirus (NPV) category with polyhedral structures were revealed in electron microscopic (EM) studies. Scanning electron microscopic (SEM) studies revealed that the OBs appeared as tetrahedral and triangular in shape and size ranging from 2.55 to 2.90 µm, with an average size of about 2.72 µm (Fig. 2). Transmission electron microscopic (TEM) studies revealed that OBs appeared as both tetrahedral and triangular (Fig. 3) in shape.

Molecular characterization of the *S. obliqua* nucleopolyhedrosis virus (*Spob*NPV) was carried out through the amplification of polyhedrin gene (*polh*) (Fig. 4). The gene sequences from the available closest representative sequences from the public database (GenBank, NCBI) were used for amplification of *polh* gene. The amplicon product obtained was 691 bp, and the sequence data generated thereof were deposited in GenBank, and accession number was obtained (MN648213). Only one open reading frames (ORF) encoding for polypeptides of 230 amino acids were detected. No stop codon was detected over the entire amino acid sequence.

Larval bioassays

The median lethal concentration (LC_{50}) values deduced from the leaf dip bioassay studies against second, third and fourth larval instars of *S. obliqua* at 72 h after treatment were 2.42×10^4, 3.09×10^6 and 5.05×10^7 POB's/ml, respectively (Table 1). The pooled data of field

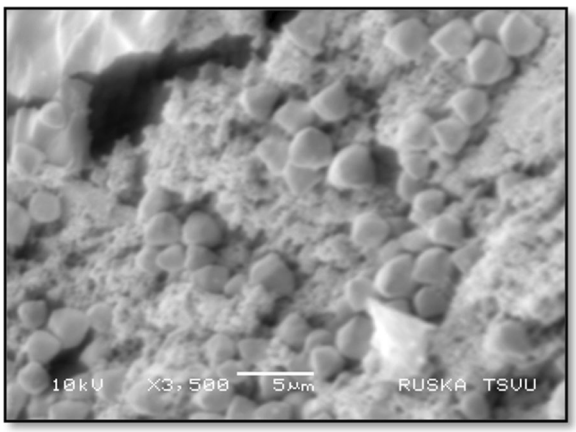

Fig. 2 Scanning electron micrographs depicting tetrahedral and triangular polyhedral OBs isolated from baculovirus-infected larvae of *Spilosoma obliqua*

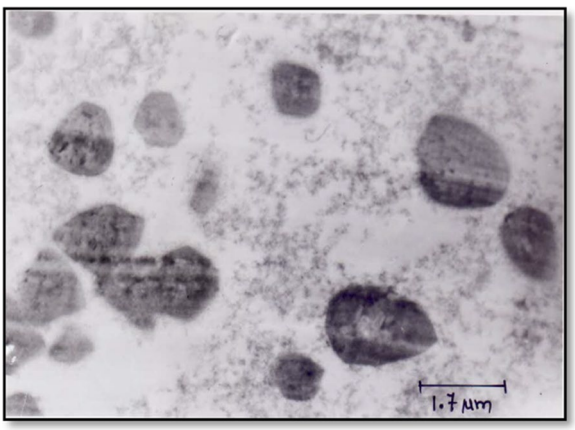

Fig. 3 Transmission electron micrographs depicting tetrahedral and triangular OBs isolated from baculovirus-infected larvae of *Spilosoma obliqua*

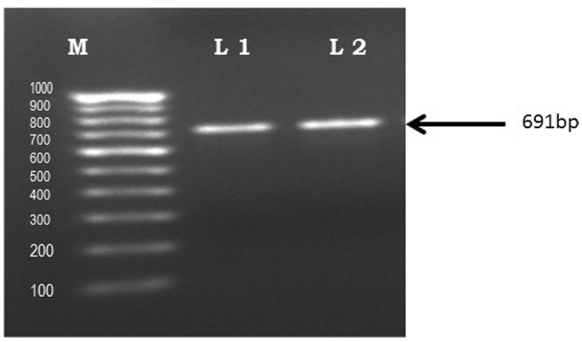

Fig. 4 *polh* gene of amplicon size 691 bp detected in PCR study; M = marker (M): 100 bp ladder; Lane 1 = *Spilosoma obliqua polh* nucleopolyhedrosis virus (NPV) gene (replication1); Lane 2 = *S. obliqua polh* NPV gene (replication 2)

Table 1 Larval bioassay with *Spilosoma obliqua* Nucleopolyhedrosis virus (*Spob*NPV) against 2nd, 3rd and 4th larval instars of *Spilosoma obliqua*

Larval instar	Number of larvae	LC_{50} (POB's/ml)	Fiducial limits		Slope	SE±	χ^2	DF
			Lower limit	Upper limit				
2nd Instar	480	2.42×10^4	1.24×10^4	4.99×10^4	0.87	0.29	3.23	6
3rd Instar	480	3.09×10^6	9.75×10^5	1.03×10^7	0.56	0.04	4.11	6
4th Instar	480	5.05×10^7	4.11×10^6	9.23×10^7	0.31	0.11	2.76	6

Table 2 Field evaluation of *Spilosoma obliqua* Nucleopolyhedrosis virus (*Spob*NPV) against third instar larvae of *Spilosoma obliqua* (pooled mean data for the years 2020 and 2021)

Treatments	No. of larvae before treatments	No. of larvae/plant		
		Days after spray		
		2	4	7
SpobNPV (3.54×10^8 POB's/ml) @ 2 ml/l	$46.67^A \pm 5.24$	$23.00^C \pm 3.46$	$18.67^B \pm 0.88$	$9.67^B \pm 1.20$
Bacillus thuringiensis kurstaki NCIM2514 @ 2 ml/l	$45.33^A \pm 4.10$	$33.67^B \pm 3.71$	$20.67^B \pm 0.88$	$13.33^B \pm 2.60$
Chlorpyriphos 20EC @ 2 ml/l	$47.67^A \pm 2.40$	$19.33^C \pm 0.88$	$11.33^C \pm 0.88$	$3.33^C \pm 0.33$
Control	$45.00^A \pm 3.51$	$47.33^A \pm 3.53$	$50.00^A \pm 3.21$	$59.33^A \pm 1.20$

Means followed by different letters within a row indicate significant differences ($P < 0.05$; LSD)

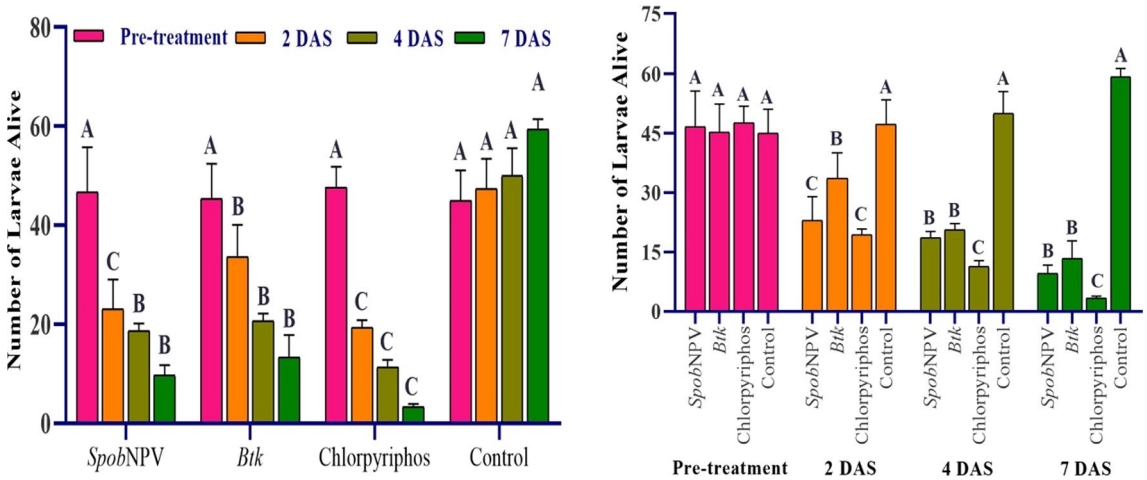

Fig. 5 *Spilosoma obliqua* third instar larvae survival at 2, 4 and 7 days after imposing of treatments

experiment conducted for two years in assessing and evaluating the efficacy of *Spob*NPV are showed below. The efficacy of the *Spob*NPV was evaluated and compared to *Bt* var. *kurstaki* strain NCIM2514 and the conventional insecticide used in farmers field, viz., chlorpyriphos 20EC as treatments under field condition on jute crop. The results revealed that at 2 day after treatments, chlorpyriphos 20EC and *Spob*NPV were on par with each other and were significantly different from *Bt* var. *kurstaki* and control treatments with respect to number of larvae per plant (Table 2). Likewise at 4 and 7 days after treatment with chlorpyriphos 20EC was significantly different from other treatments, whereas treatments *Spob*NPV and *Bt* var. *kurstaki* were par with each other but were significantly different from control treatment (Fig. 5). Chlorpyriphos 20EC caused 61.03, 78.70 and 94.68% larval reduction at 2, 4 and 7 days after spray, respectively. It was on par with *Spob*NPV at 4 and 7 days after treatment and significantly different from *Bt* var. *kurstaki* and control treatments which were envisaged

Table 3 Percent larval reduction by *Spilosoma obliqua* Nucleopolyhedrosis virus (*Spob*NPV) against third instar larvae of *Spilosoma obliqua* (pooled mean data for the years 2020 and 2021)

Treatments	Percent larval reduction		
	Days after spray		
	2	4	7
SpobNPV (3.54×10^8 POB's/ml) @ 2 ml/l	50.52^{AB}	63.25^{B}	82.91^{AB}
Bacillus thuringiensis kurstaki NCIM2514 @ 2 ml/l	27.51^{BC}	57.56^{B}	75.66^{B}
Chlorpyriphos 20EC @ 2 ml/l	61.03^{A}	78.70^{A}	94.68^{A}
Control	0.00^{C}	0.00^{C}	0.00^{C}

Means followed by different letters within a row indicate significant differences ($P < 0.05$; LSD)

with least number of larvae/plant. Though *Spob*NPV caused larval reduction of 50.52, 63.25 and 82.91% at 2, 4 and 7 days after spray, respectively, it was found to be par with *Bt* var. *kurstaki* at after the same periods of spray and was significantly different from control treatment (Table 3). *Bt* var. *kurstaki* though exhibited larval reduction by 27.51, 57.56 and 75.66%, respectively, at 2, 4 and 7 days after spray; it was on par with control treatment at 2 days after spray but significantly different at 4 and 7 days after spray (Fig. 6).

Discussion

Baculovirus encompassing nucleopolyhedrosis viruses (NPV) is empirical microbial entomopathogens characterized to cause high natural epizootics, host specific, ubiquitous, self-perpetuating, being safer to natural enemies and lastly eco-friendly. Commendable research work on varied aspects of NPV isolated from lepidopteran insect pests has been conducted globally. Research work pertaining to molecular characterization, electron microscopic studies and field evaluation of *Spob*NPV is sparsely available. Earlier works on *Spob*NPV in regard to SEM and TEM studies revealed the occlusion bodies of *Spob*NPV to be tetrahedral and triangular in shape (Sivakumar et al. 2020). The present work is in accordance and is in confirmation with the findings of previous works. The size of poly occlusion bodies in present study was 2.55–2.90 µm, while that reported earlier was 0.6 to 2.0 µm (Senthil Kumar et al. 2015), 0.466 µm × 0.363 µm (Sujayanand et al. 2019) and 1.04–1.72 µm (Sivakumar et al. 2020). Similarly works revealing the tetrahedral and hexagonal shape of poly occlusion bodies with size ranged of 1.016 to 1.596 µm were reported in NPV infecting *Euproctis chrysorrhoea* (Hussain et al. 2019). Varied shapes, viz., polyhedral, cubic, triangular, spherical and oval of *Spob*NPV poly occlusion bodies, were earlier reported by Battu et al. (1991) from India. Likewise variedly shaped occlusion bodies', viz., tetrahedral, cuboidal, globular and irregular, have been reported earlier from other NPVs (Cheng and Carner 2000). Polyhedrin gene (*polh*) in tandem with other genes, viz., late expression factor 8 (*lef8*) and late expression factor 9 (*lef9*) which are considered as the most conserved regions of baculoviral genomes provides a plausible powerful tool in phylogenetic analysis of baculoviruses (Senthil Kumar et al. 2015). PCR amplification with degenerate primers yielded amplicon product of 691 bp for the targeted polyhedrin gene (*polh*); this was in corroboration of amplicon sizes 700 bp and 738 bp for the targeted gene in earlier studies (Sivakumar et al. 2020). The median lethal concentration (LC_{50}) of SpobNPV deduced from the bioassay studies, in the present study, was 2.42×10^4, 3.09×10^6 and 5.05×10^7 POBs/ ml for second, third and fourth larval instars of *S. obliqua*, respectively, at 72 h after treatment. The LC_{50} values deduced in other experiments conducted with *Spob*NPV against second and third larval instars of *S. obliqua* were 3.7×10^4 POBs/ ml and 4.7×10^4 POBs/

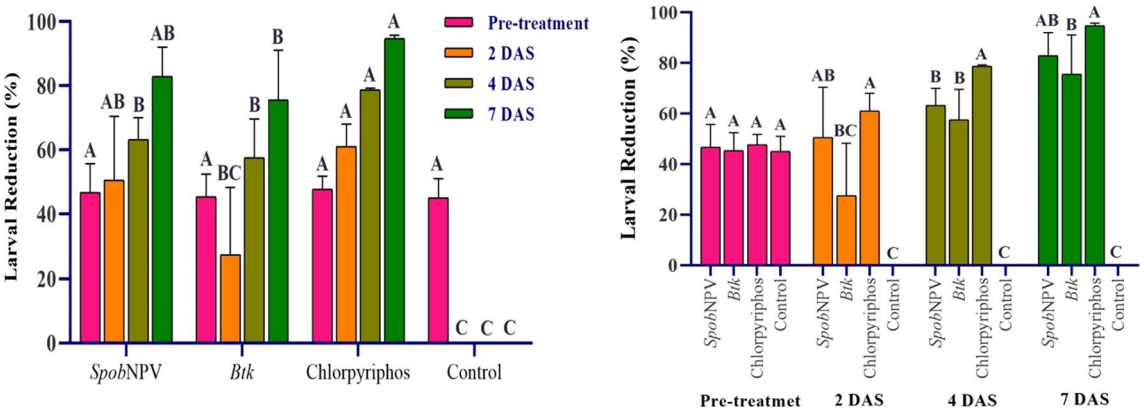

Fig. 6 *Spilosoma obliqua* third instar larvae reduction percentage at 2, 4 and 7 days after imposing of treatments. *DAS* Days after spraying

ml, respectively (Sivakumar et al. 2020). Natural epizootics of *Spob*NPV on larvae of *S. obliqua* dates back to early 1970s (Battu et al. 1977), but the studies pertaining to field efficacy and virulence testing are scanty. Field evaluation study in assessing the virulence of *Spob*NPV at concentration of 3.54×10^8 POB's/ml in the present study revealed the percentage larval reduction as 50.52, 63.25 and 82.91% at 2, 4 and 7 days post-spraying, respectively; similar type of result was reported earlier where in larval populations were reduced to the tune of 68.92, 78.59 and 93.16%, respectively, at 3, 4 and 7 days post-spray of *Spob*NPV at concentration of 1.5×10^{12} POB's/ml in jute crop (Sivakumar et al. 2020) and cowpea crop (Battu and Ramakrishnan 1989). Apart from field evaluation of *Spob*NPV studies have also been documented and reported were in *Spob*NPV viral inoculums @, 10^6 POBs/ml, 10^7 POBs/ml and 250 LE were successful in reducing the larval population of *S. obliqua* infesting several other crops by causing mortality to the 5^{th} instar larvae post 6–7 days after spraying (Veenakumari et al. 2008). In the present scenario, control management strategies for curtailing jute hairy caterpillar menace are relied mainly on insecticides. Integrated pest management mediated through microbial entomopathogenic fungi like *Beauveria bassiana* (Bals.-Criv.) Vuill. (1912) and biological control agents like the predator *Eocanthecona furcellata* (Wolff) (Kumar et al. 2001) and the parasitoids, *Meteorus spilosomae* Narendran and Rema, *Glyptapanteles agamemnonis* (Wilkinson 1932), *Protapanteles obliquae* (Wilkinson), *Blepherella lateralis* Macquart are effective against larvae of hairy caterpillar though the parasitization percentage was meagre (Sujayanand et al. 2019). Contemplating with frontier ill effects of insecticide usage, rift on microbial biocontrol agents encompassing baculoviruses is highly recommended and considered as suitable strategy of IPM in sustainable agriculture, owing with their integral features like intrinsic speed of kill, virulence, ease in field delivery and effortlessness in mass production at commercial level. Thus, *Spob*NPV strain isolated in the present study was found highly effective and has exemplary potential as biopesticide.

Conclusion

In the present investigation, highly pathogenic strain of *Spob*NPV was isolated from the natural epizootics, *i.e.,* from the farmer fields of West Bengal, India. The infestation levels and disease incidence were quantified. The *Spob*NPV was explicitly found lethal to second instar larvae based on the larval bioassays carried out in laboratory conditions. The median lethal concentrations deduced from the larval bioassays of various instars implicate the potential virulence nature of *Spob*NPV. Field experimental studies with this strain of *Spob*NPV revealed the larval mortality in short duration of time. The present study unleashes the entomopathogenic effects of *Spob*NPV in controlling the major lepidopteran defoliator, jute hairy caterpillar infesting jute. The findings from the present study authenticate the pathogenicity and virulence of *Spob*NPV on jute hairy caterpillar and thus undoubtedly can be brought out as a biopesticide thus, incorporating as a microbial biocontrol strategy in IPM of *S. obliqua.* Furthermore, commercial formulation of *Spob*NPV biopesticide would be handy in catering and deciphering the long-awaited need of farmers to curtail *S. obliqua* in a sustainable manner in the present scenario of organic farming.

Abbreviations

CRIJAF	Central Research Institute for Jute and Allied Fibres
*Spob*NPV	Spilosoma obliqua nucleopolyhedrovirus
POB's	PolyOcclusion Bodies
HAT	Hours after treatment
DAS	Days after spraying
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
ICAR	Indian Council of Agricultural Research
PCR	Polymerase chain reaction
LC_{50}	Lethal concentration
g	Gravity force or relative centrifugal force

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Author contributions

VRB and SS designed and executed the experiments and carried out the study. VRB and GS wrote the manuscript. VRB and SS analyzed the data. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Consent for publication was taken from the co-authors.

Competing interests

The authors declare that they have no competing interests.

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