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Isolation and characterization of indigenous nucleopolyhedrovirus infecting fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in India

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Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is an invasive insect pest of maize in India. Natural occurrence of nucleopolyhedrovirus (NPV) infection on *S. frugiperda* larvae was recorded in 2018 during surveys conducted in maize fields in Chikkaballapura district of Karnataka, and Coimbatore and Tirupattur districts of Tamil Nadu. A strain of *S. frugiperda* nucleopolyhedrovirus (SpfrNPV NBAIR1) infecting *S. frugiperda* was isolated from the diseased larvae; morphological and biological characteristics were studied. Electron microscopic studies showed tetrahedral-shaped SpfrNPV occlusion bodies (OBs) of size 1.64 μm. Dose–mortality bioassays revealed that first, second and third instar larvae were equally susceptible (LC_{50} 3.71–5.02 OBs/mm^2) to SpfrNPV infection. A PCR technique for detection of viral DNA in *S. frugiperda* NPV was developed by employing the polyhedrin gene (*polh*)-specific primers. The amplicon of 618 bp was amplified, sequenced and NCBI GenBank accession number was obtained (MT422725). Blast analysis revealed that SpfrNPV conserved *polh* gene sequence matched 100% with the reference GenBank sequence (J04333) from the NCBI database which confirmed the identity of the SpfrNPV.

Keywords: Insect pests, maize, nucleopolyhedrovirus, *Spodoptera frugiperda*.

MAIZE is an important cereal crop cultivated in India after rice and wheat and is valued as food, feed, fodder and industrial raw material. India is the major maize producer and contributes 2% of the world's production[1]. The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) has been reported as a devastating invasive insect pest of maize in America and Africa. *Spodoptera frugiperda* invaded India during May 2018 and then moved onto the neighbouring countries[2]. The pest is now reported in Yemen, Bangladesh, Sri Lanka, Thailand, Myanmar, China, Indonesia, Laos, Malaysia, Vietnam, Republic of Korea and Japan within a short span of time[3]. It has now established in India and causes extensive damage on maize throughout the country. The outbreak of *S. frugiperda* in India is of serious concern because of the favourable weather conditions and availability of maize throughout the year. Maximum incidence (62.5%) of the pest was reported from Hassan district, Karnataka[4]. Advisories have been issued by the Government of India for its management through the use of biopesticides and chemical insecticides to avoid extensive damage on maize (http://ppqs.gov.in). Fall armyworm feeds on above-ground parts of maize, especially on the leaf whorl of young plants, which are up to 45 days old. Larvae are voracious feeders and can destroy the whole plant in a short time[5]. Studies in Africa indicated that infestation of *S. frugiperda* on maize exceeds 94% with damage levels ranging between 25% and 50% (ref. 6).

The development of multi-insecticide resistance and resistance against transgenic *Bt* maize makes *S. frugiperda* a challenging insect pest to manage using chemical insecticides and *Bt* toxins. *Spodoptera frugiperda* nucleopolyhedrovirus (SpfrNPV) belongs to the Baculoviridae

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Figure 1. Diseased larvae of *Spodoptera frugiperda* showing characteristic viral infection symptoms.

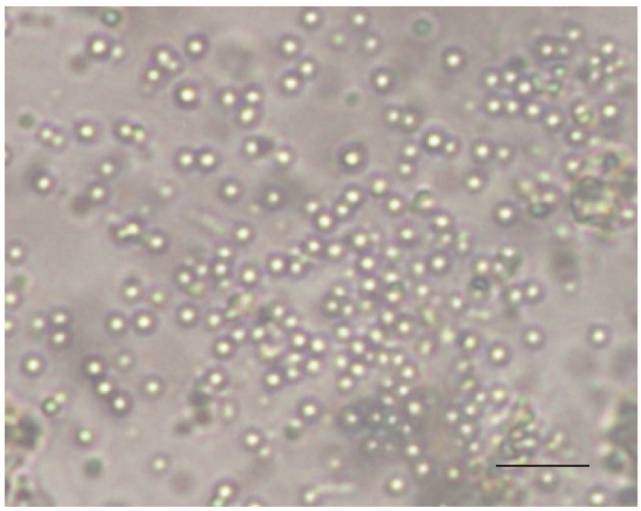

Figure 2. Light microscopy view of SpfrNPV occlusion bodies.

virus family and is recognized as an alternative method for managing insect pests effectively and in an environmentally sustainable manner. Baculoviruses are reported to be highly specific, virulent and safe to non-target organisms[7–9]. Globally, different geographical isolates of SpfrNPV have been identified and used as potential (>80% efficacies) biological control agents against FAW[10–12]. Several populations of SpfrNPV have been collected in North, Central and South America, Colombia and Brazil[13–16]. SpfrNPV is specific to FAW larvae, and 86.6–100% mortality of the larvae was recorded both in laboratory and field experiments[17,18]. During the course of the present study in maize fields, for recording the possible occurrence of FAW, few NPV-infected larvae were collected. Also, a novel virulent isolate of NPV associated with *S. frugiperda* has been characterized.

Surveys were conducted in maize fields of Chikkaballapura district in Karnataka and Coimbatore and Tirupattur districts in Tamil Nadu, and the diseased larvae of FAW showing viral infection symptoms were collected (Figure 1). *Spodoptera frugiperda* was further identified by amplification of mitochondrial cytochrome oxidase I gene (*COI*) marker. The nucleotide sequence was submitted to GenBank and the accession number was received (MK041922). The phase-contrast microscope visualization of discharged body fluids showed abundant spherical particles like occlusion bodies (OBs) of baculovirus, especially nucleopolyhedrovirus (Figure 2). The OBs of nucleopolyhedrovirus were extracted from the diseased larvae following the standard procedure[19] and they were counted using a Neubauer's haemocytometer fixed on a phase-contrast compound light microscope at 10× and 40× magnification.

The morphological characterization of the extracted OBs was done using scanning electron microscope (SEM) and transmission electron microscope (TEM). Samples of purified OBs were processed using the standard protocol described by Martins *et al.*[20]. Further, the size of OBs and nucleocapsids were measured in the electron micrographs. The SpfrNPV solution was prepared and the LC_{50} values for first, second and third instar larvae were assessed using independent leaf disc bioassay method of Magholi *et al.*[21]. The mortality of larvae was recorded on alternate days for six days post-treatment. The assays were carried out thrice and the pooled larval mortality data were subjected to probit analysis using the software POLO[22] to calculate the LC_{50} values for larval instars.

The genomic DNA of NPV was isolated by the kit method (DNeasy Blood and Tissue Kit, Qiagen, Germany) and visualized using 0.8% of agarose gel. The conserved polyhedrin gene (*polh*) of NPV was targeted as template DNA. Species-specific primers (F′ TCTAGGTTCGGTCATCAAGAAT; R′ TTGAACACGAGCGACAGTT) were designed based on the NCBI database and used for the detection of *polh* gene. PCR master mixture was used according to the protocol suggested by Thermo Scientific. The targeted marker was amplified by PCR reaction with 35 cycles of denaturation, annealing and extension. Amplified products were visualized using 1.2% agarose gel stained with ethidium bromide and compared with the GeneRuler 100 bp DNA ladder (Thermo Scientific). The eluted DNA template sequences were generated at

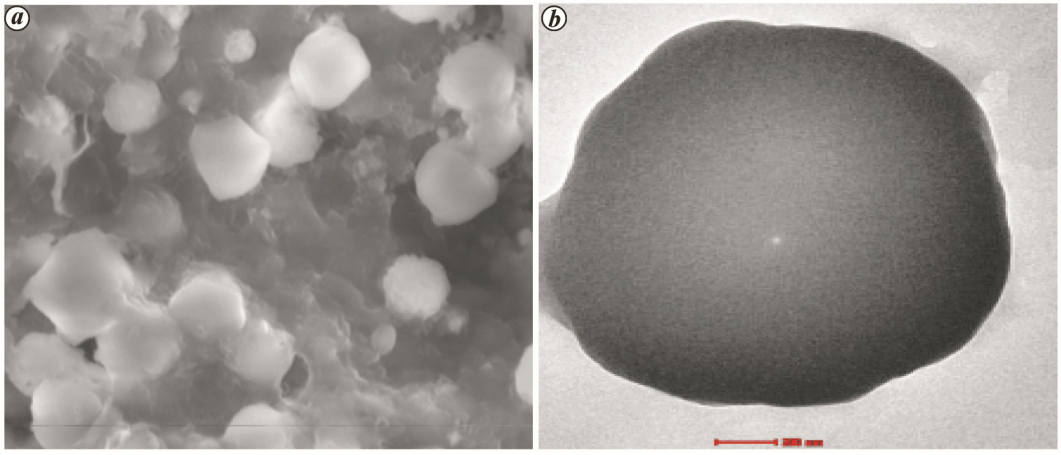

Figure 3. (*a*) Scanning and (*b*) transmission electron photomicrographs of tetrahedral occlusion bodies of SpfrNPV.

Table 1. Larval mortality in the different instars of *Spodoptera frugiperda* by SpfrNPV

SpfrNPV concentration	Number of dead larvae			Mortality (%)		
	Instars			Instars		
	First	Second	Third	First	Second	Third
1×10^8 POBs/mm^2	$45.2^a \pm 0.00$	$44.6^a \pm 0.07$	$44.4^a \pm 0.02$	$90.4^a \pm 0.71$	$89.2^a \pm 0.61$	$88.8^a \pm 1.18$
1×10^6 POBs/mm^2	$39.4^b \pm 0.06$	$38.2^b \pm 0.02$	$37.4^b \pm 0.03$	$78.8^b \pm 0.86$	$76.4^b \pm 1.32$	$74.8^b \pm 0.20$
1×10^4 POBs/mm^2	$29.2^c \pm 0.02$	$27.4^c \pm 0.02$	$27.0^c \pm 0.04$	$58.4^c \pm 0.47$	$54.8^c \pm 0.39$	$54.0^c \pm 0.06$
1×10^2 POBs/mm^2	$20.6^d \pm 0.09$	$18.2^d \pm 0.01$	$17.0^d \pm 0.00$	$41.2^d \pm 0.46$	$36.4^d \pm 0.04$	$34.0^d \pm 0.16$
Control	$4.2^e \pm 0.00$	$5.4^e \pm 0.07$	$6.2^e \pm 0.02$	$8.4^e \pm 0.20$	$10.8^e \pm 0.20$	$12.4^e \pm 0.23$
SE(d)	0.046	0.027	0.042	0.837	0.969	0.782
CD ($P = 0.05$)	0.104	0.060	0.095	1.888	2.186	1.765

Table 2. Probit regression analysis of mortality data of SpfrNPV against *S. frugiperda*

Instars	Number of larvae used	LC_{50} (POBs/mm^2)	Slope ± standard error	95% Fiducial limits		χ^2	Degrees of freedom
				Lower	Upper		
First	50	3.71	1.12 ± 0.12	2.231	8.301	1.31	4
Second	50	4.49	0.90 ± 0.11	2.832	11.205	7.20	4
Third	50	5.02	1.0 ± 0.12	3.721	13.330	3.32	4

Scigenome Laboratory, India. Confirmation of the identity of SpfrNPV was done based on nucleotide sequence homology and matching sequence similarity was done with the NCBI GenBank database using BLAST analysis. The nucleotide sequence was submitted to GenBank and accession number was assigned as MT422725.

Under SEM the OBs of SpfrNPV appeared tetrahedral in shape (Figure 3 *a*). TEM showed tetrahedral-shaped OBs of size 1.64 µm (Figure 3 *b*). Laboratory experiments revealed that the virus concentration, i.e. 1×10^8 OBs/mL caused high percentage of larval mortality of 90.4, 89.2 and 88.8 to the first, second and third instar larvae of *S. frugiperda* respectively (Table 1). The study also showed that the mortality pattern of different larval instars was based on stage and concentration of SpfrNPV, and the calculated LC_{50} values for the first, second and third instar larvae of *S. frugiperda* were 3.71, 4.49 and 5.02 OBs/mm^2 respectively (Table 2). Finally, the NPV-infected larvae dissolved and liquefied (Figure 4 *a*). Pupal infection was also noticed in some of the replications at the end of the bioassay (Figure 4 *b*). Observation of discharged liquid of infected pupae under light microscope also revealed the presence of OBs.

A PCR technique for detection of viral DNA in SpfrNPV was developed by employing the *polh* gene NPV specific primers. The amplicon of 618 bp was amplified, and nucleotide sequence was submitted and accession number was obtained from GenBank (MT422725). Blast analysis revealed that the sequence of *polh* gene of SpfrNPV matched 100% with the reference sequences in the NCBI database, and the identity of SpfrNPV was confirmed.

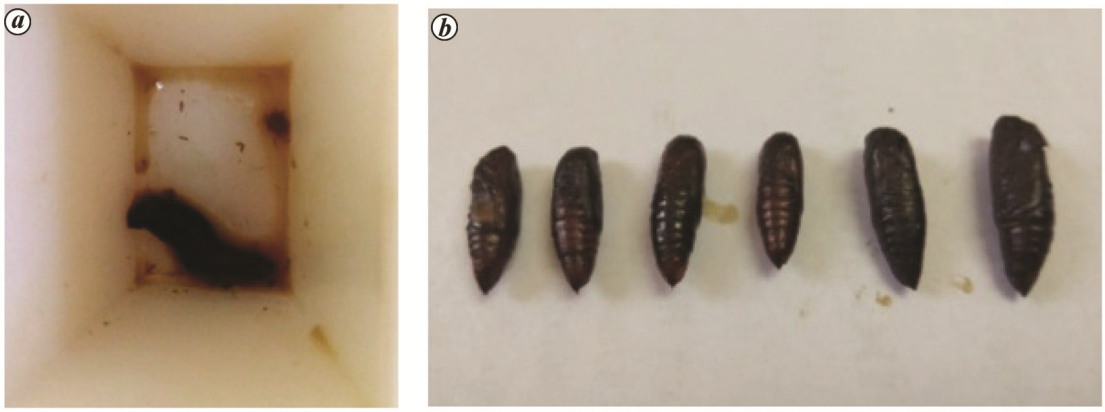

Figure 4. (***a***) Liquefaction of *S. frugiperda* larva and (***b***) liquid discharge of *S. frugiperda* pupae infected by SpfrNPV.

FAW is one of the most destructive insect pests of maize in India and its management has been done using different insecticides. However, studies have reported that continuous usage of insecticides leads to resistance development in *S. frugiperda* populations[23–25]. NPVs are important pathogens which infect and kill only the target insects. NPV is known for high epizootic levels and is naturally occurring, self-perpetuating, safe to natural enemies due to host specificity and eco-friendly. Hence, they could be potential biopesticides and an effective alternative to chemical insecticides in *S. frugiperda* management in India. Significant research has been done across the world on the natural occurrence and infectivity of NPVs in various lepidopteran insect pests. Raghunandan *et al.*[26] reported the natural occurrence of SpfrNPV from Gujarat, while Firake and Behere[27] reported it from Meghalaya. The present study has succeeded in isolating and characterising a NPV associated with FAW on maize. Electron microscopic studies revealed the tetrahedral-shaped OBs of SpfrNPV. The tetrahedral and other shapes in OBs have been described earlier in several NPVs[19,28–30]. Since, *polh* gene is highly conserved, it serves as an excellent molecular diagnostic tool to characterize and identify the baculoviruses of lepidopteran insect pests[31–33]. There is a growing concern regarding the use of hazardous chemical insecticides for the management of FAW. Thus, management of maize FAW using SpfrNPV could be an effective, promising and eco-friendly alternative to hazardous synthetic insecticides.

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