CONFIRMATION OF VERTICAL TRANSMISSION OF NUCLEOPOLYHEDROVIRUS IN THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD) USING POLYMERASE CHAIN REACTION (PCR)

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INTRODUCTION

Vertical transmission, or parent-to-offspring passage of a pathogenic microorganism, is a phenomenon commonly observed with some insect viruses. Such transmission is common in cytoplasmic polyhedrosis viruses (CPVs) (Belloncik and Mori, 1998) and nucleopolyhedroviruses (NPVs) (Kukan, 1999). The mechanisms might include transovum transmission on the egg surface, transovarian transmission within the egg and passage of latent or persistent infections. In the latter case, the virus is in a non-infective and non-replicative state in the host without causing overt disease, but it can be transformed to a replicative and infective state when the host is stressed (Fuxa *et al.*, 1992).

Vertical transmission may be potentially useful in microbial control (Fuxa and Richter, 1991). One NPV isolate which persisted for at least in seven generations of the insect host was selected by Fuxa and Richter (1992). Vertical transmission of insect pathogens could function to transport these pathogens in the ecosystem and provide foci of new infections (Fuxa *et al.*, 1992; Fuxa and Richter, 1993). Some NPVs were activated by stressors (Fuxa *et al.*, 1999), and transmitted transovarially (Fuxa *et al.*, 1999). Environmental harm is difficult to predict, even if a virus is harmless to certain non-target organisms in laboratory tests (Fuxa, 1990). Therefore, vertical transmission is pertinent to risk assessment of recombinant NPVs in pest management. Thus, a vertically transmitted virus is more likely to be transported from release sites (*i.e.* to increase the risk of environmental harm) than one that is not.

Due to the importance of *S. littoralis* as a pest insect and experimental laboratory animal (Abul-Nasr, S. 1956 & 1959; El-Dafrawi *et al.*, 1964; Shorey & Hale, 1965), its mass rearing is very important in many laboratories. Some

experiments require healthy insects for at least two and up to eight or more generations. NPV infection was the major obstacle in developing successful rearing technique for several generations.

Yet, there has been no research on vertical transmission or induction of spontaneous *Spli*NPV infection in the Egyptian cotton leafworm. The purpose of this research was to determine whether the selected *Spli*NPV is vertically transmitted in *S. littoralis*, to quantify its vertical transmission and to test some stress factors that induce spontaneous NPV disease in *S. littoralis*.

MATERIAL AND METHODS

Insect rearing

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.), was collected from various locations in Egypt. Field-collected larvae were reared in the insectary of the AGERI (Agricultural Genetic Engineering Research Institute) under highly controlled conditions to avoid contamination. Larvae were fed on either decontaminated castor bean foliage or semisynthetic diet described by Shorey and Hale (1965). The insect culture was maintained at 25 ± 2 °C, 65-70% RH and natural photoperiod.

Virus isolate

In spite of highly controlled conditions in the laboratory, typical symptoms of NPV infection (loss of appetite, slow motion, pinky rose venter, easily injured skin and completely liquefied organs) were easily observed in *S. littoralis* larvae when the colony was maintained for two or three successive generations. NPV was isolated from the diseased larvae, propagated and selected for vertical transmission studies. The virus isolate was nominated *Spli*NPV as described by Seufi and Osman (2005).

Diagnosis of the disease

The larvae were observed daily to identify the NPV infected ones based on the signs and symptoms of disease. The tissues of dead larvae were examined as soon as possible with the naked eye and tissue smears under light microscopy. If crystals resembling viral polyhedral occlusion bodies (OBs) were observed, they were dissolved in 1N NaOH and tested under phase contrast microscope to determine their viral origin (Fuxa and Richter, 1991). Furthermore, nonstainability with Sudan III, and lack of light transmission under double polarization were used as positive indications of polyhedrosis (Thomas, 1974). Insects were counted as

infected only if the OB passed all three tests. Some of the smears were stained with 0.1% bromophenol blue (Sigma) for 15 min and air dried. The polyhedra remain colourless (Seufi and Osman, 2005).

Purification of virus occlusion bodies

Larvae with symptoms of NPV infection were pooled out from the stock culture and triturated in distilled water. The homogenate was filtered through muslin cloth. The filtrate was subjected to repeated centrifugation until clear white OBs were obtained. They were also obtained directly by bleeding the haemolymph of infected larvae. The purified OBs were stored at -80 °C until their use (Khurad *et al.*, 2004).

Infection of larvae, mating of adults and histology

For vertical transmission experiment, 500 newly moulted fifth instar healthy larvae were starved for 12 h and divided into five groups, each containing 100 larvae. The larvae from four groups were inoculated individually with 10 µl virus suspension of different concentrations by droplet feeding method. The first group was inoculated with 400, second with 800, third group with 1600, fourth group was inoculated with 3200 OBs/ larva, and fifth group was fed on distilled water treated diet and used as a control. Each experiment was replicated thrice with 100 larvae per replicate (n = 300). The mortality due to infection in larval and pupal stages, percentage emergence and moth infectivity were recorded. The moths emerged from each group were provisionally regarded as infected. Those emerged from the pupae of the group inoculated with 3200 OBs/ larva, were selected for the mating experiment. Infected females (IF) were paired with healthy (uninfected) males (HM) for transovarial transmission studies and healthy (uninfected) females (HF) were paired with infected males (IM) for venereal transmission. In addition, IF and IM were also paired to assess the severity of transmission. HF and HM were paired as controls. After oviposition, all the moths, both male and female were macerated and examined for NPV infection under phase contrast microscope. The egg batches were collected, washed (4 min in 0.525% NaClO) and the insects were reared to the adult stage of the F_1 generation. Percentages of infection in F_1 insects (as determined by mortality and microscopic examination), egg productivity and hatchability were determined. The newly hatched larvae were reared on fresh semisynthetic diet and their mortality due to transmission of infection was recorded at each succeeding larval stage. The larval progeny died due to NPV infection were homogenized individually in distilled water and OBs were isolated and counted using haemocytometer. Few insects from each group were sacrificed daily for histopathological study on testes and ovaries of inoculated insects. Insects were dissected under a stereoscopic binocular microscope, testes and ovaries were fixed in Bouins

fixative for 24 h, dehydrated in alcohol series, cleared in xylene and embedded in paraffin wax. Embedded materials were sectioned at 6 μm and stained with Azan stain (Humason, 1962).

DNA extraction, PCR amplification, sequencing and sequence analyses

The viral DNA was isolated from the OBs collected from parent and F_1 offspring according to Summers and Smith (1987) with slight modifications. Briefly, the OBs were digested by proteinase K for about 4 h and DNA was extracted twice with phenol: chloroform:isoamyl alcohol (25:24:1), precipitated in 2.5 volumes of ethanol, washed with 70% ethanol, and resuspended in 10mM Tris–1mM EDTA. PCR amplification was performed according to Seufi *et al.* (2006). A DNA segment was amplified using two polyhedrin-based primers. The forward primer: GTC AAG CCG GAT ACT ATG AA and the reverse one: GTT CCG ATG TAT ACG ATT GG. Total reaction volume was 50 μl which contained 1x PCR buffer (Promega), 1.5 mM $MgCl_2$, 200 mM dNTPs, 2.5 U Taq DNA polymerase (Promega), 100 ng of each primer and 30 ng of template DNA. The amplification program used was 5 min at 94 ℃ (hot start), 1 min at 94 ℃, 2 min at 55 ℃ and 2 min at 72 ℃ for 35 cycles followed by one cycle of 72 ℃ for 7 min. PCR amplification was carried out in a DNA thermal cycler (Model 380 A, Applied Biosystems, CA, USA). The reaction product was analyzed on 1% agarose gel and eluted from the gel using GenClean Kit (Invitrogen Corporation, San Diego, CA, USA) as described by the manufacturer. For DNA sequencing, 50 ng of a highly purified PCR product was used in a sequencing reaction using M_{13} universal forward and reverse primers. Sequencing was performed using T^7SequencingT^M kit (Pharmacia, Biotech) and model 310 automated sequencer (Applied Biosystems, Foster City, CA, USA). The sequences obtained were checked against National Centre for Biological Information (NCBI) database using BLASTN search algorithm (Altschul *et al.*, 1997).

Statistical analyses

Statistical analyses for biological data were carried out using the computer program SPSS for Windows (Version 11.0). ANOVA and subsequent multiple comparison tests (Scheffé) were done to calculate the significance level between means.

RESULTS AND DISCUSSION

Diagnosis of the disease

The diseased larvae exhibited typical symptoms of NPV infection. Loss of appetite, slow motion, pinky rose venter, swelled body segments, easily injured skin

and completely liquefied organs were easily observed. At an advanced stage of infection, the body wall ruptured and a turbid haemolymph containing a large number of OBs oozed out. The cadavers became dark within 7-10 h. Infected larvae were often hanging upside down by hind prolegs in an inverted V position. Smears of the body liquid contents were examined under light microscope, dissolved in NaOH and tested under phase contrast microscope. Nonstainability with Sudan III, and lack of light transmission under double polarization were used as positive indications of polyhedrosis. After staining with 0.1% bromophenol blue (Sigma), polyhedra did not attain the stain so they appeared as polygonal unstained particles dipped in a blue matrix (Fig. 1A).

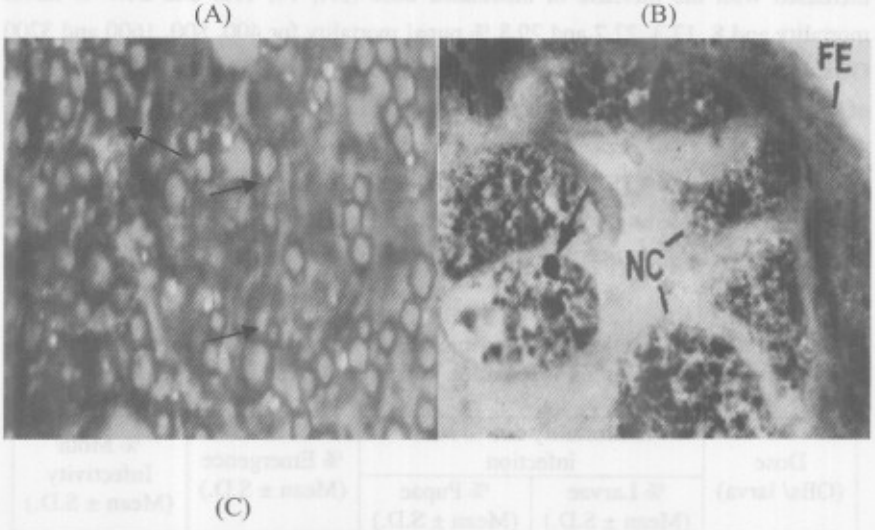

Fig. (1) A: *Spli*NPV stained with 1% bromophenol blue. B & C: NPV-infection in the rudiments of ovary and testis of *S. littoralis*. (Fig. 1B) Young oocyte follicle enveloped with follicular epithelium (FE) showing OBs (arrows) in the nuclei of nurse cells (NC) 7 days post inoculation. (Fig. 1C): Magnified view of *Spli*NPV-infected spermatocytes in the cyst containing OBs (arrows). Azan stained (oil immersion, 2500 x).

Effect of NPV-infection on *S. littoralis* after inoculation as fifth instar larvae

In cotton leaf worm, the most common mode of entry of NPV is *per os* as in the case of other lepidopteran insects. Individual feeding of 10 μl suspension of 3.2 X 10^5 OBs/ ml by droplet feeding method ensured the ingestion of the expected dosage of about 3200 OBs/ larva which was sufficient to initiate the infection since about 55 % mortality of larvae and pupae occurred prior to eclosion of adult moths and all of the adults were infected. At lower dosages of 400, 800, and 1600 OBs/ larva many individuals survived to adulthood (88.4, 68.7 and 59.2 %, respectively) and about 81.4, 91.3, and 96.8 % of these adults were infected, respectively (Table 1). The percentages of larval and pupal mortality due to NPV-infection was increased with the increase of inoculated dose (3.4, 14, 19.8 and 24.7 % larval mortality and 8, 17.3, 22.7 and 29.8 % pupal mortality for 400, 800, 1600 and 3200 OBs/ larva, respectively). Multiple comparison tests (Scheffé) and significance level of difference between means were indicated by Duncan's test (Table 1). Usually, a successful infection depends on the ingestion of sufficient virus to initiate replication in the host (Khurad *et al.*, 2004) and the virus up take is controlled by larval behavior, feeding rate, and the concentration and distribution of the viral dosage (Hunter *et al.*, 1984).

TABLE (I)

Effect of *Spli*NPV infection on larval mortality, %pupation, %emergence and moth infectivity of *S. littoralis* after inoculation as fifth instar larvae.

Dose (OBs/ larva)	% Mortality due to NPV infection		% Emergence (Mean ± S.D.)	% Moth Infectivity (Mean ± S.D.)
	% Larvae (Mean ± S.D.)	% Pupae (Mean ± S.D.)		
Control	00	00	100	00
400	3.4^{a*} ± 1.1 (2 - 5)	8.0^{a} ± 0.9 (7 – 9)	88.4^{a} ± 1.1 (87 - 90)	81.4^{a} ± 6.9 (70 – 87)
800	14.0^{b} ± 2.5 (10 - 17)	17.3^{b} ± 3.8 (11 - 22)	68.7^{b} ± 3.6 (65 - 75)	91.3^{b} ± 3.7 (87 - 97)
1600	19.8^{bc} ± 2.5 (16 – 24)	22.7^{bc} ± 4.2 (18 - 30)	59.2^{bc} ± 4.0 (54 - 66)	96.8^{bc} ± 2.2 (94 - 100)
3200	24.7^{bcd} ± 3.4 (20 - 30)	29.8^{bcd} ± 3.4 (25 - 35)	45.5^{bcd} ± 4.5 (40 - 51)	100.0^{bc} ± 0.0 (100 - 100)

*Numbers between brackets refer to the range (Min. – Max.).
Means with the same letter vertically were not different (P < 0:05, ANOVA, Scheffé).

Mating and vertical transmission of *Spli*NPV

The fecundity of *S. littoralis* was significantly reduced ($P < 0.01$) in all mating experiments (IF X IM, IF X HM and HF X IM) than controls (HF X HM). This may be attributed to improper vitellogensis in the infected oocytes (Khurad *et al.*, 2004). Meanwhile, no significant difference ($P > 0.05$) was observed between the three above mentioned mating pairs. A number of studies have shown that pupae survived exposure to NPV as larvae have reduced fecundity (Rothman and Myers, 1994 and 1996). Sublethal infection could influence the fecundity of moths by reducing the amount of fat and other nutrients available for egg or sperm production or through some associated cost of resistance mechanisms either when initially fighting off infection or in maintaining it at a nonlethal level (Myers and Kukan, 1995). However, sublethal infection could also influence fecundity through an interaction with the hormonal balance and/ or enzymatic activity of the individual during development (Myers *et al.*, 2000). Burand and Park (1992) reported that the infection of gypsy moth with NPV slowed larval growth and inhibited larval molting and pupation. Moreover, the number of hatched eggs was significantly lower in all the three mating pairs than control with significant differences between the three groups (Table 2). Furthermore, the prevalence of *Spli*NPV after decontamination of egg-surface was recorded in the larval progeny of all mating pairs. However, 100% death of larval progeny of IF X IM mating occurred at first (67.8 %) and second (32.2 %) larval instars only, reflecting the severity of virus infection. The progeny of IF X HM mating pair died at first (67.6 %), second (24.8 %) and third (7.6 %) larval instars, suggesting *Spli*NPV transmission via eggs (transovarial transmission), whereas the progeny of HF X IM mating pair died at first (5.0 %), second (11.8 %), third (26.6 %), fourth (49.8 %), fifth (4.4 %) larval instars and pupal (2.4 %) stage indicating the possibility of venereal transmission of *Spli*NPV analogous to the latent form and death occurring at later stage of their development. Statistical analyses revealed that the mortalities of first and second larval instars were significantly higher ($P< 0.01$) in transovarial mating pairs (IF X IM and IF X HM) than in venereal mating pair (HF X IM). Moreover, the second larval mortality decreased significantly ($P< 0.001$) between the three mating pairs (Table 2). The onset of mortality was observed at first instar larvae in case of transovarial mating pairs, while it was observed at fourth instar larvae in case of venereal mating pair. Furthermore, the number of OBs/ larvae was significantly lower ($P< 0.01$) in case of transovarial mating pairs when compared to venereal pair. Level of significance and multiple comparisons between groups were recorded in Table (2). These results indicate the vertical transmission of *Spli*NPV in the progeny of all the three mating pairs. The degree of infection and expression of disease in the larval or pupal stage

varied which also reflected on the recovery of OBs from the progeny of IF X IM, IF X HM, and HF X IM mating pairs (Table 2). Although the transovarial and venereal transmission of NPV in insect populations are questionable (Payne, 1982), there are some clear evidence of transovarial transmission of viruses. Recently, Sun *et al.* (2005), Zhou *et al.* (2005) and Khurad *et al.* (2004) confirmed transovarial transmission of *Ha*SNPV-WT and *Ha*SNPV-AaIT in *Helicoverpa armigera* and vertical transmission of *Bm*NPV in *Bombyx mori*, respectively. In addition, Fuxa *et al.* (2002 and 1999) reported transovarial transmission of *Tn*NPV, *Tn*CPV, and *Ac*MNPV in *Trichoplusia ni* population. Furthermore, it was reported that sublethally infected larvae are able to complete development and carry the disease to next generations either mostly by external contamination (Murray and Elkinton, 1989 and 1990), as a low level, persistent infection (Kukan and Myers, 1997) or in an inactive, latent form (Hughes *et al.*, 1993). Previously published research in certain other insect/ NPV systems reported lower prevalence rates of vertical transmission after treatment of larvae with homologous NPV: 17–57% in *Mythimna separata* (Neelgund and Mathad, 1978), 38–48% in *S. littoralis* (Abul-Nasr *et al.*, 1979), 3–8% in *Pseudoplusia includens* (Young and Yearian, 1982), 5–12% in *Lymantria dispar* (Shapiro and Robertson, 1987), 10–28% in *S. exigua* (Smits and Vlak, 1988), 4–14% in *S. frugiperda* (Fuxa and Richter, 1991, 1993), and 5–24% in *S. frugiperda* NPV selected for increased transmission (Fuxa and Richter, 1991). On the other hand, *Tn*NPV was not vertically transmitted in one study (Vail and Hall, 1969), but this was after treatment of first instars in the parental generation. In at least two systems, the rate of vertical transmission increased as the age of treated larvae increased (Young and Yearian, 1982; Fuxa and Richter, 1991). Ridhards *et al.* (1998), Kukan (1999) and Kukan and Myers (1999) reviewed that dispersal of baculoviruses by adults may occur following vertical transmission to larvae either inside the egg (transovarial) or to its surface (transovum). However, they pointed out that the dominant source of virus among generations is probably through environmental contamination.

Histopathology

Histopathological observations revealed that the infection of gonadal rudiments occurred quite late at about 9 days postinfection. In ovaries, the nuclei of nurse cells in the young oocyte follicle contained OBs. In testes, the wall (epithelial lining) of the follicle and a few primary and secondary spermatocytes in the cysts exhibited OBs in the nuclei (Figs. 1B and C). These observations clearly indicate that young oocytes leading to maturation and spermatocytes leading to reduction division harboring virions in them; and in survivors; the infection persisted in the

eggs and spermatozoa during pupal and adult stages. Similar results were presented on *S. littoralis* by Seufi (2002) and *B. mori* by Khurad *et al.* (2004).

TABLE (II)

Rate of transovarial and venereal transmission of *Spli*NPV from infected parents to the progeny of *S. littoralis*.

Parameter assayed (Mean ± S.D.)		Paired moths			
		HF X HM	IF X IM	IF X HM	HF X IM
No. of eggs laid		$480.2^{a} \pm 39.9$ (421 – 537)	$365.9^{b} \pm 36.2$ (314 – 413)	$398.1^{b} \pm 39.1$ (345 – 453)	$373.2^{b} \pm 40.7$ (315 – 427)
% Hatchability		$88.4^{a} \pm 2.3$ (85.7 – 91.3)	$38.5^{b} \pm 1.8$ (35.9 – 40.9)	$46.3^{bc} \pm 3.1$ (42.7 – 50.0)	$51.4^{bcd} \pm 2.7$ (47.9 – 55.1)
% Mortality of F_1 larvae due to NPV-infection	L_1	0.0	$67.8^{a} \pm 4.1$ (63 – 74)	$67.6^{a} \pm 7.9$ (59 – 78)	$5.0^{b} \pm 2.4$ (2 – 8)
	L_2	0.0	$32.2^{a} \pm 4.1$ (26 – 37)	$24.8^{b} \pm 4.2$ (20 -30)	$11.8^{bc} \pm 2.3$ (9 – 15)
	L_3	0.0	0.0	$7.6^{a} \pm 4.1$ (2 – 12)	$26.6^{b} \pm 3.2$ (23 – 31)
	L_4	0.0	0.0	0.0^{a}	$49.8^{b} \pm 4.5$ (43 – 56)
	L_5	0.0	0.0	0.0^{a}	$4.4^{b} \pm 3.0$ (0 – 9)
% Mortality of F_1 pupae due to NPV-infection		0.0	0.0	0.0^{a}	$2.4^{b} \pm 2.2$ (0 – 5)
No. of OBs/ larva ($X 10^5$)		0.0	$1.56^{a} \pm 0.53$ (0.9 – 2.4)	$1.7^{a} \pm 0.55$ (1.0 -2.5)	$2.61^{b} \pm 0.39$ (1.9 – 3.2)

*Numbers between brackets refer to the range (Min. – Max.).
Means with the same letter horizontally were not different ($P < 0:05$, ANOVA, Scheffé).

PCR amplification:

Previous research has indicated that adults are not killed by vertically transmitted NPV (Fuxa and Richter, 1991). Therefore, PCR was used to detect the virus in each insect that survived to the adult stage in each treatment group, including controls. DNA isolated from the polyhedra collected from both parent and offspring yielded a product of 332 bp from polyhedrin gene upon amplification as expected, while the negative control did not result in any amplification (Fig. 2). Further, sequencing of the PCR product confirmed that the amplification product was of *Spli*NPV origin. No differences in nucleotide sequence of *Spli*NPV isolated from parent and F_1 offspring were observed (Fig. 3). The BLAST results showed >98% similarity with the published *Spli*NPV sequence (GenBank Acc. # AY442260). These results confirm that the *Spli*NPV recovered from F_1 offspring are derived from parent through vertical transmission.

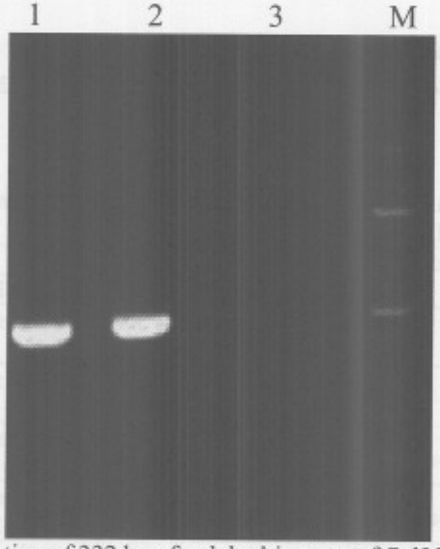

Fig. (2): PCR amplification of 332 bp of polyhedrin gene of *Spli*NPV isolated from: lane 1: infected parent, lane 2: F_1 offspring of infected parent and lane 3: negative control (DNA from freshly hatched F_1 larvae of healthy parent). M: 100 bp size marker.

F_1 offspring: GGGGCGGGCAAGAATCAGAAGTTGACTCTGTTCAAGGAGATCCGTAAC

Infected parent: GGGGCGGGCAAGAATCAGAAGTTGACTCTGTTCAAGGAGATCCGTAAC

F_1 offspring: TTGCCACCCGACACGATGAAGCTGATCGTCAACTGGAACGGCAAAGAGT

Infected parent: TTGCCACCCGACACGATGAAGCTGATCGTCAACTGGAACGGCAAAGAGT

F_1 offspring: TTCTCCGTGAGACTTGGACCCGTAATAATGGAAGACAGCTTCCCCATCGT

Infected parent: TTCTCCGTGAGACTTGGACCCGTAATAATGGAAGACAGCTTCCCCATCGT

F_1 offspring: GAACGATCGAGAAGTGATGGACGTGTTTCTGTGGTGAACATGCGTCCCAC

Infected parent: GAACGATCGAGAAGTGATGGACGTGTTTCTGTGGTGAACATGCGTCCCAC

F_1 offspring: CTAGACCGACCGGTGCTTTTAAAGTGTTGGCGCAAACGCGCAACCGTCGT

Infected parent: CTAGACCGACCGGTGCTTTTAAAGTGTTGGCGCAAACGCGCAACCGTCGT

F_1 offspring: ACGTCGGCACCAACAATGAATACCGCATCAGTCTCGCCAAGAAAGGTGGC

Infected parent: ACGTCGGCACCAACAATGAATACCGCATCAGTCTCGCCAAGAAAGGTGGC

F_1 offspring: TGTCCCGTGATGAACCTGCACGCCGAATACACCAC

Infected parent: TGTCCCGTGATGAACCTGCACGCCGAATACACCAC

Fig. (3): *Spli*NPV 332 bp sequence from *Spli*NPV isolated from infected females (IF) and F_1 offspring of IF X healthy male (HM).

The present study based on demonstration of viral DNA as shown by PCR amplification and sequencing of a viral gene in the offspring derived from the infected

parent confirms that the larvae infected by virus at sub-lethal levels can survive and the moths derived from such larvae can successfully transmit the virus infection vertically to their offspring. Hence, it is important to confirm the viral-free nature of the eggs preferably by PCR-based techniques before they are used for seed multiplication purpose or for large-scale distribution to farmers. However, further studies focusing on sensitivity of the PCR assay are needed to put this into application.

For the cotton leafworm, *S. littoralis* even though the solar ultraviolet and other environmental factors may affect or/ and destroy the OBs of NPV released by infected cotton leafworms of the previous crop, some OBs may escape inactivation and become a source of infection to the next generations due to vertical transmission of NPV both transovarially and venereally.

SUMMARY

A nucleopolyhedrovirus (NPV) isolate was selected and tested for vertical transmission in the cotton leafworm, *Spodoptera littoralis*. Fifth instar larvae were exposed to four different dosages of *Spli*NPV (400, 800, 1600, and 3200 OBs/ larva) by droplet feeding method and a dosage of about 3200 OBs/ larva was found suitable for obtaining 100% infected adults. The survivors were reared and mated then second-generation (F_1) insects were examined for infection. Mating tests of uninfected females and infected males confirmed venereal transmission as there was a significant reduction in hatching of eggs. Further, among the F_1 hybrid offspring (infected female X uninfected male) that were infected transovarially, larval progeny died at first, second and third instar larval stages, whereas those infected venereally developed acute lethal infection late and died at pupal stage. Histopathological studies revealed that spermatocytes and nurse cells in the young oocytes got the infection at later stages of infection cycle. PCR amplification and sequencing of 332 bp of polyhedrin gene of *Spli*NPV isolated from the viral-infected parent and the F_1 offspring confirmed that the viral infection is vertically transmitted to the progeny.

Keywords: Cotton leafworm; *Spodoptera littoralis* NPV; Nucleopolyhedrovirus; Vertical transmission; Latent infection; Persistent infection; Polyhedrin gene.

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