Transovarial Transmission of Nucleopolyhedrovirus in Black-Inch Looper Caterpillar, *Hyposidra talaca* (Walk.) (Lepidoptera: Geometridae)

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**ABSTRACT**

*Hyposidra talaca* is one of the main pests of north-eastern tea plantation of India. *Hyposidra talaca* nucleopolyhedroviruses (*HytaNPV*) plays a key role to control the pest outbreak. Here the investigation of transovarial transmission of *HytaNPV* in the black-inch tea looper caterpillar, *H. talaca* was carried out. The egg masses were collected from a tea garden of Dooars region of India. The larvae reared from these eggs showed typical signs of nucleopolyhedrovirus infection. These larvae pupate on the soil and crack of the tea bushes. The female moths lay eggs in clusters on the trunks and the crevices of the tea trees. After last moult, they go down to the ground to feed on the mature leaves. The first, second and third instar typically feed on the mature leaves. The fourth and fifth larval instar generally prefer to feed on the mature leaves. The fifth larval instar generally prefer to feed on the mature leaves. The fifth larval instar generally prefer to feed on the mature leaves. The fifth larval instar generally prefer to feed on the mature leaves. The fifth larval instar generally prefer to feed on the mature leaves. The fifth larval instar generally prefer to feed on the mature leaves. The fifth larval instar generally prefer to feed on the mature leaves. The fifth larval instar generally prefer to feed on the mature leaves. The fifth larval instar generally prefer to feed on the mature leaves. The fifth larval instar generally prefer to feed on the mature leaves.

**Introduction**

Baculoviruses are well-known as the largest and most widely studied virus of insects. Till now, more than 700 baculoviruses have been isolated mainly from the insect species of the orders Diptera, Hymenoptera and Lepidoptera. In the search of biopesticides, baculoviruses have been isolated from 100 species of insect pests (Moscardi, 1999). Nucleopolyhedroviruses (NPVs), which are a part of baculovirus, are pathogenic for invertebrates, particularly insects of the Lepidoptera. The virus is specified as having large double-stranded DNA genome within rod-shaped enveloped virion. The PCR technique has been shown to be suitable for virus identification (Moraes and Maruniak, 2001; Galal, 2009; Hewson et al., 2011).

In India, *Hyposidra talaca* nucleopolyhedrovirus (*HytaNPV*) is an extremely infectious natural agent causing most destructive disease to the tea pest *Hyposidra talaca*. Mukhopadhyay et al. 2010, first reported the occurrence of *HytaNPV* in Terai and the foothills of Darjeeling.

The Indian tea has been one of the favorite beverages of the world for ages. But the tea lovers might be deprived of their favorite brew if the growth of the harmful pests like *Hyposidra talaca* cannot be checked. *H. talaca* (Lepidoptera: Geometridae) is a major defoliating tea pest, it creates a periodical and regular problem in north-eastern tea plantation of India (Basu Majumder and Ghosh, 2004). In 2006, it spread all over the northern part of West Bengal (WB) and Assam (Sinu et al., 2011). During the last decade, this moth has established a population and continued to disperse throughout the tea belt. The larvae are responsible for huge economical losses and the pest has also suppressed the previous main species *Buzura suppressaria* (Anonymous, 2008). The first, second and third instar larvae of *H. talaca* feed on young leaves of tea plants and shade trees. The fourth and fifth larval instar generally prefer to feed on the mature leaves. After last moult, they go down to the ground to pupate on the soil and crack of the tea bushes. The female moths lay eggs in clusters on the trunks and the crevices of the shade trees, and each cluster contains 300-500 eggs.

**Materials and methods**

**Insect rearing**

Gravid *H. talaca* females were collected manually using light trap from the Rajabhat tea estate (Eastern Dooars, 26°39′N, 89°29′E), Jalpaiguri, West Bengal, India. The collection site was located 87 km away from North Bengal Regional Research and Development Centre, Tea Research Association (TRA), India. Individual female was maintained in each open end glass vial covered with muslin cloth. The gravid females laid their eggs in the glass vials. The freshly laid eggs were brought to laboratory and 8 % formalin was used to sterilize the surface of the eggs for 15 min at room temperature. They were reared in an insect rearing room of Plant Protection Department of TRA. The eggs were kept to a sterile Petri dish just after several cleaning with distilled water and rinsed with 70% ethanol. Eggs collected from each female were kept separately and were maintained in a wooden cage of 30 cm × 30 cm × 35 cm size. They were reared at 28 ± 2 °C, 72 ± 3 % relative humidity and a 13L:11D photoperiod. The newly hatched larvae, all from the
same parent were released onto fresh tender shoots of tea dipped in glass tubes. Each larva was reared in a separate cage, and fresh tea leaves were provided daily. Fresh tea leaves were supplied as food after sterilizing with 10% formalin for 5 min and rinsed with sterile double-distilled water. The larvae that showed typical NPV signs was collected in a 1.5 ml eppendorf tube. Later the viral DNA was isolated and the degenerate primers were used for the PCR study of the isolated DNA. Later the amplified PCR products were cloned and sequenced.

**Disease Identification**

The larvae were monitored regularly to identify the symptoms of NPV infection (Sinu et al., 2011). The larvae were dissected after the recognition of signs of NPV infection. The tissues of dissected larvae were examined through eyes. Immediately after that the smears of the tissues were examined under light microscopy. After identification of polyhedral occlusion bodies (POB) of virus, these were dissolved in 1 N NaOH and were examined under a phase-contrast microscope (Thomas, 1974). The larval progeny that showed NPV symptoms were homogenized individually in distilled water. Then polyhedral occlusion bodies were isolated and the quantification of POBs was carried out by using Neubauer haemocytometer (Marienfeld, Germany) under phase contrast microscope (Olympus BX 51).

**Viral DNA extraction and PCR amplification for detection of NPV in *H. talaca* progeny**

Larvae showed signs of NPV infection were taken for PCR study. The PCR study was performed to confirm transovarial transmission. Viral DNA was extracted individually from each larval sample and purified with QIAamp DNA Mini Kit (Qiagen) according to manufacturer’s protocol. The final DNA was obtained after several cleaning with ethanol and diluted wash solution. Later DNA was eluted and re-suspended in 20 µl molecular grade water (Himedia). The isolation of DNA was confirmed by electrophoresis in 1% agarose gel and quantified with Biophotometer (Eppendorf).

A highly conserved region of polyhedrin gene from HytaNPV was amplified. The PCR was performed using the degenerate primer (F: 5’-GGACCSSGGYAAARAYCGA AAA-3’ and R: 5’-GCRTCWGGYGCAAAYCTYTT-3’) designed according to Antony et al. (2011). The PCR reaction was carried out taking 50-100 ng of viral DNA in a 25µl reaction solution containing 1X PCR buffer (Invitrogen, USA), 1.5 mM MgCl₂, (Invitrogen, USA), 0.5 mM dNTPs (Bangalore Genei, India), 1 U Platinum Taq DNA polymerase (Invitrogen, USA), 0.5 µM of each primer. PCR amplification was performed in a DNA thermal cycler (Veriti Thermal Cycler, Applied Biosystems, CA, USA). The conditions used were initially with denaturation for 1 cycle at 94°C for 5 min; followed by 35 repeated cycles of 94 °C for 30 sec, 50°C for 30 sec, 72°C for 30 sec, and the final extension cycle at 72°C for 7 min. The PCR products were separated by 1.5% agarose gel electrophoresis with a 100-bp DNA ladder as a size marker (Genei, Bangalore).

**Cloning and sequencing of the polyhedrin gene of NPV**

The band of the expected size (527 bp) was eluted from the gel using HiPura Agarose Gel DNA Purification Spin Kit (Himedia, India) following the manufacturer’s protocol. The eluted was ligated into pGEM-T vector (Promega, UK) in a 3:1 (insert: vector) molar ratio with T4 DNA ligase (In-Taq) in a 3:1 (insert: vector) molar ratio with T4 DNA ligase (Invitrogen, USA). Following amplification of recombinant clone with M13 universal forward and reverse primers, the sequencing was performed using the BigDyeTerminator v1.0 cycle sequencing kit (Applied Biosystems) in ABI 3500 Genetic Analyzer (Applied Biosystems).

**Results**

**Disease Identification**

The larvae that were grown from the field collected *H. talaca* eggs showed typical signs of virus infection. Primary signs of infection in larval stage are loss of appetite, lethargic movement, and climb to the top of the twigs and hanging upside down by their abdominal legs. Also flaccidity and rupturing of the cuticle of larvae were noticed. These symptoms suggested nucleopolyhedrovirus (NPV) infection (Figure 1). The haemolymph contained a large number of polyhedral occlusion bodies. Later the presence of NPV was ascertained by phase contrast microscopic study (Figure 2). The number of polyhedral occlusion bodies was calculated by haemocytometer showing the presence of approx. 5.9 × 10⁹ POBs per ml of cadaver.

**Figure 1.** Clockwise representation of the transovarial transmission of *Hyposira talaca* nucleopolyhedroviruses from parent to offspring.

**Figure 2.** Polyherdra of HytaNPV in 40X under EVOS® FL Cell Imaging System (invitrogen) containing embedded virions which are released in the alkaline pH of the insect gut. Bar marker represents 100 µm.

**Diagnosis of the disease in *H. talaca* progeny by PCR amplification and polyhedrin gene sequencing**

The targeted sequence was amplified from the extracted DNA of the purified polyhedra by using the degenerate primer set. The successful amplification of the specific polyhedrin gene region of NPV confirmed the viral infection in the larvae. The bands obtained from PCR products were identical (Figure 3) and contained the nucleotide sequence of 527 base pair (bp) which confirmed the presence of NPV in the larval samples. A Blast search confirmed 100% similarity with the previously published HytaNPV sequence (Antony et al., 2011). The PCR amplification of the NPV
polyhedrin gene from larvae suggested the viral infection. These results conclude that the HytaNPV sequence amplified from the *H. talaca* progeny was derived from the parent through transovarial transmission.

**Figure 3.** Agarose gel electrophoresis of specific PCR amplified products from HytaNPV infected *H. talaca* larvae of F1 generation. Lane M: 100 bp Ladder DNA marker (Genei, Bangalore); Lane L1-L4: samples of positive PCR products. The band lies between 500-600 bp (base-pair) and the size of the band is 527 bp. This result confirms the vertical transmission of HytaNPV.

**Discussion**

Virus is the subject of importance for the development of biopesticides to maintain ecosystem in agricultural field. In this regard, baculoviruses like NPVs are considered as potential biocontrol agent and have been applied successfully against larvae of many insect pests in the world (Hu et al., 1993; Ma et al., 2007). NPV has the ability to transmit vertically from adults to their offspring through transovarial transmission (within the egg itself) and via transovum transmission (surface contamination of the eggs) (Jehle et al., 2006). In this study, the transovarial transmission of NPV in *H. talaca* was examined. The transovum transmission of HytaNPV in *H. talaca* has been reported previously by Antony et al., 2011. Here, field collected eggs were surface sterilized, and the neonates larvae were reared under laboratory conditions. The larvae showed symptoms typical of NPV infection, indicating the vertical transmission of NPV. This is the first report on the transovarial transmission of HytaNPV in *H. talaca*. Though previously published studies have been reported the vertical transmission of NPV in many lepidopteran pests (Burden et al., 2002; Fuxa et al., 2002; Petrik et al., 2003; Khurad et al., 2004; Vilaplana et al., 2009).

UV radiation is the main constrain in successful application of NPV as a biological control agent as it hinders the activity of NPV (Petrik et al., 2003). Though sustain of the NPV in the host species and vertical transmission of NPV resolve the problem. The larvae hatched from field-collected eggs showed NPV infection, and the PCR amplification of the isolated viral DNA from the infected larvae and the sequencing confirmed the presence of viral gene in the offspring. The larvae contain the HytaNPV at sub-lethal levels can survive and the moths emerge from such larvae can vertically transmit the virus to their offspring. The pest has short life cycle with multiple overlapping generations and presence in winter months (Das et al., 2010), so along with the pest HytaNPV can remain in the tea field throughout the year. Thus, the obtained result reflects a crucial prospect of HytaNPV that can be used in the biological control of *H. talaca*, specially the virus can persist in the larval population and can be transmitted from one generation to the next. These characteristics support the use of HytaNPV as a potent biological control agent and competent alternative to chemical insecticides in integrated pest management (IPM).

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**References**


