

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



Zoology

KEYWORDS : tea, *Hyposidra talaca*, nucleopolyhedrovirus, PCR, transovarial transmission.

Soma Dasgupta

Plant Protection Department, Tea Research Association, North Bengal Regional Research and Development Centre, Nagrakata, Jalpaiguri-735225, West Bengal, India

Dr. Sunil Kumar Pathak

Deputy Director, Advisory Services of Assam, Tea Research Association, Tocklai Experimental Station, Jorhat-785008, Assam, India.

Prof. Rakesh Kumar Bhola

Department of Zoology, Gauhati University, Guwahati-781014, India

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. The phase contrast microscopic view indicated the HytaNPV caused mortality of larvae with an accumulation of large number of polyhedral occlusion bodies. Later the polyhedrin gene amplified from larvae proved HytaNPV infection and presented the information of vertical transmission of virus from parent to offspring (transovarial transmission). HytaNPV can remain in larval population of *H. talaca* and be transmitted to the next generation. Hence, HytaNPV can be advantageous for longstanding control of *H. talaca*. This study reflects the important aspects of HytaNPV and proved its usefulness in biological control programme.

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 analyzed and found prospective against field and forest 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 (Morales 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 plantations 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.

Antony et al 2011, established the transovum transmission of HytaNPV by performing cloning and sequencing of a partial segment of HytaNPV polyhedrin gene. This virus has the ability to sustain in the host species at sub-lethal level and can be passed from one generation to the next (transovarial transmission). Successive generations of insect host are infected by polyhedra, thus the host serves as reservoir of inoculum. This characteristic helps in using NPV for biocontrol of the pest. The main aim is to produce a biopreparation of HytaNPV against *H. talaca*. Before that it is important to study the transovarial transmission of HytaNPV in its host. Due to the high potency of the virus as chemical alternative, finding out the the transovarial transmission of the virus is a matter of great interest. This study may play an imperative role in the programs of integrated pest management (IPM). This study focuses on the transmissibility of HytaNPV from parents to progeny one of the most important parameters to consider when using NPV in a biological control programme. This study can be useful for the improvement of HytaNPV as biological control agent.

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 larva 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'- GGACCSGGYAARAAYCAA AAA-3' and R: 5'-GCRTCWGGYGCAAAYTCYTT-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 eluent was ligated into pGEM-T vector (Promega, UK) in a 3: 1 (insert: vector) molar ratio with T₄ DNA ligase as described by the manufacturer. Ligated products were transformed into *Escherichia coli* DH10β competent cells (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. 5.9×10^9 POBs per ml of cadaver.



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

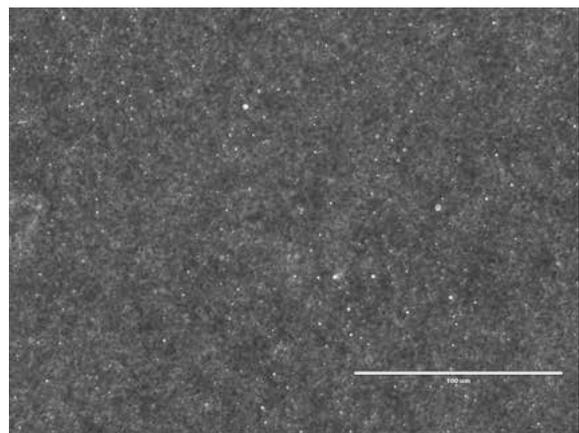


Figure 2. Polyhedra of HytaNPV in 40X under EVOS® FL Cell Imaging System (Invitrogen) containing embedded virions which are released in the alkaline pH of the insect gut. Bar marker represents 100 µm.

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.

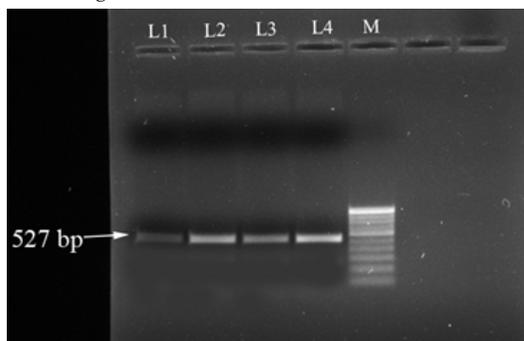


Figure 3. Agarose gel electrophoresis of specific PCR amplified products from HytaNPV infected *H. talaca* larvae of F1 generation. Lane M- 100 bp Ladder DNA marker (Genei, Bangalore); Lane L1- L4: samples of positive PCR products. The band lies between 500-600 bp (base-pair) and the size of the band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

Acknowledgements

The authors thank the Director, Tea Research Association and the Head of the Department of Plant Protection and Biotechnology for providing the necessary facilities and encouragement. The authors wish to thank Tapan Talukdar and Picklu Mandal for all their assistance during insects collection.

References

- Anonymous (2008). Quarterly Advisory bulletin: March-June. Nagrakata sub-station, Tea Research association. 1-3.
- Antony B, Sinu PA, Das S (2011). New record of nucleopolyhedroviruses in tea looper caterpillars in India. *J. Invertebr. Pathol.* 108:63-67.
- Basu Majumdar A, Ghosh P (2004). *Hyposidra talaca* (Walker) – a destructive pest of tea in Doars tea plantations. Two and a Bud. 51:49-51.
- Burden JP, Griffiths CM, Cory JS (2002). Vertical transmission of sublethal granulovirus infection in the Indian meal moth, *Plodia interpunctella*. *Mol. Ecol.* 11:547-555.
- Das S, Mukhopadhyay A, Roy S, Biswa R (2010). Emerging looper pests of tea crop from sub-Himalayan West Bengal, India. *Resistant Pest Management Newsletter.* 20:8-13.
- Fuxa JR, Richter AR, Ameen AO, Hammock BD (2002). Vertical transmission of TnSNPV, TnCPV and AcMNPV and possibly recombinant NPV in *Trichoplusia ni*. *J. Invertebr. Pathol.* 79:44-50.
- Galal F (2009). Universal primer for early and rapid detection of nucleopolyhedroviruses of multiple species using polymerase chain reaction. *Egypt. Acad. J. Biol. Sci.* 1(1):57-64.
- Hewson I, Brown J, Gitlin S, Doud D (2011). Nucleopolyhedrovirus detection and distribution in terrestrial, freshwater, and marine habitats of Appledore Island, Gulf of Maine. *Microb. Ecol.* 62(1):48-57.
- Hu ZH, Liu MF, Jin F, Wang ZH, Liu XY, Li MJ, Liang BF, Xie TE (1993). Nucleotide sequence of the *Buzura suppressaria* single nuclear polyhedrosis virus polyhedrin gene. *J. Gen. Virol.* 74:1617-1620.
- Jehle J, Blissard G, Bonning B, Cory J, Herniou E, Rohrmann G, Theilmann D, Thiem S, Vlaj J (2006). On the classification and nomenclature of baculoviruses: a proposal for revision. *Arch. Virol.* 151:1257-1266.
- Khurad AM, Mahulika A, Rathod MK, Rai MM, Kanginakudru S, Nagaraju J (2004). Vertical transmission of nucleopolyhedrovirus in the silkworm, *Bombyx mori* L. *J. Invertebr. Pathol.* 87:8-15. Ma XC, Shang JY, Yang ZN, Bao YY, Xiao Q, Zhang CX (2007). Genome Sequence and Organization of a Nucleopolyhedrovirus that Infects the Tea Looper Caterpillar, *Ectropis oblique*. *Virology.* 360:235-246.
- Moraes R, Maruniak J (2001). Detection and identification of multiple baculoviruses using the polymerase chain reaction (PCR) and restriction endonuclease analysis. *J. Virol. Methods.* 63(1-2):209-217.
- Moscardi F (1999). Assessment of the application of baculoviruses for control of Lepidoptera. *Annu. Rev. Entomol.* 44:257-289.
- Mukhopadhyay A, De D, Khewa S (2010). Exploring the biocontrol potential of naturally occurring bacterial and viral entomopathogens of defoliating lepidopteran pests of tea plantations. *J. biopesticides* 3:117-120.
- Petrik DT, Iseli A, Montelone BA, Van Etten JL, Clem RJ (2003). Improving baculovirus resistance to UV inactivation: increased virulence resulting from expression of a DNA repair enzyme. *J. Invertebr. Pathol.* 82(1):50-56.
- Sinu PA, Mandal P, Antony B (2011). Range expansion of *Hyposidra talaca* (Geometridae: Lepidoptera), a major pest, to Northeastern Indian tea plantations: change of weather and anti-predatory behaviour of the pest as possible causes. *Int. J. Trop. Insect. Sci.* 3548:1-7.
- Thomas GM (1974). Diagnostic techniques. In: Cantwell GE, editor. *Insect diseases.* Vol. I. New York, NY: Dekker. 1-48.
- Vilaplana L, Wilson K, Redman EM, Cory JS (2009). Pathogen persistence in migratory insects: high levels of vertically-transmitted virus infection in field populations of the African armyworm. *Evol. Ecol.* 24:147-160.