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S Tanuja
Department of Agricultural Microbiology, College of Agriculture, UAS, GKVK, Bengaluru, Karnataka, India

R Muthuraj
Department of Agricultural Microbiology, College of Agriculture, UAS, GKVK, Bengaluru, Karnataka, India

G Sivakumar
ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka, India

Correspondence
S Tanuja
Department of Agricultural Microbiology, College of Agriculture, UAS, GKVK, Bengaluru, Karnataka, India

Isolation and characterization of *Ha* NPV associated with *Helicoverpa armigera* insect pest

S Tanuja, R Muthuraj and G Sivakumar

Abstract
Dead larvae of *Helicoverpa armigera* were collected from different parts of Bengaluru. Some larvae were diseased and the cuticles were ruptured, discharging of white body fluid on plant parts. Nucleopoly hedroviruses were isolated from the diseased larvae of various populations of pod borer *Helicoverpa armigera*. Observation of discharged body fluid under light microscope revealed that the large number of spherical particles resembling as viral occlusion bodies (OBs). The virus was characterized as *Helicoverpa armigera* nucleopoly hedrovirus (Hear NPV) based on morphological and biological characterization. Scanning electron microscopy of OBs purified from diseased larvae revealed polyhedral particles of size approximately 0.460-1.030 µm, The transmission electron microscope (TEM) images of the OBs revealed the tetrahedral shape with dimensions of 0.831-0.990 µm, The semisynthetic diet bioassay showed LC_{50} values of NPV against 2nd instar larvae of *Helicoverpa armigera* indicated $1x10^4$ OBs/ml. Hear NPV was further mass multiplied and stored for further studies.

Keywords: Characterization of *Ha* NPV associated with *Helicoverpa armigera* insect pest

Introduction
Lepidopteran pests are of major concern since they devastate several leguminous, fibre, cereal and horticultural crops. Of the different species of *Heliothis* occurring in India, *Helicoverpa armigera* (Lepidoptera: Noctuidae) is the most devastating and widely prevalent one. Considering the reliability and suitability of HaNPV in terms of economic and ecological reasons, its utilization in pest management has received a great deal of significance.
NPV is mono specific and is extremely unlikely to present a risk to any non-target species, making it highly suitable for use as insect pest control agent, particularly in urban areas and nature reserves. The genus NPV is characterized by the presence of polyhedral-shaped viral occlusions containing randomly occluded viral particles.
The main objective of this study is characterization of *Helicoverpa armigera* NPV, Survey was conducted under natural epizootic conditions in and around Bengaluru for collection and isolation of NPV from major *Helicoverpa armigera* pests. Typical symptoms of NPV infected and dead larvae were collected and observation was done in laboratory.

Material and Methods
Collection and extraction of baculovirus
The NPV was isolated from the dead larvae of *Helicoverpa armigera* which is collected from Bendi farm, brought to NBAIR, Hebbal, Bengaluru. To release occlusion bodies (OBs), the diseased larvae were homogenised, using a sterile pestle and mortar for 4 min with 5 ml of distilled water. The suspension was filtered twice through a double-layered muslin cloth and then the filtrate was centrifuged (Remi, cC24 BL, India) at 500 rpm for 1min to remove the larger particles. The supernatant was suspended in (5ml) distilled water and centrifuged at 5000 rpm for 20 min to collect the pellet containing polyhedra. The pellet containing OBs was resuspended in (5 ml) distilled water and stored at 5 °C. The polyhedral occlusion bodies were counted using a haemocytometer.

Electron microscopy morphology
The morphological studies of the extracted OBs were carried out under a scanning electron microscope (SEM) and a transmission electron microscope (TEM). The purified OB suspensions were taken in vials, fixed in 2.5% (v/v) glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) for 2 h at 4 °C. The samples were then post-fixed in 2% (v/v) aqueous osmium tetroxide prepared in 0.1M phosphate buffer (pH 7.4) for 1 h and dehydrated in graded ethanol series (Martins *et al*., 2005) [10]. The samples were finally dried to a critical drying point. Stubs with double-sided conductivity carbon adhesive tape and

sputter coated with gold for 20 s, using an automated sputter coater (Model: EMITEC-SC 7620), were used for sample mounting. The coated samples were examined directly under a SEM (Quanta 250, FEI, Netherlands) at 10 kV with a spot size of 3.5 and a pressure of 60 Pa. The sample images were visualized and photographed at × 40,000 magnification. The amplified photographs developed by a scale were used to measure the sizes of the OBs.

For TEM studies, the pellets of OBs were initially fixed in 2.5% (v/v) glutaraldehyde in 0.05M phosphate buffer (pH 7.2) for 24h at 4 °C and again fixed in 0.5% (v/v) aqueous osmium tetroxide in the same buffer for 2h. After the post-fixation, samples were dehydrated in a series of graded alcohol; the dehydrated sample was mounted on 300 mesh carbon-coated copper grid. The sample was stained by saturated aqueous uranyl acetate and counterstained with lead citrate and viewed under TECNAI 120 Kv TEM (FEI, Netherlands). The sizes of the OBs and nucleocapsids were measured directly from the amplified photographs using a precision ruler and compared to the magnification of the photograph.

Bioassay

Median lethal concentration (LC_{50}) of *Helicoverpa* NPV to second instar larvae was estimated by using semi synthetic diet bioassay method. Viral suspensions of 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 OBs/ml were prepared in aqueous (0.05%) Tween 20 (v/v). Ten microlitres of viral suspensions was spread on semi synthetic diet, air dried and individually placed inside the multicavity trays. A blunt end polished glass rod was used to distribute the suspension containing the virus uniformly over the diet surface. Each well constituted an independent replication, and three wells were used per viral suspension, constituting 30 insects per treatment. Second instar *Helicoverpa armigera* larvae starved for about 6 h were released individually into the wells which were then covered with a lid. The larvae consumed the diet within 12 hrs. It is maintained at 26 ± 2 °C and 60-70% relative humidity. In control, larvae were allowed to feed on diet treated with aqueous (0.05%) Tween 20. Larval mortality rates were recorded at daily intervals. Mortality due to viral infection was recorded up to 9 days post inoculation.

Result and Discussion

Collection of baculovirus

Severe infestation of pod borer, *Helicoverpa armigera* was observed during the regular survey in the Bengaluru rural region. Few diseased larvae of *Helicoverpa armigera* were found to harbour the virus. The body fluid of the dead diseased larvae was found on bendi plant parts (Fig. 1). Observation of discharged body fluid under a phase-contrast microscope revealed numerous spherical particles resembling baculoviral OBs (Fig. 2).

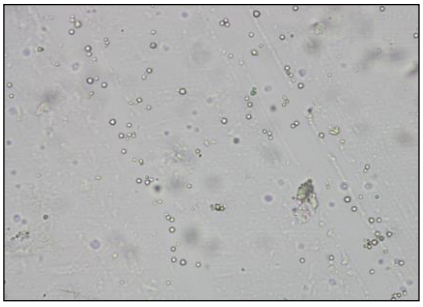

Fig 1: OBs View under compound microscope

Electron microscopic studies revealed the typical baculovirus OBs of type nucleopolyhedrovirus (NPV) with polyhedral structures. Under SEM, the OBs of *Helicoverpa* NPV appeared as crystalline structures of variable shapes and sizes ranging from 0.460-1.030 µm (Fig. 3). Most of the OBs were tetrahedral in shape. TEM studies of the OBs also revealed the tetrahedral shape (Fig. 4). The present study clearly revealed the hexagonal and tetrahedral shaped occlusion bodies. The occurrence of NPV on *Eucalptus similis* larvae with hexagonal forms of inclusion bodies was reported on mulberry plants (Chu *et al.,* 1975) [3]. On the other hand, Ishikawa *et al*. (1966) [7] found a mixture of nuclear inclusion bodies of tetragonal, triangular and pentagonal forms in one of the diseased larvae in Japan. Sridhar Kumar *et al.* (2011) [13] reported OBs of three major Lepidopteran pests revealed multiple nucleocapsids in each envelop, which were bacilliform shape. Senthil Kumar *et al*. (2015) [12] also recorded that the OBs are tetrahedral in shape in *Spilarctia oblique*.

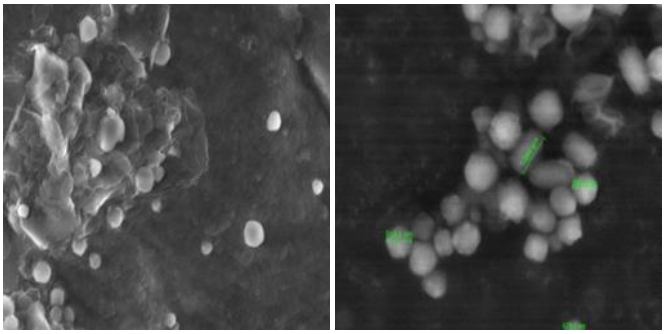

Fig 3: SEM View of *Ha*NPV

TEM of the cross-sectioned OBs revealed multiple nucleocapsids in each envelop, which were shaped of dimensions 831.0-990.0 nm for *Ha*NPV. The OBs of *Ha*NPV contained 2-6 nucleocapsids per envelope.

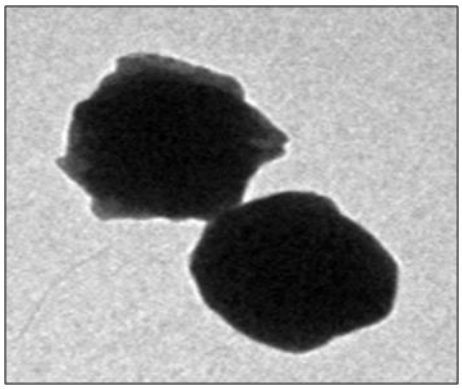

Fig 4: TEM View of HaNPV

Bioassay

Larval mortality was observed at low doses of *Helicoverpa* NPV with the LC_{50} of 1×10^4 OBs/ml against second instar larvae of *Helicoverpa armigera*. Rabindra *et al.* (1994) [11] studied influence of host plant surface environment of chickpea, pigeonpea, lablab, sunflower and different parts and species of cotton on the pathegenicity of NPV against *H. armigera.*

Table 1: Mortality percentages of the *Helicoverpa armigera*, larvae in response to nucleopolyhedrovirus

Concentration	No. of insects killed	Mortality %
$1x10^2$	7	23.3
$1x10^3$	10	33.3
$1x10^4$	13	43.3
$1x10^5$	16	53.3
$1x10^6$	20	66.6
$1x10^7$	22	73.3
$1x10^8$	22	73.3
Control	3	10.0

The pod borer, *Helicoverpa armigera* larvae infected with *Ha*NPV showed typical baculovirus OBs that have crystalline structures of variable shapes and sizes ranging from 0.460-1.030 µm. Most of the OBs were tetrahedral in shape. The semi synthetic bioassay showed the LC_{50} of $1x10^4$ OBs/ml against the second instar *Helicoverpa armigera* larvae. NPV is safe and friendly to the environment, and it could be an ideal component for the biological pest management approach to control the pod borer, which is a major insect pest of pulses and other vegetable crops.

References

1. Bartlett AC. Guidelines for genetic diversity in laboratory colony establishment and maintenance. In: Handbook on insect Rearing. P. Singh and R. F. Moore, Amsterdam: Elsevier. 1985; 1(1):7-17.
2. Christian PD, Gibb N, Kasprzak AB, Richards A. A rapid method for the identification and differentiation of *Helicoverpa* Nucleopolyhedro viruses (NPV Baculoviridae) isolated from the environment. J Virol. Methods. 2001; 96:51-65.
3. Chu GK, Xie RD, Zhang HJ, Yao YE, Fang ZJ. On a nuclear polyhedrosis virus of mulberry tussock moth, *Euproctis similis* Fuessly (Lepidoptera: Lymantridae) and field test for the moth control. Microbiol. Sin. 1975; 15:93-100.
4. Erayya Jagdish J, Sajeesh PK, Upadhyay V. Nuclear polyhedrosis virus (NPV), A Potential Biopesticide. Res. J Agriculture and Forestry Sci. 2013; 1(8):30-33.
5. Evans MF, Shapiro M. Viruses: In: Mannual of Techniques in insect pathology. Lacey L. (Ed.), Academic Press London, 1997, 17-54.
6. Grzywacz D, Rabindra RJ, Brown M, Jones KA, Parnell M. The *Helicoverpa armigera* NPV production manual. FAO, www.fao.org, 2011, 61.
7. Ishikawa Y, Tanabe H, Nakayama C, Asayama T. Studies on the nuclear polyhedrosis of *Porthesia xanthocampa*. J Ser. Sci. Japan. 1966; 35:174-180.
8. Jenny S, Mark CL, Philip H, Martin S, Speight R. Narrow host range Nucleopolyhedrovirus for control of the browntail moth (Lepidoptera: Lymantriidae). Environ. Entomol. 2000; 29(3):661-667.
9. Johannes A, Martin JL, Wang H, Wang ZHY, Hauschild RD. Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera. Virol. 2006; 346:180-193.
10. Martins T, Montiel R, Medeiros J, Oliveira L, Simoes N. Occurrence and characterization of a nucleopolyhedrovirus from *Spodoptera littoralis* (Lepidoptera: Noctuidae) isolated in the Azores. J Invertebr. Pathol. 2005; 89:185-192.
11. Rabindra RJ, Muthusami M, Jayaraj S. Influence of host plant surface environment on the virulence of nuclear polyhedrosis virus against *Helicoverpa armigera* (Hbn) (Lepidoptera: Noctuidac) larvae. J Appl. Enlomol. 1994; 118:453-460.
12. Senthil KCM, Jacob TK, Devasahayam S, Silva SD, Jinsha J, Rajna S. Occurrence and characterization of a tetrahedral nucleopolyhedrovirus from *Spilarctia obliqua* (Walker). J Invert. Pathol. 2015; 132:135-142.
13. Sridhar KCH, Ranga Rao GV, Sireesha K, Lava KP. Isolation and characterization of baculoviruses from three major lepidopteran pests in the semi-arid tropics of India. Indian J Virol. 2011; 22(1):29-36.