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Pathogenicity and Polyhedra Morphology of *Spilarctia obliqua* Nucleopolyhedrosis Virus

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Spilarctia obliqua **(Wlk.) is a serious pest of mulberry, which is naturally affected by its nucleopolyhedrosis virus (SoNPV) in field conditions. The polyhedral occlusion bodies (POB's) were hexahedron under scanning and transmission electron microscope and measured 0.42 μm to 0.67 μm in diameter. The symptoms of NPV infected *S. obliqua* larvae resembled with that of other NPVs' infected lepidopterous larvae. The pathogenicity and potentiality of this virus against *S. obliqua* was tested in the laboratory conditions and the results showed 100% mortality in larvae inoculated with SoNPV at 6.23× 10^5 POBs/ml. Therefore, SoNPV appears to have a high potential as a microbial biocontrol agent against *S obliqua* larvae.**

Key words : Nucleopolyhedrosis virus, *Spilarctia obliqua*, Pest, Biopesticide, POBs

Introduction

The production of appreciable quantity of quality mulberry foliage, which is the chief food for silkworm, *Bombyx mori* L., is often hampered by the infestation of insect pests, parasites and pathogens. Among Insects, Bihar hairy caterpillar, *Spilarctia obliqua* Wlk. is one of the major pests of mulberry, which usually infest mulberry crop from July to February in Karnataka (Ramkishore *et al.*, 1994; Shree and Manjunatha, 1998). A total foliage crop loss in mulberry by this pest was estimated around 4.90% during 1996 - 97 (Shree and Manjunatha, 1998). The larval infestation is sporadic and it is potentially destructive to a wide variety of vegetables, pulses, oilseeds and even some medicinal plants (Ahmed and Kumar, 1993). Sharma and Tara (1988) reported that an individual larva of S. *obliqua* consumes 1195.18 g of mulberry leaves in its entire larval period. Several chemical and mechanical control measures have been developed and adopted to eradicate this polyphagous pest. Still *S. obliqua* continues to be the most destructive insect pest for mulberry and other agricultural crops.

The development of alternative methods of controlling insects has become indispensable in views of incomplete control and harmful effects of chemicals insecticides to mankind. One such development in this direction is microbial control of insect pests using insect pathogens like virus, bacteria and fungi, which are effective, safe and acceptable. In sericulture it has an added importance since application of insecticides on mulberry can be hazardous for silkworm if not used judiciously. The baculoviridae is a large family of entomopathogenic viruses. Within the family there are two genera of viruses, the nucleopolyhedrosis viruses (NPVs') and the granuloviruses (GV's) (Volkman *et al.*, 1995). The effective use of baculoviruses, and in particular nucleopolyhedrosis viruses (NPVs), for control of insect pests has been known for many years (Hamm, 1994). Baculoviruses have a narrow host range, are highly pathogenic, and have occlusion bodies which make them more environmentally stable than some other families of entomopathogenic virus (Entwistle and Evans, 1985). These characteristics make baculoviruses suitable to be used as microbial control agents against insect pests, and there are many examples of their successful use in this role (Entwistle, 1998; Moscardi, 1999).

Present management of three early instars of *S. obliqua* in various agricultural crops is based on population monitoring by its two natural parasitoids *viz.*, *Apentalis* (Kalara, 1984; Katiyar and Sharma, 1987; Muthukrishnan and Senthamiznselvan, 1987; Shetgar *et al.*, 1990) and *Meteorus* (Kumar and Yadav, 1987) whereas the later stages of the pest is controlled either by chemical insec-

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ticides or by its natural microbial insecticides (Battu, 1987; Battu and Ramakrishnan, 1987; Chaudhari, 1987; Narayana, 1986). Several entomopathogenic microbes *viz.*, Microsporidia (Narayana 1985), fungi (Thontadarya *et al.*, 1973) cytoplasmic polyhedrosis virus (CPV) (Narayana 1986) and nuclear polyhedrosis virus (NPV) (Battu and Ramakrishnan, 1987) are in use for the biological control of *S. obliqua*, among which the NPVs appear to be the most potential alternative to chemical insecticides (Battu and Ramakrishnan, 1987). The present paper deals with some observations on the symptomatology and the pathogenicity of S. *obliqua* by its nuclear polyhydrosis virus (SoNPV), and morphology of its occlusion bodies using scanning electron microscope.

Materials and Methods

Insects and nucleopolyhedrosis

Last stage larvae of *S. obliqua* were observed in the mulberry germplasm of Central Sericultural Research and Training Institute, Mysore during the months of October December 1999. Dead larvae were found hanging with head downwards and attached to mulberry leaves by their abdominal legs. Haemolymph oozed out from diseased and dead larvae was also found accumulated on mulberry leaves. The suspected NPV infected larvae and dead larvae were collected from the garden for confirmation and experimental study.

Bioassay

Healthy larvae of *S. obliqua* were reared under laboratory conditions at 27.5 ± 1.0°C temperature and 71.2 ± 2.0% R.H. A test was conducted to determine the pathogenicity of SoNPV to last stage larvae. Suspension of polyhedral occlusion bodies (POBs) of NPV was sprayed on the fresh mulberry leaves and freshly moulted fifth instar larvae were fed with thsese treated leaves. After the single dose of virual inoculation the larvae were subsequently maintained on normal feeding at laboratory conditions Different dilutions of POBs were tested, each by treating a total of forty larvae in three replications. The larvae, fed with fresh mulberry leaves treated with distilled water only were also maintained as control to detect any natural mortality, under similar conditions. The mortality of larvae was recorded daily for 10 days after inoculation. The mortality data obtained were probit-analyzed and LC_{50} value for larvae was calculated (Finney, 1977; Battu and Ramakrishnan, 1987).

Purification of polyhedra

Haemolymph was collected 6 days post-infection from the diseased larvae, by making a small incision at the base of the abdominal prolegs and repeatedly centrifuged at 1000 g for 20 min. The pelleted polyhedra were suspended in aqueous sucrose [25% and 50% (w/v)] and again centrifuged repeatedly to obtain the purified polyhedra.

Preparation for scanning electron microscopy

Dried polyhedra were mounted onto the copper stubs using double-sided sticky tape and coated with gold (20 - nm thickness) under a Sputter coater (EMS - 550). The coated samples were scanned at 20 kV under ASID-4D scanner attached to JEOL 100 CX-II electron microscope.

Preparation for transmission electron microscopy

Fifth day SoNPV infected larvae were dissected and midgut tissue of the size 0.5 mm were fixed in a mixture of 2.5% glutaraldehyde and 0.2% formaldehyde, prepared in 0.2 M cacodylate buffer at pH 7.2, at 4°C for 2 hrs. The tissue was washed in cacodylate buffer and post fixed in 1% osmium tetroxide for 1 hr at 4°C, washed with triple distilled water and dehydrated in alcohol series, infiltered in a mixture of acetone and Spurr resin (1:1) (Spurr, 1969) for 1 hr and then transferred and retained in Spurr resin for overnight and the blocks were polymerised at 70°C for 9 hrs. The ultrathin (40 - 60 nm) sections were cut by Ultramicrotome (Reichert Super Nova) and collected onto formvar coated copper grids of 200 mesh size. The grids were stained in uranyle acetate for 15 min and in lead citrate for 8 min. The stained grids were observed under electron microcope (JEOL 100 CX II) at 80 kV.

Results and Discussion

While surveying the mulberry germplasm of Central Sericultural Research and Training Institute, Mysore for the incidence of pests during October-December 1999, numerous larvae of *S. obliqua* were found diseased and dead and hanging from the twigs of mulberry plants (Fig. 1). Polyhedral occlusion bodies were isolated from the haemolymph of dead larvae. The scanning and transmission electron microscopic examnination of the inclusion bodies confirmed the pathogen as nuclearpolyhedrosis virus (Fig. 2 and 3). On inoculation, SoNPV was found to highly pathogenic to fifth instar larvae of *S. obliqua*. The dosage-mortality line indicates that the larval mortality was directly related to number of POBs administered orally to *S. obliqua* (Fig. 4). LC_{50} value for fifth instar larvae was 0.1485×10^4 POBs/ml. The inoculated larvae showed similar symptoms like the field infected larvae. The larvae succumbed to death within 6 - 7 days after

Fig. 1. Dead larva of last stage of *S. obliqua* by the infection of SoNPV. Droplets (circles) of infected haemolymph oozed out from the larval body (arrow) can be seen on mulberry leaf.

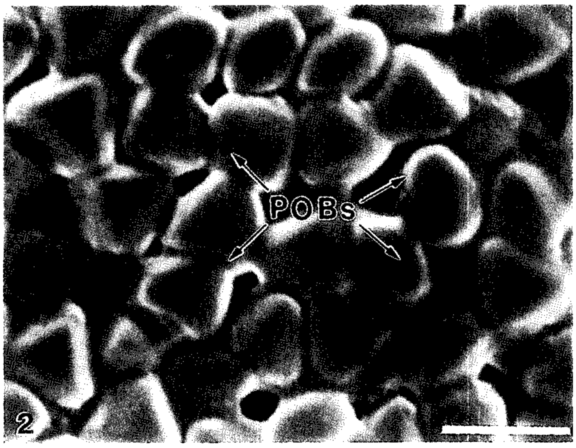

Fig. 2. Scanning electron microphotograph of occlution bodies of SoNPV (Scale bar = 1 μm).

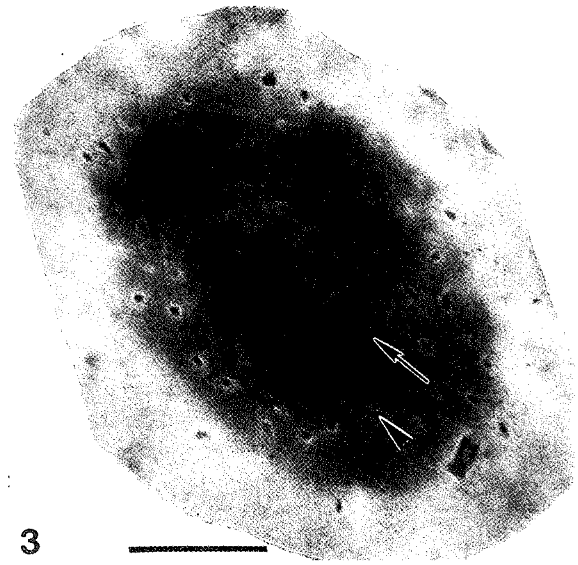

Fig. 3. Transverse section of polyhedra of SoNPV showing a number of virions cut longitudinally (arrow head) and transversally (arrow) (Scale bar = 20 μm).

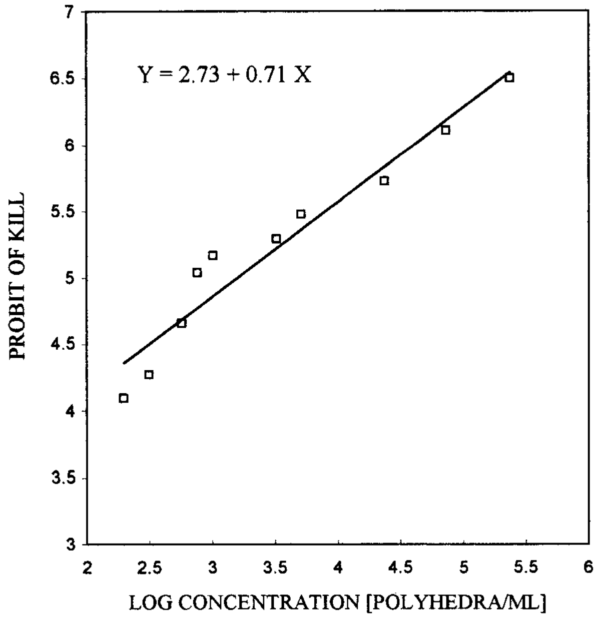

Fig. 4. Log concentration probit kill regression line of fifth instar larvae of *Spilarctia obliqua*.

inoculation in laboratory conditions. Under field conditions, SoNPV were shown to be highly effective biopesticide against *S. obliqua,* which infests mulberry. Battu (1982) and Battu and Ramakrishnan (1987) revealed that the nuclear polyhedrosis virus was found almost continuously associated with *Diacrisia* (=*Spilosoma*) *obliqua.* Further, Battu and Ramakrishnan (1987) observed that the 6-day old larvae of *D. obliqua* were most susceptible among the age group tested.

The symptoms of the NPV infected *S. obliqua* generally resembled that described for other NPV infected lepidopterous larvae (Ignoffo and Garcia, 1979; Battu, 1982; Johnson and Lewis, 1982; Attathom and Sinchaisri, 1987; ESCAP, 1990; Entwistle, 1998 Moscardi, 1999). In the present investigations, it was found that during the early phase of the disease no symptoms were noticed but with the progress of the disease the diseased larvae of *S. obliqua* were found slightly sluggish and lagged behind in their development. Initially the intersegmental lines showed oily and shining appearance. Further, the diseased larvae were also found less responsive to tactile stimuli.

The infected larvae remain undersized and shrunken even after the control larvae had completed pupation and emerged as adults. Further, in contrast to healthy larvae of *S. obliqua*, the NPV infected larvae showed clearly visible whitening of the integument on the ventral side, due to accumulation of large number of inclusion bodies. As the disease advanced, the skin became thin and fragile and the larval body observed dull in colour with intersegmental swellings as compared to the healthier larvae. The fragile skin was prone to repture easily, liberating a viscous liquid containing innumerable number of polyhedra, which become the source of secondary contamination. Another characteristic symptom of the disease was that the NPV infected larvae become restless and crawled aimlessly along the leaf ridges and plant axis, subsequently falling on the ground and dying. Diseased larvae lost the clasping power of their abdominal legs except the caudal legs by which the larvae hung with head downwards (Fig. 1). Polyhedra of *S. obliqua* were hexahedron and varied from 0.42 µm to 0.67 µm in diameter (Fig. 2 and 3), which is almost half of the size of *B. mori* nuclearpolyhedrosis virus (BmNPV) (Tewari *et al.*, 1996). Attathom and Poolpol (1984) reported the mean diameter of the polyhedra 2.7 µm which considered the biggest NPV ever isolated from lepidopterous insects in Thailand.

In conclusion, *S. obliqua* nucleopolyhedrosis virus is a baculovirus isolated from *S. obliqua* larvae and it has high virulence against its host under field conditions in mulberry, and appears to have greater potential as a microbial control agent for this insect pest than other microbial insecticide investigated to date. However, since the mulberry leaves are to be fed to silkworm, *B. mori*, the potential of this virus as a microbial biopesticide can be assured only after ensuring its host specificity.

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