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Pathogenicity and Characteristics of *Spodoptera litura* Nucleopolyhedrovirus from Peninsular Malaysia

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ABSTRAK

Satu kajian keatas ciri-ciri Sopdoptera litura *nukleopolihedrovirus (SpltNPV) telah dilakukan. Hasil kajian mendapati bahawa virus tersebut sangat patogenik dan spesifik terhadap hosnya, larva* S. litura. *Ia menyebabkan 95% kematian larva dalam masa 10 hari dengan dos 6 x 10^8PIBs/larva dan tidak menjangkiti larva lepidoptera* Spirama retorta *Clerck (Noctuidae) dan ulat bungkus,* Pteroma pendulla *Joannis (Psychidae). Polihedra mempunyai berbagai bentuk dan mengandungi banyak virion dengan lebih daripada satu nukleopkapsid setiap virion.*

ABSTRACT

A study on the characteristics of the Spodoptera litura *nucleopolyhredovirus (SpltNPV) was carried out. The result shows that the virus was pathogenic and specific to its host,* S. litura *larva. It caused 96% larval mortality within a period of 10 days at a dosage of 6 x 10^8 PIBs/larva and did not affect lepidopteran larvae,* Spirama retorta *Clerck (Noctuidae) and bagworm,* Pteroma pendula *Joannis (Psychidae). The polyhedra, varied in their shape, contained many virions with more than one nucleocapsid in a virion.*

INTRODUCTION

Spodoptera litura (Fabricius) is a cosmopolitan polyphagous insect pest of many food crops and tobacco. As in most other parts of the tropic, these armyworms are subjected to a disease caused by a *Spodoptera litura* nucleopolyhedrovirus (SpltNPV) infection in the field (Kalshoven 1981). A similar phenomenon occurred in Malaysia, but heretofore, no record of such an infection had been reported. Recently, an epizootic of NPV disease occurred in *S. litura* population attacking a tobacco crop in Kelantan, Malaysia. This incidence occurred when the population of the pest was high particularly in farms where chemical insecticides were not applied. Though the impact of the disease on the *S. litura* population in the field has yet to be known, NPV has the potential to be used as a control agent to be incorporated in management of *S. litura* in Malaysia. Many field trials carried out elsewhere had shown that NPV used either alone or in combination with chemical insecticides could bring the population of *S. litura* to below an economic level (Su 1992). The use of such viruses would not only reduce the dependency on chemical insecticides but also conserve the natural enemies.

Before field trials could be initiated, we examined the characteristics of this SpltNPV. Thus the purpose of this study was to determine (a) the relative virulence to its host, *S. litura* larvae and to non-host larvae of *Spirama retorta* Clerck (Lepidoptera: Noctuidae) and bagworm *Pteroma pendula* Joannis (Lepidoptera: Psychidae) and (b) the characteristics the of SlptNPV by restriction endonuclease analysis.

MATERIALS AND METHODS

Host Insect

Spodoptera litura pupae were collected from a tobacco field at Malaysian Agricultural Research and Development Institute (MARDI) Research Station in Kelantan, Malaysia. The pupae were surface-sterilized with 1% sodium hypochlorite and rinsed with distilled water. The pupae were sexed and five pairs were placed in an oviposition cage consisting of cylindrical wire mess, 15 x 30 cm, lined with a paper towel. Egg masses were collected, surface-sterilized with 1% sodium hypochlorite. The larvae were reared in a plastic container, 30 x 20 x 10 cm, lined at the bottom with a paper towel. Fresh castor leaves, *Ricinus communis,* were provided as their food. The leaves and the paper changed on alternate days.

Propagation of Virus

The SpltNPV was originally isolated from naturally field-infected *S. litura* larvae collected from the same field in 1995. The virus was mass-propagated in the laboratory by feeding the third instar on castor leaves dipped in NPV suspension containing 11 x 10^{10} PIBs/ml. Twenty-four hours after exposure, the larvae were then transferred to 9 cm Petri dishes in a batch of five individuals and maintained on fresh uncontaminated leaves. Diseased larvae were collected and kept in a freezer at - 20°C till further use.

Bioassay

Virus suspension was prepared from heavily viral-infected fifth instars. The larvae were homogenized in a Waring^{R} blender and filtered through double layers of muslin cloth. The homogenate was then sonicated and refiltered. Second instars (3-day old) *S. litura* were used in the bioassay. Circular castor leaf discs measuring 1.5 cm in diameter were contaminated with serial concentrations of SpltNPV. Eight concentrations of NPV ranging from 1 x 10^{1} to 1 x 10^{8} PIBs/ml were used in the study.

Sixty µl of each viral concentration was pipetted onto each leaf disc. The leaf discs were air-dried and then placed individually in a 25, 2 x 2 cm, compartmentalized plastic plate containing 2% agar. The agar maintained the humidity and thus reduced leaf desiccation. One larva was then placed in each compartment. A total of 25 larvae were used for each concentration. Larvae, having eaten the entire leaf disc, were transferred to 9 cm plastic petri dishes and fed fresh uncontaminated castor leaves. Mortality was recorded daily until pupation. The experiment was replicated four times.

For the host range, infectivity of the SpltNPV was tested against two other insect species, *Spirama retorta* Clerck (Noctuidae) and *Pteroma pendula* Joannis (Psychidae) using the same procedure prescribed for *S. litura* larvae.

The mortality data of the three insects used in the bioassay were corrected using Abbott's formula. Probit analysis of Finney (1971) was performed on the data using the generalized linear inodel of the SAS (SAS 1985) package at MARDI computer centre. The lethal doses; LD_{50} and LD_{90} and lethal times LT_{50} and LT_{90} were calculated from the regression equations.

Purification of Viral PIBs and DNA Extraction

Two batches of SpltNPV isolated separately in 1995 and 1997 were prepared for this study. These isolates were propagated in the laboratory.

The purification and DNA extraction procedure of the SpltNPV was adopted from that of Gzrywacz (pers. com.). Diseased larvae were macerated and homogenized individually in 1 ml of distilled water. The homogenate was centrifuged at 13000 *g* for 5 min and pelleted PIBs were obtained. The PIBs were resuspended in 750 µl distilled water and recentrifuged at 13000 *g* for 15 min. The PIBs were then diluted in 120 µl of distilled water to which 25 µl of 500 mM EDTA and 3 µl of 20 mg/ml protease K were added. The mixture was incubated for 1.5 h at 37°C after which 1/2 volume of 1 M Na_2CO_3 was added. The mixture was again incubated at 37°C until the mixture turned translucent. At this stage, 25 µl of 10% SDS was added and the mixture was reincubated for 30 min at 37°C and recentrifuged at 13000 *g* for 60 min. The supernatant containing DNA was extracted with an equal volume of Tris-phenol (pH 7.6), followed by Tris-phenol:chloroform: isoamyl alcohol (25:24:1, v/v/v) and chloroform:isoamyl alcohol (24:1, v/v). The DNA was precipitated twice with 1/5 volume of 3 M sodium acetate and 3 volumes of absolute ethanol at -20°C. The mixture was then centrifuged at 13000 *g* for 5 min. The pellet was washed gently in 500 µl of chilled 70% ethanol, centrifuged and dried with Speed Vac Concentrator Savant. The purified

DNA was dissolved in 20 µl of Te buffer and stored at -20°C.

Restriction Endonuclease Digestion and Agarose Gel Electrophoresis

DNA was digested with *Eco*RI, *Bam*HI and *Hind*III and *Pst*I restriction endonucleases in their respective buffers as recommended by the supplier (GIBCO BRL) for 3 h at 37°C. The DNA restriction endonucleases fragments were separated by electrophoresis in 0.6% agarose gel in 1X TAE buffer. Lambda DNA marker (GIBCO BRL) and High Molecular Weight DNA marker (GIBCO BRL) were used as standards for the determination of fragments sizes.

Electron Microscopy

Two samples were prepared for transmission electron microscopy. The pelleted polydedra and pieces of infected fat tissues were fixed in 4% glutaraldehyde in 0.1 M sodium cacodilate buffer pH 7.4, and postfixed in 1% osmium tetraoxide. The samples were dehydrated through a graded series of acetone, embedded in Epon-Araldite and sectioned using a glass knife. The thin sections were stained with uranyl acetate and lead citrate. The stained sections were examined and photographed in a Hitachi® 600 electron microscope.

RESULTS AND DISCUSSION

Bioassay

The corrected mortality percentages for the three insect pest species are given in Table 1. At the highest dosage (6 x 10^8 PIBs/larva), the NPV could cause about 96% mortality to its original host, *S. litura* larvae within a period of 10 days *(Fig. 1)*. Larval mortality rates within the same period for the doses of 6 x 10^7, 6 x 10^6 and 6 x 10^5 PIBs/larva were 80, 74 and 55%, respectively. At the lowest dosage (60 x 10^1 PIBs/larva), larval mortality was only 18% after 13 days of infection. The corrected larval mortalities for *S. retorta* and *P. pendula* from all viral concentrations were less than 1%.

The results of probit analysis showed that the values of LD_{50} and LD_{90} and for the NPV were 5.50 x 10^4 and 5.26 x 10^8. PIBs/larva, respectively. The regression equation was Y = 3.47 + 0.32X. The lethal time of the NPV at LD_{50} and LD_{90} were eight and six days, respectively. The regression equation had a value of Y = 13.59 - 9.41X.

SlptNPV evidently is very specific and pathogenic to its host, *S.litura* larvae. The NPV bioassayed against *S. retorta* and *P. pendula* showed no specific trend of mortality. However, ingestion of this NPV continuously throughout the life span of an assassin bug, *Sycanus leucomesus* Walk. did, to acertain extent impair the development of the predator (Sajap *et al.* 1999). The infection in *S. litura* and the subsequent larval mortality period were dependent on the dosage of the virus being ingested. Viral concentration and time taken to kill the larvae increased with an increase in larval age.

Restriction Endonuclease Analysis

The fragmentation profiles resulting from the digestion of the SpltNPV DNA with *BAM*HI, *ECO*RI and *Hind*III and *Pst*I, are shown in *Fig. 2.* The enzymes, *Bam*HI, *ECO*RI and *Hind*III and *Pst*I cleaved the genomes from the first isolate of SpltNPV into 9, 15, 24 and 16 but cleaved the second isolate into 10, 16, 24 and 17 fragments, respectively. Even though there were minor submolar were detected when two isolates digested DNA when they were coelectophoresed in 0.6% agarose gel. Similarities in the fragment patterns were also observed from both viral DNAs

TABLE 1
Mortality of *S. litura*, *S. retorta* and *P. pendulla* treated with SpltNPV ten days after treatment

(PIBs/ larva	Number treated	% Corrected mortality		
		S. litura	*S. retorta*	*P. pendulla*
$6X10^8$	100	95.95	0	0
$6X10^7$	100	80.81	0	0
$6X10^6$	100	69.70	0	0
$6X10^5$	100	54.55	0	0
$6X10^4$	100	49.49	0	0
$6X10^3$	100	38.38	0	0
$6X10^2$	100	28.28	0	0
$6X10^1$	100	17.17	0	1.00

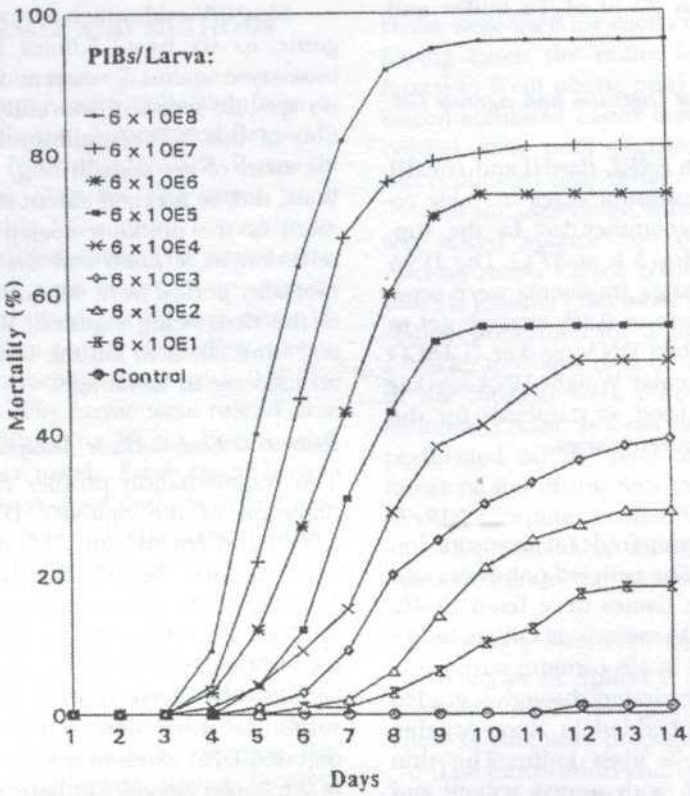

Fig. 1. Cumulative percentage mortality of S. litura larvae infected with SpltNPV

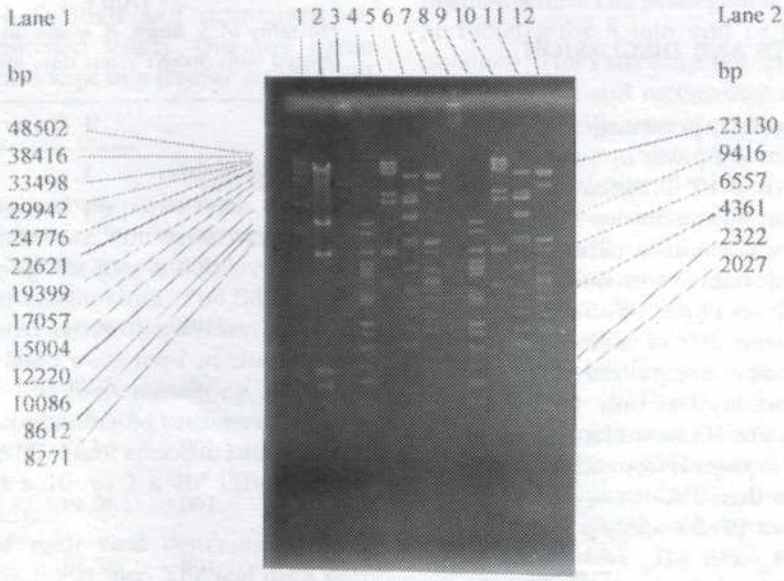

Fig. 2. Restriction endonuclease digestion of DNA fron SpltNPV

Note: lane 1, high molecular weight marker; lane 2, λDNA *Hind*III, lane 3. Undigested SpltDNA (second isolate); lane 4, *Hind*III; (second isolate);lane 5, *Bam*III (second isolate); lane 6, PstI (second isolate), lane 7, *Eco*RI (second isolate); lane 8, Undigested SpltDNA (first isolate); lane 9, *Hind*III (first isolate); lane 10, *Bam*III (first isolate); lane 11, *Pst*I (first isolate), lane 12, *Eco*RI (first isolate)

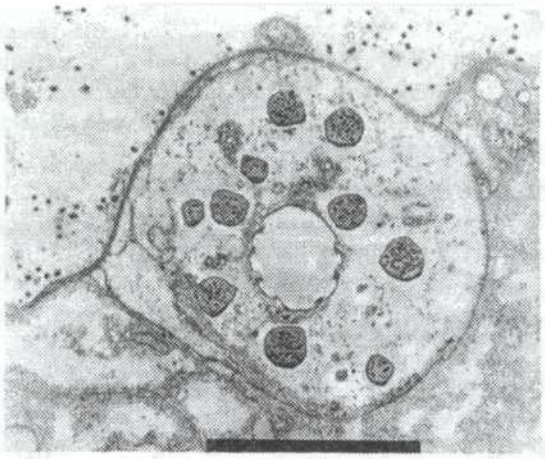

Fig. 3. SpltNPV polyhedra in the nucleus of a fat bodycell. Bar=9.3μm

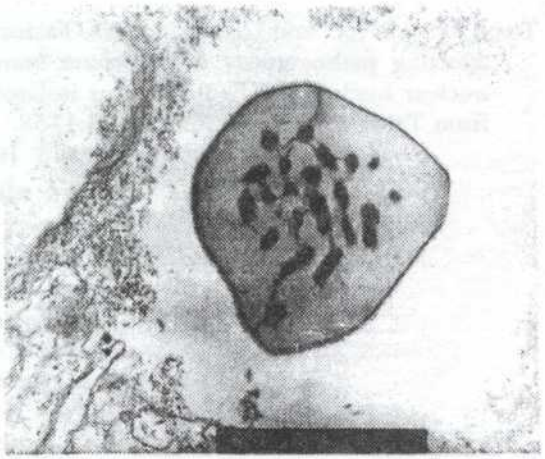

Fig. 4. A polyhedron containing a number of virions. Bar = 1.8μm

digested by *Bam*HI and *Eco*RI. Minor submolar bands, however, occurred when the two isolates were digested with *Pst*I. Such variants commonly occurred in many field-collected NPVs (Wang and McCarthy 1993). Although there was a minor difference in the restriction fragment profiles, in general both isolates exhibited similar mobility and fragmentation patterns.

Electron microscopy

The SpltNPV multiplied in the nucleus of the fat body. *Fig. 3* shows a section through an infected nucleus three days after post-infection of the larva. The polyhedra occurred in various shapes and sizes. They were round, spherical and oval, and the size ranged from 1.9 to 2.8 μm in diameter. These features resembled the polyhedra of NPVs from *S. litura* from Taiwan (Tuan *et al.* 1995), *S. exigua* (Kondo et al. 1994) and *S. exempta* NPV (Kelly 1985).

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