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ISOLATION AND AMPLIFICATION BACULOVIRUS AS A BIOCONTROL AGENT FOR BAGWORMS AND NETTLE CATERPILLARS OF OIL PALM

Keywords: Baculovirus; nucleopolyhedrosis viruses; granulosis virus; Bagworms; *Metisa plana*; Nettle caterpillars; *Setothosea asigna*; *Darna trima*; *Thosea lutae;* Amplification and *Spodoptera litura*

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Outbreaks of bagworms (Lepidoptera: Psychidae) and nettle caterpillars (Lepidoptera: Limacodidae) in oil palm occasionally affects some estates in Malaysia. Control involves mainly the use of systemic chemicals such as monocrotophos and methamidophos and the spraying of selective narrow spectrum insecticide such as triclorfon. Chemical insecticides pose several potential hazards such as residual problem, insect pest resistance to chemicals and increased frequency of pest outbreaks. In the 1991 national biological control conference, integration of selective chemicals with biological control was recommended as a strategy towards non-polluting agriculture in Malaysia. Use of baculovirus (BV) as a biological control agent supports this recommendation. The objective of this study is to report the progress made in collection, isolation and amplification of BV for the control of some oil palm insect pests.

Collections of 62 000 larvae of Metisa plana, *Wlk (Lepidoptera: Psychidae) and 1000 other pests were made in 1992–1995 from 20 estates in Selangor, Johor, Negeri Sembilan, Pahang, Sabah and Perak. The larvae were dissected, treated and examined for BV infection, particularly the subgroup A and B. The results indicated that nucleopolyhedrosis virus (NPV) was detected in* M. plana, *but the*

degree of infection was tertiary, meaning that it did not successfully infect the host. Granulosis viruses (GV) were detected more frequently than the NPV. Detection of BV was enhanced in stressed and newly dead larvae.

To date, Mahasena corbetti *NPV (McNPV),* Darna trima *GV (DtGV),* Spodoptera litura *NPV (SlNPV),* Mamestra brassicae *NPV (MbNPV),* Agrotis segetum *NPV (AsNPV),* A. segetum *GV (AsGV),* Autographa californica *NPV (AcNPV) (L1, 210, 449) and* Panolis flammae *NPV (PfNPV) have been purified. Mass production of BV for field experiment has been by* in vivo *amplification using a susceptible host. Studies on amplification of these viruses in an alternative host,* S. litura *was carried out. The amplified viruses will be used for characterization and cross-infectivity studies against oil palm insect pests.*

INTRODUCTION

Outbreaks of bagworms (Lepidoptera: Psychidae) and nettle caterpillars (Lepidoptera: Limacodidae) in oil palm (*Elaeis guineensis* Jacq.) occurred occasionally and could lead to serious damage and significant financial loses (Wood, 1977). In the past, outbreaks of bagworms, particularly *Metisa plana* Walker, were associated with the treatment of broad-spectrum, long-residual contact insecticides (Ooi and Lim., 1991; Wood, 1968; 1985). Over the last two decades, bagworm control has involved mainly the use of systemic chemicals such as monocrotophos and methamidophos (Wood *et al.*, 1974) and the use of selective narrow spectrum insecticides such as triclorfon (Wood, 1976). In the 1991 national biological control conference, the integration of these selective chemicals with biological control was recommended as a strategy towards non-polluting agriculture in Malaysia. Chemical insecticides pose several potential hazards such as chemical residues, resistance and increasing frequency of outbreaks.

Disturbances such as adverse population balance of beneficial and harmful insects or microbes are normally caused by misuse of chemical pesticides. Such an adverse balance and outbreak together can lead to a widespread infestation by the pest (Wood, 1977). Therefore, alternative control methods that are environmental friendly must be examined. Several insect pathogenic microbes for the control of oil palm insect pests, have been considered and these include the use of baculovirus (Ramlah Ali and Jalani, 1993) other insect viruses (Sipayung *et al.*, 1991; Mariau, 1990), *Bacillus thuringiensis* (Ramlah Ali and Mohd Basri, 1994; Mohd Basri *et al.*, 1994; Halim *et al.*, 1991) and entomopathogenic fungi (Ramlah Ali *et al.*, 1994; Ramlah Ali *et al.*, 1993).

Baculovirus infections are specially frequent in Lepidoptera compared to other insect viruses. Therefore, it was documented as the virus most favoured for biological control of insect pests (Evan and Entwistle, 1987). Baculovirus is pathogenic to some invertebrates and comprise diverse groups of large viruses whose particles measure 250 × 45 µm. Taxonomically, baculoviruses are divided into three subgroups (Summers, 1977; Blissard and Rohrmann, 1990). The nucleopolyhedrosis virus (NPV), subgroup A, has many virions occluded within single intranuclear polyhedral inclusion body (PIB) and measures 1–5 µm. Subgroup B, granulosis virus (GV) has single virion within each crystal or granule that measures 0.5µm (Entwistle, 1987). The subgroup C, has no inclusion body surrounding the virion (Blissard and Rohrmann, 1990).

Unlike small insect viruses, baculovirus replication is confined to certain invertebrates particularly, Lepidoptera, Hymenoptera,

Diptera, Coleoptera and Crustacea. Its infections were reported in more than 600 species of insects of these orders (Blissard and Rohrmann, 1990). No baculovirus can infect mammals, reptiles, birds, other invertebrates such as earthworms or plants (Evan and Entwistle, 1987; Merryweather *et al.*, 1990). Reviews on use of viruses such as baculovirus (Ramlah Ali and Jalani, 1993) and other small viruses (Mariau, 1990; Sipayung *et al.*, 1991) for controlling insect pests of oil palm have been documented. The objective of this study was to isolate, collect and amplify BV for the biological control of oil palm insect pests particularly, bagworms and nettle caterpillars.

MATERIALS AND METHODS

Sampling of bagworms

Sampling of bagworms was conducted weekly. Twenty estates from four states: Selangor, Johor, Negeri Sembilan and Perak were regular sampling sites for *M. plana.* Dead and live larvae of *M. plana* were collected in sterilized cylinders containing sterilized oil palm leaflet segments. While the dead bagworms were placed in the screw capped vials. In the laboratory, the larvae were sorted into various larval instars. Collections of other insects such as silkworms *Bombyx mori*, tobacco armyworm *Spodoptera litura* were obtained from MARDI. Infected *Mahasena corbetti* and *Darna trima* were collected from plantation agencies in Sabah and *Setothosea asigna* and *Thosea lutae* from estates in Pahang and Selangor respectively. While *Mamestra brassicae* NPV (*Mb*NPV), *Agrotis segetum* NPV (*As*NPV), *A. segetum* GV (*As*GV), *Autographa californica* NPV (*Ac*NPV L1, 210, 449) and *Panolis flammae* NPV (*Pf*NPV) were obtained from the Institute of Virology and Environmental Microbiology (IOVEM), Oxford. Permits for importation of larvae from Sabah and virus from IOVEM, Oxford were granted by the Plant Quarantine Department.

Smearing and staining

The dead larvae for each sampling were immediately smeared and stained for detection of nuclei of infection and inclusion bodies in the cytoplasm. While the live *M. plana* was reared under sterilized condition (Ramlah Ali *et al.*, 1993) using the indoor culture method (Mohd Basri and Kevan, 1995) under stress for one week. They were then treated and examined as for the dead larvae. Stress was in the form of overcrowding, *i.e.* forty larvae were placed in each cylinder compared with a usual number of five larvae per cylinder. All dead and live stressed larvae were smeared on microscopic slides before extraction of baculovirus. The slides were labelled according to the estates, larval stages of *M. plana,* date of sampling and sample number. They were then immersed for five minutes in naphthalene black solution at 44°C. Finally, the slides were rinsed under tap water for 5 – 10 seconds, blotted dry and examined under light microscope.

Histopathology

Twenty to forty stressed larvae were picked up randomly for study on nuclei of infection in intact tissues. The stressed live *M. plana* were dissected out of their cases, fixed, dehydrated, preinfiltrated using paraplast that has a high melting point (Humason, 1972). The embedded samples were sectioned to obtain the thinnest *ca.* 4 µm thick sections using rotary microtome. Staining was done, using naphthalene black before they were mounted and observed under the light microscope. The presence of nuclei of infections, distribution and abundance of PIB in the cytoplasm and nuclei were recorded.

Extraction and purification of nucleopolyhedrosis virus

The schemes for purification of NPV were conducted according to the method documented by Hunter *et al.*, 1984. Naked larvae were either placed in double strength plastic bags and macerated using stomacher machine or homogenised mechanically using a glass homogeniser. Homogenate obtained was filtered through muslin cloth and spunned at low speed to remove large debris and exoskeleton. Pellet was resuspended and the above centrifugation was repeated. The resulting supernatant was spun at 2500 g for 10 minutes to discard the lipid soluble materials and small contaminants. The pellet was resuspended before rate-zonal

centrifugation was conducted using 30%–80% glycerol (v/v).

The band containing virus was collected, diluted and centrifuged at 2 500 g, for 10 minutes. The resulting pellet obtained was resuspended and isopynically centrifuged in 45%–65% (w/w) sucrose. The viral band was collected and rinsed thrice using milliQ water at 2 500g, for 10 minutes.

Extraction and purification of granulosis virus

The method revised by Hunter *et al.*, 1984 was adopted for extraction of GV. The larvae were homogenized as above. Filtered homogenate was spun at 400 g, for 5 minutes for removal of large contaminants. The resulting viral pellet was resuspended before the above centrifugation was repeated. The supernatant was pooled and then spun at 10 000 g, for 30 minutes to remove soluble lipids and small contaminants. The resulting pellet was resuspended before rate-zonal centrifugation was conducted using 30%–80% glycerol (v/v) at 12 000 g, for 40 minutes. The viral band was collected, diluted and centrifuged at 50 000 g, for one hour. The pellet was then resuspended and isopycnic centrifugation was conducted using 45%–65% (w/w) sucrose at 50 000 g, for one hour. The final pellet was rinsed thrice in milliQ water.

Baculovirus

Several types of baculovirus were included in the amplification study. The local isolates were *Mc*NPV, *Sl*NPV and *Dt*GV, while the exotic viruses were the *Mb*NPV, *As*NPV, *As*GV, *Ac*NPV(L1), *Ac*NPV(210), *Ac*NPV(449) and *Pf*NPV.

Host for amplification of baculovirus

The *S. litura,* a tobacco armyworm was obtained from MARDI. This host was chosen for several reasons; first, the ease of maintaining it on commercial diets; second, its midgut pH was conducive for conversion of occluded viruses to the budded virus; third, it was not fastidious and consumed several basic artificial diets and finally, it grew into a large larva within a week to accommodate for large quantities of amplified viruses.

Inoculation of viruses

Two µl of crude virus extracts was inoculated onto the mouthpart of the *S. litura* aseptically. They were placed individually in polypots. The lids of the polypots had slits and were layered with filter paper for absorption of excess moisture. The polypots were arranged according to treatments in separate trays. The trays were filled with palm oil for trapping infected worms that escaped the polypots. At the end of one week macroscopic typical and atypical symptoms were recorded. The dead samples were smeared and examined as mentioned earlier.

RESULTS AND DISCUSSIONS

Approximately 63 000 insects were sampled between 1992–1995 (*Table 1*). They consisted of insects of various genera and species collected from Selangor, Johor, Negri Sembilan, Pahang, Sabah and Perak. The majority of the insects sampled was *M.plana*, amounting to about 62 000 since this species represents the major insect pest in oil palm in West Malaysia.

Before extraction of BV a total of 1 700 bagworms were dissected for early detection of microbes. More than 50% of the bagworms were empty cases, 25% were shrunken and the rest with flattened abdomens (*Table 2*). At the stage of rate-zonal and isopynic centrifugation, the quantity of viruses retrieved was negligible.

The dead bagworms were mostly colonized by fungi, followed by bacteria and baculovirus. The percentage detection of fungi in dead *M.plana* was as high as 62%, 11% for bacteria and 17% for NPV (*Table 3*). Percentage detection of NPV is 45.5% in freshly dead *M. corbetti* and it was undetectable in old dead larvae (*Table 3*). The degree of infection was either primary or secondary for bacteria and fungi, but tertiary for the BV. This implied that the virus was incapable of colonizing and killing the host.

In 1993, live bagworms were subjected to stress prior to death and detection for microbes. The percentage detection of BV (NPV and GV) was increased as seen in *Table 3*. Stressed *M. plana* also had reduced fungal infection of the highest 33.4% compared 61.7% for dead

TABLE 1. INSECT PESTS COLLECTED FOR DETECTION AND ISOLATION OF BACULOVIRUS IN 1992-1995

Year	Location	Pest species	Sample size	Remarks
1992	Selangor	*M. plana*	18,000	GV/NPV•
	Negeri Sembilan	*M. plana*	16,000	GV/NPV•
1993	Selangor	*M. plana*	25,507	GV/NPV•
	Johor	*M. plana*	970	GV/NPV•
		M. corbetti	726	GV/NPV•
1994	Selangor	*Manata sp.*	111	Nil*
	Selangor	*S. litura*	25	NPV*
	Trengganu	*B. mori*	65	NPV/GV*
	Selangor	*D. trima*	30	Nil*
	Pahang	*T. lutae*	150	GV*
	Sabah	*D. trima*	60	GV*
	Sabah	*M. corbetti*	50	NPV/GV*
	Perak	*M. plana*	350	NPV/GV*
1995	Perak	*M. plana*	700	NPV/GV*
	Selangor	*M. plana*	300	Nil*

• - Treated for extraction of NPV and GV, * - treated for detection of NPVand GV.

TABLE 2. DISTRIBUTION OF VARIOUS FORMS OF DEAD BAGWORMS DISSECTED FOR BACULOVIRUS

Location	Pest species and Larval stages	Sample size	Form of dead larvae (%)		
			Shrunken	Empty	Flattened
Selangor	*M.p* L1-L2	7	14.28	57.14	28.57
	M.p L3-L4	319	18.18	68.03	13.79
	M.p L5-L6	475	26.31	58.32	15.37
Johor	*M.p* L1-L2	43	2.33	69.77	27.91
	M.p L3-L4	202	15.84	72.77	11.39
	M.p L5-L6	378	30.42	41.27	28.31
	M.c L1-L5	65	18.46	70.77	10.77
	M.c L6-L9	118	4.24	22.03	72.88
	M.c L10-L13	93	2.15	50.53	47.31

M.p = *M.plana*, *M.c* = *M.corbetti* and L = larval stages of the bagworms

M.plana (*Table 3*). The fungi detected in old dead larvae were probably saprophytes. However, the baculovirus still existed in tertiary degree of infection. It probably takes more than stress to induce baculovirus infections, in *M. plana*.

The possible reason for the unsuccessful colonization of BV in *M. plana* was ascribed to the unfavourable pH of the midgut that ranged from 6–9 (Ramlah Ali and Mohd Basri, 1994). The midgut pH of most Lepidoptera is 10, in which BV polyhedral protein dissolves to

TABLE 3. PERCENTAGE DETECTION OF MICROBES IN DEAD AND STRESSED BAGWORMS

Pest species	Old dead larvae				New dead larvae			
	Fungi	Bacteria	GV	NPV	Fungi	Bacteria	GV	NPV
M.p(294•)	54.8	2.8	0	6.7	33.0	6.1	0	3.5
M.p(257•)	38.3	5.5	0.6	17.2	61.7	10.9	8.5	2.3
M.c(134*)	0	0	0	0	36.2	26.2	0.8	45.5

	Dead stressed larvae				
	Larval stages	Fungi	Bacteria	NPV	GV
M.p(300•)	L1-L3	3.3	66.7	33.3	36.1
M.p(610•)	L4-L5	33.4	54.9	36.1	25.1
M.p(167•)	L6-17	28.7	44.9	24.6	24.6

M.p = M.plana, M.c = M.corbetti, L = Larval instar and •, * - is the sample size taken from Selangor and Johor respectively.

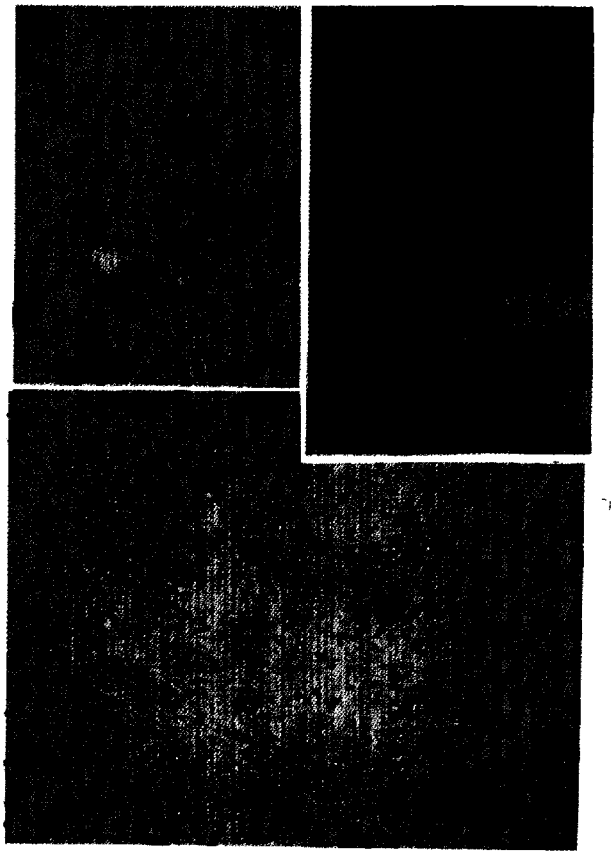

Figure 1. Histology of midgut sections of M. plana *showing the presence of polyhedra (NPV) at tertiary degree of infection in epithelial cells (a) 750X, fat body (b) 1500X and smear showing the presence of polyhedra in the cytoplasm (c), 3000X. An epithelial cell (ep), polyhedra (p), fat cell (fc) and nucleus (n).*

release the infectious virions (Blissard and Rohrmann, 1990). *Figure 1a–c* shows the section of the stressed bagworms revealing tertiary degree of infection in the midgut epithelial cell and fat body.

Amplification trials showed that most BV tested were infectious to the *S. litura* because the presence of nuclei of infection was obvious.This indicated that *S.litura* was a good host for *in vivo* amplification of PORIM collection of baculoviruses. However, an atypical viral disease symptom was noted for all BV (*Figure 2a*) except for *Sl*NPV.

The *Sl*NPV, *Mb*NPV, *As*NPV, *Ac*NPV (449) and *Pf*NPV caused primary degree of infection. Primary degree of infection was presented as many nuclei of infection and PIB in the cytoplasm, as seen in *Figure 2bi–iv*. While, *Ac*NPV (L1), *Ac*NPV (210), *Dt*GV and *Mc*NPV resulted in secondary degree of infection few nuclei of infections and some polyhedra were present in the cytoplasm (*Figure 2ci-iii*).

The percentage mortality of *S.litura* caused by the various BV ranged from 40%–100%. All BV resulted in mortality that varied from 50%–75% except *Sl*NPV and *As*NPV which led to 100% and 40% respectively. The susceptible host, *S.litura* which pupated and emerged into adults during the incubation period were harbouring amplified amount of virus. At the end of the bioassay, irrespective of the status of mortality and stage of *S.litura*, they can be

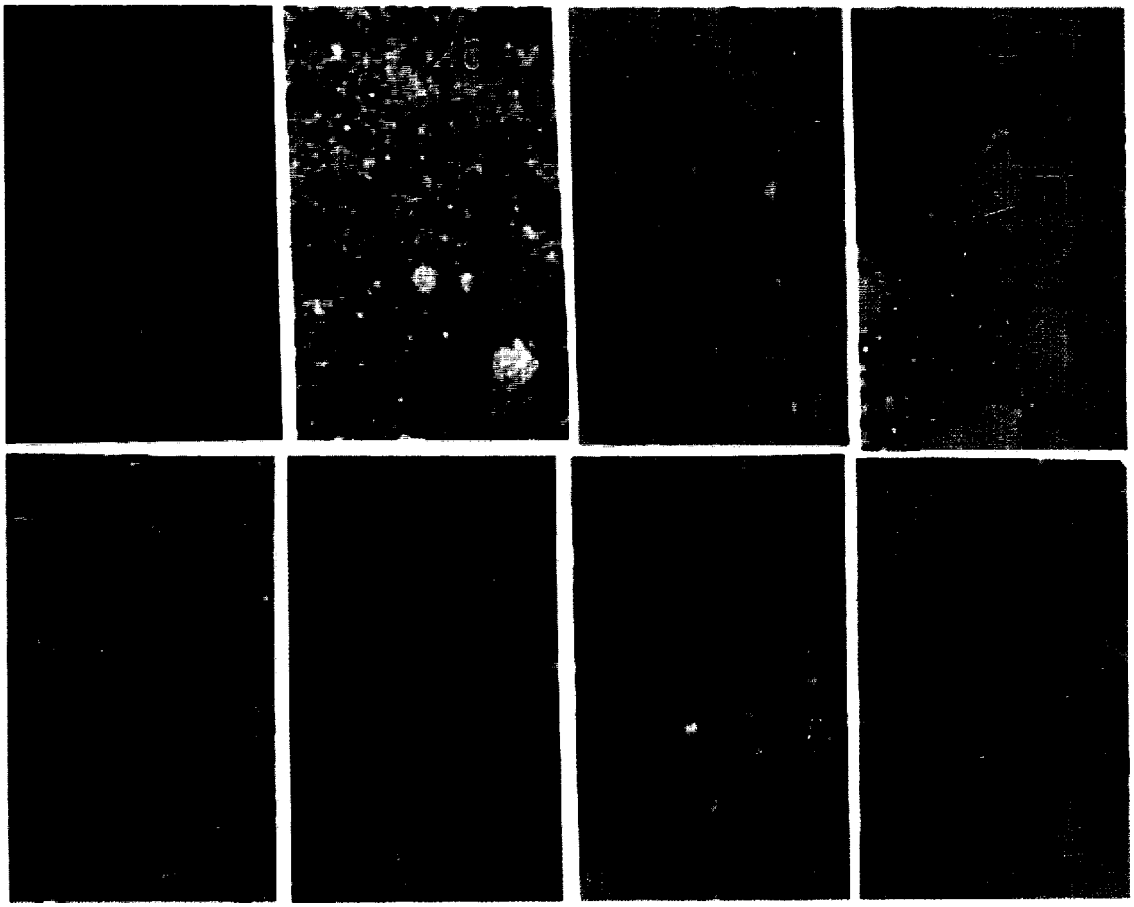

Figure 2. An atypical viral symptom in S. litura *caused by* Mb*NPV, showing darkening (a), 1X. Primary degree infection with presence of nuclei of infection and many PIBs in the cytoplasm (b), 3000X. Secondary degree infection with nuclei of infection and few PIBs in the cytoplasm (c), 3000X.* S. litura *infection due to* Sl*NPV (2bi),* Ac*NPV (449) (2bii),* As*NPV (2biii),* As*GV (2biv),* Mc*NPV (2ci),* Dt*GV/NPV (2cii) and* Ac*NPV(210) (2ciii). Nucleus of infection (ni), infection in a nucleus (in), granules (g) and nucleus (n).*

homogenized as source of inoculum for field testing. The amount of PIB obtained was 1.2×10^{10} PIB/larva for *Sl*NPV and *Ac*NPV(449), 1.8×10^{10} PIB/larva for *Ac*NPV(L1), 9.6×10^{9} PIB/larva for McNPV, 3.5×10^{8} PIB/larva for *As*NPV and 6.0×10^{8} PIB/larva for *Pf*NPV. Index of amplification, PIB obtained per larva divided by dose of PIB given per larva *per os* was between $1.73 \times 10^{3} - 1.73 \times 10^{5}$, with *Sl*NPV, *Mc*NPV and *Ac*NPV(L1) giving the highest scores.

CONCLUSIONS

The type A (nucleopolyhedrosis) and type B (granulosis) *viruses* were present in both *M.plana* and *M.corbetti*. Naturally they result in tertiary degree of infection. Stress can increase the percentage detection of baculovirus but not the degree of infection. However, stress and the application of a crude amplified virus could enhance the infectivity of some baculovirus, because crude virus in the amplified form, contains the infectious and occluded virus. Unlike *M. plana, S. litura* has conducive midgut pH of 10 for dissolution of inclusion bodies to cause infection in *in vivo* amplification of BV. For trials on bioassay, the baculovirus must first be amplified *in vivo*, in an aseptic condition or artificial diet. The infectious phenotype or budded virus helps infection in target pests such as *M. plana* and other insect pests of oil palms.

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