



UNIVERSITI PUTRA MALAYSIA

**CHARACTERIZATION OF GRANULOVIRUS AND
NUCLEOPOLYHEDROVIRUS ISOLATED FROM *SPODOPTERA LITURA***

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By

LAU WEI HONG

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

March 2002

Specially to my husband, my mother, my brothers and sister



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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NUCLEOPOLYHEDROVIRUS ISOLATED FROM *SPODOPTERA LITURA***

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March 2002

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Two baculoviruses were isolated and identified from *Spodoptera litura*; *S. litura* nucleopolyhedrovirus (SpltnPV) and *S. litura* granulovirus (SpltnGV). The polyhedra of SpltnPV were about 0.9-1.83 μm in diameter containing multiple virions measuring about 100-280 nm wide and 320-410 nm long. The SpltnPV virions contained nucleocapsids (47-60 nm wide and 300-350 nm long) within an envelope, and the size of capsids measured about 58-60 nm wide and 300-330 nm long.

The capsules of SpltnGV were about 0.2-0.3 μm wide and 0.45-0.55 μm long containing single virion (60-73 nm wide and 245-267 nm long). The SpltnGV nucleocapsids measured approximately 54-60 nm wide and 287-410 nm long, and found singly enclosed within an envelope. The SpltnGV capsids measured about 36-58 nm wide and 175-277 nm long.

The restriction endonuclease analyses (REN) revealed that these two baculoviruses did not show any identical restriction pattern. The DNA size of the SpltnPV and the



SpltGV was estimated to be 132 kb and 124 kb, respectively. The nucleotide sequence analysis of the polyhedrin gene of SpltNPV had 98% sequence identity to the known SpltNPV (accession number: AF037262); while the granulin gene of SpltGV had 81% sequence identity to the granulin gene of *Xestia c-nigrum* granulovirus (accession number: U70069). Based on the sequence analysis, the SpltNPV and the SpltGV are placed as a taxon of Group II NPV and Group GV, respectively.

Both viruses exhibited general symptoms of polyhedrosis and granulosis. The SpltNPV-infected larvae showed pinkish yellow at the dorsal and lateral sides, while the SpltGV-infected larvae exhibited whitish ventral. The SpltNPV caused a reduction in the larval size while the SpltGV-infected larvae increased in size with bloated integument when lower viral dosages were given. Both viruses infected fat bodies, Malpighian tubules, tracheal matrices, hypodermis, muscles and midguts. The SpltNPV replicated in the nucleus and spread the disease to susceptible tissues within 24-h postinoculation (p.i). The SpltGV was found replicating in both nucleus and cytoplasm, and the disease spread gradually after 48-h p.i. The LD₅₀ of both viruses in neonate larvae of *S. litura* were 9.04×10^2 polyhedra for SpltNPV and 1.26×10^4 capsules for SpltGV. The LT₅₀ of both viruses were similar when neonate larvae were fed with similar ranges of viral dosages. The SpltNPV showed a higher virulence in *S. litura* larvae than the SpltGV. The characterization of these baculoviruses is of particular interest in view of its possible use in biological or integrated control.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN GRANULOVIRUS AND NUCLEOPOLYHEDROVIRUS DARI
*SPODOPTERA LITURA***

Oleh

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Dua jenis bakulovirus telah dipencilkan and dicirikan dari *Spodoptera litura*, iaitu *S. litura* nukleopolihedrovirus (SpltnPV) dan *S. litura* granulovirus (SplgV). Saiz polibedra SpltnPV adalah lebih kurang 0.9-1.83 μm diameter dan mengandungi virion berganda yang berukuran 100-280 nm lebar and 320-410 nm panjang. Virion SpltnPV mengandungi nukleokapsid (47-60 nm lebar dan 300-350 nm panjang) dalam satu sampul dan kapsid berukuran 58-60 nm lebar dan 300-330 nm panjang.

Kapsul SplgV adalah lebih kurang 0.2-0.3 μm lebar dan 0.45-0.55 μm panjang dan mengandungi satu virion (60-73 nm lebar dan 245-267 nm panjang). Saiz nukleokapsid SplgV lebih kurang 54-60 nm lebar dan 287-410 nm panjang dan satu nukleokapsid terdapat terkurung dalam satu sampul. Kapsid SplgV adalah berukuran 36-58 nm lebar dan 175-277 nm panjang.

Analysis Pembatasan Endonuklease (REN) menunjukkan kedua-dua bakulovirus tersebut tidak mempunyai corak pembatasan yang sama. Saiz DNA SpltNPV dan SpltGV telah dianggarkan sebesar 132 kb dan 124 kb, masing-masing. Analisis jujukan nukleotida menunjukkan gen polihedrin SpltNPV mempunyai 98% homologi dengan SpltNPV yang dikenali (nombor asesi: AF037262), manakala gen granulin SpltGV mempunyai 81% jujukan sama dengan gen granulin XcGV (nombor asesi: U70069). Berdasarkan analysis jujukan tersebut, SpltNPV dan SpltGV masing-masing diletakkan sebagai satu takson dalam Kumpulan NPV II dan Kumpulan GV.

Kedua-dua virus menghasilkan simptom penyakit polihedrosis dan granulosis yang umum. Larva yang dijangkiti oleh SpltNPV menunjukkan warna kuning kemerah-mudaan pada sisi-sisi tepi dan belakang, manakala larva yang dijangkiti oleh SpltGV menunjukkan warna putih pada sisi ventral. SpltNPV menyebabkan pengurangan saiz larva, manakala larva yang dijangkiti oleh SpltGV bertambah saiz badan dengan integumen yang mengembang apabila sukatan virus yang rendah diberikan. Kedua-dua virus menjangkiti tisu lemak, tubul Malpighian, matriks trakea, hipodermis, otot dan usus tengah. SpltNPV membiak dalam nukleus dan penyakit merebak ke tisu-tisu yang mudah dijangkiti dalam masa 24 jam selepas jangkitan (p.i.). SpltGV didapati membiak dalam kedua-dua nukleus dan sitoplasma dan penyakit merebak secara perlahan-lahan selepas 48 jam jangkitan (p.i.). LD₅₀ untuk kedua-dua virus dalam larva *S. litura* yang baru lahir adalah 9.04×10^2 polihedra untuk SpltNPV dan 1.26×10^4 kapsul untuk SpltGV. LT₅₀ adalah sama untuk kedua-dua virus bila larva yang baru lahir diberi julat sukatan virus yang serupa. SpltNPV menunjukkan kevirulenan yang lebih tinggi daripada SpltGV terhadap larva *S. litura*. Pencirian

kedua-dua baculovirus tersebut adalah diminati khas disebabkan penggunaannya dalam pengawalan biologi atau bersepadu.

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I certify that an Examination Committee met on 11th March 2002 to conduct the final examination of Lau Wei Hong on her Doctor of Philosophy thesis entitled "Characterization of Granulovirus and Nucleopolyhedrovirus Isolated from *Spodoptera litura*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



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TABLE OF CONTENTS

	Page
DEDICATION	2
ABSTRACT	3
ABSTRAK	6
ACKNOWLEDGEMENTS	8
APPROVAL	10
DECLARATION	12
LIST OF TABLES	16
LIST OF FIGURES	17
LIST OF ABBREVIATIONS	19
 CHAPTER	
1 INTRODUCTION	22
2 LITERATURE REVIEW	25
2.1 <i>Spodoptera litura</i> (Fabricius)	25
2.1.1 Common Name	25
2.1.2 Geographical Distribution	25
2.1.3 Life Cycle	26
2.1.4 Host Range	26
2.2 Baculoviruses	27
2.2.1 Historical Background	27
2.2.2 Classification	28
2.2.3 Nomenclature	29
2.2.4 General Properties	31
2.2.5 Host Range	32
2.3 Occlusion Body	32
2.3.1 Size and Shape	33
2.3.2 Crystalline Lattice	34
2.3.3 Surface Structure	34
2.3.4 Dissolution of Occlusion Bodies	34
2.3.5 Mutants of Occlusion Body	35
2.4 Virion	36
2.4.1 Size and Shape	36
2.4.2 Phenotypes of Virion	37
2.5 Nucleocapsid	38
2.6 Occlusion Gene	39
2.7 Techniques Used in DNA Analysis	41
2.7.1 Restriction Endonuclease Analyses	41
2.7.2 Polymerase Chain Reaction (PCR) Method	42
2.8 Pathology Studies	44
2.8.1 Route of Infection	44
2.8.2 Symptomatology	44
2.8.3 Development of Baculoviruses in Diseased Larvae	45



2.8.4	Histopathology	49
2.8.5	Bioassay	50
2.8.6	Mixed infection	52
3	GENERAL MATERIALS AND METHODS	57
3.1	Source of Larvae	57
3.2	Source of Virus	57
3.3	Source of Chemicals and Biochemicals	57
3.4	<i>In Vivo</i> Propagation of <i>S. litura</i> NPV and GV	57
3.5	<i>In Vitro</i> Propagation of AcMNPV	58
3.6	Purification of <i>S. litura</i> NPV and GV from Infected Larvae	58
3.7	Purification of AcMNPV from Insect Cell Line	59
3.8	Electron Microscopy Negative Staining	60
3.9	Counting of Occlusion Bodies	60
3.10	DNA Extraction	61
3.11	Spectrophotometry	61
3.12	Restriction Endonucleases Analysis	62
3.13	High Pure PCR Product Purification Kit	62
3.14	TOPO TA Cloning	63
3.15	High Pure Plasmid Isolation Kit	65
3.16	Sequencing	65
4	ISOLATION AND IDENTIFICATION OF GV AND NPV FROM <i>SPODOPTERA LITURA</i>	68
4.1	Introduction	68
4.2	Materials and Methods	68
4.3	Results	69
4.3.1	Isolation of Baculoviruses	69
4.3.2	Identification of Baculoviruses	69
4.4	Discussion	72
5	MOLECULAR STUDIES OF GV AND NPV ISOLATED FROM <i>SPODOPTERA LITURA</i>	81
5.1	Introduction	81
5.2	Materials and Methods	81
5.2.1	Restriction Endonucleases Analysis	81
5.2.2	Polymerase Chain Reaction	82
5.3	Results	84
5.3.1	Comparison of Baculoviruses DNA	84
5.3.2	PCR Product	85
5.3.3	Nucleotides Sequence Analysis	86
5.3.4	Phylogenetic Analysis	87
5.4	Discussion	87
6	SYMPTOMATOLOGY OF GRANULOSIS AND POLYHEDROSIS IN <i>SPODOPTERA LITURA</i> LARVAE	103
6.1	Introduction	103



6.2	Materials and Methods	103
6.3	Results	104
6.3.1	Symptoms of Larvae Infected with Mixed Baculoviruses	104
6.3.2	Symptoms in Larvae Infected with Purified GV	104
6.3.3	Symptoms of Larvae Infected with NPV	105
6.4	Discussion	106
7	LIGHT AND ELECTRON MICROSCOPE HISTOPATHOLOGY STUDIES OF GV AND NPV	
		110
7.1	Introduction	110
7.2	Materials and Methods	110
7.2.1	Light Microscopy	110
7.2.2	Electron Microscopy	111
7.3	Results	112
7.3.1	Histopathology Study	112
7.3.2	Granulosis	112
7.3.3	Polyhedrosis	114
7.3.4	Mixed Infection	117
7.4	Discussion	118
8	DOSAGE AND TIME-MORTALITY STUDIES ON GV AND NPV	141
8.1	Introduction	141
8.2	Materials and Methods	141
8.3	Results	142
8.3.1	Bioassay with SpltNPV ^M	142
8.3.2	Bioassay with SpltGV ^M	143
8.4	Discussion	144
9	GENERAL DISCUSSION	150
10	CONCLUSION	154
	REFERENCES	157
	APPENDICES	168
	VITA	178



LIST OF TABLES

Table		Page
2.1	Size of polyhedra from different species of insect virus	53
2.2	Shape of polyhedra from different species of insect virus	53
2.3	Size of capsules from different species of insect virus	54
2.4	Size and number of virions per polyhedra of NPVs	55
2.5	Size of virion per capsule of GVs	55
2.6	Average size of NPV nucleocapsid	56
2.7	Average size of GV nucleocapsid	56
3.1	List of chemicals and biochemicals	67
5.1	List of restriction endonucleases used in this study	91
5.2	List of primers used in this study	91
5.3	Amplification program used in this study	92
5.4	Estimated size (in bp) of SpltGV ^M DNA fragments following restriction with <i>Bam</i> HI, <i>Eco</i> RI and <i>Hind</i> III	92
5.5	Estimated size (in bp) of SpltNPV ^M DNA fragments following restriction with <i>Bam</i> HI, <i>Hind</i> III and <i>Kpn</i> I	93
5.6	Nucleotide sequence identities (%) of NPV polyhedrin gene	93
7.1	Sequence of infection of tissues of <i>S. litura</i> larvae with local isolates of baculovirus	123



LIST OF FIGURES

Figure		Page
4.1	Sucrose gradient of occlusion bodies (OBs) originally isolated from diseased larvae of <i>S. litura</i>	75
4.2	Electron micrograph of occlusion bodies of mixed baculoviruses negatively-stained with 2% MAT	75
4.3	Gradient purification of occlusion bodies after a few generations of dilution and propagation in larvae. B1 and B2 were bands composed of occlusion bodies with different density gradients	76
4.4	Purification of polyhedral-shaped occlusion bodies	76
4.5	Purification of ovocylindrical-shaped occlusion bodies	77
4.6	Purification of virion of ovocylindrical-shaped occlusion body	78
4.7	Dissolution of virion of polyhedral-shaped occlusion body	79
4.8	Purification of virion of polyhedral-shaped occlusion body	79
4.9	Electron micrograph showing nucleocapsid of ovocylindrical-shaped occlusion bodies and polyhedral-shaped occlusion bodies	80
4.10	Electron micrograph showing capsid of ovocylindrical-shaped and polyhedral-shaped occlusion bodies	80
5.1	Fragments of SpltGV ^M isolate DNA produced by RENs	94
5.2	Restriction enzyme fragment patterns of SpltNPV ^M isolate	95
5.3	Agarose gel electrophoresis of SpltGV ^M , SpltNPV ^M , and AcMNPV	96
5.4	PCR amplification of the polyhedrin gene coding region of SpltNPV ^M	97
5.5	PCR amplification of the granulin gene coding region of SpltGV ^M	97
5.6	Nucleotide sequence of npv2.	98
5.7	Nucleotide sequences of gv1, gv2, and gv3	99
5.8	Alignment of the nucleotide sequences of the polyhedrin and granulin genes from SpltNPV ^M and SpltGV ^M	100
5.9	DNA distance matrix	101
5.10	Phylogenetic analysis of occlusion gene nucleotide sequences of Baculoviruses	102
6.1	Baculoviral symptoms produced in <i>S. litura</i> larvae	108
6.2	Healthy larvae of <i>S. litura</i>	108
6.3	SpltGV ^M symptoms produced in <i>S. litura</i> larvae	108
6.4	SpltNPV ^M symptoms produced in <i>S. litura</i> .	109
7.1	Midgut lumen shows the presence of ingested castor oil leaves	124
7.2	Longitudinal sections of healthy <i>S. litura</i> larval	124
7.3	Invasion of SpltGV ^M into midgut cells of <i>S. litura</i> larva	125
7.4	Section of larval malphigian tubule and trachea matrix of <i>S. litura</i> infected with SpltGV ^M	126
7.5	Sections of nucleocapsids and budded viruses in larval tissue	126
7.6	Formation of capsules in fat body and malphigian tubule	127
7.7	Section of larval hypodermis of <i>S. litura</i> infected with SpltGV ^M	128
7.8	Section of larval muscle of <i>S. litura</i> infected with SpltGV ^M	128
7.9	Disease progression of SpltGV ^M in tissues of <i>S. litura</i>	129
7.10	Sections of rupture tissue of <i>S. litura</i> after 288-h p.i. with SpltGV ^M	130



7.11	Sections of mature capsules in the cytoplasm and haemolymph	131
7.12	Section of abnormal capsules in SpltGV ^M -infected fat cells	131
7.13	Section of larval tissue of <i>S. litura</i> infected with SpltNPV ^M after 24-h p.i.	132
7.14	Infected tissues of <i>S. litura</i> after 48-h p.i. with SpltNPV ^M	132
7.15	Section of larval tissue of <i>S. litura</i> after 72-h p.i. with SpltNPV ^M	133
7.16	Sections of larval fat body and trachea matrix of <i>S. litura</i> after 120-h p.i. with SpltNPV ^M	133
7.17	Sections of infected tissue of <i>S. litura</i> after 144-h p.i. with SpltNPV ^M	134
7.18	Infection of SpltNPV ^M in larval tissue	135
7.19	Formation of SpltNPV ^M nucleocapsids within the virogenic stroma	136
7.20	Formation of SpltNPV ^M virions	137
7.21	Formation of SpltNPV ^M polyhedra	138
7.22	Section of fat cells with abundant of mature SpltNPV ^M polyhedra formed in the hypertrophied nuclei	138
7.23	Polyhedra stained in two different forms	139
7.24	Abnormal polyhedra lacking of nucleocapsids enclosed within the virions	139
7.25	Infection in fat body and hypodermis	140
7.26	Mixed infection in fat body	140
7.27	Sections of infected malpighian tubules	140
8.1	Cumulative percentage mortality of 1-day old <i>S. litura</i> larvae inoculated with five different SpltNPV ^M dosages after 3 days p.i.	147
8.2	Probit mortality plot of SpltNPV ^M on <i>S. litura</i> larvae	147
8.3	Cumulative percentage mortality of 1-day old <i>S. litura</i> larvae bioassayed against SpltGV ^M after 3 days p.i.	148
8.4	Dosage-mortality response of 1-day old <i>S. litura</i> larvae to SpltGV ^M	148
8.5	Cumulative percentage mortality of 1-day old <i>S. litura</i> larvae infected with SpltGV ^M	149
8.6	Cumulative percentage mortality of 4-day old <i>S. litura</i> larvae infected by SpltGV ^M	149

LIST OF ABBREVIATIONS

A260	absorption at 260 nm
AcfMNPV	<i>Actebia fennica</i> MNPV
AcMNPV	<i>Autographa californica</i> MNPV
AcMNPV	<i>Autographa californica</i> MNPV
AgMNPV	<i>Anticarsia gemmatalis</i> MNPV
AgNPV	<i>Anticarsia gemmatalis</i> NPV
AgurSNPV	<i>Aglais urticae</i> SNPV
AjGV	<i>Achaea janata</i> GV
AnfaMNPV	<i>Anagrapha falcifera</i> MNPV
AnpeNPV	<i>Antheraea pernyi</i> NPV
ArceMNPV	<i>Archips cerasivoranus</i> MNPV
ArceNPV	<i>Archips cerasivoranus</i> NPV
ArveGV	<i>Argyrotaenia velutinana</i> GV
BmMNPV	<i>Bombyx mori</i> MNPV
BmNPV	<i>Bombyx mori</i> NPV
bp	base pairs
BusuNPV	<i>Buzura suppressaria</i> NPV
BusuSNPV	<i>Buzura suppressaria</i> SNPV
CfNPV	<i>Choristoneura fumiferana</i> NPV
ChfuGV	<i>Choristoneura fumiferana</i> GV
ChmuGV	<i>Choristoneura murinana</i> GV
ChroMNPV	<i>Choristoneura rosaceana</i> MNPV
CpGV	<i>Cydia pomonella</i> GV
CrleGV	<i>Cryptophlebia leucotreta</i> GV
DiheSNPV	<i>Diprion hercyniae</i> SNPV
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
EcobSNPV	<i>Ectropis obliqua</i> SNPV
EDTA	ethylenediamine tetraacetic acid
EppoMNPV	<i>Epiphyas postvittana</i> MNPV
EppoNPV	<i>Epiphyas postvittana</i> NPV
ErtiSNPV	<i>Erannis tiliaria</i> NPV
EsacGV	<i>Estigmene acrea</i> GV
EuocGV	<i>Euxoa ochrogaster</i> GV
ExapGV	<i>Exartema appendiceum</i> GV
GibiGV	<i>Glena bisula</i> GV
GV	Granulovirus
h	hour
HabrGV	<i>Harrisina brillians</i> GV
HearNPV	<i>Helicoverpa armisgera</i> NPV
HycuGV	<i>Hyphantria cunea</i> GV
HycuNPV	<i>Hyphantria cunea</i> NPV
HzNPV	<i>Helicoverpa zea</i> NPV
HzSNPV	<i>Helicoverpa zea</i> SNPV
HzSNPV	<i>Helicoverpa zea</i> SNPV



JucoGV	Junonia coenia GV
kb	kilobase pairs
LD ₅₀	lethal dose 50
LdNPV	Lymantria dispar NPV
LeseNPV	Leucania seperata NPV
LT ₅₀	lethal time 50
LymoNPV	Lymantria monacha NPV
M	micromolar
MaamMNPV	Malacosoma americanum MNPV
MacoNPV	Malacosoma constrictum NPV
MadiMNPV	Malacosoma disstria MNPV
MARDI	Malaysian Agricultural Research and Development Institute
MbMNPV	Mamestra brassicae MNPV
MbNPV	Mamestra brassicae NPV
MepeGV	Melanchra persicariae GV
mm	minute
MNPV	multiple nucleocapsid polyhedrosis virus
NanaGV	Natada nararia GV
NeseSNPV	Neodiprion sertifer SNPV
NeswSNPV	Neodiprion swaini NPV
NeviSNPV	Neodiprion virginiana SNPV
nm	nanometer
NPV	Nucleopolyhedrovirus
nt	nucleotide
OpMNPV	Orgyia pseudosugata MNPV
OpNPV	Orgyia pseudosugata NPV
OranNPV	Orgyia anartoides NPV
OratSNPV	Orgyia antiqua SNPV
OrleSNPV	Orgyia leucostigma NPV
p.i.	post-inoculation
PadoNPV	Panaxia dominula NPV
PafMNPV	Panolis flammea NPV
PalaMNPV	Pandemis lamprosana NPV
PbGV	Pieris brassicae GV
PCR	polymerase chain reaction
PenuNPV	Perina nuda NPV
PhopGV	Phthorimaea operculella GV
PIB	Polyhedral Inclusion Body
PiraGV	Pieris rapae GV
PlorNPV	Plusia orichalco NPV
PlscGV	Plathypena scabra GV
Probit	probability unit
PsunGV	Pseudaletia unipuncta GV
PyanGV	Pygaera anastomosis GV
RoMNPV	Rachiplusia ou MNPV
s	second
SacaGV	Sabulodes caberata GV

SDS	sodium dodecyl sulfate
SeMNPV	Spodoptera exigua MNPV
SeNPV	Spodoptera exigua NPV
SfMNPV	Spodoptera frugiperda MNPV
SfNPV	Spodoptera frugiperda NPV
SNPV	single nucleocapsid polyhedrosis virus
SpliMNPV	Spodoptera littoralis MNPV
SpltNPV	Spodoptera litura NPV
TAE	Tris-acetate-EDTA buffer
Taq	Thermos aquaticus
ThliSNPV	Thymelicus lineola SNPV
ThorSNPV	Thysanoplusia orchalcea SNPV
TipaNPV	Tipula paludosa NPV
TnGV	Trichoplusia ni GV
TnSNPV	Trichoplusia ni SNPV
TrvmSNPV	Trichiocampus viminalis SNPV
V	volt
v/v	volume per volume
w/v	weight per volume
WisiNPV	Wiseana signata NPV
WisiSNPV	Wiseana signata SNPV
XcGV	Xestia c-nigrum GV

CHAPTER 1

INTRODUCTION

An insect is considered a pest when its presence causes an economically important loss. Various types of chemicals have been used for controlling pests. They are fast in action and can be used for a broad range of pests. The excessive use of chemical insecticides, however, can result in building up of pest resistance, side effects on beneficial and non-targeted insects, and pollution to the environment that indirectly harm public health. Chemical control has worsened the pest problem.

Integrated pest management (IPM) promotes an alternative to chemical pest control which includes the use of pest-resistant plants, cultural methods, and biological control, and recommends the application of combined methods to minimize the chance of the target insects adapting to any single tactic (Zechendorf, 1995). Biological control utilizes natural living organisms to control a particular pest. These natural enemies can be a predator or parasite (macrobial control), or pathogen (microbial control) (Burgess and Hussey, 1971). They normally occur under the conditions of pest outbreaks and are important factors in reducing the density of pest under natural population (Weiser, 1977). These biological control agents neither accumulate in the food chain nor harm the environment, but only make contact with particular targets. According to Debach and Rosen (1991), only 15% of these organisms have been discovered and identified.



The use of microbial control in insect pest is not a new concept. Microbial insecticides mainly refer to bacteria, fungi, viruses, nematodes, protozoa and rickettsiae. *Bacillus thuringiensis* toxin (Bts) is the most successful bioinsecticide and commercially available. However, resistance of pest against the Bt has been reported (Zechendorf, 1995). Fungi, protozoa and nematodes are slow in action and only effective under favorable conditions. Rickettsiae have low specificity to its host and are also pathogenic to warm-blooded animals (Burges and Hussey, 1971). Therefore, viruses are promising biological control agents since they attack the target insects and reprogram the host cells for virus production. Death is the final stage for insect with viral disease.

Baculoviruses are the most commonly and widely studied double-stranded DNA viruses that infect insects. They are divided into *Nucleopolyhedrovirus* (NPV) and *Granulovirus* (GV) based on their morphology. The baculoviruses have been found in over 600 species of arthropods (Blissard and Rohrmann, 1990) and can be as effective as chemical pesticides in controlling specific pests. They are environmentally attractive because they are highly host-specific and, in general, only infect insect species within a limited host range. They have no impact on plants, mammals, fishes, birds or even non-target insects and do not accumulate in food chains. The occluded viruses are very stable and transmitted horizontally among their host. They are ingested by the susceptible larvae, replicate in the host and finally the host dies releasing large quantities of the occlusion bodies into the environment that maximize the chance of other insects to come in contact with the virus and in turn become infected. Vertical transmission, however, occurs through contamination of

female ovipositor during egg laying. The newly hatched larvae will be infected after consuming the virus from the eggshell.

The development of baculoviruses as an ideal IPM candidate is very promising in the near future. Since people are very concerned on the impact of using chemical control, many baculoviruses have been discovered and studied. Miller and Dawes (1978) reported that HzNPV and OpNPV were registered as pesticides by the U.S. Environmental Protection Agency.

In Malaysia, NPV and GV have been found naturally in the diseased larvae of *Spodoptera litura*. A pathogenicity test of SpltNPV to *S. litura* larvae was carried out by Sajap *et al.* (2000) who reported that the larval mortality was dependent on the viral doses. The properties of NPV and GV found in *S. litura* larvae have not been fully characterized yet. The fundamental studies of these viruses are important to commercial production. Furthermore, these studies are crucial in managing and manipulating the viruses. Since insect virus classification is based on the intrinsic properties of the virus, this thesis concentrates on the basic characterization of both viruses in order to develop a better biopesticide that is environmentally friendly and highly effective for controlling *S. litura*. Therefore, the objectives to this study are to:

- 1) Isolate and characterise GV and NPV from *Spodoptera litura*.
- 2) Analyse the DNA of GV and NPV from *S. litura*.
- 3) Study the pathology of GV and NPV in *S. litura*.