UNIVERSITI PUTRA MALAYSIA

REPLICATION OF SPODOPTERA LITURA NUCLEOPOLYHEDROVIRUS IN INSECT-DERIVED CELL LINES

NORHALINA BINTI MOHD NOH

FP 2014 74

REPLICATION OF *SPODOPTERA LITURA* NUCLEOPOLYHEDROVIRUS IN INSECT-DERIVED CELL LINES

By

NORHALINA BINTI MOHD NOH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilments of the Requirements for the Degree of Master of Science

December 2014

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in the fulfilment of the requirement for the degree of Master of Science

REPLICATION OF *SPODOPTERA LITURA* NUCLEOPOLYHEDROVIRUS IN AN INSECT-DERIVED CELL LINES

By

NORHALINA BINTI MOHD NOH

December 2014

Chairman : Lau Wei Hong, PhD

Faculty : Agriculture

Spodoptera litura nucleopolyhedrovirus (SpltNPV) showed promising ability in controling *Spodoptera litura* outbreak and may play a unique role in the IPM system to control other permissive insect pests. The insect cell culture (*in vitro*) technique is the most suitable approach to be applied as it provides clean ease manipulation condition for viral gene improvement and large scale production of SpltNPV. Difficulties in the previous trials of *in vitro* propagation of SpltNPV were overcome by improvement in BV inoculums production and titration. Utilization of more cell lines from different host species and geographical isolates had provided more information concerning the SpltNPV host range. A successful *in vitro* propagation of *Spodoptera litura* nucleopolyhedrovirus (SpltNPV) was established in this study. This process was greatly influenced by the quality of the budded virus (BV) inoculum produced by *in vivo* propagation in its primary natural host, *Spodoptera litura* larvae. BV can be harvested either from infected larval haemolymph or homogenized larval tissue. Unfortunately, inocula prepared by both techniques were melanised due to oxidation of exposed larval haemolymph or tissues. This melanisation affected the *in vitro* virus-host interaction. However, the use of severe NPV-infected larvae and Grace's Insect Medium during the virus inoculum preparation and storage successfully reduced the melanisation in the virus inoculum and allowed productive SpltNPV infection in susceptible insect cells. Meanwhile, the end point dilution titration technique proved to be the most rapid, accurate and economic method to measure the BV titre in this study. Seven cell lines (Sf9, SpIm, LD, MaBr, SD, *T.ni* and SpLi) were tested for their permissibility to the infection of SpltNPV. This permissibility screening was conducted through light and electron microscopic examination for cytopathological changes caused by SpltNPV infection. Productive infection was detected in the infected permissive Sf9 cells with the production of occlusion bodies (OBs). However, the other six cell lines showed an abortive infection and no OB was detected. These six cell lines were known as semipermissive to the infection of SpltNPV as the PCR of virus essential genes (*ie-1, lef-2* and *polh*) detected the replication of SpltNPV until the tenth BV passage. An ultrastructural study further confirmed the infection in these cells. This indicated the formation of virogenic stroma and virus particles in the infected Sf9 nuclei while the other six cell lines shared some minor changes, such as vesicle formation and

hypertrophied nuclei with no formation of virus particle. Serial passage study also revealed the capability of the BV progeny to effectively infect Sf9 cells with productive infection until the tenth BV passage. Apoptosis occurrence in SpIm, SpLi cells and Sf9 cells is responsible for preventing and limiting SpltNPV propagation in these cell lines. This phenomenon was detected through the cells morphological changes assessment, AO/PI analysis and DNA fragmentation assay. Meanwhile, RT-PCR was used to detect and compare an apoptotic suppressor gene (*p49*) expression in all three cell lines. Findings showed high expression of *p49* was detected in the permissive Sf9 cells at both the first and tenth passage while low expression was detected in the SpIm and SpLi cell lines. This was evidence of *p49* involvement as virus armour to manipulate and conquer the Sf9 cell line system to allow SpltNPV replication and propagation. This research had provided important information of SpltNPV host range and its susceptibility against several Lepidoptera species and geographical isolates. Subsequent research had shown an apoptosis mechanism involvement in limiting viral replication which was defeated by viral apoptotic suspressor gene (*p49*) in permissive Sf9 cell line.

Absrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

REPLIKASI *SPODOPTERA LITURA* NUKLEOPOLIHEDROVIRUS DI DALAM SEL-SEL SERANGGA

Oleh

NORHALINA BINTI MOHD NOH

Disember 2014

Pengerusi : Lau Wei Hong, PhD

Fakulti : Pertanian

Spodoptera litura nukleopolihedrovirus (SpltNPV) telah menunjukkan kemampuan dalam mengawal pembiakan serangga perosak *Spodoptera litura* (*S.litura*) dan berkeupayaan memainkan peranan yang unik dalam sistem IPM untuk mengawal serangga perosak permisif yang lain. Teknik sel kultur serangga (*in vitro*) adalah pendekatan yang paling sesuai digunakan kerana ia menyediakan keadaan manipulasi yang mudah dan bersih untuk penambahbaikan gen virus dan penghasilan SpltNPV berskala besar. Kesukaran dalam penghasilan SpltNPV secara *in vitro* sebelum ini telah dapat diatasi dengan penambahbaikan dalam penghasilan inokulum 'budded virus' (BV) dan proses pentitratan virus. Penggunaan sel-sel serangga yang berasal dari pelbagai spesies dan kawasan geografi memberi maklumat tambahan mengenai permisibiliti setiap perumah terhadap jangkitan SpltNPV. Kajian ini telah berjaya menghasilkan SpltNPV melalui teknik *in vitro.* Keupayaan ini telah banyak dipengaruhi oleh peningkatan kualiti dalam penghasilan BV inokulum secara *in vivo* dalam sistem perumah utama, *S. litura* larva. BV inokulum boleh diekstrak sama ada dari cecair hemolim atau tisu larva yang dijangkiti. Malangnya, inokula yang dihasilkan dari kedua-dua sumber telah melanise disebabkan oleh pengoksidaan cecair hemolim yang berlaku apabila hemolim atau tisu larva terdedah pada persikitaran. Mekanisma ini telah menjejaskan interaksi *in vitro* di antara virus dan perumah. Walau bagaimanapun, pemilihan larva teruk dijangkiti dan penggunaan Grace Insect Medium semasa penyediaan dan penyimpanan inokulum telah berjaya mengurangkan masaalah melanise dalam virus inokulum dan menghasilkan produktif infeksi SpltNPV dalam sel serangga yang dijangkiti. Sementara itu, teknik pentitratan secara 'end point dilution' telah terbukti menjadi kaedah yang paling cepat, tepat dan ekonomi untuk menganggar BV titrer dalam kajian ini. Tujuh sel serangga (Sf9, SpIm, LD, MaBr, SD, *T.ni* dan SpLi) telah diuji kebolehan terhadap jangkitan SpltNPV. Jangkitan produktif telah dikesan di dalam sel-sel permisif Sf9 yang dijangkiti dengan penghasilan virus "occlusion bodies" (OBs) di dalam nukleus yang dijangkiti. Enam sel-sel yang lain menunjukkan jangkitan abortif dan tiada penghasilan OB dikesan. Perubahan cytopathological ini telah dipantau melalui pemerhatian dibawah cahaya dan elektron mikroskopik untuk perubahan yang disebabkan oleh jangkitan SpltNPV. Jangkitan produktif telah dikesan dalam sel-sel permisif Sf9 yang dijangkiti dengan penghasilan occlusion bodies (OBs). Walau bagaimanapun, enam sel yang lain menunjukkan jangkitan abortive dan tiada OB

dihasilkan. Enam sel ini kemudiannya didapati semipermissive kepada jangkitan SpltNPV apabila PCR gen-gen penting virus *(ie-1, Lef-2 dan polh*) mengesan replikasi SpltNPV sehingga siri laluan kesepuluh BV. Kajian ultrastructural pula mengesahkn lagi jangkitan virus di dalam sel-sel ini. Kajian menunjukkan pembentukan virogenic stroma dan partikal virus dalam nukleus Sf9 dijangkiti manakala enam sel yang lain berkongsi beberapa perubahan kecil, seperti pembentukan vesikel dan pembesaran nukleus tanpa pembentukan partikel virus. Kajian siri laluan juga menunjukkan lagi keupayaan keturunan BV untuk berkesan menjangkiti sel-sel Sf9 dengan infeksi produktif sehingga siri yang kesepuluh BV. Kejadian apoptosis dalam sel-sel SpIm, SpLi dan Sf9 bertanggungjawab untuk menghalang dan menghadkan penyebaran SpltNPV dalam sistem mereka. Fenomena ini telah dikesan melalui perubahan morfologi sel-sel, analisis AO/PI dan saringan pemotongan DNA. Sementara itu, RT-PCR pula digunakan untuk mengesan dan membandingkan penghasilan gen penindas apoptosis (*p49*) dalam ketiga-tiga sel. Dapatan kajian menunjukkan ekspresi gen *p49* yang tinggi telah dikesan dalam sel-sel permisif Sf9 oleh kedua-dua siri laluan pertama dan kesepuluh manakala ekspresi yang rendah telah dikesan dalam sel-sel SpIm dan dan SpLi. Ini membuktikan penglibatan *p49* sebagai perisai virus untuk memanipulasi dan menakluk Sf9 sistem seterusnya telah membenarkan SpltNPV bereplikasi dan membiak. Kajian ini telah menghasilkan maklumat yang penting menganai perumah SpltNPV dari pelbagai spesies Lepidoptera dan kawasan geografi. Penyelidikan seterusnya telah menunjukkan penglibatan apoptosis dalam menghadkan replikasi virus yang mana dapat diatasi oleh virus gen penindas apoptosis (*p49*) dalam sel-sel permisif Sf9.

ACKNOWLEDGEMENT

Alhamdulillah, thankfulness to Allah all mighty for giving me the strength, wisdom, patience and knowledge in exploring things, for the guidance to surpass all the trials encountered and for giving me determination to pursue my research and to make my study possible.

I would like to express a special appreciation and thanks to my main supervisor, Dr. Lau Wei Hong for her tremendous assistances and guidance in my research and writing. I would like to thank her for her valuable advices, trust, support and encouragement throughout the course of my study.

I also would like to express my sincere gratitude to my committee members, Professor Dr. Abdul Manaf Ali, Professor Dr. Ahmad Said Sajap and Professor Datin Paduka Dr. Khatijah Mohd Yusoff for their priceless advices, support and assistance.

I want to thank all my lab mates and officers of the Tissue Culture and Molecular Lab, UNISZA, Molecular Lab, UPM and my entire friends for their kind assistance, support and friendship throughout my research. Special appreciation goes to my special friends, Mr. Syed Ahmad Tajuddin, Mdm. Aishah Abu Bakar, Mr. Aziz, Mr. Haziq, Mr. Saiful, Mr. Mohammad and Mdm. Saleha that continually provide their sincere assistances, suggestion, advices and friendship. I would also like to dedicate my thanks to all of my friends who supported me in writing especially Mr. Fahad.

I also would like to express my appreciation to Mrs. Dayang from Insectory Lab, MARDI, Serdang, Dr. Chia Suet Lin from Faculty of Biotechnology and Biomolecular Sciences, Mr. Rafius, Mrs. Aminah, Mrs. Irmazian, Mrs. Faridah and Mrs. Farrah Deba from Microscopy Unit, IBS for their patience and kind assistance in providing research equipments, materials and services.

A special thanks to my family. Words cannot express how grateful I am to my beloved husband, son, daughter, mother, father, my sisters and brothers for all of their sacrifices. May all of everyone doa', moral and financial support will be rewarded by Allah with His greatest blessing. Those great doa's were the reason that sustained me this far.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Lau Wei Hong, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Ahmad Said Sajap, PhD
Professor
Faculty of Forestry
Universiti Putra Malaysia
(Member)

Abdul Manaf Ali, PhD
Professor
Faculty of Agriculture, Biotechnology and Food Science
Universiti Sultan Zainal Abidin
(Member)

Datin Paduka Khatijah Mohammed Yusoff, **Ph.D.**
Professor
Faculty of, Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Member)

BUJANG KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012.The thesis has undergone plagiarism detection software.

Signature: _____

Date: _____

Name and Matric No.: _____

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to..

Signature: _____	Signature: _____
Name of Chairman of Supervisory Committee: _____	Name of Member of Supervisory Committee: _____
Signature: _____	Signature: _____
Name of Member of Supervisory Committee: _____	Name of Member of Supervisory Committee: _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µm	Micro molar
A260	Absorption at 260 nm
A280	Absorption at 280 nm
AcMNPV	*Autographa californica* MNPV
AgMNPV	*Anticarsia gemmatalis* MNPV
AO	Acridine orange
BmMNPV	Bombyx mori MNPV
bp	base pair
BV	Budded virus
CABI	Centre for Agricultural Bioscience International
CD_{50}	Cytotoxic dosage
cDNA	complementary DNA
CPE	cytopathic effect
CpGV	*Cydia pomonella* GV
DIP	Defective interfering virus particle
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediamine tetraacetic acid
EPPO	European and Mediterranean Plant Protection Organization
FBS	Fetal bovine serum
FHS	fetal horse serum
FP	Few polyhedra
g	gravity
Gp64	glycoprotein 64
GV	Granulovirus
h	hour
H_2O_2	Hydrogen peroxide
hcf-1	host cell-specific factor 1 gene
HearNPV	Helicoperva armisgera NPV
hrf-1	host range factor 1gene
HycuNPV	Hyphantria cunea GV
iap	inhibitor of apoptosis gene
ie-1	immediate-early 1 gene
IPM	Integrated pest management
Kb	Kilobase pair
KCl	Kalium Cloride
LD	*Lymantria dispar* cell line
LdMNPV	*Lymantria dispar* MNPV
LdNPV	*Lymantria dispar* NPV
lef-2	late expression factor 2 gene
MaBr	*Mamestra brassicae*
MARDI	Malaysian Agricultural Research and Development Institute
$MgCl_2$	Magnesium Chloride
min	minute
mM	mili molar

MNPV	Multiple nucleocapsid polyhedrosis virus
MOI	Multiplicity of infection
MP	Multiple polyhedral
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na_2CO_3	sodium carbonate
nm	nano meter
NPV	Nucleopolyhedrovirus
OB	Occlusion body
OD	Optical density
ODV	Occlusion derived virus
OpMNPV	*Orgyia pseudotsugata* MNPV
p. i.	post inoculation
p35	anti-apoptotic gene
PBS	Phosphate-Buffered saline
PCR	Polymerase chain reaction
PD	Propotionaldistance
Pfu	Plaque forming unit
PI	Propidium iodide
polh	polyhedrin
PTU	1-phenyl-3-(2-thiazolyl)-2-thiourea (phenylthiourea)
Q-PCR	Quantitative PCR
RNA	Ribonucleic acid
RT-PCR	reverse transcription-polymerase chain reaction
SD	*Spodoptera frugiperda* clone cell line (Taiwan)
SDS	Sodium dedocyl sulfate
Sec	Second/s
SEM	Standard error mean
SeMNPV	*Spodoptera exigua* MNPV
Sf9	*Spodoptera frugiperda* cell line
SlNPV	*Spodoptera litura* NPV
SLNPV	*Spodoptera litura* NPV
SNPV	Single nucleocapsid polyhedrosis virus
SpIm	*Spilosoma imparilis* cell line
SpLi	*Spodoptera litura* cell line
Spli MNPV	*Sposoptera littoralis* MNPV
SpltNPV	*Spodoptera litura* NPV
T.ni	*Trichoplusia ni*
TAE	Tris-acetate-EDTA buffer
Taq	Thermos aquaticus
TBST	Tris-Buffered Saline-Tween 20
TCID	Tissue culture infection dosage
TE	Tris-EDTA buffer
TEM	Transmission electron microscopy
TrisHCl	Tris(hydroxymethyl)aminomethane hydrochloride.
UPM	Universiti Putra Malaysia
V	volt
v/v	volume per volume
w/v	weight per volume

CHAPTER 1

INTRODUCTION

Biological control is an approach for controlling insect pests in the agricultural field that utilize natural enemies, such as natural predators, parasitoids, pathogens and natural plant biochemical compounds. Its main application is to reduce the use of agrochemicals that raise problems, such as the emergence of resistant pests and other harmful effects on the environment and non-target organisms. This application promises a healthy and environmentally friendly agriculture practice to meet the current demand of healthy living and green technology. Most of the natural enemies used in biological control have specific and narrow host species, are safe to the environment, and help conserve the natural ecology.

Bacteria, fungi and viruses are candidates of microbial pest control agents. They usually found naturally in the environment as a fatal disease to a particular pest. Baculoviruses are well known as insect viruses through their beneficial and potential bioinsecticide for managing pest outbreaks. *Baculoviridae* is a family of rod-shaped viruses with large circular, covalently closed, double-stranded DNA. The family comprises four genera based on their genome linage. Viruses that infect Lepidoptera are divided into Alpha- and Betabaculoviruses encompassing the Nucleopolyhedroviruses (NPVs) and Granuloviruses (GVs), respectively, while those infecting Hymenoptera and Diptera are named Gamma- and Deltabaculoviruses, respectively (Jehle et al., 2006). Baculoviruses almost exclusively infect a specific insect, belonging mainly to the order Lepidoptera, Hymenoptera and Diptera (Adams and McClintock, 1991). Their application is safe and possesses no foreseeable hazard to the environment or other organisms except for their host pests due to their virulence specificity to a small number of host insects (Ignoffo, 1975). Many baculoviruses have been discovered and studied globally. The most commonly investigated baculovirus is *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). Successful baculovirus insecticides include *Anticarsia gemmatalis* multiple nucleopolyhedrovirus (AgMNPV) in Brazil to control the velvet bean caterpillar, *Anticarsia gemmatalis* (Moscardi, 1993), *Cydia pomonella* granulovirus (CpGV) for codling moth, *Cydia pomonella* has been applied in North America and Europe (Rohrmann, 2011) and *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) has been used for cotton bollworm, and *Helicoverpa armigera* in China (Sun and Peng, 2007).

In Malaysia, *Spodoptera litura* larvae have become a major pest of vegetables, profitable crops, tobacco plants and forest tree seedlings (Yahya, 1985; Sajap, 1995). This armyworm is conventionally controlled through the application of commonly used insecticides, such as methamidophos, acephate and permethrin (Yahya and Karim, 1989). In 2000, Sajap et al. reported the natural occurrence of *Spodoptera litura* nucleopolyhedrovirus (SpltNPV) in diseased larvae of *S. litura* in Malaysia. The pathogenicity test conducted demonstrated the promising ability of SpltNPV to control *S. litura* larvae by application at an appropriate viral dosage. SpltNPV may play a unique role in the IPM system to control *S. litura* and other permissive insect pests with the aim of reducing local agrochemical utilization in the field.

To date, SpltNPV is produced on a small scale through insect larvae (*in vivo*) by UPM and MARDI researchers for basic and applied research activities. The insect cell culture (*in vitro*) technique is considered to be the most suitable approach for large scale production of SpltNPV. In addition, this *in vitro* approach can assist in studies of SpltNPV to widen its host range towards the enhancement of its infectivity as an effective and versatile bioinsecticide. There are several advantages of using the *in vitro* method instead of the *in vivo* method in studying the virus-host cell interaction, such as high productivity of virus yield, reliable production of active protein of interest, simple and inexpensive harvesting of occlusion bodies (OBs), and it facilitates quality control procedures for certification of a virus product free of adventitious agents. Moreover, the culture can be easily maintained and stored in liquid nitrogen (Shuler et al., 1995).

A successful *in vitro* SpltNPV propagation may depend on the permissibility of the host cell line. Since the local *S. litura* cell line has not been established yet, commercial insect cell lines can be used for the screening of SpltNPV productive infection. A previous trial based on *in vitro* propagation of SpltNPV was conducted by Hussein (2004). The researcher managed to produce a productive infection of SpltNPV in *Spodoptera frugiperda* (Sf9) cell line but not in the *S. litura* of Japan isolate, TUAT-Spli-221 cell line. However, this productive infection was unable to be sustained in subsequent infection. Therefore, more research is required to identify and overcome the difficulties that emerged in the previous trials of *in vitro* propagation of SpltNPV. The utilization of cell lines from different host species and geographical isolates will provide information concerning the SpltNPV host range.

Apoptosis is a natural biochemical pathway controlled by an organism that plays an important role by eliminating old and unhealthy cells in maintaining the health of the organism (Kanduc et al., 2002). It is proven that apoptosis is involved in baculovirus host-range infectivity, either in a living insect (Zhang et al., 2002a) or a cell culture system (Clem and Miller, 1993). A successful virus infection may depend on the virus response to this mechanism. Thus, the objectives of this study were:

- To establish *in vitro* propagation of SpltNPV.
- To determine the susceptibility of insect cell lines from different Lepidoptera species and geographical isolates against SpltNPV.
- To identify the host cell defence mechanism that is responsible for preventing and limiting viral replication.
- To identify the possibility of apoptotic suppressor gene involvement during the replication of local SpltNPV in the insect cell lines.

REFERENCES

Adams, J.R. and McClintock, J.T. (1991). Baculoviridae: Nuclear polyhedrosis viruses. Part 1. Nuclear polyhedrosis viruses of insects. In *Atlas of Invertebrate Viruses*, eds. J.R. Adams, and J.R. Bonami, pp. 89–204. Boca Raton, Florida: CRC Press.

Adams, J.R. and Bonami, J.R. (1991). *Atlas of Invertebrate Viruses*. Boca Raton, Florida: CRC Press.

Ahrens, C.H., Russel, R.L., Funk, C.J., Evans, J.T., Harwood, S.H. and Rohrman, G.F. (1997). The sequence of the *Orgyia pseudotsugata* multinucleocapsid nuclear polyhedrosis virus genome. *Virology* 229(2): 381-399.

Aitkenhead, P., Baker, C.R.B. and Chickera, G.W.D. (1974). An outbreak of *Spodoptera litura*, a new pest under glass in Britain. *Plant Pathology* 23:117-118.

Ali, A.M., Umar-Tsafe, N., Mohamed, S.M., Inayat-Hussain, S.H., Oo, K.T., Yusoff, K., Osman, A.B. and Din, L.B. (2001). Apoptosis induction in CEM-SS T-lymphoblastic leukemic cell line by goniothalamin. *Journal of Biochemistry, Molecular Biology and Biophysics* 5(3): 227-235.

Amin, N.M. (2006). *Cloning and characterization of the apoptotic suppressor gene p49 from Spodoptera litura nucleopolyhedrovirus*, Master Thesis, Universiti Putra Malaysia, Malaysia.

Ayres, M.D., Howard, S.C., Kuzio, J., Lopez-Ferber, M. and Possee, R.D. (1994). The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. *Virology* 202: 586-605.

Barber, K.N., Kaupp, W.J. and Holmes, S.B. (1993). Specificity testing of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). *Canadian Entomologist* 125: 1055–1066.

Barlow, H.S. and D'Abrera, B. (1982). *An Introduction to The Moths of South East Asia*. Kuala Lumpur: Malayan Nature Society.

Beidler, D.R., Tewari, M., Friesen, P.D., Poirier, G., and Dixit, V.M. (1995). The baculovirus P35 protein inhibits Fas-and tumor necrosis factor-induced apoptosis. *Journal of Biology Chemistry* 270: 16526-16528.

Bilimoria, S.L., Demirbag, Z., Ng, H. and Renisch, A.J. (1992). Abortive cell culture infections of nuclear polyhedrosis viruses as model systems for host specificity. *Pesquisa Agropecuaria Brasileira* 27: 123-141.

Birnbaum, M.J. Clem, R.J. and Miller, L.K. (1994). An apoptosis inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motif. *Journal of Virology* 68: 2521-2528.

Black, B.C., Brennan, L.A., Dierks, P.M. and Gard, I.E. (1997). Commercialization of baculoviral insecticides. In *The Baculoviruses*, ed. L.K. Miller, pp. 341–387, New York: Plenum Press.

Blissard, G.W. and Rohrmann, G.F. (1990). Baculoviruses diversity and molecular biology. *Annual Review of Entomology* 35: 127-155.

Bonning, B.C. and Hammock, B.D. (1996). Development of recombinant baculoviruses for insect control. *Annual Review of Entomology* 41: 191-210.

Bradford, M.B., Blissard, G.W. and Rohrmann G.F. (1990). Characterization of the infection cycle of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus in *Lymantria dispar* cells. *Journal of General Virology* 71(12): 2841-2846.

Bump, N.J., Hackett, M., Hugunin, M., Seshagiri, S., Brady, K., Chen, P., Ferenz, C., Franklin, S., Ghayur, T., Li, P., Licari, P., Mankovich, J., Sji, L.F., Greenberg, A.H., Miller, L.K. and Wong W.W. (1995). Inhibition of ICE family proteases by baculovirus antiapoptotic protein P35. *Science* 269(5232): 1885-1888.

Carpenter, W.M. and Bilimoria, S.L. (1983). A semipermissive nuclear polyhedrosis virus infection: characterization of infection kinetics and morphogenesis. *Virology* 130(1): 227-231.

Castro, M.E.B., Souza, M.L. and Bilimoria, S.L. (1999). Host-specific transcription of nucleopolyhedrovirus gene homologues in productive and abortive *Anticarsia gemmatalis* MNPV infections. *Archives of Virology* 144(6):1111-1121.

Castro, M.E.B. and Ribeiro, B.M. (2001). Production of viral progeny in insect cells undergoing apoptosis induced by a mutant *Anticarsia gemmatalis* nucleopolyhedrovirus. *Microbiological Research* 156(4): 369-376.

Castro, M.E.B., Sauza, M.L., Araujo, S. and Bilimoria, S.L. (1997). Replication of *Anticarsia gemmatalis* Nuclear polyhedrosis virus in four Lepidopteran cell lines. *Journal of Invetebrate Pathology* 69(1): 40-41.

Chejanovsky, N. and Gershburg, E. (1995). The wild-type *Autographa californica* nuclear polyhedrosisvirus induces apoptosis of *Spodoptera littoralis* cells. *Virology* 209(2): 519-525.

Chen, C.J. (1998). *Lymantria dispar* nucleopolyhedrovirus *hrf-1* expands the larval host range of *Autographa californica* nucleopolyhedrovirus. *Journal of Virology* 72(3): 2526-2531.

Chen, Y.I. (1979). Some notes on the Lepidopteran rice pests at Java, Timur, Indonesia, National Taiwan University. *Phytopathology Entomology* 6: 38-43.

Chen, Y.R., Solter, L.F., Chien, T.Y., Jiang, M.H., Lin, H.F., Fan, H.S., Lo, C.F. and Wang, C.H. (2009). Characterization of a new insect cell line (NTU-YB) derived from the common grass yellow butterfly, *Eurema hecabe* (Linnaeus)

(Pieridae: Lepidoptera) and its susceptibility to microsporidia. *Journal of Invertebrate Pathology* 102(3): 256-262.

Clarke, T. E., and Clem, R. J. (2003). Insect defenses against virus infection: the role of apoptosis. *International Review of Immunology 22*(5-6), 401-424.

Clem, R. J. and Miller, L. K. (1993). Apoptosis reduces both the *in vitro* replication and the *in vivo* infectivity of a baculovirus. *Journal of Virology* 67(7): 3730–3738.

Clem, R. J., Fechheimer, M. and Miller, L. K. (1991). Prevention of apoptosis by a baculovirus gene during infection of insect cells. *Science* 254(5036): 1388-1390.

Clem, R.J. (2005). The role of apoptosis in defense against baculovirus infection in insects. *Current Topics in Microbiology and Immunology*. 289: 113–239.

Clem, R.J. and Duckett, C.S. (1997). The *iap* genes: Unique arbitrators of cell death. *Trends in Cell Biology* 7(9): 337-339.

Clem, R.J. and Miller, L.K. (1994). Control of programmed cell death by the baculovirus genes *p35* and *iap*. *Molecular and Cellular Biology*. 14(8): 5212–5222.

Clem, R.J., Robson, M. and Miller, L.K. (1994). Influence of infection route on the infectivity of baculovirus mutants lacking the apoptosis-inhibitting gene *p35* and adjacent gene *p94*. *Journal of Virology* 68(10): 6759-6762.

Collin, J.A., Schand, C.A., Young, K.K., Veseley, J. and Willingham M.C. (1997). Major DNA fragmentation is a late event in apoptosis. *Journal of Histochemistry and Cytochemistry* 45(7): 923-934.

Cory, J.S., Hist, M. L., Sterling, P. H. and Speight, M. R. (2000). Narrow Host range nucleopolyhedrovirus for control of the brown-tail moth, *Euproctis chrysorrhoea* (L.) (Lepidoptera: Lymantriidae). *Environmental Entomology*. 29(3): 661-667.

Crook, N.E., Clem, R.J. and Miller, L.K. (1993). An apoptosis-inhibitting gene with a zinc finger-like motif. *Journal of Virology* 67(4): 2168-2174.

Dai, X.J., Pang, Y., Nong, G. and Shi, X.Z. (1998). Is baculovirus *gp64* gene a primary factor inducing apoptosis of insect cells? *Acta Scientiarum Naturalium Universitatis Sunyatsen* 37: 7–12.

Danyluk, G.M. and Maruniak, J.E. (1987). *In vivo* and *in vitro* host range of *Autographa californica* nuclear polyhedrosis virus and *Spodoptera frugiperda* nuclear polyhedrosis virus. *Journal of Invertebrate Pathology* 50(3): 207-212.

Darzynkiewicz, Z. and Juan, G. (1997). Analysis of DNA Content and DNA Strand Breaks for Detection of Apoptotic Cells. In *Current Protocols in Cytometry*, eds. P. Robinson, Z. Darzynkiewicz, P. Dean, A. Orfao, P. Rabinovitch, C.

Stewart, H. Tanke and L. Wheeless. pp. 741-748. New York: Current Protocols/Wiley-Liss.

Das, B.K. and Roy, P. (1985). Sex pheromone of the tobacco caterpillar *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) and its use in integrated pest management. *Journal of Bengal Natural History Society* 4 (2): 127-138.

Davidson, R.H. (1966). *Insect pests of farm, garden and orchard, 6th edition*, ed. L.M. Peairs. New York: John Wiley and Sons. Inc.

Detvisitsakun, C., Hutfless, E.L., Beretta, M.F. and Passarelli, A.L. (2006). Analysis of a baculovirus lacking a functional viral fibroblast growth factor homolog. *Virology* 346(2): 258-265.

Dhir, B.C., Mohapatra, H.K. and Senapati, B. (1992). Assessment of crop loss in groundnut due to tobacco caterpillar, *Spodoptera litura* (F.). *Indian Journal of Plant Protection* 20: 215-217.

Doyle, C.J. Hirst, M.L., Cory, J.S. and Entwistle, P.F. (1990). Risk Assessment studies: detailed host range testing of wild-type cabbage moth, *Mamestra brassicae* (Lepidoptra:Nuctuidae), nuclearpolyhedrovirus. *Application Enviromental Microbiological* 56(9): 2704-2710.

Du, Q., Lehavi, D., Faktor, O., Qi, Y. and Chejanovsky, N. (1999). Isolation of an apoptosis suppressor gene of the *Spodoptera litura* nucleopolyhedrovirus. *Journal of Virology* 73(2): 1278-1285.

Du, X. and Thiem, S. M. (1997). Responses of insect cells to baculovirus infection: protein synthesis shutdown and apoptosis. *Journal of Virology* 71(10):–7872.

Elzinga, R.J. (1990). Chapter 10: Lepidoptera. In *Fundamentals of* entomology (3rd edition), pp. 391-395. New Jersey: Prentica Hall Career and Tech. Prentice-Hall, Inc.

Enstwistle, P.F. and Evans, H.F. (1985). Viral control. In *Comprehensive insect physiology, biochemistry and pharmacology*, eds. G.A. Kerkut, and L.I. Gilbert, pp. 347-412. Oxford: Pergamon Press.

EPPO/CABI, (1997). *Spodoptera littoralis* and *Spodoptera litura*. Data sheets on quarantine pests. Retrieved 2 February 2010 from http://www.eppo.org/QUARANTINE/insects/Spodoptera_litura/PRODLI_ds.pdf

Federici, B.A. (1993). Viral pathobiology in relation to insect control. In *Parasites and Pathogens of Insects. Pathogens, vol. 2*, eds. N.E. Beckage, S.N. Thompson and B.A. Federici, pp. 81–101. San Diego: Academic Press.

Francis, A. Cabbage and sawi plots ravaged: Army worms destroying crops. *New Sunday Times*, March 12, 1989, pp. 4.

Gelernter, W.D. and Federici, B.A. (1986). Continuous cell line from *Spodoptera exigua* (Lepidoptera: Noctuidae) that supports replication of nuclear

polyhedrosis viruses from *Spodoptera exigua* and *Autographa californica*. *Journal of Invertebrate Pathology* 48(2): 199–207.

Giri, L., Feiss, M.G., Bonning, B.C. and Murhammer D.W. (2012). Production of baculovirus defective interfering particles during serial passage is delayed by removing transposon target sites in *fp25k. Journal of General Virology* 93(2): 389-399.

Goodman, C.L. and McIntosh, A.H. (1994). Production of Baculovirus for insect control using cell culture. In *Insect cell Biotechnology*, eds. K. Maramorosch and A. H. McIntosh, pp. 33-56. Boca Raton, Florida: CRC Press.

Goodwin, R.H., Thompkins, G.J. and McCawley, P. (1978). Gypsy moth cell lines divergent in viral susceptibility. *In vitro* 14(6): 485-494.

Grace, T.D.C. (1962) Establishment of four strains of cells from insect tissue grown *in vitro*. *Nature* 195:788–789.

Granados, R. and Williams, K.A. (1986). *In vivo* infection and replication of baculoviruses. In *The Biology of Baculoviruses, Vol. 1*, eds. R. Granados and B. Federici, pp. 89-108. Boca Raton, Florida: CRC Press.

Guzo, D., Dougherty, E. M., Lynn, D. E., Braun, S. K. and Weiner, R. M. C. (1991). Changes in macromolecular synthesis of gypsy moth cell line IPLB-Ld652Y induced by *Autographa californica* nuclear polyhedrosis virus infection. *Journal of General Virology* 72(5): 1021-1029.

Guzo, D., Rathburn, H., Guthrie, K. and Dougherty. E. (1992). Viral and host cellular transcription in *Autographa californica* nuclear polyhedrosis virus-infected gypsy moth cell lines. *Journal of Virology* 66(5): 2966-2972.

Hay, B.A., Wolff, T. and Rubin, G.M. (1994). Expression of baculovirus *p35* prevents cell death in *Drosophila*. *Development* 120(8): 2121-2129.

Hayasaka, S. and Kawarabata, T. (1990). Cloning of a microsporidian, *Nosema bombycis* (Microsporida: Nosematidae) in insect cell cultures by a limiting dilution method. *Journal of Invertebrate Pathology* 55(1): 35-40.

Hayat, M.A. (1989). Chemical Fixation. In *Principles and Techniques of Electron Miroscopy: Biological Applications*, pp. 1–74. Boca Raton, Florida: CRC Press.

Hayflick, L. (1973). The biology of human aging. *The American Journal of Medical Sciences* 265: 432-435.

Hink, W.F. and Strauss, E. (1976). Replication and passage of alfalfa looper nuclear polyhedrosis virus plaque variants in cloned cell cultures and larval stages of four host species. *Journal of Invertebrate Pathology* 27(1): 49-55.

Hink, W.F. and Vail, P.V. (1973). A plaque assay titration of alfalfa looper nuclear polyhedrosis virus in a cabbage looper (TN-368) cell line. *Journal of Invertebrate Pathology* 22(2): 168-174.

Hiruma, K. and Riddiford L.Y. (2009). The molecular mechanisms of cuticular melanisation: The ecdysone cascade leading to dopa decarboxylase expression in *Manduca sexta*. *Insect Biochemistry and Molecular Biology* 39(4): 245-253.

Hitchman, R., Siaterli, E., Nixon, C. and King, L. (2006). Quantitative real-time PCR for rapid and accurate titration of recombinant baculovirus particles. *Biotechnology and Bioengineering* 96(4): 810–814.

Hu, H.M., O'Rourke, K., Boguski, M.S. and Dixit, V.M. (1994). A novel RING finger protein interacts with the cytoplasmic domain of CD40. *Journal of Biology Chemistry* 269(48): 30069–30072.

Hussein, S.M. (2004). *In vitro studies on the virulence of Spodoptera litura baculovirus*. Master Thesis, Universiti Putra Malaysia, Malaysia.

Iddekinge, H.V.B.J.L., Smith, G.E. and Summers, M.D. (1983). Nucleotide sequence of the polyhedrin gene of *Autographa californica* nuclear polyhedrosis virus. *Virology* 131(2): 561-565.

Ignoffo, C.M. (1975). Evaluation of *in vivo* specificity of insect viruses. In *Baculovirus for Insect Pest Control: Safety Considerations*, eds. M.D. Summers, R. Engler, L.A. Falcon and P. Vail, pp. 52-57. Washington: American Society for Microbiology.

Ikeda, M., Reimbold, E.A. and Thiem, S.M. (2004a). Fuctional analysis of the baculovirus host range gene, *hrf-1*. *Virology* 332(2): 602-613.

Ikeda, M., Yanagimoto, K. and Kobayashi, M. (2004b). Identification and functional analysis of *Hyphantria cunea* nucleopolyhedrovirus *iap* genes. *Virology* 321(2): 359-371.

Imes, R. (1992). Butterflies and moths. Order Lepidoptera. In *The Practical Entomologist*, pp. 110-121. London: AURUM Press Ltd.

Ishikawa, H., Ikeda, M., Yanagimoto, K., Alves, C.A.F., Katou, Y., Laviña-Caoili, B.A. and Kobayashi, M. (2003). Induction of apoptosis in an insect cell line, IPLB-Ld652Y, infected with nucleopolyhedroviruses. *Journal of General Virology* 84(3): 705-714.

Jacob, A. and Subramaniam, T.R. (1973). Histopathology of tobacco caterpillar *Spodoptera litura* F. Infected with a nuclear polyhedrosis virus. *Agricultural Research Journal of Kerala* 11(2): 114-118.

Janakiraman, V., Forrest, W.F., Chow, B. and Seshagiri, S. (2006). A rapid method for estimation of baculovirus titer based on viable cell size. *Journal of Virological Methods* 132(1-2): 48-58.

Jehle, J.A., Lange, M., Wang, H., Hu, Z., Wang, Y. and Hauschild, R. (2006). Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera. *Virology* 346(1): 180-193.

Kanduc, D., Mittelman, A., Serpico, R., Sinigaglia, E., Sinha, A.A., Natale, C., Santacroce, R., DiCorcia, M.G., Lucchese, A. and Dini, L. (2002). Cell death: Apoptosis versus necrosis. *Institute Journal of Oncology* 21(1):165–170.

Karkkainen, H.R., Lesch, H.P., Matta, A.I., Toivanen, P.I., Mahonen, A.J., Roschier, M.M., Airenne, K.J., Laitinen, O.L. and Herttuala, S.Y. (2009). A 96-well format for a high-throughput baulovirus generation, fast tittering and recombinant protein production in insect and mammalian cells. *Biomedical Central Research Notes* 2(1):63.

Kerr, J.F., Wyllie, A.H. and Currie, A.R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer* 26(4): 239-257.

Khurad, A.M., Kanginakurdu, S., Qureshi, S.O., Rathod, M.K., Rai, M.M. and Nagaraju, J. (2006). A new *Bombyx mori* larval ovarian cell line highly susceptible to nucleopolyhedrovirus. *Journal of Invertebrate Pathology* 92(2): 59-65.

King, L.A. and Possee, R.D. (1992). *The Baculovirus Expression System: A laboratory Guide*. London: Chapman and Hall.

Kitts, P. A. and Green G. (1999). An immunological assay for determination of baculovirus titers in 48 hours. *Analytical Biochemistry* 268(2): 173-178.

Knudson, D.L., Lescott, T. and Tinsley, T.W. (1980). Establishment of a continuous cell line of S*podoptera littoralis* (Lepidoptera: Noctuidae). *In Vitro* 16(5): 369–370.

Kool, M., Voncken, J.W., Vanlier, F.L.J., Tramper, J. and Vlak, J.M. (1991). Detection and analysis of *Autographa californica* nuclear polyhedrosis virus mutants with defective interfering properties. *Virology* 183(2): 739-746.

Kumar, M., Shanker, S., Singh, R.V. and Das, R.H. (2002). Characterization of the anti apoptotic (*p35*) gene homologue of *Spodoptera litura* nucleopolyhedrosis virus (SlNPV). *Molecular Biology Reports* 28(3): 167-173.

Kwon, M.S., Dojima, T., Toriyama, M. and Park, E.Y. (2002). Development of an antibodybased assay for determination of baculovirus titers in 10 h. *Biotechnology Program* 18(3): 647–651.

Lacey, L.A., Frutos, R., Kaya, H.K. and Vail, P. (2001). Insect pathogens as biological control agents: Do they have a future? *Biological Control* 21(3): 230–248.

Lau, W.H. (2000). *Characterization of granulovirus and nucleopolyhedrovirus isolated from Spodoptera litura*. Doctor of Philosophy Thesis, Universiti Putra Malaysia, Malaysia.

Lau, W.H. and Sajap,A.S. (2008). Susceptibility of *Putella xylostella* to infection by *Spodoptera litura* Nucleopolyhedrovirus, Proceedings “Bio agricultural input

for sustainable agriculture prospect and challenges". Medan, Indonesia. July 2008.

Lavin˜a-Caoili, B.A., Kamiya, K., Kawamura, S., Ikeda, M. and Kobayashi, M. (2001). Comparative *in vitro* analysis of geographic variants of nucleopolyhedrovirus of *Spodoptera litura* isolated from China and the Philippines. *Journal of Insect Biotechnology Sericology* 70: 199–209

Lee, H.H. and Miller, L.K. (1978). Isolation of genotypic variants of *Autographa californica* nuclear polyhedrosis virus. *Journal of Virology* 27(3): 754-767

Lin, G., Zhong, J. and Wang, X. (2000). Abnormal formation of polyhedra resulting from a single mutation in the polyhedrin gene of *Autographa californica* multicapsid nucleopolyhedrovirus. *Journal of Invertebrate Pathology* 76(1): 13-19.

Lo, H.R. and Chao, Y.C. (2004). Rapid titer determination of baculovirus by quantitative real-time polymerase chain reaction. *Biotechnology Progress* 20(1): 354–360.

Luckow, V.L. (1991). Cloning and expression of heterologous genes in insect cells with baculovirus vectors. In *Recombinant DNA Technology and Applications*, eds. A. Prokop and R.K. Bajpai and C.S. Ho, pp. 97–152. New York: McGraw-Hill.

Lynn, D.E. (1992). Improved efficiency in determining the titer of the *Autographa californica* baculovirus nonoccluded virus. *BioTechniques* 13: 282–285.

Lynn, D.E. (2001). Novel techniques to establish new insect cell lines. *In vitro Cellular and Developement Biology-Animal* 37(6): 319-321.

Lynn, D.E. (2007). Lepidopteran insect cell line isolation from insect tissue. *Methods Molecular Biology* 388:139-154.

Lynn, D.E. and Hink, W.F. (1980). Comparison of nuclear polyhedrosis virus in five Lepidopteran cell lines. *Journal of Invertebrate Pathology* 35(3):234-240.

Maeda, S. (1994). Expression of foreign genes in insect cells using baculovirus vectors. In *Insect Cell Biotechnology*, eds. K. Maramorosch and A.H. McIntosh, pp. 1-31. Boca Raton, Florida: CRC Press.

Mallikarjuna, N., Kranthi, K. R., Jadhav, D. R., Kranthi, S. and Chandra, S. (2004). Influence of foliar chemical compounds on the development of *Spodoptera litura* (Fab.) in inter-specific derivatives of groundnut. *Journal of Applied Entomology* 128(5): 21-328.

Mangalika, P.R., Kawamoto, T., Takahashi-Nakaguchi, A. and Iwabuchi, K. (2010). Characterization of cell clusters in larval hemolymph of the cabbage armyworm *Mamestra brassicae* and their role in maintainance of hemocyte populations. *Journal of Insect Physiology* 56(3):314-323.

Manji, G.A., Hozak, R.R., LaCount, D.J. and Friesen P.D. (1997). Baculovirus inhibitor of apoptosis functions at or upstream of the apoptotic suppressor *p35* to prevent programmed cell death. *Journal of Virology* 71(6): 4509-4516.

Martignoni, M.E. and Iwai, P.J. (1986). A Catalogue of Viral Diseases of Insects, Mites and Ticks, (4th ed.), General technical Report PNW-195 US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland.

Mazzacano, C.A., Du, X. and Thiem, S.M. (1999). Global protein synthesis shutdown in *Autographa californica* nucleopolyhedrovirus-infected Ld652Y cells is rescued by tRNA from uninfected cells. *Virology* 260(2): 222–231.

McClintock, J.T., Dougherty, E.M. and Weiner, R.M. (1986). Semipermissive replication of a nuclear polyhedrosis virus of *Autographa californica* in a gypsy moth cell line. *Journal of Virology* 57(1): 197-204.

Mena, J., Ramirez, O.T. and Palomares, L. (2003). Titration of non-occluded baculovirus using a cell viability assay. *BioTechniques* 34(2): 260–264.

Miller, L.K. (1995). Genetically engineered insect virus pesticides: present and future. *Journal of Invertebrate Pathology* 65(3): 211–216.

Miller, L.K. and Lu, A., (1997). The molecular basis of baculovirus host range. In *The Baculoviruses*, ed. L.K. Miller, pp. 217–235. New York: Plenum Press.

Miller, L.K., Kaiser, W.J. and Sehsagiri, S. (1998). Baculovirus regulation of apoptosis. *Seminars in Virology* 8(6): 445-452.

Mironova, E.V., Evstratova, A.A. and Antonov S.M. (2007). A fluorescence vital assay for the recognition and quantification of excitotoxic cell death by necrosis and apoptosis using confocal microscopy on neurons in culture. *Journal of Neuroscience Methods* 163(1):1-8.

Mitsuhashi, J. (1995). A continuous cell line from pupal ovaries of the common cutworm, *Spodoptera litura* (Lepidoptera : Noctuidae). *Applied Journal of Entomology Zoology* 30(1): 75-82.

Mitsuhashi, J. and Inoue, H. (1988). Obtainment of a continuous cell lines from the larval fat bodies of the Mulberry Tiger moth, *Spilisoma impairs* (Lepidoptera:Arctiidae). *Application of Entomology and Zoology* 23:488-490.

Mitsuhashi, J. and Shozawa, A. (1985). Continuous cell lines from larval hemocytes of the cabbage armyworm, *Mamestra brassicae. Development of Growth and Differentiation* 27(5):599-606.

Miyahara, Y., Wakikado, T. and Tanaka, A. (1971).Seosonal changes in the number and size of the egg masses of *Prodenia litura*. *Japanese Journal of Applied Entomology and Zoology* 15(3): 139-143.

Mochida, O. (1973). Two important pests, *Spodoptera litura* and *S. litoralis* on various crops morphological discrimination of the adult, pupal and larval stages. *Applied Entomology and Zoology* 8(4):205-214.

Moscardi, F. (1999). Assessment of the application of baculovirus for the control of Lepidoptera. *Annual Review of Entomology* 44(2): 257-289.

Moscardi, F. and Sosa,G. D.R. (1993). A case study in biological control: soybean defoliating caterpillars in Brazil. In *International crop science*, eds. D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen and R.F. Wilson, pp 115–119. Madison: Crop Science Society of America.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunology Methods* 65(1-2): 55-63.

Murhammer, D.W. (1996). Use of viral insecticide for pest control and production in cell culture. *Applied Biochemistry and Biotechnology* 59(2): 199-220.

Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A. and, Summers, M.D. (1995). Virus Taxonomy: The Classification and Nomenclature of Viruses: 6th report of the International Committee on Taxonomy of Viruses. New York: Springer-Verlag Wien.

Nakasuji, F. and Matsuzaki,T. (1977). The control threshold density of the tobacco cutworm *Spodoptera litura* on eggplants and sweet peppers in vinylhouse. *Applied Entomology and Zoology* 12(2): 184-189.

Nguyen, Q., Qi, Y.M., Wu, Y., Chan, L.C.L., Nielsen, L.K., and Reid, S. (2011). *In vitro* production of *Helicoverpa* baculovirus biopesticides: Automated selection of insect cell clones for manufacturing and systems biology studies. *Journal of Virological Methods* 175(2): 197-205.

O'Brien, V. (1998). Viruses and apoptosis. *Journal of General Virology* 79:1833–1845.

O'Reilly, D.R., Miller, L.K. and Luckow V.A. (1994). *Baculovirus expression vectors: a laboratory manual.* New York: Oxford University Press, Inc.

Palli, S.R., Caputo, G.F., Sohi, S.S., Brownwright, A.J., Ladd, T.R., Cook, B.J., Primavera, M., Arif, B.M. and Retnakaran, A. (1996). CfMNPV blocks AcMNPV-induced apoptosis in a continuous midgut cell line. *Virology* 222(1): 201-213.

Pang, Y., Yu, J., Wang, L., Hu, X., Bao, W., Li, G., Chen, C., Han, H., Hu, S. and Yang, H. (2001). Sequence analysis of *Spodoptera litura* multicapsid nucleopolyhedrovirus genome. *Virology* 287(2): 391-404.

Pant, U., Athavale, S.S. and Vipat, V.C. (2000). A new continuous cell line from larval hemocytes of *Spodoptera litura* (F.). *Indian Journal of Experimental Biology* 38(12):1201-1206.

Pant, U., Athavale, S.S., Basu, A. and Baerjee, K. (1998). A New cell line established from pupal ovaries of *Spodoptera litura* F. (Lepidoptera: Noctuidae). *Indian Journal of Experimental Biology* 36(2): 195-198.

Pant, U., Sudeep, S.B., Athawale, S.S. and Vipat, C.V. (2002). Baculovirus studies in new, indigenous Lepidopteran cell line. *Indian Journal of Experimental Biology* 40(1): 63-68.

Payne, C.C. (1986). Insect pathogenic viruses as pest control agents. In *Biological Plant and health Protection*, ed. J. M. Franz, pp. 183-20. Stuttgart: Fischer Verlag.

Pijlman, G.P., van den Born, E., Martens, D.E. and Vlak, J.M. (2001). *Autographa californica* baculoviruses with large genomic deletions are rapidly generated in infected insect cells. *Virology* 283(1): 132–138.

Popham, H.J.R. Shelby, K.S., Brandt, S.L. and Coudron, T.A. (2004). Potent virucidal activity against HzSNPV in larval *Heliothis virescens* plasma. *Journal of General Virology* 85(8): 2225-2261.

Possee, R. D. (1993). Viral approaches for insect control. In *Advanced Engineered Pesticides*, ed. L. Kim, pp. 99–112. New York: Dekker.

Potter, K., Faulkner, P., and MacKinnon, E. (1976). Strain selection during serial passage of *Trichoplusia ni* nuclear polyhedrosis virus. *Journal of Invetebrate Pathology* 18(3): 1040-1050.

Prikhod'ko, E.A. and Miller L.K. (1996). Induction of apoptosis by baculovirus transactivator IE1. *Journal of Virology* 70(10):7116-7124.

Puranam, K.L. and Boustany, R.M. (1998). Assessment of cell viability and histochemical methods in apoptosis. In *Apoptosis in Neurobiology*, ed. Y.A. Hannun and R.M. Boustany, pp. 129-152. Boca Raton, Florida: CRC Press LLc.

Rabizadeh, S., LaCount, D.J., Friesen, P.D. and Bredesen, D.E. (1993). Expression of the baculovirus *p35* gene inhibits mammalian neural cell death. *Journal of Neurochemistry* 61(6): 2318-2321.

Ramoska, W.A. and Hink, W.F. (1974). Electron microscopic examination of two plaque variants from a nuclear polyhedrosis virus of the alfalfa looper, *Autographa californica*. *Journal of Invertebrate Pathology* 23(2): 197–201.

Rao, G.V.R., Wightman, J.A. and Rao, D.V.R. (1993). World review of the natural enemies and diseases of *Spodoptera litura* (F.) (Lepidoptera: Noctuiidae). *International Journal of Tropical Insect Science* 14(3): 273-284.

Razvi, E.S. and Welsh, R.M. (1995). Apoptosis in viral infections. *Advances in Virus Research* 45(4): 1–60

Reed, J.C. (1994). Bcl-2 and the regulation of programmed cell death. *Journal of Cell Biology* 124(1): 1-6.

Reed, L. and Muench, H. (1938). A simple method for estimating fifty percent endpoints. *American Journal of Hygiene* 27(3): 493-497.

Reggiori, F. (2006). Membrane origin for autophagy. *Current Topics in Developmental Biology* 74: 1-30.

Reissig, W.H., Heinrichs, E.A., Litsinger, J.A., Moody, K., Fiedler, L., Mew, T.W. and Barrion, A.T. (1986). Illustrated guide to integrated pest management in rice in tropical Asia. Manila (Philippines). *International Rice Research Institute:* pp. 411.

Rezende, S.H.M.S., Castro M.E.B. and Souza M.L. (2009). Accumulation of few-polyhedra mutants upon serial passage of *Anticarsia gemmatalis* multiple nucleopolyhedrovirus in cell culture. *Journal of Invertebrate Pathology* 100(3):153–159.

Rohrmann, G. (2011). Introduction to the baculoviruses, their taxonomy, and evolution. In *Baculovirus Molecular Biology*: 2nd Edition, ed. G.F. Rohrmann. Bethesda (MD): National Center for Biotechnology Information.

Roldão, A., Oliveira, R., Carrondo, M.J.T. and Alves, P.M. (2009). Error assessment in recombinant baculovirus titration: Evaluation of different. *Journal of Virological Methods* 159(1): 69-80.

Sajap, A.S. (1995). A preliminary observation on microsporidian infection in the armyworm, *Spodoptera litura* (Fabricius) (Lepidoptera : Noctuidae). *Malaysian Applied Biology* 24(2): 43-48.

Sajap, A.S., Kotulai, J.R., Bakir, M.A., Lau, W.H., Samad, N.A. and Kadir, H.A. (2000). Pathogenicity and characteristics of *Spodoptera litura* nucleopolyhedrovirus from Peninsular Malaysia. *Pertanika Journal of Tropical Agricultural Science* 23(1): 23-28.

Salama, H.S., Dimetry, N.Z. and Salem, S.A. (1970). On the host preference and biology of the cotton leafworm *Spodoptera littoralis. Zeitung fur Angewandte Entomologie* 67(1-4): 261-266.

Savill, J., Dransfield, I., Gregory, C. and Haslett, C. (2002). A blast from the past: clearance of apoptotic cells regulates immune responses. *Nature Reviews Immunology* 2(12): 965-975.

Schneider, I. (1969). Establishment of three diploid cell lines of*Anopheles stephensi* (Diptera: Culicidae).*The Journal of Cell Biology* 42(2): 603–605.

Senthil-Nathan, S., Kalaivani, K., (2005). Efficacy of nucleopolyhedrovirus (NPV) and azadirachtin on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). *Biological Control* 34 (1): 93-98.

Shelby, K.S. and Popham H.J.R. (2006). Plasma phenoloxidase of the larval tobacco budworm, *Heiothis virescens*, is virucidal. *Journal of Insect Science* 6(13): 1-12.

Shen, C.F., Meghrous, J. and Kamen, A. (2002). Quantitation of baculovirus particles by flow cytometry. *Journal of Virological Methods* 105(2): 321-330.

Shih, C.J. and Chang, J.C. (1997). High Level Expression of Recombinant Protein in a Cell Line Derived from *Spodoptera litura* (Lepidoptera: Noctuidae). *Applied Entomology and Zoology* 32(1): 79-188.

Shih, C.J., Lin, R.W. and Wang, C.H. (1997). Establishment of a cell line from *Spodoptera litura* (Lepidoptera: Noctuidae) and replication of *S. litura* nuclear polyhedrosis virus *in vitro*. *Journal of Invertebrate Pathology* 69(1):1-6.

Shouche, Y.S., Patole, M.S., Pant, U., Paranjape, S. and Banerjee, K. (1999). Authentication of two cell lines developed from the larval and pupal ovaries of *Spodoptera litura* by rRNA based methods. *In Vitro Cellular and Development Biology-Animal* 35(5): 244-245.

Shuler, M.L., Wood, H.A., Granados, R.R and Hammer, D.A., (1995). Overview of baculovirus-insect culture system. In *Baculovirus Expression System and Biopesticide*, ed. M.L. Shuler, H.A. Wood, R.R. Granados and D.A. Hammer, pp. 1-11. New York: Wiley-Liss.

Silva, M.T. (2010). Secondary necrosis: the natural outcome of the complete apoptotic program. *FEBS Letters* 584(22): 4491-4499.

Silveira, E.B., Cordeiro, B.A., Ribeiro, B.M., de Castro, M.E., Soares, E.F. and Báo, S.N. (2007). An *Anticarcia gemmtalis* multiple nucleopolyhedrovirus mutant, vApAg, induces hemocytes apoptosis *in vivo* and displays reduced infectivity in larvae of *Anticarcia gemmtalis* (Hubner) (Lepidoptera: Nuctuidae). *Virus Research* 130(1-2): 182-192.

Silveira, E.B., Cordeiro, B.A., Ribeiro, B.M. and Báo, S.N. (2005). *In vivo* apoptosis induction and reduction of infectivity by an *Autographa californica* multiple nucleopolyhedrovirus *p35*− recombinant in hemocytes from the velvet bean caterpillar *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae). *Research in Microbiology* 156(10): 1014-1025.

Singh, K.R.P. (1967). Cell cultures derived from larvae of *Aedes albopictus* (Skuse) and *Aedes aegypti* (L). *Curtural Science* 3: 5-6.

Singh, S.P. and Jalali, S.K. (1997). Management of *Spodoptera litura* (Fab.) (Lepidoptera:Noctuidae). In "*Spodoptera litura in Indian Proceeding of the National Science Forum".* April 1996.

Slavicek, J. M., Plazolles, N. H. and Kelly, M. E. (1995). Rapid formation of Few Polyhedra Mutants of *Lymantria dispar* multinucleocapsid Nuclear Polyhedrosis Virus during serial passage in cell culture. *Biological Control* 5: 251-261.

Slavicek, J. M., Podgwaite, J. and Lanner-Herrera, C. (1992). Properties of two *Lymantria dispar* nuclear polyhedrosis virus isolates obtained from the microbial pesticide Gypchek. *Journal of Invertebrate Pathology* 59(2): 142–148.

Slavicek, J.M., Mercer, M.J., Kelly, M.E. and Hayes-Plazolles, N. (1996). Isolation of a baculovirus variant that exhibits enhanced polyhedra production stability

during serial passage in cell culture. *Journal of Invertebrate Pathology* 67: 153–160.

Solis-Recendez, M., Perani, A., D'Habit, B., Stacey, G.N., and Maugras, M. (1995). Hybridoma cell cultures continuously undergo apoptosis and reveal a novel 100 bp DNA fragment. *Journal of Biotechnology* 38(2): 117-127.

Stoffel, C.L., Kathy, R.F. and Rowler K.L. (2005). Design and characterization of a compact dual channel virus counter. *Cytometry* A 65(2): 140-147.

Sugimoto, A., Friesen, P.D. and Rothman, J.H. (1994). Baculovirus *p35* prevents developmentally programmed cell death and rescues a ced-9 mutant in the nematode *Caenorhabditis elegans*. *The EMBO Journal* 13(9): 2023-2028.

Summers, M.D. and Smith, G.E. (1987). A manual of methods for baculovirus vectors and insect cell culture procedures. *Texas Agricultural Experiment Station Bulletin*,pp.1555.

Sun, X.L. and Peng, H.Y. (2007). Recent advances in biological control of pest insects by using viruses in China. *Virologica Sinica* 22(2): 158-162.

Takatsuka, J., Okuna, S., Nakai, M. and Kunimi, Y. (2003). Genetic and biological comparison of ten geographic isolates of a nucleopolyhedrovirus that infect S*podoptera litura* (Lepidoptera ; Nuctuidae). *Biological Control* 26(1): 32-39.

Takei, G.H. and Tamashiro, M. (1974). Changes observed in hemolymph proteins of the lawn armyworm, *Spodoptera mauritia acronyctoides* during growth, development and exposures to a nuclear polyhedrosis virus. *Journal of Invertebrate Pathology* 26(2): 147-158.

Teodoro, J.G. and Branton, P.E. (1997). Regulation of apoptosis by viral gene products. *Journal of Virology* 71(3): 1739-1746.

Thiem, S.M., Xian-lin, D., Quentin, M.E. and Berner, M.M. (1996). Identification of a baculovirus gene that promotes *Autographa californica* nuclear polyhedrosis virus replication in a nonpermissive insect cell line. *Journal of Virology* 70(4): 2221-2229.

Thompson, C.B. (1995). *Apoptosis in the pathogenesis and treatment of disease. Science* 267(5203): 1456-*1462.*

Tramper, J. and Vlak, J. (1986). Some engineering and economic aspects of continuous cultivation of insect cells for the production of baculoviruses. *Annals of the New York Academy of Sciences* 469: 279-288.

van Oers, M. M. and Lynn, D. E. (2010). Insect Cell Culture. [Electronic version] *Encyclopedia of Life Sciences* (eLS). Wiley online Library. http://dx.doi.org/10.1002/9780470015902.a0002574.pub2

Vaughn, J.L., Goodwin, R.H., Tompkins, G.J. and McCawley, P. (1977). The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *In Vitro* 13(2): 213–217.

Vaux, D.L., Haecker, G. and Strasser, A. (1996). The molecular biology of apoptosis. *Proceedings of the National Academy of Sciences* 93(6): 2239-2244.

Venette, R.C., Davis, E.E., Zaspel, J., Heisler, H. and Larson, M. (2003). Mini Risk Assessment Rice cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). St. Paul: Department of Entomology, University of Minnesota.

Vieira, H.L., Estevao, C., Roldao, A., Peixoto, C.C., Sousa, M.F.Q., Cruz, P.E., Carrondo M.J.T. and Alves, P.M. (2005). Triple layered rotavirus VLP production: Kinetics of vector replication, mRNA stability and recombinant protein production. *Journal of Biotechnology* 120(1): 72-82.

Volkman, L. E. and Summers, M. D. (1977). *Autographa californica* nuclear polyhedrosis virus: comparative infectivity of the occluded, alkali-liberated, and nonoccluded forms. *Journal of Invertebrate Pathology* 30(1):102–103.

Volkman, L.E., Blissard, G.W. and Friesen, P. (1995). Baculoviridae: Taxonomic of structure and properties of the family. *Archives of Virology* 10: 104-113.

Washburn, J.O., Haas-Stapleton, J.A., Tan, F.A. Backage, N.E. and Volkman L.E. (2000). Co-infeection of *Maduca sexta* larvae with polydnavirus from *Cotesiacongregata* increases susceptibility to fatal infection by *Autographa californica* Multicapsid nucleopolyhedrovirus. *Journal of Insect Physiology* 46(2): 179-190.

Washburn, J.O., Kirkpatrik B.A. and Volkman L.E. (1996). Insect protection against viruses. *Nature* 383-767.

Waterhouse, D. and Norris, K. (1987). *Spodoptera litura* (Fabricius). In *Biological Control: Pacific Prospects*, pp. 250-259. Canberra: Australian Centre for International Agricultural Research.

Whitaker, A.M. (1972). Chapter 5: Culture Cells from Other Species & Chapter 6: Application of Cell culture to Virology. In *Tissue and cell culture*. Pp. 66-91. United Kingdom: Bailliere and Tindall.

Wickham, T.J., Davis, T., Granados, R.R., Hammer, D.A., Shuler, M.L. and Wood, H.A. (1991). Baculovirus defective particles are responsible for variations in recombinant proteins production as a function of multiplicity of infection. *Biotechnology Letters* 13(7): 483-488.

Wood, H.A. (1980). Isolation and replication of an occlusion body-deficient mutant of the *Autographa californica* nuclear polyhedrosis virus. *Virology* 105(2): 338-344.

Wyatt, S.S. (1956). Culture *in vitro* of tissue from the silkworm, *Bombyx mori* . *Journal of General Physiology* 39(6): 841–852.

Wyllie, A.H., Kerr, J.F. and Currie, A.R. (1980). Cell death: the significance of apoptosis. *International Review of Cytology* 68: 251–306.

Xie, W.D., Qu, S.R. and Pang, Y. (1988). The establishment of an insect cell line from *Spodoptera litura* and the study of virus infection. *Acta Scientiarum Naturalium Universitatis Sunyatseni* 45:113–116.

Xue, D. and Horvitz, H.R. (1995). Inhibition of the *Caenorhabsitis elegans* cell-death protease CED-3 by a CED-3 cleavage site in baculovirus *p35* protein. *Nature* 377: 248-251

Yahya, H. (1985). Sistem pengawalan serangga perosak tembakau. *Teknologi Tembakau* 1: 22-28.

Yahya, H. and Karim, S.A. (1989). Penilaian racun serangga untuk mengawal serangga perosak utama tanaman tembakau. *Teknologi Tembakau* 5: 19-29.

Yu, M., Li, Z., Yang, K., Lin, T., Gong, Y. and Pang, Y. (2005). Identification of the apoptosis inhibitor gene *p49* of *Spodoptera litura* multicapsid nucleopolyhedrovirus. *Virus Genes* 31(2): 145–151.

Zhang, H., Zhang, Y. A., Qin, Q., Li, X., Miao, L., Wang, Y. and Yang, Q. (2009). A cell strain cloned from *Spodoptera exigua* cell line (IOZCAS-Spex-II) highly susceptible to *S. exigua* nucleopolyhedrovirus infection. *In Vitro Cell Developement Biology Animal* 45(5-6): 201-204.

Zhang, P., Yang, K., Dai, X., Pang, Y. and Su, D. M. (2002a). Infection of wild-type *Autographa californica* multicapsid nucleopolyhedrovirus induces *in vivo* apoptosis of *Spodoptera litura* larvae. *Journal of General Virology* 83(12): 3003-3011.

Zhang, P., Yang, K., Dai, X., Pang, Y. and Su, D. M. (2002b). *Spodoptera litura* multicapsid nucleopolyhedrovirus blocks *Autographa californica* multicapsid nucleopolyhedrovirus-induced *Spodoptera litura* cell apoptosis. *Biochemistry and Biophysics* 29(5): 702-707.

Zhang, X., Lan, W.,Deng, Y., Ma, Y., Liu, K. and Peng, J. (2008). Highly passage of *Spodoptera litura* cell line causes its permissiveness to baculovirus infection. *Cytotechnology* 57(3):233–243.

Zhou, Z.S. (2009). A review on control of tobacco caterpillar, *Spodoptera litura*. *Chinese Bulletin Entomology* 46(3): 354-361.

Zoog, S.J., Bertin, J. and Friesen, P.D. (1999). Caspase inhibition by baculovirus *P35* requires interaction between the reactive site loop and the b-sheet core. *Journal of Biological Chemistry* 274(37): 25995-26002.

Zurgil, N., Shafran, Y., Fixler, D. and Deutsch, M. (2002). Analysis of early apoptotic events in individual cells by fluorescence intensity and polarization measurements. *Biochemistry and Biophysical Research Communication* 290(5):1573–1582.