EVALUATION OF BACULOVIRUSES FOR THE MANAGEMENT OF DIAMONDBACK MOTH, *Plutella xylostella* (L.) (LEPIDOPTERA: PLUTELLIDAE)

Thesis submitted in part fulfilment of the requirements for the degree of ***DOCTOR OF PHILOSOPHY IN AGRICULTURAL ENTOMOLOGY*** *to the Tamil Nadu Agricultural University, Coimbatore*

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CERTIFICATE

This is to certify that the thesis entitled **"EVALUATION OF BACULOVIRUSES FOR THE MANAGEMENT OF DIAMONDBACK MOTH, *Plutella xylostella* (L.) (LEPIDOPTERA: PLUTELLIDAE)"** submitted in part fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY IN AGRICULTURAL ENTOMOLOGY** to the Tamil Nadu Agricultural University, Coimbatore is a *record of bonafide* research carried out by **Mr.R. PARTHASARATHY** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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Abstract

ABSTRACT

EVALUATION OF BACULOVIRUSES FOR THE MANAGEMENT OF DIAMONDBACK MOTH, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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Studies were carried out in the laboratory as well as on the field to explore the potential of *Galleria mellonella* (L.) nucleopolyhedrosis virus (GmNPV) against the pests of cauliflower with special emphasis on *Plutella xylostella* (L.). Cross-infectivity studies with GmNPV revealed the susceptibility of 16 insect species out of 31 screened. It included important crop pests like *P. xylostella*, *Chilo partellus* (Swinhoe), *Chilo infuscatellus* (Snell), *Chilo sacchariphagus indicus* (Kapur), *Maruca vitrata* (Geyer), *Cnaphalocrocis medinalis* (Guen.), *Opisina arenosella* (Walk.), *Amsacta albistriga* (Walk.), *Diaphania pulverulentalis* (Hamp.), *Spodoptera exigua* (Hb.) and other pests like *Crocidolomia binotalis* (Zell), *Hellula undalis* (F.), *Pericallia ricini* (F.), *Ergolis merione* (Cram), *Exelastis atomosa* (Walsingham) and *Marasmia patnalis* (Brad.). The cross-infectivity was confirmed by DNA characterization and reciprocal inoculation studies. Serial passaging of GmNPV for five times through alternate hosts increased its virulence against *P. xylostella*.

Second, third and fourth instar larvae of *P. xylostella* were susceptible to two isolates of GmNPV *viz.*, Coimbatore (CBE) and Bangalore (BNGL). Serial passages of GmNPV through *P. xylostella* for 15 times enhanced the virulence of GmNPV to *P. xylostella* by decreasing the LC_{50} 12.76 fold. *P. xylostella* Granulosis Virus (PxGV) was found to be more effective than GmNPV but LT_{50} was higher by 23.31h than that of

GmNPV. The combination of GmNPV and PxGV did not show any synergistic effect on *P. xylostella* larvae.

C. partellus was found to be the suitable alternate host for cost effective mass production of GmNPV. Transovarial transmission of GmNPV was determined in *C. binotalis* and *P. ricini*. Passage of GmNPV through the alternate hosts reduced the size of the polyhedra. Histopathology studies revealed the infection of GmNPV in fat bodies, hypodermis, tracheal tissues, imaginal disc, salivary glands and neural ganglion of *P. xylostella*, *C. partellus* and *M. vitrata* larvae. *In vitro* studies indicated that virions from *C. partellus* larvae produced the highest per cent cell infection and yield of Polyhedral Occlusion Bodies (POB)/ml of cell culture medium in Sf21 cell line. The chitinase activity of GmNPV was lower than in *Helicoverpa armigera* NPV and *Spodoptera litura* NPV attributing to the atypical symptoms of disease.

The persistence of GmNPV on cauliflower phyllosphere was for six days. A positive correlation of persistence with relative humidity at 14.30h and negative correlation with maximum temperature and solar radiation was observed. Application of GmNPV @ 5×10^7 POB/ml was effective against the lepidopteran pest complex of cauliflower *viz.*, *P. xylostella, C. binotalis* and *H. undalis* in pot culture experiments. Four sprays of GmNPV @ 7.5×10^{13} POB/ha at eight day intervals was the best treatment next to indoxacarb in reducing the population of *P. xylostella* and *C. binotalis* under field conditions. GmNPV was safe to *Trichogramma chilonis* (Ishii), *Chrysoperla carnea* (Stephens), *Apis* spp., and six different races of *Bombyx mori* (L.). These investigations highlight the possibility of developing GmNPV as a microbial insecticide for the management of several economically important lepidopteran pests.

CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	4
III.	MATERIALS AND METHODS	27
IV.	RESULTS	51
V.	DISCUSSION	119
VI.	SUMMARY	154
	REFERENCES	
	APPENDICES	

LIST OF TABLES

Table No.	Title	Page No.
1.	Cross-infectivity of GmNPV to different lepidopteran insect pests	52
2.	Susceptibility of different insect pests to GmNPV	59
3.	Comparative virulence of GmNPV isolates to selected susceptible host species	60
4.	Comparative LC_{50} of GmNPV against *P. xylostella* upon serial passages through different susceptible hosts	64
5.	Comparative virulence of GmNPV isolates to early third instar larvae of *P. xylostella*	65
6.	Concentration mortality responses of different instars of *P. xylostella* larvae to GmNPV isolates	67
7.	Effect of serial passage of GmNPV in *P. xylostella* larvae on the concentration-mortality responses of *P. xylostella* and yield of POB	68
8.	Effect of serial passage of GmNPV in *P. xylostella* larvae on the time-mortality responses of *P. xylostella*	70
9.	Comparative concentration and time-mortality responses of *P. xylostella* larvae to GmNPV and PxGV	71
10.	Combined effect of GmNPV and PxGV on *P. xylostella* larvae	72
11.	Standardization of mass production technique for GmNPV in *G. mellonella*	74
12.	Susceptibility of different larval instars of three host insect species and yield of GmNPV	77
12a.	Production efficiency of GmNPV in three different insect species	78
13.	Concentration - mortality responses of different larval instars of three insect species to GmNPV	80

14.	Time-mortality responses of different larval instars of three insect species to GmNPV	81
15.	Transovarial transmission of GmNPV	83
16.	Comparison of the activity of horizontally and vertically transmitted GmNPV against *P. xylostella*	83
17.	Comparison of infection of GmNPV in different susceptible host tissues	84
18.	Size of polyhedra of GmNPV after passage through some alternate host insects and a cell line, Sf21.	89
19.	Permissibility of cell lines to GmNPV isolates	92
20.	Susceptibility of Sf21 cell line to GmNPV isolated from different haemolymph sources	93
21	Comparative virulence of concentration – mortality response of early third instar larvae of *P. xylostella* to *in vivo* and *in vitro* produced GmNPV from different haemolymph sources	96
22.	Comparative virulence of concentration – mortality response of early third instar larvae of *P. xylostella* to *in vivo* and *in vitro* produced GmNPV from different cell lines	97
23.	Determination of chitinase activity of GmNPV in comparison with HaNPV and SlNPV	98
24.	Persistence of GmNPV on cauliflower phyllosphere	100
25.	Effect of microclimatic conditions on the persistence of GmNPV	101
26.	Correlation matrix between microclimatic weather parameters and persistence of GmNPV	103
27.	Effect of different dosages of GmNPV on *P. xylostella* by pot culture assay	104
28.	Effect of different dosages of GmNPV on *C. binotalis* by pot culture assay	106

29.	Effect of different dosages of GmNPV on *H. undalis* by pot culture assay	107
30.	Field evaluation of GmNPV against *P. xylostella*	108
31.	Field efficacy of GmNPV against *C. binotalis*	111
32.	Effect of GmNPV on the yield of cauliflower	113
33.	Effects of GmNPV isolates on *Trichogramma chilonis* by egg treatment	114
34.	Effects of GmNPV isolates on *Trichogramma chilonis* by oral feeding	114
35.	Effect of GmNPV isolates on *Chrysoperla carnea*	115
36.	Safety of GmNPV to different races of silkworm	117
37.	Safety of GmNPV to different species of honeybees	118

LIST OF PLATES

Plate No.	Title	Page No.
1.	Polyhedral occlusion bodies of GmNPV	31
2.	Method of exposure of GmNPV to sunlight for persistence study	46
3.	Installation of thermometers for microclimatic observations	46
4.	*P. xylostella* larvae infected with GmNPV	53
5.	GmNPV infected larvae of *C. binotalis*	53
6.	Virosed larvae of *Hellula undalis* by GmNPV	53
7.	GmNPV killed larva of *Chilo partellus*	53
8.	*C. infuscatellus* larva infectd with GmNPV	54
9.	GmNPV infection in *C. sacchariphagus indicus* larva	54
10.	Cadaver of *Pericallia ricini* larva virosed by GmNPV	54
11.	Different larval stages of *Ergolis merione* infected with GmNPV	54
12.	Stages of GmNPV infection in *Maruca vitrata* larvae	55
13.	Virosed larvae of *Cnaphalocrocis medinalis* by GmNPV	55
14.	GmNPV infection in *Opisina arenosella* larvae	55
15.	Cadaver of *Amsacta albistriga* larva virosed by GmNPV	55
16.	Larvae of *Diaphania pulverulentalis* infected with GmNPV	55
17.	Restriction endonuclease analysis of DNA of GmNPV with *Pst* I-I	57
18.	Restriction endonuclease analysis of DNA of GmNPV with *Pst* I-II	57
19.	Restriction endonuclease analysis of DNA of GmNPV with *Bam*H I	57
20.	GmNPV infected larvae of *Galleria mellonella*	75
21.	Mass production of GmNPV in *G. mellonella* larvae	75
22.	Mass production of GmNPV in *C. partellus* larvae	75
23.	Vacoulation and virogenic stroma formation in the cross section of *P. xylostella* larvae during initial stages of infection of GmNPV	85

24.	Cross-section of *P. xylostella* larva showing fat bodies infection 72 h post inoculation with GmNPV	85
25.	Cross-section of refractive midgut epithelial cells to GmNPV infection in the larvae of *P. xylostella*	85
26.	Cross-section of *P. xylostella* larva showing fat bodies infection 96 h post inoculation with GmNPV	85
27.	Polyhedral occlusion bodies on the hypodermal cells of cross section of *P. xylostella* larva 120 h post inoculation with GmNPV	85
28.	Proliferation of GmNPV infection in the degenerative fat body tissues of cross-section of *P. xylostella* larva 120 h post inoculation	85
29.	Cross-section of *M. vitrata* larva showing fat body infection 96 h post inoculation with GmNPV	86
30.	Package of polyhedral occlusion bodies of GmNPV in the hypodermal cells of cross section of *M. vitrata* larva	86
31.	Proliferation of GmNPV infection in the fat body tissues of *M. vitrata* larva 144 h post inoculation	86
32.	Cross-section of *M. vitrata* larva showing GmNPV infection in the imaginal disc 144 h post inoculation	86
33.	Package of polyhedral occlusion bodies of GmNPV in the tracheal matrix of *M. vitrata* larvae	86
34.	Cross-section of *M. vitrata* larvae showing GmNPV infection in hypodermis, fat bodies and tracheal tissue matrix at advanced stages of infection	86
35.	Cross-section of *C. partellus* larva showing fat bodies infection 96 h post inoculation of GmNPV	87
36.	Cross-section of *C. partellus* larva showing GmNPV infection in fat bodies and hypodermal cells at 120 h post inoculation of GmNPV	87
37.	Infection of GmNPV in the degenerative fat body tissues of *C. partellus* larva 144 h post inoculation	87
38.	Package of Few Polyhedra (FP) and Many Polyhedra (MP) cells of hypodermis and fat bodies of *C. partellus* larva 144 h post inoculation	87
39.	Proliferation of GmNPV infection in the salivary glands of *C. partellus* larva at 168 h post inoculation	87

40.	Cross-section of *C. partellus* larva showing ganglion infection at 168 h post inoculation of GmNPV	87
41.	GmNPV (CBE isolate) infection in Sf21 cells	91
42.	Sf21 cells infection with BNGL isolate of GmNPV	91
43.	GmNPV (CBE isolate) infection in *S. litura* haemocytes	91
44.	*S. litura* haemocyte cells infected with BNGL isolate of GmNPV	91
45.	GmNPV (CBE isolate) infection in *S. litura* pupal gonad (SlPG) cells	94
46.	SlPG cells infected with BNGL isolate of GmNPV	94
47.	GmNPV (CBE isolate) infection in *H. armigera* haemocyte (HaH) cells	94
48.	HaH cells infected with BNGL isolate of GmNPV	94
49.	Field trial on the efficacy of GmNPV against *P. xylostella*	109
50.	Mass culturing of *C. partellus* larvae on artificial diet	133
51.	Few Polyhedra (FP) and Many Polyhedra (MP) mutants of GmNPV in Sf21 cell line	140
52.	Polyhedra of GmNPV liberated from SlPG cells 120 h post inoculation	140

LIST OF FIGURES

Figure No.	Title	Page No.
1.	Proportion of susceptibility of lepidopteran families to GmNPV	120
2.	Cross infectivity of GmNPV to different lepidopteran insect pests	123
3.	Median lethal concentration of GmNPV upon serial passages through alternate hosts on *P. xylostella*	126
4.	Susceptibility of Sf21 cell lines to GmNPV isolates	138
5.	Chitinase activity of GmNPV in comparison with HaNPV and SlNPV	142
6.	Persistence of GmNPV on cauliflower phyllosphere	145
7.	Field efficacy of GmNPV against *P. xylostella*	149
8.	Effect of GmNPV on the yield of cauliflower	151

LIST OF APPENDICES

Sl. No.	Title
1.	Semi-synthethic diets
2.	Buffers for restriction endonucleases
3.	Head capsule width
4.	Reagents for chitinase assay
5.	Molecular weights of GmNPV DNA
6.	Concentration - mortality responses of *P. xylostella* larvae to GmNPV serially passaged through different alternate hosts

Introduction

Chapter I

INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.), an important cosmopolitan pest, is a specialist herbivore that attacks many brassicaceae species. It is believed to be the most universally distributed of all Lepidoptera (Talekar and Shelton, 1993) and is considered the most limiting factor to successful production of cruciferous vegetables in many areas of the world (Chu, 1986). Methods used in the control of this pest include chemical, cultural and biological, where chemicals being the most problematic because of development of resistance by diamondback moth. It has an extraordinary propensity to develop resistance to every synthetic insecticide used to control it in crucifers. Efforts to control this pest solely through conventional insecticides have led to development of resistance to most of the insecticides available in India (Kalra and Chawla, 1977; Awante *et al.*, 1982; Saxena *et al.*, 1989; Mehrothra, 1993).

Bacillus thuringiensis (Berliner), a crystelliferous bacterial insecticide has been recognized as a potential alternative to chemical pesticides and several field trials have demonstrated its usefulness in the management of *P. xylostella* on crops like cabbage and cauliflower (Krishnaiah *et al.*, 1981; Chelliah and Srinivasan, 1986; Sannaveerappanavar, 1995). However, the widespread usage of *B.t.* in large quantities may lead to the development of resistance to their products by diamondback moth (Mohan and Gujar, 2000). Therefore, at present, concerted efforts are needed to devise an alternate strategy that is within the economic reach of marginal/small farmers in India.

Insect baculoviruses with no reports on the development of resistance by insect pests offer wide scope for the management of *P. xylostella*. These viruses have been isolated from over 300 insect species and have received considerable attention in the past 20 years. They are regarded as safe and selective bioinsecticides restricted to invertebrates. They have been used worldwide against many insect pests, mainly Lepidopterans (Moscardi, 1999). For the management of *P. xylostella*, the use of granulosis virus (GV) has been documented (Asayama and Osaki, 1970; Su, 1987;

Sairabanu, 2000). GVs are known for their host specificity and slow speed of kill. With the non-availability of semi-synthetic diet of *P. xylostella* under Indian conditions, the mass production of GV for large scale control of *P. xylostella* becomes difficult. The use of another group of baculoviruses namely the nucleopolyhedrosis virus (NPV) for the management of *P. xylostella* remains unexplored except for a few recent reports (Padmavathamma and Veeresh, 1991; Kariuki and McIntosh, 1999). Another possibility is the use of virus with broad-spectrum activity. The availability of baculoviruses with fairly wide host ranges, such as *Autographa californica* (Speyer) NPV and *Anagrapha falcifera* (Kirby) NPV, also has attracted increased interest (Cunningham, 1995; Grewal *et al.*, 1998).

The multiple occluded NPV from *Galleria mellonella* (GmNPV) is known to be infective to *P. xylostella* (Kadir *et al.*, 1999 a and b) besides several other insects like *Trichoplusia ni* (Hbn.), *Pseudoplusia includens* (Wlk.), *Heliothis zea* (Boddie), *Manduca sexta* (L.), *Agrotis ipsilon* (Huf.) (Witt and Janus, 1977; Kadir and Payne, 1989; Stairs, 1990). An isolate of GmNPV available at the Department of Agricultural Entomology, TNAU, Coimbatore was found to infect *P. xylostella* and a few other insects which indicated the scope of further detailed investigations on its host range and its potential for development as a microbial insecticide. A NPV with broad-spectrum activity and proven to be safe to non-target organisms will be a boon to integrated pest management of several important crop pests. With this background, the present investigations were undertaken to explore the potential of GmNPV against lepidopterous pests of cruciferous crops with special emphasis on *P. xylostella* and to study the possibility of using GmNPV for the management of economically important insect pests of other crops with the following objectives:

1. To study the effect of *in vivo* production in the alternate hosts on the virulence of GmNPV to *P. xylostella* larvae.
2. To study the virulence of GmNPV to different larval instars of *P. xylostella*.

3. To increase the virulence of GmNPV by serial passages *in vivo* in *P. xylostella* larvae.

4. To compare the virulence of GmNPV with *P. xylostella* granulosis virus (PxGV) to *P. xylostella* larvae

5. To identify an alternate host for economic production of GmNPV.

6. To study the field efficacy of GmNPV against *P. xylostella.*

Review of Literature

Chapter II

REVIEW OF LITERATURE

2.1. *Plutella xylostella* and its management

Amongst the lepidopterous pests of cruciferous crops, the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is the most serious and widely distributed pest throughout the world (CIE, 1967). The global importance of DBM is reflected in estimates that its control costs approximately US $ 1 billion annually (Talekar, 1992). In India, the DBM occurs regularly wherever crucifers are grown and often it causes serious crop damage in cole crops despite insecticide use. Krishnakumar *et al.* (1984) estimated 52 per cent loss in marketable yield due to DBM attack on cabbage.

P. xylostella has the credit of being the only pest exposed to several generation insecticides. Prior to the introduction of synthetic insecticides in the late 1940s, DBM was not reported as a major pest of crucifers. The DBM was the first crop pest in the world to develop resistance to DDT in 1953. Now in many countries this pest has developed resistance to every synthetic insecticide used against it in the field (Talekar *et al.*, 1990). In 1990's, several fourth generation insecticides like avermectins, cartap hydrochloride and insect growth regulators have been reported to be highly effective against *P. xylostella* (Bandara and Kudagamage, 1991; Rajavel and Sundarababu, 1989; Jannson *et al.*, 1997; Li, 1998; Sujatha *et al.*, 2000; Kalra and Sharma, 2000).

The alarming reports of development of resistance to new generation insecticides like avermectins (Zhang-Lue-Yen *et al.*, 2001) and insect growth regulators (Ohtsu *et al.*, 1999; Perez *et al.*, 2000) within a short period of use determine the stability of resistance in *P. xylostella*. However, in India, synthetic insecticides still remain a dominant tool in the management of *P. xylostella*.

Failure of several insecticides in managing this pest due to the development of resistance has directed the focus on the use of other agents of control. Use of plant products like neem (Meenarani *et al.*, 1999; Kumar *et al.*, 2000), garlic products (Rahman and Motoyama, 2000) and extracts of *Prosofis juliflora, Croton* sp. and *Eugenia* spp. (Torres *et al.*, 2001) has been advocated for the management of DBM. However, elaborate field studies on the efficacies of plant products are warranting.

Wyman *et al.* (1999) encouraged the use of biological control as a component of IPM with widespread control failures with *P. xylostella.* Parasitoids like *Diadegma semiclausum* (Hellen), *Cotesia plutellae* (Kurdy) and *Tomyzus sokolowskwi* (Kurdy) have been found to occur on *P. xylostella* (Delvare and Kirk, 1999). Absence of effective natural enemies, especially these parasitoids, is believed to be a major cause of the DBM's pest status in most parts of the world (Lim, 1986). Lethal effects of over use of synthetic insecticides have suppressed the potential of natural enemies of DBM. Conservation and augmentation of these natural enemies are needed for management of *P. xylostella*, but these are long-term processes.

Among the microbial agents, *Bacillus thuringiensis* (*B.t.)* (Berliner) offers tremendous scope for DBM control because of its specificity. Several insecticidal crystal proteins of *B.t.* like CryIAa, CryIAb, CryIB, CryIC and CryIF are toxic to *P. xylostella* (Ballester *et al.*, 1999). Because of widespread usage of *B.t.* in large quantities comparable to chemical insecticides, DBM have also got the distinction of being the first insect to develop resistance to this pathogen in the field (Kirsch and Schmutterer, 1988; Tabashnik *et al.*, 1990) and the resistance in the laboratory being well documented (Mohan and Gujar, 2000). Failure of genetically improved strains of *B.t.* (Talekar and Shelton, 1993) and development of resistance to transgenic broccoli plants by *P. xylostella* (Shelton *et al.*, 2000) reveal the complexity in the use of *B.t.* products against DBM.

Natural epizootics of fungal pathogens like *Beauveria bassiana* Vuill., *Zoophthora radicans* (Brefeld) Batko, (Yoon *et al.*, 1999, Gopalakrishnan *et al.*, 1999) and infectivity of *Metarhizium anisopliae* (Metchnikoff), *Nomuraea rileyi* (Farlow) Samson, *P. fumosoroseus* (Wize) (Robert and Marchal, 1980) against *P. xylostella* have been documented. Baur *et al.*,(1997) reported the invasion, development and reproduction of entomopathogenic nematode, *Steinernema carpocapsae* (Weisler) in *P. xylostella.* Environmental specificity of these pathogens limits their field use.

Other microbials for the control of diamondback moth include the baculoviruses that have been demonstrated to be pathogenic for lepidopteran insects. The potential of baculoviruses in the management of *P. xylostella* is detailed as follows.

2.1.1. Baculoviruses of *P. xylostella*

Granulovirus (GV) was the first baculovirus detected in the larvae of *P. xylostella* (PxGV). Asayama and Osaki (1970) described the symptomotologies and morphometrics of GV infection in *P. xylostella.* The pathology of the disease, host interactions, field efficacy and potential use of GV as a biocontrol agent against *P. xylostella* have been extensively reviewed (Asayama and Inagaki, 1975; Asayama, 1976; Kadir, 1986; Koshihara, 1986; Hou, 1987; Su, 1987; Yuan *et al.*, 1999 and Sairabanu, 2000).

Nucleopolyhedrovirus of *P. xylostella* (PxNPV) was first observed in the fields of cauliflower (Vail *et al.*, 1972). Observations of the polyhedral and viral morphology and results of the cross-infectivity tests indicated that the virus isolated from *P. xylostella* was the virus from the alfalfa looper used for the control of cabbage looper, *Trichoplusia ni* (Hubner). Biever and Andrews (1984) isolated a NPV from *P. xylostella* which was cross-infective to several lepidopteran pests. However the identity of the virus was later confirmed as NPV of *Galleria mellonella* (L.) (GmNPV) by characterization studies (Kadir and Payne, 1989). Kadir (1992) emphasized the potential of several baculoviruses for the control of DBM.

Grewal *et al.* (1998) reported the susceptibility of *P. xylostella* to NPV of *Anagrapha falcifera* (Kirby) (AfMNPV) and *Autographa californica* (Speyer) (AcMNPV). AcMNPV was found to be more virulent than AfMNPV for *P. xylostella.* Neonates of *P. xylostella* were found to be highly susceptible to GmNPV and a low LT_{50} was recorded with GmNPV when compared to that of AcMNPV and PxGV (Kadir *et al.* 1999b). Until recently there are no authentic reports on the NPV of *P. xylostella*. Kariuki and McIntosh (1999) isolated a new baculovirus from diamondback moth and identified as a multiple nucleopolyhedrovirus (PxMNPV). Its identity as PxMNPV was confirmed by comparing with AcMNPV and AfMNPV by restriction endonuclease (REN) analysis, hybridization and neutralization tests.

In India, there are reports on the use of PxMNPV in laboratory and field against *P. xylostella* in Punjab (Varma and Gill, 1977) and in Karnataka (Padmavathamma and Veeresh, 1991). However the validity of these reports is not confirmed by characterization studies and comparison with the other viruses causing heterologous infection as a contaminant. Rabindra *et al.* (1998b) have reported a granulosis virus from two populations of *P. xylostella* from India for the first time. The pathogenicity of PxGV, its formulation, field efficacies and interactions with other modes of control were extensively studied (Sairabanu, 2000; Rajagopalbabu, 2001).

2.2. Cross-infectivity of baculoviruses

Among insects, there are many reports of virus cross-infectivity between species of the same genus, fewer between genera of the same family, less between families of the same order and rare between orders. However, examples of virus cross transmission across all taxa including orders are reported for every group of insect virus. The relative specificity of insect virus types, based upon attempted and successful *in vivo* transmission was first summarized by Ignoffo (1968). He then, categorised the successful cross-transmission of GV and NPV as 10.71 per cent and 32.09 per cent respectively.

Bolle in 1889 was the first investigator who claimed successful transmission of an insect virus (NPV of silkworm) to other insect species and even to different orders (Prowazek, 1907). Since 1950, however, many other instances of transmission across genus, family and even order have been reported. Earlier attempts included successful transmission of *Bombyx mori* (L.) NPV to other families of Lepidoptera (Bergold, 1943). Gershenson (1955; 1962) tested the susceptibility of 60 insect species (18 lepidopteran families and one family of Hymenoptera) to seven NPVs. Five and seven of the lepidopteran families were only susceptible to viruses from *B. mori* and from *Antheraea pernyi* (Guerin-Meneville) respectively. The host recipient and virus donor systems of successful cross transmission studies are listed below.

Reports of successful attempts to cross transmit NPV to alien insect species

S.No.	Virus Donor	Host Recipient	Reference
1.	*Barathra brassicae* (L.)	*Orthosia incerta* (Huf.)	Ponsen and DeJong (1964)
2.	*B. mori*	*Samia ricini* (Drury), *Samia cynthia* (Boisd.) *G. mellonella* *Dermestes lardius* (L.) *A. pernyi, Antharaea yamamai* (Guerin-Meneville)	Aizawa (1962) Bergold (1943) Gershenson (1962)
3.	*Choristoneura pinus* (Freeman) *Choristoneura fumiferana* (Clemens)	*C. fumiferana, C. pinus*	Stairs (1960)
4.	*Colias eurytheme* (Boisd.)	*Pieris rapae* (L.)	Steinhaus (1953)
5.	*Dictyoploca japonica* (Moore)	*S. cynthia*	Ishikawa (1960)
6.	*Heliothis zea* (Boddie) *Heliothis peltigera* (Denis & Schif.) *Heliothis virescens* (Fab.)	*H. virescens, H. zea,* *Heliothis phloxiphage* (Grote & Robin.), *Helicoverpa armigera* (Hubner)	Ignoffo (1965)
7.	*Lymantria monocha* (L.)	*Dendrolimus pini* (L.)	Smith and Xeros (1952)
8.	*Malocosoma pluviale* (Dyar)	*Malocosoma disstria* (Hub.), *Malocosoma americanum* (Fab.)	Stairs (1964) Kulman and Brooks (1965)
9.	*Panaxia dominula* (L.)	*Sphinx ligustri* (L.)	Smith and Xeros (1953)
10.	*P. rapae*	*C. eurytheme*	Tanada (1954)
11.	*Porthetria dispar* (L.)	*B. mori*	Bergold (1943)
12.	*S. ricini, S. cynthia*	*B. mori*	Yamamasu *et al.* (1954)

13.	*Lymantria dispar* (L.)	*Dendrolimus spectabilis* (Butler), *B. mori*	Aratake and Kayamura (1972)
14.	*Ephestia cautella* (Wlk.)	*Plodia interpunctella* (Hub.)	Hunter *et al.* (1973)
15.	*B. mori*	*D. spectabilis, L. dispar, Malocosoma neustria testacea* (L.), *S. cynthia, Chilo suppressalis* (Wlk.), *G. mellonella*	Aratake and Kayamura (1973) Watanabe *et al.* (1975)
16.	*Thymelicus lineola* (Ochsenheiner)	*Hyphantria cunea* (Drury)	Smirnoff (1976)
17.	*Chrysodeixes chalcites* (Zenilla)	*Anadevidia peponis* (L.)	Rabindra and Subramanian (1976)
18.	*A. californica*	*Ennomus subsignacrius* (Hb.), *Eupsilia sp.* *M. americanum* *Anisota senatoria* (Smith) *H. cunea, Alsophila pometaria* (Harris) *G. mellonella, T. ni, Spodoptera exigua* (Hb.) *H. zea, Estigmene acrea* (Drury), *P. xylostella, H. virescens, Spodoptera frugiperda* (Smith) *Spodoptera littoralis* (Boisd.)	Kaya (1977) Witt and Janus (1977) Vail *et al.* (1971, 1973) Hink and Strauss (1976) Maleki - Milani and Milani - Maleki (1978)
19.	*Rachiplusia ou* (Guen.)	*Agrotis ipsilon* (Hufnagel)	Lewis and Adams (1979)
20.	*Anticarsia gemmatalis* (Hub.)	*H. zea, T. ni, Pseudoplusia includens* (Walker) *Spodoptera ornithogalli* (Guen.)	Carner *et al.* (1979)
21.	*C. fumiferana*	*T.ni, G. mellonella*	Stairs *et al.* (1981)
22.	*A. californica, T.ni*	*S. exigua*	Tompkins *et al.* (1981)
23.	*H. armigera*	*S. exigua, S. frugiperda*	Hamm (1982)
24.	*G. mellonella*	*T. ni,H. zea, Manduca sexta* (L.)	Fraser and Stairs (1982)
25.	*Orygia pseudotsugata* (McDonnough) *C. fumiferana* *L. dispar*	*E. acrea*	Shapiro *et al.* (1982)

26.	*H. armigera*	*S. littoralis*	Abou-Bakr *et al.* (1984)
27.	*Mamestra brassicae* (L.)	*Noctua pronuba* (L.)	Allaway and Payne (1984)
28.	*S. littoralis*	*H. armigera*	Abou-Bakr *et al.* (1984)
29.	*O. pseudotsugata*	*Orygia postica* (Walker)	Su (1986)
30.	*H. armigera* *Adisura atkinsoni* (Moore)	*A. atkinsoni, H. armigera*	Narayanan (1986)
31.	*Panolis flammea* (Dennis & Schif.)	*M. brassicae*	Kelley and Entwistle (1988)
32.	*G. mellonella*	*A. ipsilon, H. virescens, H. zea, P. includens, S. litura, S. ornithogalli, T.ni*	Kadir and Payne (1989)
33.	*S. littoralis*	*Locusta migratoria* (L.)	Bensimon *et al.* (1987)
34.	*A. californica*	*Boarmia selenaria* (Dennis & Schif.) *H. armigera, H. peltigera, E. cautella*	Scheepens and Wysoki (1989)
35.	*M. brassicae*	*P. xylostella*	Biache *et al.* (1989)
36.	*L. dispar*	*M. sexta*	Stairs *et al.* (1991)
37.	*A. californica*	*P. includens, R. ou A. gemmatalis*	Morales *et al.* (1993)
38.	*Porthetria sequax* (L.)	*S. frugiperda, A. gemmatalis*	Sant-Ara and Silva (1994)
39.	*Anadevidia peponis* (L.)	*Plusia eriosoma* (Doubleday) *Plusia orichalceai* (Fab.)	Rabindra *et al.* (1999)
40.	*G. mellonella*	*P. xylostella, Crocidolomia binotalis* (Zell), *H. virescens, M. brassicae*	Kadir *et al.* (1999b)
41.	*A. falcifera*	*H. zea, H. virescens, S. exigua, S. frugiperda, P. xylostella*	Grewal *et al.* (1998)
42.	*P. xylostella*	*H. virescens, T.ni, H. zea S. exigua, S. frugiperda*	Kariuki and McIntosh (1999)

However, the validity of such reports remains questionable as antipodal results are reported with same host recipient - virus donor systems. In most of the cases, the virus identity and purity, host age and sterility, inoculum form and size and route of administrations are uncertain. For instance, Biever and Andrews (1984) published the susceptibility of several lepidopterous larvae to a NPV of *P. xylostella.* Kadir and Payne (1989) disputed over this and proved that the above virus from *P. xylostella* was a contamination of the insect culture with a virus from *G. mellonella* by characterization of virus using REN analysis. This reinforced the importance of being aware of the confusion that is associated with the naming of viruses by the host from which they were isolated and also emphasized the need of characterization of virus after passage through heterologous hosts for unequivocal confirmation of cross-transmission of baculoviruses.

2.2.1. DNA characterization of NPV by restriction endonuclease analysis

REN analysis is a powerful diagnostic tool in the identification of baculoviruses. This is indispensable in cross-infectivity studies for identification of a particular viral strain after passage through alternate hosts. Initially, characterization of NPV de-oxy ribo nuclei acid (DNA) was done by methods like cesium chloride equilibrium centrifugation, thermal denaturation, natural and alkaline sucrose gradient centrifugation. These methods were used in the characterization of NPVs of *T. ni, S. frugiperda* (Smith) and *R. ou* (Guenee) (Summers and Anderson, 1973).

Baculoviruses contain circular duplex DNA genomes of molecular weights ranging from 50 to 100 x 10^6 daltons (Burges, 1977; Kelly, 1977; Summers, 1977). Analysis of baculovirus genomes with restriction endonuclease was proposed by Smith and Summers (1978). *Eco*R I restriction endonuclease fragment patterns of DNA from nine wild type insect baculoviruses were compared and genomic heterogeneity was detected. Miller and Dawes (1978) were successful in distinguishing two closely related NPVs of *A. californica* and *T. ni* by REN analysis.

Smith and Summers (1979) prepared the restriction maps of *G. mellonella* NPV with endonucleases like *Sma* I, *Kpn* I, *Bam*H I, *Sac* I, *Xho* I and *Eco*R I and concluded that GmNPV and *T.ni* NPV were variants of AcMNPV with similar genomes and protein structures based on the restriction maps.

In 1984, Biever and Andrews reported the susceptibility of several lepidopterous larvae to a PxMNPV. Later in 1989, Kadir and Payne questioned the identity of PxNPV and proved that PxMNPV used by the former authors was a contaminant of GmNPV of by REN analysis. Kadir *et al.* (1999b) determined the cross-transmission of NPVs of *G. mellonella* and *A. californica* to *P. xylostella, H. virescens, C. binotalis* and *M. brassicae* (L.). In this study, the DNA isolated from purified progeny viruses was compared by REN analysis with DNA from the inoculum viruses. The progeny virus and inoculum virus had similar DNA profiles confirming the cross-infectivity of viruses.

In another study, *H. armigera* larvae inoculated with GmNPV died showing typical virus symptoms. However, REN analysis with *Pst* I, *Hind* III, *Bam*H I and *Eco*R I revealed that the progeny virus was NPV of *H. armigera* indicating the expression of latent virus (Rabindra *et al.*, 1998a). Also, Rabindra *et al.* (1999) proved the cross-infectivity of NPV of *A. peponis* to other species of *Plusia* by REN analysis.

These studies emphasize the confirmation of cross-infectivity studies using REN analysis as a diagnostic tool. As the inoculation of alien viruses at times triggers the expression of latent virus, REN analysis of viral DNA of progeny viruses can be used as the tool for the identification and unequivocal confirmation of cross-transmission of baculoviruses.

2.2.2. GmNPV and its cross-infectivity to alternate hosts

G. mellonella NPV was studied by Stairs and Ellis (1971) using electron microscope and microfilters. The smallest infectious units in the cell cytoplasm and haemolymph of diseased larvae are rod shaped virions of 38-40 nm in diameter and 100-400 nm in length. Kozlov *et al.* (1975a,b) studied the polyhedral proteins of GmNPV and compared the properties with that of *B. mori* NPV.

Skuratovskaya *et al.* (1977) studied the supercoiled DNA of GmNPV. The rate of development of larvae and pupae of *G. mellonella* and its NPV were determined over a range of temperature from 12 to 40°C. It was reported that at 40°C, both larval development and virus disease ceased (Stairs and Milligan, 1980).

The relative infectivity of non-occluded virions, polyhedra and virions released from polyhedra of GmNPV was evaluated (Stairs, 1980). Non-occluded virions and virions occluded within polyhedra were equally effective against *G. mellonella.*

Witt and Janus (1977) were successful in replicating GmNPV in cultured cells and in larvae of *T. ni*. Chemically dissociated polyhedra of GmNPV were first reported to be infectious for TN-368 cells and *T. ni* larvae. Fraser and Stairs (1982) reported that the neonates of *H. zea, T. ni* and *M. sexta* were susceptible to GmNPV with variable mortality rates. This is believed to be the first report of a lethal virus infection in a sphingid, *M. sexta*. Stairs (1990) determined the effect of GmNPV on growth and survival of young *M. sexta* larvae. It was found that GmNPV was infectious @ 5 x 10^6 POB/cube and 2 x 10^6 POB/cube of diet to one day old and 3-day old larvae of *M. sexta* and these treated larvae weighed 40 per cent and 76 per cent less than control growth rate respectively.

NPV isolated from *P. xylostella,* reported to be cross-infective to *T. ni, P. includens, H. zea* and *A. ipsilon* (Biever and Andrews, 1984), was later identified as an isolate of GmNPV (Kadir and Payne, 1989). This proves that GmNPV has the capability to initiate heterologous infections in several lepidopteran pests. Recently, Kadir *et al.* (1999b) showed that GmNPV was cross-infective to several major lepidopterous pests of *Brassica* crops and the same was confirmed by characterization of viruses of heterologous infection by REN analysis (Kadir *et al.*, 1999a).

However, in India, there are no reports available on the use of GmNPV at laboratory or field scale except for the one (Dipankar, 2000) where GmNPV was used as a component for the management of *G. mellonella* infecting beehives in Karnataka. Also, Rabindra *et al.* (1998a) showed that larvae of *H. armigera* inoculated with GmNPV resulted in the expression of latent infection of *H. armigera* NPV (HaNPV) as revealed by the comparison of DNA profiles with that of HaNPV.

2.3. Effect of serial passage of NPV in homologous and heterologous hosts

Generally *in vivo* sequential passages of wild isolates of NPV can increase virulence and reduce the time to kill natural host as well as other species that normally have low susceptibility to a given baculovirus. Apparently, sequential passages select more active isolates present in heterogenous wild virus populations against the host or non-host species. Serial passage of NPVs through alternate host system has resulted in the selection of more virulent isolates (Veber, 1962; Smirnoff, 1963; Shapiro and Ignoffo, 1970). Vail *et al.* (1973) first attempted the study on quantitative effects of passage of *Ac*MNPV through the alternate hosts and proved that passage of AcMNPV through *T. ni, S. exigua* and *E. acrea* resulted in highest mortality of *T. ni* larvae and yield of polyhedra. Aratake and Kayamura (1973) showed that BmNPV retained its pathogenicity for *B. mori* after passage through *M. neustria testaceae* in *Samia cynthia pryeri* (Drury) larvae.

In general, NPVs adapt to the homologous or heterologous hosts upon sequential passages. However, attempts made to adapt AcMNPV to *S. littoralis* were unsuccessful even after seven passages because of latent virus infection of *S. littoralis* (Maleki-Milani and Milani - Maleki, 1978). Pavan *et al.* (1981) studied the effect of serial passage of *A. gemmatalis* NPV through an alternate host, *P. includens*. The virulence of AcMNPV remained unchanged after serial passage through homologous host while serial passage through the alternate host resulted in virus progeny having biological properties associated with wild AcMNPV and latent AcMNPV expressed during third and fifth passage in *P. includens*.

Stairs *et al.* (1981) advocated that serial passage of NPV in alternate hosts could be n useful method in obtaining more virulent baculoviruses for use against several pest species. In this study, an isolate of *C. fumiferana* NPV when passaged in *T. ni* and *G. mellonella* became increasingly virulent to homologous and heterologous hosts. In another study the infectivity of NPVs of *O. pseudotsugata, C. fumiferana* and *L. dispar* to larvae of *E. acrea* was enhanced after successive passage in the alternate hosts (Shapiro *et al.*, 1982).

Tompkins *et al.* (1988) compared the virulence of NPVs of *A. californica, T. ni, R. ou* and *H. armigera* by serially passaging numerous times in the larvae of *T. ni* and *S. exigua*. The most significant increase in virulence (12.5 fold) occurred in HaNPV after passages in *T. ni* and *S. exigua* against neonate *T. ni* larvae. Serial passage of NPV through alternate hosts not only changed the virulence of progeny virus but also affected the growth and survival of target hosts as indicated by Stairs (1990). GmNPV passaged once in *M. sexta* larvae had enhanced the activity on the growth and survival of young *M. sexta* larvae.

Kolodny-Hirsh and Van Beek (1997) proved the increase in virulence of AcMNPV following serial passage in *P. xylostella*. Serially passaged AcMNPV through *P. xylostella* larvae for 20 times increased the virulence by 15 fold to *P. xylostella.* However, the biological activity of AcMNPV passaged through *S. exigua, T. ni* and *H. virescens* remain unchanged. Hence it is important to select an alternate host which gives a variant of progeny virus upon passage with increased virulence.

Serial passage of NPV has not always resulted in the desired change of progeny viruses. Attempts to develop an isolate of AcMNPV by serial passage that would be effective against a lepidopteran complex (*A. gemmatalis, P. includens* and *R. ou*) in soybean were not successful. The virulence of AcMNPV passaged serially through each species increased dramatically after five passages with activities close to or above those of natural NPVs associated with each host, but variants obtained from each species were not effective against the other two species (Morales *et al.*, 1993).

Although baculovirus activity and host range may be improved through serial passages of wild type isolates either on host or non-host insects, this method has not been adequately explored for many viruses used as microbial insecticides (Moscardi, 1999).

2.4. Mass production of NPV

Viruses are mass-produced in larval hosts grown on artificial diets or natural host plants. Larvae are infected *per os* at suitable stage of development and reared either in-groups or individually depending on cannibalistic nature of its species. *In vivo* mass production technologies of narrow host range NPVs of *H. armigera* (Attothom *et al.*, 1990; Bell, 1991), *Spodoptera litura* (Fab.) (Huang-Yaxin and Huang, 1995), *S. exigua* (Battu, 1987; Smith and Vlak, 1988; Choi-Jac-Young *et al.* 1996), *Spodoptera exempta* (Walker) (Cherry *et al.*, 1997), *Euproctis chrysorrhoea* (L.) (Kelley *et al.*, 1989), *Diacrisia obliqua* (L.) (Battu and Ramakrishnan, 1987) have been standardized.

In the case of NPVs with broad host range, it becomes highly important to select an *in vivo* system either homologous or heterologous for mass production based on the ease of rearing of larvae and virulence and yield of progeny virus. Shapiro *et al.* (1982) compared the virulence of several NPVs against *E. acrea* and showed that *E. acrea* was ideal host for mass production of *O. pseudotsugata* NPV on the basis of both virus yield and activity.

A NPV of *P. flammea* was mass produced *in vivo* in an alternative host, *M. brassicae* and in the same host. It was found that homologous NPV/host system was more efficient production system than the heterologous one (Kelley and Entwistle, 1988). Another study by Grzywacz *et al.* (1998) indicated that homologous host was most suitable for *in vivo* production of *S. littoralis* NPV. Infectiousness of HaNPV in homologous and heterologous host, *S. exigua* was established by multiplication bioassays. Larvae of *S. exigua* exhibited less susceptibility to HaNPV than *H. armigera* based on the yield of POB (Maraccaja *et al.*, 1998).

Also, the effect of diet, temperature and method of inoculation on *in vivo* mass production of NPVs has been studied elaborately. The optimum temperature was 24-27°C when fourth instar larvae of *S. litura* were infected and the yield of *S. litura* NPV (SlNPV) was higher from larvae reared on an artificial diet than from a natural diet (Huang-Yaxin and Huang, 1995). The leaf dip method resulted in the highest mortality of

S. littoralis to its NPV and net yield/larva. Natural diet (castor leaves) gave the best result when compared with semi-synthetic diet (El-Saadany *et al.*, 1992).

Currently, viruses used for pest control are produced and harvested from diseased insects. However, mass production of viruses *in vitro* using insect cell lines has been reviewed extensively (Ignoffo, 1966; Rivers, 1976). Recent improvements in cell yields and reproducibility of cell growth in large volume cultures indicate the feasibility of NPV production in cell culture (Weiss *et al.*, 1992). These cell lines and techniques are often proclaimed as the future source for mass-produced viral insecticides. The production technology of viruses in cell culture is highlighted by the development of bioreactor by Tramper *et al.* (1990) which support the continuous cell culture of *S. frugiperda* and viral infection process of AcMNPV. *In vitro* studies of NPV are reviewed in detail later in this chapter.

2.5. Histopathology studies of NPV in homologous and heterologous hosts

Histopathology studies determine the extent of infection of viruses in the various tissues of the host. These provide cytological evidence for the replication of baculoviruses in insect hosts. The mechanisms and pathway of infection of baculoviruses can be documented by studying the histopathology and ultrathin structures of host tissues.

The histopathology of NPV infection in homologous hosts has been studied in detail in insects like *H. armigera* (Jacob and Subramanian, 1972; Rabindra and Subramanian, 1974) *S. exempta* (Odindo, 1977), *Heliothis zea* (Granados, 1978), *Mythimna separata* (Walker) (Shreesam *et al.*, 1983), *S. litura* (Im *et al.*, 1988), *S. frugiperda* (Hamm and Styler, 1985), *S. exigua* (Tuan-Shujen *et al.*, 1999), *A. gemmatalis* (Matos *et al.*, 1999) and *Choristoneura rosaceana* (Clem.) (Pronier *et al.* 2000).

The first tissues affected by replicating virus in treated larvae were the fat bodies, hypodermis, hypodermal glands and tracheal matrix. The wing-buds, imaginal discs, ovary epithelial cells, testes, silk glands, malpighian tubules and sarcolemma were infected heavily in due course and finally the infection occurred in the neurolemma, frontal ganglion, thoracic ganglion and cerebral complex. The epithelial layers of the fore-gut and hind-gut were rarely infected (Shreesam *et al*., 1983).

For heterologous infections, histopathology studies are used for comparing the rate of infection of tissues of original and alternate hosts. The histopathology of AcMNPV was studied by examining 13 tissues in the original and six alternate hosts including *P. xylostella*. In all the hosts, the hypodermal glands, tracheal matrix and fat body cells were infected (Vail and Jay, 1973). Hunter *et al.* (1973) described the viral replication of *Cadra cautella* (Wlk.) NPV in hypodermis, tracheal matrix, fat bodies, muscles and midgut columnar epithelial cells of *P. interpunctella*. It was observed that the infection in the alternate host developed more slowly and the tissue tropisms were less apparent. Studies on histopathology of *O. postica* infected with NPV of *O. pseudotsugata* revealed the break down of nuclear materials in fat body cells a day after inoculation and increase in size of infected cells three days after inoculation (Su, 1986). Stairs *et al.* (1991) used the ultra structures and electron microscopy to study the infection of *P. dispar* (L.) NPV on *M. sexta* larvae. Electron micrographs showed both cytoplasmic and nuclear polyhedrosis virions in fat bodies, tracheal epithelium and epidermis of diseased larvae. However, more of the infected cells contained very large numbers of virions or polyhedra in this heterologous infection.

2.6. Vertical transmission of NPV

There are two major modes of transmission of NPV *viz.*, horizontally in the environment and vertically from an adult to its own offspring, while horizontal transmission is fairly well understood there are studies on vertical transmission of few NPV. Vertical transmission of NPV takes place through the virus that may be contained within the eggs (transovarial) or on the exterior of the egg (transovum) (Martignoni and Milstead, 1962).

Hamm and Young (1974) proved that NPV of *H. zea* was transmitted by surface contamination of the eggs with polyhedral occlusion bodies (PIB) that had passed through the digestive tract of adult *H. zea* fed with PIB. The transovarial transmission of NPV of *B. mori* was confirmed by the location of antigen of the virus in the amniotic fluid and embryo (Larinov and Bakhvalor, 1974). Nordin (1976) found that adults of *H. cunea* contaminated with dust containing the virus effectively infected their progeny by transovum transmission. Survivors of virus treatments in the sixth instar stage of *P. includens* resulted in the virosis of progeny larvae (Young and Yearian, 1982). El-Nagar *et al.* (1982) demonstrated that the surface contamination of egg-masses of cotton pest, *S. littoralis* with NPV resulted in transmission of the disease to the progeny larvae. Surface sterilization of the eggs prevented the transmission .

In a review, Fine (1984) concluded that although vertical transmission had been frequently reported for insect pathogens, its quantitative importance in relation to other transmission pathway was not well known. Transmission of NPV of *Spodoptera mauritiana* (Biosd.) through egg was studied by Nair and Jacob (1985). Both virus fed moths and virus treated moths could transmit the virus to their progeny and the route of transmission being either transovum or transovarial.

Survivors of virus treated population resulted in 4.7 - 11.5 per cent virus - induced mortality in progeny of *L. dispar* and a high positive correlation was observed between mortality in progeny and NPV-induced mortality in the parental generation (Shapiro and Robertson, 1987).

Olofsson (1989) advocated a new route of transmission of NPV of *Neodiprion sertifer* (Geoffrey) through the meconia. Virus was transmitted more often by newly emerged females than by females having voided their meconia. Transovum or transovarial transmission of NPV in other insects like *S. litura* (Santharam and Jayaraj, 1989), *L. dispar* (Murray and Elkinton, 1989; Ilyinykh *et al.*, 1997), *M. separata* (Rahman *et al.*, 1997) were also observed.

2.7. *In vitro* studies with NPV

Use of cultured cells for the study of insect viruses has become increasingly important. Over sixty insect cell lines have been established from major lepidopteran pests of agriculture and forestry (Hink and Bezanson, 1985). The usage of lepidopteran cell lines has increased during the past 10 years as a result of development of baculovirus expression vectors (BEV) that are directly applicable to biomedical and agricultural research. Insect cell lines offer an attractive alternative for *in vivo* production of NPV in susceptible hosts and many studies has shown the replication of NPV in established insect cell lines (Ignoffo *et al.*, 1971; Vail *et al.*, 1973) and feasibility of large-scale cell culture production (Weiss and Vaughn, 1986; Maiorella *et al.*, 1988 and Agathos, 1994).

2.7.1. GmNPV

Witt and Janus (1977) determined the capability of an established *T. ni* cell line (Tn-368) to support the replication of GmNPV. The successful large-scale production of GmNPV in *S. frugiperda*, IPL-ZIAE cells grown in roller bottles was described by Dougherty *et al.* (1982).

Several plaque assay techniques employing TN-368 or PLB-SF-21 AE cells were evaluated for their usefulness in detecting and distinguishing many polyhedra (MP) and few polyhedra (FP) plaque variants of GmNPV (Fraser and Hink, 1982a). Moreover, MP and FP mutants were isolated and characterized. It was found that FP variant is less virulent, devoid of nucleocapsid and is a staple genotypic variant which arises from spontaneous mutation of the MP variants (Fraser and Hink, 1982b).

2.7.2. Other viruses

Several cell lines have been tested for their permissiveness towards specific viral strains

Cell line sources	Organ / explants	Virus source	Reference
M. disstria	Haemocytes	*Lambdina fiscellaria sommaria* (Hulst.)	Sohi and Cunningham (1972)
M. brassicae	Fat bodies	*A. californica*	Mitsuhashi (1981)

T. ni, S. frugiperda	Imaginal disc	*A. californica*	Lynn *et al.* (1983)
S. littoralis	-	*A. gemmatalis*	Lynn and Oberlander (1983)
S. frugiperda		*Xestia-c-nigrum* (L.)	Tsuda *et al.* (1984)
S. exigua	Epithelial cells	*S. exigua*	Gelernter and Federici (1986)
S. frugiperda	-	*A. californica,* *S. littoralis*	Kislev, 1986
P. dispar	-	*A. californica,*	McClintock *et al.* (1986)
H. zea	-	*H. zea*	Gettig *et al.* (1987)
B. mori	Embryo	*B. mori*	Inoue and Mitsuhashi (1988)
A. gemmatalis	Embryo	*A. gemmatalis,* *A. californica*	Seibuith and Maruniak (1988)
T.ni, S. frugiperda, *M. separata,* *H. armigera*	-	*H. armigera*	Qui *et al.* (1988)
T.ni, S. frugiperda, *H. virescens,* *P. xylostella,* *A. gemmatalis*	-	*A. california*	McIntosh and Ignoffo (1989)
H. subflexa	-	*A. falcifera*	McIntosh (1991)
T. ni	-	*T. ni*	Granados *et al.* (1994)
H. zea	-	*H. zea, H. armigera*	Chakraborty *et al.* (1995)
S. litura	Pupal ovary	*S. litura*	Shih *et al.* (1997)
T. ni	Embryo	*A. californica, T. ni*	McKenna *et al.* (1988)
H. virescens, T. ni	-	*P. xylostella*	Kariuki *et al.* (2000)

The virus produced *in vitro* must be as effective as the virus obtained from *in vivo* propagation if it is to be used successfully as a control agent. Ignoffo *et al.* (1974) demonstrated that AcMNPV and TnNPV produced in cell culture were as effective as that produced in larvae by laboratory bioassays and field trials. Summers and Vokman (1976) advocated the use of lepidopteran cell lines for the study of the kinetics and biochemistry of baculovirus replication. NPVs of *R. ou* and *A. californica* were studied to clarify the nature of nonoccluded infectious virus by making biophysical and morphological comparisons.

Though many cell lines are permissive for a particular viral strain, it is important to compare the efficiency of viral replications in cell lines from different insect species.

Comparative studies were performed on the replication of AcMNPV in cell lines from *E. acrea, L. dispar, M. brassicae, S. frugiperda* and *T. ni* . *M. brassicae* and *T. ni* produced more polyhedra than the other lines (Lynn and Hink, 1980). Danyluk and Maruniak (1987) determined *in vitro* host range of AcMNPV and *S. frugiperda* NPV in five different insect cell lines of *P. interpunctella, S. frugiperda, T. ni* and *A. californica*. AcMNPV replicated in all the cells while SlNPV did not replicate in the lines of *T. ni*.

The effect of serial passage of NPVs of *A. californica, T. ni, R. ou, H. armigera* were determined in cell lines of *S. frugiperda* and *T. ni* (Tompkins *et al.*, 1988). McIntosh and Ignoffo (1989) studied the population doubling time, virus yield and susceptibility of five lepidopteran cell lines to AcMNPV. *P. xylostella* cell line produced the greatest number of POB while POB from *T. ni* and *H. virescens* lines had the highest and lowest virulence respectively.

P. xylostella NPV replicated in six lepidopteran cell lines with *H. virescens* and *T. ni* cells produced the highest viral titers and greatest number of occlusion bodies (Kariuki *et al.*, 2000). Hence, selection of a cell line that will produce a high concentration of POBs equivalent in activity to that produced in larvae is an important aspect of *in vitro* produced viral insecticides.

2.8. Effect of weather factors on the persistence of NPV

2.8.1. Sunlight

Inactivation of NPV by ultraviolet (UV) radiation of sunlight has been reviewed extensively. Studies by Young and Yearian (1974) demonstrated that HaNPV on the upper leaf surface of cotton, soybean and tomato was rapidly inactivated in the field. Yearian and Young (1974) reported that viral deposits in shaded areas on cotton plants were protected from UV radiation, resulting in greater persistence of the virus. Effects of sunlight and artificial UV radiation on the infectivity of NPV of *H. cunea* were determined in the laboratory (Nordin, 1976). Exposure of suspensions of the virus on microscopic slides to direct sunlight for about 195 min resulted in 50 per cent reduction of the infectivity.

Narayanan *et al.* (1977) reported that SlNPV could not tolerate the outdoor exposure for more than eight days without severe loss in activity. AcMNPV with apparently increased resistance to inactivation by near UV radiation was isolated by Witt and Hink (1979). El-Nagar *et al.* (1980) studied the effect of direct sunlight on the virulence of NPV of *S. littoralis* by comparative tests in sunny and shady conditions. The virus sprayed on to cotton plants in the field lost much of its virulence against *S. littoralis,* the day after application due to the effect of sunlight.

The effect of sunlight on the infectivity of NPV of *M. separata* (Manjunath and Mathad, 1981; Parameshwar - Hugar *et al.*, 1996); *O. pseudotsugata* (Griego *et al.*, 1985), *T. ni* (Biever and Hostetter, 1985) and *S. litura* (Kulkarni *et al.*, 1999) has been documented.

Witt and Stairs (1975) studied the effects of UV radiation on GmNPV. The virus was inactivated by exposure to an UV radiation (253.7 A^{o}) and to near UV radiation (3000-3800 A^{o}). The virus inactivation was found to depend on virus concentration, whereas the mode of action for each type of radiation was found to be constant.

2.8.2. Temperature and relative humidity

Nuclear polyhedrosis viruses are inactivated rapidly at temperatures above 50°C, but only slowly at lower temperatures. There is some variability, however, between viruses from different hosts and even between unicapsid and multicapsid strains (Martignoni and Iwai, 1977). The nonoccluded virions from *G. mellonella* are inactivated rapidly at temperatures as low as 40°C. However, occluded virions were found to be 3.5 times more thermostable than nonoccluded virions. The inactivation threshold was 42°C and 359-degree minutes caused 50 per cent inactivation of GmNPV (Stairs and Milligan, 1980).

The rates of development of larvae and pupae of *G. mellonella* and its NPV were determined over a range of temperature of 18-40°C. Both the extremes of temperature ceased the larval development and virus disease. The degree of disease development depended on the time of exposure to the high temperature (Stairs, 1978).

The influence of temperature and relative humidity on the interaction of NPV of *S. littoralis* was studied by Moawad (1986). High temperatures decreased the incubation period but did not increase overall mortality due to the virus. However, high relative humidity resulted in a high percentage of mortality among larvae at all the temperatures tested.

These studies emphasize the effect of weather factors on the persistence of NPV. For any pathogen, it is desired to persist for a prolonged period under field conditions to bring out significant effect on the target pest and serve as an effective microbial insecticide.

2.9. Field evaluation of NPV

There are no reports on the use of NPV against *P. xylostella* under field conditions. Though GmNPV has been found to be cross-infective to different economically important crop pests, it has not been field tested against alternate hosts so far. Hence the field use of other baculoviruses in other crop ecosystems is reviewed.

In field studies in Taiwan in 1987-88, *Artogeia rapae* (L.) GV. *P. xylostella* GV and SlNPV gave effective control of the respective pest (Su, 1989). Geissler (1995) reported the successful biological control of *M. brassicae* in cabbage with the use of its NPV under field condition. The mortality rates ranged between 68.9-100 per cent. Field trails conducted in United Kingdom to assess the efficacy of a wild type AcMNPV and a genetically modified NPV from same host containing a gene for an insect-selective scorpion toxin revealed that *T. ni* and *M. brassicae* were effectively controlled. The susceptibility of these two insects did not differ between conventional and genetically modified (GM) viruses but the rate of hill of *T. ni* was faster with the GM virus (Hernandez-Crespo, *et al.* 1999).

However in India, NPVs of *H. armigera* and *S. litura* are chiefly used against the respective pests. Field efficacy of HaNPV against *H. armigera* was evaluated in different crop ecosystems like chickpea (Santharam and Balasubramanian, 1982; Rabindra and Jayaraj, 1988), cotton (Dhandapani, 1990; Rabindra and Jayaraj, 1995), pigeonpea (Vijayakumar, 1980; Muthiah and Rabindra, 1991), sunflower (Rabindra *et al.*, 1990), pulses (Srinivas, 1986; Jayaraj *et al.*, 1987), tomato (Narayanan and Gopalakrishnan, 1990) and groundnut (Dhandapani *et al.*, 1993). In the late 1990s, HaNPV was reported to be effective in controlling *H. armigera* in conjunction with other chemicals (Geetha, 1997; Sivaprakasam, 1998; Sajjanar *et al.*, 1999; Satpathy *et al.*, 2000; Loganathan *et al.*, 2000; Sathiah, 2000).

Field efficacy of SlNPV has been proved in many crops like banana (Santharam *et al.*, 1978), tobacco (Ramakrishnan *et al.*, 1981), groundnut (Muthuswami *et al.*, 1993), castor (Mahadevan, 1978), crucifers (Pawar *et al.*, 1991), cotton (Santharam, 1985). A dose of 1.5 x 10^{12} POB/ha of HaNPV and SlNPV was found to be effective against the respective target pests (Geetha, 1997; Subramanian, 1998).

Though there are several reports on the efficacies of NPVs of *P. xylostella, G. mellonella, A. californica* and *A. falcifera* based on laboratory bioassays, none has been used against this pest under field conditions so far. However, the GV of *P. xylostella* has been found to be effective under field conditions in Taiwan (Su, 1986, 1987, 1988). Sairabanu (2000) reported that PxGV @ 3 x 10^{13} OB/ha effectively checked the population of *P. xylostella* under field conditions. The effect of PxGV in combination with insecticides like indoxacarb, abamectin and cartap hydrochloride, *B. thuringiensis* and neem products against *P. xylostella* was documented (Rajagopalbabu, 2001).

2.10. Safety tests on non-target organisms

Accrued data from specific safety testing gives increasing confidence to use NPV in pest management. In the late 1960's several NPVs underwent a series of tests by the Environmental Protection Agency (E.P.A.) including the broad host range NPV of *A. californica* and proved harmless in extensive tests, including long term tests. The

NPVs proved harmless and unable to replicate in microorganisms, non-insect invertebrate cell lines, vertebrate cell lines, vertebrates, plants and non-arthropod invertebrates (Burges *et al.*, 1980).

Several NPVs have been tested against beneficial organisms and natural enemies of insect pests. Inclusion bodies from *M. brassicae* NPV and *Cydia pomonella* (L.) GV were found to be safe for honey bees, *Apis mellifera* L. (Groner *et al.*, 1978). Dhaduti and Mathad (1981) studied the effect of NPV of *M. separata* on eri silkworm, *Philosamia ricini* (Huff.) and indicated that the virus was safe to the silkworm.

A broad host range NPV of *A. gemmatalis* was shown to be non-pathogenic to *A. mellifera* (Alves *et al.*, 1990). Jayanthi and Padmavathamma (1996) proved that SlNPV was safe to predators (*Coccinella septempunctata* (L.), *Menochilus sexmaculatus* (F.), *Chrysoperla carnea* (Stephens) and a few parasitoids. The adverse effect of NPVs of *H. armigera* and *S. litura* on the growth, development and survival of *B. mori* was recorded by Ramarethinam (1998). A reduction in the net weight gain and growth was observed in *B. mori* fed with HaNPV and SlNPV and virus treatments induced microsporidian infection. Safety of other baculoviruses to non-target organisms has been recorded by many workers (Arif and Dobos, 1978; Doller and Groner, 1981; Young and Hamm, 1985; Battu, 1987, Geetha, 1997).

Materials and Methods

Chapter III

MATERIALS AND METHODS

3.1. Mass culturing of host insects

Different insect species which were reared, field collected or obtained from various sources are listed below:

Crucifer pest complex
Diamondback moth - *Plutella xylostella* L.
Leaf webber - *Crocidolomia binotalis* Zell.
Borer - *Hellula undalis* F.
Leaf caterpillar - *Spodoptera litura* F.
Spodoptera exigua Hb.

Sugarcane borer complex
Early shoot borer - *Chilo infuscatellus* Snell.
Internode borer - *Chilo sacchariphagus indicus* Kapur.

Pulse pod borer complex
Spotted pod borer - *Maruca vitrata* Geyer
Pod borer - *Helicoverpa armigera* Hubner.
Plume moth - *Exelastis atomosa* Walsingham

Rice leaf folder complex
Leaf folders - *Cnaphalocrocis medinalis* Guen.
Marasmia patnalis Bradley

Millet pests
Stem borer - *Chilo partellus* Swinhoe
Leaf feeder - *Mythimna separata* Walk.

Cucurbit pest complex
Pumpkin caterpillar - *Diaphania indica* Saund.
Snake gourd semilooper - *Anadevidia peponis F.*

Stored product pests
Rice moth - *Corcyra cephalonica* Stainton.
Greater wax moth - *Galleria mellonella* L.

Potato pest
Black cutworm, *Agrotis ipsilon* Hufnagel

Mulberry silkworm
Silkworm - *Bombyx mori* L.

Castor leaf feeder complex
Woolly bear - *Pericallia ricini* F.
Semilooper - *Achaea janata* L.
Butterfly - *Ergolis merione* Cram.

Oilseed pests
Black headed caterpillar - *Opisina arenosella* Walker
Red hairy caterpillar - *Amsacta albistriga* Walker

Jasmine pest
Leaf folder - *Nausinoe geometralis* Kuen

Cotton / Okra borer
Spotted bollworm - *Earias vittella* Fb.

Teak pest complex
Defoliator - *Hyblaea puera* Cr.
Skeletonizer - *Eutectona macheralis* W.

Moringa pest
Hairy caterpillar - *Eupterote mollifera* W.

Mulberry pest
Leaf webber - *Diaphania pulverulentalis* Hampson

Curry leaf pest
Leaf roller - *Psorosticha zizyphi* Staint.

3.1.1. *P. xylostella*

The Bangalore strain of *P. xylostella* larvae maintained in the Department of Entomology was reared on cauliflower leaves. The larval stages were maintained in cauliflower leaf bouquets in wire mesh fitted larval rearing cages of 45 x 45 x 45 cm size for easy handling. The larvae were maintained @ 200-300/leaf upto early third instar stage and later reduced to 25-50/leaf. The pupae were collected and transferred to oviposition cages (30 x 30 x 30 cm) periodically.

The adults upon emergence were provided with 10 per cent sucrose solution fortified with vitamins (ABDEC) for feeding and mustard seedlings for ovipositing. Mustard seeds, treated with Carbendazim (Bavistin) 50% WP (2g/kg) for atleast 24 h, were soaked overnight in water and sown in cups (5.5 cm height, 10 cm dia) filled with sterilized soil. Two to three days after germination, the seedlings were used for oviposition. The second instar larvae upon emergence from the leaf mines were transferred to cauliflower leaves for rearing of late instars.

3.1.2. *G. mellonella*

The larvae of *G. mellonella* were reared on semi-synthetic diet described by Singh (1994) (Appendix I). The pupal cases formed in the galleries of the diet were collected and kept separately for adult emergence. The adults, upon emergence, were released @ 5 pairs/plastic jar of size 10 cm dia and 19 cm height. The adults were provided with 10 per cent sucrose solution containing vitamins and beeswax - dipped cards (2.5 x 7.5 cm) for oviposition. The egg cards were transferred to freshly prepared diet.

3.1.3. *C. binotalis* and *H. undalis*

The larval stages of *C. binotalis* and *H. undalis* were reared on cauliflower leaves. The leaf bouquets were used for oviposition of the adults.

3.1.4. *C. partellus*

The larvae of *C. partellus* were reared on sorghum leaf powder - based semi-synthetic diet (Sharma *et al.*, 1992) (Appendix I). The larvae were reared on plastic boxes

(27 x 17 x 5 cm) containing the artificial diet upto third instar stage. Then the larvae were transferred to glass vials with diet individually to facilitate boring activity. The adults were released in plastic containers (24 cm dia, 18 cm height) lined with butter paper. The egg masses laid on the paper were surface - sterilized with 10% formaldehyde solution and transferred to fresh diet boxes.

3.1.5. *P. ricini*

Castor leaves were used for rearing of larvae of *P. ricini.* The larvae which pupated on the edges of the cages were collected and kept separately for adult emergence. Adults were released in oviposition cages (45 x 45 x 45 cm) @ 10 pairs/cage. Fresh castor leaves were provided for oviposition.

3.1.6. *M. vitrata*

The larval stages of *M. vitrata* were reared on semi-synthetic diet as described in Appendix I. Adults were released in plastic buckets (20.5 cm dia. and 24.5 cm height) covered with muslin cloth. Eggs laid on cloth were surface sterilized with 10% formaldehyde solution and the neonates, upon hatching, were released in freshly prepared semi-synthetic diet.

3.1.7. *O. arenosella*

Coconut fronds were used for rearing the coconut black headed caterpillars. Pupae formed in the galleries of the leaves were collected and kept in oviposition chamber (45 x 45 x 45 cm size). Coconut fronds with intact terminal ends were provided for oviposition. The larval instars were reared on fresh fronds (15 x 3 cm) placed inside plastic jars (10 cm dia. and 19 cm height) lined with a pad of moist cotton at the bottom.

3.1.8. Other insects

The following insects were reared on the respective natural host and the F_1/F_2 progenies were used for the experiment.

Ergolis merione	- Castor
Diaphania pulverulentalis	- Mulberry
Nausinoe geometralis	- Jasmine
Achaea janata	- Castor
Agrotis ipsilon	- Artificial diet (Appendix I)
Anadevidia peponis	- Bottle gourd
Diaphania indica	- Pumpkin

Laboratory cultures of the following insects were used for cross-infectivity studies.

Insect sp.	Source
H. armigera, S. litura *C. cephalonica*	Biocontrol laboratory, Department of Agricultural Entomology, TNAU, Coimbatore
C. medinalis, M. patnalis	Paddy Breeding Station, TNAU, Coimbatore
B. mori	Department of Sericulture, TNAU, Coimbatore.
E. vittella	Monsanto India Pvt. Ltd. Bangalore
C. infuscatellus, *C. sacchariphagus indicus*	Sugarcane Breeding Institute, Coimbatore
H. puera, E. macheralis	Forest Research Institute, Nilambur, Kerala
A. albistriga	Project Directorate of Biological Control, Bangalore

Besides, the larval stages of insects like *M. separata, E. atomosa, E. mollifera* and *P. zizyphi* collected from the fields were also used for the experiments.

3.2. Mass production of GmNPV and semipurification

GmNPV (Plate 1) maintained in the Department of Agricultural Entomology, TNAU, Coimbatore was designated as Coimbatore (CBE) isolate. Another isolate obtained from *G. mellonella* larvae from Bangalore was designated as Bangalore (BNGL)

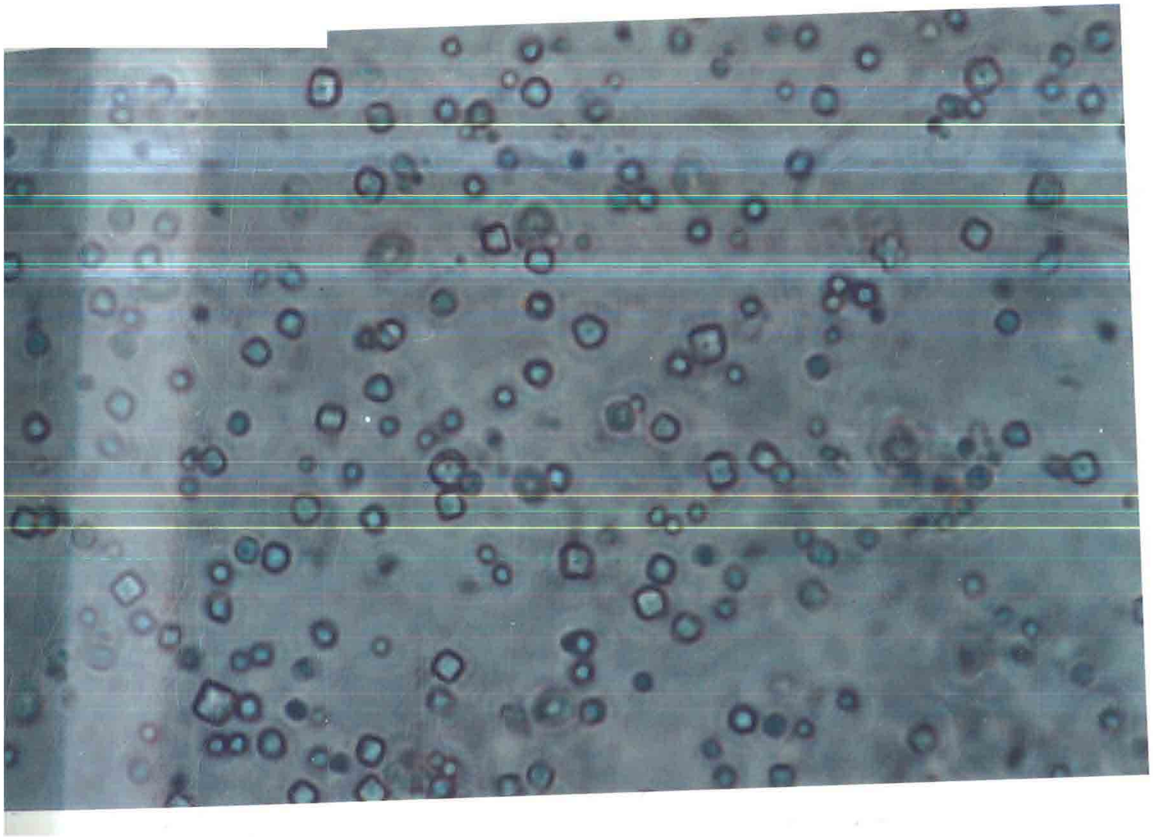


Plate 1. Polyhedral Occlusion Bodies (POB) of GmNPV (100X)

isolate. The original stocks of these isolates had a load of 2.54 x 10^8 POB/ml and 3.44 x 10^7 POB/ml respectively. Both the isolates were mass-produced *in vivo* using *G. mellonella* third instar larvae under laboratory conditions (28 ± 2°C) by the following procedure.

An aliquot of 100 µl of viral suspension (2 x 10^7 POB/ml) of each isolate was blended with 1.0 g of semi-synthetic diet using a sterile glass rod and allowed to shade-dry. The diet was pelleted (0.2 g) and placed inside a polypot (4 cm dia.) @ 5 pellets/pot. Pre-starved (4 h) third instar larvae of *G. mellonella* were released @ 5/polypot and allowed to feed on the contaminated pellets for 24 h. The larvae were transferred to plastic jars (10 cm dia, and 19 cm height) @ 50/jar containing the semi-synthetic diet to a thickness of 250 mm at the bottom. Mortality of larvae was observed daily for 15 days post treatment. The cadavers collected were stored in sterile glass vials under frozen conditions.

The cadavers were homogenized in a sterile glass pestle and mortar and the concentrate was diluted with distilled water at the ratio of 1:10 (w/v). The homogenate was filtered through a prewashed, sterile double layered muslin cloth into a 250 ml Erlenmeyer flask. The residue settled on the cloth was washed repeatedly with distilled water and finally squeezed gently for maximum extraction of the polyhedra. The extract was semipurified by differential centrifugation using a Unipan high speed centrifuge type 310. Host debris was removed as pellet by centrifuging at 500 rpm for 1 min. Then the supernatant was spun @ 10,000 rpm for 5 min. The pelleted virus was washed thrice by repeated centrifugation with distilled water and suspended in sterile distilled water. The viral suspension was stored at -20°C.

3.2.1. Enumeration of POB/larva

The diluted viral suspensions obtained from cadavers of different insect species were used for the enumeration. The cadavers were macerated in a sterile glass pestle and mortar and diluted 10 folds with sterile distilled water. An aliquot of 10 µl of the suspension was added on to the chamber of the improved Neubauer haemocytometer

(Weber, England) with a depth of 0.1 mm. Two minutes were allowed for settling of POBs before counting under a phase contrast microscope. Ten samples were used per insect species and four counts were made to arrive at the average POB/larva. The suspensions were stored in refrigerator at 4°C till use.

3.3. Cross-infectivity of GmNPV to different lepidopteran insect pests

Leaf disc bioassay using GmNPV (CBE isolate) was carried out with respective natural host plants for insects like *P. xylostella, C. medinalis, H. undalis, S. exigua* (cauliflower), *P. ricini, E. merione, A. janata* (castor), *D. indica, A. peponis* (bottle gourd), *N. geometralis* (jasmine), *O. arenosella* (coconut), *D. pulverulentalis,* (mulberry), *A. albistriga* (castor) *C. medinalis, M. patnalis* (rice), *E. mollifera* (moringa), *P. zizyphi* (curryleaf) and *E. macheralis* (teak). In case of moringa, jasmine and curry leaf, leaflets were treated with virus, while leaf bits were used for rice and coconut with the dimensions of 4 x 0.7 cm and 2.5 x 1.5 cm respectively.

Leaf discs of respective natural host of insect species were cut to a size of 33 mm diameter. GmNPV suspension (2x 10^7 POB/ml) containing 0.01 per cent Tween 80 was applied at the rate of 10 μl at the centre of the leaf disc and spread uniformly over the entire surface of the disc using the rounded and polished end of a sterile glass rod. After the suspension had dried off, the discs were turned and the lower surfaces treated similarly. Control discs were treated with distilled water containing 0.01% Tween 80 only. The leaf disc was placed in cheese cup (8 cm dia. and 5 cm depth) lined with moist filter paper discs. The larvae released on the discs were allowed to feed for 24 h. The larvae were then transferred to fresh untreated leaf bouquets contained in plastic jars. The leaf bouquets were changed periodically during the period of observation. The cheese cups and plastic jars with larvae were maintained at 25 ± 1°C in an incubator. Mortality of larvae was recorded from 4-10 days after treatment.

A diet surface contamination bioassay method was followed for insects like *M. vitrata, H. armigera, S. litura, E. atomosa, M. separata, H. puera* and *A. ipsilon* with respective semi-synthetic diet (Appendix I). The semi-synthetic diet lacking formaldehyde was dispensed to a thickness of 10 mm in 5 ml glass vials. GmNPV suspension containing 5 x 10^7 POB/ml was applied @ 10 µl/vial on to the diet. The viral suspension was distributed uniformly over the diet surface using the blunt end of polished glass rod (6 mm). Larvae were released singly into the glass vials after inoculation and plugged with cotton and incubated at 25°C in an incubator (Biotron). When the larvae consumed the diet completely, fresh untreated diet was provided till the period of observation. For neonates bioassay diet filled to a thickness of 5 mm in polypots were used with ten neonates per polypot.

Direct *per os* treatment by the pin head method was done for *C. infuscatellus, C. sacchariphagus indicus, C. partellus, B. mori, E. vittella* and *C. cephalonica.* The head of an ordinary pin (1") was dipped in GmNPV viral suspension (2 x 10^8 POB/ml) and larvae were allowed to engulf the suspension collected on the pin head (ca.1 µl). Care was taken to avoid the regurgitation by the larvae. The larvae were then released on to the respective semi-synthetic diets (*C. infuscatellus* and *C. partellus*), sugarcane shoot bits (*C. sacchariphagus indicus*), mulberry leaf bits (*B. mori*), bhendi fruit bits, (*E. vittella*) and sorghum grains (*C. cephalonica*).

In all the cases, third instar larvae were used. Other instars were tested for insects which were not susceptible to GmNPV in the third instar stage. In the preliminary tests, 10-15 larvae were used per insect species. For the bioassays, with susceptible insect species three replications were maintained with 30 insects in each replication for determining the mortality of the larvae. Ten cadavers of each insect were used for determining the yield of POB. In the case of BNGL isolate, susceptible insects like *P. xylostella, C. binotalis, H. undalis, C. partellus, M. vitrata, P. ricini, C. infuscatellus* and *C. medinalis* were bioassayed as mentioned above for comparison with CBE isolate.

3.3.1. DNA characterization by restriction endonuclease analysis

Restriction endonuclease analysis of the DNA of GmNPV was carried out to confirm the cross-infectivity of GmNPV in alternate hosts using *Pst* I and *Bam*H I by following the basic methods of Smith and Summers (1979). The reagents and buffers required for the DNA extraction are listed in Appendix II.

3.3.1.1. Extraction and dissolution of virus

An aliquot of 120 µl of GmNPV suspension containing ca 1 x 10^9 POB was taken in Eppendorf tubes (1.5 ml) and 25 µl of 0.5 M EDTA and 3 µl of proteinase K (20 mg/ml) were added and incubated at 37°C for 1 h 30 min in a Techne Dri-Block DB-2A heater. To this 75 µl of 1 M sodium carbonate was added and incubated at 37°C for 15 minutes to release the virions from the POB. After adding 25 µl of 10 per cent sodium dodecyl sulphate, the samples were incubated at 37°C for 30 min and centrifuged at 10,000 rpm for 3 min to remove undissolved polyhedra.

3.3.1.2. Phenol extraction of DNA and dialysis

To each Eppendorf tube containing the dissolved polyhedra, an equal volume of tris-saturated phenol was added and tubes were agitated gently for 5 min and centrifuged at 10,000 rpm for 5 min. The supernatant containing DNA was transferred to fresh tubes and equal volume of 25: 24: 1 tris-saturated phenol: chloroform: isoamyl alcohol mixture was added and the extraction was repeated. Finally the DNA was extracted with an equal volume of 24: 1 chloroform: isoamyl alcohol mixture. The extracted DNA was dialysed in 1x tris acetate EDTA buffer at 4°C for 36 h changing the buffer thrice.

3.3.1.3. Ethanol precipitation of DNA

Ethanol precipitation was done for samples of DNA which were insufficient after dialysis. To the extracted DNA solution, 1/5th volume of 3M sodium acetate and 2.5 volumes of cold absolute ethanol were added. The samples were placed in the freezer for one hour to ensure complete precipitation. The precipitate was pelleted by centrifuging at 10,000 rpm for 5 min. After removing the ethanol, the pellet was washed gently with 500 µl of 70 per cent ethanol and centrifuged again as described above. The ethanol was pipetted out and the samples were left for atleast one hour for evaporation of residual ethanol. The dry pellet of DNA was resuspended in 20 µl 1x tris buffer solution and used for digestion.

3.3.1.4. Digestion and Electrophoresis

Samples of 20 µl of DNA were digested with 1.5 µl of *Pst* I and *Bam*H I separately in appropriate buffer at 37°C for 4 h and the digestion was halted by adding stop mix. Electrophoresis was carried out using a BIORAD DNA subcell electrophoresis system using 0.6 per cent agarose gel at 35 V (22 mA current) overnight. Lambda DNA cut with *Hind*III and a 1 kb molecular weight marker were used as standards. DNA bands stained with ethidium bromide were photographed on a UV-transilluminator (UVP) using a Poloroid Camera (Copal, DS34).

3.3.1.5. Estimation of molecular size

The molecular size of the fragments was calculated on log molecular weight of co-migrating lambda marker and 1 kb ladder fragments and their migration distances were measured using Adobe Photoshop 5.0 software package. Curves were fitted and equations were generated using SPSS software to deduce the molecular weight of fragments.

GmNPV infection in the heterologous hosts were confirmed by comparing the molecular weights of the fragments of DNA of GmNPV passed through different hosts with that of the GmNPV original stock.

3.3.2. Effect of serial passage of GmNPV (CBE isolate) through different susceptible hosts against *P. xylostella* larvae

The susceptible insect species in this experiment included *P. xylostella, C. binotalis, H. undalis, M. vitrata, E. atomosa, C. infuscatellus, C. sacchariphagus indicus, C. partellus, C. medinalis, P. ricini, E. merione* and *D. pulverulentalis*. For comparison, GmNPV passaged through *G. mellonella* larvae was also included. GmNPV was serially passed through these susceptible hosts five times as per the methods described vide section 3.3. At the end of each passage through different susceptible hosts, the virus was extracted, semipurified, enumerated and bioassayed against early third instar larvae of *P. xylostella*. Six concentrations of GmNPV passaged through susceptible hosts *viz*, 5 x 10^7, 1 x 10^7, 2 x 10^6, 4 x 10^5, 8 x 10^4 and 1.6 x 10^4 POB/ml were used for the leaf disc bioassay. Thirty three larvae were used per dose and the mortality was recorded at 24 h intervals. A control was maintained for each bioassay which contained 0.01 per cent Tween 80 alone.

3.4. Bioefficacy of GmNPV isolates against *P. xylostella larvae*

Six different doses of GmNPV isolates of CBE and BNGL were evaluated against the early third instar larvae of *P. xylostella*. The treatments included were 2 x 10^9, 2 x 10^8, 2 x 10^7, 2 x 10^6, 2x 10^5 and 2 x 10^4 POB/ml and a control receiving only 0.01% Tween 80 solution. Each treatment was replicated three times with 30 larvae per replication. A Leaf disc bioassay technique was followed as described in section 3.3.

3.4.1. Age related response of *P. xylostella* larvae to GmNPV isolates

Leaf disc bioassay procedure was followed with six different concentrations of GmNPV of CBE and BNGL isolates. The doses were prepared by making five fold dilutions with the starting dose of 5 x 10^7 POB/ml to give 1 x 10^7, 2 x 10^6, 4 x 10^5, 8 x 10^4, and 1.6 x 10^4 POB/ml and tested against second, early third, late third and fourth instar larvae of *P. xylostella.* The stages were determined by the width of the head capsule (Sairabanu, 2000). Thirty-three larvae were used for each dose and a control was maintained which received only 0.01% Tween 80. The mortality of larvae was observed at 24 h interval for a period of eight days after treatment. The larvae inoculated in the final instar (fourth instar) were observed for mortality during pre-pupal and pupal stages also.

3.4.2. Effect of serial passage of GmNPV through *P. xylostella* larvae

GmNPV (CBE isolate) @ 2 x 10^7 POB/ml was tested against early third instar larvae of *P. xylostella* by serially passaging the virus through the same host 15 times. At the end of each passage, the mortality percentage and yield of POB/larva was determined. Thirty larvae per replication were used for each passage and replicated three times. The average yield of POB/larva was determined using ten cadavers at the end of each passage.

Leaf disc bioassay with six different concentrations *viz.*, 5 x 10^7, 1 x 10^7, 2 x 10^6, 4 x 10^5, 8 x 10^4 and 1.6 x 10^4 POB/ml was carried out at the end of each passage to determine the LC_{50} and LT_{50}. Thirty three early third instar larvae were used for each dose. Mortality was observed for eight days after treatment at 12 h interval. For comparison, *P. xylostella* granulosis virus (PxGV) (CBE isolate) passed once through *P. xylostella* larvae was tested with the same concentrations mentioned above.

3.4.3. Combined effect of GmNPV and PxGV on *P. xylostella*

LC_{75}, LC_{50} and LC_{25} doses of GmNPV and PxGV determined in the previous experiments were used. In order to study the interaction effects, if any, different combinations *viz.*, LC_{50} of GmNPV (2.34x10^6 POB/ml) + LC_{25} of PxGV (2.14 x 10^4 OB/ml), LC_{25} of GmNPV (6.41x10^4 POB/ml)+ LC_{50} of PxGV (2.28x10^5 OB/ml), LC_{25} of GmNPV + LC_{25} of PxGV, were used against *P. xylostella* and compared with the individual effects. Three replications were maintained with thirty insects per replication. The number of insects died during the period of 4-10 days post treatment was estimated for each treatment. A control was maintained separately.

3.5. Mass production of GmNPV

3.5.1. Standardization of mass production of GmNPV in *G. mellonella*

In order to standardize the mass production of GmNPV in *G. mellonella* larvae, different techniques were followed.

a) Larval head dip: GmNPV viral suspension (2 x 10^8 POB/ml) was taken in the well of cut eppendorf caps. The head of larvae of *G. mellonella* was dipped in the viral suspension for 60 sec. The larvae were then released on the semi-synthetic diet @ 30/plastic jar.

b) Pin head method : The head of an ordinary pin (1") was dipped in GmNPV viral suspension (2 x 10^8 POB/ml) and larvae of *G. mellonella* was allowed to engulf the suspension collected on the pin head (ca.1 μl).Care was taken to avoid regurgitation of the larvae by handling the larvae using soft tip forceps.

c) **Diet surface contamination:** Semi-synthetic diet of *G. mellonella* was pelleted (0.2 g each) and placed inside the polypot @ 5 pellets/pot. To the surface of the pellet, 10 µl of the viral suspension (2 x 10^7 POB/ml) was added and shade-dried. Larvae of *G. mellonella* (1 larva/pellet) were allowed to feed on the diet for 24 h and then transferred to fresh diet.

d) **Whole diet contamination**: Hundred µl of viral suspension (2 x 10^7 POB/ml) was blended with 1 g of semi-synthetic diet and shade dried for few minutes. Larvae of *G. mellonella* were fed with the contaminated diet @ 5 larvae/g of the diet for 24 h. After consumption of the contaminated diet, the larvae were fed with fresh diet.

In all the above methods, third instar larvae numbering 30 were used along with control. Both the isolates, CBE and BNGL were tested against *G. mellonella* larvae.

3.5.2. Mass production of GmNPV in three insect species

Laboratory reared culture of *P. ricini, C. partellus* and *G. mellonella* were used for the mass production of GmNPV. In order to select a suitable host stage for maximizing the yield of POB, a leaf disc bioassay was conducted using castor leaves for *P. ricini* and diet contamination bioassays for *C. partellus* and *G. mellonella.* GmNPV was used at a concentration of 5 x 10^7 POB/ml. Second, early and late stages of third, fourth and fifth instars of *P. ricini* were tested. The stages were determined by the width of the head capsule (Appendix III). Thirty larvae were used per stage with three replications in each. The per cent mortality due to virosis was determined during the period of observation of 4-12 days after treatment. Average yield of POB/larva was estimated with ten cadavers in each stage. Also concentration and time - mortality responses of above mentioned stages were determined with concentrations of 5 x 10^7, 1 x 10^7, 2 x 10^6, 4 x 10^5, 8 x 10^4 and 1.6 x 10^4 POB/ml of GmNPV suspension. Thirty three larvae were used per dose to calculate the LC_{50} and LT_{50}.

3.6. Transovarial transmission of GmNPV

Transovarial transmission of GmNPV was studied in *P. ricini* and *C. binotalis*. The larvae of these insect species which had escaped GmNPV infection (CBE isolate) in the previous experiments were allowed to pupate and maintained separately. The moths were sexed based on the difference in the banding patterns of wings of *C. binotalis* and abdominal segment markings of *P. ricini* and genitalia of both the sexes. The adult moths were caged in oviposition chamber @ 5 pairs/cage and respective natural hosts (cauliflower and castor) were provided for oviposition. The eggs/egg masses were surface - sterilized with 10% formaldehyde and the larvae upon hatching were reared on the respective natural hosts. A similar set-up was maintained with normal healthy adults as control. The mortality of the larvae was observed during rearing. The virosis of the cadavers was confirmed by taking a smear from the infected cadaver and observing for presence of POB. The yield of POB/larva was also determined as per the methods described vide section 3.2.1. Leaf disc bioassays were conducted with different concentrations of 5 x10^7, 1 x 10^7, 2 x 10^6, 4 x 10^5, 8 x 10^4 and 1.6 x 10^4 POB/ml of transovarially transmitted GmNPV and compared with that of the non-transmitted virus.

3.7. Histopathology studies

Yield of POB/larva of GmNPV showed variation among the susceptible insect species tested. In order to determine the course of infection of GmNPV in different susceptible hosts, histopathology studies were conducted to find out if there were any variations in the rate of infection of tissue in different insects. Three insect species *viz., P. xylostella, M. vitrata* and *C. partellus* were selected based on the variation in the mortality rates and yield of POB/larvae for this study.

Thirty numbers of third instar larvae of each insect species were inoculated with GmNPV (CBE isolate, 2 x 10^8 POB/ml) as per the methods described earlier. Three larvae were sampled at 12 h interval from 72 h post inoculation onwards and fixed in alcoholic Bouin's fixative for 24 h. Then the specimens were washed in 70 per cent alcohol for several days till the traces of picric acid were removed. This was indicated by the disappearance of yellow colour from the specimens. The specimens were dehydrated in tertiary butyl-alcohol - ethanol series, infiltrated and embedded in paraffin wax of melting point, 58-60°C. The cross-section of the specimens cut by a rotary microtome (Spencer AOB 180) was stained by the modified Azan staining method (Hamm, 1966). The series of steps for staining followed are as follows:

Xylene - 3 changes - 3 min.
Xylene - Alcohol series - each 3 min.
Alcohol - 100, 60, 30% - each 2 min.
Glacial Acetic acid, 50% - 5 min
Distilled water rinse, 2 changes - 1 min. each
Azocarmine - 45 min
Distilled water rinse, 2 changes - 1 sec. each
Alcohol 50, 70%- each 2 sec.
Aniline 0.5% in 95% alcohol - 1 sec.
Distilled water rinse - 2 changes - 1 sec.
Counter stain (orange G, Aniline Blue, Fastgreen FCF and Phospho tungstic Acid) - 15 min
Alcohol, 10, 30, 50, 75% - 1 sec. each
Absolute alcohol - 30 sec
Xylene, 3 changes - 10 min

The specimens were mounted in DPX mounting medium on clean microscopic slides and air dried. Microscopic observations on the total number of adipose tissue (fat body) present, the number of cell nuclei of adipose and hypodermis showing infection were recorded. The number of cells showing infections in the tissues of three insects was compared.

3.8. Morphometrics of GmNPV passaged through different hosts

GmNPV upon passage through different susceptible hosts showed variations in the size and shape of the polyhedra. Hence morphometric studies were undertaken using a filar micrometer for measuring the diameter of the POB. GmNPV viral suspensions (CBE isolate) obtained from different hosts were diluted 100 folds. The polyhedra were observed under 100 x magnification in a phase contrast microscope. The size of fifty polyhedra selected at random was determined. For comparison, GmNPV passed through different susceptible larvae were used. Also, morphometrics of GmNPV produced *in vitro* were determined.

3.9. *In vitro* studies with GmNPV

3.9.1. Cell lines and subculturing

The insect cell lines available at the Department of Agricultural Entomology, Coimbatore *viz.*, SlH (*S. litura* larval haemocytes), SlPG (*S. litura* pupal gonads). HaH (*H. armigera* larval haemocytes) and Sf21 clonal line (*S. frugiperda*) were used for the study. TNM-FH medium and Grace's medium were used for culturing of cells. The subculturing was done at the confluence stage of the cell culture. From the established culture flasks, one ml. of spent medium was retained and the remaining was removed. Then four ml of fresh medium was added and the attached cells were scraped to dislodge the attached cells with a silicone rubber policeman. Then five ml. more of fresh medium was added thoroughly mixed and split into two culture flasks. The flasks were incubated in cooled incubators (Ms/Labline) at 27.5°C. All the operations were carried under sterile condition in laminar hood.

3.9.2. Susceptibility of different cell lines to GmNPV (CBE isolate)

3.9.2.1. Preparation of inoculum

Fourth instar larvae of *C. partellus, H. undalis* and early fifth instar larvae of *P. ricini* were inoculated with a dose of 2 x 10^8 POB/ml of GmNPV suspension as per the method described earlier. After three days of inoculation, the larvae were washed in 70 per cent ethanol and twice with sterile distilled water. The haemolymph was drawn by cutting the prolegs and collected in TNM-FH medium containing 0.5 per cent cysteine to prevent melanization. Forty, thirty and twenty numbers of larvae of *H. undalis, C. partellus* and *P. ricini* respectively were used to collect the haemolymph in 5 ml of the medium. The medium with the haemolymph was passed through a 0.45 μm millipore membrane filter to remove the impurities if any. Filtrate containing virions were used to challenge the cell lines.

3.9.2.2. Challenging cell lines

At log phase of the different cell lines, i.e., 2-3 days after subculturing, the spent medium was removed and 5 ml of medium containing the inoculum was added and an adsorption time of two hours was allowed with periodical rocking of flasks at an interval of 15 minutes. The medium was then removed and the cells washed twice gently with sterile Hank's balanced salt solution (HBSS). After removing the HBSS completely, five ml of fresh TNM-FH medium was added. The culture flasks were incubated at 27.5°C and observed for virus infection under an inverted phase contrast microscope.

3.9.2.3. Harvesting of *in-vitro* cultured virus

The virus infected cells were harvested 10 days post inoculation. The cells were sonicated in ice bath using a Branson sonifier 450 to disrupt the cells and release the polyhedra. The cell debri were removed by centrifugation at 3000 rpm for one minute. The supernatant was again centrifuged at 10,000 rpm for five minutes to pellet the virus. The polyhedra were suspended in distilled water and the concentration assessed with the help of a haemocytometer under phase contrast microscope.

3.9.2.4. Biological activity of *in-vitro* cultured virus

The per cent cells infected and yield of POB/ml were determined. Five culture flasks were used for each cell line and insect species. The *in vitro* produced GmNPV were bioassayed against early third instar larvae of *P. xylostella* by leaf disc bioassay. The concentrations of virus and number of insects used were same as mentioned earlier. The activity of the *in vitro* produced GmNPV passaged through *C. partellus, M. vitrata, H. undalis* and *P. ricini* was compared with *in vivo* produced GmNPV. Also, CBE and BNGL isolates of GmNPV passaged through *C. partellus* larvae were compared for their efficacies in infecting the cell lines.

3.10. Estimation of chitinase activity in GmNPV infected larvae

3.10.1. Enzyme extraction

Fifty larvae of *C. partellus* (fourth instar) and *P. ricini* (early fifth instar) were treated with GmNPV (CBE isolate) viral suspension @ 2 x 10^8 POB/ml as per the methods described earlier. One g of larvae / cadavers was extracted in 2 ml of 0.1 M sodium citrate buffer (pH 5.0) on an ice bath at 24, 48, 72, 96, 120, 144 h post-inoculation. The homogenate was centrifuged for 20 min. at 10,000 rpm. The supernatant was used as crude enzyme source for assaying chitinase activity. For comparison, thirty larvae of *H. armigera* and *S. litura* (early fifth instar) were treated with 1 x 10^6 POB/ml of HaNPV and SlNPV, respectively, and the enzyme extract was prepared as described above at different time intervals.

3.10.2. Assay of chitinase

The colorimetric assay of chitinase was carried out as per Boller and Mauch (1988). Reagents used were colloidal chitin, snail gut enzyme, dimethyl amino benzaldehyde (DMAB) and buffer (Annexure IV).

The reaction mixture consisted of 10 ml of 0.1 M sodium acetate buffer (pH 4.0), 0.4 ml enzyme solution and 0.1 ml colloidal chitin (10 mg). After incubation for 2 h at 37°C, the reaction was stopped by centrifugation at 10,000 rpm for 3 min. An aliquot of 0.3 ml of the supernatant was pipetted into a glass reagent tube containing 30 ml of 1 M potassium phosphate buffer (pH 7.0) and incubated with 20 ml of 3% (w/v) snail gut enzyme for 1 h. After 1 h, the reaction mixture was brought to pH 8.9 by the addition of 70 ml of 0.1 M sodium borate buffer (pH 9.8). The mixture was incubated in a boiling water bath for 3 min. and rapidly cooled on an ice water bath. After addition of 2 ml of DMAB, the mixture was incubated for 20 min. at 37°C. Immediately the absorbance was measured at 585 nm. N-acetylglucosamine (GlcNAc) was used as a standard. The enzyme activity was expressed as µg/g/h of GlcNAc released.

3.11. Studies on effect of weather factors on persistence of GmNPV

3.11.1. Effect of sunlight

The persistence of GmNPV (CBE isolate) was assessed by exposing the virus to sunlight for different periods of time. Circles of dia 33 mm were marked on the leaves of potted cauliflower plant. An aliquot of 10 µl of GmNPV suspension (2 x 10^8 POB/ml) containing 0.01 per cent Tween 80 was applied on the centre of the marked circles and spread uniformly using the polished blunt end of a sterile glass rod (Plate 2). Leaves were sampled from 1 to 6 days after exposure to sunlight. The leaf discs were cut from the already marked and treated circles and assayed against early third instar larvae of *P. xylostella.* Thirty larvae were maintained per replication with five replications per day. Larvae fed on leaf discs without virus treatment were maintained as control (0 h). The mortality was recorded 4-10 days post inoculation. The percentage original activity remaining (% OAR) on the foliage was determined as follows:

$$\text{Per cent OAR} = \frac{\text{Per cent larval mortality after exposure}}{\text{Per cent larval mortality before exposure}} \times 100$$

The GmNPV obtained from virosed cadavers as a result of feeding larvae with the virus-treated leaves after five days of exposure were again used for the persistence experiment following the same procedure. The exposure was repeated over five cycles to see if by repeated exposure, a strain of virus tolerant to UV light can be selected.

3.11.2. Influence of temperature and relative humidity

Cauliflower seedlings raised in pots were used for this study at 50 - 60 days after transplanting. The methodology for persistence study as described in section 3.11.1 was followed at monthly intervals for a period of eight months (September 2001 - April 2002). As GmNPV was found to persist for five days after inoculation on cauliflower phyllosphere, the meteorological data on the maximum temperature, minimum temperature, and relative humidity were recorded at monthly intervals during the period of exposure (5 days) using maximum, minimum, wet bulb and dry bulb thermometers. The thermometers were installed in the pot culture yard one hour prior to the period of

Plate 2. Method of exposure of GmNPV to sunlight

Plate 3. Installation of thermometers for microclimatic observations

observations, i.e., 7.30 am and 2.30 pm daily for 5 days to calibrate the thermometers to pot culture microclimatic conditions (Plate 3). Data on maximum and minimum temperatures and relative humidity were recorded periodically. The persistence of the virus was assayed by leaf disc bioassay using early third instar larvae of *P. xylostella* as mentioned in section 3.11.1. Correlation between the temperature, relative humidity and persistence of virus was worked out at the end of each exposure period and compared.

3.12. Pot culture experiment on the efficacy of GmNPV against lepidopterous pests of cauliflower

Cauliflower seedlings (var. Double Cross) were raised in pots. At primordial leaf initiation stage (60 - 70 days after sowing) the potted plants were used for the assay. GmNPV suspension (CBE isolate) containing 0.01 per cent Tween 80 were diluted to make concentrations of 1.00 x 10^8, 5.00 x 10^7, 2.50 x10^7, 1.25 x 10^7, 6.25 x 10^6 and 3.13 x 10^6 POB/ml. Early third instar larvae of *P. xylostella* were released @ 30 larvae/plant. The above concentrations were sprayed at evening hours using a hand sprayer @ 10 ml/plant. As GmNPV persisted only for five days, a second spray was given five days later. Four replications were maintained per concentration. The number of larvae present in the treated plants was observed at two days interval upto 14 days post treatment. Similar procedure was followed for the larvae of *C. binotalis* and *H. undalis*.

3.13. Field evaluation of GmNPV

An exploratory field trial was conducted on cauliflower (var. Paswan) to evaluate the efficacy of GmNPV (CBE isolate) against *P. xylostella*. Since the LC_{50} of GmNPV was high for *P. xylostella* larvae, higher doses were used. The treatments included

1	GmNPV	-	7.50 x 10^{13} POB/ha
2.	GmNPV	-	3.75 x 10^{13} POB/ha
3.	GmNPV	-	1.88 x 10^{13} POB/ha
4.	PxGV'	-	1.50 x 10^{13} OB/ha
5.	*B. thuringiensis* var *kurstaki* (Btk-Delfin ®)	-	0.5 kg/ha
6.	Indoxacarb	-	29 g a.i./ha
7.	Control		

The experiment was laid out, in a farmer's field at Kuppanur, Coimbatore district during January - April 2002 in a randomized block design with a plot size of 5 x 4 m with three replications per treatment.

Four rounds of sprays were given using a spray fluid volume of 450 lit/ha at eight day intervals commencing from 60 days after transplanting with a back-pack hydraulic sprayer (Aspee®, Bombay). Tween 80 @ 0.01% was used as a surfactant for all the treatments except chemical insecticide. Observations on the number of larvae were recorded on the fourth, sixth and eighth day after each spray on fifteen plants selected at random from each plot. The leaf webber, *C. binotalis* was found to occur along with *P. xylostella*. Hence, observations on the number of larvae of *C. binotalis* were also recorded as mentioned above. At the time of harvest, data on mean diameter of flower head, mean weight of flower head and yield were recorded.

3.14. Safety tests with GmNPV on non-target organisms

The effect of GmNPV was tested on the following beneficial organisms.

1.	Silkworm	- *Bombyx mori* (L.)
2.	Honey bees	- *Apis* spp. and *Trigona iridipennis* (Prov.)
3.	Egg parasitoid	- *Trichogram ma chilonis* Ishii
4.	Predator	- *Chrysoperla carnea* (Stephens)

3.14.1. *B. mori*

Five different races of silkworm, *B. mori viz.,* TN white, AE40032, Mysl, Pure Mysore, Thai and CSRI 4 x 2 were used for the study. GmNPV suspension (CBE isolate, 2×10^9 POB/ml) was fed orally to silkworm larvae by pinhead method as described earlier. Ten third instar larvae were used per replication with three replications per race/cross. In each race/cross, a control was maintained with larvae not fed with the virus. The larvae after inoculation were reared on chopped mulberry leaves. The leaves were changed four times a day and the larvae were fed mulberry leaves of appropriate age depending upon the larval stage. The larvae in final instar stage were released on bamboo trays for cocoon formation. Observations on the weight of the larvae, mortality of larvae, fresh weight of cocoon, pupal period, per cent pupation and adult emergence were recorded.

3.14.2. *Apis* spp.

Different species of *Apis viz., A. cerana indica* (Fab.), *A. mellifera* (L.), *A. florae* (Fab.) and *T. iridipennis* were used for the study. Newly emerged worker bees were caged (30 x 30 x 30 cm) at the rate of 30 bees/cage without the queen. The bees were fed with 2 ml. of 2 x 10^9 POB/ml of GmNPV suspension (CBE & BNGL isolates) mixed in 50 per cent sucrose solution for the first three days and with 50 per cent sucrose alone on the subsequent days. In control, the bees were fed with sucrose solution alone. The mortality of bees was observed daily until all the bees died.

3.14.3. *T. chilonis*

Freshly laid eggs of *C. cephalonica* were glued over strips of cards (2.5 x 7.5 cm). Egg card bits containing 100 eggs each were used for the study. They were exposed to UV light for 20 min. to kill the developing embryos. GmNPV suspensions (CBE and BNGL isolates) were applied on these egg cards with the help of an atomizer using a spray fluid volume of 1 ml (2 x 10^9 POB/ml). The cards were shade-dried for 10 min. and placed in glass tubes and exposed to *T. chilonis* at the rate of 25 parasitized eggs/tube. The parasitoids upon emergence were allowed to parasitize the treated egg cards for 48 h and kept separately for adult emergence. Data on parasitism, emergence, duration of total life cycle and adult longevity were recorded. A control was maintained with untreated eggs simultaneously.

3.14.4. *C. carnea*

Corcyra egg cards prepared as mentioned above were sprayed with 1 ml. of GmNPV suspensions (CBE and BNGL isolates) @ 2 x 10^9 POB/ml. The treated egg card bits containing 40 - 50 eggs were transferred to sterile polypot. One destalked egg of *C. carnea* was transferred to each polypot and reared. Ten eggs were used per replication with five replications per isolate. The grubs after consuming the treated eggs were fed with untreated eggs until pupation. After pupation, the pupae were transferred to sterile plastic jars and a mixture of honey, protein hydrolysate, fructose, yeast and water was provided for emerging adults. The eggs laid on a plastic sheet kept at the bottom of the jars were collected. Observations on hatching, larval duration, larval mortality, pupal period and adult longevity were determined.

3.15. Statistical Analysis

The data obtained in percentages from different experiments were transformed to corresponding angles (Arc sine percentage) and numbers to log values. The larval counts in the field experiments were transformed to $\sqrt{x + 0.5}$ values as per the method developed by Poisson for statistical analysis (Snedecor and Cochran, 1967). Larval mortality in control was corrected using Abbott's formula (Abbott, 1925). The concentration and time-mortality responses of various experiments were subjected to probit analysis using a Statistical Package for Social Sciences (SPSS), ver. 6.0, SPSS Inc., USA. The analysis of variance in different experiments were carried out in IRRISTAT ver 3.1., Biometric Unit, IRRI, Philippines and the means were separated by Duncan's new Multiple Range Test (DMRT) available in the package.

Results

Chapter IV
RESULTS

4.1. Cross - infectivity of GmNPV

Results on the cross-infectivity studies with GmNPV (CBE isolate) to several economically important lepidopteran insect pests are furnished in Table 1. Among the 31 insect species screened by different bioassay techniques, 16 were found to be susceptible to GmNPV. The susceptible insects included *P. xylostella* (Plate 4), *C. binotalis* (Plate 5), *H. undalis* (Plate 6), *S. exigua, C. partellus* (Plate 7), *C. infuscatellus* (Plate 8), *C. sacchariphagus indicus* (Plate 9), *P. ricini* (Plate 10), *E. merione* (Plate 11), *M. vitrata* (Plate 12), *E. atomosa, C. medinalis* (Plate 13), *M. patnalis, O. arenosella* (Plate 14), *A. albistriga* (Plate 15) and *D. pulverulentalis* (Plate 16). These insects were susceptible in the dose range of 2 x 10^7 - 2 x 10^8 POB/ml. Mostly second and third instar larvae of insect species were used in the study.

The period for initiation of mortality varied among the susceptible insects. In the case of *P. xylostella, E. atomosa* and *D. pulverulentalis,* the mortality occurred 4 days post inoculation while it took 5 days to initiate mortality in *C. binotalis, H. undalis, M. vitrata* and *S. exigua. Chilo* spp. (6-10 days), *C. medinalis* (6-12 days) and *E. merione* (6-10 days) took longer time to die due to GmNPV.

It was interesting to note that the host range of GmNPV spanned several families of the order Lepidoptera. It included Plutellidae, Pyralidae, Arctiidae, Cryptophasidae, Pterophoridae, Nymphalidae and Noctuidae. Also, all the species of the same family were not susceptible. For example, GmNPV was infectious to *S. exigua* belonging to Noctuidae but not to other noctuids like *H. armigera, S. litura* and *M. separata.* Among the susceptible insects, pyralidae accounted for 62.50 per cent, Arctiidae for 12.50 per cent and other families accounted for only 6.67 per cent.

GmNPV was non-infectious to *H. armigera, S. litura, E. vittella, A. peponis, M. separata, B. mori, A. janata, E. mollifera, N. geometralis, D. indica, P. zizyphi,*

Table 1. Cross infectivity of GmNPV to different lepidopteran insect pests

S.No	Insect pests	Family	Dose (POB/ml)	Instars used	Method	Cross-infective
1	*Plutella xylostella*	Plutellidae	$2x10^7$	II, III, IV	Leaf disc	+
2	*Crocidolomia binotalis*	Pyralidae	$2x10^7$	II,III,IV	Leaf disc	+
3	*Earias vittella*	Noctuidae	$2x10^8$	II,III	*Per os*	-
4	*Pericallia ricini*	Arctiidae	$2x10^7$	III,IV ,V	Leaf disc	+
5	*Spodoptera litura*	Noctuidae	$5x10^7$	Neonate, I,II,III	Diet surface	-
6	*Maruca vitrata*	Pyralidae	$5x10^7$	II,III,IV	Diet surface	+
7	*Nausinoe geometralis*	Pyralidae	$2x10^7$	II,III	Leaf disc	-
8	*Anadivedia peponis*	Noctuidae	$2x10^7$	IV	Leaf disc	-
9	*Helicoverpa armigera*	Noctuidae	$5x10^7$	Neonate, I,II,III	Diet surface	-
10	*Mythimna separata*	Noctuidae	$5x10^7$	III	Diet surface	-
11	*Bombyx mori*	Bombycidae	$2x10^8$	III,IV	*Per os*	-
12	*Opisina arenosella*	Cryptophasidae	$2x10^7$	II,III	Leaf strips	+
13	*Chilo partellus*	Pyralidae	$5x10^7$	II,III	Diet surface	+
14	*Chilo infuscatellus*	Pyralidae	$2x10^8$	II,III	*Per os*	+
15	*Chilo sacchariphagus indicus*	Pyralidae	$2x10^8$	II,III	*Per os*	+
16	*Cnaphalocrocis medinalis*	Pyralidae	$2x10^7$	III	Leaf bits	+
17	*Marasmia patnalis*	Pyralidae	$2x10^7$	III	Leaf bits	+
18	*Exelastis atomosa*	Pterophoridae	$5x10^7$	II,III	Diet surface	+
19	*Ergolis merione*	Nymphalidae	$2x10^7$	II,III	Leaf disc	+
20	*Achaea janata*	Noctuidae	$2x10^7$	II	Leaf disc	-
21	*Diaphania pulverulentalis*	Pyralidae	$2x10^7$	II,III	Leaf disc	+
22	*Spodoptera exigua*	Noctuidae	$2x10^7$	II	Leaf disc	+
23	*Eupterote mollifera*	Eupterotidae	$2x10^7$	Neonate, I, II,III	Leaflet dip	-
24	*Diaphania indica*	Pyralidae	$2x10^7$	II, III, IV	Leaf disc	-
25	*Psorosticha zizyphi*	Oecophoridae	$2x10^7$	II,III	Leaflet dip	-
26	*Hyblea peura*	Pyralidae	$2x10^7$	II,III	Diet surface	-
27	*Eutectona macheralis*	Noctuidae	$2x10^7$	II,III	Leaf disc	-
28	*Hellula undalis*	Pyralidae	$2x10^7$	II,III	Leaf disc	+
29	*Corcyra cephalonica*	Pyralidae	$2x10^8$	II,III	*Per os*	-
30	*Agrotis ipsilon*	Noctuidae	$5x10^7$	II,III	Diet surface	-
31	*Amsacta albistriga*	Arctiidae	$2x10^7$	IV	Leaf surface	+

GmNPV infection in alternate hosts

Plate 4. *Plutella xylostella*

Plate 5. *Crocidolomia binotalis*

Plate 6. *Hellula undalis*

Plate 7. *Chilo partellus*

GmNPV infection in alternate hosts

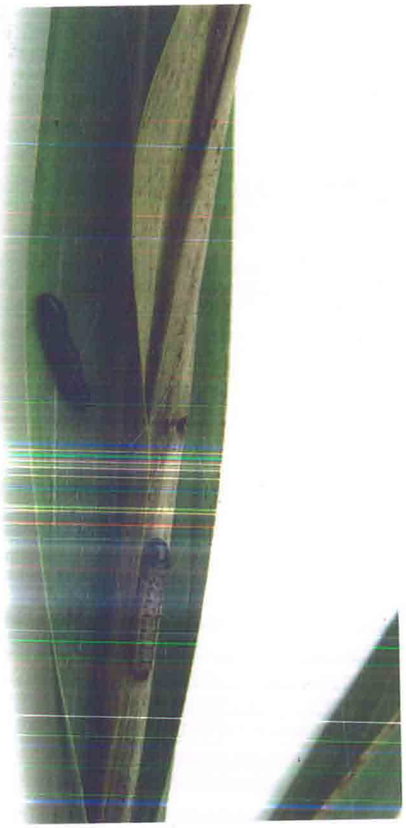


Plate 8. *Chilo infuscatellus*

Plate 9. *Chilo sacchariphagus indicus*

Plate 10. *Pericallia ricini*

Plate 11. *Ergolis merione*

GmNPV infection in alternate hosts

Plate 12. *Maruca vitrata*

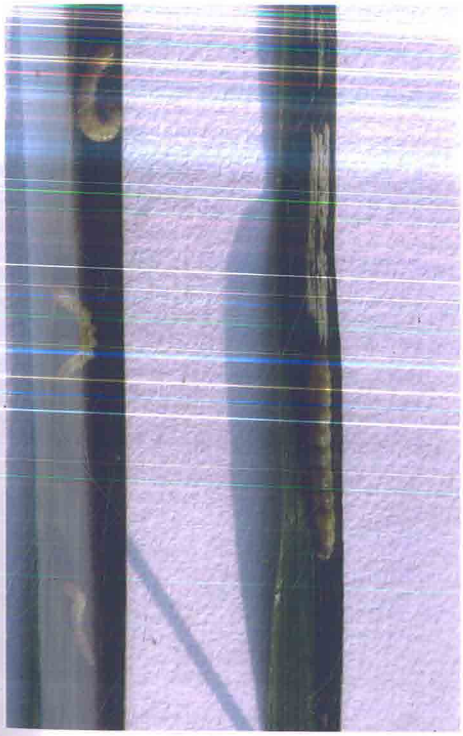
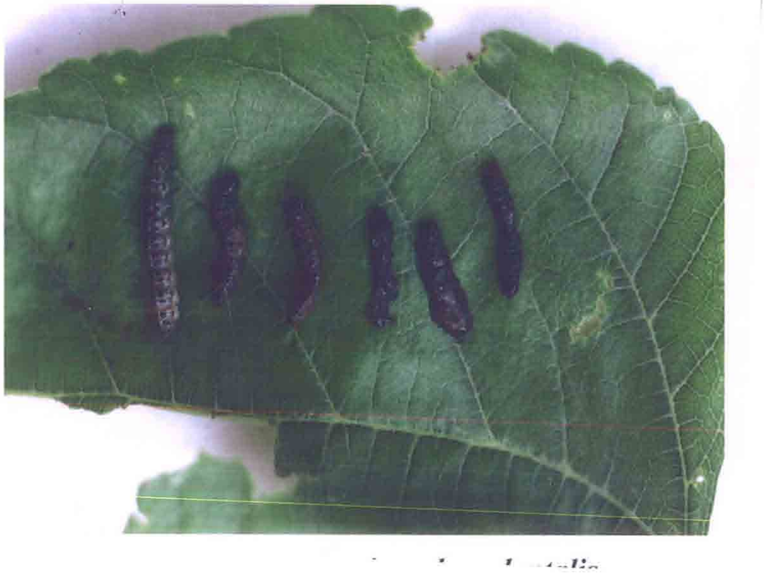


Plate 13. *Cnaphalocrocis medinalis*

Plate 14. *Opisina arenosella*



H. puera, E. macheralis, C. cephalonica and *A. ipsilon*. For non-susceptible insect species like *H. armigera* and *S. litura*, instars other than third including neonates tested against GmNPV and were not found to be susceptible.

4.1.1. DNA characterization of GmNPV

The restriction profiles of GmNPV DNA samples from inoculum and progeny viruses are shown in Plates 17-19. *Pst* I enzyme profile revealed that the progeny viral DNA from *P. xylostella* (Lane 3) and *M. vitrata* (Lane 4) contained the same fragment profiles as detected in the inoculum GmNPV viral DNA (Lane 2; Plate 17). The molecular weights of the fragments were determined with the help of co-migrating fragments of 1 Kb ladder and λ– DNA (Appendix V-A). Out of the total ten fragments of the viral DNA, the maximum molecular size fragment of 25.13 Kbp and minimum molecular size fragment of 2.46 Kbp were identified. However, in the progeny viral DNA profile from *P. xylostella,* the fourth fragment with molecular size of 17.08 Kbp was distinct from the other viral DNA fourth fragment (12.86 Kbp). The molecular size of inoculum GmNPV DNA was 116.82 Kbp and it was the same for progeny viral DNA from *M. vitrata* while the molecular size of progeny viral DNA from *P. xylostella* was 121.04 Kbp. In the other lanes, progeny viral DNA from *C. binotalis, P. ricini, H. undalis, C. infuscatellus and C. partellus* did not produce any profiles because of insufficient quantity of DNA. Hence they were subjected to ethanol precipitation of DNA.

The GmNPV DNA profiles and progeny viral DNA profiles from *C. binotalis, P. ricini, H. undalis, C. infuscatellus* and *C. partellus* were identical (Plate 18). The restriction endonuclease activity with *Pst* I generated a total of ten fragments. The molecular size of the fragments was 115.96 Kbp (Appendix V-B). However the viral DNA from *G. mellonella* had a total molecular weight of 111.62 Kbp because of slight variation in the migration when compared with the co-migrating bands. DNA profiles generated from inoculated viral DNA and progeny viral DNA from *D. pulverulentalis* cut with *Bam*H I enzyme were similar (Plate 19). There were seven fragments with maximum molecular size of 20.96 Kbp and minimum molecular size of 2.60 Kbp. The molecular

Restriction endonuclease analysis of DNA of GmNPV

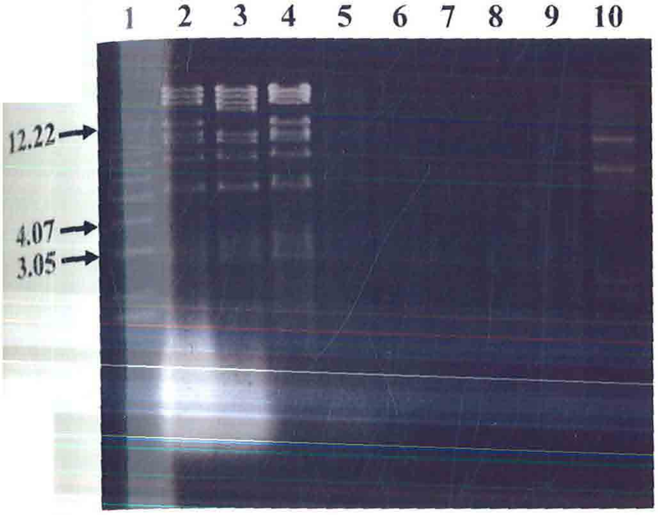


Plate 17. With *Pst* 1

1. 1 kb Marker
2. GmNPV inoculum
3. Progeny virus from *P. xylostella*
4. Progeny virus from *M. vitrata*
5. Progeny virus from *C. binotalis*
6. Progeny virus from *P. ricini*
7. Progeny virus from *H. undalis*
8. Progeny virus from *C. infuscatellus*
9. Progeny virus from *C. partellus*
10. Lambda DNA

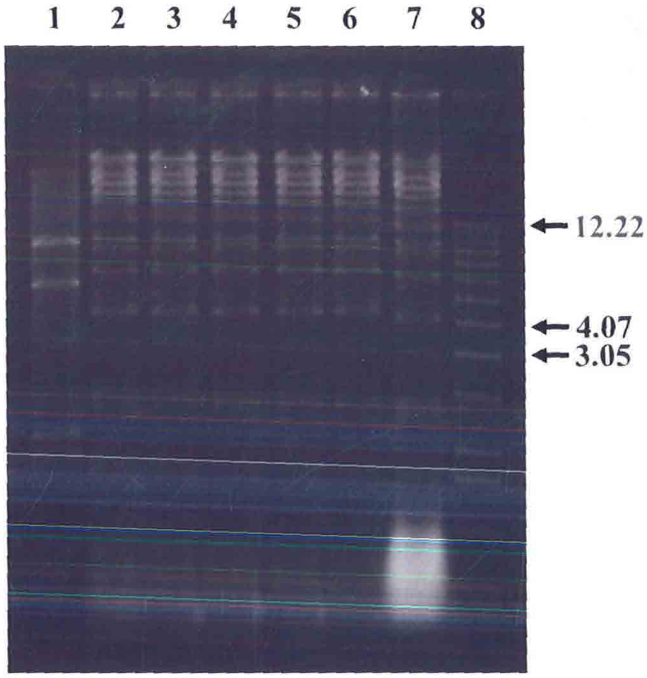


Plate 18. With *Pst* 1

1. Lambda DNA
2. Progeny virus from *C. binotalis*
3. Progeny virus from *P. ricini*
4. Progeny virus from *H. undalis*
5. Progeny virus from *C. infuscatellus*
6. Progeny virus from *C. partellus*
7. GmNPV inoculum
8. 1 kb Marker

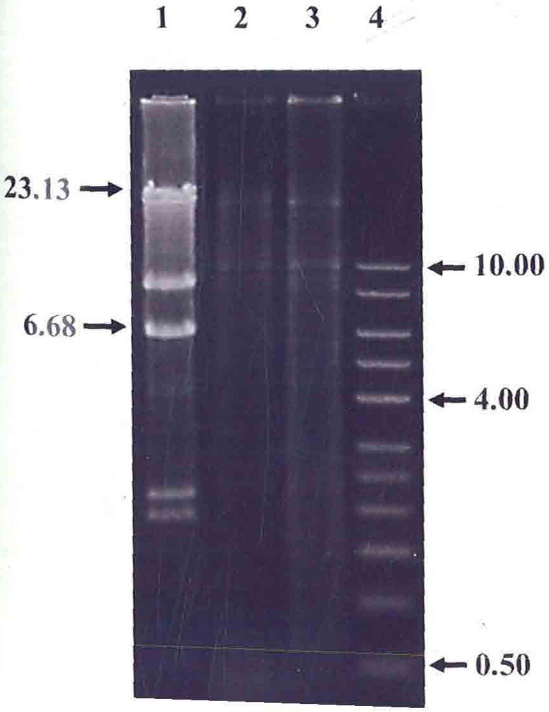


Plate 19. With *Bam*H1

1. Lambda DNA
2. GmNPV inoculum
3. Progeny virus from *D. pulverulentalis*
4. 1 kb Marker

size of DNA fragments was 62.20 Kbp (Appendix V – C). DNA characterization with *Pst* I and *Bam*H I restriction enzymes thus confirmed the cross-infectivity of GmNPV in *P. xylostella, M. vitrata, C. binotalis, P. ricini, H. undalis, C. infuscatellus, C. partellus* and *D. pulverulentalis.*

4.1.2. Susceptibility and yield of GmNPV in alternate hosts

Data on the susceptibility and yield of GmNPV revealed variation among the alternate hosts (Table 2). *C. infuscatellus* was the most susceptible with a mortality of 94.4 per cent followed by *M. vitrata* (87.8 per cent), *P. ricini* (80.0 per cent) and *D. pulverulentalis* (80.0 per cent). *P. xylostella, H. undalis* and *C. sacchariphagus indicus* were on par with the mortality of 78.9 per cent, 71.1 per cent and 68.9 per cent respectively. *C. binotalis* (66.7 per cent), *C. partellus* (64.5 per cent), *E. merione* (66.7 per cent), *E. atomosa* and *M. patnalis* each with 51.1 per cent followed next. *C. medinalis, O. arenosella* and *S. exigua* were the least susceptible with a mortality range of 20.0 - 35.3 per cent.

The yield of GmNPV was highest in *P. ricini* larvae (21.86 x 10^7 POB/larva) followed by *C. infuscatellus* (14.90 x 10^7 POB/larvae), *C. partellus* (8.90 x 10^7 POB/larva) and *D. pulverulentalis* (8.24 x 10^7 POB/larva). *C. sacchariphagus indicus* and *E. merione* with a yield of 4.83 x 10^7 and 3.34 x 10^7 POB/larva respectively followed next. The yield of POBs was on par for *M. vitrata* 3.89 x 10^7 POB/larva and *H. undalis* (3.43 x 10^7 POB/larva) followed by *C. binotalis* (2.59 x 10^7 x POB/larva) and *S. exigua* (2.42 x 10^7 POB/larva). The yield of POB/larva in *P. xylostella, E. atomosa, C. medinalis* and *M. patnalis* did not differ significantly. The lowest yield was obtained from *O. arenosella* (0.74 x 10^7 POB/larva). On comparing both the attributes, *P. ricini* was highly susceptible with highest yield/larva followed by *C. infuscatellus, D. pulverulentalis* and *C. partellus. A. albistriga* with the lowest mortality of 15.00 per cent and highest yield of 86.04 x 10^7 POB/larva was not included in the comparison as late fifth instar larvae were used.

4.1.3. Comparison of efficacies of GmNPV isolates in alternate hosts

The efficacies of GmNPV isolates were compared in terms of per cent mortality and yield of POB/larva (Table 3). In general, CBE isolate was more effective than BNGL

Table 2. **Susceptibility of different insect pests to GmNPV (CBE isolate)**

S.No	Insects tested	Per cent mortality	Yield ($x10^7$ POB /larva)
	Crucifer lepidopteran complex		
1.	*Plutella xylostella*	78.89 cd	1.82 fg
2.	*Crocidolomia binotalis*	66.67 e	2.59 ef
3.	*Hellula undalis*	71.11 cde	3.43 de
4.	*Spodoptera exigua*	20.00 h	2.42 f
	Pulse pod borer complex		
5.	*Maruca vitrata*	87.78 b	3.89 de
6	*Exelastis atomosa*	51.11 f	1.04 fg
	Sugarcane borer complex		
7.	*Chilo infuscatellus*	94.44 a	14.90 b
8.	*Chilo sacchariphagus indicus*	68.89 de	4.83 d
	Millet stem borer		
9.	*C. partellus*	64.45 e	8.90 c
	Rice leaf folder complex		
10.	*Cnaphalocrocis medinalis*	35.33 g	1.49 fg
11.	*Marasmia patnalis*	51.11 f	1.10 fg
	Coconut blackheaded caterpillar		
12.	*Opisina arenosella*	33.33 g	0.74 g
	Hairy caterpillar		
13.	*Pericallia ricini*	80.00 bc	21.86 a
	Castor butterfly		
14.	*Ergolis merione*	66.67 e	3.34 e
	Mulberry leaf webber		
15.	*Diaphania pulverulentalis*	80.00 bc	8.24 d
	Red hairy caterpillar		
16	*Amsacta albistriga* *	15.00	86.04

* Late fifth instar larvae used and hence not included in the statistical analysis

In a column, means followed by the same letter do not differ significantly by DMRT (P=0.05)

Table 3. **Comparative virulence of GmNPV isolates to selected host species at a dose of 467.91 POB/mm^2**

Insect species	Per cent mortality		Yield (x10^7 POB/larva)	
	CBE	BNGL	CBE	BNGL
P. xylostella	81.1 c	74.5 c	1.81 e	1.28 e
C. binotalis	65.6 d	81.1 c	2.54 e	2.06 e
H. undalis	76.7 c	66.7 d	3.39 d	1.71 e
C. partellus	87.8 b	76.7 c	8.84 c	2.59 e
M. vitrata	81.1 c	64.4 d	3.80 d	2.31 e
P. ricini	80.0 c	68.9 d	21.58 a	16.19 b
C. infuscatellus	95.6 a	82.2 bc	14.75 b	10.80 c
C. medinalis	35.6 e	30.0 e	1.40 e	1.10 e

In a column, means followed by same letter do not differ significantly by DMRT (P=0.05) with higher mortality rates for all the insects tested. CBE isolate was highly infectious to *C. infuscatellus* (95.6 per cent) followed by *C. partellus* (87.9 per cent), *P. xylostella* (81.1 per cent) and *M. vitrata* (81.1 per cent). While BNGL isolate was highly effective against *C. infuscatellus* (82.2 per cent) followed by *C. binotalis* (81.1 per cent), *C. partellus* (76.7 per cent) and *P. xylostella* (74.5 per cent). *P. ricini* and *H. undalis* were moderately susceptible to both the isolates with per cent mortality of 80.0, 68.9 and 76.7 and 66.7 respectively. Both the isolates were least effective against *C. medinalis* (35.6 per cent and 30.0 per cent mortality).

The yield of CBE isolate was higher than that of BNGL isolate in all the insects tested. The yield of POB/larva was 1.33, 1.36, 3.41, 1.98 and 1.65 fold higher for CBE isolate than BNGL isolate in *P. ricini, C. infuscatellus, C. partellus, H. undalis* and *M. vitrata* respectively. The yield from other insects like *P. xylostella, C. binotalis* and *C. medinalis* were on par for both the isolates.

4.1.4. Concentration - mortality responses of *P. xylostella* larva to GmNPV passaged through alternate hosts

4.1.4.1. First passage (Appendix VI-A)

The concentration mortality response of *P. xylostella* larvae to GmNPV passaged once through susceptible alternate hosts showed variation as revealed by LC_{50} values. The lowest LC_{50} of 15.16 POB/mm^2 was recorded after passage through the original host, *G. mellonella*, while the highest LC_{50} of 339.70 POB/mm^2 was observed upon passage through *C. medinalis*. LC_{50} was on par for passages through *P. xylostella* and *P. ricini*. Passage through *C. infuscatellus* recorded a LC_{50} of 24.91 POB/mm^2 followed by that of *H. undalis*. LC_{50} for passages through *E. merione* and *D. pulverulentalis* were 8.39 and 6.96 fold higher than that passaged through original host. GmNPV passaged through *C. binotalis*, *C. partellus* and *E. atomosa* recorded higher LC_{50} of 314.56, 215.26 and 272.64 POB/mm^2 respectively.

4.1.4.2. Second passage (Appendix VI-B)

Lowest LC_{50} of 12.15 POB/mm^2 was observed when GmNPV was passaged through *C. infuscatellus* followed by that passaged through original host (21.83

POB/mm^2) and *H. undalis* (23.06 POB/mm^2). Highest LC$_{50}$ of 612.52 POB/mm.^{2} was recorded with passage through *C. medinalis* followed by passage through *E. atomosa* (217.52 POB/mm^2). LC$_{50}$ of GmNPV passaged through *P. xylostella, M. vitrata, C. sacchariphagus indicus* and *D. pulverulentalis* were in the range of 42.96 - 88.17 POB/mm^2. GmNPV passaged through *C. binotalis, E. merione, C. partellus* and *P. ricini* recorded 7.10, 5.82, 5.37 and 4.83 fold higher LC$_{50}$ when compared to passages through the original host.

4.1.4.3. Third passage (Appendix VI-C)

GmNPV passaged through the original host recorded the lowest LC$_{50}$ of 8.73 POB/mm^2 against *P. xylostella* followed by the passages through *P. xylostella* (33.59 POB/mm^2) and *C. infuscatellus* (34.08 POB/mm^2). LC$_{50}$ of GmNPV passaged through *H. undalis, M. vitrata* and *C. sacchariphagus indicus* were on par with overlapping fiducial limits. Likewise, passage through *P. ricini, C. partellus* and *D. pulverulentalis* recorded LC$_{50}$ of 70.72 - 97.18 POB/mm^2 and were on par. LC$_{50}$ of GmNPV passaged through *C. binotalis, E. atomosa* and *E. merione* was 23.86, 22.29 and 14.56 fold higher than that of the passages through original host. Passage through *C. medinalis* recorded the highest LC$_{50}$ of 362.63 POB/mm^2.

4.1.4.4. Fourth passage (Appendix VI-D)

The lowest LC$_{50}$ of 6.65 POB/mm^2 was seen with the passage of GmNPV through the original host followed by the passage through *P. xylostella* (18.04 POB/mm^2). LC$_{50}$ of all the other passages through *P. ricini, H. undalis, M. vitrata, C. infuscatellus, C. sacchariphagus indicus* and *C. partellus* were on par with overlapping fiducial limits. Passage of GmNPV through *C. binotalis* recorded the highest LC$_{50}$ of 138.98 POB/mm^2.

4.1.4.5. Fifth passage (Appendix VI-E)

GmNPV passaged five times through the original host recorded the lowest LC$_{50}$ of 5.88 POB/mm^2 followed by the passage through *P. xylostella* (8.02 POB/mm^2) and *C. infuscatellus* (10.86 POB/mm^2). GmNPV passaged five times through *P. ricini, H. undalis, M. vitrata, C. sacchariphagus indicus* and *C. partellus* produced LC$_{50}$ in the range of 22.87 - 46.02 POB/mm^2 against *P. xylostella* and they did not differ significantly with overlapping fiducial limits. Highest LC$_{50}$ of 120.10 POB/mm^2 was observed with GmNPV passaged through *C. binotalis.*

On comparing the LC$_{50}$ of GmNPV passaged subsequently through alternate hosts five times against *P. xylostella* larvae, it is evident that the LC$_{50}$ decreased with increasing number of passages through alternate hosts (Table 4). There was a drastic reduction in the LC$_{50}$ for passages through *C. partellus* and *P. xylostella* while there was not a clear trend for passages through *G. mellonella, C. binotalis, P. ricini, M. vitrata* and *C. sacchariphagus indicus.* LC$_{50}$ upon passages through *G. mellonella* and *C. infuscatellus* did not differ significantly with overlapping fiducial limit. However the serial passage of GmNPV through *H. undalis* has no significant effect on LC$_{50}$ against *P. xylostella.* In general there was a decrease in LC$_{50}$ by 2.58, 6.84, 2.62, 2.36, 2.52, -0.99, 2.29, 4.68 and 2.42 fold after five subsequent passages through *G. mellonella, P. xylostella, C. binotalis, P. ricini, M. vitrata, H. undalis, C. infuscatellus, C. partellus* and *C. sacchariphagus indicus* respectively when compared to the activity after first passage against *P. xylostella* larvae.

4.2. Comparative virulence of GmNPV isolates to *P. xylostella*

Mortality rates of early third instar larvae fed with different concentrations of GmNPV isolates *viz.*, Coimbatore and Bangalore are presented in Table 5. Both the isolates showed an increasing trend in mortality rates with increase in dose. The mortality of the larvae commenced four days post inoculation for both the isolates and increased with period of time upto 10 days post inoculation.

Highest mortality of 96.85 per cent was recorded with highest concentration of 2 x 10^9 POB/ml followed by the subsequent concentrations of 2 x 10^8 and 2 x 10^7 POB/ml with 84.32 and 75.76 per cent mortality respectively for CBE isolate. Mortality rates were less than 50 per cent *ie.*, 39.08 per cent and 21.21 per cent with lowest concentrations of 2 x 10^5 and 2 x 10^4 POB/ml respectively. A similar trend was observed with BNGL isolate with highest and lowest mortality of 93.42 per cent and 18.18 per cent at highest and lowest concentrations of 2 x 10^9 POB/ml and 2 x 10^4

Table 4. Comparative LC_{50} of GmNPV against *P. xylostella* upon serial passages in different susceptible hosts

GmNPV passed through	**LC_{50} (POB/mm^2) after passage**				
	I	**II**	**III**	**IV**	**V**
G. mellonella	15.16	21.83	8.73	6.65	5.88
P. xylostella	54.84	48.35	33.59	18.04	8.02
C. binotalis	314.56	154.89	208.30	138.98	120.10
P. ricini	54.02	105.34	70.72	41.47	22.87
H. undalis	31.61	23.06	43.55	40.94	32.06
M. vitrata	98.59	42.96	54.61	59.26	39.15
C. infuscatellus	24.91	12.15	34.08	34.60	10.86
C. partellus	215.26	117.30	84.31	51.83	46.02
C. sacchariphagus indicus	68.68	48.19	42.57	31.46	28.40
C. medinalis	339.70	612.52	362.63	-	-
E. merione	127.14	127.13	127.10	-	-
E. atomosa	272.64	217.52	194.62	-	-
D. pulverulentalis	105.47	88.17	97.18	-	-

Table 5. **Comparative virulence of GmNPV isolates to early third instar larvae of *P. xylostella***

Dose (POB/ml)	Per cent mortality (days after inoculation)							
	CBE				BNGL			
	4	6	8	10	4	6	8	10
$2x10^9$	51.52	66.67	90.91	96.85 a	42.26	54.56	78.64	93.42 a
$2x10^8$	45.62	51.58	66.67	84.32 b	33.33	45.62	66.67	80.81 b
$2x10^7$	42.25	51.47	51.47	75.76 b	39.40	45.38	54.48	66.67 c
$2x10^6$	15.36	27.23	30.28	51.48 c	18.12	21.18	39.34	54.55 c
$2x10^5$	9.04	15.12	33.33	39.08 c	9.04	18.15	21.18	33.33 d
$2x10^4$	6.02	18.15	18.15	21.21 d	6.02	9.09	15.12	18.18 e

In a column, means followed by same letters do not differ significantly by DMRT (P=0.05)

POB/ml respectively. The mortality rate ranged between 33.33 - 80.81 per cent with the concentrations range of 2×10^5 - 2×10^8 POB/ml.

CBE and BNGL isolates of GmNPV did not differ significantly in their virulence at all doses tested except 2×10^7, 2×10^5 and 2×10^4 POB/ml where the mortality rates were not on par. Mortality above 65 per cent was witnessed at a dose of 2×10^7 POB/ml for both the isolates. Hence, for subsequent bioassays with these isolates, the highest dose used was 5×10^7 POB/ml with five-fold dilutions.

4.2.1. Response of different larval instars of *P. xylostella* to GmNPV isolates

Data on concentration-mortality responses of different larval instars of *P. xylostella* to GmNPV isolates revealed that all the instars were susceptible to both CBE and BNGL isolates (Table 6). The degree of susceptibility of larval instars varied significantly as evidenced by the variation in the LC_{50} with non-overlapping fiducial limits among the instars in consideration. Second instar larvae were the most susceptible with the lowest LC_{50} of 4.55 POB/mm^2 while the fourth instar larvae were least susceptible with highest LC_{50} of 588.20 POB/mm^2 for CBE isolate. LC_{50} were 11.55 and 33.76 fold higher in early third and late third instars when compared to that of second instar larvae.

A similar trend was observed with Bangalore isolate with lowest LC_{50} of 6.32 POB/mm^2 in second instar and highest LC_{50} of 391.08 POB/mm^2 in fourth instars. LC_{50} for early and late third instars were 12.84 and 29.14 fold higher respectively than that of second instar larvae. Though the CBE isolate recorded a lower LC_{50} than that of BNGL isolate for second, early third and late third instars, the variations were not significant because of the overlapping fiducial limits.

4.2.2. Effect of serial passage of GmNPV through *P. xylostella*

The yield of POB/larva of CBE isolate was more or less the same upto 13 passages. A significant increase in POB yield was observed at the 14th and 15th passages (Table 7).

Table 6. Concentration - mortality response of different instars of *P. xylostella* larvae to GmNPV isolates

Instars used	Isolates	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LC_{50} (POB/mm^2)	Fiducial limits
II	**CBE**	187	1.798	0.623 ± 0.146	4.55	1.77 - 9.60
	BNGL	197	0.669	0.559 ± 0.141	6.32	2.41 - 13.90
Early III	**CBE**	197	0.839	0.480 ± 0.149	52.57	19.00 – 180.68
	BNGL	196	2.540	0.458 ± 0.155	81.12	31.34 – 294.29
Late III	**CBE**	198	0.473	0.464 ± 0.161	153.64	58.36 - 648.47
	BNGL	197	0.552	0.439 ± 0.159	184.16	62.53 –1046.39
IV	**CBE**	198	3.629	0.570 ± 0.209	588.20	226.51 - 2863.03
	BNGL	196	4.141	0.550 ± 0.195	391.08	155.04 – 1704.48

* All lines are significantly a good fit ($P<0.05$)

Table 7. **Effect of serial passage of GmNPV in *P. xylostella* larvae on the concentration-mortality responses of third instar *P. xylostella* larvae**

No. of passages	**No. observed**	$\chi^2_{(n-2)}$*	**Slope** b ± S.E.	**LC_{50}** (POB/mm2)	**Fiducial limits**	**Yield (x 10^7 POB/larva) $**
1	197	0.282	0.446 ± 0.153	54.84	21.03 – 173.05	1.01 h
2	198	1.059	0.406 ± 0.150	57.06	19.99 – 208.72	1.30 b-f
3	193	2.831	0.508 ± 0.162	85.09	35.88 – 256.20	1.22 b-g
4	196	1.250	0.463 ± 0.148	18.04	6.46 –46.71	1.19 d-g
5	198	4.270	0.331 ± 0.139	8.02	2.28 – 11.03	1.08 gh
6	196	0.612	0.433 ± 0.139	8.90	2.65 – 23.94	1.20 c-g
7	193	1.128	0.528 ± 0.141	4.72	1.56 – 10.97	1.16 e-h
8	198	3.383	0.372 ± 0.138	5.05	1.05 – 14.99	1.23 b-g
9	193	5.034	0.489 ±0.140	6.41	2.00 – 16.11	1.14 fgh
10	197	4.704	0.466 ± 0.137	5.85	1.15 – 18.31	1.39 b
11	194	0.414	0.498 ± 0.140	6.74	2.26 – 16.20	1.20 b-e
12	193	0.351	0.489 ± 0.142	10.39	3.72 – 20.46	1.36 bcd
13	195	0.514	0.5067 ± 0.142	11.31	4.00 – 28.24	1.39 bc
14	197	0.332	0.504 ± 0.138	5.40	1.78 – 12.83	1.73 a
15	194	2.562	0.537 ± 0.139	4.47	1.58 – 10.03	1.68 a

*All lines are significantly a good fit ($P<0.05$)

$ In a column, means followed by the same letter do not differ significantly by DMRT ($P=0.05$)

4.2.2.1. Concentration and time-mortality responses of *P. xylostella* larvae to serially passaged GmNPV

Concentration-mortality responses of early third instar larvae of *P. xylostella* to serially passaged GmNPV (CBE isolate) were compared (Table 7). The susceptibility of the larvae increased with subsequent passages of GmNPV through the same host as revealed by the decreasing trend of LC_{50}. It was highest at 54.84 POB/mm^2 after 1st passage and was on par in the subsequent two passages. However, there was a significant decrease in LC_{50} by 4.72 and 10.61 fold after fourth and fifth passages when compared to LC_{50} after third recorded passage. There was a decreasing trend in LC_{50} in the subsequent passages except after 12th and 13th passages where there was a slight increase with LC_{50} of 10.39 and 11.31 POB/mm^2 respectively. However, the LC_{50} after 5-15 passages did not differ significantly. There was 12.27-fold decrease in LC_{50} after 15 passages, compared to LC_{50} after first passage.

The serial passage of GmNPV through *P. xylostella* larvae however had no significant effect on the LT_{50} which ranged from 108.65 to 144.99 h (Table 8)

4.2.3. Responses of *P. xylostella* larvae to GmNPV and PxGV

GmNPV and PxGV passaged once through *P. xylostella* larvae were compared for their efficacies against third instar larvae of *P. xylostella* (Table 9). Though LC_{50} of GmNPV was 10.31 fold higher than that of PxGV, GmNPV recorded a lower LT_{50} (133.25 h) when compared to that of PxGV (157.06 h). LC_{50} of the viruses differed significantly with non-overlapping fiducial limits.

4.2.4. Combined effect of GmNPV and PxGV on *P. xylostella* larvae

Mixtures of GmNPV and PxGV were used at different ratios to determine the interaction effects on *P. xylostella* larvae, if any. From Table 10, it is evident that GmNPV and PxGV when combined together in any ratio did not produce synergistic effect. The effect of GmNPV and PxGV combined together in the ratio of LC_{25}: LC_{50}, LC_{50}: LC_{25} and LC_{25}: LC_{25} produced mortality of 61.67 per cent, 64.17 per cent and 34.87 per cent respectively which were all lower when compared to that of respective single effects. The treatments in combination produced an effect lower than the algebraic sum of

Table 8. Effect of serial passage of GmNPV in *P. xylostella* larvae on the time - mortality responses of third instar *P. xylostella* larvae

No. of passages	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LT_{50} (hours)	Fiducial limits
1	197	1.113	0.297 ± 0.174	133.25	114.63 – 148.43
2	198	0.489	0.338 ± 0.273	135.42	117.25 – 150.82
3	193	2.751	0.741 ± 0.177	132.38	116.84 – 145.38
4	196	0.333	0.548 ± 0.178	119.48	101.04 –132.68
5	198	0.218	0.178 ± 0.184	108.65	88.92 – 121.90
6	198	6.615	0.625 ± 0.164	143.38	130.76 – 159.56
7	196	1.348	0.619 ± 0.153	121.34	70.31 – 170.58
8	198	1.225	0.427 ± 0.136	112.54	92.16 – 132.42
9	193	0.745	0.255 ± 0.132	127.79	87.72 –130.42
10	197	0.079	0.494 ± 0.139	123.37	107.00 – 141.07
11	194	1.129	3.334 ± 2.060	135.71	113.28 – 158.32
12	193	0.840	3.089 ± 2.046	133.73	108.08 – 157.74
13	195	0.226	2.211 ± 2.030	135.06	86.42 – 182.69
14	197	0.258	2.704 ± 2.032	144.99	118.16 – 187.82
15	194	0.492	2.288 ± 2.009	126.31	70.88 – 156.94

*All lines are significantly a good fit ($P<0.05$)

Table 9. Comparative concentration and time – mortality responses of *P. xylostella* larvae to GmNPV and PxGV

Virus	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	Responses	Fiducial limits
				LC_{50} (OB/mm^2)	
GmNPV	197	0.282	0.446 ± 0.153	54.84	21.03 – 173.04
PxGV	196	0.854	0.349 ± 0.047	5.32	1.855 – 14.72
				LT_{50} (h)	
GmNPV	197	1.113	0.297 ± 0.174	133.25	114.63 – 148.43
PxGV	196	0.805	0.241 ± 0.132	157.06	118.19 – 182.21

*All lines are significantly a good fit ($P<0.05$)

Table 10. **Combined effect of GmNPV and PxGV against *P. xylostella* larvae**

Treatments	Per cent mortality (Days after inoculation)*			
	4	6	8	10
LC_{50} GmNPV	17.50 b	32.50 c	50.83 c	52.50 c
LC_{75} GmNPV	31.67 a	62.49 a	73.33 a	74.17 a
LC_{25} GmNPV + LC_{50} PxGV	30.83 a	53.33 b	58.34 b	61.67 b
LC_{50} GmNPV + LC_{25} PxGV	35.83 a	50.00 b	60.83 b	64.17 b
LC_{25} GmNPV + LC_{25} PxGV	8.36 d	15.83 e	26.67 e	34.87 d
LC_{50} PxGV	13.33 c	23.33 d	43.34 d	54.17 c
LC_{75} PxGV	16.67 bc	31.67 c	61.67 b	76.17 a

* In a column, means followed by the same letter do not differ significantly by DMRT (P=0.05)

single effects revealing the absence of any interaction between the components. However, it is interesting to note that treatment of GmNPV alone initiated the infection at a greater pace and the expected effects were observed eight days post treatment. The rate of infection of PxGV was rather at a slow pace and the expected effects were observed 10 days post treatment.

4.3. Mass production of GmNPV

4.3.1. Standardization of mass production technique for GmNPV in *G. mellonella*

Among the methods of inoculation tested, whole diet contamination and direct *per os* methods gave the highest larval mortality of 42.50 per cent and 43.33 per cent respectively for CBE isolate and 42.50 and 52.50 per cent respectively for BNGL isolate (Table 11) (Plate 20, 21). Both the methods were equally effective as the mortality rates of larvae were on par. These were followed by the diet surface contamination method with mortality rates of 11.67 per cent and 18.34 per cent for CBE and BNGL isolates respectively. Larval head dip method was the least effective with the lowest mortality of 7.5 per cent and 13.34 per cent for CBE and BNGL isolates respectively. However the susceptibility of *G. mellonella* larvae to BNGL isolates was significantly higher than that of CBE isolate in diet surface contamination, direct *per os* and larval head dip methods.

The yield of CBE isolate did not differ significantly with the methods of inoculation and it was in the range of 76.60 - 81.70 x 10^7 POB/larva. BNGL isolate recorded around 10 fold lower yield than CBE isolate irrespective of the method of inoculation. The yield of this isolate was highest in larvae inoculated by whole diet contamination method (8.33 x 10^7 POB/larva) followed by larvae inoculated by larval head dip method (7.30 x 10^7 POB/larva). The yield was on par in larvae inoculated by diet surface contamination and direct *per os* methods.

4.3.2. Susceptibility of different host insect species and yield of GmNPV

Three insect species *viz.*, *G. mellonella*, *P. ricini* and *C. partellus* were selected for mass production of GmNPV on the basis of production of virulent progeny virus, higher yield of POB/larva and ease of mass culturing respectively from the previous

Table 11. **Standardization of mass production technique for GmNPV in *G. mellonella***

Method of bioassay	**Per cent mortality**		**Yield (x 10^7 POB/larva)**	
	CBE	**BNGL**	**CBE**	**BNGL**
Larval head dip	7.50 b	13.34 b	78.82 a	7.30 ab
Diet surface contamination	11.67 b	18.34 b	78.88 a	6.58 b
Whole diet contamination	42.50 a	42.50 a	81.70 a	8.33 a
Direct *per os*	43.33 a	52.50 a	76.60 a	6.76 b

In a column, means followed by same letters do not differ significantly by DMRT (P=0.05)

Plate 20. GmNPV infection in *Galleria mellonella*

Plate 21. Mass production of GmNPV in *G. mellonella*

experiments. The susceptibility of different larval instars and yield of GmNPV in these selected insect species were determined to standardize the host instar for mass production of GmNPV (Table, 12).

Among the insect species tested, the yield of GmNPV was highest in the early fifth instar larvae of *P. ricini* (10.19 x 10^8 POB/larva) and the susceptibility was also high with 76.57 per cent mortality. Though the susceptibility was highest in the early instars the yield of GmNPV was low. Hence early fifth instar larvae of *P. ricini* was found to be the ideal stage for mass production of GmNPV.

The yield of GmNPV in late fourth and early fifth instars of *G. mellonella* was on par with that of *P. ricini* larvae. However the susceptibility of fourth and fifth instars of *G. mellonella* larvae was low in the range of 20.0 - 39.4 per cent. The yield of GmNPV (7.45 - 8.20 x 10^8 POB/larva) with moderate per cent mortality of 55.16 - 58.19 in the early and late third instar stages can also be considered suitable for mass production of GmNPV in *G. mellonella* larvae.

In the case of *C. partellus* larvae, second and third instar larval stages recorded the highest mortality in the range of 89.46 - 98.18 per cent but the yield of GmNPV was poor to the tune of 0.12 - 1.14 x 10^8 POB/larva. This was followed by early and late fourth larval stages. Though highest yield of GmNPV (3.38 x 10^8 POB/larva) was recorded in the early fifth instar, the susceptibility was below 50 per cent. Hence fourth instar larval stages of *C. partellus* were optimum for mass production of GmNPV.

4.3.2.1. Production efficiency ratio of GmNPV in three different insect species

The production efficiency ratio of GmNPV in *G. mellonella, P. ricini* and *C. partellus* was compared (Table 12a). The production efficacy was highest in the third instar larvae of *G. mellonella* (433.52-452.31), early fifth instar larvae of *P. ricini* (780.25) and fourth instar larvae of *C. partellus* (234.14 - 238.09) (Plate 22). The production virulence ratio was highest in *G. mellonella* larval stages because of lowest LC_{50} (5.88 POB/mm^2) against *P. xylostella* larvae. This was followed by *P. ricini* larval stages

Table 12. Susceptibility of different larval instars of three insect species and yield of GmNPV

Instars tested	Per cent mortality			Yield (x10^8 POB/larva)		
	G. mellonella	*P. ricini*	*C. partellus*	*G. mellonella*	*P. ricini*	*C. partellus*
II	63.03 aC	92.24 aB	98.18 aA	3.57 eA	1.87 dB	0.12 dC
Early III	58.19 bC	87.03 aB	93.94 bA	7.45 dA	2.21 dB	0.83 cC
Late III	55.16 abC	80.45 abB	89.46 bA	8.20 cA	3.35 cB	1.14 cC
Early IV	39.40 cB	79.18 abA	83.03 cA	8.99 bA	6.70 bB	2.82 bC
Late IV	39.40 cB	76.24 bA	81.82 cA	9.24 abA	9.66 aA	2.91 bB
Early V	20.00 dC	76.57 bA	45.45 dB	9.42 aA	10.19 aA	3.38 aB

In a column, means followed by same small letter do not differ significantly by DMRT (P=0.05)
In a row, means followed by same capital letter do not differ significantly by DMRT (P=0.05)

Table 12a. Production efficiency ratio of GmNPV in three different insect species

Instars	Per cent mortality (a)			Yield (x10^8 POB/larva) (b)			Production efficacy (a x b)			Production / virulence *			Production efficiency ratio	
	X_1	X_2	X_3	Y_1	Y_2	Y_3	X_1Y_1	X_2Y_2	X_3Y_3	Z_1	Z_2	Z_3	$Z_2 : Z_1$	$Z_3 : Z_1$
II	63.0	92.24	98.18	3.57	1.87	0.12	225.02	172.49	11.78	38.27	7.54	0.26	0.098:1	0.004:1
Early III	58.9	87.03	93.94	7.45	2.21	0.83	433.52	192.34	77.97	73.73	8.41	1.69	0.109:1	0.022:1
Late III	55.16	80.45	89.46	8.20	3.35	1.14	452.31	269.51	101.98	76.92	11.78	2.22	0.153:1	0.029:1
Early IV	39.40	79.18	83.03	8.99	6.70	2.82	354.21	530.51	234.14	60.24	23.20	5.09	0.302:1	0.066:1
Late IV	39.40	76.24	81.82	9.24	9.66	2.91	364.06	736.48	238.09	61.91	32.20	5.17	0.419:1	0.067:1
Early V	20.00	76.57	45.45	9.42	10.19	3.38	188.40	780.25	153.62	32.04	34.12	3.34	0.444:1	0.043:1

X_1, Y_1, Z_1 : *Galleria mellonella*

X_2,Y_2,Z_2 : *Pericallia ricini*

X_3,Y_3,Z_3 : *Chilo partellus*

* Virulence determined by LC_{50} of hosts against *P. xylostella* (Table 4)

with LC_{50} of 22.87 POB/mm^2. The ratio was lowest in *C. partellus* larval stages with highest LC_{50} (46.02 POB/mm^2). On comparing the production efficiency ratio of *P. ricini* and *C. partellus* with *G. mellonella*, the ratio was highest in the early fifth instar stages of *P. ricini* (0.444) and it was 2.25 fold lower than *G. mellonella.* The ratio was highest in fourth instar larval stages of *C. partellus* (0.067) but it was 14.93 fold lower than *G. mellonella.* Hence GmNPV requires more serial passages through *P. ricini* and *C. partellus* larvae to enhance the virulence against *P. xylostella.*

4.3.3. Relative susceptibility of different larval instars of *G. mellonella, P. ricini* and *C. partellus* to GmNPV

Different instars of *G. mellonella, P. ricini* and *C. partellus* differed in their responses to GmNPV as revealed by the variation in LC_{50} (Table 13). In general, LC_{50} was positively correlated with the age of all the insects tested. The LC_{50} for late instars of *G. mellonella i.e.* IV and V instars was not determined as the mortality rates were below 50 per cent with the concentrations tested. Among the insects tested, *C. partellus* recorded the lowest LC_{50} in all the stages except the second and fifth instars. The responses of second instars of all the three insects were on par with overlapping fiducial limits. For the early and late third instars, LC_{50} in *G. mellonella* and *P. rinici* did not differ significantly. However LC_{50} in *C. partellus* was significantly lower than those of the other two species. LC_{50} of early and late fourth instars of *C. partellus* were 2.08 and 2.96 fold lower than that of *P. ricini* larvae. However, in the early fifth instar stage, *C. partellus* recorded a higher LC_{50} (496.62 POB/mm^2) than that of *P. ricini* (284.31 POB/mm^2).

The time-mortality responses of the three insects tested *viz.*, *G. mellonella, C. partellus* and *P. ricini* differed significantly between the insects for the same age group of larvae with non-overlapping fiducial limits (Table 14). LT_{50} for *G. mellonella* were not determined for fourth and fifth instar larvae as the mortality rates were below 50 per cent. Upto the third instars, *C. partellus* recorded the lowest LT_{50} of 177.98 h followed by *G. mellonella* (215.64 h) and *P. ricini* (252.75 h). *C. partellus* recorded 1.38, 1.35 and 1.19 fold lower LT_{50} than *P. ricini* larvae for early fourth, late fourth and early fifth instar stages respectively.

Table 13. Concentration- mortality responses of different larval instars of three insect species to GmNPV (CBE isolate)

Instar	Insects	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LC_{50} (POB/mm^2)	Fiducial limits
II	*G. mellonella*	197	1.016	0.594 ± 0.089	11.12	5.27 - 26.98
	P. ricini	198	0.261	0.633 ± 0.089	20.89	10.08 – 43.29
	C. partellus	198	2.720	0.862 ± 0.105	12.80	7.15 – 22.48
Early III	*G. mellonella*	198	1.689	0.745 ± 0.114	55.97	27.76 – 149.73
	P. ricini	196	1.624	0.892 ± 0.177	22.33	12.80 – 39.12
	C. partellus	198	1.180	0.709 ± 0.939	18.35	9.43 – 35.48
Late III	*G. mellonella*	198	1.909	0.712 ± 0.113	68.08	32.18 – 203.99
	P. ricini	195	1.166	0.645 ± 0.169	53.85	26.79 – 118.69
	C. partellus	197	1.007	0.679 ± 0.092	21.76	11.00 – 48.41
Early IV	*P. ricini*	196	4.222	0.640 ± 0.179	94.91	46.62 – 228.48
	C. partellus	198	0.992	0.550 ± 0.086	45.68	20.63 – 113.49
Late IV	*P. ricini*	198	0.770	0.525 ± 0.170	160.08	67.07 – 549.31
	C. partellus	198	1.027	0.518 ± 0.085	54.16	23.47 – 146.83
Early V	*P. ricini*	198	1.246	0.699 ± 0.221	284.31	136.88 – 790.56
	C. partellus	197	1.896	0.619 ± 0.014	496.62	208.19 – 1952.77

*All lines are significantly a good fit ($P<0.05$)

Table 14. Time- mortality responses of different instars of three insect species to GmNPV (CBE isolate)

Instar	Insects	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LT_{50} (hours)	Fiducial limits
II	*G. mellonella*	197	0.763	6.331 ± 1.043	195.86	182.79 – 218.39
	P. ricini	198	1.052	9.899 ± 1.362	212.76	202.13 – 222.69
	C. partellus	198	4.514	7.970 ± 1.086	159.09	148.80 – 168.48
Early III	*G. mellonella*	198	1.497	7.882 ± 1.243	210.37	198.07 – 228.13
	P. ricini	196	5.088	9.29 ± 0.428	252.52	239.98 – 269.39
	C. partellus	198	2.546	7.437 ± 0.919	172.46	161.81 – 182.57
Late III	*G. mellonella*	198	1.149	8.041 ± 1.290	215.64	202.86 – 234.99
	P. ricini	195	4.747	9.047 ± 4.259	252.75	239.91 – 270.33
	C. partellus	197	1.395	7.223 ± 0.904	177.98	167.72 – 188.65
Early IV	*P. ricini*	196	2.775	8.890 ± 4.330	261.33	247.59 – 283.26
	C. partellus	198	1.463	6.994 ± 0.905	189.55	176.32 – 199.26
Late IV	*P. ricini*	198	0.746	4.817 ± 4.415	264.27	251.31 – 284.93
	C. partellus	198	1.200	7.478 ± 0.950	195.67	184.79 – 207.55
Early V	*P. ricini*	198	2.907	11.972 ± 5.118	268.96	257.42 – 286.90
	C. partellus	197	2.770	7.306 ± 1.057	225.72	212.56 – 243.88

*All lines are significantly a good fit ($P<0.05$)

4.4. Transovarial transmission of GmNPV

Table 15 depicts the transovarial transmission of GmNPV in *C. binotalis* and *P. ricini*. The parameters of the first generation of virus treated larvae *viz.* fecundity, fertility, per cent larval mortality, per cent pupation and per cent adult emergence revealed that the virus treated generation had significantly higher per cent mortality of larvae of *C. binotalis* (92 per cent) and *P. ricini* (79.8 per cent) than the untreated generation. However other parameters like fecundity, fertility, per cent adult emergence did not differ significantly between treated and untreated populations.

4.4.1. Activity of horizontally and vertically transmitted GmNPV against *P. xylostella*

The activity of horizontally and vertically transmitted GmNPV against *P. xylostella* larvae was compared in terms of LC_{50} (Table 16). Though the LC_{50} of horizontally transmitted GmNPV were 1.26 and 1.32 fold less than that of the vertically transmitted GmNPV for *C. binotalis* and *P. ricini* respectively, however, it did not differ significantly because of the overlapping fiducial limits

4.5. Comparison of infection of GmNPV in different susceptible host tissues

The infection of GmNPV in different susceptible host tissues of *P. xylostella* (Plate 23-28), *M. vitrata* (Plate 29-34) and *C. partellus* (Plate 35-40) was compared (Table17). The per cent infection of nuclei of fat bodies of insect species increased significantly with increase in time after treatment. The presence of polyhedra was observed 48 h post inoculation in *P. xylostella* and 72 h post inoculation in *C. partellus and M. vitrata*. At 120 h post inoculation, 93.28 per cent nuclei were found to contain the POB in *P. xylostella*, while 93.19 per cent and 81.38 per cent of cell nuclei were packed with POB at 158 h for *C. partellus* and *M. vitrata* respectively. The rate of infection did not differ significantly between 72 h and 144 h post treatment in fat bodies of *M. vitrata* larvae.

The polyhedra were assembled in hypodermal cells only in the advanced stage of infection for all the three insect species. The number of polyhedra visible per cell at the

Table 15. Transovarial transmission of GmNPV

Insects		No. of eggs or egg masses per female $	Per cent hatchability $	Per cent larval mortality*	Per cent pupation*	Per cent adult emergence $
C. binotalis	Treated	2.80 ± 0.20**	93.0 ± 1.09	92.0 ± 2.92	8.00 ± 1.30	89.80 ± 4.26
	Control	3.00 ± 0.00**	94.2 ± 0.86	1.80 ± 1.78	98.20 ± 0.80	97.10 ± 1.29
P. ricini	Treated	128.00 ± 2.02	100.00 ± 0.00	79.80 ± 1.77	20.20 ± 1.77	81.39 ± 4.04
	Control	128.40 ± 3.60	100.00 ± 0.00	0.40 ± 0.90	99.60 ± 0.40	85.76 ± 2.26

** Average of 40 eggs/egg mass
$ In a column, the means do not differ significantly at t = 0.05
* In a column, the means differ significantly at t = 0.05

Table 16. Comparison of the activity of horizontally (HT) and vertically transmitted (VT) GmNPV against *P. xylostella*

Category	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LC_{50} (POB/mm^2)	Fiducial limits
C. binotalis					
HT	196	0.413	0.448 ± 0.838	94.13	35.79 – 358.63
VT	197	1.441	0.512 ± 0.087	118.65	49.62 – 390.98
P. ricini					
HT	194	0.444	0.519 ± 0.085	38.28	16.41 – 98.24
VT	195	0.356	0.579 ± 0.088	50.47	23.44 – 121.85

* All lines are significantly a good fit (P<0.05)

Table 17. Comparison of infection of GmNPV (CBE isolate) in different susceptible host tissues

Hours after inoculation	Per cent infection of nuclei of fat bodies *			No. of polyhedra per cell ± S.E.					
				Hypodermis			Fat bodies		
	P. xylostella	*C. partellus*	*M. vitrata*	*P. xylostella*	*C. partellus*	*M. vitrata*	*P. xylostella*	*C. partellus*	*M. vitrata*
48	53.06 c	-	-	-	-	-	7.87 ± 1.99	-	-
72	59.60 bc	36.54 c	33.13 b	-	-	-	10.93 ± 1.84	20.21 ± 2.36	8.87 ± 3.24
96	71.43 b	40.01 c	37.11 b	-	-	-	12.40 ± 1.46	22.17 ± 2.74	10.57 ± 0.85
120	93.28 a	77.75 b	48.41 b	10.93 ± 1.15	14.8 ± 2.95	-	16.47 ± 2.50	20.23 ± 2.54	14.93 ± 2.17
144	-	78.98 b	52.67 b	-	12.1 ± 2.39	13.67 ± 2.23	-	20.40 ± 1.74	18.37 ± 2.30
168	-	93.19 a	81.38 a	-	14.1 ± 1.62	12.33 ± 1.54	-	25.37 ± 4.70	25.17 ± 3.75

* In a column, means followed by the same letter do not differ significantly by DMRT (P=0.05)

Microsections of GmNPV infected *Plutella xylostella* larvae

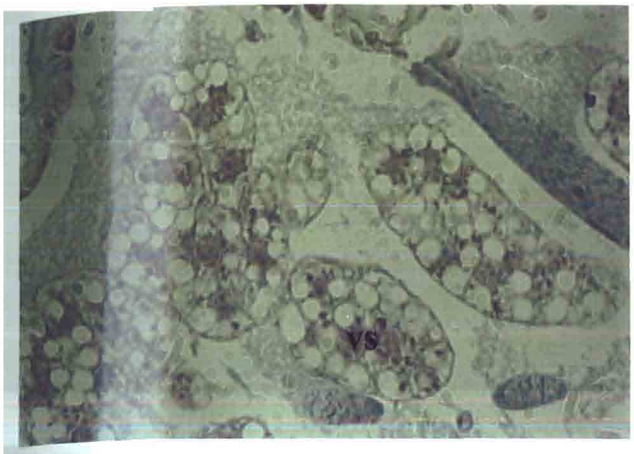


Plate 23. Vacoulation and virogenic stroma (vs)

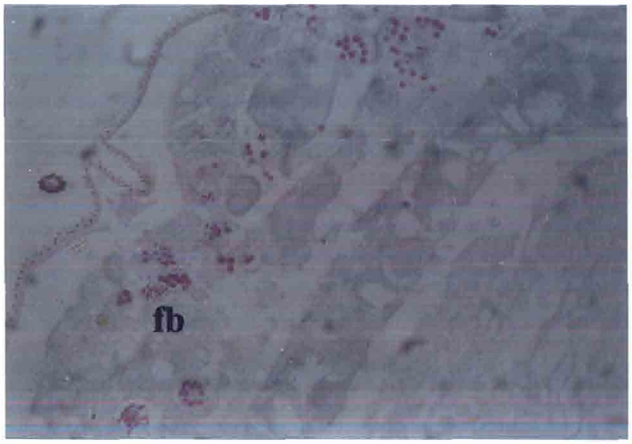


Plate 24. Fat body (fb) infection (72 h)

Plate 25. Refractive midgut epithelium (me)

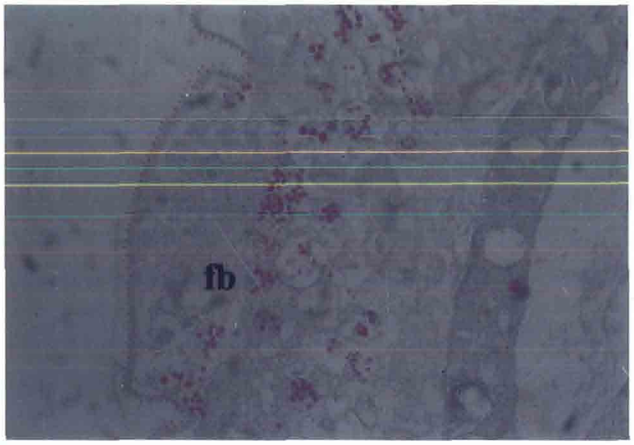


Plate 26. Fat body (fb) infection (96 h)

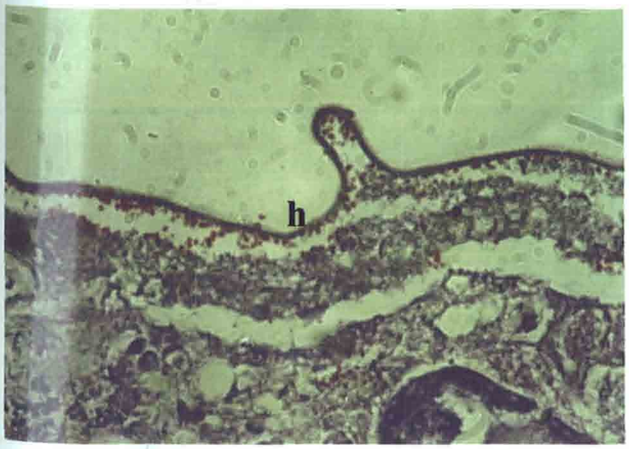


Plate 27. Hypodermal (h) infection (120 h)

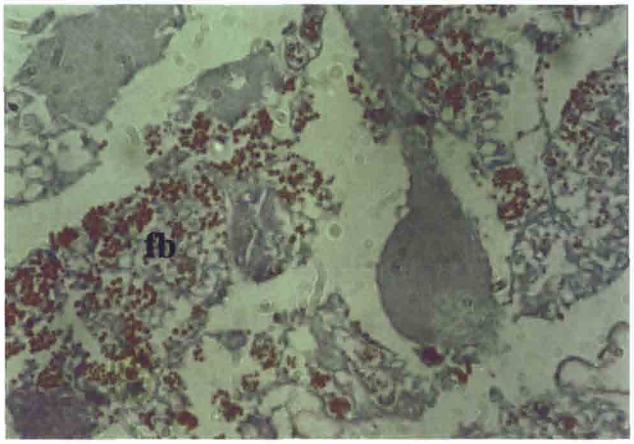


Plate 28. Fat body (fb) infection (120 h)

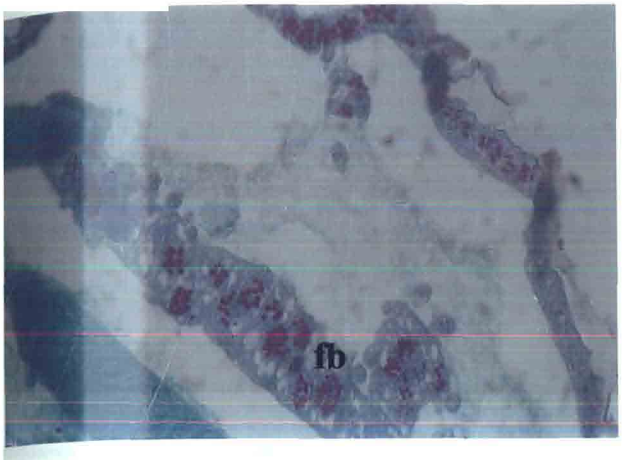


Plate 29. Fat body (fb) infection (96 h)

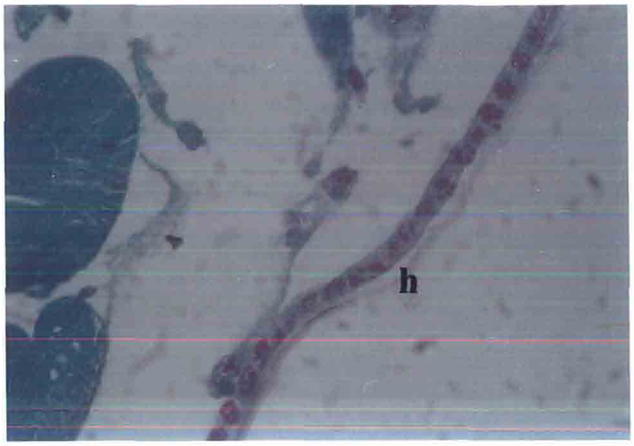


Plate 30. Hypodermal (h) infection

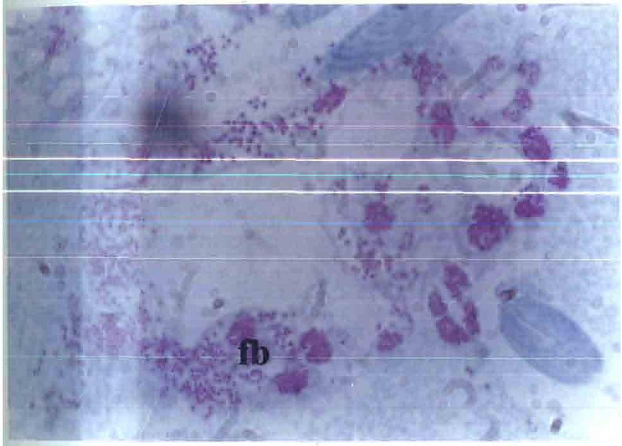


Plate 31. Fat body (fb) infection (144 h)

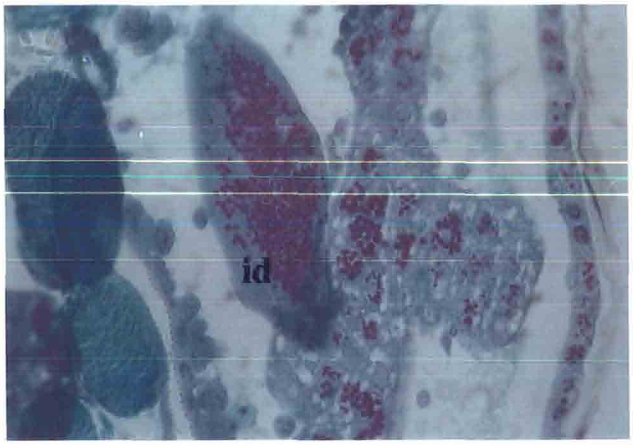


Plate 32. Imaginal disc (id) infection (144 h)

Plate 33. Tracheal matrix (tm) infection

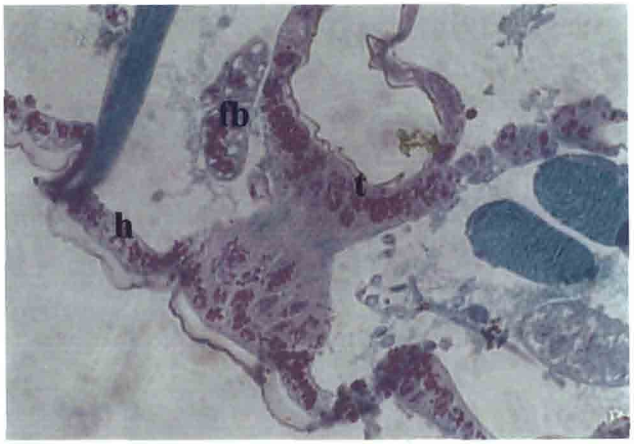


Plate 34. Hypodermal (h), Fat body (fb),

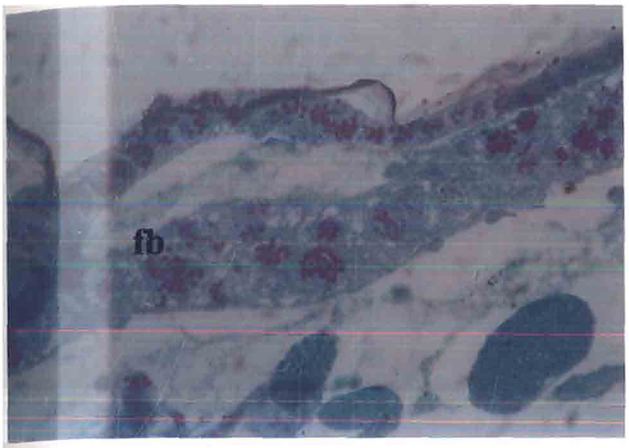


Plate 35. Fat body (fb) infection (96 h)

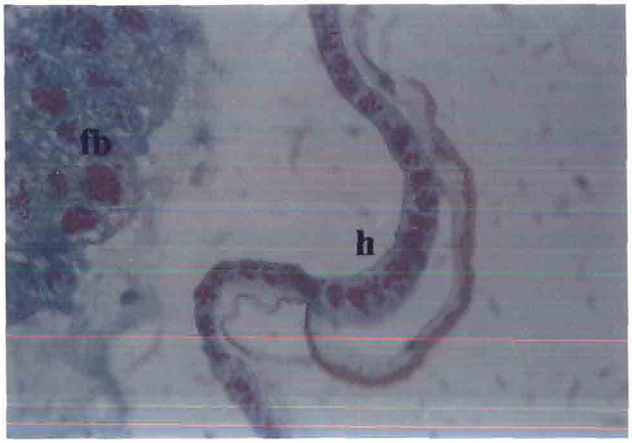


Plate 36. Fat body (fb) and Hypodermal (h) infections (120 h)

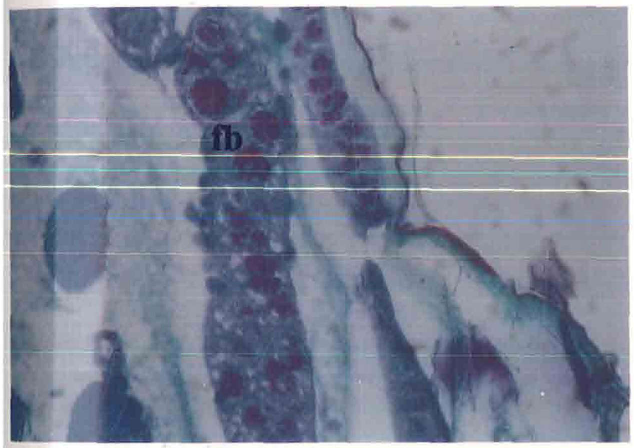


Plate 37. Fat body (fb) infection (144 h)

Plate 38. Few Polyhedra (FP) and Many Polyhedra (MP) cells

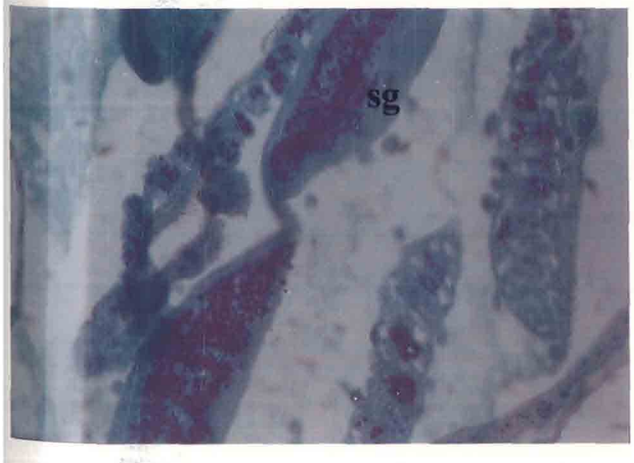


Plate 39. Salivary glands (sg) infection (168 h)

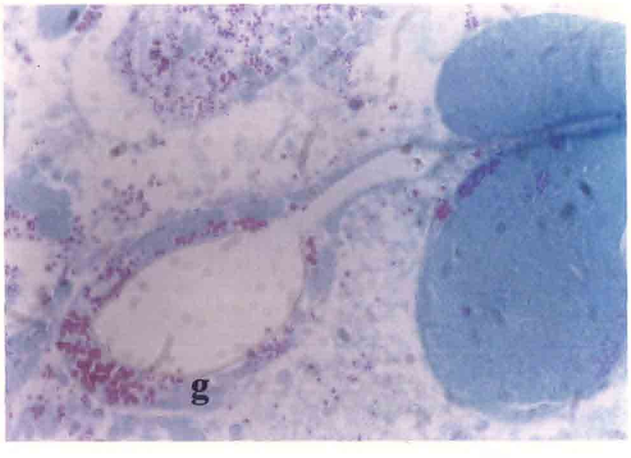


Plate 40. Ganglion (g) infection (168 h)

plane of cutting was 10.9 at 120 h after inoculation for *P. xylostella*, 14.1 and 12.3 at 168 h for *C. partellus* and *M. vitrata* larvae respectively. The polyhedra were observed in the fat body cells in the early stage of infection for insect species tested *i.e.* 48 h post treatments in *P. xylostella* and 72 h in *C. partellus* and *M. vitrata* larvae. The number of polyhedra per cells of fat bodies was highest in *C. partellus* larvae followed by *P. xylostella* and *M. vitrata* upto 120 h after inoculation. However the number of polyhedra per cell of fat bodies did not differ significantly during the period of observation in all the insect species tested. High standard error values in *C. partellus* and *M. vitrata* reveal the presence of few polyhedra cells more frequently in these insects when compared to *P. xylostella*.

4.6. Size of polyhedra of GmNPV after passage through some alternate host insects and *in vitro* through cell line

The size of the polyhedra of GmNPV upon passage through homologous and heterologous hosts showed variation as depicted in Table 18. The passage of GmNPV through the homologous host, *G. mellonella* resulted in the biggest mean polyhedra of size 2.90 µm. This was followed by the size of polyhedra produced *in vitro* in Sf21 cell line (2.75 µm). The difference between the smallest and biggest polyhedra was also maximum in homologous passage to the tune of 2.88 µm.

In general, passage of GmNPV through heterologous hosts yielded smaller polyhedra than when passaged through *G. mellonella*. Among the heterologous hosts, *P. ricini* larvae yielded polyhedra of biggest size (2.29 µm) followed by that of *C. partellus* (2.16 µm) and *C. binotalis* (2.07 µm). The size of polyhedra of GmNPV obtained from *H. undalis* (2.05 µm), *S. exigua* (2.05 µm), *C. sacchariphagus indicus* (2.01 µm) and *C. medinalis* (1.98 µm) were on par. Passage of GmNPV through *M. vitrata*, *A. albistriga*, *P. xylostella* and *D. pulverulentalis* yielded polyhedra of size 1.95, 1.93, 1.90 and 1.91 µm respectively and they did not differ significantly. Polyhedra obtained from *C. infuscatellus* and *O. arenosella* were of the size of 1.85 µm. Passage of GmNPV through *E. atomosa* and *E. merione* had the lowest size of 1.63 µm and 1.67 µm respectively. Based on the upper and lower limits, the maximum and minimum size of

Table 18. Size of polyhedra of GmNPV after passage through some alternate host insects and *in vitro* passaged through Sf21 cell line

GmNPV passed through	Size of polyhedra * µm ± S.E.	GmNPV passed through	Size of polyhedra * µm ± S.E.
G. mellonella	2.90 a ± 0.245 (2.07 – 4.95)	*P. ricini*	2.29 c ± 0.164 (1.70 – 2.98)
P. xylostella	1.90 gh ± 0.110 (1.29 – 2.40)	*E. merione*	1.67 I ± 0.068 (1.35 – 1.91)
C. binotalis	2.07 de ± 0.125 (1.65 – 2.88)	*C. medinalis*	1.98 efg ± 0.078 (1.70 – 2.45)
H. undalis	2.05 ef ± 0.141 (1.42 – 2.83)	*O. arenosella*	1.85 h ± 0.098 (1.33 – 2.33)
M. vitrata	1.95 fgh ± 0.134 (1.44 – 2.53)	*D. pulverulentalis*	1.91 gh ± 0.084 (1.59 – 2.36)
E. atomosa	1.63 i ± 0.121 (1.18 – 2.28)	*S. exigua*	2.05 ef ± 0.120 (1.45 – 2.58)
C. partellus	2.16 d ± 0.105 (1.60 – 2.64)	*C. infuscatellus*	1.85 h ± 0.148 (1.07 – 2.98)
C. sacchaiphagus indicus	2.01 efg ± 0.126 (1.44 – 2.71)	*A. albistriga*	1.93 fgh ± 0.102 (1.26 - 2.13)
***In vitro* passaged Sf21 cell line**	2.75 b ± 0.124 (2.04 – 3.26)		

In a column, means followed by the same letter do not differ significantly by DMRT (P=0.05)

* Figures in parenthesis denote the upper and lower limit

polyhedra of 2.98 μm and 1.07 μm were witnessed with the passage through *P. ricini* and *C. infuscatellus* respectively among the heterologous hosts.

4.7. *In vitro* studies with GmNPV

4.7.1. Permissibility of cell lines to GmNPV isolates

All the four cell lines tested *viz.*, SlH, SlPG, Sf21 and HaH were found to be susceptible to both the isolates of GmNPV passaged through *C. partellus* with varied frequencies (Table 19). Among the cell lines, Sf21 was the most susceptible with 92.99 per cent and 84.12 per cent infection for CBE and BNGL isolate respectively (Plate 41 and 42). The susceptibility of SlH and SlPG was on par for CBE isolate but differed significantly for BNGL isolate (Plate 43 – 46). HaH was least susceptible with 12.07 per cent and 5.32 per cent cell infection respectively for CBE and BNGL isolate respectively (Plate 47 and 48).

The yield of POB/ml from cell lines followed the same trend. In both the virus isolates highest yield was obtained in Sf21 cell line while the lowest yield was recorded in HaH cell line. Infection rates as well as yield of virus did not differ significantly between the isolates.

4.7.2. Susceptibility of Sf21 cell line to GmNPV obtained from the haemolymph of different insects

Virus from *C. partellus* and *P. ricini* recorded 81.48 per cent and 75.61 per cent infection respectively of Sf21 cell line and they were on par. This was followed by that of *M. vitrata* (47.72 per cent) and *H. undalis* (35.65 per cent) for CBE isolate. Virions from *C. partellus* recorded the highest cell infection of 82.48 per cent for BNGL isolate followed by that of *P. ricini* (70.42 per cent) and *M. vitrata* (40.46 per cent). Haemolymph source from *H. undalis* recorded the lowest infection of 25.34 per cent (Table 20).

The yield of POB/ml of cells was highest for virus from *C. partellus* which was on par with *P. ricini* for both the isolates. This was followed by *M. vitrata.* Virions from

GmNPV infection in Sf21 cell line

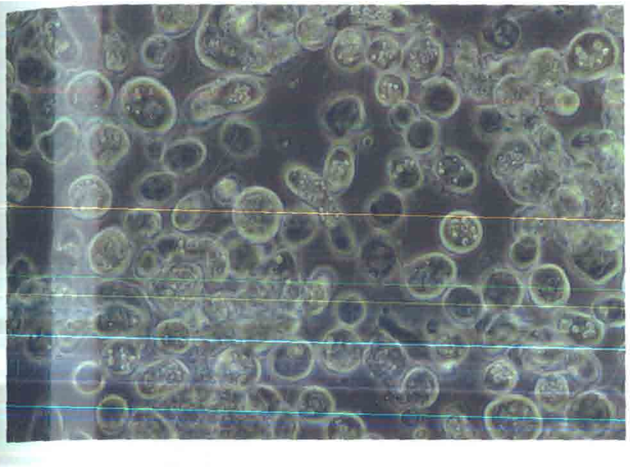


Plate 41. CBE isolate

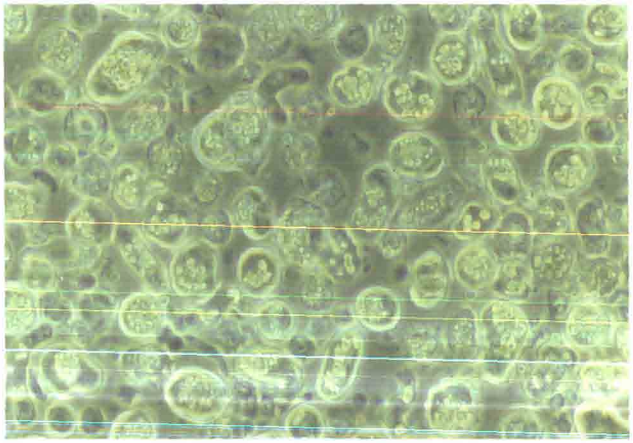


Plate 42. BNGL isolate

GmNPV infection in *Spodoptera litura* larval Haemocyte (SlH) cell line

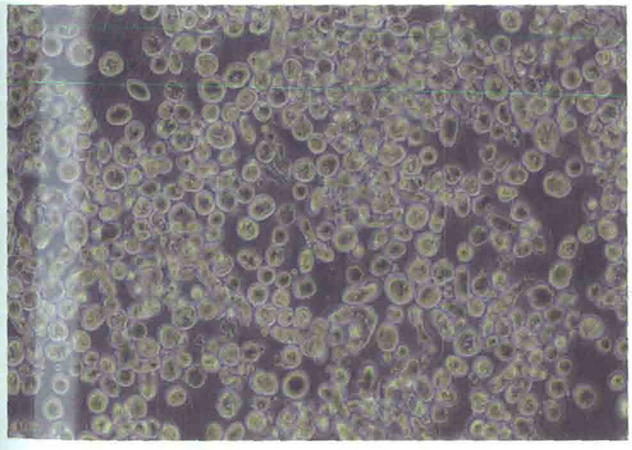


Plate 43. CBE isolate

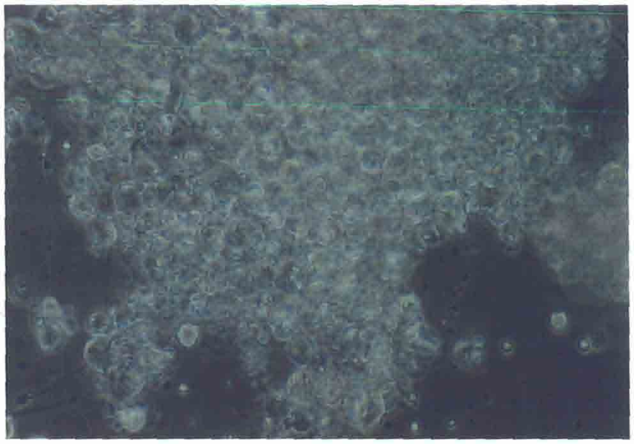


Plate 44. BNGL isolate

Table 19. Permissibility of cell lines to GmNPV isolates

Cell lines	Per cent cells infected ± SE		Yield ± SE (x 10^7 POB/ml)	
	CBE	BNGL	CBE	BNGL
SlH	44.99 b ± 3.452	42.37 b ± 3.246	3.25 b ± 0.061	3.14 b ± 0.072
SlPG	36.48 b ± 2.287	25.12 c ± 1.944	2.01 b ± 0.063	1.68 c ± 0.047
Sf21	92.99 a ± 1.496	84.12 a ± 1.874	28.24 a ± 3.435	25.40 a ± 1.499
HaH	12.07 c ± 2.815	5.32 d ± 1.771	0.04 c ± 0.013	0.03 d ± 0.014

In a column, the means followed by same letter do not differ significantly by DMRT (P=0.05)
In a row, the means of the two isolates do not differ significantly at t=0.05

Table 20. Susceptibility of Sf21 cell line to GmNPV isolates from different insect haemolymph sources

Haemolymph source	Per cent cell infected ± SE		Yield ± SE (x 10^7 POB/ml)	
	CBE	BNGL	CBE	BNGL
P. ricini	75.61 a ± 2.879	70.42 b ± 2.379	22.37 ab ± 2.017	19.81 a ± 2.576
C. partellus	81.48 a ± 1.685	82.48 a ± 2.683	26.72 a ± 2.733	20.46 a ± 1.847
H. undalis	35.65 c ± 2.671	25.34 d ± 2.111	5.82 c ± 0.941	1.88 c ± 1.530
M. vitrata	47.72 b ± 2.620	40.46 c ± 2.239	16.52 b ± 2.249	11.74 b ± 1.860

In a column, the means followed by same letter do not differ significantly by DMRT (P=0.05)

In a row, the means of the two isolates do not differ significantly at t=0.05

GmNPV infection in *Spodoptera litura* Pupal Gonad (SlPG) cell line

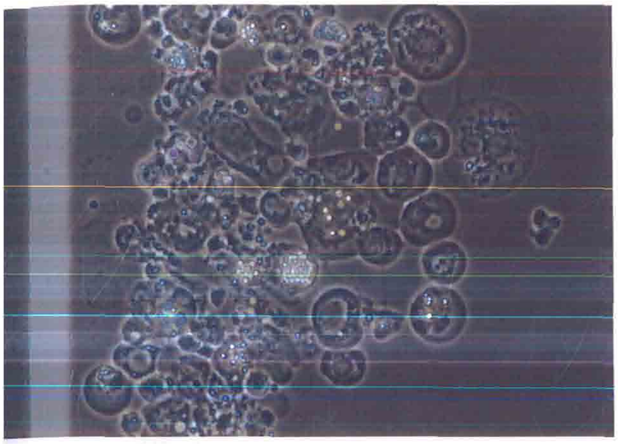


Plate 45. CBE isolate

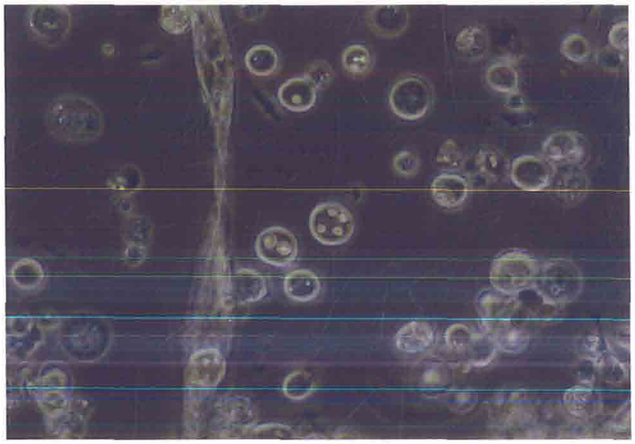


Plate 46. BNGL isolate

GmNPV infection in *Helicoverpa armigera* Haemocyte (HaH) cell line

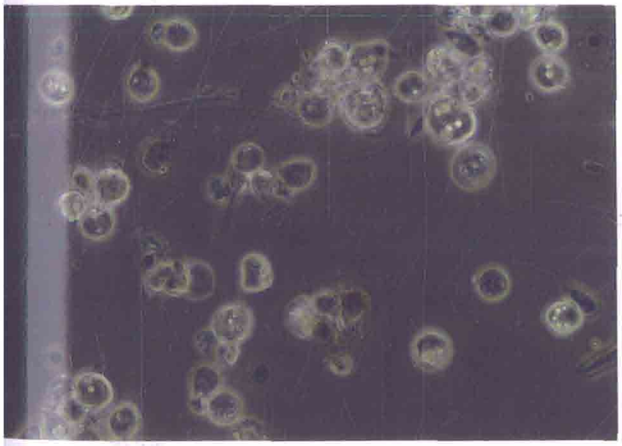


Plate 47. CBE isolate

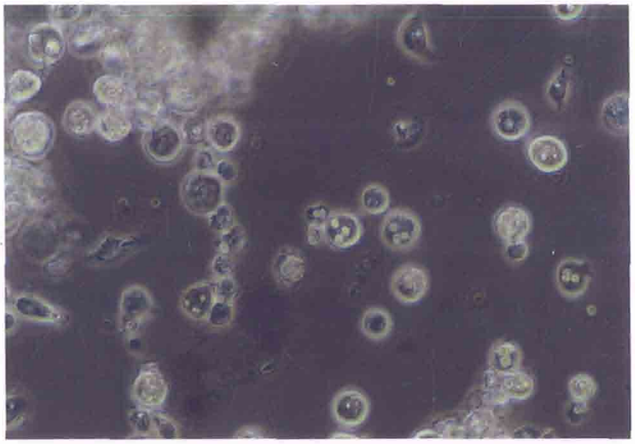


Plate 48. BNGL isolate

H. undalis larvae produced the lowest yield of virus in both CBE and BNGL isolates. Irrespective of haemolymph source, the isolates did not differ significantly for both the parameters (per cent cell infection and yield).

4.7.3. Comparison of *in vivo* and *in vitro* produced GmNPV

The LC_{50} of *in vitro* and *in vivo* produced GmNPV did not differ significantly irrespective of the insect haemolymph source as seen by the overlapping fiducial limits against *P. xylostella* larvae (Table 21). However, in the case of *H. undalis*, *in vitro* produced GmNPV recorded a 1.27 fold increase in LC_{50} when compared to that of *in vivo* produced GmNPV.

4.7.4. Comparison of *in vitro* produced GmNPV from different cell lines

GmNPV produced in Sf21 cell line recorded the lowest LC_{50} of 42.13 POB/mm^2 followed by SlH (57.36 POB/mm^2) and SlPG (63.11 POB/mm^2) (Table 22). On comparing with LC_{50} of *in vivo* produced GmNPV (54.81 POB/mm^2), it is clear that LC_{50} did not differ significantly between the *in vivo* and *in vitro* produced GmNPV as evidenced by the overlapping of fiducial limits.

4.8. Determination of chitinase activity of GmNPV

The chitinase activity in terms of N-acetyl glucosamine released in larvae of *G. mellonella* and *C. partellus* infected with GmNPV was compared with that of HaNPV and SlNPV at hourly intervals (Table 23). In all the three NPVs tested, the chitinase activity increased with increase in time after treatment to a certain period and stabilized thereafter. Among the three NPVs tested, HaNPV infection showed significantly highest chitinase activity at the end of each observation at 24 h interval. The activity was minimum (94.55 μg/g) at 24 h and it steadily increased and the maximum of 151.11 μg/g was witnessed at the end of 192 h post treatment. The activity got stabilized after 144 h post treatment. A similar trend was observed with SlNPV where the maximum chitinase activity was recorded at 168 h post treatment (148.89 μg/g). Though the activity of SlNPV was slightly lower than that of HaNPV, it was on par at 120, 168 and 192 h post inoculation.

Table 21. Concentration mortality response of early third instar larvae of *P. xylostella* to *in vivo* and *in vitro* produced GmNPV from different insect haemolymph sources

Category	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LC_{50} (POB/mm^2)	Fiducial limits
P. ricini					
In vivo	195	0.431	0.573 ± 0.088	47.687	21.991 – 115.25
In vitro	197	0.162	0.620 ± 0.089	38.120	18.416 – 84.151
C. partellus					
In vivo	196	0.245	0.451 ± 0.083	50.604	19.479 –161.552
In vitro	195	0.164	0.451 ± 0.082	40.198	15.331 – 122.086
H. undalis					
In vivo	194	0.509	0.529 ± 0.086	37.684	16.367 – 95.213
In vitro	196	0.600	0.494 ± 0.085	47.947	19.880 – 135.226
M. vitrata					
In vivo	195	0.295	0.598 ± 0.088	38.444	18.174 – 87.386
In vitro	197	0.191	0.649 ± 0.091	33.293	16.458 – 70.403

* All lines are significantly a good fit ($P<0.05$)

Table 22. **Concentration mortality response of early third instar larvae of *P. xylostella* to *in vivo* and *in vitro* produced GmNPV from different cell lines**

Cell line	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LC_{50} (POB/mm^2)	Fiducial limits
SlH	196	0.483	0.459 ± 0.082	57.36	22.46 – 184.29
SlPG	195	0.747	0.521 ± 0.086	63.11	27.26 – 174.95
Sf21	196	0.640	0.467 ± 0.083	42.13	16.49 – 126.01
In vivo	196	0.802	0.412 ± 0.082	54.81	19.47 – 203.57

* All lines are significantly a good fit ($P<0.05$)

Table 23. **Determination of chitinase activity of GmNPV in comparison with HaNPV and SlNPV**

Hours after treatment	**N-acetyl glucosamine released (μg/g/h)***			
	GmNPV passed through		**HaNPV**	**SlNPV**
	G. mellonella	*C. partellus*		
24	BDL**	BDL	94.55 dA	54.44 gB
48	13.33 gC	7.45 eD	114.45 cA	68.89 bB
72	16.11 gC	8.89 eD	132.22 bA	96.67 dB
96	22.67 fC	18.89 dC	112.22 cA	82.22 cB
120	95.55 dB	37.78 cC	131.11 bA	125.00 cA
144	121.67 cC	56.67 bD	147.78 aA	135.55 bB
168	84.44 eB	46.67 bC	145.56 aA	148.89 aA
192	128.89 bcB	55.55 bC	151.11 aA	145.56 abA
216	146.67 aA	97.78 aB	-	-
240	136.66 bA	88.89 aB	-	-

* In a column, means followed by same small letters do not differ significantly by DMRT at P=0.05

* In a row, means followed by same capital letters do not differ significantly by DMRT at P=0.05

** Below detectable level

On the whole, GmNPV recorded the lowest chitinase activity among the three NPVs tested. The activity commenced only after 48 h post treatment and it was as low as 13.33 µg/g and 7.45 µg/g respectively in homologous (*G. mellonella*) and heterologous host (*C. partellus*) at the beginning. The activity reached its peak only after 216 h post treatment with 146.67 µg/g and 97.78 µg/g respectively for homologous and heterologous hosts. At the end of each observation the chitinase activity of GmNPV was significantly higher in homologous host than heterologous host. It is interesting to note that the chitinase activity of GmNPV lowered at 168 h post treatments irrespective of hosts and stabilized thereafter.

4.9. Persistence of GmNPV on cauliflower phyllosphere

The activity of GmNPV exposed to sunlight for different periods of time against *P. xylostella* larvae is presented in Table 24. The activity of GmNPV showed a declining trend with increase in exposure to sunlight and it persisted for six days after exposure. However, the virus lost 50 per cent of its activity at the end of two days of exposure to sunlight. The per cent Original Activity Remaining (OAR) was low at 16.67 after five days of exposure while it was meager (2.22 per cent) at the end of six days of exposure.

Passage of persisted virus through *P. xylostella* and subsequent exposure to sunlight for five times did not enhance the persistence of GmNPV significantly except at the end of three and six days of exposure. The per cent OAR was significantly greater at the end of six days of exposure in the fourth and fifth passages than that of the previous passages. At the end of one and five days post exposure, the per cent OAR was on par for all the passages. The rate of increase of per cent OAR was rather gradual at the end of three days of exposure.

4.9.1. Effect of microclimatic conditions on the persistence of GmNPV

The influence of microclimatic conditions on the persistence of GmNPV was determined for a period of eight months (Sep 2001 - April 2002) (Table 25). During the period of observation, GmNPV was found to persist on cauliflower leaf phyllosphere for five days. The persistence of GmNPV, measured in terms of its activity against

Table 24. **Persistence of GmNPV (CBE isolate) on cauliflower phyllosphere**

Days of exposure	Passages									
	I		II		III		IV		V	
	Per cent mortality	% OAR	Per cent mortality	% OAR	Per cent mortality	% OAR	Per cent mortality	% OAR	Per cent mortality	% OAR
0	96.00 aA	-	96.00 aA	-	94.00 aA	-	98.00 aA	-	98.00 aA	
1	68.00 bA	71.11 aA	70.00 bA	72.89 aA	70.00 bA	74.56 aA	74.00 bA	75.78 aA	78.00 bA	79.78 aA
2	48.00 cC	50.45 bB	52.00 cBC	54.00 bB	64.00 bA	68.78 aA	60.00 bAB	61.33 bAB	62.00 cAB	63.56 bAB
3	40.00 cdAB	41.78 bcAB	34.00 dB	35.33 cB	46.00 cAB	49.11 bAB	52.00 cA	53.11 bA	54.00 cdA	55.56 bcA
4	28.00 deB	29.11 cdB	36.00 dAB	39.78 cAB	40.00 cA	42.89 bA	38.00 cAB	38.67 cAB	42.00 dA	42.89 cA
5	16.00 eA	16.67 dA	20.00 eA	20.44 dA	18.00 dA	19.44 cA	22.00 dA	22.67 dA	26.00 eA	26.89 dA
6	2.00 fBC	2.22 e BC	0.00 eC	0.00 eC	6.00 eB	6.22 dB	16.00 dA	16.44 dA	20.00 eA	20.44 dA

In a column, mean followed by same small letter do not differ significantly by DMRT (P=0.05)
In a row, mean followed by same capital letter do not differ significantly by DMRT (P=0.05)
OAR - Original Activity Remaining

Table 25. Effect of microclimatic conditions on the persistence of GmNPV (CBE isolate)

Months	Microclimatic parameters*					Per cent mortality of *P. xylostella* days after exposure**				
	Max T°C	Min T°C	RH 1 (%)	RH 2 (%)	Solar radiation (cal./m^2)	1	2	3	4	5
September	32.1 ± 0.06	23.0 ± 0.06	86.6 ± 0.58	61.8 ± 0.51	373.5 ± 9.00	69.0 b	48.6 cd	40.0 c	19.4 cd	12.6 b
October	31.0 ± 0.18	22.3 ± 0.12	90.6 ± 0.35	68.8 ± 0.79	358.8 ± 7.88	72.0 ab	52.8 bc	44.8 c	21.4 c	11.4 b
November	28.5 ± 0.08	20.6 ± 0.13	93.2 ± 0.09	78.8 ± 1.23	368.1 ± 11.14	74.2 a	58.0 b	50.8 b	30.6 b	19.2 a
December	26.2 ± 0.23	20.8 ± 0.03	93.6 ± 0.09	83.8 ± 1.10	225.7 ± 11.52	76.8 a	66.2 a	57.0 a	44.4 a	25.0 a
January	31.8 ± 0.09	28.6 ± 0.07	90.4 ± 0.23	48.8 ± 0.52	403.0 ± 5.65	54.4 c	44.4 de	34.0 d	13.0 cd	3.8 c
February	33.1 ± 0.06	17.1 ± 0.17	82.0 ± 0.50	18.4 ± 0.53	513.1 ± 1.13	48.0 d	28.8 f	21.2 e	7.8 e	1.4 cd
March	35.1 ± 0.06	23.0 ± 0.12	88.2 ± 0.53	38.8 ± 0.82	423.5 ± 3.77	52.2 cd	41.2 e	29.2 d	16.2 d	9.4 b
April	36.7 ± 0.10	24.3 ± 0.07	83.2 ± 0.52	35.4 ± 0.80	439.8 ± 3.71	48.8 cd	39.0 e	18.6 e	9.0 e	0.4 d

* Average of five days period of exposure ± S.E.

** In a column, mean followed by the same letters do not differ significantly by DMRT (P=0.05)

P. xylostella, declined with increase in time of exposure after treatment. The activity of GmNPV was highest in the month of December with significantly higher mortality rate of more than 57 per cent at the end of three days of exposure and the activity was maintained with mortality of 25 per cent at the end of five days of exposure. GmNPV was relatively more persistent in the month of November and December as evidenced by higher activity against *P. xylostella* than that of September.

The low persistence of GmNPV was witnessed in the month of February and April with a loss of 52 per cent activity at the end of the one day of exposure. During January and March, the activity of GmNPV was on par upto four days after exposure and the activity diminished by 93 per cent and 82 per cent at the end of five days of exposure when compared to the activity at the end of one day of exposure.

The correlation of microclimatic weather parameters with the persistence of GmNPV is depicted in Table 26. There was correlation between maximum temperature, relative humidity at 14.30 h solar radiation and per cent mortality of *P. xylostella*. The relative humidity at 7.30 h and minimum temperature did not have any effect on the persistence of GmNPV. The maximum temperature and solar radiation were negatively correlated with the per cent mortality of *P. xylostella* while relative humidity at 14.30 h was positively correlated with the same.

4.10. Efficacy of different dosages of GmNPV in pot culture assay

4.10.1. *P. xylostella*

The mortality of *P. xylostella* larvae increased with increase in dose of GmNPV and period of exposure (Table 27). Observations on the per cent mortality of *P. xylostella* larvae taken at four, six and eight days post treatment showed significant differences among the treatments on eight days post treatment. Highest mortality of 60 per cent was observed with the highest dose of 1 x 10^8 POB/ml followed by the dose of 5 x 10^7 POB/ml (56.67 per cent). The effects of doses of 2.50 x 10^7, 1.25 x 10^7 and 6.25 x 10^6, 3.13 x 10^6 POB/ml were on par. However all the treatments were effective in debilitating the larval population of *P. xylostella* when compared to the control.

Table 26. Correlation matrix of microclimatic weather parameters with the persistence of GmNPV

	Maximum T^{o}C	Minimum T^{o}C	RH (7.30 h)	RH (14.30 h)	Solar radiation	Per cent mortality
Maximum T^{o}C	1.000	0.219	-0.510	-0.738	0.491	-0.394*
Minimum T^{o}C	0.219	1.000	0.319	0.080	-0.141	0.045
RH (7.30 h)	-0.510	0.139	1.000	0.672	-0.422	0.234
RH (14.30 h)	-0.738	0.080	0.672	1.000	-0.739	0.437**
Solar radiation	0.491	-0.144	-0.422	-0.739	1.000	-0.434**
Per cent mortality	-0.398*	-0.045	0.234	0.437**	-0.434**	1.000

* Correlation significant at the 0.05 level

** Correlation significant at the 0.01 level

Table 27. Effect of different dosages of GmNPV (CBE isolate) on *P. xylostella* by pot culture assay

Treatments (POB/ml)	Per cent mortality of larvae (Days after treatment)		
	4	6	8
1.00×10^8	28.33 a	49.17 a	60.00 a
5.00×10^7	25.85 a	48.34 a	56.67 ab
2.50×10^7	21.67 a	42.50 ab	50.00 bc
1.25×10^7	13.33 b	35.00 bc	43.33 b
6.25×10^6	10.83 bc	27.50 cd	32.50 d
3.13×10^6	6.67 c	25.00 d	28.33 d
Control	1.67 d	2.50 e	2.50 e

In a column, means followed by the same letter do not differ significantly by DMRT (P=0.05)

4.10.2. *C. binotalis*

Similar trend was observed with the population of *C. binotalis* (Table 28). There was moderate increase in per cent mortality of *C. binotalis* larva with all the doses tested a day after first spray. Due to the subsequent spray at five days interval, there was a drastic increase in per cent mortality of the larvae on 11th and 13th day observations. Plants sprayed with GmNPV @ 1 x 10^8 POB/ml recorded the highest larval mortality of 70 per cent followed by doses of 5 x 10^7 (63.33 per cent) and 25 x 10^7 POB/ml (54.17 per cent). The doses of 6.25 x 10^6 and 3.13 x 10^6 POB/ml were equally effective against *C. binotalis*.

4.10.3. *H. undalis*

Application of GmNPV @ 1 x 10^8 POB/ml and 5 x 10^7 POB/ml was the most effective with on par mortality rates of 59.17 per cent and 55.83 per cent respectively at the end of 11 days post treatment (Table 29). Subsequent doses of 2.5 x 10^7 POB/ml and 1.25 x 10^7 POB/ml followed suit and their effects were on par. The effect of doses of 6.25 x 10^6 POB/ml and 3.13 x 10^6 POB/ml did not differ significantly with on par mortality rates of 31.67 per cent and 27.50 per cent respectively. However drastic increase in the mortality rates of *H. undalis* was not observed in the subsequent count after second spray at five days interval as that of *C. binotalis*.

4.11. Field efficiency of GmNPV

4.11.1. *P.xylostella*

Pre-treatment counts showed the larval population in the range of 19.56-23.42 per 10 plants. Observations on larval population taken on four, six and eight days post treatment showed significant reduction in all the treatments (Table 30; Plate 49). Eight days after first spray, indoxacarb was found to be the most effective in reducing the larval population (9.03 larvae/10 plants) followed by *B.t.* (14.20 larvae/10 plants). GmNPV @ 7.5 x 10^{13} and 3.75 x 10^{13} POB/ha were as effective as PxGv (1.5 x 10^{13} OB/ha) followed by GmNPV @ 1.88 x 10^{13} POB/ha.

Table28. **Effect of different dosages of GmNPV (CBE isolate) on *C. binotalis* by pot culture assay**

Treatments (POB/ml)	Per cent mortality of larvae (Days after treatment)				
	5	7	9	11	13
1.00×10^8	15.00 a	29.99 a	31.67 a	55.83 a	70.00 a
5.00×10^7	15.83 a	25.00 ab	30.00 ab	48.34 ab	63.33 b
2.50×10^7	14.17 a	25.33 ab	25.00 abc	42.50 b	54.17 c
1.25×10^7	13.33 a	20.00 bc	22.50 bcd	33.33 c	41.67 d
6.25×10^6	9.17 b	15.83 c	20.00 cd	27.50 cd	34.17 e
3.13×10^6	3.33 b	14.17 c	15.84 d	23.33 d	31.67 e
Control	0.00 c	0.83 d	2.00 e	2.50 e	7.50 f

In a column, means followed by the same letter do not differ significantly by DMRT ($P= 0.05$)

Table 29. **Effect of different dosages of GmNPV (CBE isolate) on *H. undalis* by pot culture assay**

Treatments (POB/ml)	**Per cent mortality of larvae (Days after treatment)**			
	5	**7**	**9**	**11**
1.00×10^8	29.17 a	34.17 a	45.83 a	59.17 a
5.00×10^7	26.67 a	30.00 ab	44.17 a	55.83 a
2.50×10^7	21.67 ab	24.17 bc	39.17 ab	46.67 b
1.25×10^7	17.50 bc	20.00 cd	31.67 bc	41.67 b
6.25×10^6	14.17 cd	17.50 cd	26.67 c	31.67 c
3.13×10^6	10.00 d	15.00 d	24.17 c	27.50 c
Control	0.96 e	2.50 e	5.00 d	7.50 d

In a column, means followed by the same letter do not differ significantly by DMRT (P= 0.05)

Table 30. **Field efficacy of GmNPV against *P. xylostella***

Treatments	Pre count	Number of larvae per ten plants (Days after treatment)												% reduction
		I spray			II spray			III spray			IV spray			
		4	6	8	4	6	8	4	6	8	4	6	8	
GmNPV 1.88×10^{13} (POB/ha)	23.05 b	19.40 c	17.27 d	15.73 d	14.47 d	11.47 c	11.17 b	15.50 d	13.07 d	11.70 bc	9.47 c	8.20 bc	8.90 c	60.84
GmNPV 3.75×10^{13} (POB/ha)	22.67 b	19.00 c	17.27 d	14.40 c	13.40 c	11.40 c	11.70 bc	13.67 c	12.20 c	11.27 bc	9.10 c	8.53 bc	8.70 c	61.72
GmNPV 7.50×10^{13} (POB/ha)	22.45 b	18.90 c	16.33 c	15.17 c	13.47 c	10.63 b	9.93 a	14.33 c	11.27 b	10.93 b	7.80 b	7.60 b	7.80 b	65.68
PxGV 1.5×10^{13} (OB/ha)	20.82 a	21.33 d	17.17 d	15.00 c	15.47 e	14.37 d	9.60 a	16.40 d	11.97 bc	11.14 b	12.33 d	10.50 d	7.77 b	65.82
B.t. (0.5 kg /ha)	19.56 a	10.40 b	12.33 b	14.20 b	12.00 b	14.37 d	11.93 c	9.60 b	11.70 bc	12.10 c	7.33 b	9.47 cd	9.23 c	59.39
Indoxacarb (29 g a.i./ha)	20.95 a	4.27 a	6.60 a	9.03 a	7.03 a	7.57 a	9.63 a	7.10 a	6.53 a	8.80 a	3.93 a	5.70 a	5.93 a	73.91
Control	23.42 b	23.60 e	22.10 e	25.67 e	20.30 f	22.43 e	21.50 d	22.07 e	21.50 e	21.13 d	21.20 e	20.50 e	22.73 d	-

* In a column, mean followed by the same letter do not differ significantly by DMRT ($P=0.05$)

Plate 49. Field trial on efficacy of GmNPV

Observation on the larval count eight days after second spray showed that GmNPV @ 7.5 x 10^{13} was as effective as PxGV and indoxacarb in reducing the larval population followed by GmNPV @ 1.88 x 10^{13} and 3.75 x 10^{13} POB/ha. The plots treated with *B.t.* stood next.

In the subsequent two sprays also indoxacarb was the most effective in reducing the larval population by 73.91 per cent over control. Among the other treatments, GmNPV @ 7.5 x 10^{13} POB/ha and PxGV were equally effective in reducing the larval population by 65.68 and 65.82 per cent respectively. GmNPV @ 3.75 x 10^{13} and 1.38 x 10^{13} POB/ha stood next with the total reduction of approximately 61 per cent of larval population. These were on par with *B.t.* recording 59.39 per cent reduction in larval population over control. Control plots recorded significantly the highest larval population in the range of 20.30 - 25.67 larvae/10 plants at the end of each spray.

4.11.2. *C. binotalis*

The larval population of *C. binotalis* in pre-treatment count was in the range of 9.45-11.22/10 plants (Table 31). Observations on the larval population on four, six and eight day post treatment in all the sprays revealed that indoxacarb was the most effective in checking the population of *C. binotalis*. The reduction in larval population in this treatment was 70.95, 73.65, 84.67 and 64.22 per cent over control eight days after I, II, III and IV sprays respectively. The highest dose of GmNPV @ 7.5 x 10^{13} POB/ha stood next to indoxacarb with the larval population of 5.37, 4.07, 4.93 and 4.10/10 plants eight day after I, II, III and IV sprays respectively. The reduction in larval population was 62.39 per cent over control at the end of four subsequent sprays.

GmNPV @ 3.75 x 10^{13} and 1.88 x 10^{13} POB/ha exhibited equal efficacy. The plots treated with these doses of GmNPV recorded larval population of 4.27 and 4.33/10 plants at eight days after fourth spray respectively. The larval population in these plots did not differ significantly from those of *B.t.* - treated plots at the end of I spray while they were superior over the *B.t.* application at the end of II, III and IV sprays. Based on the debilitation of larval population, *B.t.* was more effective than GmNPV only at four

Table 31. Field efficacy of GmNPV against *C. binotalis*

Treatments	Pre count	Number of larvae per ten plants days after treatment *												% reduction
		I spray			II spray			III spray			IV spray			
		4	6	8	4	6	8	4	6	8	4	6	8	
GmNPV 1.88×10^{13} (POB/ha)	10.36 a	10.40 d	7.70 c	6.40 c	6.43 d	5.37 dc	4.63 c	6.00 c	5.46 c	5.13 bc	5.20 d	4.63 c	4.33 b	60.28
GmNPV 3.75×10^{13} (POB/ha)	9.45 a	9.45 c	7.80 c	6.07 c	5.87 cd	4.97 d	4.67 c	6.13 c	5.27 c	5.23 bc	5.00 cd	4.27 c	4.27 b	60.82
GmNPV 7.50×10^{13} (POB/ha)	10.22 a	9.87 c	7.67 c	5.37 b	5.60 c	4.47 c	4.07 b	6.07 c	5.13 c	4.93 b	4.77 c	4.47 c	4.10 ab	62.38
PxGV 1.5×10^{13} (OB/ha)	10.56 b	10.87 d	10.67 d	10.67 d	10.53 e	10.33 e	10.80 e	10.67 d	10.77 d	10.93 d	10.73 e	10.77 d	10.70 d	1.83
***B.t.* (0.5 kg /ha)**	9.67 a	4.33 b	4.33 b	5.97 c	3.27 b	3.17 b	5.40 d	3.37 b	3.57 b	5.37 c	3.60 b	3.77 b	5.33 c	51.92
Indoxacarb (29 g/ha)	11.22 b	2.20 a	2.50 a	3.10 a	1.40 a	1.53 a	2.97 a	1.00 a	1.20 a	1.87 a	3.12 a	2.97 a	3.90 a	64.22
Control	10.47 b	10.50 d	10.10 e	10.67 d	11.23 e	11.17 f	11.27 f	12.40 e	12.00 e	12.20 e	11.93 f	11.67 d	10.90 d	-

* In a column, mean followed by the same letter do not differ significantly by DMRT (P=0.05)

and six days post treatment, while at eight days post treatment GmNPV was found to perform better than *B.t.*

4.11.3. Yield parameters

Observations on the mean diameter of flower heads and yield per hectare showed that all the treatments were significantly better than control (Table 32). Application of indoxacarb resulted in the highest mean diameter of flower head (15.37 cm) and yield of 26.64 t/ha. GmNPV @ 7.5 x 10^{13} POB/ha and PxGV at 1.5 x 10^{13} OB/ha were found to be the next best in increasing the yield of cauliflower followed by GmNPV @ 3.75 x 10^{13} POB/ha. Plots treated with GmNPV @ 1.88 x 10^{13} POB/ha and *B.t.* were next in order. Control plots recorded the lowest mean diameter of flower head (11.53 cm) and yield (19.70 t/ha).

4.12. Safety tests of GmNPV isolates on non-target organisms

4.12.1. *T. chilonis*

The per cent parasitization, adult emergence, adult duration and total life cycle of *T. chilonis* exposed to GmNPV (CBE and BNGL isolates) through *C. cephalonica* eggs or through adult feed did not differ significantly with those of untreated parasitoids (Table 33 and 34).

4.12.2. *C. carnea*

There were no harmful effect on hatchability, larval period, per cent pupation, pupal period and per cent adult emergence of *C. carnea* fed with *C. cephalonica* eggs treated with GmNPV (CBE and BNGL isolates) when compared to that of control (Table 35). Though, 8-10 per cent mortality of grubs was observed in virus treatments, there existed no significant difference from control.

4.12.3. *B. mori*

Observations on the larval weight of III, IV and V instars, per cent pupation, pupal period, cocoon weight, per cent adult emergence and per cent larval mortality of six different races of *B. mori* compared with those of respective control are given in Table

Table 32. Effect of GmNPV on the yield of cauliflower

Treatments	Mean diameter (cm)	Yield /ha (tonnes)
GmNPV 1.88×10^{13} (POB/ha)	13.33 d	21.67 cd
GmNPV 3.75×10^{13} (POB/ha)	14.30 c	22.73 c
GmNPV 7.50×10^{13} (POB/ha)	14.93 ab	24.40 b
PxGV 1.5×10^{13} (OB/ha)	14.43 bc	23.13 bc
B.t. (0.5 kg/ha)	13.57 d	20.50 de
Indoxacarb (29 g a.i./ha)	15.37 a	26.64 a
Control	11.53 e	19.70 e

In a column, means followed by the same letter do not differ significantly by DMRT (P=0.05)

Table 33. Effect of GmNPV isolates on *T. chilonis* by egg treatment

Parameters *	GmNPV treated		Control
	CBE	BNGL	
Parasitization (%)	73.8 ± 5.32	76.6 ± 4.61	70.6 ± 4.66
Adult emergence (%)	93.1 ± 2.92	91.5 ± 2.44	94.7 ± 1.61
Total life cycle (days)	9.10 ± 0.31	9.20 ± 0.20	9.10 ± 0.31
Adult duration (days)	2.80 ± 0.25	3.00 ± 0.26	3.50 ± 0.22

* In a row, the means do not differ significantly by DMRT (P=0.05)

Table 34. Effect of GmNPV isolates on *T. chilonis* by oral feeding

Parameters *	GmNPV fed		Control
	CBE	BNGL	
Parasitization (%)	68.1 ± 4.52	66.1 ± 2.70	67.7 ± 2.50
Adult emergence (%)	93.9 ± 1.59	92.7 ± 1.96	94.3 ± 2.08
Total life cycle (days)	9.10 ± 0.23	9.00 ± 0.30	9.10 ± 0.35
Adult duration (days)	3.00 ± 0.30	3.10 ± 0.28	3.10 ± 0.23

* In a row, the means do not differ significantly by DMRT (P=0.05)

Table 35. Effect of GmNPV isolates on *C. carnea*

Parameters *	GmNPV treated		Control
	CBE	BNGL	
Hatchability (%)	94.00 ± 2.44	96.00 ± 2.45	96.00 ± 2.45
Larval mortality (%)	10.00 ± 3.16	8.00 ± 2.00	8.00 ± 3.74
Larval period (days)	10.84 ± 0.16	10.96 ± 0.12	11.02 ± 0.17
% pupation (days)	80.17 ± 3.99	80.22 ± 4.28	80.11 ± 2.60
Pupal period (days)	10.78 ± 0.07	10.89 ± 0.05	10.95 ± 0.02
Adult emergence (%)	92.14 ± 3.23	92.06 ± 3.29	95.28 ± 2.90

* In a row, the means do not differ significantly by DMRT (P=0.05)

36. The above parameters did not differ significantly between the virus treated and control. However, a higher larval mortality of 26.70 per cent and 20 per cent less pupation were observed in CSRJ 4 x 2 race treated with GmNPV compared to control (6.67 per cent). This was probably due to triggering of latent infection of the homologous *B. mori* NPV.

4.12.4. Honey bees

The mortality rates of adult bees of four species *viz*., *Apis mellifera, A. cerana indica, A. florea* and *Trigona iridipennis* are given in Table 37. GmNPV @ 2 x 10^9 POB/ml fed with sucrose solution did not cause any deleterious effect in all the species tested as the mortality rates between treated and control were on par at the end of each observation period. Moreover, there were no significant differences in the mean number of days of survival between treated and control for all the four species tested.

Table 36. Safety of GmNPV to different races of *Bombyx mori*

Parameters	TN white		AE40032		Mysl		CSRI 4x2		Pure Mysore		Thai	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Larval weight (g) $												
III instar	0.32 ± 0.14	0.32 ± 0.25	0.28± 0.09	0.29± 0.08	0.31±0.16	0.32± 0.32	0.37± 0.17	0.36± 0.24	0.35± 0.05	0.37± 0.07	0.34 ± 0.05	0.34 ± 0.07
IV instar	1.02 ± 0.03	1.01 ± 0.02	0.99 ± 0.01	0.64 ± 0.02	1.06 ± 0.14	1.07 ± 0.13	1.12 ± 0.25	1.11 ± 0.16	1.01 ± 0.34	1.01 ± 0.07	0.98 ± 0.09	0.97 ± 0.14
V instar	2.10 ± 0.24	2.12± 0.18	2.43 ± 0.15	2.41± 0.03	2.20 ± 0.02	2.31± 0.44	2.18 ± 0.56	2.18 ± 0.34	2.34 ± 0.36	2.21 ± 0.42	2.28 ± 0.22	2.29 ± 0.57
Pupation (%) $	93.3 ± 0.01	93.3 ± 0.02	93.3 ± 0.06	90.0 ± 0.08	100 ± 1.02	100 ± 0.06	73.3 ± 0.97*	93.3 ± 1.21*	93.3 ± 0.88	96.7 ± 0.75	96.7 ± 0.86	96.7 ± 1.09
Pupal period (days) $	11.9 ± 0.02	12.0 ± 0.03	12.6 ± 0.03	12.9 ± 0.02	11.7 ± 0.01	11.9 ± 0.02	12.0 ± 0.04	12.7 ± 0.02	13.2 ± 0.06	13.2 ± 0.04	12.2 ± 0.04	13.0 ± 0.06
Coccoon weight (g) $	1.14± 0.01	1.28 ± 0.01	1.32 ± 0.01	1.39 ± 0.02	1.18 ± 0.03	1.28 ± 0.05	1.05 ± 0.06	1.14 ± 0.08	1.42 ± 0.02	1.38 ± 0.06	1.29 ± 0.04	1.32 ± 0.01
Adult emergence % $	92.0 ± 0.12	95.0 ± 0.15	90.0 ± 0.24	90.0 ± 0.18	96.0 ± 0.97	95.0 ± 1.12	94.0 ± 0.86	96.0 ± 1.07	100 ± 0.00	100 ± 0.00	95.0 ± 1.24	95.0 ± 1.34
Larval mortality (%) $	6.67 ± 0.15	6.67 ± 0.34	6.67 ± 0.53	10.0 ± 1.12	-	-	26.7 ± 0.78*	6.67 ± 0.65*	6.67 ± 1.32	3.33 ± 0.21	3.33 ± 0.43	3.33 ± 0.76

$ In a row, the means do not differ significantly at t = 0.05

* In a row, the means differ significantly at t = 0.05

Table 37. Safety of GmNPV (CBE isolate) to different species of honeybees

Species*	Category	Per cent bees dead (Days after feeding)					Mean no. days survived
		4	8	12	16	20	
Apis mellifera	Treated	26.67	53.33	60.00	73.33	96.67	11.5 ± 1.11
	Control	33.33	46.67	66.67	83.33	100.00	11.8 ± 1.10
A. cerana indica	Treated	33.33	40.00	56.67	80.00	100.00	11.6 ± 1.16
	Control	36.67	50.00	63.33	86.67	93.33	10.6 ± 1.14
A. florea	Treated	43.33	60.00	86.67	100.00	100.00	8.4 ± 0.82
	Control	40.00	53.33	93.33	100.00	100.00	8.5 ± 0.68
Trigona iridipennis	Treated	33.33	56.67	96.67	100.00	100.00	8.5 ± 0.76
	Control	26.67	66.67	93.33	100.00	100.00	8.3 ± 0.67

The means of each category do not differ significantly at t=0.05 for a species

Discussion

Chapter V

DISCUSSION

In the past 50 years, the diamondback moth, *P. xylostella* has become one of the most difficult insects in the world to control because of its intrinsic biology and ecology and its large host range. Failure to control is not an indication of lack of efforts but it is rather the ability to develop resistance by diamondback moth to every insecticide that has been widely used against them, including, *B. thuringiensis*. New products such as microbial insecticides to complement our traditional strategies of pest management are sorely needed. The present study investigates the potential of nucleopolyhedrosis virus of *G. mellonella* for the management of *P. xylostella* and explores the possibility of developing GmNPV as a bioinsecticide.

5.1. Cross-infectivity of GmNPV

GmNPV was found to be cross-infective to 16 insect species out of 31 species tested. Several of the economically important pests like *P. xylostella*, *C. partellus*, *C. infuscatellus*, *C. sacchariphagus indicus*, *M. vitrata*, *C. medinalis*, *M. patnalis*, *O. arenosella*, *A. albistriga* and *D. pulverulentalis* were susceptible to GmNPV (Table 1). The host range of GmNPV covered several families of the order Lepidoptera, of which Pyralids dominated followed by Arctiids (Fig 1). Not all the species of the same family were susceptible to GmNPV. Some of the pyralids like *D. indica*, *H. puera*, *C. cephalonica*, *N. geometralis* did not get infect with GmNPV. Likewise, except *S. exigua*, other noctuids like *H. armigera*, *S. litura*, *E. vittella*, *E. macheralis*, *A. peponis*, *A. janata*, *A. ipsilon* and *M. separata* were refractive to GmNPV infection. Also, the host range of GmNPV covers pests of different crop ecosystems like paddy, sugarcane, pulses, crucifers, oilseeds and mulberry.

Interestingly, more than one insect pest colonizing the same crop *viz.*, *P. xylostella*, *C. binotalis*, *H. undalis*, *S. exigua* (crucifers), *C. infuscatellus*, *C. sacchariphagus*, indicus (sugarcane), *M. vitrata*, *E. atomosa* (pulses), *C medinalis*, *M. patnalis* (rice) and *P. ricini* and *E. merione* (castor) were susceptible to GmNPV. This

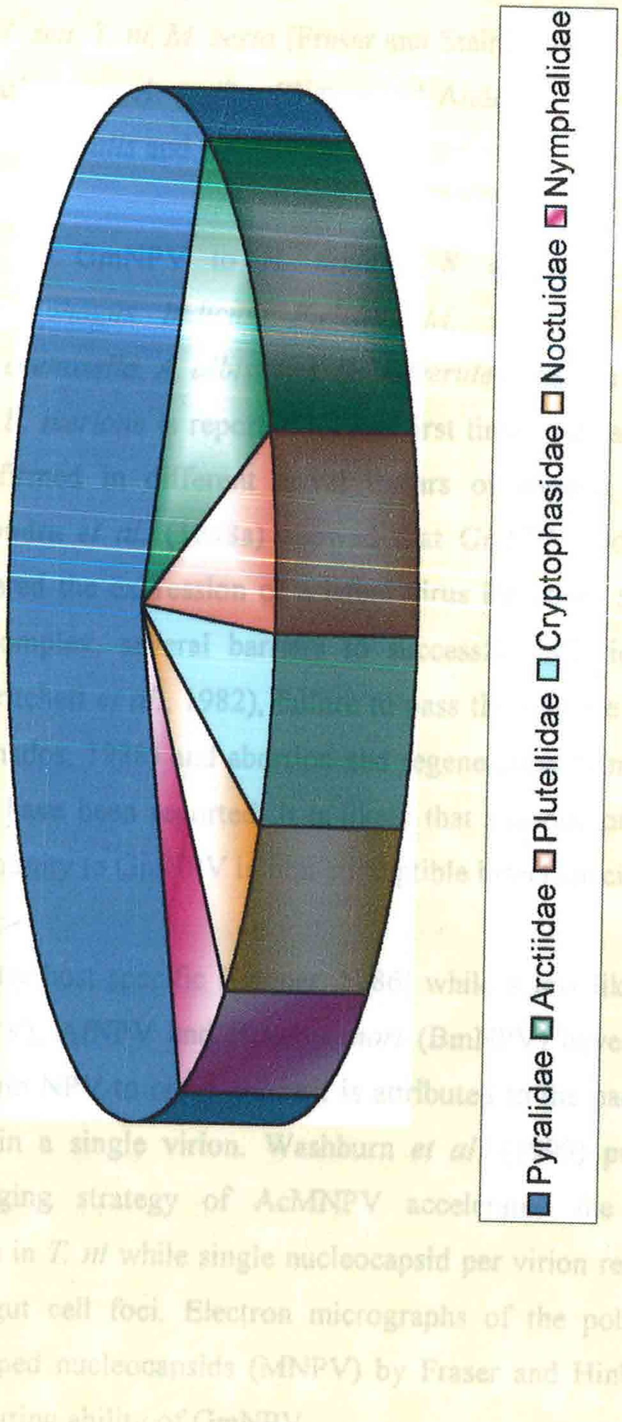


Fig 1. Proportion of susceptibility of lepidopteran families to GmNPV

would be of great economic significance in pest management in different crop ecosystems.

The susceptibility of several lepidopteran species to NPV from GmNPV has been reported earlier. It included *H. zea, T. ni, M. sexta* (Fraser and Stairs, 1982), *H. virescens, S. frugiperda, Plathypena includens*, , *A. ipsilon* (Biever and Andrews, 1984; Kadir and Payne, 1989), *P. xylostella, C. binotalis* and *M. brassicae* (Kadir *et al.* 1999b).

The cross-infectivity of GmNPV to *H. undalis, S. exigua, C. partellus, C. infuscatellus, C. sacchariphagus indicus, P.ricini, M. vitrata, E. atomosa, C. medinalis, M. patnalis, O. arenosella, A. albistriga, D. pulverulentalis* and to a distinct relative nymphalid butterfly, *E. merione* is reported for the first time. Refractiveness to GmNPV infection was confirmed in different larval instars of another 15 species including *H. armigera.* Rabindra *et al.* (1998a) showed that GmNPV did not infect *H. armigera* larvae but triggered the expression of a latent virus infection. Since insect host-virus interactions are complex, several barriers to successful infection such as deactivation in the midgut (Pritchett *et al.*, 1982), failure to pass through the peritrophic membrane (Derkson and Granados, 1988) and abortion and regeneration of midgut cells (Inoue and Miyagawa, 1978) have been reported. It is likely that any one of the above factors might have caused immunity to GmNPV in non-susceptible insect species.

Baculoviruses are mostly host-specific (Groner, 1986) while a few like AcNPV, *Anticarsia gemmatalis* (AgNPV), AfNPV and *Bombyx mori* (BmNPV) have a broader host range. The ability of certain NPV to cross-transmit is attributed to the packaging of redundant nucleocapsids within a single virion. Washburn *et al.* (1999) proved that multiple nucleocapsid packaging strategy of AcMNPV accelerated the onset of irreversible systemic infections in *T. ni* while single nucleocapsid per virion restricted to primary infection in the midgut cell foci. Electron micrographs of the polyhedra of GmNPV with multiply enveloped nucleocapsids (MNPV) by Fraser and Hink (1982b) substantiates the cross - transmitting ability of GmNPV.

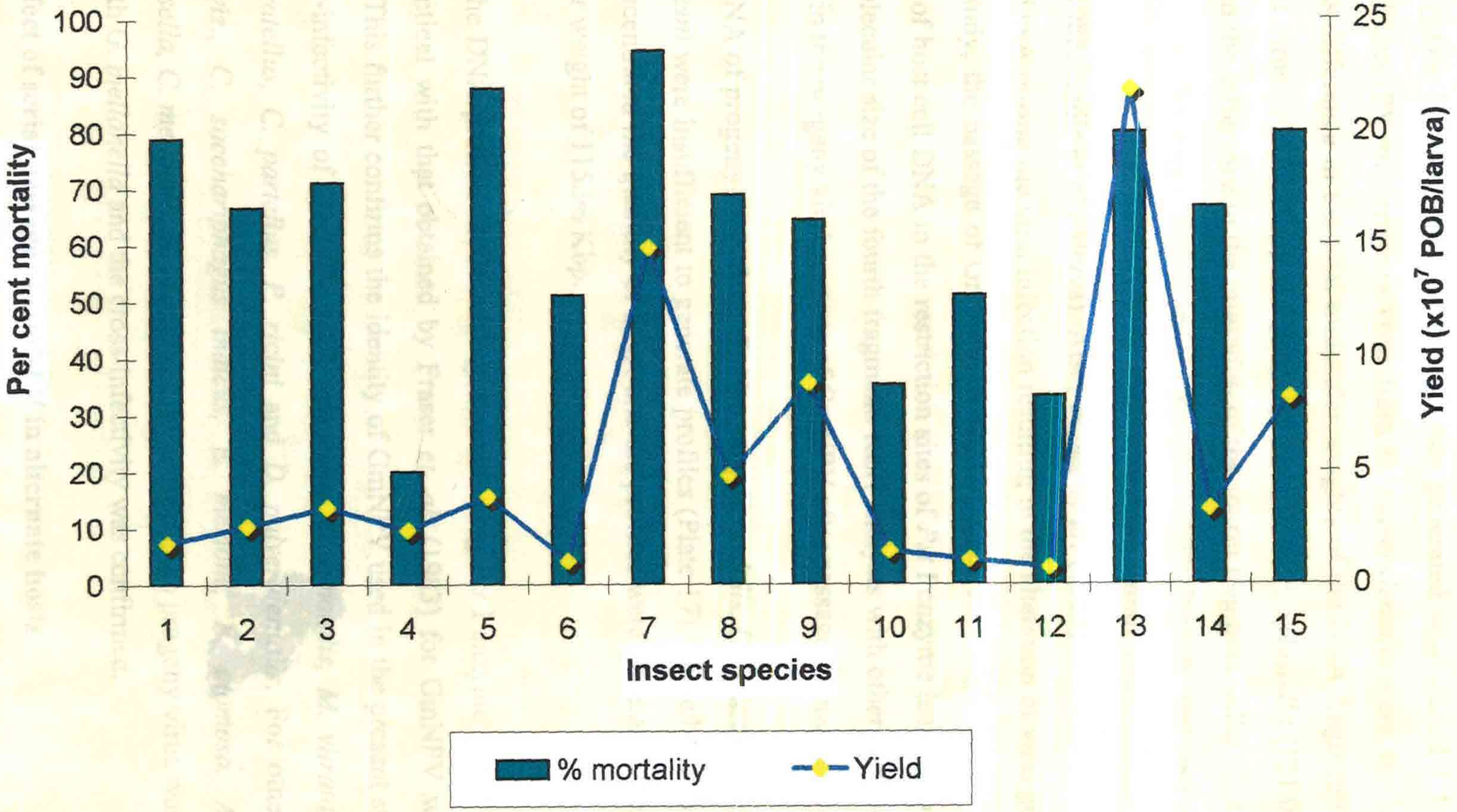
The degree of susceptibility of insect species to GmNPV varied significantly in the present study. Among the 16 susceptible insect species, three different levels of susceptibility were observed (Fig 2.). *C. infuscatellus, M. vitrata, P. ricini* and *D. pulverulentalis* were highly susceptible group with the mortality range of 80-95 per cent. *P. xylostella, H. undalis, C. binotalis, C. sacchariphagus indicus, C. partellus, E. atomosa, E. merione* and *M. patnalis* could be grouped together with the moderate mortality range of 51-79 per cent. *C. medinalis, O. arenosella, S. exigua* and *A. albistriga* were least susceptible group with the mortality range of 15-40 per cent. The variation in the degree of susceptibility of GmNPV (CBE isolate) is supported by the view of Fraser and Stairs (1982) that among the three alternate species tested with GmNPV, *T. ni* was the most susceptible followed by *H. zea* and *M. sexta* was the least susceptible. Grewal *et al.* (1998) ranked the insect species from most to least susceptible as *H. virescens* > *H. zea* > *S. exigua* > *S. frugiperda* > *P. xylostella*, based on relative susceptibility to AfNPV. The order of susceptibility of seven insect species *viz.*, *P. xylostella, H. virescens, T. ni, H. subflexa, H. zea, S. exigua* and *S. frugiperda* varied significantly for three viruses tested (PxMNPV, AcMNPV and AfMNPV) (Kariuki and McIntosh, 1999).

The yield of GmNPV also varied significantly among the susceptible insect species (Table 2). The yield of GmNPV was highest in the highly susceptible species like *P. ricini* (21.86 x 10^7 POB/larva), *C. infuscatellus* and *D. pulverulentalis*. The yield was lowest (0.74 x 10^7 POB/larva) in the least susceptible species, *O. arenosella*. Highly susceptible species yielding average POB/larva (*M. vitrata*) and least susceptible species yielding highest POB/larva (*A. ablistriga*) suggest that size of the host species influence the yield of POB/larva irrespective of degree of susceptibility. Also moderately susceptible and medium sized species yielding high POB/larva (*C. partellus*) reveals the involvement of other factors like rate of invasion and spread of systemic secondary infection.

5.1.1. Confirmation of cross-infectivity of GmNPV

DNA profiles generated by restriction endonuclease analysis with *Pst* I revealed identical patterns of fragments of inoculated GmNPV and progeny virus from

Fig 2. Cross infectivity of GmNPV to different lepidoteran insect pests



1 - *P.xylostella*; 2 - *C. binotalis*; 3 - *H. undalis*; 4 - *S. exigua*; 5 - *M. vitrata* 6 - *E. atomosa*; 7 - *C. infuscatellus*; 8 - *C. s. indicus*; 9 - *C. partellus*; 10 - *C. medinalis*; 11 - *M. patnalis*; 12 - *O. arenosella*; 13 - *P. ricini*; 14- E. *merione;* 15 - D. *pulverulentalis*

P. xylostella, C. binotalis, M. vitrata, H. undalis, C. infuscatellus, C. partellus and *P. ricini* (Plate 17 and 18). Also the profiles generated with *Bam*H I from DNA of inoculated GmNPV and progeny virus from *D. pulverulentalis* were similar (Plate 19). There was difference in the total molecular weights of the DNA fragments between the inoculated virus (116.82 Kbp) and progeny virus from *P. xylostella* (121.04 Kbp). This was due to the difference in the migration of the fourth fragment. Alteration in molecular weight of a few fragments during serial passage through alternate hosts is fairly typical of most wild-type baculoviruses which usually exhibit some heterogeneity (Smith and Crooks, 1988, Kadir *et al.*, 1999a). Also, Fraser *et al.* (1983) reported the acquisition of host cell DNA during the viral infection resulting in the alteration of viral genome. In the present study, the passage of GmNPV through *P. xylostella* might have resulted in the insertion of host cell DNA in the restriction sites of *Pst* I enzyme leading to the variation in the molecular size of the fourth fragment. REN analysis with other enzymes may show variation in the progeny viral genome of GmNPV after passage through alternate hosts.

DNA of progeny virus from *C. binotalis, H. undalis, C. infuscatellus, C. partellus* and *P. ricini* were insufficient to generate profiles (Plate 17). The ethanol precipitaion of DNA concentrated the quantity of DNA and the profiles were generated thereafter with a molecular weight of 115.96 Kbp.

The DNA profiles of GmNPV generated using *Pst* I enzyme in the present study were identical with that obtained by Fraser *et. al.* (1983) for GmNPV with the same enzyme. This further confirms the identity of GmNPV used in the present study and also the cross-infectivity of GmNPV to *P. xylostella, C. binotalis, M. vitrata, H. undalis, C. infuscatellus, C. partellus, P. ricini* and *D. pulverulentalis*. For other susceptible insects *viz., C. sacchariphagus indicus, E. merione, E. atomosa, A. albistriga, O. arenosella, C. medinalis, S. exigua* and *M. patnalis*, the progeny virus was reciprocally tested with *G. mellonella* and the cross-infectivity was confirmed.

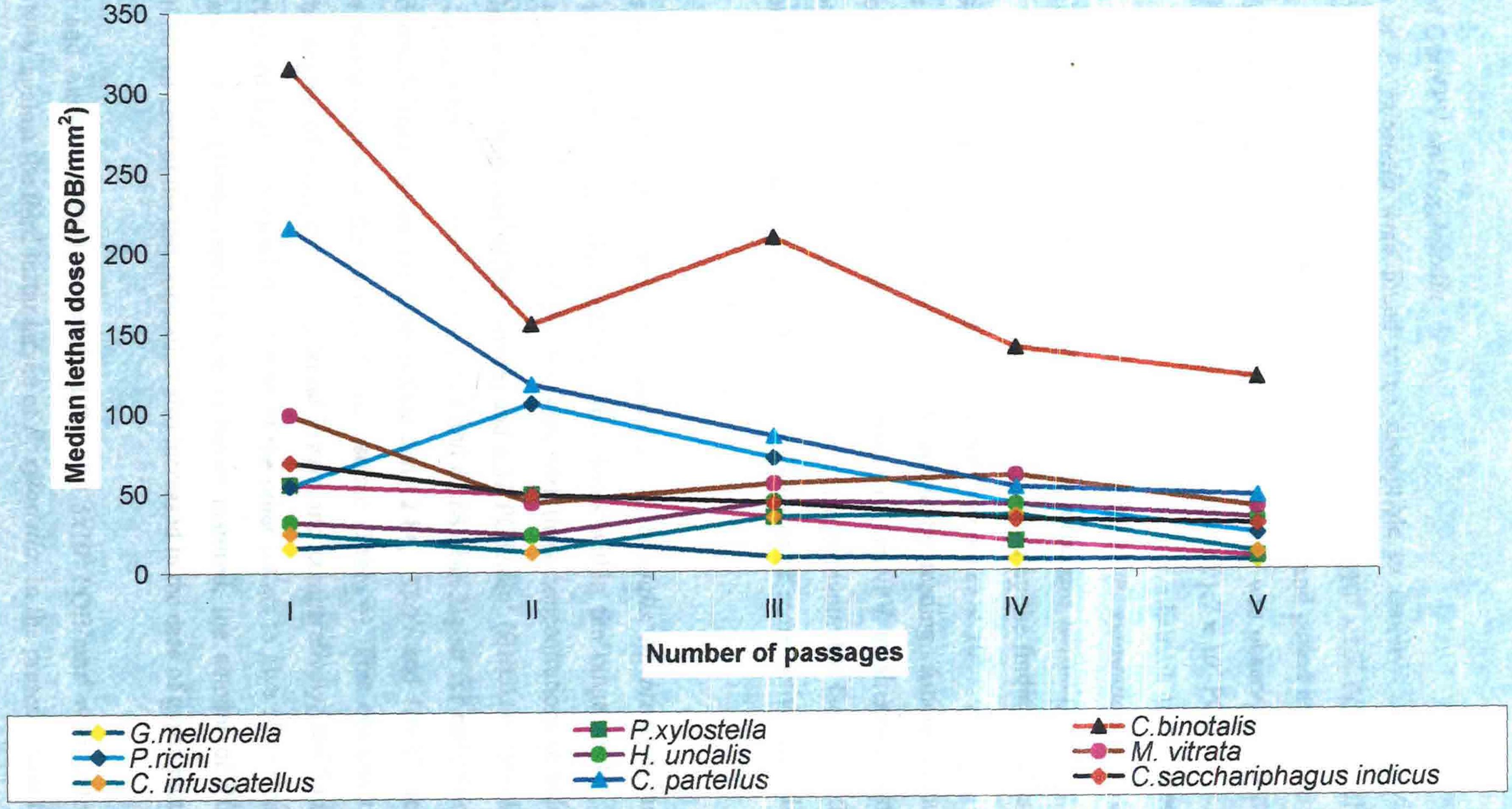
5.1.2. Effect of serial passage of GmNPV in alternate hosts

The virulence of GmNPV against *P. xylostella* larvae increased by serially passaging through the alternate hosts as evidenced by the decrease in LC_{50} after successive passages (Appendix VI A-E, Fig 3.). The lowest LC_{50} for *P. xylostella* was witnessed with the subsequent passages through the original host, *G. mellonella*. This is in agreement with the view of Stairs (1990) who reported that progeny virus of GmNPV from *M. sexta* larva when passaged back through the original host, *G. mellonella* retained its virulence for this species.

A drastic reduction in LC_{50} by 6.84 fold was recorded with five subsequent passages of GmNPV through *P. xylostella* against the same. Tompkins *et al.* (1988) recorded a 12.5 fold increase in virulence of HaNPV to neonate *T.ni* after three passages in *T. ni* larvae. Serially passaged AcMNPV through *P. xylostella* larvae for 20 times increased the virulence by 15 fold against the pest (Kolodny - Hirsch and Van Beek, 1997). These reports support the view that passage of NPV through an alternate host but same as the test insect enhance the virulence of NPV against the test insect, as observed in this case with *P. xylostella.*

Passage of GmNPV subsequently through other hosts like *C. binotalis, P. ricini,M. vitrata, C. infuscatellus, C. partellus* and *C. sacchariphagus indicus* resulted in reduction of LC_{50} for *P. xylostella* larvae thereby revealing the increase in virulence. It seems NPVs adapt to the homologous or heterologous hosts upon sequential passages. Passage of virus through alien insects produces changes in growth and virulence of NPV (Stairs *et al.*, 1981). Tompkins *et al.* (1981) showed that passage of AcMNPV through alternate hosts changed the size of the polyhedra and these polyhedra contained more virions per cross section of PIB and greater mean and mode of nucleocapsids per virion and were more virulent to neonate *T. ni.* As discussed earlier, increase in nucleocapsids per virion upon serial passages through alternate hosts contributes to the increase in virulence of passaged virus against the target pests. Hence, it can be assumed that GmNPV should be serially passaged through the test insect subsequently followed by a passage through the original host, *G. mellonella* for selection of a more virulent strain against a test insect.

Fig 3. Median lethal concentration of GmNPV upon serial passages through alternate hosts on *P. xylostella*



5.2. Efficacy of GmNPV on *P. xylostella*

Larvae of *P. xylostella* were found to be susceptible to GmNPV isolates *viz.* Coimbatore (CBE) and Bangalore (BNGL) in the dose range of 10^4 - 10^9 POB/ml. The mortality rates of larvae were positively correlated with the dose and period of time after inoculation (Table 5). However, more than 50 per cent mortality was observed with the dose of 2 x 10^6 POB/ml in the present study, as against the dose of 1.7 x 10^3 POB/ml with PxNPV for third instar larvae of *P. xylostella*, reported by Padmavathamma and Veeresh (1991). This may be attributed to the heterologous infection of *P. xylostella* larvae by NPV of alien insect source, *G. mellonella.* Heterologous infections normally require a high dose to produce an expected effect. This view is supported by the finding of Fraser and Stairs (1982) that extremely large doses of GmNPV @ 4.125 x 10^6 - 4.125 x 10^7 POB/ml were required to infect neonate *M. sexta* larvae by feeding. Among certain conditions shown to favour the successful transmission of GmNPV to alternate hosts, requirement of higher doses is advocated to overcome some unfavourable factors in the midgut environment of alternate hosts such as pH differences or more active digestive enzymes.

Assays of GmNPV with neonate *P. xylostella* , *C. binotalis, M. brassicae* and *H. virescens* larvae were done using half-log virus doses within the range of 10^4 to 10^8 POB/ml (Kadir *et al.*, 1999b). Leaf disc bioassay with these concentrations revealed the lowest LC_{50} of 4.55 POB/mm^2 (CBE isolate) and 6.32 POB/mm^2 (BNGL isolate) for second instar larvae while LC_{50} were 11.55, 29.14 and 61.88 fold higher for early third, late third and fourth instar larvae for CBE isolate and 12.84, 33.77 and 129.27 fold higher for the above instars in the case of BNGL isolate (Table 6). This exhibits the variation in the degree of susceptibility of larval instars and comparatively, the fourth instar larvae required higher POB/ml than larvae of the other instars to give 50 per cent mortality. Shapiro *et al.* (1986) reported that as larvae matured, the amount of POB required to kill 50 per cent of the test population also increased in the case of gypsy moth.

Biever and Andrews (1984) recorded a LC_{50} of 1.5x10^3 POB/mm^2 with Oxford isolate of GmNPV against the third instar larvae of *P. xylostella.* In the present study the

LC_{50} for *P. xylostella* were low in the range of 52.57 - 81.12 POB/mm^2 (early third) and 153.64 - 184.16 POB/mm^2 (late third instar). From this it is evident that both the Coimbatore and Bangalore isolates tested are more virulent than the Oxford strain of GmNPV to *P. xylostella.*

LC_{50} obtained with these isolates for *P. xylostella* larval instars corresponding to 3.88×10^5 to 5.02×10^7 POB/ml are corroborative with the report of Kadir *et al.* (1999b) who determined the LC_{50} in the range of 3.7×10^6 to 1.2×10^7 POB/ml for GmNPV against *P. xylostella* larval instars from eight bioassays. In both the dose-mortality assays (Table 5 and 6), CBE and BNGL isolates did not differ significantly in their activity against *P. xylostella* larvae. Brown *et al.* (1981) identified five European isolates of *M. brassicae* MNPV with no significant differences in virulence between the isolates against *M. brassicae*. Also, Beretta *et al.* (1988) characterized four geographical isolates of *S. frugiperda* but the biological activity of the different isolates did not differ significantly against the same.

5.2.1. Effect of serial passage of GmNPV in *P. xylostella*

Alternate hosts are often less susceptible to heterologous infection (Allaway and Payne, 1984). The virulence of baculoviruses can be increased by serial passage in homologous or heterologous hosts (Shapiro and Ignoffo, 1970; Maleki-Milani and Milani-Maleki, 1978; Shapiro *et al.* 1982; Martignoni and Iwai, 1986). Hence GmNPV was serially passaged through *P. xylostella* larvae subsequently for 15 times to determine the increase in virulence, if any.

Subsequent passages of GmNPV in *P. xylostella* for 15 times resulted in substantial decrease in the LC_{50} by 12.76 fold (Table 7). LC_{50} for early third instar was 54.84 POB/mm^2 at the end of first passage while it was as low as 4.47 POB/mm^2 after 15 successive passages through the same host. There was a sharp decline in LC_{50} by 4.72 and 10.61 fold after fourth and fifth passages when compared to LC_{50} after third passage. LC_{50} in the subsequent passages did not differ significantly. However there was a slight increase in LC_{50} at the end of 12 and 13 passages, which may be attributed to the variation in the host insect population tested. This clearly indicates that GmNPV needs to be passaged through *P. xylostella* for a minimum of seven times for maximizing the virulence against the pest.

This is in conformity with earlier observations by Kolodny - Hirsch and Van Beek (1997) that OBS from AcMNPV passaged serially 20 times through *P. xylostella* were approximately 15 times more virulent to second instar *P. xylostella* larvae than the wild type virus. The basis for increased virulence of serially passaged virus is selection of genotypic variants characterized by OBs occluding a greater number of virions containing fewer nucleocapsids per envelope. OBs with greater number of virions would be expected to be more virulent than with fewer virions because there is more number of virus particles to initiate infection. It is suggested that virions containing fewer nucleocapsids could be more infectious, mainly because they would be more efficient in passing through the peritrophic membrane and binding to midgut cells.

GmNPV isolate used in the present study might have had a similar variation in the number of virions per OB and nucleocapsids per virion before (wild type) and after passages contributing to the enhancement of virulence against *P. xylostella*. Besides morphological differences, the increased infectivity could be due to (1) the presence of a factor affecting infectivity, which is embedded in the OB matrix (Derksen and Granados, 1988) or (2) the speed of release of virions in the midgut (Ignoffo *et al.*, 1995).

5.2.2. Comparison of efficacies of GmNPV and PxGV against *P. xylostella*

PxGV recorded a lower LC_{50} of 5.32 OB/mm^2 than GmNPV (54.84 POB/mm^2) indicating that PxGV is more virulent than GmNPV to *P. xylostella* (Table 9). Similar observations were also made by Kadir *et al.* (1999b). LC_{50} corresponding to 4.5 x 10^5 OB/ml observed in the present study is nearly equivalent to the LC_{50} of 5.5 x 10^5 OB/ml recorded for third instar larvae of *P. xylostella* by the above authors. However, by serially passaging GmNPV four times through *P. xylostella*, LC_{50} of GmNPV was on par with that of PxGV against third instar larvae of *P. xylostella* (Table 7).

However, GmNPV recorded a lower LT_{50} (133.25 h) than PxGV (157.06 h). This corresponds to 5.55 days (GmNPV) and 6.54 days (PxGV). Kadir *et al.* (1999b) recorded the LT_{50} of 4.24 days for GmNPV against neonate larvae of DBM while PxGV had a LT_{50} of 5.51 days for third instar larvae of DBM. The explanation for high LT_{50} of PxGV could be that each GV OB is likely to contain only one virion, whereas each NPV OB contains many virions which can infect many cells leading to an earlier death of the larvae.

5.2.3. Combination of GmNPV and PxGV against *P. xylostella*

Mixtures of GmNPV and PxGV in different ratio *viz.* LC_{50}: LC_{25}, LC_{25} : LC_{50} and LC_{25} : LC_{25} did not produce any synergistic effect as the mortality rates in the combined treatments were less than that of the respective individual effects (Table 10). On the contrary, synergistic effects of enhancing gene (enhacin) of *T.ni* GV (Gallo *et al.*, 1991; Lepore *et al.*, 1996; Wang and Granados, 1997) and *H. armigera* GV (Whitlock *et al.*, 1996) on the respective NPVs have been demonstrated. Shapiro (2000) studying the effect of two GVs, HaGV and *S. frugiperda* GV on gypsy moth NPV (LdMNPV) found that HaGV reduced the LC_{50} of LdMNPV while SfGV at a dilution of 10^{-5} had no effect on NPV activity. It was concluded that enhancin or an enhancin like gene may not been present in SfGV. The same may be the case with PxGV used in the present study.

It was interesting to note that the rate of infection in GmNPV treated larva was higher than that of combined treatment or PxGV treatments (Table 10). This is in accordance with the findings of HunterFujita *et al.* (1997) that survival of larvae of *S. littoralis* following mixed infection of GV and NPV was longer than that following infection with NPV alone and shorter than that following infection with GV alone. Moreover, the number of individual cells in the larva that become infected at the start of the infection process will be greater in NPV infected larvae (more virions per OB) and therefore the infection will proceed at a greater pace, leading to an earlier death of the larva.

5.3. Mass production of GmNPV

Although susceptible to infection by GmNPV, *P. xylostella* is not an ideal production host because of its small size and low yield of virus (1.01 - 1.30 x 10^7 POB/larva). Any insect pathogenic virus for mass production requires a suitable host, which can give a high yield of POB with good virulence. The host insect should also be amenable for economic mass rearing technology. Keeping in view the above facts, *G. mellonella* producing virulent progeny virus (low LC_{50}: 15-16 POB/mm^2), *P. ricini* yielding highest POB/larva (2.19 x 10^8) and *C. partellus* (easy mass rearing technique on artificial diet) were selected for studying the production efficiency.

In order to standardize the host instar for maximizing the production, the susceptibility of different instars of these three insect species to GmNPV was studied. As the neonates of *G. mellonella* are difficult to handle, neonates were excluded from the study. Based on the susceptibility and yield of POB/larva, late third instar larvae of *G. mellonella*, early fifth instar of *P. ricini* and fourth instar larvae of *C. partellus* were found to be best for mass production of GmNPV (Table 12).

The maturation immunity of late instars of *G. mellonella* and *C. partellus* observed in this study is supported by the view of Gitanjali *et al.* (2000) that the high level of phenoloxidase activity probably acted as gut barrier to secondary NPV infection in late instars of *S. litura*. Morobrullah and Nagata (2000) showed development of resistance in orally inoculated mature larvae of *S. litura* to its NPV. However, in the present study, the late instars of *P. ricini* larvae were susceptible to GmNPV producing 76.57 per cent mortality. *P. ricini*, being a voracious feeder, probably ingested more quantity of virus resulting in GmNPV infection.

Furthermore, concentration and time mortality responses of different instars of three insect species revealed lowest LC_{50} and LT_{50} for *C. partellus* in all the instars tested (Table 13, 14). It is obvious that, among the three insect species, *C. partellus* requires the lowest concentration of GmNPV to produce the expected effect and the mortality was observed in relatively shorter period than *G. mellonella* and *P. ricini*. These results suggest that C. *partellus* larvae are ideal for mass production of GmNPV. In this study, GmNPV was found to be more infectious to the heterologous host, *C. partellus* than the homologous host. In general, alternate hosts are often less susceptible, but this is not true in all cases as suggested by Allaway and Payne (1984). Dose-time mortality responses of different host species to *M. brassicae* NPV revealed that MbNPV was more infective to *Plusia gamma* (LC_{50}: 3.5 OB, LT_{50}: 8.7 days) and *Noctua promba* (LC_{50}: 10 OB, LT_{50}: 8.7 days) than to *M. brassicae* (LC_{50}: 21 OB, LT_{50}: 9.8 days).

The production efficiency ratio of GmNPV was highest in *G. mellonella* larvae when compared to the other hosts, *P. ricini* and *C. partellus* (Table 12a). This is mainly due to the production of virulent progeny virus after passage of GmNPV through the homologous host (*G. mellonella*). Hence, in terms of virulence against *P. xylostella* GmNPV requires more serial passages through *P. ricini* and *C. partellus* to increase the virulence.

However, high cost of artificial diet of *G. mellonella* (Rs. 135/kg) and pupal diapause and allergenic indicating setae of *P. ricini* make them unsuitable for mass production of GmNPV. *C. partellus* can be reared throughout the year on inexpensive diet (Rs. 20/litre) (Plate 50). Although the virus could be produced in the alternate host, the extracted virus must also be virulent for natural host. Shapiro *et al.* (1982) recommended *O. pseudosugata* NPV - *E. acrea* host system for production of OpNPV on the basis of both virus yield and activity. Previous studies indicated the production of virulent strains of GmNPV upon serial passage through *C. partellus*. Hence mass production of GmNPV using *C. partellus* larvae as *in vivo* system on large scale is recommended.

5.4. Transovarial transmission of GmNPV

Survivors of late instar larvae of *C. binotalis* and *P. ricini* treated with GmNPV successfully completed the life-cycle but larval mortality by virosis was observed in the subsequent generation. This prompted to study the transmission of GmNPV through eggs in these insects.

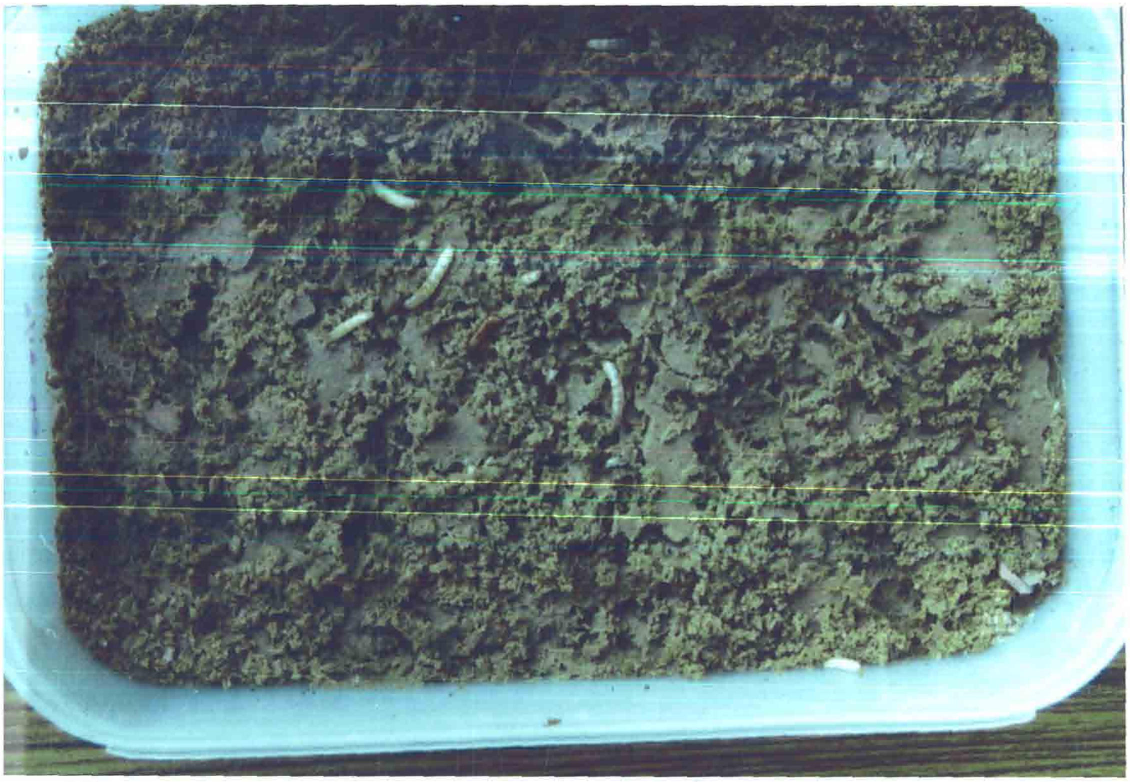


Plate 50. Mass culturing of *Chilo partellus* on semi-synthetic diet

Virus treated generation had significantly higher per cent mortality of larvae of *C. binotalis* (92%) and *P. ricini* (79.8%) than untreated generation (Table 15). Surface sterilization of egg/egg masses before hatching ruled out the possibility of transovum transmission but confirmed the transovarial transmission of GmNPV. Roegner-Austs (1950) observed dissolution of polyhedra in the lymph of *B. mori* pupa and concluded that such freed virus particles could penetrate the eggs in ovaries resulting in transovarial transmission. Vail and Gough (1973) detected viral particles in the ovaries of virus infected cabbage looper larvae. Nair and Jacob (1985) indicated low per cent mortality of *S. mauritiana* by transovarial transmission. The evidence gathered in the present investigation with GmNPV substantiated the above findings.

Moreover, fecundity, hatchability and per cent adult emergence did not differ significantly between GmNPV treated and untreated populations. Vertically transmitted GmNPV was equally virulent as that of horizontally transmitted GmNPV against *P. xylostella* larvae (Table 16). This is in accordance with the findings of Hamm and Young (I974) who observed no adverse effects on mating, oviposition or egg hatch of adult *H. zea* fed with PIB. The absence of adverse effects due to feeding PIB on fecundity, hatchability, adult emergence and virulence of progeny viruses is an important consideration if PIB fed adults are to be released to disseminate the virus in a natural population.

One of the principal methods of dissemination of insect pathogens is by the movement of healthy carriers and infected hosts (Tanada, 1964). This concept was recognized by Knipling (1960), when he suggested the release of insects to spread pathogenic organisms as one of four ways to utilize insects for their own destruction. In the present study, transovarial transmission of GmNPV was tested as it is more advantageous than transovum transmission. In transovum transmission, surface contamination of eggs occurs via adults and the persistence of virus inoculum on egg surface till consumption by emerging larvae under natural condition is critical. While in transovarial transmission, viral inoculum contained within the egg is protected and remains active. Transmission of virus through egg provides an economical and self perpetuating method of insect control and knowledge of this mode of transmission of GmNPV will be of much practical utility.

5.5. Histopathology of GmNPV infection

Microsections of tissues of *P. xylostella, M. vitrata* and *C. partellus* revealed the susceptibility of different tissues of the insect species for the production of POB of GmNPV (Table 17). In *P. xylostella* the polyhedra were observed on the tissue of fat bodies 48 h post inoculation. The per cent infection of nuclei of cells increased with increase in time and the maximum of 93.28 per cent was witnessed in the sections taken 120 h post inoculation. Virus replication was observed mainly in fat bodies and hypodermis. In the initial stages of infection vacoulation and hypertrophied nuclei with virogenic stroma were observed (Plate 23). The spread of secondary infection in hypodermis was noted at the advanced stages of infection (Plate 27). Vail *et al.* (1973) described large cuboidal polyhedra of AcMNPV in nuclei of the hypodermis, tracheal matrix and fat body cells of infected larvae of *P. xylostella.*

In the present study, midgut epithelial tissues of *P. xylostella* larvae were refractive to GmNPV infection (Plate 25). Livingston and Yearian (1972) reported no polyhedra formation in the midgut epithelium of *P. includens* larvae. Tompkins *et al.* (1969) in a histological study of the *T. ni* single embedded virus (SEV), failed to find polyhedra in the midgut cells. However, Vail and Jay (1973) recorded infection of the midgut columnar epithelium by AcNPV in alternate hosts.

Variation in the number of polyhedra per cell in different tissues of three insect species tested indirectly indicates the variation in the susceptibility of these species to GmNPV. Presence of more number of polyhedra per cell in *C. partellus* than in *M. vitrata* explains the higher yield in *C. partellus.* However the same cannot be explained for *P. xylostella* larva. In the advanced stages of infection, the spread of secondary infections in salivary glands, neural ganglion, tracheal matrix and tissues of imaginal disc were observed in C. *partellus* and *M. vitrata* larvae (Plates 39, 40, 33, 32). *C. cautella* NPV replication in *P. interpunctella* occurred in cell nuclei of the hypodermis, tracheal matrix, fat body cells, malphighian tubules and in tissue associated with muscles (Hunter *et al.*, 1973). Histopathology studies aid in the elucidation of rate of infection of GmNPV in alternate hosts and by the way confirmed the viral infection by GmNPV in larvae of *P. xylostella*, *C. partellus* and *M. vitrata.*

5.6. *In vitro* studies with GmNPV

The use of cultured cells for the study of insect viruses has become increasingly important as a result of the development of baculovirus expression vectors (BEV). The successful replication of nucleopolyhedrosis viruses in insect organ explants or primary cell cultures has been documented extensively. Before cell technology can be developed as a viable alternative to *in vivo* production of viral insecticides, the conditions for *in vitro* production must be systematically evaluated and optimized (McIntosh and Ignoffo, 1982). One important parameter in this evaluation is selection of a cell line that is a producer of virulent, polyhedral inclusion bodies. Hence, in the present investigation four different cell lines *viz.* SIH, SIPG, Sf21 and HaH were compared for their ability to grow and replicate GmNPV.

Among the cell lines tested, Sf21 was the most susceptible with infection range of 84.12 - 92.99 per cent followed by SIH and SIPG. HaH was least susceptible with 5.32 - 12.07 per cent infection for CBE and BNGL isolates of GmNPV. The yield of POB/ml of culture medium had the same trend (Table 19). This in accordance with the findings of McIntosh and Ignoffo (1989) that susceptibility of cell lines from *T.ni, S. frugiperda, H. virescens, P. xylostella* and *A. gemmatalis* to AcMNPV varied. Based on the virus yield, *P. xylostella* cell line was the best with 1.20 x 10^8 POB/ml while *S. frugiperda* cell line recorded a lowest yield of 1.13 x 10^7 POB/ml. Based on the variation in per cent cell infection of PxMNPV, cell lines HsAMI, PxEMI, HVAMI and TNMCL 1 recorded highest per cent infection in the range of 82.5 - 98.1 while HZFB 33 had a meagre of 1.5 per cent infection.

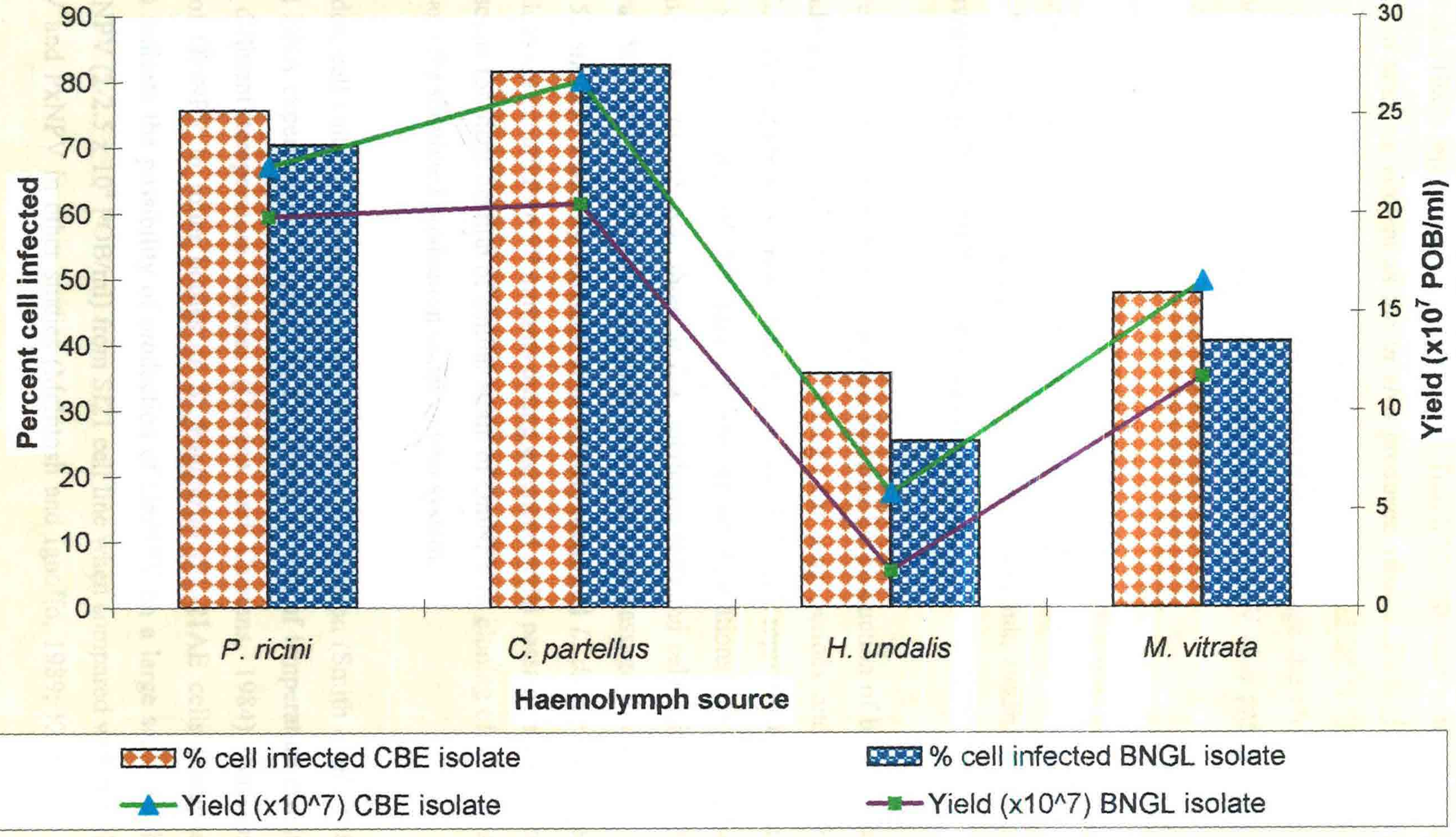
Moreover, cell lines established from *S. litura* and *H. armigera* were permissive to GmNPV infection while *in vivo* counterparts were refractive to infection. It is known that under *in vitro* conditions, viruses are not specific. Cell lines from *M. brassicae* (Mitsuhashi, 1981), *T. ni* (McKenna *et al.* 1988), *S. frugiperda* (Bonning *et al.*, 1995) and *H. virescens* (McIntosh and Ignoffo, 1983) were susceptible to the heterologous virus, AcMNPV. Similarly, *T. ni, S. frugiperda* and *M. separata* cell lines replicated the heterologous virus, HaNPV (Qui *et al.*, 1988). These facts widen the scope of using cell lines from diverse species for replication of GmNPV.

Haemolymph source of the virus is also as important criterion as observed with the variation in the susceptibility, and yield of GmNPV from four different sources *viz.*, *C. partellus, P. ricini, M. vitrata* and *H. undalis* (Fig 4.). Virus source from *C. partellus* and *P. ricini* were more virulent than that of from *M. vitrata* and *H. undalis*. The same trend was observed with yield of POB/ml. There are no reports so far on this aspect and hence further studies on this line are required for selection of insect host with high per cent infection and yield of GmNPV.

Another important aspect of *in vitro* produced viral insecticides is the proper selection of a cell line that will produce a high concentration of occlusion bodies (OBs) equivalent in activity to that produced in larvae. GmNPV produced in Sf21 cell line from different haemolymph source was compared with the respective *in vivo* produced counterpart. The LC_{50} of *in vitro* and *in vivo* produced GmNPV did not differ significantly irrespective of the insect source (Table 21). Laboratory bioassays and field trials demonstrated that the AcMNPV and TnMNPV produced in cell culture were as effective as that produced in larvae (Ignoffo *et al.*, 1974). No significant differences in virulence were noted between *in vitro* and *in vivo* produced GmNPV against *G. mellonella* (Dougherty *et al.*, 1982).

Also, the activity of GmNPV produced in different cell lines was on par with that of *in vivo* produced GmNPV against *P. xylostella* (Table 22). The LC_{50} of GmNPV for *P. xylostella* larvae did not vary significantly between the sources of cell line or larvae. Jegan Mohanambal (1998) observed that ICXNPV after passage through HaE1 and E7 were found to be as virulent as the *in vivo* produced virus to larvae of *H. armigera.*

Fig 4. Susceptibility of Sf21 cell lines to GmNPV isolates



McKenna *et al.* (1988) reported that AcMNPV and TnSNPV replicated in the embryonic cell line of *T.ni* were as virulent as the *in vivo* produced viruses. Passaging AcMNPV through the cell line Sf21 and Tn531-4 (Bonning *et al.*, 1995) and IPLB-SF-21, TN-368 and BCRL. HNU 3 (McIntosh and Ignoffo, 1989) did not change the virulence of the virus. Geethalakshmi (2001) observed similar effects with SlNPV after passage through *in vivo* and *in vitro* systems.

In this study, the GmNPV passaged only once through *in vivo* and *in vitro* systems were compared. However, serial passages in cell line generate few-polyhedra mutants (FP) with reduced activity as observed in GmNPV (Fraser and Hink, 1982b) and in other viruses (Brown and Faulkner, 1975; Hink and Strauss, 1976).

There are several advantages of *in vitro* systems for production of baculoviruses. These include purity of viral preparations, storage for long periods and less labour intensive. *In vitro* system is more flexible, controlled and reproducible. Cost of production and generation of FP mutants are major limitations. In the present investigation, cells with one, two, three or few polyhedra (FP) and cells with more than 30 polyhedra (MP) of GmNPV were observed even after one passage through the cell lines (Plate 51 and 52). Recently Slavicek *et al.* (2001) identified *L. dispar* NPV isolate (122b) that does not accumulate FP mutants during extended serial passage in cell culture. This offers scope for identification of similar strain of GmNPV or cloning of MP mutants of GmNPV and for stabilized production under *in vivo* system.

Besides, cell cultures favour the study of DNA transfection (Smith *et al.*, 1983), recombinant DNA experiments (Fraser *et al.* 1985) and growth of temperature sensitive mutants at different temperature regimes (Gorden and Carstens, 1984). Successful production of GmNPV in roller bottles on *S. frugiperda* IPL-21AE cells (Dougherty *et al.*, 1982) indicate the possibility of production of GmNPV on a large scale. Highest yield of GmNPV (2-2.5 x 10^8 POB/ml) from Sf21 cell line when compared with the yield of AcMNPV and PxNPV in other studies (McIntosh and Ignoffo, 1989; Kariuki *et al.*,

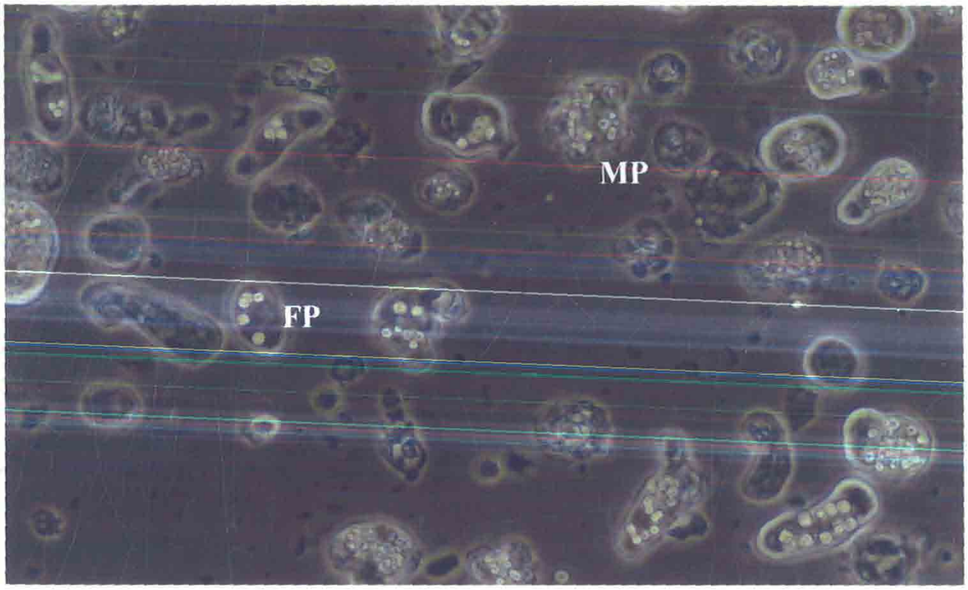


Plate 51. Few Polyhedra (FP) and Many Polyhedra (MP) mutants of GmNPV in Sf21 cell line

Plate 52. Polyhedra of GmNPV liberated from SlPG cell line at 120 h post inoculation

2000) indirectly indicates the production of high level of protein. Hence, GmNPV offers a wide scope in baculovirus expression vector system.

5.7. Chitinase activity of GmNPV

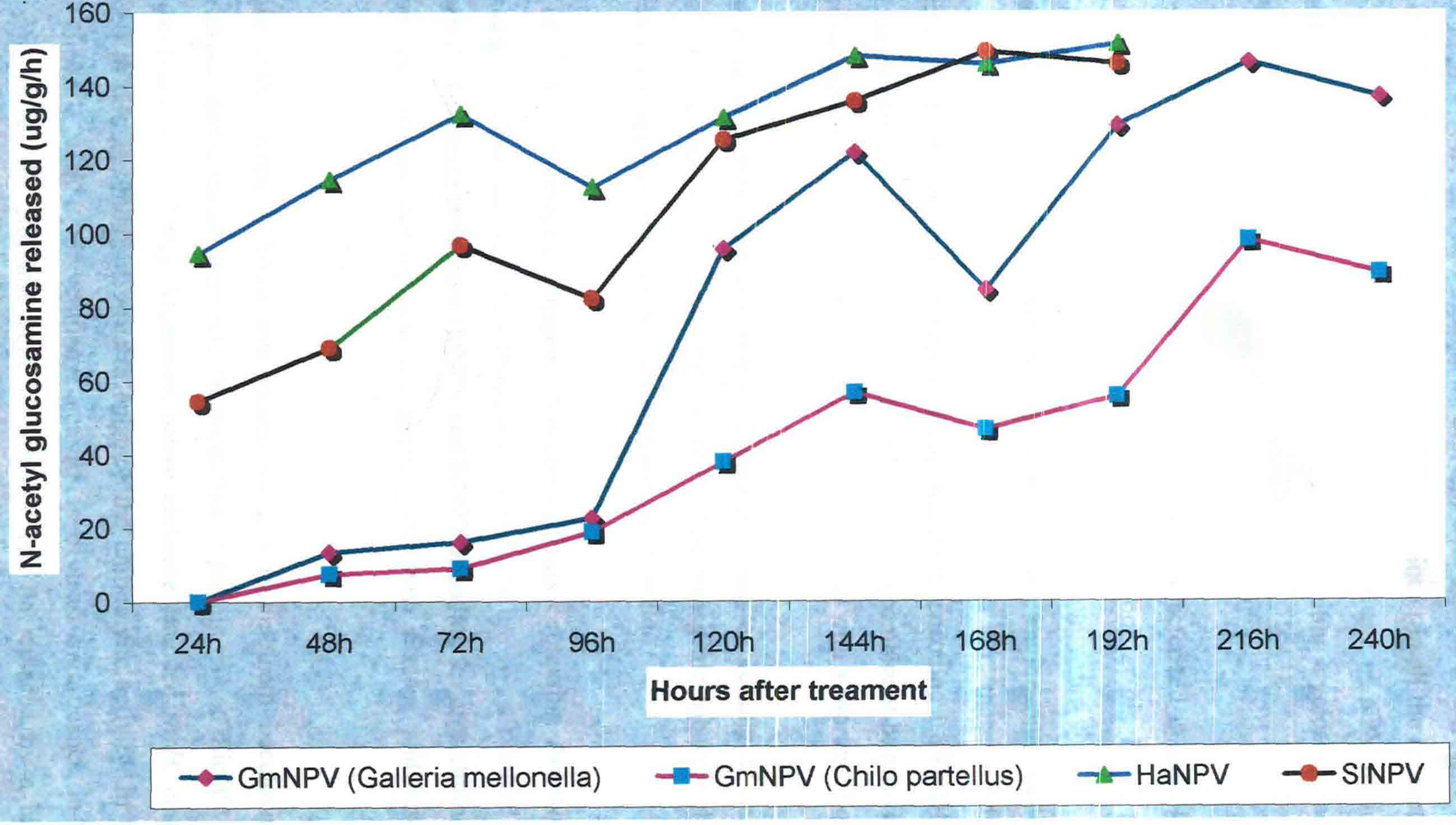
The heterologous hosts died of GmNPV infection showed atypical symptoms of disease. Even in the homologous host, *G. mellonella*, the integument of the cadavers remained intact 48 hrs post death and liquefaction of the body contents occurred thereafter. Since chitinase enzyme is involved in the degradation of cuticular chitin, the main constituent of the integument, the activity was determined for GmNPV in the homologous host and a heterologous host, *C. partellus*. The activity was compared with that of HaNPV and SlNPV which showed typical polyhedrosis against the respective pests.

GmNPV recorded the lowest chitinase activity among the three nucleopolyhedrosis viruses tested (Fig 5.). The activity measured in terms of N-acetyl glucosamine released (µg/g/h) was highest with HaNPV followed by SlNPV. However, the activity was on par after 168 h post treatment for both the viruses. Though SlNPV recorded a lower chitinase activity, the liquefaction of body contents of *S. litura* larvae occurred soon after the death. This may be due to the thin integument with less amount of chitin. *H. armigera* larvae with thick and tough integument probably require more quantity of chitinase to degrade the chitin.

The same can be argued for GmNPV where liquefaction of the homologous host, *G. mellonella* with a thin skin, occurs at a faster pace than the heterologous host. In the heterologous host, the chitinase activity did not reach the levels as observed in *G. mellonella* and it was nearly half the level at the end of each observation. The chitinase activity of GmNPV being lowest could be attributed for the atypical symptoms in *C. partellus* and delayed rupture of body wall in *G. mellonella*.

Tanada (1954) showed that the pathology produced by NPV isolated from *Pieris rapae* was atypical in an alternate host, *C. eurytheme*. Passage of *A. gemmatalis*

Fig 5. Chitinase activity of GmNPV in comparison with HaNPV and SINPV



NPV through *S. exigua, T. ni, H. zea, P. indudens, S. frugiperda* including *A. gemmatalis* produced non-wilt symptom wherein the integument remained intact in all the respective inoculated larvae. These studies substantiate the finding that passage of NPV through alternate hosts results in change in the virus chitinase activity exhibiting atypical diseased symptom of cadavers.

The gene responsible for chitinase enzyme has been characterized in the NPV genome of *A. californica*, *B. mori* and *H. cunea* (Gong-ChengLiong *et al.*, 1999) and *C. pomonella* granulosis virus (Kang-Wonkyug, *et al.* 1998). Recently, the chitinase of NPV has become the molecule of interest in many genetic engineering studies. Baculoviruses serve as the chief expression vector system in these studies. Gopalakrishnan *et al.* (1995) constructed a recombinant AcMNPV encoding 1.8 kb DNA fragment of *M. sexta* chitinase. Insect cell line infected with the recombinant baculovirus expressed *M. sexta* chitinase and was effective against *S. frugiperda* with a shorter LT_{50}.

Similar technology was used to construct transgenic plants expressing *M. sexta* chitinase protein via recombinant *Agrobacterium* strain. The transgenic tobacco plant suffered less tissue damage and inhibited the larval growth of *M. sexta*.

Shi *et al.* (2000) developed transgenic tobacco cultivars expressing AcMNPV derived chitinase gene. These cultivars were found to be resistant to brown spot caused by *Alternaria alternata*. Chitinase gene from HaNPV and SlNPV encoding for high chitinase activity can be used in the development transgenic plant with disease and insect resistance.

Chitinase cDNA from *S. litura* was cloned using a recombinant baculovirus lacking the virus-encoded chitinase gene and the recombinant protein named 'Sichi' was characterized (Shinoda *et al.*, 2001). Moreover, active chitinase encoded in *H. cunea* NPV was cloned in this recombinant virus to enhance the chitinolytic activity. In the present study, GmNPV with a low chitinase activity is of least significance in its biological activity. Hence the chitinase gene in GmNPV can be replaced by a high chitinase expression gene either from an insect or another NPV like HaNPV or SlNPV with high chitinase activity. Such recombinants would shorten the LT_{50} of GmNPV against the pest and make them more effective in the field.

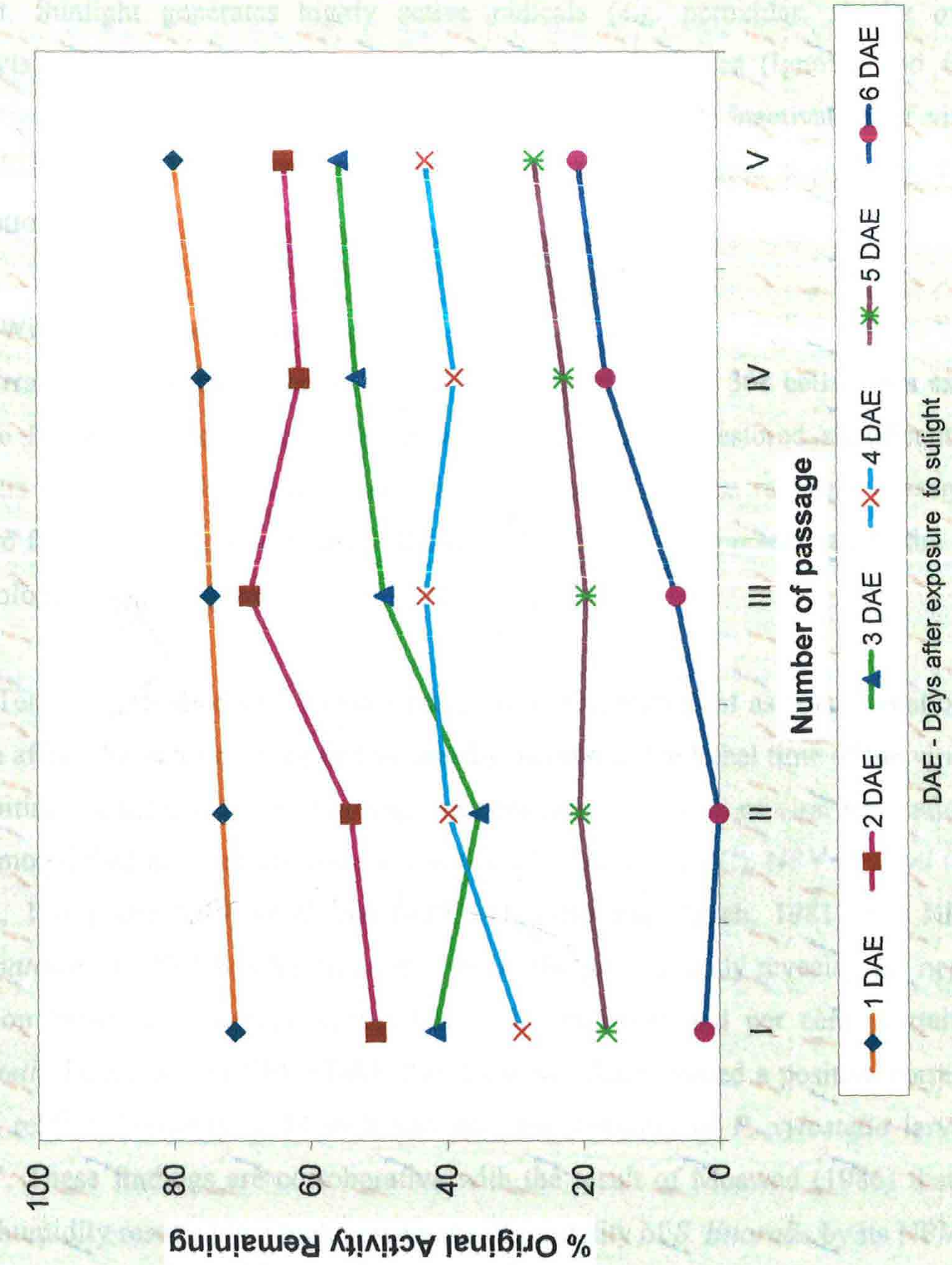
5.8. Persistence of GmNPV

One of the drawbacks of the use of entomopathogenic viruses is their relative sensitivity to UV light. Insect viruses are known to be inactivated by solar radiation (Bullock, 1967; Cantwell, 1967; Morris, 1971; Smirnoff, 1972). Inactivation of insect viruses by radiation has important consequences in terms of their effectiveness as microbial insecticides (Burges and Hussey, 1971; DeBach, 1964; Stairs, 1972). Although the predominant type of radiation penetrating the atmospheric ozone of the earth occurs in the near UV range (3000-3800 A), a significant amount of far UV radiation (1900-3000 A) may periodically reach the surface of the earth (Barker, 1968). Hence an attempt was made to study the effect of UV light by exposing GmNPV to sunlight continuously for a period of time.

The persistence of GmNPV decreased considerably with increase in exposure time to sunlight. It persisted for 6 days with 50 per cent loss in activity at the end of 2 days of exposure (Table 24). Witt and Stairs (1975) showed that NPV of *G. mellonella* was inactivated by exposure to both UV and near UV radiation. El-Nagar *et al.* (1980) reported that the per cent originally activity remaining (OAR) of purified *S. littoralis* NPV on cotton plants was 44 per cent after one day of exposure.

Carter (1984) suggested that viral isolates with high resistance to UV inactivation can be developed by manipulation of the existing isolate. In the present study, selection of UV tolerance by passaging the persisted virus serially through the host and exposing the progeny virus to sunlight to extended period was attempted. The persistence of GmNPV could not be enhanced by serial passages and exposures to sunlight as the per cent OAR was on par even after five serial passages (Fig. 6). This is in conformity with the result obtained by Witt and Hink (1979). Repeated selection treatment did not yield AcMNPV

Fig 6. Persistence of GmNPV on cauliflower phyllosphere



with increased resistance to UV radiation. Moreover the virulence of the virus after selection was significantly reduced in comparison with that of wild virus.

Several factors have been identified for the mechanism of inactivation of NPVs by sunlight. Sunlight generates highly active radicals (e.g. peroxides, singlet oxygen, hydroxyls) which are involved in degradation of baculoviruses (Ignoffo and Garcia, 1994). Pyrimidine dimers are the major factors responsible for inactivation of virus by UV radiation. If other photolesions occur, they are irreparable accounting for the inactivation of virus.

Witt (1984) developed photoreactivation and ultra violet - enhanced reactivation of UV irradiated NPV by insect cells. GmNPV cultured in TN - 368 cells when exposed to white fluorescent and black light or to far UV radiation restored significantly the infectious capacity of UV- irradiated virions of GmNPV. Since repeated passage and exposure failed to select UV tolerant strain of GmNPV in the present study, the above methodology can be adopted.

Temperature effects on baculoviruses are not as important as solar radiation, but they can affect the success of applied viruses by increasing the lethal time of the virus and by inhibiting the infection at low or high temperatures. Inhibition of viral replication has been demonstrated in some insects, such as GV of *P. rapae* (36°C), NPV of *T. ni* (39°C) (Tanada, 1963), the NPV of *H. zea* (40°C) (Ignoffo and Couch, 1981) and NPV of *A. gemmatalis* (10-40°C) (Johnson *et al.,* 1982). The present study revealed the negative correlation between maximum temperature, solar radiation and per cent mortality of *P. xylostella* larvae by GmNPV (Table 27). However there existed a positive correlation between relative humidity at 14.30 h and per cent mortality of *P. xylostella* larvae by GmNPV. These findings are corroborative with the result of Moawad (1986) that high relative humidity resulted in a high percentage of mortality of *S. littoralis* by its NPV.

The persistence of GmNPV was highest in the month of October, November and December with low maximum temperature (26.2 - 31°C), low solar radiation (225.7 -

358.8 calories/m^2) and high relative humidity (68.8 - 83.8%) while the persistence of GmNPV was lowest in the month of February and April when a higher maximum temperature, solar radiation and low RH prevailed. Manjunath and Mathad (1981) reported that the reduction in virus infectivity of NPV of *M. separata* was more rapid in summer than in winter which was in conformity with the results obtained in the present study.

Many substances like uric acid, folic acid, Tinopal DCS, dyes have been tested as sunscreening agents to extend viral activity in the field (Jaques, 1985; Shapiro, 1995). Recently research with flourescent brighteners of the stilbene group showed that they are efficient protectants of baculoviruses against solar radiation as they absorb UV radiation (Shapiro and Hamm, 1999; Farrar and Ridgway, 2000; Young, 2001).

Several other protectants advocated were copper ammonium nitrate, copper sulfate (Arivudainambi *et al.,* 2000), robin blue (Reddy *et al.,* 2001) lampblack, typtophan, lyrosine (Sairabanu, 2000) for NPVs and GVs. This offers scope for enhancing the persistence of GmNPV under field conditions particularly in the months of January-April by adding UV protectants.

5.9. Field efficacy of GmNPV

Normally NPVs are used in the dose range of 1.5 - 3.0 x 10^{12} POB/ha under field conditions (Geetha, 1997; Subramanian, 1998; Sathiah, 2000). Being a heterologous infection and based on the laboratory experiment results, GmNPV was used in the high dose range of 1.88 - 7.50 x 10^{13} POB/ha against *P. xylostella* on cauliflower in the present study.

The results of the field trial indicated that the chemical insecticide indoxacarb was the most effective with a total reduction of 73.91 per cent in larval population over control after four rounds of sprays (Table 30). The efficacy of chemical insecticide can be attributed to its quick knock down effect. Rajagopalbabu (2001) reported that indoxacarb was one among the best treatment against *P. xylostella* in field. GmNPV @ 7.5 x 10^{13}

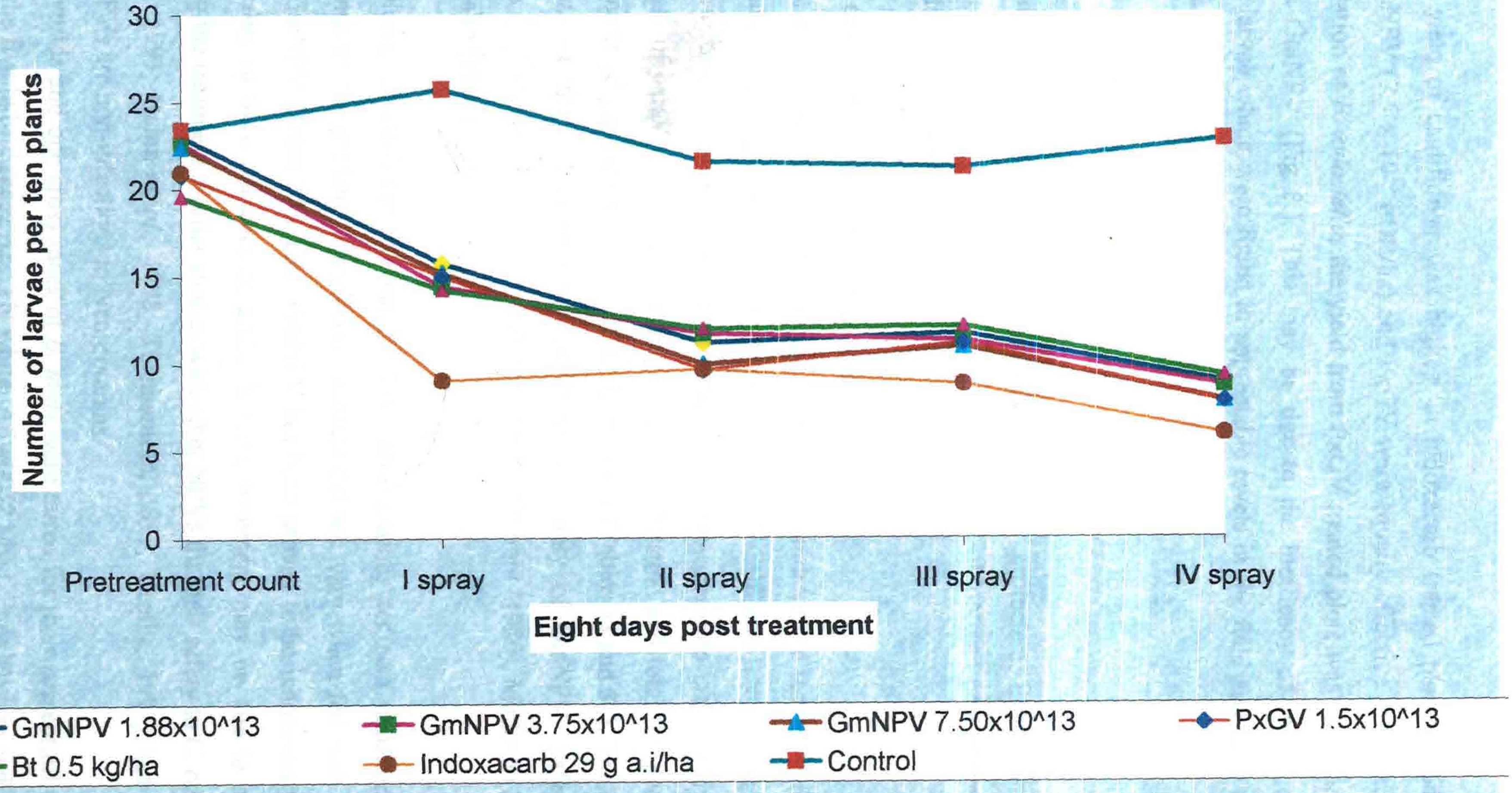
POB/ha stood next and was as effective as PxGV applied @ 1.5 x 10^{13} POB/ha. The subsequent doses of GmNPV (1.88 x 10^{13} and 3.75 x 10^{13} POB/ha) were on par with *B.t.* In all the virus treatment, a total reduction of 60-75 per cent of larval population over control was observed at the end of four subsequent days at eight day intervals (Fig 7.). This proves that GmNPV is effective under field conditions against *P. xylostella.*

Though GmNPV is persistent for 6 days under natural condition, spraying was done at 8 days interval to cover the period from primordial initiation to heading stages of cauliflower where the peak incidence of *P. xylostella* occurs. *B.t.* was found to be effective only upto four days after spray and the larval population increased six days after each spray. Kreig *et al.* (1981) demonstrated that inactivation by short wave and long wave UV radiation was higher in *B.t.* than NPV. Hence it can be presumed that poor persistence of *B.t* on cauliflower foliage reflected in the less efficacy of *B.t.* than GmNPV and PxGV in the present study. The order of efficacy of treatments did not vary at the end of each spray.

The efficacies of above treatments followed the same order as those on the larval population of *C. binotalis*. The plots treated with GmNPV @ 7.5 x 10^{13} POB/ha recorded the lowest population of *C. binotalis* and it was equivalent to that of indoxacarb (Table 31). GmNPV applied at the rate of 1.88 - 3.75 x 10^{13} POB/ha was more effective than *B.t.* However, the larval population of *C. binotalis* treated with PxGV @ 1.5 x 10^{13} OB/ha was on par with control. As PxGV is highly host specific, it was not effective in checking the larval population of *C. binotalis.* Kadir *et al.* (1999b) reported that neonate larvae of *C. binotalis* were more susceptible to GmNPV than those of *P. xylostella.* This substantiates the results in the present study that the effect of GmNPV at highest dose is comparable with that of chemical insecticide.

There are no reports on the field efficacy of GmNPV in the control of crop pests except the one by Dougherty *et al.* (1982). The authors have used GmNPV for the management of *G. mellonella* by spraying on bee hives under field conditions.

Fig 7. Field efficacy of GmNPV against *P. xylostella*



The yield of cauliflower was highest in indoxacarb treated plots (26.64 t/ha). Though GmNPV (7.5 x 10^{13} POB/ha) and PxGV were equally effective in reducing the larval population of *P. xylostella*, the yield from PxGV treated plots was less than those treated with GmNPV (Fig 8.). This may be due to the inefficacy of PxGV against *C. binotalis* larvae which were found in appreciable levels in the field and have caused high damage to the flower heads in PxGV treated plots.

Field studies indicate that GmNPV @ 7.5 x 10^{13} POB/ha was as effective as PxGV against *P.xylostella*. GmNPV has an added advantage over PxGV that it is highly effective against *C. binotalis* also. Moreover addition of UV protectants like the stilbene compounds may improve the efficacy of GmNPV against lepidopteran pest complex of crucifers under field conditions.

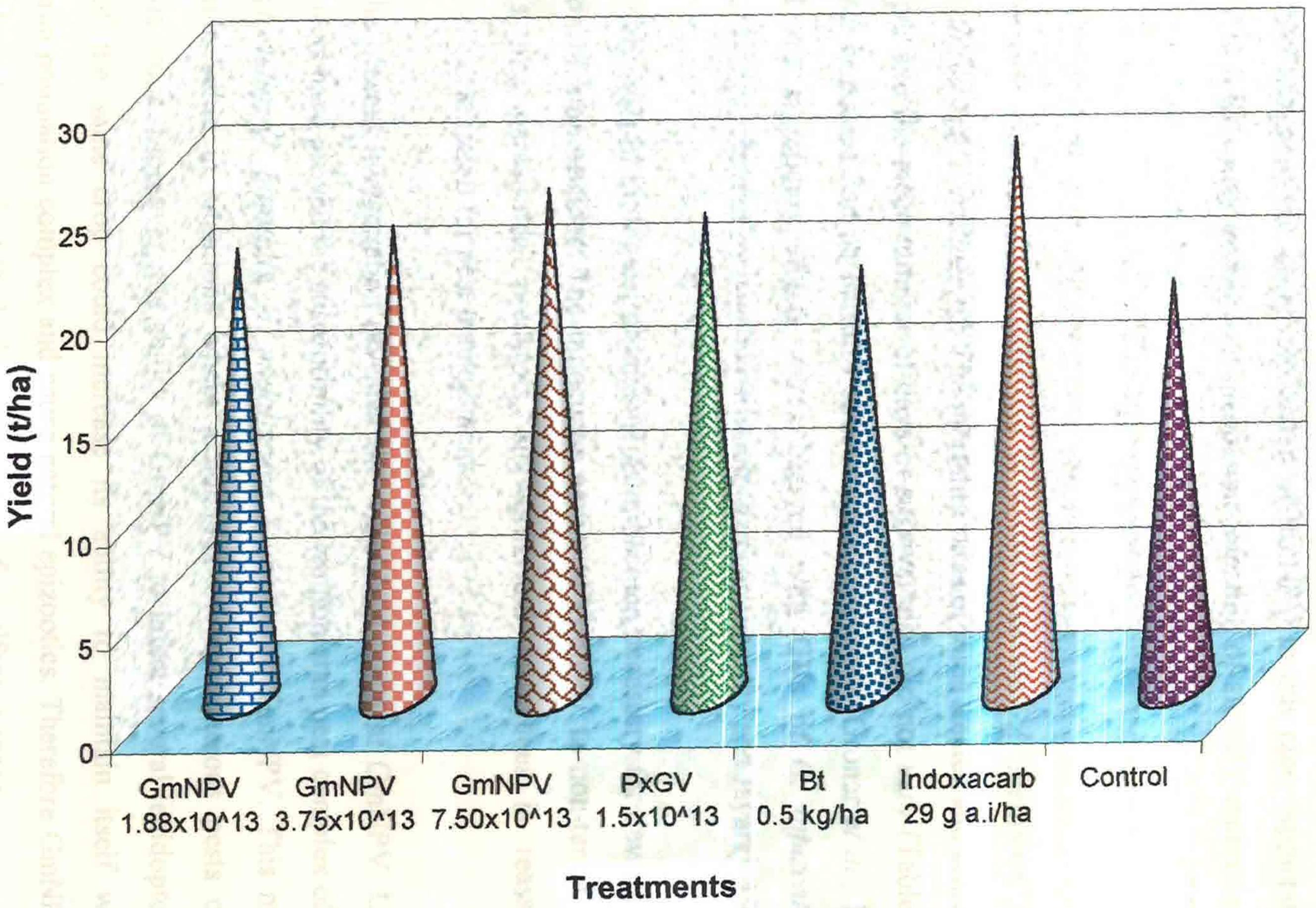
5.10. Safety of GmNPV to non-target organisms

Being a virus with a broad host range, it becomes mandatory to test the safety of GmNPV to non-target organisms. In the present study, both CBE and BNGL isolates of GmNPV were found to be safe to the egg parasitoid *Trichogramma chilonis* by egg treatment. Rate of parasitization, adult emergence, adult duration and total life cycle of treated and untreated population did not differ significantly (Tables 33 and 34). Treatment of *T. chilonis* adults by oral feeding confirmed the safety of GmNPV. Safety of baculoviruses to *T. chilonis* has been already reported by Ethiraju (1986), Muthiah (1988) and Maheshbabu (1991).

The biological attributes like hatchability, larval period, per cent pupation and adult emergence of the predator *Chrysoperla carnea* did not vary when fed with eggs treated with GmNPV (Table 35). Thus GmNPV has been proved to be innocuous to *C. carnea*. Mortality of 8-10 per cent of grubs in virus treatment may be due to stress induced during the treatment. Earlier studies with other NPVs for their safety to *C. carnea* (Maheshbabu, 1991; Heinz *et al.*, 1995; Thennarasan, 1997; Geetha, 1997) had also proved that NPVs are non-infectious to Neuropterans.

Five hybrids and one cross races of *B. mori* were tested for their susceptibility to GmNPV. All the five hybrid races *viz.*, TN white, AE 40032 Mysl, Pure Mysore and Thai

Fig 8. Effect of GmNPV on the yield of cauliflower



were resistant to GmNPV infection. The larval weight, duration of life stages, cocoon weight and adult emergence were normal (Table 36). However, a mortality of 26.70 per cent of larvae of cross race, CSRI 4 x 2 was observed. The virus extracted from cadavers was confirmed as BmNPV by physical examination. The inoculation of GmNPV probably had triggered the latent expression of BmNPV in this race. Application of an alien NPV in a host may activate an inapparent infection of an NPV native in that host (Burges *et al.*, 1980). Also, Rabindra *et al.* (1998a) showed that inoculation of GmNPV in *H. armigera* triggered the latent expression of HaNPV.

GmNPV did not induce infection in honey bees *viz.*, *A. mellifera*, *A. cerana indica*, *A. florea* and *T. iridipennis*. The mortality rates of virus treated and untreated bees were on par and the mean number of days of survival also did not vary (Table 37). The NPV of *M. brassicae* had no harmful effect on the honeybees (Groner *et al.*, 1978). No abnormal and significant effects were observed with GVs of *C. infuscatellus* and *C. sacchariphagus indicus* by cage experiment (Easwaramoorthy and Jayaraj, 1987)

Safety tests of biological organisms have become necessary for development and registration of biopesticides. The innocuous nature of GmNPV to non-target beneficial organisms like parasitoids, predators, silkworm and honeybees is reassuring its commercial exploitation for pest management

The present investigation revealed the wider host range of GmNPV. Laboratory and field tests have proved the susceptibility of the lepidopteran pest complex of crucifers *viz. P. xylostella*, *C. binotalis*, *H. undalis* and *S. exigua* to GmNPV. This may be of considerable value in situations where a complex of lepidopterous pests occurs on cruciferous crops. Moreover, the ability of GmNPV to infect several lepidopterous pest species of the same crop could increase its ability to maintain itself within the lepidopteran population complex and cause natural epizootics. Therefore GmNPV would be an ideal candidate against the lepidopteran pests of cruciferous crops.

The key pests of sugarcane *viz. C. infuscatellus* and *C. sacchariphagus indicus* were found to be susceptible to GmNPV. Though there are reports of granulosis virus infections in these pests, difficulty in mass culturing the borers under laboratory conditions hampers the economical production of GVs, which are highly host specific. On the other hand, GmNPV can be mass produced economically in *C. partellus*, as evidenced in the present study, and this would be of greater significance for use against these pests in the sugarcane ecosystem. Also, there are no reports on the viral infection of lepidopteran pest complex of rice ecosystem. There are several lepidopteran pests like stem borers, horned caterpillar, skipper, case worm, armyworm and cutworms besides the GmNPV susceptible leaf folder complex. The cross-infection of GmNPV to these pests needs to be tested by experimentation. The same can be extended to the pests of pulses where GmNPV is infectious to spotted pod borer and plume moth.

Based on the results of the present investigation the following are the future lines of research:

1. Cross-infectivity tests on other lepidopteran pests that produce more and high yield of polyhedra per larva
2. Characterization of chitinase gene of GmNPV, cloning of hyperactive chitinase genes either from insects or other baculoviruses like HaNPV and SlNPV.
3. Cloning of small POB from a cell line and passages through *in vivo* for the development of a strain of GmNPV that produce small and many polyhedra.
4. Development of IPM module with GmNPV as a component against insect pests of different crop ecosystems *viz.* crucifer, sugarcane, rice and pulses.

The inherent characteristics of GmNPV *viz*, the wide host range, increase in virulence by serial passages, capacity to replicate in cell lines, amenability to economic mass production in *C. partellus* larvae, ability to transmit transovarially and to persist on phyllosphere, potentiality to reduce larval populations under field conditions and safety to non-target organisms, make it a potential candidate for development as bioinsecticide for use against several economically important lepidopteran pests in different crops.

Summary

Chapter VI

SUMMARY

Laboratory and field investigations were carried out to explore the potential of GmNPV as a microbial insecticide against important pests of crops with special focus on the pests of cauliflower. Safety of the virus to silkworm, honeybees and entomophages was also studied. The results of the findings are summarized below:

1. GmNPV was cross-infective to *C. binotalis, H. undalis, S. exigua, C. partellus, C. infuscatellus, C. sacchariphagus indicus, P. ricini, E. merione, M. vitrata, E. atomosa, C. medinalis, M. patnalis, O. arenosella, A. albistriga* and *D. pulverulentalis*, besides *P. xylostella*. Insect species like *H. armigera, S. litura, E. vittella, A. peponis, M. separata, B. mori, A. janata, E. mollifera, N. geometralis, D. indica, P. zizyphi, H. puera, E. macheralis, C. cephalonica* and *A. ipsilon* were not susceptible to GmNPV infection. The cross- infectivity tests reveal the broader host range of GmNPV and its infection in 14 insect species are reported for the first time.
2. The cross-infectivity of GmNPV was confirmed in *P. xylostella, M. vitrata, H. undalis, P. ricini, C. partellus, C. infuscatellus* and *D. pulverulentalis* by restriction endonuclease (REN) analysis of GmNPV DNA before and after passage through alternate hosts. In the insect species with insufficient DNA for REN analysis because of poor yield/ larva, cross-infectivity was confirmed by reciprocal inoculation studies. Thus DNA characterization studies unequivocally confirm the GmNPV infection in these insects.
3. *C. infuscatellus* was the most susceptible alternate host and *S. exigua* was the least susceptible host to GmNPV. The yield of GmNPV was highest in *P. ricini* larvae and lowest in *O. arenosella* larvae. CBE isolate was more virulent than BNGL isolate against *H. undalis, C. partellus, M. vitrata, P. ricini* and *C. infuscatellus*. This study indicates the variation in the susceptibility of alternate hosts to the heterologous GmNPV infection.

4. Serial passages of GmNPV through alternate hosts like *P. xylostella*, *C. binotalis*, *P. ricini*, *H. undalis*, *M. vitrata*, *C. infuscatellus*, *C. partellus*, *C. sacchariphagus indicus* and homologous host, *G. mellonella* for five times decreased LC_{50} of GmNPV against *P. xylostella.* The reduction was significant by 6.84 fold in passages through the same host, *P. xylostella*. Hence, GmNPV requires serial passages through the homologous or heterologous hosts to be more virulent against *P. xylostella.*
5. Second, third and fourth instar larvae of *P. xylostella* were susceptible to CBE and BNGL isolates of GmNPV with no variation in the virulence between the isolates. LC_{50} for second instar larvae was in the range of 4.55- 6.32 POB/mm^2 while third and fourth instars had 11.55 and 129.27 fold higher LC_{50} for CBE isolate. More than 50 per cent mortality of early third instar larvae was recorded with a dose of 46.79 POB/mm^2 of GmNPV. GmNPV was found to be effective against all the larval stages of *P. xylostella.*
6. The median lethal concentration of GmNPV against *P. xylostella* larvae decreased by 12.76 fold when serially passaged through *P. xylostella* larvae for 15 times, while LT_{50} did not change upon passages. Though GmNPV recorded 10.31 fold high LC_{50} for *P. xylostella* than PxGV, LT_{50} was short by 23.31 h. LC_{50} of GmNPV was comparable with that of PxGV after four subsequent passages in *P. xylostella*. The combination of GmNPV and PxGV did not have any synergistic effect on *P. xylostella* larvae. Serial passages of GmNPV through *P. xylostella* larvae aids in the isolation of virulent strains of the virus for use against the pest.
7. Whole diet contamination method with 100 µl of GmNPV suspension ($2x10^7$ POB/ml) in 1.0 g of artificial diet was found to be the best method for mass production of GmNPV in *G. mellonella* larvae. The yield of POB/larva of CBE isolate of GmNPV was 10 fold higher than that of BNGL isolate.
8. Fourth instar larvae of *C. partellus* was found to be the suitable host for mass production of GmNPV with high mortality (81.82-83.03%) and yield (2.82-$2.91x10^8$ POB/larva) and ease of mass culturing on inexpensive semi-synthetic diet. Other hosts studied *viz.*, *G. mellonella* and *P. ricini* were found unsuitable because of the presence of maturation immunity and allergenic urticating setae

respectively. This imparts the importance of selection of suitable host for mass production of GmNPV with high susceptibility, good yield of POB and economic mass culturing technology.

9. Transovarial transmission of GmNPV determined in *C. binotalis* and *P. ricini* did not affect the fecundity, hatchability and adult emergence of the treated generations. The horizontally and vertically transmitted GmNPV did not vary in their activity against *P. xylostella* larvae. Thus, transmission of virus through eggs provides an economical and self-perpetuating method of insect control.

10. Passage of GmNPV through alternate hosts influenced the morphometrics of GmNPV as revealed by decrease in size of polyhedra when compared to that of passages through homologous host.

11. Histopathology studies confirmed the GmNPV replication in fat body tissues of *P. xylostella, C. partellus* and *M. vitrata* during the initial stages of infection and in hypodermal cells, tracheal tissue matrix, salivary glands, ganglion and imaginal disc at the advanced stages of infection. Variations in the rate of infection of host tissues were observed among the three insects. Microsections of different tissues of insect species elucidate the course of GmNPV infection.

12. *In vitro* studies revealed the permissibility of cell lines *viz.* SlH, SlPG, Sf21 and HaH to GmNPV infection. Sf21 cell line was the most susceptible with high per cent infection and yield of POB/ml of culture medium while HaH was the least susceptible cell line. The virions from *C. partellus* were more virulent than virions from *P. ricini, M. vitrata* and *H. undalis*. The *in vitro* and *in vivo* produced GmNPV did not differ in their activity against *P. xylostella* larvae irrespective of inoculum from haemolymph or cell lines. Selection of virulent haemolymph source and cell line supporting the maximum polyhedra production is of great economic significance in the baculovirus expression vector system studies.

13. GmNPV recorded the lowest chitinase activity when compared to HaNPV and SlNPV attributing to the atypical symptoms of disease. Between the homologous (*G. mellonella*) and heterologous host (*C. partellus),* the chitinase level was higher in the former than the latter at the end of each observation period. Determination

of chitinase level of GmNPV explores the possibility of future cloning studies with this virus.

14. The persistence of GmNPV on cauliflower phyllosphere under natural conditions was six days post exposure to sunlight. Subsequent passages of persisted virus and exposure to sunlight did not enhance the persistence of GmNPV. The persistence of GmNPV was positively correlated with relative humidity at 14.30 h and negatively correlated with maximum temperature and solar radiation. This study shows the significance of microclimatic weather parameters on the persistence of GmNPV. Application of GmNPV @ 5×10^7 POB/ml caused more than 50 per cent mortality of lepidopteran pest complex of crucifers *viz*., *P. xylostella*, *C. binotalis* and *H. undalis* in pot culture assays.

15. GmNPV @ 7.5×10^{13} POB/ha was found to be the most effective in debilitating the larval population of *P. xylostella* and *C. binotalis* under field conditions. GmNPV at this dose performed better than *B.t.* but only next to the chemical insecticide, indoxacarb for *P. xylostella* while it was equivalent to the effect of chemical insecticide in the case of *C. binotalis*. The yield of cauliflower heads was highest in indoxacarb treated plots followed by that of GmNPV treatments. Four rounds of GmNPV spray at eight day intervals commencing from 60 DAT were required. Thus GmNPV was found to be effective in reducing the larval population of *P. xylostella* and *C. binotalis* on cauliflower under field conditions.

16. The innocuous nature of GmNPV to natural enemies and beneficial organisms was ascertained by safety tests with *T. chilonis*, *C. carnea*, four species of honey bees and six different races of *B. mori*. Safety of GmNPV to non-target organisms gives assurance for developing it as a bioinsecticide for use against several economically important crop pests.

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Appendices

Appendix I

Semi-synthethic diets

Galleria mellonella (Singh, 1994)

Fraction A:		**Fraction B**	
Wheat flour	200g	Honey	375 ml
Corn flour	400g	Glycerol	375 ml
Wheat germ	200g		
Milk powder	200g		
Yeast	100g		

Mix fraction A and B separately and blend together into a dough.

Chilo partellus (Sharma *et al.* 1994)

Fraction A		**Fraction B**	
Water	2000 ml	Agar-agar	40.8 g
Chickpea flour	438.4 g	Water	1600 ml
Yeast	32.0 g	Formaldehyde (40%)	3.2 ml
Sorbic acid	4.0 g		
Vitamin E	4.6 g		
Methyl p-hydroxy benzoate	6.4 g		
Ascorbic acid	10.4 g		
Sorghum leaf powder	160.0 g		

Blend A without sorghum leaf powder. For 1 min soak sorghum leaf powder in 70°C water. Boil agar for 2 min., cool, add formaldehyde and A. Blend for 3 min. 300g diet - 1 lit plastic jar.

Maruca vitrata/Agrotis ipsilon/Helicoverpa armigera/ Spodoptera litura/Exelastis atomosa/Mythimna separata **(Singh, 1994)**

Chickpea seeds (White kabuli)	:	100.00g
Distilled water	:	400.00ml
Agar agar	:	12.80g
Distilled water	:	400.00ml
Methy p hydroxy benzoate	:	2.00g
Sorbic acid	:	1.00g
Wesson's salt	:	7.20g
Carbendazim	:	0.50g
Formaledhyde 40%	:	1.00ml
Yeast (Brewer's)	:	30.00g
Choline chloride 10%	:	7.20ml
Vitamin (ABDEC)	:	2.00ml
Ascorbic acid	:	3.20g
Streptomycin sulphate	:	0.04g

Appendix II

Buffers for restriction endonucleases

PRODUCT : PROMEGA

Pst I, Buffer H : 10 mM Tris-HCl pH 7.4, 50 mM NaCl, 0.1 mM EDTA, 1 mM DIT, 0.15% Triton x 100, 0.5 mg/ml BSA, 50% glycerol

BamH I, Buffer E : 10 mM Tris-HCl pH 7.4, 300 mM KCl, 0.1 mM EDTA, 1 mM DIT, 5 mg/ml BSA, 50% glycerol

Appendix III

Head capsule width

Instar	Width of the head capsule (μ) ± S.E. *		
	G. mellonella	*P. ricini*	*C. partellus*
II	64.4 ± 1.54	191.82 ± 1.56	87.40 ± 0.86
Early III	100.74 ± 2.52	243.80 ± 1.14	105.34 ± 1.20
Late III	120.98 ± 1.02	262.20 ± 3.98	129.26 ± 0.73
Early IV	159.36 ± 3.56	354.60 ± 5.45	145.82 ± 1.47
Late IV	184.00 ± 2.74	409.20 ± 2.96	163.18 ± 2.34
Early V	223.62 ± 1.23	424.00 ± 7.85	196.88 ± 2.69

*** Mean of 30 observations**

Appendix IV

Reagents for chitinase assay

1. 1M CH3COONa buffer pH 4.0

Fourty one ml of 1 M CH3COO and 9 ml of 1 M CH3COONa are added to make 50 ml of 0.2 M buffer. From this stock, the buffer of 1 M concentration was made.

2. 1M potassium phosphate buffer, pH 7.1

Thirty five ml of 0.2 M KOH and 15 ml of 0.2 M KH_2PO_4 are added and the volume is made upto 100 ml to arrive 0.1 M of potassium phosphate buffer.

3. 0.05M Na-borate buffer, pH 9.8

Fifty ml of 0.05 M borax and 34 ml of 0.2 N NaOH are added and the volume is made to 200 ml to arrive 0.05 M borate buffer.

4. Colloidal chitin substrate : 10 mg/ml

Appendix V-A

Molecular weights (Kbp) of GmNPV DNA fragments

Restriction endonuclease analysis – *Pst* I

Fragment number	Lane 1	Lane 2	Lane 3
1	25.13	25.13	25.13
2	21.52	21.52	21.52
3	19.11	19.11	19.11
4	12.86	17.08	12.86
5	10.67	10.67	10.67
6	9.56	9.56	9.56
7	7.48	7.48	7.48
8	5.13	5.13	5.13
9	2.89	2.89	2.89
10	2.46	2.46	2.46
	116.82	121.04	116.82

Lane 1 – inoculated virus of GmNPV

Lane 2 – progeny virus from *P. xylostella*

Lane 3 – progeny virus from *M .vitrata*

Appendix V-B

Restriction endonuclease analysis – *Pst* I

Fragment number	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
1	25.47	25.47	25.47	25.47	25.47	25.12
2	21.93	21.93	21.93	21.93	21.93	21.46
3	17.13	17.13	17.13	17.13	17.13	16.58
4	13.39	13.39	13.39	13.39	13.39	11.86
5	10.48	10.48	10.48	10.48	10.48	10.02
6	9.08	9.08	9.08	9.08	9.08	8.72
7	7.34	7.34	7.34	7.34	7.34	7.23
8	5.53	5.53	5.53	5.53	5.53	5.26
9	2.85	2.85	2.85	2.85	2.85	2.75
10	2.76	2.76	2.76	2.76	2.76	2.62
	115.96	115.96	115.96	115.96	115.96	111.62

Lane 1 – progeny virus from *C. binotalis*

Lane 2 – progeny virus from *P. ricini.*

Lane 3 – progeny virus from *H. undalis*

Lane 4 – progeny virus from *C. infuscatellus*

Lane 5 – progeny virus from *C. partellus*

Lane 6 – inoculated virus of GmNPV

Appendix V-C

Restriction endonuclease analysis – *Bam*H I

Fragment number	**Lane 1**	**Lane 2**
1	20.96	20.96
2	10.50	10.50
3	9.18	9.18
4	8.61	8.61
5	5.39	5.39
6	4.96	4.96
7	2.60	2.60
	62.20	62.20

Lane 1 – inoculated virus of GmNPV

Lane 2 – progeny virus from *D. pulverulentalis*

APPENDIX VI

Comparison of concentration - mortality responses of *P. xylostella* larvae to GmNPV (CBE isolate) passaged through different susceptible hosts

VI. A) I Passage

GmNPV passed through	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LC_{50} (POB/mm^2)	Fiducial limits
G. mellonella	192	0.768	0.918 ±0.183	15.16	8.59 – 27.35
P. xylostella	197	0.282	0.564 ±0.153	54.84	21.03 – 173.04
C. binotalis	198	0.212	0.408 ± 0.165	314.56	107.11 – 1929.99
P. ricini	198	0.440	0.578 ± 0.161	54.024	25.221 – 131.188
H. undalis	197	0.459	0.365 ±0.139	31.61	8.72 – 138.73
M. vitrata	197	0.212	0.351 ±0.141	98.59	23.95 – 117.28
C. infuscatellus	195	1.598	0.561 ± 0.152	24.91	11.11 – 57.35
C. partellus	197	2.049	0.546 ±0.179	215.26	90.72 – 763.43
C. medinalis	197	3.605	0.548 ±0.189	339.70	136.38 – 1411.43
C. sacchariphagus indicus	195	0.278	0.514 ± 0.159	68.68	29.39 – 196.67
E. atomosa	195	1.009	0.582 ± 0.191	272.64	116.84 – 948.46
E. merione	192	1.041	0.522 ± 0.0893	127.14	52.82 – 428.70
D. pulverulentalis	195	0.629	0.544 ± 0.089	105.47	46.37 – 309.14

***All lines are significantly a good fit ($P<0.05$)**

VI. B) II Passage

GmNPV passed through	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LC_{50} (POB/mm^2)	Fiducial limits
G. mellonella	198	0.424	0.569 ± 0.151	21.83	9.65 – 49.37
P. xylostella	198	0.564	0.416 ± 0.148	48.35	17.33 – 169.71
C. binotalis	196	1.185	0.405 ±0.147	154.89	53.05 – 794.75
P. ricini	197	1.410	0.341 ± 0.144	105.34	30.65 – 783.72
H. undalis	198	0.564	0.448 ± 0.143	23.06	8.47 – 64.56
M. vitrata	194	0.767	0.662 ± 0.167	42.96	21.54 – 93.08
C. infuscatellus	195	4.413	0.605 ± 0.146	12.15	5.49 – 25.65
C. partellus	195	0.664	0.450 ± 0.159	117.30	44.13 – 481.33
C. medinalis	197	2.618	0.548 ± 0.204	612.52	226.67 – 3292.34
C. sacchariphagus indicus	194	1.154	0.553 ± 0.159	48.19	21.66 – 120.31
E. atomosa	195	0.607	0.417 ± 0.158	217.52	72.80 – 1316.32
E. merione	195	0.407	0.440 ± 0.0836	127.13	46.68 – 552.52
D. pulverulentalis	194	0.280	0.527 ± 0.087	88.17	38.16 – 258.07

***All lines are significantly a good fit ($P<0.05$)**

VI. C) III Passage

GmNPV passed through	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LC_{50} (POB/mm^2)	Fiducial limits
G. mellonella	198	1.354	0.645 ± 0.133	8.73	4.53 – 17.54
P. xylostella	198	0.212	0.378 ± 0.149	33.59	10.62 – 125.63
C. binotalis	196	0.842	0.415 ± 0.158	208.30	70.03 – 1244.88
P. ricini	196	0.236	0.511 ± 0.157	70.72	30.21 – 205.99
H. undalis	196	1.869	0.378 ± 0.146	43.55	14.02 – 173.54
M. vitrata	196	0.472	0.513 ± 0.155	54.61	23.35 – 153.11
C. infuscatellus	194	1.559	0.659 ± 0.166	34.08	17.32 – 70.86
C. partellus	197	0.505	0.442 ± 0.152	84.31	31.92 – 319.19
C. medinalis	197	1.420	0.696 ± 0.229	362.63	169.39 – 1102.87
C. sacchariphagus indicus	196	1.895	0.617 ± 0.164	42.57	20.55 – 94.84
E. atomosa	194	0.502	0.436 ± 0.161	194.62	67.59 – 1080.52
E. merione	193	0.407	0.440 ± 0.086	127.10	46.68 – 552.52
D. pulverulentalis	195	0.292	0.593 ± 0.910	97.18	45.32 – 253.66

***All lines are significantly a good fit ($P<0.05$)**

VI. D) IV Passage

GmNPV passed through	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LC_{50} (POB/mm^2)	Fiducial limits
G. mellonella	196	0.410	0.571 ± 0.142	6.65	2.60 – 14.45
P. xylostella	196	1.250	0.463 ± 0.148	18.04	6.46 –46.71
C. binotalis	197	1.260	0.491 ± 0.163	138.98	55.83 – 511.09
P. ricini	197	1.081	0.346 ± 0.141	41.47	12.06 – 193.50
H. undalis	192	0.122	0.488 ± 0.148	40.94	15.49 – 197.91
M. vitrata	193	0.607	0.535 ± 0.158	59.26	25.66 – 162.47
C. infuscatellus	197	0.771	0.594 ± 0.158	34.60	12.23 – 78.28
C. sacchariphagus indicus	195	1.037	0.570 ± 0.155	31.46	14.21 – 73.31
C. partellus	194	0.441	0.420 ± 0.082	51.83	18.56 – 185.75

***All lines are significantly a good fit ($P<0.05$)**

VI. E) V Passage

GmNPV passed through	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LC_{50} (POB/mm^2)	Fiducial limits
G. mellonella	195	2.434	0.574 ± 0.141	5.88	2.29 – 12.75
P. xylostella	198	4.270	0.331 ± 0.139	8.02	2.28 – 11.03
C. binotalis	197	1.230	0.158 ± 0.164	120.10	50.73 – 390.27
P. ricini	192	0.732	0.414 ± 0.146	22.87	7.48 – 69.31
H. undalis	194	1.180	0.502 ± 0.151	32.06	13.25 – 83.77
M. vitrata	196	0.851	0.589 ± 0.158	39.15	18.37 – 90.06
C. infuscatellus	198	2.394	0.444 ± 0.139	10.86	3.52 – 28.75
C. sacchariphagus indicus	196	0.469	0.484 ± 0.148	28.40	11.34 – 75.16
C. partellus	195	0.318	0.464 ± 0.083	46.02	17.91 – 139.06

*** All lines are significantly a good fit ($P<0.05$)**