Chapter: 1
INTRODUCTION

The cabbage white, *Pieris brassicae* (L.) is an important pest, known to infest 83 species of food plants belonging to the families such as Cruciferae, Tropaeolaceae, Capparaceae, Resedaceae and Papilionaceae (Feltwell, 1982). It is a cosmopolitan species, found wherever cruciferous vegetables are grown (Atwal and Dahliwal, 1990). In India this pest has been observed in the entire sub Himalayan region and also up to the plains of Punjab, Haryana, Himachal Pradesh, Madhya Pradesh, West Bengal and north-eastern states of India (Thakur and Deka, 1996; Bhalla et al., 1997). They feed and breed on the cole crops and cause appreciable crop loss to the extent of 75%. The damage is primarily caused by the caterpillars by virtue of their voracious feeding habit (Sachan and Gangwar, 1990). In the present study, larvae of *P. brassicae* were observed at an appreciable density on the cabbage and cauliflower plants especially during January to July under the climatic conditions of Manipur (750-900m MSL).

Parasitoids such as *Cotesia glomeratus* L. (Braconidae); *Campoletis* sp. (Ichneumonidae); *Brachymeria bengalensis* (Cameron) and *B. lasus* (Walker) (Chalcididae) parasitize the larval stages of *P. brassicae* to some extent under the field condition. However, effective control was not achieved with the help of parasitoids because the parasitic activity was found maximum only in March-April. But the early stages of the crop suffer a setback due to the pest species such as *Pieris brassicae* (L.), *P. canidia* (Sparrman) and *Pontia daplidice moorei* (Rober) (=*Pieris daplidice*). Therefore, an alternate eco-friendly method becomes imperative to keep the pest under check. During the routine study on the larval density of *P. brassicae* some larvae were found affected and they exhibited typical symptoms of viral infection. A close examination of the infected larvae reflected that the causative agent was a baculovirus (Ingobi, et al., 2006). Preliminary investigations revealed that this viral pathogen (*Pieris brassicae* Granulosis Virus, *PibrGV*) would be an ideal candidate for the control *P. brassicae*. Further, the review of literature pertaining to *PibrGV* gave a strong indication that this pathogen has not been studied and evaluated in India. Therefore, looking at the prospects of *PibrGV* as a potential microbial agent, a detailed investigation has been attempted in the present work.
Review of Literature

The word virus is derived from Latin word meaning slimy liquid with poison on stench. “A virus is a set of one or more nucleic acid template that is able to organize its own replication only within an ideal host cell (Mathews, 1991). Virus replication within the cell depends on the host’s protein-synthesizing machinery and it gives rise to variants through various kinds of changes in the viral nucleic acid”. The viral particle consists of a protein shell or capsid that surrounds the nucleic acid. The capsids together with nucleic acid form the nucleocapsid. In some, the nucleocapsid is surrounded by envelopes that are lipid bilayer and may be related to components of the cell membrane. Some insect viruses are occluded in proteinaceous bodies and are referred as viral occlusion or inclusion bodies. Occlusion body is more appropriate for the body containing virion and the inclusion body should be a general term referring to a body with or without virion (Goodwin, 1968).

Holmes (1948) was the first to classify insect virus and listed them under two genera–Borrelina and Morotar. The Genus Borrelina consisted of virus that cause polyhedrosis, wilt and other diseases in Lepidoptera, while the genus Morator has only one virus that can cause the sac brood disease in honey bee. The study of insect virus was initiated by Smith in United Kingdom during 1950. More than 20 groups of viruses are known to infect the insects based on the Martigoni and Iwai (1986). However, they broadly grouped the insect virus under the following: (a) Occluded virus; (b) Non-occluded virus; (c) Unclassified virus (Fijita et al., 1998b).

**Occluded virus**

- Family Baculoviridae
  - Eg. NPV- Nuclear
  - Polyhydrolysis virus
  - & Granulosis virus-GV

- Family Reoviridae
  - Eg. Cypovirus-CPV

- Family Entomopox viridae
  - Eg. Entomopox virus
The occluded viruses belong to three families such as Baculoviridae, Reoviridae and Entomopoxviridae. These three families are unique because of the presence of occlusion bodies in which virions at a certain stage in their development are occluded or embedded within a proteinaceous body, i.e. they are capable of producing occlusion bodies consisting of largely of a single protein. Within each family, the occlusion body protein from different viruses is related but there appears to be no homology between proteins from different families (Fujita et al., 1998b). The occlusion body proteins contribute to the stability and persistence of the virus in the environment. It protects them from sunlight, pH and helps in the survival for months in the absence of susceptible host.

The non-occluded viruses are classified as below

```
Non-occluded virus (10 Families)

- Ascoviridae
- Birnaviridae
- Caliciviridae
- Iridoviridae
- Nodaviridae
- Paroviridae
- Picornaviridae
- Polynaviridae
- Rhabdaviridae
- Tetraviridae
```
Non-occluded virus:

The non-occluded virus particles occur freely in the tissue (Smith, 1976). The virions occur freely or occasionally form paracrystalline arrays of virions. The families of non-occluded virus along with their examples are given below:

<table>
<thead>
<tr>
<th>Family</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ascoviridae</td>
<td>Ascoviruses</td>
</tr>
<tr>
<td>2. Birnaviridae</td>
<td>Birnaviruses</td>
</tr>
<tr>
<td>3. Caliciviridae</td>
<td>Caliciviruses</td>
</tr>
<tr>
<td>4. Iridoviridae</td>
<td>Iridoviruses</td>
</tr>
<tr>
<td>5. Nodaviridae</td>
<td>Nodaviruses</td>
</tr>
<tr>
<td>6. Paroviridae</td>
<td>Paroviruses</td>
</tr>
<tr>
<td>7. Picornaviridae</td>
<td>Picornaviruses</td>
</tr>
<tr>
<td>8. Polydnaviridae</td>
<td>Polydnaviruses</td>
</tr>
<tr>
<td>9. Rhabdoviridae</td>
<td>Rhabdaviruses</td>
</tr>
<tr>
<td>10. Tetraviridae</td>
<td>Tetraviruses</td>
</tr>
</tbody>
</table>

Unclassified Virus:

The viruses that are neither occluded nor non-occluded are placed under this group. There was no genetic morphology with those of the two types.

Ex. (i) *Oryctes* virus isolated from *Oryctes rhinoceros*.
(ii) Virus associated with the salivary gland of *Merodon equestris*.
(iii) Virus that cause sterility in adults of Tse tseflies.

The baculovirus is predominant over all other insect viruses for pest control and therefore, they have appreciable economic importance. Hence, a detailed account of baculovirus is given below. The Baculoviruses are rod shaped particles and are packed into a large protein crystal variously termed as polyhedra, capsules, granules. The family Baculoviridae is divided into 3 genera or subgroups on the basis of their structure (Mathews, 1982a). They include:
A. Nuclear Polyhedrosis Virus (NPVs).
B. Granulosis Virus (GVs).
C. Non occluded, rod shaped nuclear virus.

The third subgroup, non occluded rod shaped nuclear virus is no longer included under Baculoviridae as they show no similarity to this family and also fail to synthesis polyhedra (Huger, 1966; Kelly 1975). Therefore, these viruses never become packed in protein crystals. Currently the Family Baculoviridae comprises only two genera. NPVs and GVs and the terms nuclear polyhedrosis virus and granulosis virus have been replaced by *nucleopolyhedrovirus* and *granulovirus* respectively (Murphy et al., 1995). The genus NPV is characterized by the presence of polyhedral-shaped viral occlusions containing randomly occluded viral particles. Nucleo- Polyhedrosis Virus (NPV) has two sub genera. They are:

(i) Single-nucleocapsid NPVs (SNPVs) in which only one nucleocapsid is found per envelope Ex. *Bombyx mori* SNPV (*Bm* SNPV).

(ii) Multinucleocapsid NPVs (MNPVs) in which several nucleocapsid (as many as 39) are enclosed in a common envelope. [ Ex. *Autographa Californica* MNPV (*Ac* MNPV).

The genus GV has one nucleocapsid per envelope and has granular shaped viral occlusion (capsule) containing one or rarely two or more virions as in the case of *Plodia interpunctella*. The sequence homologies of the DNAs indicate that the members of baculoviridae are genetically related (Smith and Summer, 1982). The NPVs and GVs appear to have an ancient association with their host insect and may have evolved with them during 40 to 60 million years of existence (Rhromann et al., 1981). The occurrence of only SNPV in the older insect order and the existence of MNPVs and GVs only in the more recently evolved Lepidoptera suggest that SNPV is the progenitor of the family Baculoviridae (Rohrmann, 1986b). Through host-dependent evolution, the SNPVs gave rise to the MNPVs and GVs infecting Lepidoptera.

**General Morphology of Occlusion Bodies:**

The proteinaceous crystals which occlude the viral particles are the most conspicuous element of the baculovirus structure (Kelly, 1985). These polyhedral structures were observed
firstly in the nuclei of virus infected cells of gypsy moth caterpillars suffered from wilt (Glaser, 1915). The occlusion bodies of NPV or Polyhedra occur in different shapes and sizes and may vary in different insect or even in the same insect but tends to be of the same size in the same cell. This difference may be governed by the virus rather than by the host cell (Gershenson, 1959). The polyhedra are large crystals with the size ranging from 0.5-15 (Smith, 1976). Their shape may be cubic or quasi-spherical and sometimes crescent shaped as in Tipula paludasa NPV (Bergoin and Guelpa, 1977). The polyhydra are also rectangular in the larvae of Panaxia dominula, while others may take the shape of rectangular, hexigonal or segment of an orange. In Bombyx mori the polyhedra may take the shape of dodecahedron, tetrahedra in Lymantria monarca and irregular in Porthetria dispar (Smith, 1976). The size of polyedra may vary inversely with the number formed in a nucleus. The OBs of GVs were first observed by Paillot (1926) in the infected cells of diseased cabbage caterpillar. The capsules are ovo-cylindrical in shape. Their shape has been described as oval, ellipsoidal ovoid or egg shaped. The granulosis affecting Persectania ewingii were sub ovoid, elongate bodies with more or less parallel sides (Lower, 1954).

**General Properties of Polyhedra & Granules:**

The Polyhedra are heavier than water and settle at the bottom in aqueous solution. The polyhedra matrix protein (polyhedrin) makes up about 95% and the virion about 5% of the protein content (Bergold, 1963). This protein forms a distinct crystalline lattice that is not distorted by the presence of occluded virion (Morgan et al., 1955). Federici (1986) stated that polyhedrin is the only single protein that constitutes the matrix of occlusion bodies. This protein is approximately a six armed or nodal unit and is oriented in face centered 4nm cubic lattice. It has a marked tendency to aggregate and also contains octamers of polypeptide as sub unit (Rohrmann, 1977). The virus particles lie at random within the lattice and do not distort the lattice (Kelly, 1985). The polyhedrin has a molecular weight of 27,000 to 34,000 Da, varying with the isolate (Kelly, 1985). It is a phosphorylated molecule of 25 to 31 kDa and commonly reported as 29 kDa (Marunaik, 1986). Polyhedra are stable at moderate pH and they dissolve at pH 9 to 11. Kawanishi et al (1972) tested various solvents and found that Na₂CO₃ originally proposed chemical by Bergold (1947) was the only one producing intact free virion.
This closely resembled with those processed in the insect’s midgut. In the insect midgut with high pH, the alkaline protease present in the polyhedra gets activated and help in dissolution of occlusion bodies (Eppstein et al., 1975). Faust and Adams (1966) have also suggested that in the insects mid gut where the juices are alkaline and contain proteases leading to similar breakdown of occlusion bodies. Mature polyhedrons are surrounded by an outer morphologically distinct layer referred as the polyhedron envelope (Minion et al., 1979). Its function and composition have not been established but it may play a role with the interaction of the polyhedron with surface. Maruniak et al, (1979); Zummer and Faulkner (1979) suggested that protease is absent in polyhedra collected from tissue culture cells. This indicated that protease is a contaminant because the polyhedra are collected usually by macerating dead or live-infected larvae and are exposed to the larval digestive enzymes. The tissue-cultured polyhydra, lacking the protease are as infectious for larvae as larva produced polyhedra (Faulkner and Henderson, 1972) and both types of polyhedra are equally effective in the field (Ignoffo et al., 1974). This indicated that protease is not an essential component of polyhedra but it may help in the dilution of polyhedra in the insect’s mid gut (Granados and Williams, 1986).

**Capsule Size:**

Granules or capsules are comparatively smaller than the polyhedra. They are 0.3 to 0.5 µm in length and 0.1 to 0.4 µm in width (Kelly, 1985) or 120 to 300 and 300 to 500 nm (Tanada and Kaya, 1993). In contrast to polyhedron containing numerous virus particles, a capsule has one envelope nucleocapsid. But Crook et al., (1982) reported that 2 to 5 virus particles are present in the granules of some GV. Granulin form a crystalline lattice and its molecular weight range from 25,000 to 30,000 Da. similar to those of polyhedrin (Tweeten et al., 1981). Capsules of GV are insoluble in water, alcohol, acetone, xylene and ether but soluble in Na₂CO₃, NaOH, KOH, NH₄ OH, H₂SO₄ and CH₃ COOH (Huger, 1963). They are not destroyed by natural putrefaction process and they are heavier than water.
**Genome of baculovirus:**

The genome of baculovirus is highly complex and occurs as circular, super coiled, ds DNA of 88 to 160 Kbp (Mathews, 1982b, Blissard and Rohrmann, 1990). When the DNA is treated with the restriction endonuclease, each enzyme produces a unique profile of a DNA fragment pattern for a specific virus. Different enzymes act on the viral DNA producing different profiles. DNA fragments with common mobility in gel electrophoresis indicate high genetic relatedness of the virus while the completely different mobility’s indicate different virus (Tanada and Kaya, 1993). Double digestion using two different enzymes ensure the similarity between the position of restriction site as well as the fragment sizes. Physical mapping determines the exact location of the restriction sites in the viral DNA. The physical maps have been used to locate the changes in the DNA genomic variants’ (Miller and Dawes, 1979) and to identity the recombination of events between closely related baculoviruses (Miller, 1986). It also helps in the establishment of DNA restriction fragment libraries (Cochrah *et al.*, 1986). Such collection can provide DNA fragments for the purpose of transcription and translation mapping and for the development of functional maps by techniques such as marker residue for the characterization of mutants. The DNA of AcMNPV was the first constructed physical map. (Miller and Dawes, 1979; Smith and Summer 1979) and others include:

(i) NPVs of *Spodoptera frugiperda* (Loh *et al.*, 1981; Maruniak *et al.*, 1984)  
(ii) NPV of *Heliothis Zea* (Knell and Summer, 1984)  
(iii) GV of *Cydia pomonella* (Crook *et al.*, 1985)  
(iv) NPV of *Amsacta albistriga* (Premkumar, 1997)  
(v) NPV of *Helicoverpa armigera* (Sudhakar and Mathavan, 1999) etc.

The orientation of the circular genome of *Autographa californica* MNPV was accepted as the standard (Vlak and Smith, 1982). The zero point of the genome is the location of one of the restriction site closest to the 5’ end of the polyhedrin gene and *EcoR1* is used for its location. This facilitates comparison of data from different laboratories on the physical, genetical,
transcriptional and functional maps of AcMNPV variants, mutants and related NPVs. The maps are arranged in linear or circular form.

The genome of AcMNPV is 128 Kbp or from 82 to 88 × 10⁶ Da (Smith and Summer, 1978 and Miller, 1981b) and contains 100 different genes (Miller, 1988; Blissard and Rohrmann, 1990). The gene for polyhedrin (p29) in AcMNPV is an uninterrupted, single copy gene about 1200 bp with 7.3 kb fragment of Eco RI (Smith et al., 1983) and is conserved in many NPVs. About 30% of the genome has been sequenced and characterized but the functions of many genes on the sequences that apply in controlling their expression during infection are not known (Miller, 1988; Blissard and Rohrmann, 1990). Repeated sequences in the genome function enhance the expression of other genes (Guarino et al., 1986). Variants of AcMNPV were isolated from successive plaque purification of each type of clones in a few polyhedra variants (Lee and Miller, 1978; Fraser and Mc Carthy, 1984) and from wild isolates of the virus (Smith and Summer, 1978). Genomic variants were also isolated from many wild isolates of NPVs collected from different geographical regions. Ex. (i) Viruses of Mamestrabrassicae (Vlak and Groner, 1980; Brown et al., 1981); (ii) Heliothis spp. (Getting and Mc Carthy 1982; Hughes et al., 1983); (iii) Spodoptera littoralis (Kislew and Edelman, 1982); (iv) Agrotis segetum (Allaway and Payne, 1983). (v) S. litura (Maeda et al., 1990). Of these genomic variants, some show high DNA homology whereas others do not. Also completely different species of NPV may occur in the same host insect (Maeda et al., 1990).

Baculovirus possesses regions of repeated sequences in the genome (Cochran et al., 1986) as instances like AcMNPV and O. pseudotsugata MNPV (Leisy et al., 1984) &AcMNPV and C. fumiferana MNPV (Arif et al., 1985). Intragenic sequence homology has been found in the genome of AcMNPV variants (Smith and Summer, 1979; Brown et al., 1984) and with the genome of Choristoneura funiferana MNPV (Arif and Doerfler, 1984). Homology in the intergenic sequence also occur in the MNPV of different species of Spodoptera (Kelley, 1977; Maeda et al., 1990); in AcMNPV, Heliothis zea SNPV (Knell and Summer, 1984) and some DNA of nearly 18 baculoviruses (Smith and Summer, 1982).

Recombination of AcMNPV and virus Galleria mellonella is formed by feeding these two viruses on the larva of Galleria mellonella (Croizier et al., 1980; Croizier and Quiot, 1981). Such recombination gives new viral infection and is not uncommon with virus of tissue culture
like temperature sensitive mutant (Brown and Faulkner, 1980; Miller, 1981a) but genetically
distinguishable variants (Summer et al., 1980; Crozier et al., 1980). When insect cell line was
co-infected with variants of Bombyx NPV and Autographa NPV, a recombinant virus was
obtained with wider host range than the two parent virus.

The genome of GV falls within the range of 90-160 Kbp similar with those of NPVs. Physical maps of GVs are a few as like (i) GV of Cydia pomonella (Crook et al., 1985); (ii) Artogeia rapae (Dwyer and Gravados, 1987; Smith and Crook, 1988). Basic protein, VP 12, responsible for DNA condensation (Tweeten et al., 1980b; Wilson and Consigle, 1985) may be acted upon by a kinase during condensation in the capsid via phosphorylation or during the release of DNA from a nucleocapsid i.e. uncoating (Wilson and Consigle, 1985, b). As granulin is closely related in structure and function to polyhedrin (Rohrmann, 1986a; 1986b) its proteins are also almost similar. The main difference is that granulin has two or three additional amino acid and a conserved cystine at the N-terminus while polyhedrin lacks these amino acids and presence of conserved proline. Isolates of GVs collected from different areas have revealed differences in their genomes as examples like, GV of Pieris brassicae and Artogeia rapae (Crook, 1981) and among variants of the GV of the codling moth, Cydia pomonella (Harvey and Volkmann, 1983) and A. rapae (Smith and Crook, 1988; Bellonicik et al., 1988).

Sciocco-cap et al (2001) isolated the DNA of Epinotia aporema GV, and the DNA genome size was estimated to be 120 Kbp. The occlusion bodies were 466 by 296 nm in size and prominent protein had a molecular mass of 29 kDa. Its amino-terminal sequence was remarkably homologous to that of the granulin of other GVs. The GV isolated from diseased tea caterpillar of Arctornis submarginata was analysed for the protein present on the occlusion bodies by SDS-PAGE. The molecular weight of the protein was found to be 31 kDa, which is a characteristic for granulin (Mukhopadhyay and Damayanti, 2009).

Harrap and Payne (1979) stated that the biophysical and biochemical properties of baculovirus can be used as a tool to identify and differentiate the subgroups of baculovirus. This aspect was studied by Manickavasagan et al (1992) where the genome size of NPVs of Amsacta albistriga (AaNVP); Spodoptera litura (SlNPV) and Spilosoma obliqua (SoNPV) were compared through restriction endonuclease analysis. After treatment of respective DNA
with Hind III, the SlNPV, AaNPV and SoNPV generated 12, 6 and 17 fragments respectively. The genome size of the DNA fragments were also estimated by comparison with lambda DNA Hind III + EcoRI marker and found to be 134.6, 109.6 and 103.0 Kb for SlNPV, AaNPV and SoNPV respectively. The granulosis virus of Achaea janata was analysed by Singaravelu and Ramakrishnan (1998) and found that the capsule size as 463 ± 25 × 280± 22 mm with a single virus particle. The molecular wt. of granulin protein was 28.9 ± 0.5 kDa and associated with other 2 proteins having molecular wt. of 58.2 ± 2.3 and 55.13 kDa.

The genome size determined from re-association kinetics was 92 Kbp for the Cydia pomonella granulosis virus (CpGV), isolated from seven different sources in Europe, America and New Zealand and they were also compared by restriction enzyme analysis (Crook et al., 1985). Here most of the isolates were indistinguishable from Mexican isolate (CpGV-M) and isolates from Russia (CpGV) and England (CpGV-E) showed small genomic differences. CpGV-E was shown to be a mixture of 2 variants E₁ and E₂. CpGV-E₁ was indistinguishable from CpGV-M. A physical map was constructed for the enzymes EcoR1, BanHI, HindIII, Sma1 and APaI and oriented by locating the granulin gene using the cloned granulin gene from TnGV as a probe. There was no significant difference between the infectivity’s of Mexican, Russian and English isolates from neonate larvae.

Structure of virus particles:

The nucleocapsid along with its envelope in the virus particle, which is rod shaped in structure and contains > 30 nucleocapsids per envelope in MNPV and only one nucleocapsid in one envelope as in SNPV and some GVs. The envelope surrounding the nucleocapsid may be acquired either de novo within the nucleus (Stoltz et al., 1973) or by budding at the plasma membrane (Hess and Falcon, 1978). In the case of MNPVs, the multiple nucleocapsid virus particles are formed in the nucleus (Knudson and Harrap, 1976; Kelley 1982; Adams et al., 1977). Viruses occluded within polyhedra are destined to infect another insect and play no role in the pathogenesis within the host insect. Virus acquiring plasma membrane envelopes infect additional cells in the original host at neutral and slightly acidic pH. Virus occluded within polyhedra has the ability to fuse with plasma membrane of the gut at alkaline pH (Kelly, 1985).
Some proteins of the envelope are glycoxylated (Goldstein and Mc Intosh, 1980) for assembling at the envelope (Kelly and Lescott, 1983).

**Structure of Nucleocapsid:**

Nucleocapsid is a cylindrical core of DNA and protein. They are rod shaped, 4nm in diameter and 350 nm long (Entwistle and Robertson, 1968). Outer shell or sheath is the capsid and is a structure of rings of sub-unit in a stacked series (Federici, 1986). Burley et al., (1982) reported that the cylindrical portion of the capsid surface is composed of 12 sub-units in each capsid ring. Within the nucleocapsid, dsDNA associates heterogeneously with a highly basic protein to form a cylindrical core. This basic protein is a major nucleocapsid protein and DNA binding protein (Monsarrat et al., 1975; Tweeten et al., 1980a). The gene encoding this basic protein is located on the viral genome (Wilson et al., 1987). The capsid is made up of a major protein of 31 kDa and its gene has been located on the viral genome (Pearson et al., 1988) and occurs between envelope and basic DNA binding protein complex. Kozlov and Alexeenko (1967) reported that DNA within the capsid is in the form of a helix, coil or super coil. The width and length of nucleocapsid are fairly constant for each type of baculovirus. Long forms occur during replication indicating the expandable nature of nucleocapsid (Tanada and Kaya, 1993).

**Infectious Elements:**

Isolated DNA, unenveloped virion (nucleocapsid), nuclear enveloped virion, occluded nuclear enveloped virion, and plasma enveloped virion may initiate viral infections but among these only the plasma envelope virion and the occluded nuclear envelope virion were considered as infectious agents (Tanada and Kaya, 1993).

**Influence of Plant host on viral infection:**

Plants provide thousands of compounds like terpenoids, phenolics, proteinase inhibitors, nitrogen compounds, alkaloids and growth regulators including hormonomimetic compounds
which defend against insect attack. Secondary plant chemicals or allelochemics play vital role in insect behaviour and are non-nutritional compounds which interfere with insect feeding and reproduction, besides growth and development of insect (Ananthakrishnan, 1992). According to Alanwood, (1995), the plant chemicals may alter the efficacy of virus infection and the larva may not get infected at all. It was believed that the alkaline nature of the surface of cotton plant was the cause of the loss of Heliothis NPV activity. Investigation revealed that the presence of small salt glands having the trichomes may excrete salt complexes (Elleman, 1983). The divalent cation Ca\(^{2+}\), Mg\(^{2+}\) are well represented and alter the solubility characteristic of OB in vivo, thus reducing infectivity. Under the \textit{in vitro} condition this effect can be reversed by chelating agent- EDTA. However, OBs are exposed to salt gathered from the leaf surface; EDTA is not effective presumably due to interaction of other unidentified substances which inactivates the virus (Elleman and Entwistle, 1982).

The impact of salt secretion is not uniform across the cotton cultivation zone of the world which may be associated with the influence of soil nutrient status on the composition of salt gland exudates (Elleman, 1983). Influence of tannins on the infectivity of NPV has been studied in relation to the Gypsy moth \textit{L. dispar}. Infectivity falls with increasing hydrolysable tannin whether in food plants or added under controlled condition to synthetic diet (Keating \textit{et al.}, 1988; 1990). Phenol catechol also influences NPV infectivity (Felton \textit{et al.}, 1987). Tannins bind with proteins as a result, the solubility of OBs matrix protein could be affected and the number of virions available for infection might be reduced. The interaction of \textit{Spodoptera frugiperda} NPV and host plant (signal grass and soybean) was studied by Richter \textit{et al.}, (1987). The LC\(_{50}\) on signal grass was 142 OBs/ml and on soybean it was 13 OBs/ml. Acidity in food also reduces baculovirus infectivity due to adverse effect on OBs dissolution and virion release. A comparative study was made on the influence of selected host plant on the larval mortality of Gypsy moth caused by Baculovirus. Larvae of Gypsy moth were fed with NPV of Gypsy moth on the plant host red oak, red mapal, pitch pine and quacking aspen and the mean mortality was found to be 32.0; 35.5; 44.4 and 49.7\% respectively (Keating and Yendol, 1987).

Susceptibility of gypsy moth \textit{Lymantria dispar} to gypsy moth NPV revealed that the susceptibility was significantly altered when the larvae were fed with oak species and aspen species (Keating \textit{et al.}, 1988). Viral pathogenicity was correlated with increase in the acidity
and hydrolysable tannin content of the leaf materials. The result indicated that increased acidity could reduce baculovirus activity. Hoover et al., (1998) investigated the influence of damage induced plant phenolic oxidases in cotton and tomato on the mortality caused by two baculoviruses in their respective host, *Heliothis virescens* and *Helicoverpa zea*. The POD (peroxidase) and phenolic level in both the plants were predictive of the fact that as the POD activity went up; the larval mortality got reduced drastically. Phenolics such as monohydroxyphenolics, catecholic in combination with POD affect the viral activity.

The compatibility of NPV with host plant resistance was studied by Rabindra and Jayaraj (1992). The mortality caused by NPV in *H. armigera* larvae was much higher on a susceptible chickpea than on a resistant variety ICC506. The effect of host plant on virus infected larval mortality was assessed by Rabindra et al., (1994). Individuals of *Helicoverpa armigera* were allowed to feed the *Ha*NPV on different host plants like chickpea, Pigeon pea, Lablab, Sunflower and Cotton. They found that cotton, chickpea foliage environment was detrimental to virus. The activity of virus was effective on Lablab that the LC$_{50}$ was very low. The LC$_{50}$ value of *Ha*NPV on sunflower was also similar to Lablab and the Pigeon pea leaf environment was intermediate between the two groups. The variation on susceptibility to virus and larval mortality due to food plant was reported by Santiago-Alvarerz and Ortiz-Garcia (2009). Influence of plants such as castor, bean, alfalfa, mulberry, cotton and potato on the susceptibility of cotton leaf worm, *Spodoptera littoralis* (Boisd) to NPV revealed that third instar larvae were highly susceptible to *Sl*NPV when the larvae were allowed to feed on mulberry, cotton and potato. Less susceptibility was also observed in the larvae that fed the NPV on alfalfa, followed by those larvae that were allowed to take NPV on the host plant castor and bean.

**Purification of Occlusion Bodies/Capsules:**

Purification of OBs can be done mainly by differential centrifugation. The virus infected dead larvae were putrefied and the capsules settled at the bottom were purified by differential centrifugation (Huger, 1963). OBs of *Spodoptera litura* GV were purified from diseased larvae which were macerated, filtered and centrifuged at 25,000 rpm for 15 minutes. After discarding the supernatant, the pellet was diluted and centrifuged at 16,000 rpm for 1
hour (Narayanan, 2003a). Sanjeev et al., (2005) also purified the OBs of Hyblaea puera NPV by washing the macerated dead larvae with 0.1% SDS and further filtered. Finally the filtrate was centrifuged at different speed (1000 rpm for 10 minutes) and settled pellets at 7000 rpm for 25 minutes. Sucrose gradient centrifugation was also adopted for the pure purification of OBs (Fujita et al., 1988a). Centrifugation at 3000-4000g x 25 mints followed by suspending in small volume of Tris/HCl buffer and finally centrifuged through 30-58% (w/w) sucrose gradient at 4°C for one hour at 50000g. The band sedimented at 55 – 58 % zone could be collected and stored. Easwaramorthy et al., (2001) isolated the OBs of Chilo infuscatellus and C.sacchariphagus GV by maceration and filtration of the diseased larvae in water. But purification was done using differential centrifugation and isopycnic centrifugation on continuous 30-60% (w/w) sucrose gradient at 50,000g for 60 min using 0.1% sodium dodecyl sulphate. Similarly for the ultra purification of OBs, the virus infected dead larvae were homogenized and followed by two cycles of centrifugation on continuous 40-60% (wt/wt) sucrose gradient at 100,000g for 1 hour (Sciocco-cep et al., 2001). Rao and Meher (2004) isolated the OBs of Helicoverpa armigera NPV by the process of differential centrifugation. After filtering the macerated larvae, the filtrate was centrifuged at 1000 rpm for 30-60sec. and then the precipitate was again centrifuged at 5000 rpm for 15 minutes. Similarly OBs of Spodoptera litura NPV were purified by differential centrifugation of the supernatant obtained by filtering the virus infected insect cadaver (Subramanian et al., 2006).

**Symptoms:**

When larvae were fed with OBs, the larvae exhibited certain symptoms. At the early stage of the disease, the caterpillar became sluggish and avoided food intake and cuticle became oily (Smith, 1976). Finally the cuticle turned fragile and ruptured liberating a milky fluid which consisted of thousand of polyhedra. The restless movement of the infected caterpillars made them to occupy the apical region of the twig and hung head downwards by their prolegs. Similar symptoms were also observed by Huger (1963). The initial symptoms will be the loss of appetite, slowly ventral side turn pale whitish or milky yellow. Change in colour appeared to depend on the larval age at the time of infection (Wilson, 1960). The infected larvae moulted like its counterpart of healthy larvae and grew larger than normal
healthy larvae and finally died (Tanada, 1959a). In some cases, they swell up with or without slightly increased turgidity (Tanada 1953a; Todd, 1960). The change in body colour is followed by weakening and lethargic. At the final stage diarrhoea may occur by bacterial infection (Tanada 1953b) and remain suspended from leaves or twig by the caudal prolegs in the form of inverted V shaped. Sometimes they die in the prepupal and pupal stage (Huger, 1963). Generally liquefaction of the internal tissue takes place soon after death and the cuticle becomes fragile and gets easily ruptured liberating the inner body contents. These symptoms of GV infection were similar to NPV. In both the granulosis and nuclear polyhedrosis, the infected larvae became sluggish, their integument colour changed and prone to damage. On rupturing, a whitish or grayish white fluid would liberate which would have innumerable viral inclusion bodies. The infected dead larva hanged in an inverted V-shaped position from a leaf on branch (Mazzone, 1985).

**LT$_{50}$ and LC$_{50}$:**

The occlusion body when ingested by the caterpillar eventually kills the host. Therefore, OBs are used as viral pesticide. But prior to field use, it becomes essential to know the lethal concentration and lethal time for field application. Once the virus has been administered, the larvae must be monitored daily until first larva dies and then subsequently at 8 hourly intervals or until all the larvae die. The median survival time (ST$_{50}$) also known as the LT$_{50}$ is calculated (Fujita *et al.*, 1998b). The toxicity of an insecticide to a particular organism is usually expressed in terms of LD$_{50}$ (Median Lethal Dose) i.e. the amount of insecticide/unit weight which will kill 50% of the particular population of the animal species employed in the test. The value will be expressed as mg/kg body weight, mg/body or mg or µg/larva or adult. But when the exact dose initially given to the insect cannot be determined, the concentration of the insecticide can be used as LC$_{50}$ (Ragupathy and Dhamu, 1990). Though both (LD$_{50}$ and LC$_{50}$) carry different meaning, LC$_{50}$ is used to express the dipping method. Pawar and Ramakrishnan (1975) reported the LC$_{50}$ and LT$_{50}$ value of S/NPV for the newly hatched as well as five day old larvae of *Spodoptera litura* under laboratory condition and the LC$_{50}$ value was $0.089 \times 10^6$ and
7.97 x 10^6 PIB/ml respectively. The LT_{50} value for the newly hatched larvae ranged from 5.41 to 9.95 days.

Artificial infestation of *L. oleracea* in green house was controlled by GV of *L. oleracea* at a spray volume of 10^8 to 10^9 capsules per ml (Crook *et al.*, 1982). Occurrence of *HaNPV* has been found in *Helicoverpa assulta*, tobacco budworm in People’s Republic of China (Tsai and Ding, 1982). The lab bioassay of this isolate was conducted and found that when 1 x 10^6 OBs/ml was exposed, the average LT_{50} was observed as 2.8 days (Hussey and Tinsley, 1981). Another assay was carried out on the first and second instar larvae of *H. armigera* using the same concentration. Larval mortality of 80-85% was observed on the seventh day. When *Peiris rapae* GV was applied at 9.8 x 10^2 and 9.8 x 10^4 OBs/ml, the LT_{50} was 5.5-6.7 days. This PrGV was first found in China in 1960s and formally described by Su (1982).

The LC_{50} of *HaNPV* was calculated on different host plants (Rabindra *et al.*, 1994). Plants like chick pea, cotton and pigeon pea reduced the activity of the virus whereas lablab and sunflower showed low LC_{50} value. The LC_{50} of untreated NPV was found to be 8.26 x 10^4 OBs/ml. Jayachandran and Chaudhari (1996) conducted the bioassay to determine the pathogenicity of NPV on different larval ages of *Spodoptera litura*. LC_{50} value increased with host age. Increase in larval growth & development, body weight showed enhanced value of LC_{50} by several thousand times. Murali Baskaran *et al.*, (1997) experimented with *Spodoptera litura* NPV by comparing the LC_{50} of SiNPV by mixing with UV protectants. The LC_{50} with water alone was given 26,779 POBs/ml i.e., 2.6 x 10^4 OBs/ml. The dosage-mortality data of SoNPV was calculated against the larvae of *Spilosoma obliqua*. The LC_{50} value for 4, 12 & 16 days old larvae was found to be 2.6 x 10^4, 1.1 x 10^6 & 3.16 x 10^5 PIB/ml respectively (Chaudhari, 1997).

Further studies on the LT_{50} value of granulosis virus of other insect species also revealed similar trend. For example, LT_{50} value of *PxGV* experimented at S.E. Asia was 4.9 – 5.4 days when it was inoculated to different larval stages of *Plutella xylostella* (Jones *et al.*, 1998). Application of PibrGV @ 10^6 OBs/ml against *P. brassicae* reduced the population in the field up to 72-95% in USSR. Similar trend was also observed when *Agrotis segetum* GV was applied @ 10^7 OBs/ml under the field condition. The population of *A. segetum* was checked up to 80%. Efficacy of *Cydia pomonella* GV was also tested under the field condition
by applying @ 9 x 10^6 – 2.9 x 10^{11} OBs/ml. The pest population was reduced to 74.88% (Lipa, 1998). Again in the Indian context, use of *Chilo infuscatellus* GV @ 1 x 10^7 – 1 x 10^3 OBs/ml drastically reduced the incidence of *Chilo infuscatellus* below the economic injury level of 20% (Easwaramoorthy, 1998). Sciocco-cap *et al* (2001) reported similar bioassay. The median survival time using the lowest dose of *Epinotia aporema* GV (EaGV) gave cent percent mortality in 90 hours and 105± 16 hours for the fourth instar larvae. Again the dose as well as age related mortality of *Agrotis segetum* was studied with AsNPV by Narayanan and Sarma (2002). They reported that larval mortality began on the third to fifth day after inoculation depending on age of the instar. Cent percent mortality occurred in the first and second instars within six days after inoculation, while 80% mortality resulted in third and fourth instar larvae. The LD_{50} required for the third instar larvae of *A. segetum* was found to be 1.06 x 10^7 POB. The LT_{50} of SoNPV for the third, fourth and fifth instar larvae of *Spilarctia obliqua* was found to be 5.73, 6.43 and 6.96 days respectively (Varatharajan and Singh, 2003). The GmNPV was found infected with the *Maruca vitrata*. When bioassay of the GmNPV was carried out against *Maruca vitrata*, the LT_{50} of second instar was found to be 108 hours. The thirdand fourth instar larvae had greater LT_{50} value of 139 and 144 hours respectively (Parthasarathy. *et al.*, 2004). When several noctuid species were exposed to HaNPV, the neonate larvae of bollworm were found most susceptible at LC_{50}=10^5 OBs/ml. On the other hand, the tobacco budworm and the beet armyworm (*S. littura*) were found less susceptible (LC_{50}=about 10^5 – 10^6 OBs/ml) (Narayanan and Shetty, 2007). Damayanti *et al.*, (2006) isolated NPV from the diseased caterpillar of *Buzura suppressaria*. The bioassay of BsNPV showed that the LC_{50} value was 1.23×10^6 POBs/ml. The LT_{50} value was 5.11 days for 1×10^5 POBs/ml, 5.06 days for 1×10^6 POBs/ml and 5.04 days for 1×10^7 POBs/ml. The bioassay of baculovirus, *Arctornis submarginata*, AsGV was carried out in the tea fields of Darjeeling by Mukhopadhyay and Damayanti (2009). It was found that the LC_{50} value of AsGV for the second instar was 4.46×10^4 OBs/ml and the LT_{50} value being 6.6, 5.9 and 4.45 days for 1×10^4, 1×10^5 and 1×10^6 OBs/ml respectively i.e. larval mortality depended on the concentration of the OBs. Similarly, the Characteristics and virulence of nucleopolyhedrovirus isolated from *Hyposidra*
talaca (Lepidoptera: Geometridae), a pest of tea in Darjeeling was studied by Ananda Mukhopadhyay et al (2011).

**Dispersal and transmission of baculovirus:**

Transmission of baculovirus can be either biotic or abiotic, of which the latter process is mediated through air and water. The spread of *N. sertifer* NPV was found associated with wind blow (Olofsson, 1988) and that of *O. pseuotsugata* with the dust generated by cattle driven through the forest (Thompson, 1978). Rain plays a major role in the initiation of epizootics through the mobilization and spread of inoculums. This was proved by Hoffmaster and Ditman (1961) in the NPV of cabbage looper, *T. ni*. They stated that the epizootics of NPV disease in *T. ni* are rain correlated. This observation was reviewed by Entwistle (1986; 1987). Substantial run off of *N. sertifer* NPV from pine trees was demonstrated by Kaupp (1981) who collected OBs in pots containing washed sand beneath the trees. The spread of an NPV of *Colias eurytheme* in irrigation water was noted as a major cause of epizootics in alfalfa fields in California (Steinhaus and Thompson, 1949). Spread between spruce trees in *G. hercynial* NPV disease by rain splash was identified by Bird (1961) in Canada.

The **biotic agents** responsible for the spread of viral pathogen include predators and parasitoids. Among the vertebrates, birds play an important role as effective dispersal agents. The food and the virus may begin to pass through the gut in less than 30 minutes but virus may continue to present in the fecal matter for several days (Entwistle, 1982; Kaya, 1982 and Andrealis 1987). In Scotland, willow warbler *Phylloscopus trochilis* migrating south to east to winter quarters carried NPV of *P. flamenea*. Entwistle *et al.*, (1993) reported that the time of the year *P. flammea* was in the pupal stage in forest soil and that the bird therefore probably acquired the virus by feeding on larvae. Similar observation was also recorded with birds in spruce forest which were trapped in January carrying *G. hercyniae* NPV and birds could disperse this NPV for about 10 months of the year (Entwistle, *et al.*, 1977).

Interaction of parasitoids and baculovirus diseases in an epizootic was proposed by Entwistle (1982). This interaction stated that parasitoids and baculovirus compete for host. Therefore, as the larvae of *Cotesia sp* are easily killed and only survive minimal infection of
host by baculovirus, they tend to attack the younger host individuals of which the proportional disease in epizootics tends to be low. This is strong and effective competition. Such parasitoids may be termed “isolationists” from their tendency to select young host individuals linked to a positive avoidance of diseased host or oviposition sites. Some tachinids tolerate infection in host (e.g. *Voria ruralis* -Vail, 1981) or even host death from baculovirus infection (e.g. *Drino bihemica*; Bird, 1961) and do not need to compete for host. These are termed as non-isolationist. When baculovirus is used as biological insecticide, usually applied when host larvae are young, the population of parasitoids becomes depressed. This was observed in *Pieris rapae* on cabbages (Kelsey, 1960) and the orchard pest *A. orana* (Shiga et al., 1973). The transmission of the granulovirus of *Pieris rapae* by *Cotesia glomeratus* was investigated by Levin et al., (1983). Nearly 84% of the parasite *C. glomeratus* that developed to maturity in *P. rapae* infected larvae transmitted the GV pathogen to the larvae of *P. rapae* in which they subsequently oviposited as adult. The adult *C. glomeratus* did not exhibit a statistically significant discrimination between GV treated and non-treated larvae of *P. rapae*, although healthy larvae were attacked first with a greater frequency than GV treated larvae. *C. glomeratus* could distinguish between the parasitised and non-parasitised healthy host, but did not discriminate between parasitised and non-parasitised GV infected host. They concluded that *C. glomeratus* may play significant role in the transmission of GV in the field population of its host (*P. rapae*).

In another experiment, transmission of OBs by parasitoids was evaluated by Caballero *et al* (1991) and they observed the involvement of three parasitic species such as *Cotesia telengai*, *Aleipodes gasteratus* and *Compoletis annula* parasitizing *Agrotis segetum* help in transmitting the granulosis virus. Females of the three parasitic species that oviposited previously in the GV infected *A. segetum* larvae transmitted the virus to healthy *A. segetum* in subsequent oviposition, but males did not transmit the GV. Transmission of *Spodoptera litura* NPV from generation to generation was studied by Santharam and Jayaraj (1989). NPV infected adults were produced by feeding fifth instar larvae with sub normal concentration of NPV. Such transmission was transovarial or transovum as eggs and larval mortality were observed in the progeny from infected adult. Normal adult was able to transmit NPV to their progeny by the egg surface coating when fed with NPV orally, more so when both the sexes were fed. The larval mortality was more in the case of eggs laid on the first day and declined
from second and third day laid eggs. Kalia and Chaudhari (2004) reported that the infection of HaNPV through the trachea via spiracular opening was more rapid than the oral route. Ten days old larvae highly refractory to oral infection were made susceptible by direct tracheal infection. The incubation period was also reduced from 5 days in oral infection to 2-3 days through tracheal infection.

**Effect of Storage and Temperature:**

The storage effect on the infectivity of viruses is important especially when OBs are used as biopesticide for field use. Kelsey (1958) reported cent percent mortality in the laboratory experiment with capsules of *P. rapae* kept under room temperature for nearly 16 months. One year storage of PiragGV was also successful in getting 100% mortality (Rivers, 1959). *P. rapae* granuloviruses when kept in dry state for one year under room temperature remained infectious (Tanada, 1956). The infectivity after one year of storage was proved for the following capsules: *Pseudaletion unipuncta* (Tanada, 1955); *Eucosyma griseana* (Martigoni and Auer, 1957); *Hyphantria cunea* (Drury) (Schmidts, 1959).

It is not only the period of storage, the temperature at which the capsules are stored also affect the infectivity. Tanada (1953a) found that GV of *P. rapae* survived up to the temperature of 75°C for 10 minutes. Similar observation was also reported by Tanada (1959a). The granulovirus of *Pseudaletia unipuncta* was inactivated when heated at 75°C for 10 minutes and also at 70°C for 40 minutes. Tanada (1953a) showed that the larvae of *P. rapae* succumbed to granulosis to a lesser extent when they were reared and infected at 36°C. In another experiment, there was no larval death from the granulosis virus of *Peridroma margaritosa* when the larvae were reared at 37°C (Steinhaus and Dineen, 1960). In addition to inactivation of the capsules by high temperature, the heat inactivated capsules (80°C for 10 minutes) retains the synergistic property of enhancing the virulence of unheated nuclear polyhedrosis virus (Huger, 1963). There was no loss of virulence when granulosis virus of *Pieris rapae* was stored at 4 - 6°C over 12 months (Huger, 1963). No larval mortality was recorded for the capsules stored for two years of storage in freezing compartment of a household refrigerator for the two cases- (i) *Argyrotaenica veletinana* (Glass 1958) & (ii) *C. murianana*. It has been noticed that capsules keep their infectivity at least for 2 years under room condition but storage of capsules
at low temperature is surely advantageous and possibly lengthens the period of infectivity (Huger, 1963).

When a highly purified preparation of *PibrGV* of *P. brassicae* was exposed to direct sunlight, on the upper surface of cabbage leaves, the virus was rapidly inactivated. The suspension gave very low larval mortality than a non-irradiated control suspension, 1/10 of its concentration after an exposure period of three hours. Total inactivation of the virus under these circumstances took between 12 and 19 hours. These results discourage from the point of view of bio-control and emphasize the importance of finding for protecting the virus from UV radiation (David et al., 1968). Singh et al., (2007) reported that the virus showed appreciably good effect up to 2 years of storage under room temperature. The long term stability of baculovirus under various conditions was investigated. The result demonstrated that the major factor contributing the loss of viral infectivity was exposure to light. Virus stocks stored at temperature ranging from -85 to -37°C were quite stable as long as they are protected from light with little long term loss of infectivity (Jarvis and Garcia, 1994).

**Cross Infectivity:**

There were a number of reports of cross infectivity that showed certain NPVs being not species specific but have a narrow insect host range. This property therefore helps in optional use for insect pest control. The infectivity of NPVs to alternate host is typically evaluated on the basis of mortality of the test insect caused by the virus and the detection of polyhedra in tissue of the test host by light microscopy. Similarly the granulosis virus infecting *Pieris brassicae* has been found infecting *P. rapae* and *P. napi* (Kelsey, 1958; Smith, 1959; Smith 1960). The GV’s of *Chilo infuscatus* (sugarcane shoot borer) could infect the sugarcane internode borer, *Chilo sacchariphagus* (Easwaramoorthy and Jayaraj, 1987a). In another cross infectivity test, the granulosis virus of *Agrotis segetum* (AsGV) was found effective in a spray formulation against both *A. segetum* and *A. ipsilon* (Shah, et al., 1979 and Zethner, et al., 1987).

Baculovirus infecting *Bombyx mori* (BmNPV) was infectious to *Galleria mellonella* (Stairs, 1991). Again Parthasarathy (2002) investigated the cross infectivity of *GmNPV* and the study revealed that it was susceptible to 16 insect species such as *Plutella xylostella; Chilo*
partellus; Chilo infuscatus; Chilo sacchariphagus; Maruca vitrata; Cnaphalocrocis medinalis; Opisina arenosella; Amsacta albistraga; Diaphania pulverulentalis; Spodoptera exigua; Crocidolomia binotalis; Hellula undalis; Pericallia ricini; Ergolis merione; Exelastis almosa and Marasmia patnalis. The cross infectivity was confirmed by DNA characterization and reciprocal inoculation studies. The study also indicated that serial passage of GmNPV five times through alternate hosts increases its virulence to P. xylostella. Narayanan (2003b), reported that the cross infectivity of granulovirus of Adisura atkinsoni, field bean pod borer to Helicoverpa armigera. GV of H. armigera can be multiplied in large scale as H. armigera has short larval period (12–14days) with the biomass of 407mg without undergoing any dormancy, while A. alkinsoni has longer larval period of 20-22 days with an average weight of 350mg and undergoing pupal diapauses.

The susceptibility and yield of NPV of Galleria mellonella was screened against Galleria mellonella, Pericallia ricini and Chilo partellus. Third instar larvae of G. mellonella, early fifth instar of P. ricini and late fourth instar of C. partellus were found suitable for mass production of GmNPV. Among the insect examined, C. partellus recorded the lowest LC$_{50}$ of 14.68 POB/mm and LT$_{50}$of 177.98 h for the third instar larvae, (Parthasarathy and Rabindra, 2003). Infection of Maruca vitrata (legume pod borer) by the NPV of Galleria mellonella was investigated by Parthasarathy et al., (2004). They reported that the second instar larvae of M. vitrata recorded the highest mortality of 94.67% and the percentage mortality declined with increasing age of the larvae. The LC$_{50}$ and LT$_{50}$ of second instar larvae were found to be 10.29 POB/ml and 108 hrs respectively.

Narayanan (2005) reported that Bombyx mori nucleopolyhedrosis virus was cross infective to cabbage leaf webber, Crocidolomia binotelis. Again Narayanan (2006) tested the efficacy of Crocidolomia binotelis NPV against late second instar larvae with varying concentration from $10^5$ to $10^9$ POBs/ml. The result indicated 33 to 90% mortality with an incubation period of 4 to 6days. The NPV was found infecting Plutella xylostella, Hellula undalis, Trichoplusia ni, Helicoverpa armigera, Spodoptera litura, S. exigua, Chilo partellus and Corcyra cephalonica. Also C. binotelis larvae were susceptible to Bombyx mori NPV. Varatharajan et al., (2006) reported that the mulberry pest, Porthesia xanthorrhoea was susceptible to NPV of Spilarctia obliqua (SoNPV). Narayanan and Veenakumari (2007)
studied the occurrence of NPV of *Cadra cautella* (Almond moth) for the first time in India. They reported that this NPV was found to infect the larvae of greater wax moth larvae, *Galleria mellonella*.

**Host range of Baculovirus:**

Baculoviruses are generally said to be host specific, capable of infecting only the caterpillar species from which they could have been isolated. It means that each caterpillar species has its own virus. However, recent investigation states that some of the viruses can extend their host range. As for instance Mc Intosh et al., (1985) tested the host range of five baculoviruses in Lepidopteran cell lines. Multiple enveloped baculovirus of *Autographa californica* MNPV, *Trichoplusia ni* MNPV, *Galleriamellonella* MNPV replicated in cell lines of *Trichoplusia ni*, *Spodoptera frugiperda* and *Heliothis virescens* to a litre of $10^7$ TCID 50/ml. The multiple envelope baculovirus of *S frugiperda* (SfMNPV) replicate only in *S.frugiperda* cells. The single enveloped baculovirus of *H.zea* NPV replicate in cells of *H.zea* but not in cells of *H. armigera*, *T. ni* or *S. frugeperda*. Low levels of replication of *AcMNPV*, *TnMNPV* and *SfMNPV* in culture of *H. zea*, *H. virescens*, *T. ni* respectively could be detected by using a sensitive tritiated thymidine technique. However, two characteristically labeled peak at densities of 1.145 and 1.245 g/ml were obtained in *H.virescens* cells inoculated with *AcMNPV*. 60 minutes of post inoculation with *AcMNPV*, showed the presence of virus inside the *S. frugiperda* cells but were not observed in *H. zea or H armigera* cells none of the five baculoviruses replicated in *H. armigera* cells. The mechanism of host specificity of nuclear polyhedrosis virus (NPV) was analyzed after co-infection of *Bombyx mori* NPV (*BmNPV*) and one of the four distinct groups of *Spodoptera litura* NPV (*SlNPV*) including an *AcNPV* variant into various Lepidopteran cell lines. Replication of *BmNPV* in non-permissive cell (TN-386, SF-21 and CLS-79) was induced by co infection with *AcNPV* but not with other *SlNPV* groups. These induced progeny NPVs were plaque purified in BmN cells which were susceptible to only *BmNPV*. Most of these isolates did not replicate in the cell lines indicating the existence of a helper function of *AcNPV* for *BmNPV* replication. Some of the isolates were able to replicate in cell lines non permissive to *BmNPV* indicating the appearance of a new virus with wider host specificity. DNA restriction endonuclease analysis showed that the isolate
exhibiting wider host range were recombinant viruses between the parents (Kondo and Maeda, 1991).

Similarly the host range of Autographa californica nuclear polyhedrosis virus was studied by Maeda et al., (1993). They isolated a hybrid baculovirus of B.mori NPV and A. californica NPV capable of replicating in both BmN cell (not susceptible to AcNPV) and SF-21 cell lines (not susceptible to BmNPV) (Kondo and Maeda, 1991). Repeated backcross infection of one of these recombinant isolates with AcNPV generated eh-AcNPV, a virus with restriction endonuclease pattern of genomic DNA nearly identical to those of AcNPV but capable of replicating in both BmN and SF-21 cells with expanded host range. Such viruses were also isolated following co-transfection of AcNPV DNA with eh-AcNPV DNA cleaved with either Hind III or Pst I. Subsequent cotransfection of AcNPV DNA with plasmid from an eh-AcNPV DNA fragment library identified an 11 Kbp Hind III fragment that could expand the host range of AcNPV. Sub cloning and cotransfection analyses localized at 572 bp and Sac I- Hind III fragment within its 11 Kbp fragment which could alone expand the host range of AcNPV. Mapping and nucleotide sequencing analysis revealed that this fragment was identical to the corresponding 572-bp fragment (BmScH of BmNPV). Further, this fragment originated from the coding region of the putative DNA helicase gene. Cotransfection of AcNPV DNA with BmScH also generated a host range expanded virus eh 2-AcNPV. This result indicated that the expanded host range characteristics of eh 2 AcNPV were solely the result of recombination with the coding region of the putative DNA helicase.

The host range and virulence of five baculoviruses (2 MNPVs, one each from Agrotis segetum and Mamestra brassicae; one SNPV from Plusia gamma and 2 granulosis virus from A. segetum and)were studied for seven lepidopterous pest of temperate agriculture (A. segetum, A. exclamationis, Lacanobia oleracea, M. brassicae, Noctua pronuba, P. gamma & P rapae). None of the virus killed the above pests but M. brassicae MNPV failed to infect only P. rapae. The other viruses were restricted to the homologous host or members of its genus or sub family. In all the examples, except A segetum GV, the median lethal dose for the most susceptible host was less than 22 virus inclusion bodies and median lethal time for all infection ranged from 5.5 to 16.6 days (Allaway and Payne, 2008).
Safety evaluation of baculovirus:

It is mandatory to evaluate the viral pesticide for its safety or else it will give undesirable effect to the organism as well as ecosystem as a whole. Keeping this view in mind, a number of safety evaluations were attempted. The safety of NPV of European skipper, *Thymelicus lineola* against non target organisms has been evaluated by Smirnoff and Ackeman (1977). The experiment indicated that *Tl* NPV was safe on mammals, fish and beneficial insects and therefore recommended for the control of *T.lineola*. Experiments were conducted to study the effect of NPV of snakegourd semilooper, *Anaduridia peponis* on white rats, white mice and chick embryos. The result showed that the virus neither infected the animals nor did it cause any abnormality in their physiology and behaviour. Histopathological studies of different organs did not show any evidence of tissue damage. The virus did not affect the development of the embryo or the growing tissue on embyronated chick eggs. Post mortem examination of the embryo indicated no gross pathological changes in both the group. The result indicated that nuclear polyhedrosis virus of *A. peponis* was safe to vertebrates and can be recommended for field use (Philip et al., 1987).

The GVs infecting sugarcane shoot borer, *Chilo infuscatellus*, (*CiGV*) and *C. sacchariphagous* (*CsGV*) were tested against the Indian honey bee, *Apis cerana indica*; silk worm, *Bombyx mori* and albino rats. *CiGV* and *CsGV* were found safe to *Apis cerana indica* (Easwaranmorthy and Jayaraj, 1987b) and *Bombyx mori* (Easwaranmorthy and Jayaraj, 1988) and albino rats (Easwaranmorthy and Jayaraj, 1990). In another experiment, *HaNPV* was given in the form of PIBs to silkworm, *Bombyx mori*, *Samia ricini*, *Apis cerana indica*, and entomogenous nematode (*Steinernema feltiae*). In all the trials, the tested organisms compared with respective control showed normal development and there wasn’t any sign of mortality attributable to *HaNPV*. Thus *HaNPV* was found safe to the tested organisms (Bijjur et al., 1991). Ramakrishnan et al., (1992) evaluated the safety of NPV against non-target organisms. The *SnNPV* was administered with single as well as multiple doses to albino rats, rabbits and guinea pigs. The virus treated animals did not show any abnormal behaviour and there was no difference in food consumption, body weight, urology or any chemical parameters investigated between treated and untreated animals.
The safety of NPV of European skipper, *Thymelicus lineola* against non target organisms has been evaluated by Smiroff and Ackeman (1977). The experiment indicated that *Tln*NPV was safe on mammals, fish and beneficial insects and therefore recommended for the control of *T. lineola*. Rabindra *et al.*, (1998) reported that the GV of *Plutella xylostella (PxGV)* was found safe to beneficial insects like honey bees and silk worms and entomogenous insects like *Chrysoperla carnea* and parasitoids *Trichogramma chilonis*. Again NPVs of *Helicoverpa* were evaluated in the laboratory for safety to a parasitoid, *Trichogramma Chilonis* and a predator, *Chrysoperla carnea*. The dose of the virus tested was $2 \times 10^7$ POBs/ml which is ten times the normal field dose ($1.5 \times 10^{12}$ POBs/ml in 500 l of water). There was no deleterious effect on parasitization, parasitoid emergence, adult duration and total life cycle of *T. chilonis* when exposed to the virus by egg treatment and adult feeding methods. The virus did not have any harmful effect on the eggs, grubs and adults of *C. carnea*. The hatchability, incubation period, larval mortality, larval duration, percent pupation, pupal period, adult emergence, fecundity and total life cycle were not significantly different among untreated and treated *C. carnea* (Geeta and Rabindra, 2001). Polyhedral occlusion bodies of *Spilarctia obliqua (SoNPV)* was given to mulberry and tasar silkworm larvae @ $10^6$ OBs/ml. Both the species of larvae completed their development successfully and emerged as normal adults (Varatharajan, 2003). All these safety bioassays revealed that no measurable virus replication could be detected in the test animals and has been reported to be safe for use under the field condition.

**Field evaluation of baculovirus:**

The most adverse effect of solar UV radiation is the induction of molecular changes in DNA. Two adjacent thymine bases on a DNA strand may fuse by the formation of a cyclobutane ring between the bases thus creating a block in the synthesis of normal DNA and high rate of mutation. Strand breakages may also occur (Harm, 1980). Since the baculovirus has DNA as genome, the viral DNA is bound to undergo changes when exposed to sunlight. When a highly purified preparation of the known virus of *P. brassicae* was exposed to direct sunlight by applying on the upper surface of cabbage leaves, the virus was rapidly inactivated. After an exposure of 3 hours, the suspension applied on the leaves gave significantly less kill of larvae than a non irradiated control suspension. Total inactivation of the virus under these
circumstances took between 12 and 19 hours. These results emphasize importance of finding some means of screening or protecting the virus from UV radiation (David et al., 1968).

David (1969) exposed the PibrGV to UV radiation from a germicidal lamp (predominantly 253.7 nm) and he found that it was much more rapidly inactivated in pure films than in film of crude virus, where coloured and solid impurities were present. In both cases inactivation was more rapid in wet film than in dry films. When the inactivating effect of UV of different wavelength was compared, using a highly purified preparation, it was observed that this property decreased progressively as the wavelength was increased but that inactivation was still brought about by at any rate, the shorter wavelength radiation (291.5-310nm) present in sunlight as it reached the earth. Optical brighteners can provide protection against UV light by absorbing UV radiation and emit light in the blue region of the spectrum (Topper et al., 1984).

Nair and Paulose (1988) made a field trial of Anadevidia peponis NPV on snakegourd semilooper (Anadevidia peponis) at the College of Agriculture, Kerala for two seasons. The result showed that the virus was highly pathogenic and better in controlling the pest @12 x 10^5 PIBs/ml and 6 x 10^5 PIBs/ml. Field experiments were carried out to evaluate certain adjuvants to improve the efficacy of NPV for the control of H. armigera on sunflower and pigeon pea. The result revealed that application of NPV@500 LE/ha + boric acid 0.1% or NPV@ 500LE/ha + jaggery 0.5% reduced larval population significantly over the application of NPV without any adjuvant. These applications also reduced the head damage in sunflower, pods and seed damage in pigeon pea & thus increased the yield (Bijjur et al., 1991). Ramakrishnan and Chaudhari (1991) reported that addition of folic acid, pyridoxine, riboflavin, charcoal black ink and urea provided protection for SlNPV against the far and near UV light. In case of far UV, the protection was concentration dependent and 1% folic acid and charcoal resulted in 100% mortality. Though the relative efficacy was low in case of direct exposure to sunlight compared to germicidal lamp, riboflavin and folic acid protected the virus by a factor of 4.9 and 3.6 respectively. In another experiment, different leaf extract of Vitex negundo, Tajetus patula, Prosopis juliflora and Argemone mexicana with HaNPV and another treatment with HaNPV suspension alone were used in the field experiment to control H. armigera. All the botanicals when combined with the virus recorded significantly more number of healthy pods than virus alone. NPV with V. negundo showed higher yield than NPV alone (Rabindra et al., 1991).
In yet another experiment, different concentrations of boric acid were tested along with S/NPV. The LC<sub>50</sub> value for virus alone, virus with 1% boric acid and virus with 0.5% boric acid was 8.27 x 10<sup>6</sup>, 4.25 x 10<sup>5</sup> and 8.32 x 10<sup>5</sup> PIB/ml. The virus formulated with boric acid was found more effective than virus alone and it reduced the LC<sub>50</sub> ten times less than virus alone. There was no significant difference between 1% and 0.5% boric acid formulation. LT<sub>50</sub> values were also reduced from 153 hrs in virus alone to 97 hours in these formulations (Chaudhari, 1992). Another field trial was made on a naturally occurring NPV for the control of the Brown tail moth, *Euproctis chrysorrhoea* during autumn on a non-outbreak population of second instar larvae on the bushes of *Rubus sp.* in Southern England. Despite erratic droplet coverage generated by a hand held, fan assisted ULV sprayer (Turbair Fox) a series of doses produced a clearly defined primary infection response peaking at ca. 70% at 1 x 10<sup>9</sup> PIB/ml. The development of this infection was slow and mostly mortality occurred during the winter diapause. Secondary infection peaks in the spring were unpredictable but occurred in many plots across the dose range. The data suggested that the virus would be useful and environmentally acceptable insecticide for controlling this insect in sensitive area especially during pest outbreak (Speigt, *et al.*, 1992). Shapero and Robertson (1992) reported that the addition of stilbene brighteners to *L. dispar* NPV reduced the LC<sub>50</sub> in the lab bioassays from 18.000 OB/ml to 10 – 44 OB/ml and the LT<sub>50</sub>. Successful results were also obtained in the field trials allowing considerable reduction in virus dosage (Webb *et al.*, 1994; Cunningham *et al.*, 1997). Activity of AcMNPV increased to 41 folds in *Trichoplusia ni*, when AcMNPV was used in combination with brightener (Daugherty *et al.*, 1996)

Aerial application of three baculoviruses stock was tested against *Orgyia psuedolsugata* in British Columbia by Stelzer *et al.*, (1977) @100 billion polyhedral/acre formulated in a molasses and non molasses formulation applied at 1 or 2 gal/acre, and it provided, appreciable population control and excellent foliage protection. Larvae collected from the treated areas 5 days after treatment showed virus infection ranging from 60-80%. With the exception of one treatment that was applied under extreme adverse meteorological conditions, there were no significant differences between virus treatments. Population densities were reduced by more than 90% within 21 days of post treatment and no evidence of survival of pupal stage in the virus treated areas. A six step sequence of selection of the GV of *Laspeyresia pomonella* has resulted in identification of a strain with increased resistance to sunlight. Each step consisted of
irradiation of dry deposits of the virus on apples with near range ultraviolet light, infection of freshly enclosed codling moth larvae with the irradiated virus and propagation of the virus thus produced in the large fifth instar larvae. Compared with the original virus, the new strain was 5.6 more resistance to artificial UV irradiation in the lab and remained twice as long infective in the field (Brassel and Benz, 1979).

**Protection of baculovirus from UV:**

Monobrulla (2000) used optical brighteners to protect the baculovirus from UV radiation. He stated that optical brighteners are chemicals used in laundry detergents to make fabrics appear brighter by absorbing energy from UV light and emitting it as visible light. These optical brighteners have been reported as UV protectants for entomopathogens to extend their effectiveness in the field of microbial agents. Some brighteners have demonstrated not only as UV protectants but have also shown the potential to enhance virus efficacy against forest and agricultural pests. The enhanced infectivity produced by optical brighteners generally resulted not only enhanced the larval mortality (reduced LD$_{50}$ value) but also hastened larval death (reduced LT$_{50}$ value). Moreover in some cases extended infectivity of the virus to older larvae has been observed. Efficacy of HaNPV with and without adjuvants and UV protectants was field evaluated against *H. armigera* in chickpea crop. All the treatments registered significantly lower larval population and higher seed yield as compared to untreated control. Significantly higher seed yield of 1612.5 and 1550.0 Kg/ha was observed in the treatment of NPV 250 LE + milk powder (1.0%) and NPV 250 LE + Ranipal (0.5%) respectively and both were on par (Rawat and Shukla, 2001). Four isolates of NPV of *Amsacta albistriga* collected from Dharwad, Pavagado, Madurai and Narikudi were bio-assayed with II, III and IV instars and all the isolates were effective against the larvae (Mulari Baskaran, *et al.*, 2001).

**Formulations of baculovirus:**

Mulimani and Kulkarni, (2003) reported that addition of Boric acid, Jaggery @ 1% and 0.5% respectively to 2 x $10^9$OBs/ml of *Achaea janata* GV gave a larval mortality of 97.60 and
96.11% after 10 days of treatment on second instar larvae. They also reported that jaggery can be used as UV protectant for GV in the management of *A. janata* which will be cost effective. Field experiment with *PxGV* on *P. xylostella* revealed that this virus is effective at a concentration of $1.5 \times 10^{13}$ OBs/ha. Plots sprayed with GV had more of parasitoid activity with lesser damage by diamond black moth. Glycerol based liquid formulation of GV performed better than unformulated virus (*Kennedy et al.*, 2003). The efficacy of NPV and neem product (nimbitor) alone, in combination and sequences were evaluated against *Helicoverpa armigera* on chick pea by *Mote and Satpute* (2003). The sequences with *HaNPV* Nimbitor-Nimbitor were found effective in terms of efficacy, yield and economics.

**Variation in virulence:**

*Muniraj et al.*, (2006) reported that Hisar isolates of *HaNPV* was more virulent than Sri Ganganagar, Sirsa, Faridabad, Coimbatore, Guntur and Nagpur isolates. In sunflower, field application of 2 spray of *HaNPV* @250 LE/ha +2 times application of 5% neem seed kernel extract (NSKE) + imidaclorpid @5g/ha caused a statistically significant decline in the population of leafhopper, thrips, aphids and defoliators as compared to insecticidal check and untreated control. In addition, lower incidence of *H. armigera* and highest grain yield were obtained in the above said treatment (*Jagadish, et al.*, 2007). The susceptibility of 7 geographic isolates of *H armigera* NPV to sunlight was evaluated by subjecting them to different exposure time (0, 3, 6, 12, 24 and 36 hours). The viral dose of exposure was $1 \times 10^7$ POB/ml and bioassay was performed at dose of $1 \times 10^5$ POB/ml of each virus isolates. The study showed that exposure to natural sunlight affected the activity of *HaNPV* isolates. About 6hrs of exposure reduced the activity of the viral isolates by nearly 50%. By 36 hours all the isolates had lost their activity by about 70%. There were no significant differences in the susceptibility of different isolates to natural sunlight. However (Negamum) NGM isolates showed the lowest inactivation after 36 hrs of exposure. Time mortality response of *Helicoverpa armigera* isolates indicated that exposure to natural sunlight beyond 12 hours declined the viral activity to the extent of 50%. The order of LT$_{50}$ for the isolates was Negamum > Ooty > Coimbatore > Mumbai > Parbhani > Hyderabad > Rahuri (*Mehrvar, et al.*, 2007). Such studies on virulence will pave way to use the potent virus for the control of insect pests.
Scope of the present study

The perusal of the literature pertaining to baculovirus in general and granulovirus in particular infecting different species of caterpillars revealed appreciable amount of works being carried out by various researchers on diverse aspects of host-pathogen interactions. However, with respect to the GV infecting, not much work has been made but for the study undertaken by eminent researchers like Paillot, Smith, David and Gardiner who have contributed significantly on the morphology, biochemistry and efficacy of the PibrGV virus. But, in the Indian scenario, occurrence of PibrNPV and PibrGV has been just reported and no work has been initiated. Therefore, considering this lacuna, an attempt has been made in the present thesis on Pieris brassicae Granulosis Virus (PibrGV). The works presented in this thesis are based on the following objectives.

Objectives:

1. Bioassay of PibrGV against the caterpillars of P. brassicae.
2. Standardizing the methods of extraction, semi-purification, inoculation and augmentation of occlusion bodies of PibrGV.
3. Pathogenic effect on the mid gut, haemocyte and cuticle
4. Field evaluation of PibrGV
5. Cross infectivity with target pests and safety evaluation against non-target organisms.

Accordingly the chapters were categorized in the following manner

CHAPTER – I                    INTRODUCTION AND REVIEW OF LITERATURE
CHAPTER – II                   MATERIALS AND METHODS
CHAPTER – III                  RESULTS
CHAPTER – IV                   DISCUSSION
REFERENCES
APPENDICES – Published papers