Chapter: 4
DISCUSSION

The caterpillars of *Pieris brassicae* are invariably prone to viral infections caused either by nucleo polyhedrovirus or granulovirus (Paillot, 1926). In India, incidence of viral diseases of the cabbage butterfly is known through the reports of Battu (1995) and Ingobi *et al* (2006). But for this record no detailed investigations were carried out on the GV infecting the cabbage butterfly. Observations on the symptoms of the infected larvae, spherical shape of the capsule and presence of unique protein [granulin] present in the matrix of the occlusion bodies have clearly revealed that the virus infecting *P. brassicae* is a baculovirus belonging to the family baculoviridae. Yet another feature of this virus is its propensity to use midgut epithelial cell as the prominent site for multiplication and the virion eventually infects other cells leading to cell lysis (Smith, 1976). This was also evident in the present system where the viral infection began at the midgut cells and ended after lysis of haemocytes besides infecting cells of the integument (Figs– 9 to 11). The above evidences have clearly confirmed that the pathogen taken up for the present investigation is a granulovirus and more precisely that of *Pieris brassicae* granulovirus (*PibrGV*) as it has the ability to infect only the *Pieris* sp.

Capsule size:

The purified capsules of *PibrGV* appeared spherical and highly reflective particles showing “Brownian motion” when observed under the light microscope especially at the high resolution. They could easily be distinguished from the haemocytes in terms of size. SEM studies also confirmed the spherical shape of the capsule that being unique for GVs, with the diameter ranging from 200-300nm (Fig - 7). The GVs isolated from *Agrotis segetum* and *Cydia pomonella* were also found to have almost the same morphological size. On the other hand, the size of nucleo polyhedrovirus and cypovirus range from 0.9 to 1.45 μm and 8 to 12.2 μm respectively and they are significantly bigger than the granulovirus (Smith, 1976).
Granulin protein:

The virions are usually suspended on the protein matrix known as “polyhedrin” in NPVs and CPVs, while “Granulin” in the case of Granulovirus (Tweeten et al., 1981; Maruniak, 1986). The granulin protein has been isolated from the OBs of the infected larvae of *Pieris brassicae* and run through the SDS PAGE. This has indicated the presence of granulin protein, with the molecular weight of 28kDa. The electrophoretic profile presented below confirms the presence of granulin protein (28kDa) in the undiluted samples of OBs extracted from the virus infected *P. brassicae* and the rest include non specific proteins.

The granulovirus isolated from the castor semi-looper, *Achea janata* composed of capsule with single virus particle and the SDS–PAGE electrophoresis of capsule matrix protein revealed the presence of granulin with the molecular weight of 28.9±0.5 kDa (Singaravelu and Ramakrishnan, 1998). Similar results were also reported by Easwaramoorthy *et al* (2001) when they tried to distinguish GVs infecting the sugar cane shoot borer, *Chilo infuscatus* and *Chilo sacchariphagus* in terms of protein profile, wherein the polypeptides of the GVs extracted from *C. infuscatus* had a molecular weight of 30, 500 Da, and that of *C. sacchariphagus* being 30, 000 Da. These observations support the present study on granulin protein of *Pibrgv*. In the present analysis in addition to the 30kDa, a number of minor bands were also found associated with granulin which might have resulted from the activity of an alkaline protease associated with GV. This is also evident in electrophoretic profiles
of GVs isolated from the two sugar can shoot borers. Thus it became clear that the viral pathogen taken for the present study has been proved to be \textit{PiibrGV}.

**Mode of viral infection:**

The occlusion bodies of the virus enter into the body system of caterpillars by the following ways: (Fig. 14)

a) Oral ingestion

b) Passage through egg/egg surface contamination (Transovarial transmission)

c) Injury/insertion in to the larval haemocoel by the parasitoid

**Oral ingestion:** Among the above said methods, normal mode of entry of virus is by ingestion of virus contaminated food (Mazzone, 1985). The virus particles of baculovirus exist in two morphological forms and that includes 1. Polyhedra derived virus or Occluded derived virus (PDV/ODV) and 2. Non-occluded virus or budded virus (NOV/BV). Those within OBs are known as PDV or ODV and are responsible for initiating infection in the epithelial cells of the mid gut. But the NOV/BV is responsible for the spread of secondary infection within the insect body. The envelope of PDV is produced within the nucleus during morphogenesis of OBs, whereas those of budded viruses are acquired from the cytoplasmic membrane of the host cell and are eventually released into the haemolymph (Fujita \textit{et al.}, 1998).

On entering the lumen of mid gut, the capsule or occlusion body is affected by the alkaline medium, which dissolves the protein matrix and releases the free virion. The virus particle liberated from the OBs enters the peritrophic membrane with the help of yet another protein, \textit{enhancin} (Gijzen \textit{et al.}, 1995) and subsequently reaches the epithelial cell by fusion. This association allows the naked nucleocapsid to enter the cytoplasm of columnar cells. The virus uncoats before injecting the DNA into the nucleus as in GV or after entering the nucleus as in NPV. Primary infection begins by undergoing one round of DNA replication where the newly formed nucleocapsid comes out through the nucleus and gains envelope from the nuclear membrane and eventually enters the cytoplasm of the columnar cells (Mazzone,
Fig 7. Possible pathway of virus infection in *Pieris brassicae* larvae

- **OBs contaminated leaf**
- **OBs being ingested by caterpillar**
- **MID GUT**
- **OBs solubilised in the alkaline condition of mid gut**
- **Virus particles pass through peritrophic membrane with the help of enhancin**
- **Virus particles fuse with the apical microvillar membrane and enter the midgut epithelial cells**
- **Uncoate before reaching the nuclear pore, inject DNA into the nucleus**
- **Virus undergoes one round of replication in the mid gut cell**
- **Newly formed virus particles are non occluded**
- **Budded virus enters cell of various tissues by endocytosis and replicates**
- **OBs are produced from virus particles**
- **Infected larvae disintegrates releasing OBs**
- **OBs contaminated leaf**

*Parasitoids*

- **Infect another healthy larva by oviposition**

**PRIMARY INFECTION**

**SECONDARY INFECTION**

Possible pathway of virus infection in *Pieris brassicae* larvae
These budded viruses later enter haemocoel where secondary infection occurs in tracheal cells and fat bodies (Xeros, 1956). It was also observed in the histological study of the infected *Pieris brassicae* that the OBs initially infected the midgut epithelial cells, followed by haemocytes of the haemocoel and eventually cuticular cells of the integument. (Fig. 14)

**Transovarial transmission:**

In this method, the pathogen enters into egg or being coated on the surface of the egg, thereby the next generation of the host larva gets viral infection. David (1965) demonstrated the transmission of GV from infected *P. brassicae* to healthy larvae of the next generation. This is quite possible because once the pathogen reaches haemocoel, it gets ample chances to get coated over the surface of oocytes or the virions possibly enter into the developing oocytes, resulting in infection. Although in the present investigation transovarial transmission of pathogen was not observed, still its possibility cannot be ruled out.

**Injury/insertion into the larval haemocoel by the parasitoid:**

Field observation revealed that *P. brassicae* is parasitized by a number of hymenopteran parasitoids. As they oviposit their eggs inside the haemocoel of the host larva, the pathogen present in a larva can easily be transmitted to other healthy larvae through the ovipositor. Such incidence has been noticed in the present study, wherein the parasitoid, *Cotesia (=Apanteles) glomeratus* not only parasitized the caterpillars of *P. brassicae*, but also disseminated PibrGV pathogen as is evident from the photo plate of the fig – 13B. Influence of parasitoids in transmitting the pathogen has been well documented in a number of caterpillars. As for instance, the Bihar hairy caterpillar, *Spilarctia obliqua* gets SoNPV infection through parasitoids (Battu & Ramakrishnan, 1989). Similar cases of parasitoid disseminated viral infection were also recorded in the European spruce saw fly (Buse, 1977) and also in *Trichoplusia ni* (Beegle and Oatman, 1975).
Lethal time of *PibrGV*:

The lethal time to kill 50% (LT$_{50}$) of the test insects of II, III and IV instars was respectively 93, 125, and 139 hours when they are inoculated with OBs @ $7.9 \times 10^4$ per ml. The LT$_{90}$ also showed similar trend of increase from II to IV instar larvae. Table- 3 provides regression equation, LT$_{50}$ and LT$_{90}$ values, and corresponding fiducial limits for the three larval stages. From this bioassay, it became evident that 4 to 5.75 days would be required for 50% larval mortality. This observation is in tune with the GVs that infect *Chilo infescatellus* Snell and *Chilo sacchariphagus indicus* (Kapur) (Easwaramoorthy and Jayaraj, 1987; 1993) and also the NPVs that attack caterpillars in general (Ramakrishnan and Kumar, 1981). The present experiment has also proved unequivocally that the efficacy of *PibrGV* was appreciably better at the early stage than the advanced stage of the caterpillar, indicating the view that spraying OBs at the early stage of the caterpillar would lead to appreciable control of the pest especially under field conditions. Almost similar range of LT$_{50}$ i.e., 5.5-6.7 days was observed by Entwistle (1998), when *PibrGV* was applied against the larvae of *Pieris rapae* (L.) in China. Further studies on LT$_{50}$ of GVs infecting other insects’ also revealed similar trend on lethal time. For example, LT$_{50}$ values of *PxGV* were 4.9-5.4 days when individuals of different larval stages of *Plutella xylostella* (L.) were inoculated with *PxGV* (Jones *et al*., 1998).

Lethal concentration of *PibrGV*:

When each larval stage was individually exposed to different concentrations of *PibrGV* ranging from $10^4$ to $10^6$ OBs/ml separately, it was found that LC$_{50}$ exhibited an increasing trend, i.e., $7.9 \times 10^4$, $1.3 \times 10^5$, and $3.2 \times 10^6$ OBs/ml respectively for III, IV, and V instar larvae of , indicating a typical dose dependent mortality with respect to larval stages (Table-5). Based on the lab bioassay, large scale field
trials were also initiated in a cabbage field wherein $7.5 \times 10^8$ OBs/ml was sprayed against II and III instar larvae of *P. brassicae*. The trials conducted for two consecutive seasons (2007 and 2008) under the climatic conditions of the valley areas of Imphal gave an average of 63 and 100% larval mortality respectively on 7th and 12th DAT (Table-9). Earlier studies showed that application of *PibrGV* @ $10^6$ OBs/ml reduced 72-95% populations of *P. brassicae* in USSR. Similarly application of *Agrotis segetum* granulosis virus @ $10^7$ OBs/ml also effectively checked the density of *A. segetum* up to 85%. Almost the same trend was observed with *Cydia pomonella* GV wherein, application of OBs @ $9 \times 10^6 - 2.9 \times 10^{11}$ per ml reduced 74-88% pest population (Lipa, 1998). Again in the Indian context, use of *Chiloinfuscatellus* GV @ $1 \times 10^7 - 1 \times 10^9$ OBs/ml reduced the incidence of *C. infuscatellus* below the economic injury level of 20% (Easwaramoorthy, 1998). While referring to the above examples, it is evident that although there appears to be slight variation in terms of LC$_{50}$ of GV infecting different insect species, their relative efficacy seems to be almost at par with each other. Minor variation if any could be attributed to factors such as climatic condition, geographical location, age and stage of test insect etc.

**Impact of viral infection on the host larvae:**

Comparative assessment of the developmental period between normal and virus infected larvae of *P. brassicae* indicated that the infected larvae took longer time than that of their normal counterpart. For instance, the normal third instar larva required 72 hours to moult into fourth instar. On the other hand, the moulting process was significantly delayed (120 hours) in the virus infected larvae of the third instar. Similar result was also evident in the fourth instar. In other words, 6 days are normally required for the development of third and fourth instar larvae, whereas the virus infected larvae have been found to delay their development by another 3 days. Requirement of additional period may be utilized by the virus for their multiplication with the help of ecdysteroid Uridine 5- di phosphate glucosyl transferase gene - egt gene present in the viral genome. The egt gene of the baculovirus was first identified in *Autographa californica* nucleo polyhedrosis virus (AcNPV) that codes for the
enzyme, ecdysteroid Uridine 5-di phosphate glucosyl transferase. This enzyme catalyses the conjugation of ecdysteroids with glucose or galactose (Reilly and Miller, 1991), thereby limiting the presence of ecdysteroid in the haemocoel. It is well known that presence of ecdysteroid in the haemolymph is vital for ec dysis (Koolman and Karlson, 1985). Expression of egt gene allowed the virus to interfere with the normal insect development so that moulting is blocked in the infected larvae. This is the reason for delayed development in the virus infected larvae. As early as 1991, Reilly and Miller compared the growth and development of insects infected with virus having egt gene and also egt deleted mutant virus. Their data also revealed the delay in insect moulting especially with the former, while infection with mutant virus showed normal period of insect development prior to death. Further, their study also inferred that the delay in insect development of the infected larvae could be to exploit the larval host for virus multiplication.

The pathways of OBs after ingestion by the host larvae have been depicted in the flow chart. Infection and multiplication of the virus take place at different sites with the beginning from mid gut to end up to the integument. The infection is accelerated when the virions reach haemocoel, wherein they infect haemocytes. Fig - 11 provides evidence for the haemocytes as one of the sites of multiplication resulting in lysis of the cell and also reduction in total haemocytes (Table - 16). The virus reaches haemolymph even within 12 hours of ingestion as in the case of forest tent caterpillar, *Malacosoma disstria* (Reheja and Brooks, 1971). Once it enters haemolymph, virtually the circulatory fluid enables the virus to circulate throughout the body and infect each and every organ systems. The duration on LT_{50} depends on ingestion of OBs and its infectivity at different organs and sites of multiplication especially the haemolymph.

**Viability of the OBs:**

Viability of *PibrGV* was tested by storing the pellets of OBs for 2½ years and evaluating them at regular intervals against III instar larvae of *P. brassicae*. The OBs stored for 12, 24 and 30 months gave a mean larval mortality of 95, 88 and 76%
respectively on 7\textsuperscript{th} day after virus treatment, reflecting a gradual reduction in viability of OBs as the storage period went up (Table – 13). But, significance of the study is that the farmers can store and use the viral pesticide at least for two years under room temperature. Since \textit{P. brassicae} is a regular seasonal pest, farmers can use the OBs of the virus every year and augment it by serial passage once again from the field collected larvae to replenish their stock. This observation supports the view of Hunter-Fujita \textit{et al} (1998) who are of the view that dry formulations of the virus can be stored for 18 months. However, the temperature at which the OBs are stored will determine the period of storage viability. In yet another experiment, the field viability of \textit{PibrGV} [exposure to sunlight] was also evaluated by applying \textit{PibrGV} suspension @ 7.5x10\textsuperscript{8} OBs/ml on the cabbage plant. In this trial, the average viability of OBs was same for first five days [of field exposure to sunlight] since the average larval mortality on 10\textsuperscript{th} DAT was 94%. But 7\textsuperscript{th} and 9\textsuperscript{th} day exposure of OBs gave only 50% larval mortality (Table -11). This trial revealed that OBs remained viable up to 5\textsuperscript{th} day under field condition and subsequently showed a reduction in their viability. The literature concerned with the response of insect virus to UV irradiation is quite voluminous (Fujita \textit{et al}., 1998) and that pertains to \textit{PibrGV} explains that its biological half life is more during winter months than summer (Richards and Payne, 1982). Since these cruciferous vegetables are predominantly cultivated during autumn and winter, \textit{PibrGV} can be used for the field regulation of \textit{Pieris brassicae}.

Field evaluation:

Field evaluation was conducted with different aqueous formulations of \textit{PibrGV} @ 7.5x10\textsuperscript{8} OBs/ml against the caterpillars of \textit{P. brassicae}. Different adjuvants like glycerol, ranipal, charcoal, and crude sugar solution were individually mixed with \textit{PibrGV} under respective treatment schedule (T1 – T7) as mentioned in the protocol. Pre treatment assessment on larval density was made prior to the application of OBs. Observations revealed that all the treatments (T1 – T6) were found superior to untreated control (T7). The treatment T1 (\textit{PibrGV} + crude sugar + glycerol + ranipal)
exhibited a maximum larval mortality of 67% on 7th DAT, while T5 (PibrGV + Charcoal) showed a minimum larval mortality of 49% as compared to other treatments. But no incidence on larval death was observed in the control plots (Table – 10). It is already known that substances like crude sugar, glycerol, ranipal, promote the retaining ability of OBs on the foliage; crude sugar acts as phagostimulant, glycerol reduces bacterial contamination and ranipal functions as reflectant, thereby GV can be protected from UV radiation. Due to such features, perhaps the larval mortality was more than 55% in those treatments as compared to PibrGV alone. However, among all, (T6) with PibrGV + charcoal exhibited a low mortality rate even though charcoal is one among the UV protectants. But it is presumed that some larvae might have avoided feeding the charcoal sprayed foliage and that being the reason for low larval mortality. By and large, combination of these substances with PibrGV gave appreciable control of caterpillars on 7 DAT and their infestation was almost nil on 12 DAT especially in all the chemical treated plots. Influence of such substances along with NPVs has also been documented especially in the context of field control of varied insect pests (Hunter-Fujita et al., 1998; Jayaraj, 2001). But the present trial will add additional information, since these substances have been tried for the first time against the cabbage butterfly.

Mass production of viral pathogen:

In case of in vivo production of viral pesticide, individual caterpillar is considered as a bioreactor. This is because appreciable turn over in the production of OBs has been observed in many cases (Granados and McKenna, 1995). For instance, the third instar larvae of P. brassicae are capable of producing an average of 5.5×10^9 OBs/larva when inoculated with PibrGV @ 7.7×10^4 OBs/ml (Sangeeta et al., 2009). Based on this, an attempt has been made here to produce PibrGV pathogen by in vivo method. In one batch at least 200 larvae could be inoculated and reared on cabbage leaf as stated under the materials and method [on insect rearing]. As the mean yield of OBs is estimated to be 5.5×10^9 per individual, inoculating 200 larvae will give rise to OBs of 200 larval equivalents which in turn can be used for spraying at least one
hectare area of the crop (Varatharajan, 2008). Like the general methodology highlighted by Jayaraj (2001) for the production of viral pesticide, mass production of PibrGV is also cost effective and required quantity of OBs can be produced through any one of the Pieris sp. available during the season concerned.

Cross infectivity of PibrGV:

PibrGV was tested against two other closely related species namely Pieris canidia and P. daplidice and it became evident that they were found susceptible to PibrGV. This was confirmed by inoculating each species with the OBs obtained from other species of Pieris and vice versa. Both the siblings showed same symptoms of viral infection as shown by P. brassicae (Fig. 12). The infected larvae were found hanging with their abdominal legs on the leaf of the plant host. The bio-assay data of III instar larvae of P. brassicae, P. canidia and P. dapladice reflected that LC50 value of the pathogen-PibrGV was 1.3 x 10^5, 4.6 x 10^5, and 8.0 x 10^5 OBs/ml and the LT50 being 140, 135 and 145 hours respectively. Further, the infectivity of PibrGV on P. rapae was already known through the works of Entwistle (1998). The cross infectivity of PibrGV on different siblings of Pieris species would be, by far beneficial to the farmers by virtue of the fact that all the Pieris species that were examined being the pests of cruciferous crops and therefore, spraying PibrGV could minimize the incidence of all the Pieris species present in the same agro ecosystem.
SUMMARY

The granulovirus (*PibrGV*) infecting the cabbage butterfly, *P. brassicae* has been studied in detail from November 2005 to June 2011 and the results of the research works are given below:

1. Seasonal incidence of the cabbage butterfly has indicated that *Pieris brassicae* (L.) infest cabbage and cauliflower from January to July with maximum density in April under the climatic condition of Imphal. During routine sampling, some viral infected larvae were collected at random from the field and used for further augmentation (Fig.1 & Table -1).

2. Virus infected larvae showed symptoms like pale body colour with shiny and loose cuticle, weak body, restless movement, haemolymph oozing through the mouth and hanging upside down at the advanced stage of infection. Body weight of the infected larvae was significantly more than that of their normal counterpart (Fig. 5 & 6).

3. Although Sucrose Gradient Ultra Centrifugation (SGUC) will give highly purified occlusion bodies (OBs), still partial purification of OBs as adopted in the present study also showed the Brownian motion of OBs under the light microscope and gave a spherical shape with an average size of 300nm especially when viewed under the SEM.

4. The virus pathogen was confirmed as granulovirus based on the symptoms of the infected larvae, spherical shaped capsule as revealed by SEM, presence of granulin protein in the capsule (Protein profile : page - 69) and histopathological studies of the mid gut and the cuticle.

5. Lab bioassay of *PibrGV* to calculate LC$_{50}$ and LT$_{50}$ using Finney’s method of Probit analysis indicated that LC$_{50}$ exhibited an increasing trend, i.e., 7.9x10$^4$, 1.3x10$^5$, and 3.2x10$^6$ OBs/ml respectively for III, IV, and V instar larvae of , indicating a typical dose dependent mortality with respect to larval stages (Table-5). On the other hand, the lethal time to kill 50% (LT$_{50}$) of the test insects of II, III and
IV instars of was respectively 93, 125, and 139 hours (Table -3 & 4). LT$_{50}$ value of *PibrGV* under the field condition was 124, 178 and 187 hours respectively for the II, III and IV instars of *P. brassicae* (Table -7).

6. The virus infected larvae were reared at two different temperature schedules: Schedule-a) 10±1.4 (minimum); 22±15$^\circ$C (maximum); RH = 81±5% Schedule-b) 15±1.2 (minimum); 29±2$^\circ$C (maximum); RH = 75±4%.

The LT$_{50}$ value of *PibrGV* was significantly higher when the larvae were reared at low temperature in comparison to the larvae cultured at higher temperature.

7. Field trial conducted at the cabbage as well as cauliflower field for two consecutive years gave an average of 60 % mortality on 7$^{th}$ day after virus treatment (DAT) and 100% percent mortality on 15$^{th}$ day after treatment (Table -9).

8. Field experiment on different formulations prepared by mixing the viral suspension with adjuvants like crude sugar, glycerol and ranipal indicated nearly 60 % mortality on 7$^{th}$ DAT and 100 % mortality on 10$^{th}$ DAT. This result has indirectly proved the adhesiveness of OBs on the foliage surface besides retaining viability against UV radiation. On the other hand, application of *PibrGV* suspension alone resulted in 50 and 95% larval mortality on 7$^{th}$ and 10$^{th}$ DAT respectively (Table – 10).

9. The viability of *PibrGV* [exposure to sunlight] under field condition was also evaluated by applying *PibrGV* suspension @ 7.5x10$^8$ OBs/ml on the cabbage plant. In this trial, the viability was appreciably good for the first five days of field exposure and that being evident in terms of larval mortality which was found to be 94% on 10$^{th}$ DAT. But 7$^{th}$ and 9$^{th}$ day exposure of OBs gave only 50% larval mortality (Table -11). This experiment revealed that OBs remained viable up to 5$^{th}$ day under the field condition.
10. Viability of *PibrGV* under different period of storage reflected that OBs could be stored for about two years. However, gradual reduction in viability was evident as time progressed, but this could also be overcome through serial passage (Table -13).

11. Screening the plant to find out the host suitability to rear virus infected larvae revealed that the cabbage leaf was found better than cauliflower and mustard foliage since the larval mortality was 78, 75 and 43% on the respective plant hosts on 6th DAT (Graph - 4).

12. The third instar larva of *P. brassicae* has been able to produce an average of 5.5×10⁹ OBs/larva when inoculated with *PibrGV* @ 7.7×10⁴ OBs/ml.

13. Assessment on optimum larval density to rear virus infected larvae in the wooden cage of 30 x 30 x 30 cm, indicated that rearing 200 larvae / culture was found ideal (Table – 15). There wasn’t any cannibalism among the infected larvae.

14. Artificial diet for mass rearing the larvae has given a hope that virus infected larvae can be reared on the semi synthetic diet when there is a scarcity of foliage (Fig – 8).

15. Larval duration of virus infected and normal larvae of indicated that the former took 3 days more than that of latter especially during third to fifth instar stage. [Requirement of this additional period may be utilized by the virus for their multiplication with the help of ecdysteroid glucosyl transferase gene – (*egt gene*) present in the viral genome].

16. Comparison of the body weight of the healthy as well as virus infected larvae clearly reflected that the body weight of the infected larvae was greater than that of normal larvae (Graph- 6).

17. Cross section of the cuticle as well as the mid gut tissue of the virus infected larvae depicted the discontinuity of the cuticular layer and the peritrophic membrane of the mid gut besides, hyper-trophid mid gut epithelial cells (Fig – 9 and 10).
18. Total haemocyte count of healthy and virus infected larval haemolymph revealed significant variation between them in terms of their count. Virus infected fourth instar larvae had appreciably more number of haemocytes than their normal counterpart. On the contrary, haemocyte count of virus infected fifth instar larvae was significantly less than that of normal individuals (Fig – 11 and Table – 16).

19. Cross infectivity experiment unambiguously demonstrated the infectivity of the pathogen, PibrGV to the closely related siblings namely, Pieris canidia and Pieris daplidice in addition to Pieris brassicae (Fig – 12).

20. Safety evaluation of PibrGV against albino rats, Cotesia (=Apanteles) glomeratus (Parasitoid), Bombyx mori (mulberry silkworm) and Antheraea proyli (Tasar silkworm) showed that PibrGV was not harmful to the above organisms and therefore, safe to use this pathogen for the field control of cabbage butterflies (Fig – 13).