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# Natural incidence of *Pieris brassicae* Granulosis virus among the population of *Pieris brassicae*

**Ramandeep Kour, RK Gupta, Simranjeet Kour, Suheel Ahmad Ganai, Sonika Sharma and Shahida Ibrahim**

**Abstract**
Isolating GVs from naturally infected insects provides insight into the biodiversity of these naturally occurring pathogens and provides a pool of potential biocontrol agents. Since native strains are always preferred for sustainability, adaptability and efficacy in given agroecosystem. Therefore, exploratory surveys carried out to isolate naturally occurring strains of *Pieris brassicae* Granulosis virus in the population proved the existence of Granulosis virus in the natural populations of *P. brassicae* in Jammu & Kashmir range of North-Western Himalayas. The natural incidence of GV in *P. brassicae* larvae is very low in the natural population of Jammu and Kashmir. A total of 3 isolates were isolated from Jammu and Kashmir range of North –Western Himalayas in the present study. The natural incidence of GV in *P. brassicae* larvae varied from 0.95 % – 6.88 %. The highest and lowest incidence of 6.88 per cent and 0.95 per cent was observed on cabbage crop from district Udhampur (Sudhmahadev) and Jammu (Chatha), respectively. However, no natural incidence of GV was observed in Kashmir division of Jammu and Kashmir.

**Keywords:** granulosis virus, biodiversity, natural incidence, sudhmahadev, exploratory surveys

## Introduction
Cabbage white butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae), is one of the most destructive and cosmopolitan insect pest which can cause extensive damage at all the growing stages of Cole crops such as seedling, vegetative and flowering stages (Hasan and Ansari, 2010 and Ullah *et al*., 2016) [12, 25]. At pre-heading stage, the caterpillars may guttle the whole plant while at head formation stage, they damage the head by feeding on it and by contaminating it with faeces, whereas, after head formation, they feed mainly on the inflorescence reducing the seed yield. Utmost infestations of *P. brassicae* completely destroy the plant foliage leading to annual loss of 40% in India (Ali & Rizvi, 2007; Hasan and Ansari, 2010) [1, 12]. The damage is primarily caused by the fourth and fifth instar caterpillars by virtue of their voracious feeding habit in the temperate, tropical and subtropical regions of the entire sub Himalayan region and north-eastern states of India (Younas *et al.*, 2004; Bhandari *et al.*, 2010) [26, 2].

During the past decades the efforts to manage this insect pest was dominated by chemical insecticides but their use is becoming less appropriate due to ecological, social and economical problems (Ndakidemi *et al*., 2016; Hikal *et al.*, 2017) [17, 13]. Moreover, the availability of many chemical pesticides is declining as a result of the evolution of resistances (Tabashnik *et al*., 2009; Mahmood *et al*., 2016) [24, 16] and legislation (Chandler *et al.*, 2011) [4]. Thus, there is an urgent need to develop eco-friendly pest management tactics alternative to chemical insecticide such as biopesticides' for suppression of this pest. Unlike other natural enemies, biopesticides can be used in a manner similar to the familiar chemical pesticides as they can be produced, stored and made available to the farmers at short notice due to their longer shelf life (Roberts *et al*., 1991, Ravishankar, 2010) [20, 19]. In recent years several organizations have also been promoting the use of eco-friendly biopesticides including baculovirus. Baculoviruses are attractive alternatives for biological control under IPM. They possess distinct advantages over other microbial bioagents because of their horizontal and vertical transmission from one generation to the next host generation. Moreover, these microbes also establish themselves in the pest population and exert long-term protection in sprayed areas even after initial application (Gupta *et al.*, 2016) [11]. However, to best of our knowledge, the present study established clearly for the first time that the *Pieris brassicae* Granulosis virus exists in the natural population of Jammu. Since native strains are always preferred for sustainability, adaptability and efficacy in given agroecosystem,

the present study was planned to select an GV isolate for use against the host species in a control program designed for vegetable growers in J & K.

## Material and Method

### The virus

The virus used in this study was isolated from *Pieris brassicae* larvae showing typical disease symptoms of GV collected from different ecosystems in the Jammu & Kashmir region of North-western Himalaya's viz., Jammu, Poonch, Udhampur and Kashmir during during intensive exploratory surveys (Table 1). During the survey, live and dead specimens were collected individually. The dead larvae were placed individually in sterile glass vials and capped with ventilated lids to permit exchange of gases and prevent condensation of moisture and were kept at -20°C until till further use. However, the Himachal Pradesh isolate of *Pieris brassicae* granulosis virus was isolated from infected larvae of *P. brassicae* (Sood, 2004) [23]. The extraction, purification and standardization of OBs were done as per the methods given by Moore, 2002 [22]. Preliminary baculovirus identification was done by light microscopy by spreading smears from infected larvae thinly across a microscope slide followed by Giemsa staining and then examined through a phase-contrast microscope at ×1000 magnification under oil immersion (Lacey, 2012) [15]. While surveying, Disease recognition was done at several steps. Movement and irritability, discolouration, changes in body size and shape and physiological disturbances were the symptoms of diseases when live insects were collected. In the third stage, the causal organisms after passing through susceptible healthy individuals were reisolated for experimental investigations to satisfy Koch's postulates.

**Table 1:** Locations of collecting diseased insects with their altitude, latitude and longitude.

| S. No. | Collection area | Altitude (ft) | Latitude/Longitude |
|---|---|---|---|
| 1 | Jammu | 1148 | 32° 44' N/74° 54' E |
| 2 | Udhampur | 2477 | 32° 54' N/75° 7' E. |
| 3 | Poonch | 3212 | 33° 46' N/74° 5' E. |
| 4 | Kashmir | 5201 | $34^0$ 01'N/ $74^0$ 47'E |

### Establishment of laboratory culture of *P. brassicae*

The larvae collected from cole crops (cabbage, cauliflower, knol-khol, kale) of the different locations were monitored and reared in the laboratory at SKUAST- J, FOA- Chatha. The larvae were fed with fresh cabbage leaves surface sterilized with aqueous solution of sodium hypochlorite (0.05%). The cabbage leaves were kept in ethanol washed and UV sterilized glass jars (50×30 cm diameter) lined with filter paper and covered with muslin cloth to permit exchange of gases and prevent condensation of moisture. Caterpillars in cages were provided with surface sterilized fresh cabbage leaves daily. Uneaten food along with faeces was removed regularly in order to maintain hygiene in the rearing containers. The feed was changed daily and rearing space was increased regularly by using more number of jars for avoiding overcrowding of the larvae for promoting uniform growth and development of the larvae. Larvae were regularly monitored for their growth and the larvae, which appeared to be symptomatically infected with a baculovirus, were removed from the culture, placed individually in marked microtubes, and frozen at -20°C.

### Estimation of natural incidence of the virus

To estimate the natural incidence of the virus, ten plants of respective crop (cabbage, cauliflower, knol-khol, kale) were randomly selected from an infested area at each location. Diseased and healthy larvae present on each leaf were collected and reared in the laboratory. The larvae showing typical symptoms of GV infection were counted with the help of Petroff-Hausser counting chamber and the GV infection was confirmed by microscopic examination of the larval smear through Giemsa staining. The percentage infection was calculated by the following formulae:

The per cent infection was calculated by the following formula:

$$\frac{\text{No. of larvae infected}}{\text{Total no. of larvae (healthy + infected)}} \times 100$$

## Result

### Estimation of natural incidence of the Granulosis virus

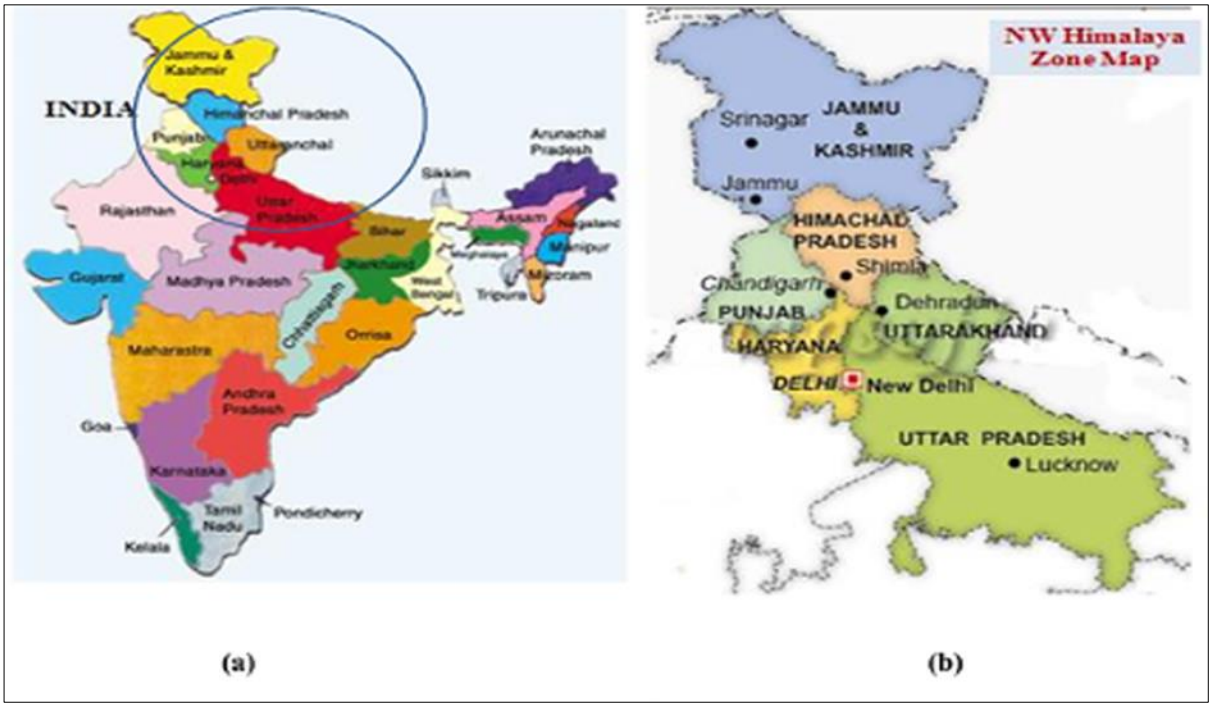
Exploratory surveys were conducted at different locations viz., Poonch, Udhampur Jammu and Kashmir district of Jammu and Kashmir. During the survey, only 2-3 % larvae were found to be infected with disease. On close scrutiny, larvae were found to be infected by the pathogen and it was found to be a granulosis virus (Figure 1). Most of the larval population of *P. brassicae* did not harbour the signs of viral disease under natural field conditions. However, when they were transferred to the laboratory for monitoring and rearing, they died showing viral infection symptoms. Microscopic examination through giemsa staining of the haemolymph and tissue smears of the larvae showing typical GV symptoms collected from all the locations revealed the presence of OBs as observed under a phase contrast microscope at × 1000 magnification using a haemocytometer.

The natural incidence of GV in *P. brassicae* larvae varied from 0.95 % – 6.88 %. The highest and lowest incidence of 6.88 and 0.95 per cent was observed on cabbage crop from Sudhmahadev and Chatha, respectively. However, no natural incidence of GV was observed in Kashmir division of Jammu and Kashmir (Table 2).

**Table 2:** Natural incidence of GV in *P. brassicae* larvae collected from different host crops from different zones of J & K

| Location | Host tree | No. of larvae collected | GV infected Larvae | Per cent GV infection |
|---|---|---|---|---|
| Poonch | Cabbage | 95 | 1 | 1.05 |
| | Cauliflower | 120 | 3 | 2.50 |
| | Knol-Khol | 165 | 7 | 4.24 |
| | Broccoli | 0 | 0 | 0.00 |
| | Kale | 0 | 0 | 0.00 |
| Udhampur | Cabbage | 160 | 11 | 6.88 |
| | Cauliflower | 85 | 0 | 0.00 |
| | Knol-Khol | 105 | 6 | 5.71 |
| | Broccoli | 0 | 0 | 0.00 |
| | Kale | 0 | 0 | 0.00 |

| | | | | |
|---|---|---|---|---|
| Jammu | Cabbage | 105 | 1 | 0.95 |
| | Cauliflower | 120 | 3 | 2.50 |
| | Knol-Khol | 120 | 2 | 1.66 |
| | Broccoli | 0 | 0 | 0.00 |
| | Kale | 150 | 6 | 4.00 |
| Kashmir | Cabbage | 95 | 0 | 0.00 |
| | Cauliflower | 140 | 0 | 0.00 |
| | Knol-Khol | 125 | 0 | 0.00 |
| | Broccoli | 0 | 0 | 0.00 |
| | Kale | 0 | 0 | 0.00 |



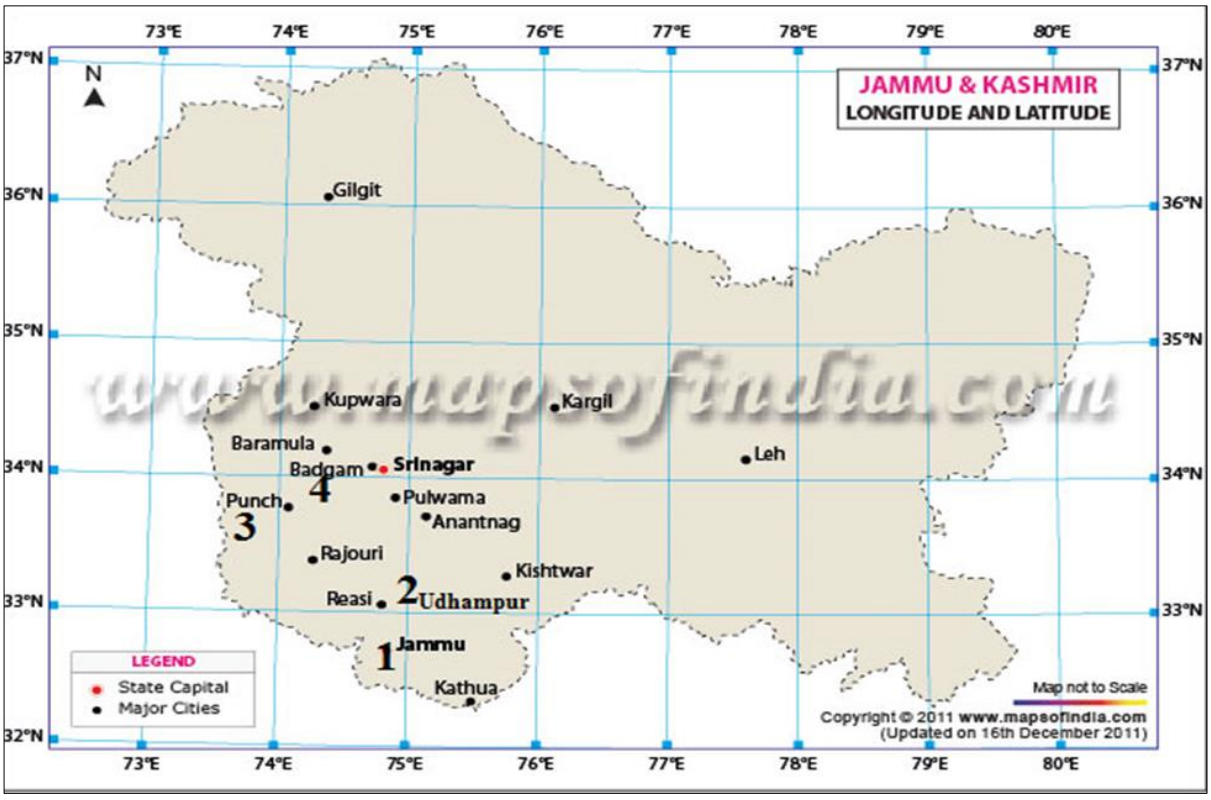
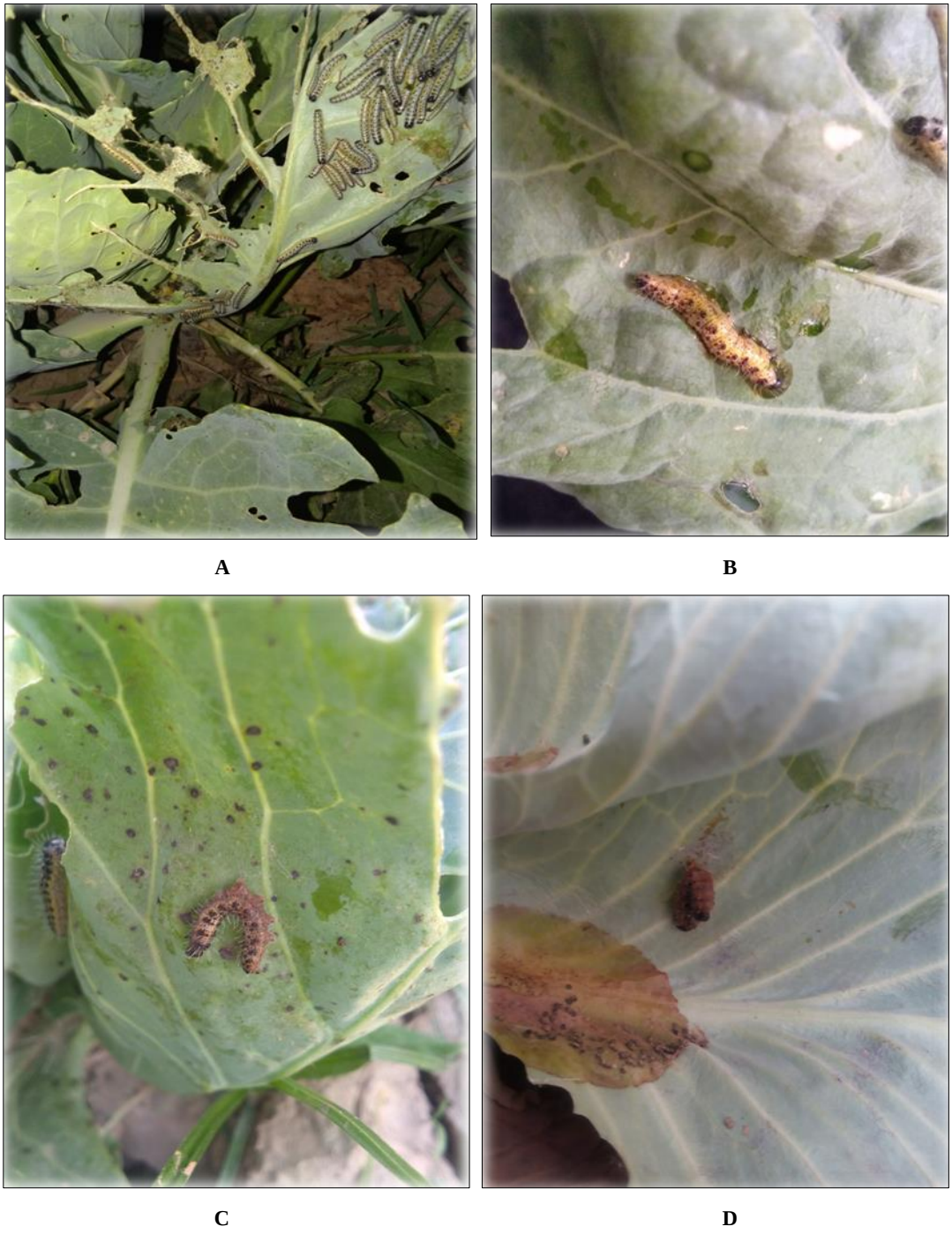




**Fig 1:** Exploratory surveys were conducted in different ecosystems in the Jammu & Kashmir region of North-western Himalayas for the presence of native GV isolates of *Pieris brassicae*



**A** **B**

**C** **D**

**Fig 2A:** Damage caused by larvae of *Pieris brassicae.*
**Fig 2 B, C, D:** Granulosis Virus infection in larvae of *Pieris brassicae* observed under field conditions

**Nomenclature of Baculovirus isolates**

In order to name the four P. *brassicae* GV isolates recovered, the most commonly used methods based on naming the virus directly after the host from which they were isolated, and secondly on the type of OBs (NPV or GV) associated with it were adopted (OECD, 2002; Bonsall, 2004; Fauquet & Fargette, 2005; Erlandson, 2009) [18, 3, 8, 7]. The first two letters of the genus and species name of the host were used for abbreviated versions of the virus isolate. The country from which the virus was recovered was added as an acronym or suffix to distinguish baculoviruses recovered from the same host species from different countries (Fritsch, 1989; Singh *et al.*, 2003) [9, 22]. Further, the name of the geographic region from which an isolate was recovered was also considered for the naming of the isolates. The rationale for adopting this method is that, each isolate consists of more than one genotype and in future these genotypes can be distinguished from each other using numbers. *In vivo* and *in vitro* cloning techniques can be used to distinguish between the genotypes within each isolate (Crook, 1986; Erlandson, 2009) [5, 7]. Therefore, taking into consideration all these facts the H.P, Poonch, Sudmahadev (Udhampur) and Chatha isolates of P. *brassicae* were referred to as PibrGV-IND HP, PibrGV-IND PH, PibrGV-IND SD and PibrGV-IND CH.

**Discussion**

The exploratory survey conducted for the isolation of local strains of PibrGV led to the recovery of this virus from different locations of Jammu & Kashmir. During the survey, only 2-3 % larvae were found infected with virus. Most of the larval population of *P. brassicae* did not harbour the signs of viral disease under natural field conditions. However, when

they were transferred to the laboratory for monitoring and rearing, few of them died showing viral infection symptoms. Morphological, symptomatogical and microscopic examination through giemsa staining of the haemolymph and tissue smears of the larvae showing typical GV symptoms confirmed the pathogen as granulovirus. The occlusion bodies of naturally infected larvae resembled to that of experimentally infected larvae thus confirming the Koch's postulates. Natural incidences of viral diseases of the cabbage butterfly were previously reported in India (Sood, 2004; Ingobi *et al* 2006) [23, 14]. However, the present study established clearly for the first time that the *Pieris brassicae* Granulosis virus exist in the natural population of *P. brassicae* collected from different locations of Jammu and Kashmir. Nevertheless, the natural incidence of GV in *P. brassicae* larvae is very low in the natural population of Jammu and Kashmir. The natural incidence of GV in *P. brassicae* larvae varied from 0.95 % – 6.88 %. The highest and lowest incidence of 6.88 per cent and 0.95 per cent was observed on cabbage crop from Sudhmahadev and Chatha, respectively. However, no natural incidence of GV was observed in Kashmir division of Jammu and Kashmir. Variation in magnitude of natural incidence of GV indicated that different agro climatic conditions might have played important role in natural occurrence of GV. The baculovirus epizootics have been recorded in the natural field conditions due to certain stress factors like high population density. For instance Gupta *et al*. (2016) [11] reported that the prevalence of baculovirus is density dependent phenomenon. In Jammu division a warm spring might have favoured outbreaks of *P. brassicae* and as such the chances of lethal epizootic diseases are more. However, in Kashmir valley inclement early spring weather might be expected to have the greatest influence on the survival of young larvae, hence there are minimum chances of lethal epizootics. In present study, fluctuation in the population density of *P. brassicae* is attributed to temperature, which concurred with conclusions of Devi (2011) [16] and Sharma *et al.* (2017) [21] that weather influence is a likely explanation for fluctuation of population density.

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