Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(4): 1293-1298

Received: 19-05-2018
Accepted: 20-06-2018

**Ramandeep Kour**
Division of Entomology, SKUAST-J, FOA- Chatha, Jammu and Kashmir, India

**RK Gupta**
Division of Entomology, SKUAST-J, FOA- Chatha, Jammu and Kashmir, India

**Hafeez Ahmad**
Division of Entomology, SKUAST-J, FOA- Chatha, Jammu and Kashmir, India

**Suheel Ahmad Ganai**
Division of Entomology, SKUAST-J, FOA- Chatha, Jammu and Kashmir, India

**Nadeya Khaliq**
Division of Entomology, SKUAST-J, FOA- Chatha, Jammu and Kashmir, India

**Simranjeet Kour**
Division of LPT, NDVSU, F.V.Sc & A.H., Jabalpur, Madhya Pradesh, India

**Correspondence**
**Ramandeep Kour**
Division of Entomology, SKUAST-J, FOA- Chatha, Jammu and Kashmir, India

# Biological characterization of different isolates of *Pieris brassicae* Granulosis virus

**Ramandeep Kour, RK Gupta, Hafeez Ahmad, Suheel Ahmad Ganai, Nadeya Khaliq and Simranjeet Kour**

**Abstract**
To determine the biological characteristics of different isolates of *Pieris brassicae* Granulosis virus, bioassays were carried out against the third instar larvae of *Pieris brassicae* under laboratory conditions. The four different GV isolates of *P. brassicae* isolated from different geographical regions, exhibited differential degree of pathogenicity in terms of both $LD_{50}$ and $ST_{50}$. The $LD_{50}$ values of different GV isolates of *P. brassicae* against 3rd instar larvae of *P. brassicae* varied from 6.31 x $10^3$ to 1.22 x $10^4$ OBs/larvae with non significant differences between the isolates. However, significant differences were observed in the $ST_{50}$ estimates of four different isolates of PibrGV against 3rd instar larvae of *P. brassicae* which varied from 5.90 to 7.43 days. The Sudhmahadev isolate exhibited 11.01- 20.59% reduction in $ST_{50}$ as compared to *Poonch*, *Chatha* and Himachal Pradesh isolates. Among all these isolates, Sudhmahadev isolate of PibrGV was found to be most effective.

**Keywords:** Baculoviruses, characterization, isolates, pathogenicity, virulence

## 1. Introduction
India is bestowed with varied agro-climate, which is highly favourable for cultivation of vegetable crops which are the important components of our daily diet. However, Insect herbivory is one of the major factors responsible for the reduction in vegetable production and yield [1]. Among these herbivores, cabbage white butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae), is one of the most serious insect pest which can cause extensive damage at all the growing stages of Cole crops such as seedling, vegetative and flowering stages [2, 3]. Utmost infestations of *P. brassicae* completely destroy the plant foliage leading to annual loss of 40% in India [4, 5]. 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 [6, 7].

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 the evolution of resistances [8, 9] and legislation [10] in addition to apart from ecological, social and economical problems caused by them [11, 12]. 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. In recent years several organizations have also been promoting the use of eco-friendly biopesticides including baculovirus. Baculoviruses possess distinct advantages over other microbial bioagents because of their horizontal and vertical transmission from one generation to the next host generation. Unlike other natural enemies, baculoviruses 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 [13, 14]. Baculoviruses are known to be highly variable, with isolates collected from the same species in different geographical locations frequently showing genetic variation and differences in their biology [15]. To determine their biological characteristics particular baculovirus strains were screened by conducting bioassays or biological assays in order to recover the most virulent baculovirus isolates for use in the development of biopesticides as there is a urgent need to generate the information regarding virulence of GV before they can be used against the host species in a control program designed for vegetable growers in J & K.

## 2. Material and Methods

### 2.1 The virus strain

The virus used in this study was originally isolated from *Pieris brassicae* larvae showing typical disease symptoms of GV infection collected from different zones of J & K during intensive exploratory surveys by differential centrifugation method. The extraction, purification and standardization of OBs were carried out with the help of protocol given by [16]. 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 1000X magnification under oil immersion [17].

### 2.2 The host insect

The laboratory culture of *P. brassicae* was established from the eggs collected from different cole crops (cabbage, cauliflower, knol-khol, kale) and reared in the laboratory. The eggs were kept in sterilized Petri-plates (7.5 cm diameter) over a UV irradiated filter paper moistened with sterile distilled water (SDW) to prevent desiccation under laboratory conditions (Temp. 25 ± $2^0$C and RH 75–80%). The neonate larvae were collected after hatching and transferred to fresh cabbage leaves kept 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. The full grown healthy caterpillars were transferred to the new jars for pupation. Two days old pupae were detached from the leaves or walls of jars and were kept in a batch of 20 pupae in each jar over a thick layer of UV irradiated filter paper for adult emergence. The colony was reared at temperature of 26 ± $2^0$ C and 70 ± 10% RH and L: D (16:8) photoperiod. After emergence adults were transferred in Nylon mesh cages (10×9×7 ft) to provide the natural conditions where they could also receive sun from early morning until late afternoon. Potted cabbage, cauliflower, Knol-khol plants were kept in mesh cage for their mating and oviposition. Some flowering plants of *Nasturtium sp* were also provided as nectar source. The larvae emerged from eggs were reared together and later used in the experiments

### 2.3 Dose response and time response bioassays: selection of a potent isolate

Selection of a more virulent NPV isolate is a key to the development of effective viral insecticides and could be of vital importance for eco-friendly management of *P.brassicae* in various cropping systems of J&K. For strain selection mortality caused by different geographical isolates of PibrGV to the laboratory reared host larvae were evaluated by leaf disc bioassay. The virus isolates were bio assayed on laboratory reared freshly moulted third-instar host larvae via leaf disc method. Circular leaf discs measuring 1.5 cm in diameter were cut from fresh cabbage/cauliflower leaves. Six different virus doses viz., $8\times10^2$, $3.6\times10^3$, $7.2\times10^4$, $1.44\times10^5$, $2.88\times10^6$ and $5.76\times10^7$ OBs/larvae were used in the study. For time-response larvae were inoculated with a dose of $1.44\times10^5$ OBs/larva to produce similar mortality rates in order to facilitate comparison among the isolates. Serial dilutions with distilled water were performed to achieve the desired doses. Ten µl aliquots of each viral dose from each viral isolate were spread evenly onto each leaf disc with the help of brush. Control larvae were fed a leaf disc inoculated with distilled water. The leaf discs were air-dried and then placed individually in a 25, 2 x 2 cm, compartmentalized plastic plate containing 2% agar. The agar maintained the humidity and thus reduced leaf desiccation. Larvae were pre-starved for 12 hours before inoculation of virus. A total of 30 larvae were used for each concentration. Larvae, having eaten the entire leaf disc, were transferred to fresh uncontaminated leaves and reared at 28 ± 2ºC, 60% RH, and 16L: 8D photoperiod. Larvae that did not consume the entire disc were discarded. Bioassay of each viral dose and a corresponding experimental control group was replicated three times in case of dose-response and 7 times in case of time-response. Larvae were observed daily for virus induced mortality until pupation. Mortality caused by virus was diagnosed from typical virus disease characteristics (soft, flaccid body) and confirmed by microscopic examination through Giemsa staining. Larval mortality was analysed using Probit analysis to estimate the median lethal dose ($LD_{50}$) values. Median survival time ($ST_{50}$) was calculated using log rank test under Kaplan-Meier analyses.

## 3. Result

### 3.1 Dose response and time response bioassays: selection of a potent isolate

Bioassays of different geographical isolates/strains of PibrGV carried out against the third instar larvae of *Pieris brassicae* under laboratory conditions revealed a range of variation in their biological activity which was taken as the measure for strain selection. The mortality of 3rd instar *P. brassicae* at a dosage of $1.8\times10^2$, $3.6\times10^3$, $7.2\times10^4$, $1.44\times10^5$, $2.88\times10^6$ and $5.76\times10^7$ OBs/larva ranged from 23.33-100.00%, with significant differences between the doses (HP, F= 478.2, df=6, 14, p=0.00; PH, F= 532.6, df=6, 14, p=0.00; CH, F= 393.2, df=6, 14, p=0.00; SD, F=770.25, df=6, 14, p=0.00). Sudhmahadev isolate caused highest (100%) mortality while Himachal Pradesh strain inflicted minimum (95%) mortality at a dose of $5.76\times10^7$ OBs/larva. No mortality was observed in the larvae in controls. It was evident that the percent mortality was highest in larvae inoculated with Sudhmahadev isolate followed by Chatha, Poonch and H.P, the differences being significant between the isolates ($\chi^2$ = 11.112; df = 4; P = 0.025) (Table 1).

Probit analysis of the mortality data of *P. brassicae* larvae showed median lethal dose ($LD_{50}$) estimates of $1.22\times10^4$, $1.03\times10^4$, $1.19\times10^4$ and $6.31\times10^3$ OBs/larvae against H.P, Poonch, Chatha and Sudhmahadev isolate respectively, the differences being non-significant (F = 263.30; df = 3, 8; P = 0.16) (Table 3). The effect of dose on the mortality of *P. brassicae* larvae varied up to the magnitude of 95 –98%. The $LD_{50}$ value of H.P isolate was highest while the isolate with lowest $LD_{50}$ value was Sudhmahadev isolate. The order of $LD_{50}$ values was: Sudhmahadev isolate < Poonch isolate < Chatha isolate < H.P isolate. It was also found that the Sudhmahadev isolate exhibited a 38.74 – 48.28% reduction in the $LD_{50}$ as compared to other isolates. Thus Sudhmahadev isolate of PibrGV was most infective among the four isolates (Table 2).

The time response bioassays of four geographic isolates PibrGV against 3rd instar larvae of *P. brassicae* with the dose

of $1.44 \times 10^5$ OBs/larva are summarized in Table 6. The first virus induced mortality was observed on day 4 and it continued up to day 10 post-inoculation. After day 10 no further mortality was recorded. The larval mortality percent value varies from 79 – 86% in 3rd instar larvae of *P. brassicae*. Significant differences were observed between the treatments and control (HP, F = 19.3, df = 1, 12, P = 0.00; CH, F = 29.4, df = 1, 12, P = 0.00; PH, F = 5.93, df = 1, 12, P = 0.00 and SD, F = 14.9, df = 1, 12, P = 0.00). The results also revealed that the speed of kill in *P. brassicae* larvae was highest with the Sudhmahadev isolate followed by Poonch, Chatha and Himachal Pradesh (Table 3).

Significant differences were observed in the $ST_{50}$ values of four different isolates of PibrGV against 3rd instar ($\chi^2$ = 22.220; df = 3; P = 0.00). The $ST_{50}$ was lowest in Sudhmahadev isolate (5.90 days) followed by Poonch (6.63 days), Chatha (6.80 days) and H.P (7.43 days). It was found that the Sudhmahadev isolate exhibited 11.01- 20.59% reduction in $ST_{50}$ as compared to *Poonch*, *Chatha* and Himachal Pradesh isolates against 3rd instar. The effect of dose on the mortality of *P. brassicae* larvae varied up to the magnitude of 94 – 99% (Table 4).

**Table 1:** Mortality of 3nd instar *P. brassicae* larvae with the four different geographical isolates of PibrGV

| Isolate | Dose (OBs / larva) | No. of larvae/replicate | Larval death due to GV (Mean ± SE) | Percent larval mortality (Mean ± SE) |
|---|---|---|---|---|
| Himachal Pradesh | Control | 30 | 0.00$^a$ | 0.00$^a$ |
| | $1.8 \times 10^2$ | 30 | 7.67 ± 0.33$^b$ | 25.55 ± 1.11$^b$ |
| | $3.6 \times 10^3$ | 30 | 11.00 ± 0.58$^c$ | 36.66 ± 1.93$^c$ |
| | $7.2 \times 10^4$ | 30 | 15.33 ± 0.33$^d$ | 51.11 ± 1.11$^d$ |
| | $1.44 \times 10^5$ | 30 | 23.00 ± 0.58$^e$ | 76.66 ± 1.93$^e$ |
| | $2.88 \times 10^6$ | 30 | 26.33 ± 0.88$^f$ | 87.77 ± 2.93$^f$ |
| | $5.76 \times 10^7$ | 30 | 28.67 ± 0.33$^g$ | 95.55 ± 1.11$^g$ |
| Poonch | Control | 30 | 0.00$^a$ | 0.00$^a$ |
| | $1.8 \times 10^2$ | 30 | 8.33 ± 0.33$^b$ | 27.77 ± 1.11$^b$ |
| | $3.6 \times 10^3$ | 30 | 10.66 ± 0.67$^c$ | 35.55 ± 1.11$^c$ |
| | $7.2 \times 10^4$ | 30 | 16.00 ± 0.58$^d$ | 53.33 ± 1.92$^d$ |
| | $1.44 \times 10^5$ | 30 | 23.00 ± 0.58$^e$ | 76.66 ± 1.93$^e$ |
| | $2.88 \times 10^6$ | 30 | 26.67 ± 0.67$^f$ | 88.88 ± 2.22$^f$ |
| | $5.76 \times 10^7$ | 30 | 29.00 ± 0.58$^g$ | 96.66 ± 1.93$^g$ |
| Chatha | Control | 30 | 0.00$^a$ | 0.00$^a$ |
| | $1.8 \times 10^2$ | 30 | 7.00 ± 0.58$^b$ | 23.33 ± 1.92$^b$ |
| | $3.6 \times 10^3$ | 30 | 10.66 ± 0.33$^c$ | 35.55 ± 1.11$^c$ |
| | $7.2 \times 10^4$ | 30 | 17.00 ± 0.58$^d$ | 56.66 ± 1.93$^d$ |
| | $1.44 \times 10^5$ | 30 | 22.00 ± 0.58$^e$ | 73.33 ± 1.93$^e$ |
| | $2.88 \times 10^6$ | 30 | 27.00 ± 0.58$^f$ | 89.99 ± 1.93$^f$ |
| | $5.76 \times 10^7$ | 30 | 29.67 ± 0.33$^g$ | 98.89 ± 1.11$^g$ |
| Sudhmahadev | Control | 30 | 0.00$^a$ | 0.00$^a$ |
| | $1.8 \times 10^2$ | 30 | 7.00 ± 0.58$^b$ | 23.33 ± 1.93$^b$ |
| | $3.6 \times 10^3$ | 30 | 11.67 ± 0.33$^c$ | 38.86 ± 1.11$^c$ |
| | $7.2 \times 10^4$ | 30 | 19.00 ± 0.58$^d$ | 63.33 ± 1.92$^d$ |
| | $1.44 \times 10^5$ | 30 | 26.33 ± 0.67$^e$ | 87.78 ± 2.22$^e$ |
| | $2.88 \times 10^6$ | 30 | 28.67 ± 0.33$^f$ | 95.55 ± 1.11$^f$ |
| | $5.76 \times 10^7$ | 30 | 30.00 ± 0.00$^f$ | 100.00 ± 0.00$^f$ |

Means ± SE followed by different letters within the same column within the same isolate are significant at $p<0.05$; SE = Standard error of mean; Control = Distilled water

**Table 2:** Median lethal dose ($LD_{50}$) of four different isolates of PibrGV against *P. brassicae* larvae

| Host population | $LD_{50}$ (OBs/larva) | 95% confidence interval | $\chi^2$/df | Regression equation y = bx + a | $R^2$ |
|---|---|---|---|---|---|
| Himachal Pradesh | $1.22 \times 10^4$ | 5938.32 - 23052.34 | 13.43/19 | Y = 1.57x – 1.35 | 0.95 |
| Poonch | $1.03 \times 10^4$ | 5096.23 – 19158.08 | 15.16/19 | Y = 1.59x – 1.30 | 0.96 |
| Chatha | $1.19 \times 10^4$ | 6387.88 – 20937.88 | 9.78/19 | Y = 1.65x – 1.96 | 0.98 |
| Sudhmahadev | $6.31 \times 10^3$ | 3560.82– 10575.99 | 16.95/19 | Y = 1.73x – 1.59 | 0.95 |

Regression equation; Y = mortality percentage and X = dose

$r^2$ is a correlation determination between the dose and mortality value

**Table 3:** Mortality of 3rd instar *P. brassicae* larvae with the four different geographical isolates of PibrGV

| Isolate | Time after treatment (days) | Larval death due to GV | Daily mortality (%) | Cumulative mortality (%) |
|---|---|---|---|---|
| Himachal Pradesh | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| | 4 | 0 | 0 | 0 |
| | 5 | 1 | 3 | 3 |
| | 6 | 3 | 10 | 13 |
| | 7 | 7 | 23 | 36 |
| | 8 | 9 | 30 | 66 |
| | 9 | 3 | 10 | 76 |
| | 10 | 1 | 3 | 79 |
| Poonch | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| | 4 | 1 | 3 | 3 |
| | 5 | 5 | 17 | 20 |
| | 6 | 7 | 23 | 43 |
| | 7 | 8 | 27 | 70 |
| | 8 | 2 | 7 | 77 |
| | 9 | 0 | 0 | 77 |
| | 10 | 1 | 3 | 80 |
| Chatha | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| | 4 | 0 | 0 | 0 |
| | 5 | 2 | 7 | 7 |
| | 6 | 5 | 17 | 24 |
| | 7 | 10 | 33 | 57 |
| | 8 | 5 | 17 | 74 |
| | 9 | 0 | 0 | 74 |
| | 10 | 1 | 3 | 77 |
| Sudhmahadev | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| | 4 | 2 | 7 | 7 |
| | 5 | 6 | 20 | 27 |
| | 6 | 10 | 33 | 60 |
| | 7 | 4 | 13 | 73 |
| | 8 | 6 | 20 | 83 |
| | 9 | 1 | 3 | 86 |
| | 10 | 0 | 0 | 86 |

**Table 4:** Median survival time ($ST_{50}$) of four different geographical isolates of PibrGV against 3rd instar *P. brassicae* larvae

| Isolate | $ST_{50}$ (days) | 95% confidence interval | $\chi^2$/df | Regression equation y = bx + a | $r^2$ |
|---|---|---|---|---|---|
| Himachal Pradesh | 7.43 | 7.39 – 7.47 | 14.36/13 | Y = 6.79x + 0.56 | 0.94 |
| Poonch | 6.63 | 6.45 – 6.82 | 14.64/13 | Y = 7.78x - 0.99 | 0.98 |
| Chatha | 6.80 | 6.63 – 6.97 | 14.51/13 | Y = 7.55x - 0.68 | 0.96 |
| Sudhmahadev | 5.90 | 4.70 – 7.09 | 14.11/13 | Y = 9.11x – 3.36 | 0.99 |

Regression equation; Y = mortality percentage and X = time

$r^2$ is a correlation determination between time and mortality value

## 4. Discussion

The selection of a new natural isolate with better bio pesticidal properties is an important aspect in biological control programmes. In this regard, the different native geographical isolates required to be tested against insect populations from the locality of the program. The biological activity is an important element in the evaluation of the potential of the virus as a biocontrol agent (Geetha and Rabindra, 2000) [18]. Consequently, bioassays were used to measure dose-response and time-response relationships, for assessing the biological activity of baculoviruses (Hughes & Shapiro, 1997; Jones, 2000) [19, 20]. Through bioassays the infectivity (dose-response relationship) and virulence (time response relationship) of virus isolates against host larvae was determined. It has been shown by this study that the geographical isolate tested influence the effectiveness of the virus as a biopesticide. The four different GV isolates of *P. brassicae* isolated during the present study from different geographical regions, exhibited differential degree of pathogenicity. The $LD_{50}$ values of four different GV isolates of *P. brassicae* against 3rd instar larvae of *P. brassicae* varied from $6.31x10^3$ to $1.22x10^4$ OBs/larvae with non significant differences between the isolates. However, significant differences were observed in the $ST_{50}$ estimates of four different isolates of PibrGV against 3rd instar larvae of *P. brassicae* which varied from 5.90 to 7.43 days. When compared to other isolates Sudhmahadev isolate is the most infective isolate in terms of both $LD_{50}$ and $ST_{50}$. The different

geographic isolates of baculoviruses isolated from the same species at different sites revealed vast differences in their pathogenicity and virulence against the natural populations of the pest all over the world (Battu and Arora, 1996; Cory and Myers, 2003; Erlandson *et al*., 2007; Mehrvar *et al.*, 2008; Gupta *et al.*, 2016) [21, 22, 23, 24, 25]. For instance, Rezapanah, 2015 [26] who reported that the $LC_{50}$ values of six isolates of *Cydia pomonella* granulovirus (CpGV) against neonate larvae of *C. pomonella* varied from 2277 to 4454 OBs/ml. Similarly Crook (1986) [27] reported differences in the $LD_{50}$ values of 13 different isolates of *Artogeia* (= *Pieris*) *rapae* granulosis virus (ArGV1 to ArGV13) varying from $10^{2-3}$ to $10^{2-6}$ capsules. However, only two isolates (ArGV1 and ArGV2) had significant infectivity for third instar *P. brassicae*. Similarly, Mehrvar *et al*. (2008) [28] and Gupta *et al*. (2010) [29] reported variation in the dose and time response mortality among different isolates of *Helicoverpa armigera* nucleopolyhedrovirus. Several of the AcMNPV variants also possess different levels of infectivity for particular host species (Harrison and Bonning, 2003) [30]. Such differences are not unusual among virus isolates collected from the same species from different geographical locations (Cory *et al.*, 2005) [31]. Furthermore, the native isolates tend to be more pathogenic to local populations in comparison with exotic isolates (Cabodevilla *et al.*, 2011, Barrera *et al.*, 2011) [32, 33] owing to an adaptive advantage to retain high infectivity toward the local population via the process of host–pathogen coevolution (Barrera *et al.*, 2011) [34]. In other instances, baculovirus isolates with varying genotypes particularly the presence or absence of key genes (Crook, 1981) [35] can display differences in viral virulence (Eberle *et al*., 2008; Jehle *et al*., 2008 and Eberle *et al.*, 2009) [36, 37, 38].Thus, it is suggested that the virus isolate collected from Sudhmahadev might be genetically heterogeneous with other wild-types from other geographic regions. Further studies on their polypeptide profiling and gene sequencing may reveal deeper insights on this attribute.

## 5. References


1. Tolman JH, McLeod DGR, Harris CR. Cost of crop losses in processing tomato and cabbage in south-western Ontario due to insects, weeds and/or diseases. Canadian Journal of Plant Science. 2004; 84:915-921.
2. Hasan F, Ansari MS. Effect of different crops on the biological parameters of *Pieris brassicae* (L.) (Lepidoptera: Pieridae) under laboratory conditions. Journal of Crop Science and Biotechnology. 2010; 13(3):195-202.
3. Ullah MI, Arshad M, Ali S, Iftikhar Y, Mahmood SU, Arshad M. Effect of thiamethoxam and some botanical extracts on *Cotesia glomerata* L. (Hymenoptera: Braconidae): an endoparasitoid of *Pieris brassicae* L. (Lepidoptera: Pieridae). Egyptian Journal of Biological Pest Control. 2016; 26(3):545-549.
4. Ali A, Rizvi PQ. Developmental response of cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae) on different crops under laboratory and field condition. Asian Journal of Plant Sciences. 2007; 6(8):1241-1245.
5. Hasan F, Ansari MS. Effect of different crops on the biological parameters of *Pieris brassicae* (L.) (Lepidoptera: Pieridae) under laboratory conditions. Journal of Crop Science and Biotechnology. 2010; 13(3):195-202.
6. Younas M, Naeem M, Rquib A, Masud, S. Population dynamics of *Pieris brassicae* on five cauliflowers at Peshawar. Asian Journal of Plant Science. 2004; 3:391-393.
7. Bhandari K, Sood P, Mehta PK, Choudhary A. Effect of granulosis virus infection on food consumption and utilization by *Pieris brassicae* (linnaeus). Journal of biological control. 2010; 24(1):65-69.
8. Tabashnik BE, Van-Rensburg JBJ, Carrière Y. Field-evolved insect resistance to BT crops: definition, theory and data. Journal of Economic Entomology. 2009; 102(6):2011-2025.
9. Mahmood I, Imadi SR, Shazadi K, Gul A, Hakeem KR. Effects of pesticides on environment. In: Hakeem, K., Akhtar, M. and Abdullah, S. (eds.). Plant, Soil and Microbes, Springer, Cham, 2016, 253-269.
10. Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP. The development, regulation and use of biopesticides for integrated pest management. Philosophical Transactions of the Royal Society B: Biological Sciences. 2011; 366(1573):1987-1998.
11. Ndakidemi B, Mtei K, Ndakidemi P. Impacts of Synthetic and Botanical Pesticides on Beneficial Insects. Agricultural Sciences. 2016; 7:364-372.
12. Hikal WM, Baeshen RS, Said-Al HA. Botanical insecticide as simple extractives for pest control. Cogent Biology. 2017; 3(1). https://doi.org/ 10.1080/23312025. 2017.1404274.
13. Roberts DW, Fuxa JR, Gaugler R, Goettel M, Jaques R, Maddox J. Use of pathogens in insect control. In: Pimentel, D. (eds.). CRC Handbook of Pest Management in Agriculture, CRC Press, Boca Raton, Florida, 1991, 243-278.
14. Ravishankar BS, Venkatesha MG. Effectiveness of SlNPV of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) on different host plants. Journal of Biopesticides. 2010; 3:168-171.
15. Cory JS, Green BM, Paul RK, Hunter-fujita F. Genotypic and phenotypic diversity of a baculovirus population within an individual insect host. Journal of Invertebrate Pathology. 2005; 89:101-111.
16. Moore SD. The development and evaluation of *Cryptophlebia leucotreta* granulovirus (CrleGV) as a biological control agent for the management of false codling moth, *Cryptophlebia leucotreta*, on citrus. Ph.D. thesis, Rhodes University, Grahamstown, South Africa, 2002.
17. Gupta RK, Gani M, Jasrotia P, Srivastava K. Development of the predator *Eocanthecona furcellata* on different proportions of nucleopolyhedrovirus infected *Spodoptera litura* larvae and potential for predator dissemination of virus in the field. BioControl. 2013; 58(4):543e552.
18. Geetha N, Rabindra RJ. Genetic variability and comparative virulence of some geographic isolates of nuclear polyhedrosis virus of *Helicoverpa armigera* Hub. In: Ignachimuthu, S., Sen, A. and Janarthanan, S. (eds.). Biotechnological Applications for Integrated Pest Management, Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, 2000, 65-80.
19. Hughes E, Shapiro M. Viruses. In: Lacey, L. (eds.). Manual of techniques in insect pathology, Academic Press, London, 1997, 40-48.
20. Jones KA. Bioassay of entomopathogenic viruses. In: Navon, A. and Ascher, K. R. S. (eds.). Bioassays of

entomopathogenic microbes and nematodes, CABI publishing, U.K, 2000, 95-141.
21. Battu GS, Arora R. Genetic diversity of baculoviruses-implications in insect pest management. In: Ananthakrishnan, T. N. (eds.). Biotechnological perspectives in chemical ecology of insects, Oxford and IBH publication Company, New Delhi, 1996, 170-200.
22. Cory JS, Myers JH. The ecology and evolution of insect baculoviruses. *Annual* Review of Ecology, Evolution and Systematics. 2003; 34(1):239-272.
23. Erlandson M, Newhouse S, Moore K, Janmaat A, Myers J, Theilmann D. Characterization of baculovirus isolates from *Trichoplusia ni* populations from vegetable greenhouses. Biological Control. 2007; 41(2):256-263.
24. Mehrvar A, Rabindra RJ, Veenakumari K, Narabenchi GB. Molecular and biological characteristics of some geographic isolates of nucleopolyhedrovirus of Helicoverpa armigera (Lep.: Noctuidae). Journal of Entomological Society of Iran. 2008; 28(1):39-60.
25. Gupta RK, Gani M, Kaul V, Bhagat RM, Bali K, Samnotra RK. Field evaluation of *Lymantria obfuscata* multiple nucleopolyhedrovirus for the management of Indian gypsy moth in Jammu & Kashmir, India. Crop Protection. 2016; 80:149-158.
26. Rezapanah M. Screening *Cydia pomonella* granulovirus (CpGV) isolates via comparative bioassays. Iranian Journal of Virology. 2015; 9(3):1-5.
27. Crook NE. Restriction analysis of granulosis virus isolated from *Artogeia rapae* and *Pieris brassicae*. Journal of General Virology. 1986; 67:781-787.
28. Mehrvar A, Rabindra RJ, Veenakumari K, Narabenchi GB. Molecular and biological characteristics of some geographic isolates of nucleopolyhedrovirus of Helicoverpa armigera (Lep.: Noctuidae). Journal of Entomological Society of Iran. 2008; 28(1):39-60.
29. Gupta RK, Amin M, Bali K, Monobrullah M, Jasrotia P. Vertical transmission of sublethal granulovirus infection in the tobacco caterpillar *Spodoptera litura*. Phytoparasitica. 2010; 38(3):209-216.
30. Harrison RL, Bonning BC. Comparative analysis of the genomes of *Rachiplusia ou* and *Autographa californica* multiple nucleopolyhedroviruses. Journal of General Virology. 2003, 84(7):1827-1842.
31. Cory JS, Green BM, Paul RK, Hunter-fujita F. Genotypic and phenotypic diversity of a baculovirus population within an individual insect host. Journal of Invertebrate Pathology. 2005; 89:101-111.
32. Cabodevilla O, Ibanez I, Simon O, Murillo R, Caballero P, Williams T. Occlusion body pathogenicity, virulence and productivity traits vary with transmission strategy in a nucleopolyhedrovirus. Biological Control. 2011; 56(2):184-192.
33. Barrera G, Simon O, Villamizar L, Williams T, Caballero P. *Spodoptera frugiperda* multiple nucleopolyhedrovirus as a potential biological insecticide: Genetic and phenotypic comparison of field isolates from Colombia. Biological Control. 2011; 58(2):113-120.
34. Barrera G, Simon O, Villamizar L, Williams T, Caballero P. *Spodoptera frugiperda* multiple nucleopolyhedrovirus as a potential biological insecticide: Genetic and phenotypic comparison of field isolates from Colombia. Biological Control. 2011; 58(2):113-120.
35. Crook NE. A comparison of the granulosis viruses from *Pieris brassicae* and *Pieris rapae*. Virology, 115:173-181.
36. Eberle KE, Asser-Kaiser S, Sayed SM, Nguyen HT, Jehle JA. Overcoming the resistance of codling moth against conventional *Cydia pomonella* granulovirus (CpGV-M) by a new isolate CpGV-112. Journal of Invertebrate Pathology. 2008, 98(3):293-298.
37. Jehle JA, Eberle KE, Asser-Kaiser S. CpGV resistance of codling moth: new isolates with improved efficacy. Proceeding of *23rd* International Congress of Entomology. Durban, South Africa, 2008.
38. Eberle KE, Sayed S, Rezapanah M, Shojai-estabragh S, Jehle JA. Diversity and evolution of the *Cydia pomonella* granulovirus. Journal of General Virology. 2009; 90:662-671.