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# Synergistic effect of naturally occurring Granulosis virus isolates (*PbGV*) with phagostimulants against the cabbage butterfly, *Pieris brassicae* (L.) for its eco-friendly management

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## Abstract

**Background:** To manage the cabbage butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae), it is not wise to use insecticides on leafy vegetables which are eaten mostly fresh. During the past decades, the efforts to manage the pest, through chemical insecticides have raised serious health. Investigations were carried out to isolate naturally occurring GVs (*PbGV*) as a potent biopesticide against *P. brassicae* and to explore their efficacy with the application of phagostimulants.

**Results:** Among the four naturally occurring isolates obtained from Northwestern Himalayas, Sudhmahadev isolate was found to be the most promising based on virulence and speed of kill against all the instars tested in the laboratory, showing the natural incidence of *PbGV* infection in field conditions. In concentration and time–response bioassay, all the isolates of *P. brassicae* Granulosis virus were found high virulent against second instar larvae of cabbage butterfly. Therefore, for enhanced efficacy of *PbGV*, its combined application with phagostimulants (*Lepidium sativum* + Teepol + jaggery) or sticker (Teepol + jaggery), applied in field trials, resulted into greater mortality of larval instars than the single one. Overall, the results indicated that the introduction of a more isolates *PbGV* strain into populations of *P. brassicae* could be of vital importance for eco-friendly suppression of this pest globally with the combination of phagostimulants. The application virus alone with the pre-standardized concentration of  $1 \times 10^{12}$  OBs/ha did not reduce the larval population density to the desirable extent in the greenhouse chamber and therefore was not included in field experiments. Overall, the most promising treatments in reducing the larval population of the pest were *PbGV* + Teepol + *B. thuringiensis* (93.49 and 91.39%) and *PbGV* + Teepol + *L. sativum* (88.79 and 86.97%) over control in both greenhouse and field trials, respectively.

**Conclusions:** In this study, the native isolates of *PbGV* from different target locations to test their efficacy against different instars of *P. brassicae* were explored. Using native *PbGV* isolates with phagostimulant combinations played an important role for regulating the pest effectively. These phagostimulants not only protected the OBs from degradation in the presence of sunlight but also increased the speed of killing. The biocontrol potential of *PbGV* in both laboratory and field conditions indicated that baculoviruses are sustainable alternative to chemical insecticides.

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## Background

The cabbage butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae) is a cosmopolitan species with Palearctic distribution and causes extensive damage at all the growing stages of Cole crops due to their voracious feeding habit (Ohno et al. 2020).

During the past decades, the efforts to manage this insect pest were dominated by chemical insecticides. However, increasing failures of chemical pesticides and the health hazard problems posed by their indiscriminate use in the field reaffirmed the need to focus attention on alternative and eco-friendly pest management tactics. Entomopathogenic viruses (EPVs) appear to be an ideal candidate as biological control agent against insect pests (Lacey 2012). Moreover, baculoviruses are naturally involved in the regulation of insect populations and individual isolates often have a very narrow host range and, therefore, no side effects or harmful effects on mammals, birds, other animals, aquatic systems and beneficial insects. Also, these microbes may establish themselves in the pest population and exert long-term protection. Therefore, their use is being encouraged by international concerns for a reduction in pesticides within the environment, also as by the event of resistance to chemical insecticides by target pests. However, their usage is hampered due to the inactivation of OBs in the presence of sunlight and the slow speed of action to kill the insect. Nearly 50% of the viral activity of HaNPV was reduced when exposed for 6 h. However, by 36 h all the isolates had lost their activity by about 70% (Mehrvar et al. 2007). Similarly, when a highly purified preparation of *P. brassicae* virus was exposed to direct sunlight, total inactivation of the virus occurred between 12 and 19 h (David et al. 1968).

These viruses have greater significance for bioprospecting as most of these baculoviruses are registered and used mainly in many countries. Despite the successes of the past, there is a continuous need to discover and develop new strains/races of baculovirus due to vast differences in their pathogenicity and virulence (Gupta et al. 2013; Cory and Myers 2003). In this regard, the different native geographical isolates must be tested against insect populations from the locality of the program to meet the future needs of food and fiber production and concurrent reduction in the use of chemical pesticides. Since native isolates are always preferred for sustainability, adaptability, and efficacy in a given agro-ecosystem owing to an adaptive advantage to retain high infectivity toward the local population via host–pathogen co-evolution process (Barrera et al. 2011).

Therefore, the present study aimed to isolate and identify the *P. brassicae* Granulosis virus from different geographical regions of Jammu and Kashmir, India, and to assess their potential under field conditions.

## Methods

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

The nucleus culture of *P. brassicae* was maintained in the Biocontrol laboratory, Division of Entomology at SKUAST-J, FOA-Chatha, India, by collecting the adults from the field. The adults were kept in Nylon mesh cages (10 × 9 × 7 ft) provided with potted cabbage, cauliflower, Knol khol, and some flowering plants of *Nasturtium* sp. Leaves with eggs were kept in sterilized glass jars (50 × 30 cm diameter) covered with muslin cloth for further rearing. Uneaten food and fecal matter were removed daily to maintain the hygiene conditions. The colony was maintained at a temperature of 26 ± 20 °C and 70 ± 10% RH and L/D (16:8) photoperiod.

### Virus-isolation, purification, and standardization

Intensive exploratory surveys were conducted from different zones of J & K, viz. Chatha, Poonch, Sudhmahadev, and Kashmir, India, during 2014–2020 for the presence of native GV isolates of *Pieris brassicae*. During the survey, live and dead specimens were collected individually in sterile glass vials and were kept at –20 °C till further use. It is pertinent to mention here that the Himachal Pradesh (H.P) isolate of PbGV was procured from Dr. Pankaj Sood, Principal Scientist, KVK Mandi, Himachal Pradesh, India. The extraction, purification, and standardization of OBs were done as per the methods given by Moore (2002) and Hussain et al. (2020). Preliminary baculovirus identification was done by a 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 (Gupta et al. 2013). Enumeration of OBs in the viral suspension was done with the help of the Petroff-Hausser counting chamber (Lacey 2012). The virus was also identified through electron microscopy as per the methods of Sood and Prabhakar (2012) and Hussain et al. (2019).

### Concentration–response and time–response bioassays: selection of a potent isolate

For selection, the virus isolates were bio-assayed on 12-h pre-starved, 30 freshly molted first-, second-, and third-instar host larvae via leaf disc method. Six different

virus concentrations, viz.  $8 \times 10^2$ ,  $3.6 \times 10^3$ ,  $7.2 \times 10^4$ ,  $1.44 \times 10^5$ ,  $2.88 \times 10^6$  and  $5.76 \times 10^7$  OBs/larvae, were used in the study. Serial dilutions with distilled water were performed to achieve the desired concentrations. For time–response, the second, third, and fourth larval instars were inoculated with a concentration of  $1.44 \times 10^5$  OBs/larva. Ten  $\mu$ l aliquots of each viral concentration from each viral isolate were spread evenly onto each leaf disc with the help of a brush. Control larvae were fed a leaf disc inoculated with water. Larvae ate the whole leaf disc, were transferred to fresh uncontaminated leaves and reared at  $28 \pm 2$  °C, 60% RH, and 16L/8D photoperiod. Bioassay of each viral concentration and a corresponding experimental control group was replicated 3 times in case of concentration–response and 7 times in case of the time–response. Larvae were observed daily for virus-induced mortality until pupation.

### Virus propagation

For in vivo production of PbGV, laboratory-reared third instar healthy larvae were inoculated by  $7.2 \times 10^4$  OBs/larva (optimum inoculum concentration). To avoid contamination, only moribund larvae were harvested into plastic bags and held at  $-20$  °C till further use. Frozen larvae were thawed for 24 h at 4 °C and then blended with water at high speed for 10 s to release the OB's. The crude concentrate was filtered through a three-layered muslin cloth to remove debris and stored at  $-20$  °C for further studies.

### Field trails

#### Greenhouse pot experiment

A laboratory pot experiment was conducted to evaluate the efficacy of PbGV against *P. brassicae* on cabbage by preparing different aqueous formulations of PbGV with different substances. The plants were grown in pots under lab conditions encaged in nylon mesh for aeration. The virus @  $1 \times 10^{12}$  OBs/ml was sprayed on the plants uniformly with a hand sprayer. All the additives were added to GV before spraying. Third instar larvae (50) released on plants immediately after drying of spray fluid on plants. The larval mortality was observed after 1, 2, and 3 weeks of application. Each experiment was replicated 5 times with 5 plants (6 weeks old) per replicate, and mean larval mortality at different intervals was recorded.

The different formulations used in the experiment are as follows:

- PbGV @  $1 \times 10^{12}$  OBs/ha + 0.1% Teepol + 2.5% *Lepidium sativum* seeds.
- PbGV @  $1 \times 10^{12}$  OBs/ha + 0.1% Teepol + 0.5% jaggery.

- PbGV @  $1 \times 10^{12}$  OBs/ha + 0.1% Teepol + Bt @ 250 g a.i/ha.
- PbGV alone.
- Control—water spray.

### Field efficacy

To check whether the performance of an isolate in the laboratory is entirely reflective of its performance, 2 trials (I and II) were conducted in the field to generate the efficacy data of the most virulent strain.

#### Trial 1

A small-scale field trial was conducted at the Experimental Farm of the Division of Entomology, FOA-Chatha. The trial was conducted on cabbage in RBD comprising 5 treatments, replicated four times:-

- PbGV @  $1 \times 10^{12}$  OBs/ha + 0.1% Teepol + 2.5% *Lepidium sativum* seeds.
- PbGV @  $1 \times 10^{12}$  OBs/ha + 0.1% Teepol + 0.5% jaggery.
- PbGV @  $1 \times 10^{12}$  OBs/ha + 0.1% Teepol + Bt @ 250 g a.i/ha.
- Control.
- Spinosad @ 3 ml/lit.

#### Trial 2

Another trial was conducted at Farmer's field at Marh area of Jammu, Jammu, and Kashmir on Knol khol, which is another major host of *P. brassicae*. Five treatments (as applied in Trail 1) were assigned to 4 replicates in randomized block design. A crude preparation of the virus from infected larval cadavers was applied at dusk with the motorized knapsack sprayer, while the control plots were sprayed with water in both trails. Five plants of cabbage were randomly selected from each plot and observations were recorded after first, second, and third weeks of spray.

### Data analysis

Data were subjected to one-way ANOVA using statistical software SPSS on the personal computer. The data on mortality were analyzed through one-way ANOVA and further subjected to post hoc tests for comparison of means. The mortality among the isolates was compared through the nonparametric Kruskal–Wallis H test. Larval mortality was analyzed using Probit analysis to estimate the median lethal concentration ( $LC_{50}$ ) values that cause 50% mortality in test population. Median survival time ( $ST_{50}$ ) was calculated using a log-rank test under Kaplan–Meier analyses.

### Results

A microscopic examination through Giemsa staining of the tissue smears and hemolymph of the infected larvae collected from all the locations revealed the presence of OBs. The amplification of total DNA with *gran* gene-specific primers resulted in successful amplification of all the isolates of PbGV with a readable sequence of 500 bp. As such, the Himachal Pradesh, Poonch, Chatha, and Sudhmahadev isolates were named as PbGV-IND HP, PbGV-IND PH, PbGV-IND CH, and PbGV-IND SD, respectively, by adopting the most commonly used methods (Erlandson 2009). The pathogenicity of all the isolates was confirmed through Koch's postulates, and symptoms typical to GV were observed. The natural incidence of GV in *P. brassicae* larvae varied from 0.95 to 6.88%. The highest incidence and lowest incidence of 6.88 and 0.95% were reported on cabbage from Sudhmahadev and Chatha, respectively. However, no natural incidence of GV was observed in the Kashmir division of Jammu and Kashmir.

In concentration–response bioassay, all the strains of *P. brassicae* Granulosis virus were found to be highly virulent against second (PbGV-IND HP,  $F = 348.2$ ,  $df = 6, 14$ ,  $p = 0.00$ ; PbGV-IND PH,  $F = 352.6$ ,  $df = 6, 14$ ,  $p = 0.00$ ; PbGV-IND CH,  $F = 433.2$ ,  $df = 6, 14$ ,  $p = 0.00$ ; PbGV-IND SD,  $F = 680.25$ ,  $df = 6, 14$ ,  $p = 0.00$ ), third (PbGV-IND HP,  $F = 478.2$ ,  $df = 6, 14$ ,  $p = 0.00$ ; PbGV-IND PH,  $F = 532.6$ ,  $df = 6, 14$ ,  $p = 0.00$ ; PbGV-IND CH,  $F = 393.2$ ,  $df = 6, 14$ ,  $p = 0.00$ ; PbGV-IND SD,  $F = 770.25$ ,  $df = 6, 14$ ,  $p = 0.00$ ) and fourth instar (PbGV-IND HP,  $F = 581.2$ ,  $df = 6, 14$ ,  $p = 0.00$ ; PbGV-IND PH,  $F = 536.2$ ,  $df = 6, 14$ ,  $p = 0.00$ ; PbGV-IND CH,  $F = 375.82$ ,  $df = 6, 14$ ,  $p = 0.00$ ; PbGV-IND SD,  $F = 680.25$ ,  $df = 6, 14$ ,  $p = 0.00$ ) *P. brassicae* larvae. Although nonsignificant differences were observed in median lethal concentration ( $LC_{50}$ ) of all the isolates ( $F = 263.30$ ;  $df = 3, 8$ ;  $p = 0.16$ ), Sudhmahadev isolate exhibited a 57.79–70.49%, 38.74–48.28% and 77.29–80.36% reduction in the  $LC_{50}$  in second, third and fourth larval instars of *P. brassicae*, respectively, as compared to other isolates (Table 1).

In time–response bioassays of second instar larvae, per cent mortality varied from 83 to 97%, for PbGV-IND HP and PbGV-IND SD, respectively; differences being significant in treatment over control (PbGV-IND HP,  $F = 8.60$ ,  $df = 1, 12$ ,  $p = 0.00$ ; PbGV-IND CH,  $F = 11.9$ ,  $df = 1, 12$ ,  $p = 0.00$ ; PbGV-IND PH,  $F = 22.6$ ,  $df = 1, 12$ ,  $p = 0.00$  and PbGV-IND SD,  $F = 15.4$ ,  $df = 1, 12$ ,  $p = 0.00$ ). Similarly, in third instar larvae, 77–86% between PbGV-IND HP –PbGV-IND SD, variation in the larval mortality was observed which further decreased to 63–80% for PbGV-IND HP and PbGV-IND SD, respectively; in fourth instar larvae of *P. brassicae*. Significant differences were also observed in treatments and control in third (PbGV-IND HP,  $F = 19.3$ ,  $df = 1, 12$ ,  $p = 0.00$ ; PbGV-IND CH,  $F = 29.4$ ,  $df = 1, 12$ ,  $p = 0.00$ ; PbGV-IND PH,  $F = 5.93$ ,  $df = 1, 12$ ,  $p = 0.00$  and PbGV-IND SD,  $F = 14.9$ ,  $df = 1, 12$ ,  $p = 0.00$ ) and fourth instar larvae (PbGV-IND HP,  $F = 13.9$ ,  $df = 1, 12$ ,  $p = 0.00$ ; PbGV-IND CH,  $F = 33.3$ ,  $df = 1, 12$ ,  $p = 0.00$ ; PbGV-IND PH,  $F = 10.06$ ,  $df = 1, 12$ ,  $p = 0.00$  and PbGV-IND SD,  $F = 17.2$ ,  $df = 1, 12$ ,  $p = 0.00$ ). The speed of kill in *P. brassicae* larvae was the highest with the Sudhmahadev isolate followed by Poonch, Chatha and Himachal Pradesh in all the instars tested (Table 2).

The analysis related to the median survival time ( $ST_{50}$ ) revealed significant differences in  $ST_{50}$  values of H.P., Poonch, Chatha and Sudhmahadev isolates against second ( $\chi^2 = 19.30$ ;  $df = 3$ ;  $p = 0.00$ ), third ( $\chi^2 = 22.220$ ;  $df = 3$ ;  $p = 0.00$ ) and fourth instars ( $\chi^2 = 25.06$ ;  $df = 3$ ;

**Table 2** Mortality of *P. brassicae* larvae on 12th-day post-treatment with the four different geographical isolates of PbGV in time–response bioassays

Isolates	Mortality (%)		
	Second instar	Third instar	Fourth instar
PbGV-IND HP	83	79	63
PbGV-IND PH	87	80	66
PbGV-IND CH	86	77	70
PbGV-IND SD	97	86	80

**Table 1** Median lethal concentration ( $LC_{50}$ ) of different isolates of PbGV against *P. brassicae*

Isolate	Second instar		Third instar		Fourth instar	
	$LC_{50}$ (OBs/larva)	$r^2$	$LC_{50}$ (OBs/larva)	$r^2$	$LC_{50}$ (OBs/larva)	$r^2$
PbGV-IND HP	$6.61 \times 10^3$ (2497.727–14,892.99)	0.97	$1.22 \times 10^4$ (5938.32–23,052.34)	0.95	$5.55 \times 10^4$ (21,929.00–74,815.587)	0.92
PbGV-IND PH	$4.62 \times 10^3$ (2165.66–8799.336)	0.96	$1.03 \times 10^4$ (5096.23–19,158.08)	0.96	$4.80 \times 10^4$ (21,034.78–67,058.06)	0.94
PbGV-IND CH	$4.86 \times 10^3$ (2314.049–9156.770)	0.99	$1.19 \times 10^4$ (6387.88–20,937.88)	0.98	$5.10 \times 10^4$ (13,658.26–65,891.21)	0.94
PbGV-IND SD	$1.95 \times 10^3$ (917.61–3644.59)	0.98	$6.31 \times 10^3$ (3560.82–10,575.99)	0.95	$1.09 \times 10^4$ (6210.57–18,527.88)	0.94

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

Figures in the parenthesis represent 95% confidence interval

$p=0.00$ ) larvae of *P. brassicae*. It was found that the Sudhmahadev isolate exhibited a 31.64–44.47% reduction in  $ST_{50}$  values against second instar *P. brassicae* larvae as compared to Poonch, Chatha and Himachal Pradesh isolates. Similarly, the  $ST_{50}$  values of H.P, Poonch, Chatha, and Sudhmahadev isolates against third instar *P. brassicae* larvae were 7.43, 6.63, 6.80, and 7.73 days, respectively, with an 11.01- 20.59% reduction. All the tested isolates took a long time to kill fourth instar *P. brassicae* larvae as compared to second and third larval instars. The  $ST_{50}$  values of Sudhmahadev isolate were found to be the lowest (7.73 days) followed by Poonch (9.67 days), Chatha (10.76 days), and H.P (11.83 days). In all the tested instars, the order of  $ST_{50}$  values was: Sudhmahadev isolates < Poonch isolate < Chatha isolate < Himachal Pradesh isolates. Therefore, the Sudhmahadev isolate of PbGV was

selected for further study because of high virulence and low  $ST_{50}$  against all the tested instars (Table 3).

The results obtained from pot experiments under greenhouse conditions revealed that the treatment of *P. brassicae* Granulosis virus ( $T_1$ ) when applied alone caused 71.6% mortality; however, when combined with *Bacillus thuringiensis* and *Lepidium sativum* mortality rate can further be enhanced up to 97.6 and 94.4%, respectively. All the treatments were significantly different from control after first ( $F=53.63$ ,  $df=4$ , 20,  $p=0.00$ ), second ( $F=107.70$ ,  $df=4$ , 20,  $p=0.00$ ) and third ( $F=433.51$ ,  $df=4$ , 20,  $p=0.00$ ) week of spray PbGV + Teepol + Bt ( $T_4$ ) and PbGV + Teepol + Lepidium ( $T_2$ ) being the superior among all the treatments (Table 4).

The results obtained from the experimental trials of SKUAST-J revealed nonsignificant differences in the pre-treatment population of *P. brassicae* in both trials. However, all the treatments were proved significantly superior

**Table 3** Median survival time ( $ST_{50}$ ) of four different geographical isolates of PbGV against *P. brassicae* larvae

Isolate	Second instar		Third instar		Fourth instar	
	$ST_{50}$ (days)	$r^2$	$ST_{50}$ (days)	$r^2$	$ST_{50}$ (days)	$r^2$
PbGV-IND HP	5.33 (4.74–5.93)	0.98	7.43 (7.39–7.47)	0.94	11.83 (11.66–12.00)	0.92
PbGV-IND PH	4.33 (3.99–4.67)	0.99	6.63 (6.45–6.82)	0.98	9.67 (9.62–9.71)	0.98
PbGV-IND CH	4.90 (4.30–5.49)	0.99	6.80 (6.63–6.97)	0.96	10.76 (10.68–10.85)	0.96
PbGV-IND SD	2.96 (2.62–3.30)	0.99	5.90 (4.70–7.09)	0.99	7.73 (7.56–7.90)	0.99

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

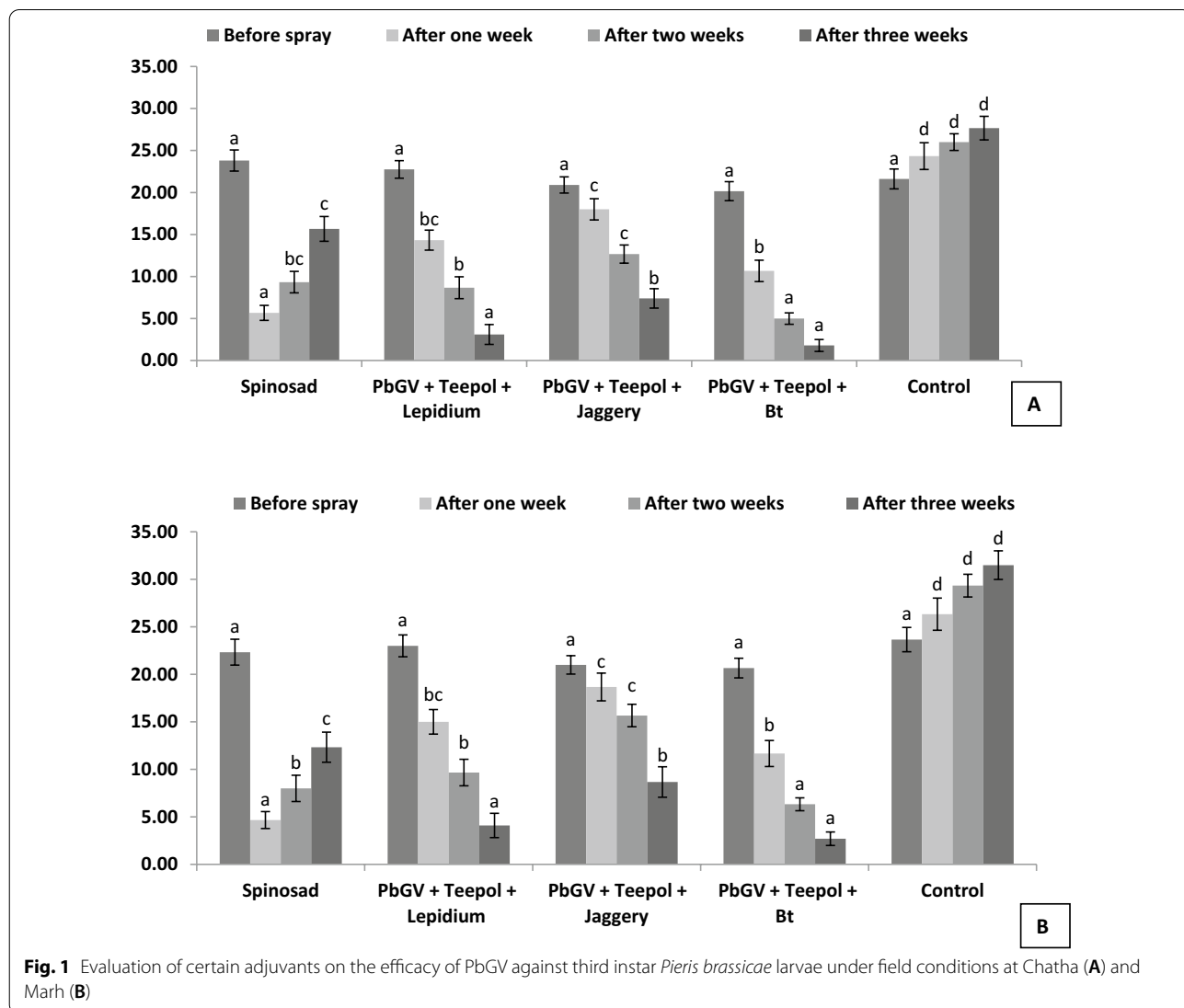
Figures in the parenthesis represent a 95% confidence interval

**Table 4** Evaluation of certain adjuvants on the efficacy of PbGV against third instar *Pieris brassicae* larvae under greenhouse conditions

S. no.	Treatment	Time after treatment (weeks)	Larval death due to GV	Cumulative mortality (%)
T1	PbGV alone	1	12.6	25.2
		2	26.8	53.6
		3	35.8	71.6
T2	PbGV + Teepol + Lepidium	1	28	56
		2	37.4	74.8
		3	47.2	94.4
T3	PbGV + Teepol + Jaggery	1	15.6	31.2
		2	29.2	58.4
		3	39.8	79.6
T4	PbGV + Teepol + Bt	1	30.8	61.6
		2	41.0	82.0
		3	48.8	97.6
T5	Control	1	0	0
		2	0	0
		3	0	0

over control after first ( $F=12.28$ ,  $df=4$ ,  $15$ ,  $p=0.00$ ), second ( $F=16.88$ ,  $df=4$ ,  $15$ ,  $p=0.00$ ) and third larval instars of *P. brassicae* ( $F=19.28$ ,  $df=4$ ,  $15$ ,  $p=0.00$ ) week of spray in trial I at Chatha. Following 7 days post-treatment application, Spinosad 20 ES (4.6 larvae/plant) and PbGV + *Bacillus thuringiensis* (10.67 larvae/plant) were found to be the most effective treatment in reducing the larval population. However, the efficiency of Spinosad decreased with time and a drastic increase in the population dynamics of *P. brassicae* was observed after the second and third weeks of spray. In contrast, 60.2–93.9% and 49.32–88.79% reduction in the larval population density in the PbGV + Teepol + *Bacillus thuringiensis* and PbGV + Teepol + Lepidium, respectively, were observed over control after first to third weeks of spray (Fig. 1A).

Similarly, in trail II at Marh significant differences were observed after first ( $F=15.81$ ,  $df=4$ ,  $15$ ,  $p=0.00$ ), second ( $F=21.69$ ,  $df=4$ ,  $15$ ,  $p=0.00$ ) and third ( $F=47.16$ ,  $df=4$ ,  $15$ ,  $p=0.00$ ) week of spray. A similar trend of decreased in the Spinosad efficacy was observed after 7 days post-spray. Similarly, PbGV + Teepol + *Bacillus thuringiensis* and PbGV + Teepol + Lepidium were found superior from all other treatments. The magnitude of reduction increased from 36.60 to 86.97% and 59.57 to 91.39%, respectively, over control after the first to third week of spray, respectively (Fig. 1B).



## Discussion

Interest in baculoviruses as potential biopesticides has certainly increased the screening of insect populations for new and potentially more efficacious isolates or strains. It is a well-known fact that the geographical isolate with different biological activities influences the effectiveness of the virus as a biopesticide. In this regard, 4 different GV isolates of *P. brassicae* were screened against the *P. brassicae* population of Jammu and Kashmir during the present study, which exhibited a differential degree of pathogenicity. Although non-significant differences were observed in median lethal concentrations ( $LC_{50}$ ), Sudhmahadev isolate exhibited a 38.74–48.28% reduction in the  $LC_{50}$ , as compared to other isolates. The  $ST_{50}$  estimates of 4 different isolates of PbGV against second, third, and fourth larval instars of *P. brassicae* varied (from 2.96 to 5.33), (5.90 to 7.43) and 7.73 to 11.83 days, respectively. Therefore, in all the tested isolates, the Sudhmahadev isolate of PbGV was selected for further study because of its high infectivity and low  $ST_{50}$  values. Marked differences within the virulence of baculoviruses isolated from different sites are reported by several researchers (Gupta et al. 2013). Mehrvar et al. (2007) reported variation in the concentration and time–response mortality among different isolates of *Helicoverpa armigera* (Hb.) nucleopolyhedrovirus. Such differences are not unusual among virus isolates collected from the same species and from different geographical locations (Cory et al. 2005). Furthermore, native isolates tend to be more pathogenic to local populations in comparison with exotic ones (Barrera et al. 2011) owing to an adaptive advantage to retain high infectivity toward the local population via the process of host–pathogen coevolution (Barrera et al. 2011). In other instances, baculovirus isolates with varying genotypes particularly the presence or absence of key genes (Crook 1981) can display differences in viral virulence (Eberle et al. 2009). In contrast, Ali et al. (2018) reported nonsignificant differences between 2 strains of *S. litura* (SpltnNPV-Pak-BNG and SpltnNPV-G1 strain) in terms of concentration–mortality response ( $LC_{50}$ ). However, time-to-death ( $LT_{50}$ ) was significantly shorter for SpltnNPV-Pak-BNG than for SpltnNPV-G1, thus suggesting that it may 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. In addition, it was also evident from the results that the median survival time ( $ST_{50}$ ) increased with the age of larvae in all the isolates tested, *i.e.*, susceptibility of the *P. brassicae* larvae is inversely proportional to the age of the insect. A rapid decrease in larval susceptibility was reported by Payne

(1982) against the biggest larval instars in the case of both NPVs and GVs. These age-dependent differences in susceptibility were also reported by many researchers (Sood and Prabhakar 2012). This may be due to the less developed defense system of insects at their young stage than at the oldest stage and midgut-based barriers to infection in older larvae such as lesser permeability of the midgut peritrophic membrane,

The speed of kill of baculoviruses is of great concern because the chemical insecticides produce quicker results. Another constrain in the usage of baculovirus is the inactivation of OBs in the presence of Sunlight. When a highly purified preparation of the known virus of *P. brassicae* was exposed to direct sunlight, only 7–33% of the initial virus deposits remained 1 day after application (Tatchell and Payne 1984). Many fields and laboratory studies have demonstrated that microbial insecticides are inactivated by solar UV radiation (Mehrvar et al. 2007). These results emphasize the importance of finding some means of screening or protecting the virus from UV radiation. Therefore, small-scale trials were conducted in the laboratory and field to evaluate the efficacy of PbGV in combination with certain botanical and microbial insecticides against *P. brassicae*. Several materials have been tested for the ability to protect deposits of viruses from ultraviolet light thereby extending the activity of the virus after application. In the present study, PbGV + *Bacillus thuringiensis* and PbGV + *Lepidium* + Jaggery were found to be the most effective treatments in reducing the larval population of *P. brassicae*. Enhanced efficacy of PbGV in combination with *B. thuringiensis* might be due to the latent infections, *i.e.*, due to stress imparted by *B. thuringiensis* on *P. brassicae* larvae resulting in inactivated infection of PbGV. These results are in line with Aruga (1963) who reported stress as the main cause of activation of ingested viruses which results in the outbreak of the disease in the host insect populations. The role of stressors in activating latent viral infection has also been reported earlier (Williams et al. 2017). For instance, *S. exempt* larvae infected by bacteria were markedly more susceptible to SpexNPV than bacteria-free larvae (Graham et al. 2012; Mahmoud et al. 2012). Enhanced effectiveness of NPV/GV with the addition of *B. thuringiensis* was reported by Sood et al., (2011). The synergetic effect of *Lepidium sativum* might be due to hydro colloidal–encapsulation of the baculovirus, where OBs were embedded within micro granules which protect the OBs from degradation in the presence of sunlight (Alfazairy et al. 2014). To improve the efficacy of baculovirus certain experiments were carried out by several researchers (Devi 2011).

Overall, the results indicated that the introduction of a more virulent GV strain into populations of *P.*

*brassicae* could be of vital importance for suppression of this insect in various cropping systems. But, even though the native viruses are permanent regulators of the population density of insect pests, there is also the high propensity of the development of resistance by the local population against the native isolate. Therefore, multiple isolates of baculoviruses from different geographic regions can be used concurrently in field applications to avoid resistance development against the native isolates in the future. However, the inactivation of OBs in the presence of sunlight is one of the major constraints which restrict their proper utilization. On that account, PbGV can be used in combination with certain microbial insecticides and phagostimulants, viz. *B. thuringiensis*, *Lepidium sativum*. They not only protect the OBs from degradation in the presence of sunlight but also increase the speed of killing. The bio-control potential of PbGV in both laboratory and field conditions indicated that baculoviruses can be used as a promising viable alternative to chemical insecticides. Also, the application of this virus in the primary outbreak sites (wild hosts) could be a more viable option as it may help to prevent population outbreaks and population dispersal to the croplands.

## Conclusions

In this study, native isolates of PbGV from different target locations were tested against different instars of cabbage butterfly *P. brassicae*. Suppression of this pest, using native PbGV isolates with phagostimulant combinations can play an important role for regulating the pest effectively. Therefore, the use of insect viruses is vital and can be used in combination with other bioagent (*B. thuringiensis*), along with various combinations of phagostimulants for the control of cabbage butterfly organically.

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## Authors' contributions

RK carried out all the experimental work for her doctoral research. RKG conceptualized the idea and carried out the research work with first author. BH wrote the paper, interpreted and analyzed the data and designed the experiment. RK and BH wrote the paper. SK conducted and analyzed the data statistically. All authors read and approve the final manuscript.

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All data generated during this study are included in the manuscript and doctoral work of first author.

## Declarations

### Ethics approval and consent to participate

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### Consent for publication

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### Competing interest

We the authors cited in the manuscript do declare that we do not have any competing interests.

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