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Research Article

Field evaluation of an indigenous granulosis virus isolate for *Pieris brassicae* (Linnaeus) management under north western Himalayan conditions

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ABSTRACT: A granulosis virus strain infecting *Pieris brassicae* (PbGV) was isolated from the dry temperate region of North-western Himalayas as a potential microbial agent for the management of *P. brassicae*. Host specificity and safety to parasitoid *Cotesia glomerata* (L.) revealed high host specificity and safety to the most prevalent natural enemy. Field evaluation of PbGV was carried out alone and in combination with another microbial pesticide formulation pathogenic to lepidopteran pests viz. *Bacillus thuringiensis* (DIPEL®) and neem seed kernel extract (NSKE) on cole crops (cabbage, cauliflower and broccoli) at two geographically isolated locations *viz*. Palampur (sub tropical) and Sangla (dry temperate) in Himachal Pradesh, India in order to design ecofriendly management modules in the hill state, which is fast transforming in to organic farming. The studies revealed that the PbGV isolate alone was quite effective against *P. brassicae* larvae at higher dose of 1.12 x 10^{12} OBs/ha per hectare whereas at lower dose of 5.58 x 10" LE/ha, PbGV was effective when combined with Bt or NSKE. Single foliar application of PbGV was sufficient to suppress the pest at higher altitude areas (dry temperate region) while two applications at 15 days interval were needed at low altitude areas. The findings are of great significance for exploitation of its full potential

KEY WORDS: PbGV, *Pieris brassicae*, granulosis virus, cabbage butterfly

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INTRODUCTION

Cabbage butterfly, *Pieris brassicae* (Linn.) (Lepidoptera: Pieridae), is a serious pest of cabbage, cauliflower and many crucifers distributed along temperate, tropical and subtropical regions of the world (Feltwell, 1978). In Indian subcontinent, it is distributed along Himalayan region including Pakistan, Nepal and throughout the plains except southern states of India (Lal and Ram, 2004; Younas *et al.,* 2004). In India, it passes winter in the plains and migrates to hilly regions during summer (Gupta, 1984). It breeds on the rapeseed- mustard in the month of September and remains active till April. It causes extensive damage at all the growing stages such as seedling, vegetative and flowering stage (Sachan and Gangwar, 1980; Younas *et al,* 2004; Ali and Rizvi, 2007). The young caterpillars feed gregariously on leaves, defoliating the plants (Younas *et al.,* 2004; Ali and Rizvi, 2007) making insecticidal application mandatory for the cultivation of the cole crops. Entomopathogenic viruses possess distinct advantages over other microbial bio-agents owing to their horizontal as well as vertical transmission. They cause high mortality thereby making them very effective at all the stages of the host even at sub lethal dose of the pathogen (Bruden *et al.,* 2002).

A granulosis virus strain infecting *P. brassicae* (PbGV) isolated from the dry temperate region (situated at an altitude of 2590m above msl, 31°25′56 N latitude and 78°15′ 4″ E longitudes) of North-western Himalaya is a potential microbial agent for the management of *P. brassicae* (Sood, 2004). Some local botanical extracts were also found effective in reducing incidence of *P. brassicae* (Mehta *et al.*, 2005) in cole crops. Under laboratory conditions at Palampur, the LC_{50} of PbGV against 2nd instar *P. brassicae* larvae was 1.85×10^4 OBs/ ml, whereas against 3rd and 4th instars the values were 1.86×10^6 and 5.89×10^7 OBs/ ml, respectively (Bhandari *et al.,* 2009). Laboratory evaluations of sub lethal infection to 3rd instar larvae also suggested that this virus is vertically transmitted to the next generation of the host (Sood *et al.,* 2010). In order to assess the field potential of this novel GV isolate for successful management of *P. rapae*, host specificity/ cross infectivity

to other major lepidopterous pests of cole crops, safety to the natural enemy and field evaluation of PbGV alone and in combination with commercial formulation of *Bacillus thuringiensis* (DIPEL®) and NSKE (earlier reported effective against the pest) were carried out at two geographically isolated locations in Himachal Pradesh.

MATERIALS AND METHODS

Rearing of host and test insects

The initial culture of *P. brassicae* was established from field-collected eggs. 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°C and 75–80% R.H.). Newly hatched larvae were transferred to fresh cabbage leaves, surface-sterilized with aqueous solution of sodium hypochlorite (0.05%) followed by several washings with SDW. The cabbage leaves were kept in an ethanol-washed and UV-sterilized cage (15×15×15 cm^3). The first three larval instars were reared in the small cages (15×15×15 cm^3) while the later instars were reared in large cages (45×45×55 cm^3). Caterpillars in cages were provided with surface sterilized fresh cabbage leaves daily. The fully grown caterpillars were transferred to the new cages for pupation. Two day-old pupae were detached from the walls of the cage and kept separately in a batch of 20 pupae per cage (60×60×70 cm^3) over a thick layer of UV-irradiated filter paper for adult emergence. The adults were held in cages (60×60×70 cm^3) provided with cotton swabs soaked in honey solution and SDW. A few flowering shoots of mustard were also provided as pollen source. Potted cabbage plants were kept in each cage for egg laying whenever needed. The diamond back moth (DBM), *Plutella xylostella* L. larvae were used from the culture being maintained in the post graduate laboratory of the department of Entomology, CSK HPKV, Palampur whereas field collected larvae of semilooper, *Thysanoplusia orichalcea* (Fab.) were used in the present study.

Preparation of PbGV OBs suspension

The *P. brassicae* granulovirus (PbGV) used in the study was the local strain isolated and characterized from the dry temperate region of Himachal Pradesh by Sood (2004). The PbGV was produced in the larvae of *P. brass*icae in the Postgraduate Laboratory of the Department of Entomology, CSK HP Agricultural University, Palampur, India (1200 msl). The purified virus was used in the present studies and the concentration of OBs in stock suspensions was determined by direct count using a Helber bacteria haemocytometer (0.02 mm depth). A stock solution of PbGV with the strength of 2×10^{12} OBs/ml was prepared for bioassay studies and stored in amber coloured bottles at 4°C. Serial dilutions (10^3–10^9 OBs /ml) were prepared from the quantified virus stock solutions in SDW.

Cross infectivity of PbGV

The purified PbGV was tested for cross infectivity against *P. xylostella* and *T. orichalcia*. Three doses (one above and one below LC_{50} value and one LC_{50} dose for PbGV) along with control using SDW were evaluated in laboratory bioassay (leaf disc assay) following Ballard *et al*. (2000). Fifty pre-starved larvae (24 hr) of 4th instars of *P. xylostella* and *T. orichalcia* each were released on treated cabbage leaf discs for each treatment separately. Each treatment was replicated three times. After 24 hours of feeding, the larvae were transferred to 70% ethanol washed untreated cabbage leaves. The mortality was recorded after 120 hours of release and thereafter by identifying the peculiar virosis symptoms.

Safety to natural enemies

The safety of PbGV was evaluated against the *Cotesia glomerata* (L.) (Ichneumonidae: Hymenoptera) under laboratory conditions. Cocoons of *C. glomerata* were treated with PbGV at a concentration lethal to the fourth instar larvae. An untreated control treatment was also maintained using SDW spray only. Observations were recorded on per cent emergence of *C. glomerata* from cocoons. Thereafter, the adults were provided with the healthy *P. brassicae* larvae (3rd instar) inside the glass chimmenies covered with muslin cloth to ascertain their parasitisation potential. A total of five *P. brassicae* larvae were provided for two pairs of *C. glomerata* adults. Data were recorded on the emergence, life span and parasitization. The data obtained were subjected to analysis of variance.

Field evaluation of PbGV against *P. brassicae*

Bioefficacy studies of PbGV at different concentrations against *P. brassicae* alone and in combination with *Bacillus thuringiensis* (DIPEL® 8L), neem seed kernel extract (NSKE) and control (cypermethrin as positive and untreated check as negative control) were carried out at two geographically isolated locations *viz.* Palampur 1290.8 msl, 32°6′37″ N and 76°32′47″ E) and Sangla (2590 msl, 31°25′56″ N and 78°15′ 4″ E). The treatments were PbGV alone ($5.58\text{x}10^{11}$ and $1.12\text{x}10^{12}$ OBs/ha), PbGV in combination with *B. thuringiensis* (DIPEL® 8L) & neem seed kernel extract (NSKE) and control (cypermethrin as positive and untreated check as negative control). At Palampur, studies were carried out during *rabi*, 2006-07 and 2007-08. Three main cole crops

viz. cabbage, cauliflower and broccoli were raised in the experimental field of the Department of Entomology, CSK HPKV Palampur following recommended package of practices, except insecticide application. The foliar application twice (at 15 days interval) of PbGV and other treatment combinations was applied with a knapsack sprayer using tritonX100 as surfactant. Same set of experiment was repeated at Sangla on off-season crop during *kharif* 2007 and 2008, however, only one spray of the treatments was applied. Pre- treatment (1 day before) and post- treatment (7 & 14 days after) larval count of *P. brassicae* were recorded on five randomly selected plants in each plot. The pre-treatment counts were, however, almost same in all the plots as the differences were non-significant at both the locations. The data was analysed through ANOVA.

RESULTS AND DISCUSSION

Cross infectivity

Data (Table 1) revealed that PbGV did not impart any lethal effect in *P. xylostella* and *T. orichalcea* at either of the PbGV doses tested under laboratory conditions indicating high host specificity of the virus isolate.

Safety to natural enemies

Data (Table 2) revealed that PbGV did not result in any negative effect on the emergence of *C. glomerata* from the cocoons sprayed with PbGV suspension. Emergence was observed to be 85.23 per cent in treated *C. glomerata* which was statistically on par with untreated control (87.67%). After emergence the adults were provided with *P. brassicae* 3rd instar larvae for parasitisation which was also observed not to be affected. The total period of egg and larval development was observed to be 12.24 days in case of treated individuals which was statistically on par with untreated control (12.55 days). The corresponding pupal period was 4.55 and 4.77 days, respectively. Adult lived for 5.12 days in treated and 5.00 days in untreated. Parasitization rate was found to be 76.41 (treated) against 77.58 per cent in untreated control.

Field evaluation of PbGV against *P. brassicae*

At Palampur, the treatment with cypermethrin resulted in minimum number of *P. brassicae* larvae initially, however, its efficacy decreased at 7 days after first spray (DAFS) (45.27 larvae/5 plants). Lowest number of *P. brassicae* larvae (30.33) were recorded in PbGV (5.58×10^{11} OBs)+ NSKE though being statistically on par with PbGV (1.12 x 1012 OBs) (Table 3). Second foliar application also resulted in almost similar trends. At 7 days after second spray (DASS) PbGV (5.58×10^{11} OBs)+ Bt was the best treatment in reducing larval population to 4.33 larvae/ 5 plants and PbGV (5.58×10^{11} OBs), PbGV (1.12×10^{12} OBs) and PbGV (5.58×10^{11} OBs)+ NSKE being statistically on par with it. Egg hatching was observed to be normal in all the treatments. In cauliflower, the insect pressure was comparatively more than cabbage and broccoli. At 7 DASS PbGV (1.12×10^{12} OBs) was highly effective (5.33 larvae/5 plants) followed by (5.58×10^{11}OBs), PbGV (5.58×10^{11} OBs) + Bt and PbGV (5.58×10^{11} OBs) + NSKE though all being statistically at par with each other. At 7 DAFS & DASS, the pest populations were 46.33 and 18.36 larvae/5 plants, respectively in cypermethrin treated plots (Table 1). In broccoli also, PbGV (5.58×10^{11} OBs) + Bt was more effective (9.33 larvae/ 5 plants) compared to other treatments *viz*. PbGV (1.12×10^{12} OBs) and PbGV (5.58×10^{11} OBs) + NSKE. Larval populations in these treatments were 7.34 and 9.33 larave/5 plants, respectively while it was 130.3 larvae/ 5 plants in control.

At Sangla, minimum number of *P. brassicae* larvae (21.00) in cabbage were recorded in plots treated with PbGV (1.12×10^{12} OBs) at 7 DAFS being statistically on par with PbGV (5.58 x 1011 OBs) + Bt followed by

Table 1. Cross infectivity of PbGV against *Plutella xylostella* and *Thysanoplusia orichalcea*

PbGV concentration (OB/ml)	No. treated	Number of larvae alive after 5 days	
		Plutella xylostella	*Plusia orechalcia*
1×10^6	50	38.25±0.02	39.22±0.04
1×10^7	50	39.62±0.01	38.85±0.01
1×10^8	50	38.42±0.03	38.54±0.20
Untreated Control	50	38.22±0.02	37.44±0.03
CD (*P*=0.05)		NS	NS

Table 2. Effect of PbGV (@ 2.0X10^7) on *Cotesia glomerata*

Parameters	Treated	Untreated	CD (p=0.05)
Adult emergence (%)	85.23±0.12	87.67±0.12	NS
Egg + Larval period (days)	12.24±0.12	12.55±0.21	NS
Pupal period (days)	4.55±0.11	4.77±0.24	NS
Adult longevity(days)	5.12±0.17	5.00±0.21	NS
Parasitization (%)	76.41±0.21	77.58±0.08	NS

Table 3. Bioefficacy of PbGV against *Pieris brassicae* on cole crops at Palampur

Treatments	Pest population after indicated days of spray								
	Cabbage			Cauliflower			Broccoli		
	7DAFS	14DAFS*	7DASS	7DAFS	14DAFS*	7DASS	7DAFS	14DAFS*	7DASS
5.58x10^{11} OBs/ ha	60.67 (7.79)	72.00 (8.48)	7.00 (2.64)	108.33 (10.55)	75.33 (8.75)	16.34 (4.04)	110.33 (10.50)	115.67 (10.75)	16.34 (4.04)
1.12x10^{12} OBs/ ha	50.33 (7.09)	70.00 (8.36)	5.66 (2.23)	73.33 (8.66)	50.25 (7.04)	5.33 (2.30)	75.67 (8.69)	80.00 (8.94)	7.34 (2.70)
T1+Bt (1.5 ml/l)	40.47 (6.36)	75.67 (8.69)	4.33 (2.08)	72.33 (8.56)	52.34 (7.30)	10.24 (3.20)	70.33 (8.38)	75.67 (8.69)	6.33 (2.51)
T1+NSKE (3.00%)	30.33 (5.50)	80.00 (8.94)	6.33 (2.51)	95.00 (9.29)	50.44 (7.06)	12.33 (3.51)	65.00 (8.06)	70.33 (8.38)	9.33 (3.05)
Cypermethrin (0.001%)	45.27 (6.72)	85.33 (9.23)	18.66 (4.31)	46.33 (6.87)	80.00 (8.87)	18.36 (4.28)	30.00 (5.47)	32.00 (5.65)	12.33 (3.51)
Untreated control	65.67 (8.10)	95.67 (9.78)	120.33 (10.96)	158.33 (12.61)	175.33 (13.67)	180.95 (13.45)	110.67 (10.51)	120.67 (10.98)	130.33 (11.41)
CD (p=0.05)	1.09	1.30	0.90	0.60	1.24	2.12	2.01	0.98	0.98

Values in parentheses are square root transformed values; DAFS: Days after first spray DASS: Days after second spray

PbGV (5.58 x 10^{11} OBs) and PbGV (5.58 x 10^{11} OBs) + NSKE recording 31.67 and 35.00 larvae/5plants, respectively (Table 4). Pest population in untreated control was 171.67 larvae/5 plants. In case of cauliflower and broccoli also similar trends were observed with respect to larval populations. In cauliflower, at 14 DAS it was PbGV (5.58 x 10^{11} OBs) + Bt which was most effective (9.33 larvae/5 plants) though statistically on par with PbGV (1.12 x 10^{12} OBs). In broccoli, however, PbGV (1.12 x 10^{12}OBs) was more effective (11.67 larvae/ 5plants) and PbGV (5.58 x 10^{11} OBs) + Bt recording 12.00 larvae/5plants being statistically on par with it.

The present studies revealed that all the treatments were superior over control (both positive and negative control). Increased efficacy of PbGV in combination with Bt might be due to the stress imparted by Bt on *P. brassicae* larvae resulting in activated infection of PbGV. Enhancement of virulence of PbGV or disease occurrence in the host larvae suggested latent infections meaning that ingested viruses are sometimes activated by stress, both biotic and abiotic, resulting in the outbreak of the disease in the host insect populations (Aruga, 1963). Similar findings were observed by Komalpith and Ramakrishnan (1978) and Kamala Jayanthi and Padmavathamma (2001) who found that combination of NPV + *B. thuringiensis* resulted in enhanced effectiveness against *Spodoptera litura* (Fab.). In the present case, PbGV + NSK was also found as effective as PbGV alone and PbGV + Bt. The role of stressors in activating latent viral infection has also been reported earlier by Steinhaus (1958). Muralibaskaran

Table 4. Bioefficacy of PbGV against *Pieris brassicae* on cole crops at Sangla

Treatments	Pest population after indicated days of spray					
	Cabbage		Cauliflower		Broccoli	
	7DAS	14DAS	7DAS	14DAS	7DAS	14DAS
$5.58x10^{11}$ OBs/ ha	31.67 (5.71)	20.00 (4.58)	29.67 (5.53)	22.00 (4.79)	30.0 (5.57)	24.00 (4.99)
$1.12x10^{12}$ OBs/ ha	21.00 (4.69)	11.00 (3.46)	17.33 (4.27)	10.67 (3.41)	16.67 (4.19)	11.67 (3.56)
T1+Bt (1.5 ml/l)	22.67 (4.86)	10.00 (3.31)	19.33 (4.49)	9.33 (3.15)	20.33 (4.59)	12.00 (3.59)
T1+NSKE (3.00%)	35.00 (5.99)	23.33 (4.93)	28.67 (5.43)	24.67 (5.07)	29.33 (5.49)	24.67 (5.07)
Cypermethrin (0.001%)	34.66 (5.97)	45.00 (6.78)	38.00 (6.23)	51.06 (7.25)	41.00 (6.46)	55.00 (7.48)
Untreated control	150.00 (12.28)	171.67 (13.13)	160.66 (12.68)	181.67 (13.51)	173.33 (13.19)	185.67 (13.66)
CD (*P*=0.05)	0.32	0.30	0.74	0.49	0.83	0.33

Values in parentheses are square root transformed values

et al. (1999) observed that suspending nuclear polyhedrosis virus in different concentrations (0.10–1.00%) of neem oil and neem seed kernel extract reduced the LC_{50} value of *S. litura* nuclear polyhedrosis virus by 1.05 to 1.43 folds and 1.03 to 1.33 folds, respectively. Reduction in LC_{50} and LT_{50} of the HaNPV while combining neem products with different formulations of *Helicovera armigera (Hubner)* NPV have been reported (Muthiah *et al.,* 1988; Rabindra *et al.,* 1994 and Kumar *et al.,* 2008. Lingappa *et al.,* (2000) also reported that econeem (a neem based formulation) in combination with HaNPV reduced the damage to an extent of 70.36 to 71.99 per cent and recorded maximum cotton yield. Murugan *et al.,* (1999) observed that combining neem products with *Sl*NPV resulted in three fold enhancement of per cent mortality even at reduced concentration. The present findings suggested that application of GV in combination with other microbial or botanical insecticides will give better management of the pest than GV alone at lower concentration. However, more experimentation on its formulation that would include search for ideal carriers, protectants (brighteners) against sunlight, spreaders, stickers etc is warranted for successful management of *P. brassicae* using granular virus .

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