J. Biol. Control, 13: 79-84, 1999

Cross infectivity and effect of environmental factors on the infectivity of granulosis virus of *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)

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ABSTRTACT: Studies on granulosis virus (GV) of *Phthorimaea operculella* (Zeller) were carried out to determine the LC_{50}, cross-infectivity and effect of sunlight, heat and rearing temperature on the infectivity. The LC_{50} value of *Phthorimaea operculella* GV (*Po*GV) for the neonate larvae was 9.08 x 10^4 occlusion bodies (Obs) /ml. The virus did not show cross-infectivity to other eleven lepidopterous insect species. The stability of GV was affcted by sunlight, heat and rearing temperature. The virus could withstand exposure to sunlight for 4h without any significant loss of its infectivity. The infectivity gradually declined with increase in sunlight exposure period from 8 to 18h and total inactivation took place beyond 24h. The infectivity of *Po*GV was not affected significantly up to 40° C for 10 minutes exposure to heat and the thermal inactivation point (TIP) was in between 90 and 100° C. Though the rearing temperatures of 20 to 35° C had no significant effect on infectivity of *Po*GV, rearing of the virus inoculated larvae of *P. operculella* at 25° C would be optimum for development of granulosis.

KEY WORDS: GV, LC_{50}, infectivity, *Phthorimaea operculella*

Potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) is the serious pest of potato (*Solanum tuberosum* Linn.) and causes losses to the extent of 20 to 70 per cent in field and storage (Lal, 1993). In order to alleviate the pest ravages, synthetic insecticides as well as parasitoids have been tried which gave substantial control of PTM. The granulosis virus (GV) of *P. operculella* was isolated and described by Amonkar *et al.* (1979). The potential of *P. operculella* GV (*Po*GV) in Australia has been demonstrated under field conditions (Reed and Springet, 1971). Hence, prior

to use of *Po*GV in field as component of IPM programme in potato cultivation, investigations on bioassay studied to determine LC_{50} for *P. operculella,* cross-infectivity and stability of *Po*GV as influenced by various environmental factors like sunlight and temperature were investigated.

MATERIALS AND METHODS

Mass rearing of *P. operculella* and production of *Po*GV

Mass rearing of PTM was undertaken on potato tubers following the method described by Platner and Oatman (1968). The nucleus culture of *Po*GV (1.1 x 10^9 Obs/ml) was obtained from the Bhabha Atomic Research Centre, Mumbai. The virus was propagated, semi-purified and counts of Obs were made as per the procedure followed by Amonkar *et al.* (1979).

Bioassay of virus activity

The stock culture of *Po*GV (1.1 x 10^9 Obs/ml) was further subjected to 8 serial dilutions from 1.1 x 10^9 to 1.1 x 10^2) Obs/ml. The neonate larvae of PTM were inoculated by smearing one ml virus suspension from each dilutions to punctured tubers using camel hair brush. These tubers were dried under a fan. Five neonate larvae of PTM were placed on each treated tuber and allowed to develop for 15 days. Thirty larvae constituted 3 replications for each of the serial dilutions. Similarly, control was maintained with untreated tubers. Larval mortality was recorded by cutting open the tubers with a knife. The data were then subjected to Probit analysis for determination of LC_{50} value (Finny, 1971).

Cross-infectivity test

The cultures of eleven lepidoptern insects were obtained from field and insectaries and reared on their respective hosts. Lists of the insect species and their hosts are given in Tale 1. The second instar test larvae were inoculated with *Po*GV (1.1 x 10^9 Obs/ml) by smearing one-ml virus suspension on to the plant parts at their feeding sites. The larvae were exposed to treated-food for 48h and thereafter allowed for further development. A control was maintained for every test species by rearing them separately on their own untreated food. Besides, the neonate larvae of PTM were also inoculated with one -ml virus as described in bioassay test. The data on larval mortality or pupation were recorded by infecting 30 larvae in 3 replications for each insect species.

Effect of sunlight on infectivity of *Po*GV

Aliquots of one- ml virus containing 1.1 x 10^9 Obs were spread homogeneously in a flat bottom and top of Petri-dish (95mm diam) and air dried. The dried films of viral suspension were then exposed directly to sunlight for 2, 4, 6, 8, 10, 12, 18, 24, 48 and 72h during second fortnight of September, 1995. The Obs from each exposure periods were re-suspended in one ml sterile distilled water to get the original concentration. These virus suspensions were inoculated to neonate larvae of PTM and further reared till death or pupation.

The infectivity of each test sample was tested against 30 larvae in 3 replications. Similarly the larvae inoculated with unexposed virus constituted treated control.

Effect of heat on infectivity of *Po*GV

Aliquots of one ml virus (1.1 x 10^9 Obs/ml) pipetted separately into each of the sterilized thin walled glass test tubes and exposed to 40, 50, 60, 70, 80, 90 and 100°C temperatures for 10 minutes in the serological hot water bath. Unexposed virus suspension at normal temperature (30°C) was considered for treated control. After heat treatment, the test tubes were cooled under slow jet of cold water and the suspensions were inoculated to neonate larvae of PTM. These larvae were further reared on tubers till death or pupation. In all 30 larvae in 3 replications were exposed to each test samples.

Effect of rearing temperature on infectivity of *Po*GV

The neonate larvae of PTM were reared on punctured tubers surface contaminated with the virus suspension (1.1x10^9 Obs/ml) at 20, 25, 30 and 35°C temperatures in BOD incubators till death or pupation. Control was maintained with untreated tubers. Fifty larvae in 5 replications constituted a treatment.

Data on larval mortality due to infectivity of *Po*GV influenced by sunlight, heat and rearing temperature were subjected to analysis of variance.

RESULTS AND DISCUSSION

The log concentration- Probit kill regression line for neonate larvae of PTM was derived as $Y = 3.81 + 0.24X$. The LC_{50} value was observed to be 9.08 x 10^4 Obs/ml with upper and lower fiducial limits of 4.9 x 10^9 and 1.69 x 10^4 Obs/ml, respectively. Cross-infectiivity test indicated that PoGV was highly specific to P. operculella. While the virus did not show infectivity to the larvae of other eleven lepidoptern insect species (Table 1).

Table 1. Cross-infectivity of *P. operculella* GV to different lepidopterous insect species

Insect species	Host	Larval mortality due to virus (%)	Pupation (%)	Infectivity (%)
Helicoverpa armigera (Hub.)	Chickpea leaves	0.0	100.00	-ve
Spodoptera litura (Fabr.)	Castor leaves	0.0	100.00	-ve
Spilosoma obliqua (Walk.)	Sunflower leaves	0.0	100.00	-ve
Achaea janata (Linn.)	Castor leaves	0.0	100.00	-ve
Prospalta capensis (Guen.)	Safflower leaves	0.0	100.00	-ve
Plutella xylostella (Linn.)	Cabbage leaves	0.0	100.00	-ve
Bombyx mori (Linn.)	Mulberry leaves	0.0	100.00	-ve
Corcyra cephalonica (Staint.)	Sorghum grains	0.0	100.00	-ve
Earias vittella (Fabr.)	Okra fruits	0.0	100.00	-ve
Leucinodes orbonalis (Guen.)	Brinjal fruits	0.0	100.00	-ve
Chilo partellus (Swinh.)	Sorghum stem	0.0	100.00	-ve
Phthorimaea operculella (Zell.)	Potato tubers	83.33	16.67	+ve

Infectivity of *Po*GV was influenced significantly by sunlight. The larval mortality data revealed that there was no significant loss in infectivity of the virus after sunlight exposure upto 4h compared to unexposed virus (Table 2). There was gradual reduction in larval mortality due to granulosis when the obs exposed for 8 to 18h. Where as total inactivation of the virus took place with exposures of 24 to 72h.

Effect of heat on *Po*GV showed that there was no significant loss in infectivity of the virus on exposure to 40°C temperature compared to unexposed virus (Table 3). The larval mortality recorded through an exposure of the virus at 50°C was statistically at par with those at 40°C. Thereafter, it declined gradually with the increase in temperature from 60 to 90° C and total inactivation of the virus was noticed at 100°C. Thus, the thermal

Table 2. Effect of sunlight on infectivity of GV of *P. operculella*

Period of exposure (h)	Larval mortality due to granulosis (%)	Pupation (%)
2	80.00 (63.44)	20.00
4	76.66 (61.22)	23.34
6	53.33 (47.00)	46.67
8	30.00 (33.00)	70.00
10	26.66 (30.78)	73.34
12	23.33 (28.77)	76.67
18	16.66 (23.85)	83.34
24	0.00 (0.81)	100.00
48	0.00 (0.81)	100.00
72	0.00 (0.81)	100.00
Control (Unexposed virus)	83.33 (66.14)	16.67
Distilled water (without virus)	0.00 (0.81)	100.00
SEM±	(2.62)	
CD (P=0.05)	(7.62)	

Figures in parentheses are arcsine- transformed values.

inactivation point (TIP) of the virus when heated for 10 min, appeared to be between 90 and 100° C. The TIP of *Pericallia ricini* GV to be between 95° and 100° C as reported by Philip and Jacob (1979) supported the present observations.

Table 3. Effect of heat on infectivity of GV of *P. operculella*

Temperature (°C)	Larval mortality due to granulosis	Pupation (%)
40	76.66 (61.22)	23.34
50	70.00 (56.79)	30.00
60	56.66 (48.84)	43.34
70	43.33 (41.15)	56.67
80	23.33 (28.77)	76.67
90	6.66 (12.56)	93.34
100	0.00 (0.81)	100.00
Control (Unexposed virus)	83.33 (66.14)	16.67
Distilled water (without virus)	0.00 (0.81)	100.00
SEM±	(2.71)	
CD (P=0.05)	(8.12)	

Figures in parentheses are arcsine transformed values.

Rearing of *P. operculella* larvae inoculated with own GV at test temeprature did not show significant differences in larval mortality due to granulosis (Table 4). The higher mortality was recorded at 25°C followed by 30°C, whereas, it was least at 35°C. Liang (1983) indicated the optimum temperature for infection of *Pieris rapae* GV as 21 to 28.1° C, which is corroborative to the present findings.

Table 4. Effect of rearing temperature on infectivity of GV of *P. operculella*

Temperature (°C)	Larval mortality due to granulosis	Pupation (%)
40	76.66 (61.22)	23.34
20	55.55 (48.20)	44.45
25	84.00 (66.88)	16.00
30	62.25 (52.12)	27.75
35	40.00 (39.10)	30.00
SEM±	(39.00)	
CD (P=0.05)	(NS)	

Figures in parentheses are arcsine- transformed values.
NS = Non-significant

Thus, it is evident from the present investigations that the isolate of GV was infective to the neonate larvae of *P. operculella* and highly host specific. Sunlight, heat and rearing temperatures influenced stability of *Po*GV. Hence, evening applications of *Po*GV could protect the virus from longer exposure to sunlight and day-temperature and could give protection from *P. operculella* in potato field.

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