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Research Article

Effect of granulosis virus infection on food consumption and utilization by *Pieris brassicae* (Linnaeus)

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ABSTRACT: The early fourth instar larvae of *Pieris brassicae* were fed on cabbage leaves treated with PbGV at three concentrations (2×10^7, 1×10^7 and 2×10^6 occlusion bodies ml^{-1}) to ascertion the food consumption and its utilization by *P. brassicae* larvae upon infection under laboratory conditions. It was found that the mean consumption index decreased to the tune of 4.39% at 24 hours to 67.19% at 120 hours over control after PbGV infection. Approximate digestibility also reduced from 4.22 to 80.78% at 24 and 120 hours, respectively over control. Food consumption of virus-treated larvae decreased with passage of time as compared to the healthy larvae. Conversion of ingested and digested food to body substance also reduced in virus infected larvae to different degrees which ranged from 2.77 to 96.04% for ingested food and 4.55 to 92.16% for digested food. The differences among various concentrations with respect to consumption index, approximate digestibility, conversion of ingested and digested food were, however, statistically non-significant. The impact of virus infection on food consumption and utilization by *P. brassicae* larvae was enhanced with time after infection.

KEY WORDS: Nutritional requirement**,** food consumption, utilization, PbGV, *Pieris brassicae*

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INTRODUCTION

Amongst many insect species associated with cole crops, cabbage butterfly, *Pieris brassicae* (L.) (Pieridae) is an important pest in hill regions of north-western Himalayas, apart from DBM, *Plutella xylostella* (L.) and cabbage aphid, *Brevicoryne brassicae* (L.) (Anonymous, 1989). In Indian subcontinent, *P. brassicae* is distributed the along the Himalayan region including Pakistan, Nepal and throughout the plains except southern states of India (Vevai, 1973; Lal and Ram, 2004; Younas *et al*., 2004). The damage done by the voraciously feeding fourth and fifth instar caterpillars is a function of crop stage and the level of infestation. At pre-heading stage, the caterpillars may gormandize the whole plant but at head formation stage, they damage by feeding on head and also by contaminating it with faeces, whereas, after head formation, the caterpillars feed mainly on the inflorescence reducing the seed yield. The virus that infects *P. brassicae* results in slow death of the host causing various physiological changes after infection till death. The quantitative aspects of nutrition concerning the intake, digestibility and efficiency of conversion of diets as affected by virus infection in *P. brassicae* are little known. The present studies were, hence, undertaken to study the effects of PbGV infection on physiological attributes like feeding activity, approximate digestibility (AD), efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) in *P. brassicae*.

MATERIALS AND METHODS

Experimental insects and virus

The initial culture of *P. brassicae* was started 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% RH). 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 ethanol washed and UV sterilized cages (15 x 15 x 15 cm). The first three larval instars were reared in small cages (15 x 15 x 15 cm), while the later instars were reared in larger cages (45 x 45 x 55 cm). The caterpillars in cages were provided with surface sterilized fresh cabbage leaves daily. The full grown caterpillars were transferred to new cages for pupation. Two days old pupae

were detached from the walls of the cages and were kept separately in batches of 20 pupae in each cage (60 x 60 x 70 cm) over a thick layer of UV irradiated filter paper for adult emergence. The adults were held in cages (60 x 60 x 70 cm) provided with cotton swabs soaked in honey solution and SDW. Some flowering shoots of mustard were also provided as pollen source. Potted cabbage plants were kept in each cage for egg laying whenever needed (Bhandari *et al.,* 2009). Pre-starved (24 hr) healthy larvae of 4th instar surface sterilized with 70% ethanol were selected for the bioassay to facilitate feeding and reducing the chances of mortality due to other factors (Bhandari *et al.,* 2009).

The *P. brassicae* granulovirus (PbGV) isolated from dry temperate region of Himachal Pradesh (Sood, 2004) was used in the present studies. The putrefied larvae of *P. brassicae* (infected with granulosis virus) were homogenized in a homogenizer and the homogenates containing the viral inclusions were filtered through four layers of muslin cloth. The filtrate was spun at 1000 rpm for 5 min to remove the pelleted larval cells and tissues. The relatively clear supernatant was then centrifuged at 8000 rpm for 20 min. The viral capsules pelleted were retained after discarding the supernatant. The virus pellet was resuspended in appropriate volume of distilled water and stored at 4°C. The virus capsules per larvae were counted using Helber bacteria haemocytometer (0.02 mm depth).

Test procedure

Three dosages near to median lethal concentrations of PbGV, 2×10^7, 1×10^7 and 2×10^6 OBs ml^{-1}, were prepared from the stock. Fresh cabbage leaves were cut into discs (diameter 4.5 cm) and treated with 100 µl suspensions from each of the PbGV concentrations. In the control, the cabbage leaf discs were treated with equal quantity of sterilized distilled water only. Fourth instar larvae (n = 10), pre-starved for 24 hours were released on the treated cabbage leaves in separate cages (15 x 15 x 15 cm) for each concentration as they were large enough to survive for 5-6 days after chronic virus infection. The experiment was replicated four times. After 24 hours of feeding, the larvae were transferred to untreated fresh cabbage leaves in new cages and observations on the weight of cabbage leaves consumed, faecal matter produced and larval weight were recorded. The cabbage leaf discs were weighed on an electronic balance (Denver APEX series APX-200) before and 24 hours after treatment. The leaf discs were replaced daily and observations on the amount of food consumed during every 24 hours and weight of larvae as well as their excreta produced were taken daily till 120 hours of treatment.

The data taken were used to work out the impact of PbGV on quantitative nutritional requirements (approximate digestibility, efficiency of conversion of ingested food, efficiency of conversion of digested food) of cabbage caterpillars on fresh (wet) weight basis (Waldbauer, 1968; Jalali *et al.*, 1988). Correction for water evaporation was obtained by holding 10 cabbage leaf discs under identical conditions.

$$\text{Consumption Index (CI)} = \frac{F}{T \times A}$$

where, F = fresh weight (wt.) of food eaten (gms); T = duration of feeding period (days); A = mean fresh weight of animal during feeding.

$$\text{Approximate Digestibility (AD)} = \frac{\text{Wt. of food ingested} - \text{Wt. of faeces}}{\text{Wt. of food ingested}} \times 100$$

$$\text{Efficiency of Conversion of Ingested food to body substance (ECI)} = \frac{\text{Wt. gained}}{\text{Wt. of food ingested} - \text{Wt. of faeces}} \times 100$$

$$\text{Efficiency of Conversion of Digested food to body substance (ECD)} = \frac{\text{Wt. gained}}{\text{Wt. of food digested} - \text{Wt. of faeces}} \times 100$$

RESULTS AND DISCUSSION

In order to understand the effect of PbGV infection on the quantitative nutritional requirements of *P. brassicae* larvae, fourth instar larvae infected with PbGV at three concentrations (2 x 10^7, 1 x 10^7 and 2 x 10^6) were observed for changes in leaf uptake for up to 120 hours after treatment. The data presented in Table 1 reflect an increasing trend in per cent reduction of consumption index with passage of time after virosis. A gradual increase in the per cent reduction in consumption index over control was observed in all the three treatments evaluated. The mean per cent reduction in consumption index at three dosages varied non-significantly from 27.77–31.24 per cent. However, there was a significant variation in the pattern of reduction in the consumption index of treated larvae over control at variable time intervals (4.39 to 67.19%). These results draw considerable support from the report of Hua Lu *et al*. (2001), who reported that *Plutella xylostella* GV caused significant reduction in consumption rate of *P. xylostella* larvae which varied from 99, 77, 53 and 46 per cent for second to fifth instar larvae, respectively. Tatchel (1981) also reported 99.6 per cent reduction in the potential food consumption of *Pieris rapae* due to PrGV infection. The results are also similar to earlier reports in *P. rapae*, *Ectropis obliqua hypulina* and *Trichoplusia ni* (Wang and Hu, 1986; Hu *et al.*, 1990; Harper, 1973). The per cent reduction in approximate digestibility in virus inoculated fourth instar larvae of *P. brassicae* increased gradually in comparison to control (Table 2). The mean per cent reduction in approximate digestibility at three dosages varied non-significantly from 41.30 to 43.74 per cent. However, the pattern of reduction in approximate digestibility of treated larvae over control was observed to vary significantly with time (4.22 to 80.78%).

The efficiency of conversion of ingested food to body substance of virus fed fourth instar larvae of *P. brassicae* (Table 3) revealed significant differences. It varied from 2.77 to 96.04 per cent at 24 to 120 hours of treatment, respectively. However, no significant difference in reduction of efficiency of conversion of ingested food to body substance (43.39 to 43.73%) was observed among the larvae treated with different concentrations.

A gradual increase in per cent reduction in efficiency of conversion of digested food to body substance in virus inoculated *P. brassicae* larvae in comparison to control was observed. The mean per cent reduction in efficiency of conversion of digested food to body substance at three dosages varied non-significantly from 46.47 to 47.84 per cent. The pattern of reduction in efficiency of conversion of digested food to body substance of treated larvae (Table 4) over control, however, varied significantly at variable time (4.55 to 92.16 %). Interaction effects of time and concentrations were, however, found to be non-significant in all the parameters observed.

Table 1. Effect of PbGV infection on consumption index of fourth instar *P. brassicae* larvae

Concentration (OBs ml^{-1})	Per cent reduction in consumption index over control					
	Time after treatment					
	24 hr	48 hr	72 hr	96 hr	120 hr	Mean
2 x 10^7	5.77 (11.91)*	11.07 (17.11)	23.53 (26.68)	45.00 (42.02)	70.83 (57.62)	31.24 (31.47)
1 x 10^7	4.11 (9.45)	10.75 (17.11)	22.09 (26.53)	47.46 (43.41)	67.12 (55.11)	30.30 (30.32)
2 x 10^6	3.31 (7.67)	8.93 (14.94)	20.22 (25.05)	42.76 (40.78)	63.63 (52.98)	27.77 (28.29)
Mean	4.39 (9.68)	10.25 (16.39)	21.94 (26.75)	45.07 (42.07)	67.19 (55.23)	

*Figures in parentheses are arcsine transformed values

	CD (5%)
Time after treatment (df = 4)	4.1
Concentration (df = 3)	NS
Time after treatment x concentration (df = 12)	NS

Table 2. Effect of PbGV infection on approximate digestibility of fourth instar *P. brassicae* larvae

Concentration (OBs ml^{-1}	Per cent reduction in approximate digestibility over control					
	Time after treatment					
	24 hr	48 hr	72 hr	96 hr	120 hr	Mean
2x10^7	4.36 (9.19)*	16.14 (21.88)	47.97 (43.78)	65.06 (53.81)	82.27 (65.18)	43.16 (38.77)
1 x 10^7	3.90 (7.72)	16.55 (22.45)	46.54 (49.27)	61.47 (51.65)	78.04 (62.27)	41.30 (37.42)
2 x 10^6	4.40 (7.60)	16.67 (22.14)	50.23 (45.11)	65.40 (54.09)	82.03 (65.28)	43.74 (38.84)
Mean	4.22 (8.17)	16.45 (22.16)	48.24 (43.95)	63.97 (53.19)	80.78 (64.24)	

*Figures in parentheses are arcsine transformed values

	CD (5%)
Time after treatment (df = 4)	3.9
Concentration (df = 3)	NS
Time after treatment x concentration (df = 12)	NS

Table 3. Effect of PbGV infection on efficiency of conversion of ingested food to body substance of fourth instar *P. brassicae* larvae

Concentration (OBs ml^{-1})	Per cent reduction in efficiency of conversion of ingested food to body substance over control					
	Time after treatment					
	24 hr	48 hr	72 hr	96hr	120 hr	Mean
2 x 10^7	3.56 (8.47)*	15.52 (22.84)	38.31 (38.17)	64.50 (53.44)	95.06 (79.39)	43.39 (40.46)
1 x 10^7	2.16 (5.67)	15.08 (22.31)	36.48 (37.05)	67.61 (55.41)	96.35 (82.27)	43.53 (40.54)
2 x 10^6	2.60 (7.08)	15.45 (22.69)	37.86 (37.89)	66.02 (54.37)	96.72 (81.54)	43.73 (40.72)
Mean	2.77 (7.07)	15.35 (22.61)	37.55 (37.70)	66.04 (54.41)	96.04 (81.07)	

*Figures in parentheses are arcsine transformed values

	CD (5%)
Time after treatment (df = 4)	2.8
Concentration (df = 3)	NS
Time after treatment x concentration (df =12)	NS

Table 4. Effect of PbGV infection on efficiency of conversion of digested food to body substance of fourth instar *P. brassicae* larvae

Concentration (OBs ml^{-1})	Per cent reduction in efficiency of conversion of digested food to body substance over control					
	Time after treatment					
	24 hr	48 hr	72 hr	96hr	120 hr	Mean
2 x 10^7	5.30 (10.20)*	21.19 (27.19)	44.69 (41.91)	75.56 (60.44)	92.49 (76.00)	47.84 (43.15)
1 x 10^7	3.87 (7.9)	17.64 (24.48)	41.14 (39.82)	76.92 (61.46)	92.81 (75.87)	46.47 (41.92)
2 x 10^6	4.48 (9.60)	18.65 (25.21)	45.99 (42.66)	75.48 (60.62)	91.18 (73.80)	47.15 (42.38)
Mean	4.55 (9.24)	19.16 (25.62)	43.94 (41.46)	75.98 (60.84)	92.16 (75.22)	

*Figures in parentheses are arcsine transformed values

	CD (5%)
Time after treatment (df = 4)	3.0
Concentration (df = 3)	NS
Time after treatment x concentration (df = 12)	N

It is evident from the data that PbGV infection exerted significantly negative influence on the efficiency of conversion of ingested food to body substance and the efficiency of digested food to body substance of treated larvae over control. The present findings that PbGV infection reduced food consumption and utilization by *P. brassicae* larvae after virosis are of great significance in pest management. It may be concluded that the infected larvae cause less damage to plants after infection during the virus incubation period in larvae (until host death) contrary to the general belief that the infected larvae continue to feed and damage the host plants.

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