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Studies on the Pathogenicity of Granulosis Virus of the Sugarcane Shoot Borer, *Chilo infuscatellus* Snellen.

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ABSTRACT

The granulosis virus of the sugarcane shoot borer, *Chilo infuscatellus* Snell., was found effective when applied as egg treatment. The mortality of larvae hatched from virus-coated eggs ranged from 26.34 to 81.17 per cent at 10^5 to 10^9OB (occlusion bodies)/ml respectively. The first and second instar larvae were found highly susceptible when fed with virus-contaminated food. The mortality varied from 69.05 to 96.67 per cent at 10^3 to 10^7 OB/ml. When microfed with the virus, third, fourth and fifth instar larvae were killed to an extent of 81.44, 64.15 and 54.62 per cent respectively, when the different doses were considered together. At the highest dose tried (10^7 OB/larva), the virus caused 100.0, 81.90 and 72.01 per cent mortality in third, fourth and fifth instar larvae respectively. The median lethal dose (LD_{50}) of the virus was 1061.2 and 8128.3 OB in third and fourth instar respectively. The LT_{50} decreased with increase in age of the larvae or decrease in the dosage of the virus.

Key words: *Chilo infuscatellus*, Granulosis virus, pathogenicity, LD_{50} and LT_{50}

The sugarcane shoot borer, *Chilo infuscatellus* Snell., is distributed in all the sugarcane growing areas of India and is a key pest in the early stages of crop growth causing economic losses (Avasthy and Tiwari, 1986). A granulosis virus is found to infect the larvae of shoot borer (Easwaramoorthy and David, 1979) which is widely distributed in the different agroclimatic zones surveyed in Tamil Nadu and Pondicherry (Easwaramoorthy and Jayaraj, 1987). Studies carried out at Coimbatore indicated the occurrence of the virus under natural conditions throughout the year (Easwaramoorthy, 1984). However, no information is available on the virulence of the virus and hence the present study was carried out to determine the pathogenicity of the virus to different instars of shoot borer larvae.

MATERIALS AND METHODS

Field-collected shoot borer larvae were reared on sugarcane shoot bits (Easwaramoorthy and Jayaraj, 1987). The pupae obtained were disinfected with one per cent sodium hypochlorite and kept on moist synthetic sponge in emergence boxes. The adults emerged were released into egg-laying cages, which consisted of a glass jar closed at the top with muslin cloth. The egg masses laid on sugarcane leaves and trashes were collected when they turned black and were disinfected. The larvae 12-24 h after moulting to the respective instars were used for different studies.

The original stock culture of the virus maintained in the laboratory was multiplied by microfeeding third to fifth instar shoot borer larvae and collecting the diseased larvae. The virus was purified and finally pelleted by centrifugation at 17,000 rpm for 30 min. at 5°C in a refrigerated centrifuge. The capsules were counted, using a Petroff Hauser and Helber counting chamber (depth 0.02 mm) under a phase contrast microscope.

Shoot borer eggs of 2 and 4 days age were selected for the study. The egg masses were painted *in situ* with different concentrations of the virus using a camlin hair bursh. Teepol (Sodium secondary alkyl (C 10 - C 18) sulphate) 0.5% was added to the virus suspension as a wetting agent. Suitable control was also maintained. The egg masses when turned to 'black-head stage' were clipped along with a bit of leaf and kept in the laboratory at 28 ± 1°C. The larvae that hatched were transferred to sugarcane leaf whorls for feeding. The leaf whorls were changed daily four days after the start of the experiment and mortality of the larvae was recorded. The experiment was terminated 15 days after the start.

Pathogenicity of the virus to first and second instar larvae was tested by shoot contamination method. The shoot pieces (5-6 cm length) were split open at both the ends and the whorls were loosened before treatment. They were dipped in the virus suspension of concentration varying from 1.1

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Table 1. The effect of GV treatment of eggs on shoot borer larval mortality

Dose (OB/ml)	% larval mortality		
	2 days old egg	4 days old egg	Mean
1.1×10^9	82.46 (67.24)	79.88 (64.32)	81.17 (65.78)
1.1×10^8	65.14 (54.01)	65.22 (54.08)	65.18 (54.05)
1.1×10^7	49.31 (44.59)	49.42 (44.67)	49.37 (44.63)
1.1×10^6	45.00 (42.03)	42.81 (40.81)	43.91 (41.42)
1.1×10^5	26.85 (31.14)	25.82 (29.48)	26.34 (30.31)
Mean	53.75 (47.80)	52.63 (46.67)	

(Figures in parentheses are arc sine $\sqrt{\text{percentage}}$ values

	S.E.	C.D. (P = 0.05)
Dose	3.47**	10.14
Age of eggs	2.00^{NS}	-
Dose x age of eggs	4.91^{NS}	

x 10^3 to 10^7 OB.ml for 2 min. Teepol 0.5% was added as a wetting agent. The shoot pieces were dried under shade and transferred to plastic boxes (7 cm dia x 7.5 cm ht) @ one piece/box. Single host larva of the particular instar was allowed to feed on the virus-contaminated shoot for 48 h. After that, the shoot was removed and uncontaminated shoot piece was provided as feeding material. The control larvae were fed with uncontaminated shoot pieces from the beginning of the experiment. The shoot pieces and filter paper were changed once a day after recording data on mortality of larvae. The treatments were replicated thrice with 50 larvae per replication and terminated 15 days after start.

Pathogenicity of the virus to freshly moulted third to fifth instar larvae was tested by microfeeding 0.1 µl of the virus suspension using an Agla micrometer syringe (Wellcome Reagents Ltd, Beckenham, England). Care was taken to discard those larvae which failed to ingest the entire quantity of the inoculum. The control larvae were fed with an equal quantity of distilled water. The treatments were replicated 5 times with 30 larvae/replication. The larva after microfeeding were reared on shoot pieces and observed for mortality as described earlier.

To determine the LD_{50} and LT_{50}, freshly moulted third and fourth instar larva were microfed with 6 different doses viz., 1.1×10^1, 10^2, 10^3, 10^4, 10^5 and 10^6 OB/larvae. Suitable control was also maintained. One hundred larvae were used for each treatment. The mortality was recorded daily. The LD_{50} of the virus was determined by probit analysis (Finney, 1962) and the LT_{50} for the various doses was assessed following the procedure of Biever and Hostetter (1971).

RESULTS AND DISCUSSION

The data on mortality of larvae hatched from eggs painted with different doses of the virus are presented in Table 1. At 10^9 OB/ml, significantly high mortality of 81.17 per cent was observed. The mortality decreased with decrease in the virus dose and the least mortality of 26.34 per cent was observed at the dose of 10^5 OB/ml. There was no significant variation in the mortality of larvae hatched from eggs treated on the second and fourth day after egg laying at any of the doses tested. The study revealed the possibility of virus infection, when the larvae cut and ingest the virus contaminated chorion during eclosion. Yamada and Oho (1973) observed that dipping egg masses of *Adoxophyes orana* F. von R in a GV suspension resulted in high percentage of infection when the hatched larvae were reared *en masse*. Philip and Jacob (1979) also found that treatment of eggs of *Pericallia ricini* F. in GV suspension caused almost complete mortality of hatched larvae. The mortality of young larvae hatching from eggs having virus contamination on the shell surface, acquired from the mother during oviposition, or from the environment was noticed in *Zeiraphera diniana* (Gn.) (Schmid, 1976) and *Corpocapsa pomonella* (L.) (Etzel and Falcon, 1976).

Table 2 Mortality of first and second instar larvae due to GV fed by food contamination method

Dose (OB/ml)	% mortality		
	I instar	II instar	Mean
1.1×10^7	93.33 (81.35)	100.00 (90.00)	96.67 (85.57)
1.1×10^6	93.33 (81.15)	93.33 (81.15)	93.33 (81.15)
1.1×10^5	80.95 (68.95)	80.95 (64.41)	80.95 (66.68)
1.1×10^4	76.19 (61.04)	66.67 (54.81)	71.43 (57.92)
1.1×10^3	76.19 (61.04)	61.90 (51.94)	69.05 (56.49)
Mean	84.00 (70.67)	80.57 (68.46)	69.05 56.49)

	S.E.	C.D.(P = 0.05)
Dose	4.21**	12.29
Instar	2.43^{NS}	-
Dose x Instar	5.95^{NS}	-

This will have practical significance in the applied control of the borer, as it is an internal tissue feeder. The shoot borer larvae after hatching remains outside the plant only for a few days before entering into the shoot/cane, and once they enter the sugarcane plant, the chance of contracting the virus is only during their migration from one plant to another. So, when the virus application is also directed to the egg masses, the emerging offsprings will contract the virus in the early stages of their development and die quickly without causing much damage to the crop.

Significantly high mortality of first and second instar larvae was noticed when they were fed with shoots dipped in virus at 10^7 (96.67%) and 10^6 (93.33%) OB/ml (Table 2). At 10^5 OB/ml, 80.95 per cent mortality was observed and it was further reduced at 10^4 and 10^3 OB/ml. However, the differences observed among the latter three treatments were not significant. The mortality of first and second instar larvae did not vary significantly at any of the doses tested. Similar results were obtained with *Pseudaletia unipuncta* (Haworth) (Tanada, 1956) and *Chilo sacchariphagus indicus* (K.) (Easwaramoorthy, 1984). In peroral infection experiments, the average mortality of first and second instar larvae of *P. unipuncta* was 80.8 and 92.7 per cent respectively.

The third, fourth and fifth instar larvae when microfed with virus at the dose of 10^7 OB/larva showed 100.0, 81.90 and 72.01 (mean 84.64) per cent mortality respectively (Table 3). At 10^6 OB/larva, the mean mortality observed was 74.53 per cent. However, the differences were not statistically significant. Similarly, there was no significant difference in the mean mortality observed at 10^5 (66.68) and 10^4 (60.63) OB/larva. At 10^3 OB/larva, significantly low mortality of 45.62 percent was observed.

When all the doses were considered together, significantly high mortality of 81.44 per cent was observed in the third instar. The mortality observed in the fourth instar was 64.15 percent and in fifth instar significantly low mortality of 54.62 per cent was observed. However, the differences observed in mortality of different instars at a particular dose and mortality of a particular instar fed with different doses were not significant.

Melamed-Madjar and Raccah (1979) working with GV infection in *Sesamia nonagrioides* (Lef.) reported that the rate of disease and death in larvae on 7th and 14th days were high, only 19 and 16 per cent respectively emerging as adults. On the other hand, the death rate of larvae inoculated on the 21st day, though having a similar disease rate as larvae inoculated on earlier age, was significantly low and the pupation rate was almost double. Boucias and Nordin (1977) also observed a decrease in the susceptibility of *Hyphantria cunea* (Drury) to GV infection as the larvae developed. But mortality data expressed in terms of dose/mg of body weight demonstrated that this decrease in susceptibility was due only in part to differences in weight of the larvae tested.

Table 3. Mortality of third to fifth instar larvae microfed with GV

Dose (OB/larva)	% Mortality				Time taken for kill (days)			
	III instar	IV instar	V instar	Mean	III instar	IV instar	V instar	Mean
1.1×10^7	100.00 (90.00)	81.90 (69.52)	72.01 (58.80)	84.64 (72.77)	10.8	11.9	13.5	12.1
1.1×10^6	91.07 (75.69)	73.81 (59.44)	58.72 (50.19)	74.53 (61.77)	12.1	12.5	13.8	12.8
1.1×10^5	85.19 (71.77)	63.18 (53.04)	51.39 (54.84)	56.59 (56.88)	13.4	14.6	15.5	14.5
1.1×10^4	78.57 (67.59)	56.67 (59.22)	46.67 (43.04)	60.63 (53.30)	13.8	15.8	16.0	15.2
1.1×10^3	52.38 (46.36)	45.18 (42.23)	39.29 (38.81)	45.62 (42.47)	14.1	16.8	18.0	16.4
Mean	81.44 (70.28)	64.15 (54.69)	54.62 (47.34)		12.8	14.3	15.4	

	Mortality		Time taken for kill	
	S.E.	C.D. (P = 0.05)	S.E.	C.D. (P = 0.05)
Dose	3.63**	10.43	0.50**	1.4
Instar	2.57**	7.38	0.43**	1.1
Dosex instar	6.29 NS	-	0.86 NS	-

The time taken for mortality decreased significantly with increase in the dose of the virus (Table 3). It was significantly low in the third instar (10.8-14.1 days) compared to fourth (11.9-16.8 days) and fifth (13.5-18.0 days) instars. Boucias and Nordin (1977) also reported that the incubation time of GV in *H. cunea* was dependent on the age of the larvae and the dose of the virus assayed. Similar results were obtained in *P. ricini* (Philip and Jacob, 1979).

Interestingly this experiment also revealed that the incubation period in fourth and fifth instar larvae varied from 11.9 to 16.8 and 13.5 to 18.0 days respectively, though freshly moulted normal larvae required only 9 to 18 and 6 to 12 days respectively to pupate. This clearly indicates that the GV infection prolonged the larval period. Whitlock (1977) also reported that the larval period in *Heliothis armigera* Hbn. was increased from the normal (18-22 days) to an average of 27 days and could be as long as 41 days due to GV infection.

The median lethal dose of the virus was 1061.2 OB/larva in the third instar (Fig.1) which increased to 8128.3 OB/larva in the fourth instar. Such variation in LD_{50} was observed in other insects. For instance, the median lethal doses for *P. brassicae* ranged from 66 OB in first instar to 2.3×10^7 OB in fifth instar (Payne *et al.*, 1981).

The time taken for initial mortality and LT_{50} increased as the instar advanced from third to fourth at all the doses tested (Table 4). Philip and Jacob (1979) reported that the LT_{50} increased from 3.37 days in first instar to 8.52 days in fifth instar

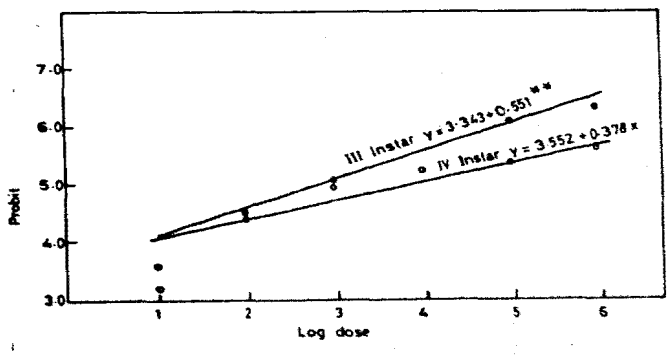

Fig. 1. Dosage - mortality curve for third and fourth instar larvae of shoot borer

Table 4. LT_{50} for third and fourth instar larvae due to GV

Dose (OB/larva)	LT_{50} (Days)	
	III instar	IV instar
1.1×10^6	9.6	9.8
1.1×10^5	9.7	10.1
1.1×10^4	10.3	11.5
1.1×10^3	10.8	12.6
1.1×10^2	11.9	13.8
1.1×10^1	12.8	15.3

of *P. ricini* infected by GV. Similar results were obtained by Payne *et al.* (1981) in *P. rapae* (Linn.) and *P. brassicae*. In a particular instar, the LT_{50} decreased as the dosage was increased. The LT_{50} was 12.8 days at the lowest dose and 9.6 days at the highest dose in the third instar and the differences observed between the lowest and highest dose was 5.5 days in fourth instar.

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