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Environmental Persistence of Granulosis Virus Infecting Sugarcane Shoot Borer, *Chilo infuscatellus* Snellen

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ABSTRACT

Thermal inactivation of the granulosis virus (GV) of *Chilo infuscatellus* Snellen occurred in the temperatures between 80 and 90°C. The pH 4 to 6 and 10 showed adverse effect on the virus infectivity and the ultraviolet light inactivated the virus completely within 20 minutes of exposure. The dry deposit of the virus was not inactivated as readily as virus in aqueous suspension. The persistence of shoot borer GV was inversely related to the exposure time and the half life was found to be 6.8 days.

Key words : *Chilo infuscatellus*, GV, environmental persistence

INTRODUCTION

A granulosis virus (GV) has been isolated from sugarcane shoot borer, *Chilo infuscatellus* Snellen (Crambidae, Lepidoptera) a serious pest of sugarcane in the early stages of crop growth by Easwaramoorthy and David (1979). The virus was found naturally occurring in the sugarcane growing belts of Tamil Nadu and Pondicherry states in south India. The natural mortality due to viral infection varied from 2.6 to 14.6 per cent (Easwaramoorthy and Jayaraj, 1987b). The virus was found active throughout the year irrespective of the season. The laboratory studies showed that the virus was highly pathogenic and effective in checking the pest under pot culture (Easwaramoorthy, 1984) and field conditions (Easwaramoorthy and Jayaraj, 1987a). The results of studies on some of the environmental persistence characteristics of the GV are reported in this paper.

MATERIALS AND METHODS

i. Virus suspension

The virus was harvested from larvae of *C. infuscatellus*, killed in the laboratory by GV. The virus suspension used in the studies was purified several times using alternate cycles of low (500 rpm) and high speed (10,000 rpm) centrifugation. Finally, the virus was sedimented by centrifugation at 17,000 rpm for 30 min at 5°C in a refrigerated centrifuge. Counting of occlusion bodies (OB) was done using a Petroff Hauser and Helbar counting chamber with 0.02 mm depth under a phase contrast microscope and number of OBs in original stock suspension was calculated using the formula given by Kolmer and Boener (1945).

ii. Rearing of test insects

The healthy larvae 12-24 h after moulting to third instar were used in the studies. They were reared on sugarcane shoot bits (5-6 cm length) split open at one end, at the rate of five larvae in plastic boxes (7.0 cm dia x 7.5 cm height) provided with filter paper at the bottom to absorb excess moisture. The filter paper and shoot bits were changed daily after recording mortality due to virus and other causes.

iii. Persistence on foliage

To determine the persistence of the GV on sugarcane foliage, aliquots of 0.5 ml of virus suspensions were painted on 25 cm^2 of leaf area marked with a ball-point pen on the upper surface of leaves of sugarcane plants (variety Co 6304). The deposits were exposed for 1, 2, 3, 4, 6, 8 and 10 days during December 1983. At each sampling, the entire 25 cm^2 area was cut from representative leaves and resuspended in 5 ml of sterile water. Then the virus suspension was microfed (using Alga micrometer syringe, Wellcome Reagents Ltd, Beckenham, England) to third instar larvae at the dose of 1.1×10^6 OB/larva. Unexposed virus served as control to determine the pathogenicity of the GV while untreated larvae served as overall control. The experiment was replicated three times with 25 larvae/replication. Viral persistence was calculated as per cent original activity remaining (OAR) (Ignoffo *et al*, 1977). Using the logarithmic values of OAR,

the half life of the virus was calculated with the formula:

$$t\ 1/2 = \log^2/b$$

iv. **Effect of UV light**

Aliquots of 1 ml virus containing 1.1 x 10^9 OB/ml were taken in plastic petri dishes (15 x 60 cm) with lids removed. In one set, the aliquots were dried under shade and in another they were retained as suspensions. A germicidal lamp (253.7 nm) was used for inactivation of the virus. Dry deposits and aqueous suspensions of the virus were placed at 15 cm distance from the lamp during exposure which varied from 1 to 20 min. The exposed dry deposits were suspended in sterile water and microfed 1.1 x 10^5 OB/ larva. The treatments were replicated five times with 20 larvae in each replication.

v. **Determination of thermal inactivation point**

Aliquots of 1 ml virus suspensions containing 1.1 x 10^9 OB in corning bottles were exposed to 40, 50, 60, 70, 80 and 90°C temperatures for a period of 10 min in a well stirred thermostatically controlled water bath. After this period, the bottles were removed, ice-cooled and stored in a refrigerator. The virus suspensions were microfed at the dose of 1.1 x 10^5 OB/larva within 48 hour after heat treatment. Suitable control was also maintained. The treatments were replicated five times with 20 larvae per replication.

vi. **Effect of pH**

The pH of virus suspension was adjusted to 4, 5, 6, 7, 8, 9 and 10 using a Systronics pH meter with standard buffer tablets of pH 4.8, 7 2, and 9 2 and then with N/10 hydrochloric acid or N/10 sodium hydroxide. The virus-buffer mixture was incubated at 28°C for 24 hour and then microfed to larvae at a dose of 1.1 x 10^5 OB/ larva. Suitable control was also maintained. The experiment was replicated thrice with 25 larvae in each replication.

For statistical analysis, the percentage mortality due to virus infection corrected using Abbott's formula was converted into corresponding angles and subjected to analysis of variance.

RESULTS

The results obtained in our studies indicated that the unexposed virus caused 86.7 per cent mortality of third instar shoot borer larvae and there was only a gradual loss in the virus activity upto 6 days after exposure as the differences in the mortality observed were not statistically significant ($P = 0.05$) But when the virus was exposed to 8 days, significantly ($P > 0.01$) low mortality of 33 9 per cent was observed and when exposed to 10 days, the virus lost its infectivity (Fig. 1). The virus retained about 82% OAR even on 6th day, but it was significantly reduced to 39.1 per cent on 8th day. The half-life of the virus was determined as 6.841 days. There was no significant variation in the incubation period observed (14.7 to 15.5) on 0, 1 and 2 days after exposure. But significantly longer incubation periods were noticed when the virus was exposed for three or more days.

Several studies have demonstrated that deposits of viral inclusion bodies are inactivated within a few days after

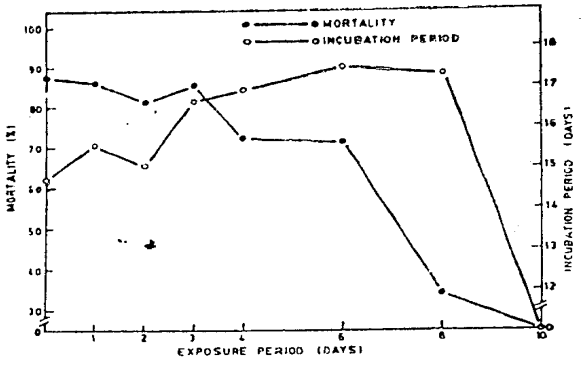

Fig. 1. Persistence of shoot borer GV on the upper surface of foliage

application to foliage of crops or trees (Tanada, 1973; Yendol and Halmen, 1973). The present study was conducted during December, when the sky was partially overcast and so the half life of the virus may be still less during periods of bright sunshine. But the virus deposited on the inner surface of leaf sheaths where the newly hatched larvae feed before boring into the plant may retain its activity for a longer period as virus sprays given at an interval of 15 days offer good protection (Easwaramoorthy and Jayaraj, 1986b). There is considerable evidence accumulated in the literature that it is the ultraviolet portion of sunlight that inactivates insect viruses (David, 1969; Jaques, 1972; Keller, 1973; Schmid, 1974; Injac, 1977). In the present study the virus in suspension was inactivated more quickly than in dry deposit.

When the virus was exposed for 1 min as dry deposit to the UV light, the mortality was reduced from 94.3 to 73.0 per cent only, whereas in the case of aqueous suspension, it was reduced greatly from 92 1 to 32.6 per cent. The virus exposed as suspension was inactivated rapidly and there was only 8.7 per cent mortality at 5 min exposure time and there was no mortality at 10 min exposure time. But in the case of dry deposit, even at 10 min, 30.2 per cent mortality was observed. The virus exposed as dry deposit was completely inactivated when it was exposed to ultraviolet light for 20 min (Fig. 2). Similar observation were made ins the case of *Pieris brassicae* GV (David *et al*, 1968; David, 1969).

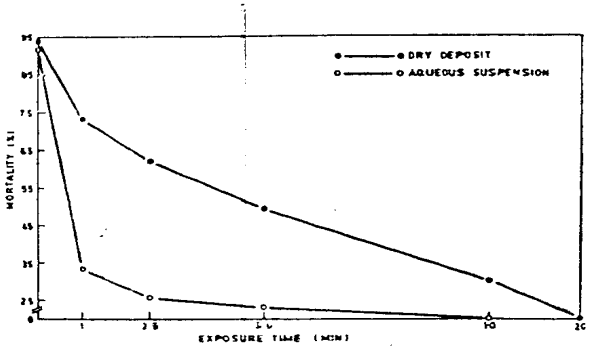

Fig. 2. Effect of UV light on the infectivity of shoot borer GV

When the virus exposed to 40°C for 10 min was fed to the larvae, 63.3 per cent mortality was observed (Fig.3). There was a progressive decrease in the mortality of the larvae with increase in temperature upto 80°C. At 90°C, there was no mortality of

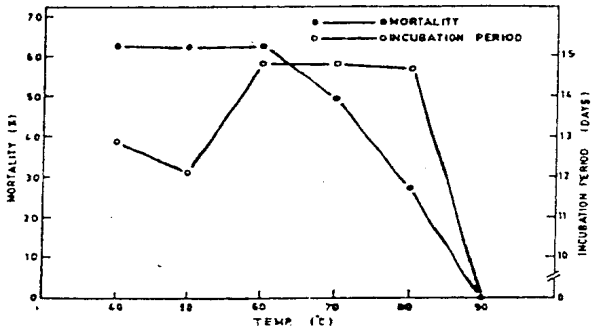

Fig. 3. Thermal inactivation point of shoot borer GV

larvae indicating that the virus was completely inactivated at this temperature. The incubation period was significantly low ($P > 0.01$) at 40 and 50°C compared to other temperatures. At higher temperatures, it ranged from 14.7 to 14.8 days.

The effect of temperature on viruses is significant with regard to their stability in storage and in the field following application. Whereas exposure to low temperatures had little effect on activity of insect viruses, high temperatures caused inactivation as reviewed by Jaques (1977). The shoot borer GV is able to tolerate a higher temperature range than the known bio-control agents of shoot borer, as the thermal inactivation occurs only at the temperatures between 80 and 90°C and thus the virus has a distinct advantage.

The mortality of larvae was significantly higher (92.4%) when fed with virus incubated at pH 8 (least effect of pH). There was no significant difference in the mortality ($P < 0.05$) at pH 9, 8 and 7. At higher pH of 10, significantly low ($P > 0.01$) mortality of 34.5 per cent was observed. Similarly, the lower pH range from 6 to 4 showed gradual adverse effect on the mortality of larvae (Fig 4). The differences observed in the incubation period were not statistically significant.

The shoot borer GV was found more stable at pH 7 to 9. Bergold (1958) stated that in order to prevent dissolution of NPVs, the pH of the suspending medium must not drop below 5 or go above 8.5. This generalization has been confirmed in

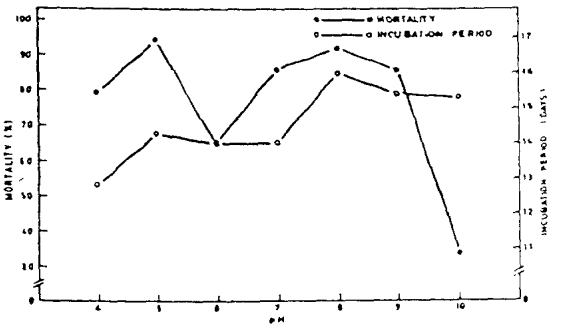

Fig. 4. Effect of pH on shoot borer GV infection

GVs also (Huger, 1963). This is of considerable importance with regard to accumulation of virus in the soil. The stability of virus in soil is an important factor in the ecology of baculoviruses and therefore in the maintenance of a virus reservoir for the initiation of natural epizootics (Tinsley, 1979). It is evident that the virus can remain active in soils suitable for sugarcane cultivation.

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Biochemical Potentiation of Nuclear Polyhedrosis Virus of *Spodoptera litura (F.)*

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ABSTRACT

Studies were conducted to potentiate NPV of *Spodoptera litura* by biochemical adjuvants for maximisation of control of the pest in FCV tobacco nurseries and field crop. The studies established that addition of tannic acid 0.25% and boric acid 0.35 % gave maximum efficacy. The significance of this finding is that boric acid and tannic acid, the commonly used chemicals in tobacco culture, are not only compatible with NPV but also potentiate it, thereby satisfying the basic concept of compatibility of entomoviruses with agricultural chemicals.

Key words : *Spodoptera litura* NPV, potentiation, boric acid, tannic acid