J. Biol. Control, 21(1): 91-96, 2007

Susceptibility of crude and semi-purified extracts of nucleopolyhedrovirus isolates of *Helicoverpa armigera* (Hübner) to simulated sunlight

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ABSTRACT: **The stability of both crude and semi-purified extracts of different *Ha*NPV isolates under simulated sunlight was studied. It was realized that the persistence of both crude and semi-purified suspensions of Negamum isolate collected from Tamil Nadu was most tolerant to simulated sunlight among all the isolates tested in this study. The highest larval mortality (70.12%) was obtained from crude extract of the Negamum isolate as compared with that of semi-purified suspension (54.44%). The order of inhibition of crude viral extracts of *Ha*NPV isolates under simulated sunlight irradiation at 500 W/m² for 90 minutes was Negamum< Ooty< Coimbatore< Mumbai < Hyderabad< Parbhani< Rahuri, which was similar with that of the semi-purified suspensions with the exception of Parbhani < Hyderabad.**

KEY WORDS: Geographic isolates, *Helicoverpa armigera*, Nucleopolyhedrovirus, persistence, simulated sunlight

INTRODUCTION

Sunlight is the primary factor limiting viral persistence in the environment. Inactivation by sunlight is mainly due to ultraviolet light (280-400 nm) (Young, 2000). This limitation has prevented the utilization of microbial pesticides on a large scale. In many cases, baculoviruses exposed to sunlight are rapidly inactivated with a half-life of hours (Morris, 1971; Smirnoff, 1972; Ignoffo and Garcia, 1992). Several attempts have been made to increase the field persistence of insect pathogens by using different chemical and organic additives (Jacques, 1971; Rabindra and Jayaraj, 1989; Shapiro and Robertson, 1990). Shapiro (1984) suggested that use of larval tissues of gypsy moth as an adjuvant offered protection against UV radiation by increasing larval mortality by two-fold. David *et al.* (1971) and Jacques (1971) have also reported that impure viral suspension acted as UV-protectant.

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In the present investigation, susceptibility of processed and crude extracts of different geographical isolates of *Ha*NPV to simulated sunlight was evaluated using the Atlas CPS+ Suntest machine.

MATERIALS AND METHODS

Healthy culture of *H. armigera* was maintained in the laboratory on a semi-synthetic diet (Shorey and Hale, 1965). Seven isolates of *Ha*NPV from the microbial repository of the Project Directorate of Biological Control (PDBC), Bangalore, were used in this study. These isolates were passaged through early fifth instar larvae of host insect at 25±1°C to get uniformity in their virulence since they were stored for unequal time at 3±2°C in a refrigerator. Bioassays were conducted in a facility far away from the healthy colony (Bio-Control Research Laboratories (BCRL), Bangalore) during January-April 2006. The viral isolates used in the study and their abbreviations are listed in Table 1. A sunlight simulator, Atlas Suntest CPS+/ XLS+ (Atlas Material Testing Technology GmbH, Vogelsbergstra âe, 22), was used as a source of simulated sunlight, which mimicked the natural sunlight both in intensity as well as spectrum. This device which is available in PDBC, uses a xenon lamp (ranges from 250 W/m² up to 765 W/m2) with a filter to illuminate a chamber of 20×28×21cm area with light similar to the solar spectrum at the Earth's surface, from UV-B through the visible portion of the spectrum (Farrar *et al.*, 2003; Lacey and Arthurs, 2005). McGurie *et al.* (2000) tested this machine and found that the rate of degradation of *Anagrapha falcifera* NPV under simulated sunlight was similar to that under natural sunlight when exposure was expressed as cumulative total energy (Joules/m²). They also stated that the solar simulator provided light at a flux level higher than sunlight (*i.e.*, greater intensity of irradiance). This enables faster exposure in the simulator to equivalent amounts of accumulated were radiant energy in the environment.

Five hundred micro-liters of each viral isolate (1×10^7 POB/ml) in 0.1% Teepol, both unprocessed (crude) and processed (semi-purified), were applied onto the surface of plastic sheets (6×12 cm) using a micropipette. The suspension was spread uniformly over the sheets with the polished blunt end of a sterile 6 mm glass rod and air-dried before exposing to simulated sunlight. These sheets were irradiated in a suntest machine at 500 W/m² for 90 minutes. Temperatures during exposure were maintained at 35°C.

Table 1. *Ha*NPV isolates studied for strain selection

Sl.no.	Origin	Abbreviation
1	Parbhani, Maharashtra	PRB
2	Ooty, Tamil Nadu	OTY
3	Coimbatore, Tamil Nadu	CMB
4	Negamum, Tamil Nadu	NGM
5	Mumbai, Maharashtra	MUM
6	Rahuri, Maharashtra	RHI
7	Hyderabad, Andhra Pradesh	HYD

After exposure, the virus deposits were eluted with distilled water and collected in eppendorf tubes, labeled, and re-enumerated. Bioassays were performed with a concentration of 1×10^5 POB/ml of each isolate on second instar larvae of *H. armigera*. The larvae after inoculation were incubated at 25±1°C in an incubator. Each treatment was replicated thrice, with 30 larvae per replication. Non-irradiated virus for each isolate served as a control in addition to a general check. Larval mortality was recorded daily for a period of 10 days and per cent corrected mortality (CM%), per cent original activity remaining (OAR%), and per cent inhibition of viral activity (IVA%) were computed for each viral isolate based on the following formula. Larval mortality obtained with both crude and semi-purified NPV was subjected to Student's t test, whereas the differences were evaluated by DMRT within the groups.

$$\text{OAR}\,(\%) = \frac{\text{NPV caused larval mortality Post-UV exposure}}{\text{NPV caused larval mortality Pre-UV exposure}} \times 100$$

$$\text{IVA}(\%) = \frac{\text{Per cent larval mortality (before exposure)} - \text{Per cent larval mortality (after exposure)}}{\text{Per cent larval mortality (before exposure)}} \times 100$$

RESULTS AND DISCUSSION

The per cent larval mortality of *H. armigera* larvae due to crude extracts of all *Ha*NPV isolates was significantly higher (Student's T test, p=0.05) than that of the semi-purified extracts (Table 2). The highest mortality was obtained with crude extracts of NGM isolate (70.1%), whereas, it was 54.4 per cent in the case of the semi-purified extracts. The order of inhibition of crude viral extracts was NGM< OTY< CMB< MUM< HYD< PRB< RHI, which was similar with that of the semi-purified suspensions with the exception of PRB< HYD. The per cent original activity remaining after exposure to simulated sunlight in of NGM isolate was 79.4 and 54.5 for crude and semi-purified extracts, respectively. The order of original activity remaining for semi-purified extracts of virus was NGM< OTY< CMB< MUM< PRB< HYD< RHI. But the order of original activity remaining for crude extracts was same as that of semi-purified virus except RHI> HYD (Fig. 1).

Data on the stability of both crude and semi-purified suspensions of *Ha*NPV isolates under simulated sunlight condition showed that crude

Table 2. Effect of simulated sunlight on the virulence of crude and semi-purified extracts of *Ha*NPV isolates against second instar larvae of *H. armigera*

*Ha*NPV† isolates	Treatment	Per cent larval mortality	
		Crude(Unprocessed) NPV	Semi-Purified(Processed) NPV
NGM	Simulated sunlight	70.1 ± 0.5**a (20.5)	54.4 ± 0.4a (45.5)
	Unexposed	88.4	99.9
OTY	Simulated sunlight	69.6 ± 1.0**a (21.2)	54.0 ± 0.3a (45.8)
	Unexposed	88.2	99.5
CMB	Simulated sunlight	67.0 ± 1.2**b (21.9)	53.4 ± 0.5a (46.5)
	Unexposed	85.7	99.9
MUM	Simulated sunlight	66.9 ± 0.6**b (23.3)	52.2 ± 0.3ab (47.5)
	Unexposed	87.2	99.5
PRB	Simulated sunlight	67.0 ± 0.7**b (24.3)	51.7 ± 0.2b (47.8)
	Unexposed	88.4	99.1
HYD	Simulated sunlight	61.7 ± 1.3**c (23.5)	50.1 ± 0.8bc (49.8)
	Unexposed	85.2	99.7
RHI	Simulated sunlight	62.6 ± 1.2**c (27.5)	49.8 ± 0.9c (50.0)
	Unexposed	86.4	99.5

† All the treatments contained NPV at 1×10^5 POB/ml.

In parentheses, figures present per cent inhibition of viral activity.

In a column means followed by the same letters are not significantly different (p=0.05) by DMRT.

**: Highly significant between the extracts by Student's "T" test.

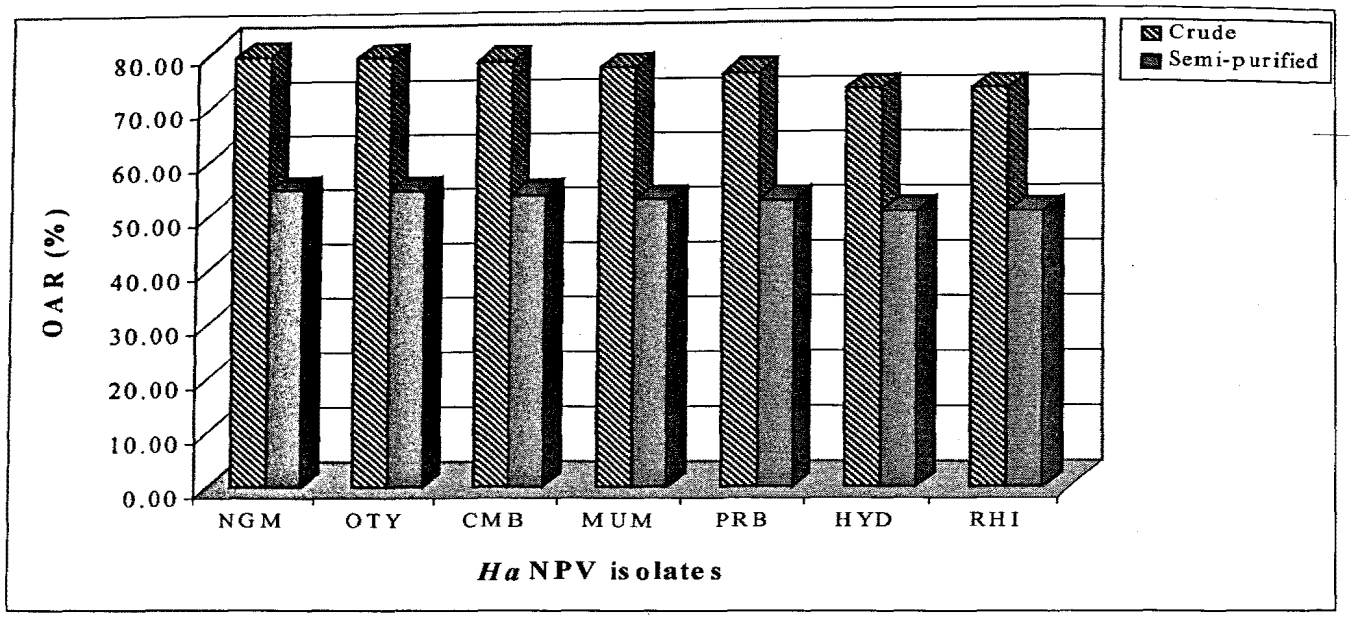

Fig. 1. Per cent original activity remaining (OAR%) of crude and semi-purified extracts of *Ha*NPV (1×10^5 POB/ml) isolates after exposure to simulated sunlight (at 500 W/m² for 90 minutes) against second instar larvae of *H. armigera*

extract of NGM isolate was more tolerant to simulated sunlight in all the isolates tested in this study (Table 2). Larval body fluid and debris in the crude extracts of the isolates probably acted as a UV-protectant and increased the larval mortality in significant proportions.

These findings are in accordance with those of David *et al.* (1971) and Jacques (1971) who reported that insect body impurities absorbed UV radiation and protected the virus. Shapiro (1984) also could increase virus-caused mortality of *Lymantria dispar* NPV using tissue extracts of the host larvae which acted as UV-protectant. Larval extracts also can act as a phagostimulant adjuvant to enhance the activity of the virus (Rabindra and Jayaraj, 1989).

The findings indicate that it is not necessary to subject the virus to an expensive processing to produce an extremely pure form of virus for making a viral formulation. Retention of some quantity of larval debris in the formulation in fact may enhance the activity of the virus on host plants. However, care should be taken to ensure that a semi-purified product does not have secondary microbial contaminants above the levels prescribed by the regulatory authorities.

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(Received: 07.06.2006; Revised: 05.01.2007; Accepted: 15.01.2007)