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Effect of natural sunlight on the activity of different geographic isolates of nucleopolyhedrovirus of *Helicoverpa armigera* (Hübner)

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ABSTRACT: **Susceptibility of seven geographic isolates of *Helicoverpa armigera* NPV to sunlight was evaluated by subjecting them to different exposure times (0, 3, 6, 12, 24 and 36 hours). The viral dose of exposure was 1×10^7 POB/ml and bioassays were performed at a dose of 1×10^5 POB/ml of each virus isolate. The study showed that exposure to natural sunlight affected the activity of the different HearNPV isolates. About six hours of exposure reduced the activity of the viral isolates by nearly 50 percent. By 36 hours, all the isolates had lost their activity by about 70 percent. There were no significant differences in the susceptibility of different isolates to natural sunlight. However, NGM isolate showed the lowest inactivation after 36 hours of exposure. Time-mortality response of HearNPV isolates indicated that exposure to natural sunlight beyond 12 hours declined the viral activity to the extent of 50 per cent. The order of IT_{50} values for the isolates was Negamum >Ooty >Coimbature >Mumbai >Parbhani >Hyderabad >Rahuri.**

KEY WORDS: Geographic isolates, *Helicoverpa armigera*, natural sunlight, nucleopolyhedrovirus, persistence

INTRODUCTION

The nucleopolyhedrovirus (NPV) has been found to be effective in the control of *Helicoverpa armigera* (Hübner) on crops like chickpea, pigeonpea, soybean, sunflower, cotton, tomato and sorghum (Jayaraj *et al.*, 1989; Rabindra and Jayaraj, 1995). A major problem in the effective utilization of NPVs is the lack of persistence in the field (Moscardi, 1999). Among the factors influencing the effectiveness of baculoviruses, solar radiation is the primary limiting activity in the environment. Inactivation by sunlight is mainly due to its ultraviolet (UV) spectrum (Young and Yearian,

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1974). However, different geographic isolates of baculoviruses show different responses to solar UV. Witt and Stairs (1975) reported a thousand-fold difference in susceptibility to UV light in the virion population of *Galleria mellonella* NPV. The present investigation was, therefore, undertaken to evaluate the relative susceptibility of different geographic isolates of HearNPV to natural sunlight radiation.

MATERIALS AND METHODS

A laboratory culture of *H. armigera* was maintained on hydrated chickpea seeds based semi-synthetic diet (Shorey and Hale, 1965). The HearNPV isolates used in this study were obtained from PDBC, Bangalore (Table 1). Since the samples of these isolates had been stored under refrigerated condition for various periods, initial serial passages of the viral isolates (1×10^7 POB/ml) were made in early fifth instar larvae of *H. armigera* incubated at 25±2°C.

Table 1. HearNPV isolates studied for strain selection

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

A dose of 1×10^7 POB/ml of each viral isolate was prepared in Teepol (0.1%) and 500 µl of the virus was applied onto the surface of plastic sheets (6×12 cm) using a micropipette. The suspension was spread uniformly over the sheets with the blunt end of a sterile 6 mm polished glass rod. After air-drying, the sheets were exposed to direct sunlight for 0, 3, 6, 12, 24, and 36 hours during the month of March 2006. The virus isolates were exposed daily from 10.00 AM to 4.00 PM (6 hours per day). Hence, in the case of 6, 12, 24, and 36 hours of exposure period, viruses were exposed for 1, 2, 4, and 6 days, respectively. After exposure, the virus deposits on the sheets were eluted with distilled water and the suspensions collected in eppendorf tubes, re-counted, and kept in a refrigerator. The activity of the virus was determined by bioassays adopting diet surface treatment method. Serial dilutions were made to reach the concentration 1×10^5 POB / ml and an aliquot of 10µl virus was applied on the diet surface filled in 5 ml glass vials for each isolate. The droplets were spread uniformly over the diet surface with the aid of the rounded and polished tip of a sterile glass rod. Second instar *H. armigera* larvae of uniform age and size were individually released into the glass vials containing a semi-synthetic diet and plugged with sterile cotton. Each treatment was replicated three times with 30 larvae in each replication. Untreated control was maintained with the same number of larvae for each isolate. Mortality data were recorded daily for a period of 10 days. Corrected mortality (CM%), inhibition of viral activity (IVA%), and median inhibitory time (IT_{50}) were computed for each viral isolate.

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

RESULTS AND DISCUSSION

Studies conducted with virus exposed to direct sunlight at different exposure periods indicated that viral inactivation rate under direct sunlight increased as the period of exposure prolonged. After six days (36 hours) of exposure, larval mortality due to the virus showed insignificant differences among the isolates (Table 2). A significant difference ($P = 0.05$) in larval mortality was seen between days of exposure, in which the virus-caused mortality was the highest in NGM isolate (Table 3). The inactivation of viral isolates (IVA%) was the highest at sixth day and ranged from 73.4 (HYD) to 70.3 (NGM) per cent under direct sunlight. Also, NGM isolate was found to be the most UV-tolerant isolate as its IT_{50} value

Table 2. Effect of natural sunlight on the virulence of HearNPV isolates against second instar larvae of *H. armigera*

HearNPV† isolates	Per cent larval mortality after different hours of exposure‡					
	0	3	6	12	24	36
NGM	98.6[a]	78.9[a]	51.1[a]	46.3[a]	37.7[a]	29.3[a]
OTY	99.3[a]	80.0[a]	50.7[a]	42.7[ab]	37.3[a]	28.8[a]
CMB	99.1[a]	80.7[a]	48.6[a]	40.1[ab]	36.3[a]	28.5[a]
MUM	98.8[a]	77.3[ab]	48.2[a]	39.7[ab]	35.9[a]	27.9[a]
PRB	99.4[a]	76.5[ab]	47.9[a]	39.3[ab]	35.3[a]	27.7[a]
HYD	98.1[a]	74.7[b]	46.3[a]	37.9[b]	33.7[a]	26.1[a]
RHI	98.6[a]	74.3[b]	46.7[a]	37.7[b]	34.2[a]	26.4[a]

Means followed by the same letter in a column are not significantly different (P = 0.05) by DMRT; († All the treatments contained HearNPV @ 1×10^5 POB / ml; ‡ The virus isolates were exposed daily from 10.00 AM up to 4.00 PM (6 h per day)).

Table 3. Relative inactivation of viral isolates (IVA%) after exposure to natural sunlight against second instar larvae of *H. armigera*

HearNPV† isolates	Per cent larval mortality after different hours of exposure‡				
	3	6	12	24	36
NGM	20.0[d]	48.2[c]	53.0[c]	61.8[b]	70.3a
OTY	19.5[d]	49.0[c]	57.0[b]	62.4[b]	71.0[a]
CMB	18.6[d]	51.0[c]	59.6[b]	63.4[b]	71.3[a]
MUM	21.7[d]	51.2[c]	59.8[b]	63.7[b]	71.7[a]
PRB	23.1[d]	51.9[c]	60.4[b]	64.5[b]	72.2[a]
HYD	23.8[d]	52.8[c]	61.4[b]	65.6[b]	73.4[a]
RHI	24.6[d]	52.6[c]	61.8[b]	65.4[b]	73.2[a]

Means followed by the same letter in a column are not significantly different (P = 0.05) by DMRT;

(12.1 hours) was the highest (1.27 fold) among the isolates tested (Table 4). The order of IT_{50} values was NGM>OTY>CMB>MUM>PRB>HYD>RHI.

The results of this study showed that exposure to direct sunlight affected the activity of the different viral isolates (Tables 2 and 3). About six hours of exposure reduced the activity of the viral isolates by nearly 50 per cent. By 36 hours, all the isolates had lost their activity by about 70 per cent. There were no significant differences in the susceptibility of different isolates to natural sunlight. However, NGM isolate showed the lowest inactivation after 6 days of exposure.

Table 4. Probit analysis of exposure time-inhibitory response of second instar larvae of *H. armigera* to direct sunlight

HearNPVIsolates	IT_{50} values (h)	Fiducial limit		Slope "b"±SE	χ^{2*}(n-2)	Relative activity†
		Lower	Upper			
NGM	12.1	8.4	18.8	1.32±0.10	4.58	1.27
OTY	11.1	7.7	17.2	1.31±0.01	3.69	1.17
CMB	10.7	7.1	17.4	1.32±0.10	5.53	1.12
MUM	10.4	7.0	16.6	1.30±0.10	6.74	1.09
PRB	9.9	6.7	15.4	1.30±0.10	4.63	1.04
HYD	9.7	6.7	15.0	1.29±0.10	5.26	1.02
RHI	9.5	6.5	14.7	1.28±0.10	4.11	1.00

* All lines are insignificant at $P<0.05$; † All lines were compared with RHI as the lowest IT_{50}.

Exposure time-inhibitory response of HearNPV isolates indicated that exposure to natural sunlight beyond 12 hours reduced the viral activity to the extent of 50 per cent (Table 4). Inactivation of HearNPV on host plants exposed to direct sunlight has been reported earlier by Rabindra and Jayaraj (1988) on chickpea. Cantwell (1967) reported that *Trichoplusia ni* NPV was completely inactivated by direct sunlight within 3 hours. Morris (1971) found that the *Lambdina fiscellaria lugubrosa* NPV lost most of the activity beyond one day of exposure to sunlight. Similar findings on inactivation of *Neodiprion swainei* NPV under solar sunlight were reported by Smirnoff (1972). These studies indicate that in order to enhance the persistence of the virus in the field, future studies on selection of strain for enhancing the persistence should involve natural sunlight. Utilization of a strain tolerant to sunlight in order to increase the persistence of baculoviruses on the crop foliage to provide prolonged suppression of the pest would be crucial to the successful deployment of baculoviruses in pest management.

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