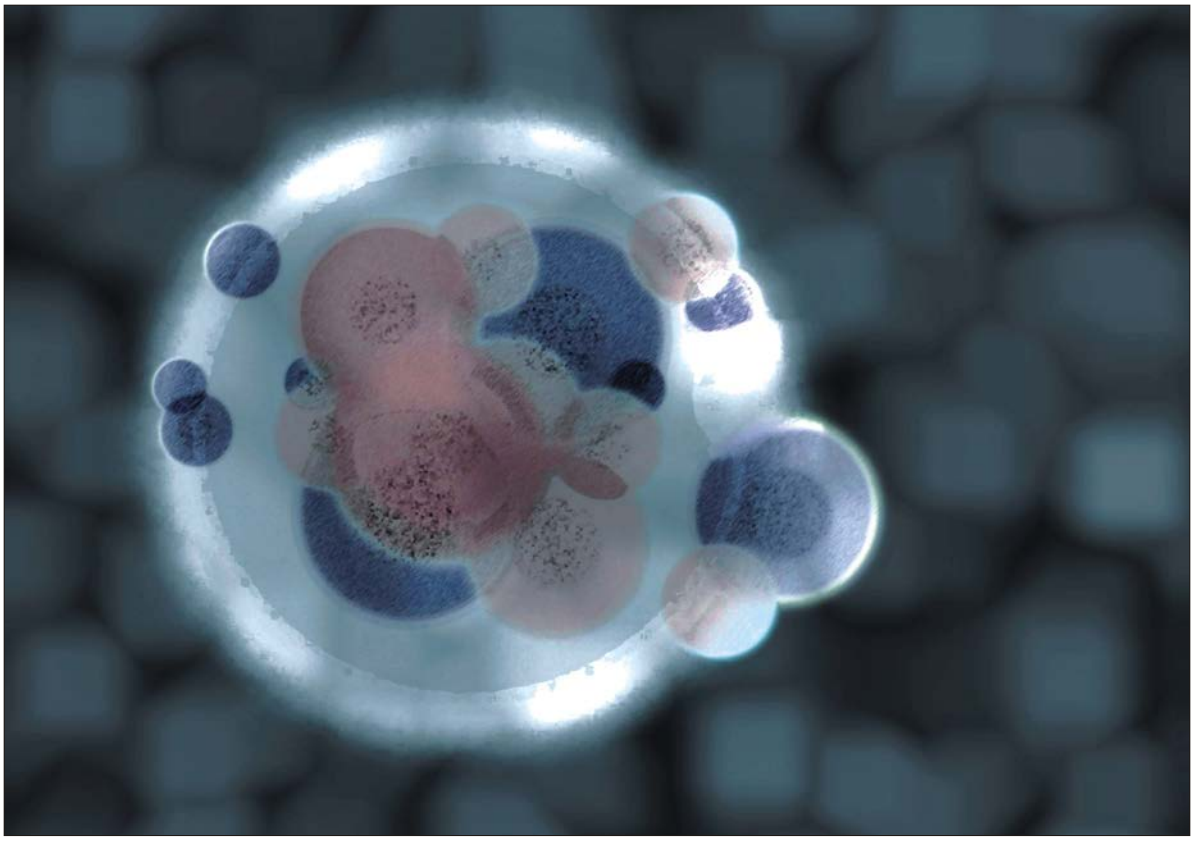

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Persistence of Different Geographical Isolates of *Helicoverpa armigera* Nucleopolyhedrovirus in Two Types of Soils under Different Conditions

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Abstract: An experiment was conducted to evaluate the persistence of *Helicoverpa armigera* NPV after 6 months of storage in two types of soil viz., black soil and red soil under different conditions to screen the relatively persistent HearNPV geographical isolate. The isolates of the virus used in this study were CMB (Coimbatore), NGM (Negamum) and OTY (Ooty) all collected from Tamil Nadu, India. Two types of soils viz., black soil and red soil were selected to study the persistence of HearNPV. Each virus isolate (1×10^9 OB mL^{-1}) was added to 2 kg of each soil and kept in indoor and outdoor conditions in plastic troughs for 6 months. Assays were carried out with extracted viruses from each soil at monthly intervals against early second instar larvae of *H. armigera*. Results indicated that as the period of storage of virus in soil was advanced the mean mortality percentage declined. Also, the comparison of results showed that the virus treated black soil kept indoor could relatively retain its infectivity (52.66, 58.56 and 54.35% OAR with CMB, NGM and OTY isolates, respectively) longer period compared to the soil kept outdoor. Similar kind of trend was also noticed with red soil. However, the infectivity was relatively more in the case of HearNPV stored in red soil compared to those stored in black soil. This high amount of persistence in red soil may be due to the fact that adsorption rate was higher in red soil than black soil. However, among the isolates evaluated in this study, NGM isolate was found to be the most relatively tolerant to inactivation through the storage time.

Key words: HearNPV, geographical isolates, soil persistence, red soil, black soil

INTRODUCTION

The occlusion bodies of insect viruses are known to persist in soil for longer period of time without loss of infectivity. This property is of much practical significance as soil borne virus can initiate epizootics in subsequent seasons (Mohamed *et al*., 1982).

The remarkable stability of baculoviruses in soil has been reported by several researchers. Studies demonstrated that appreciable quantities of active virus accumulated in the upper 1 cm of the soil in plots sprayed with 10, 20 and 100 LE $acre^{-1}$ as well as unsprayed plots. After completion of the spray program soil from plots treated with 8 foliar applications of 10 LE $acre^{-1}$ contained only slightly more virus than accumulated in plots treated with 20 and 100 LE $acre^{-1}$. The accumulation of virus continued after completion of spraying and considerable quantities of active virus persisted through winter (Thomas *et al*., 1972; Thompson and Scott, 1979). Strong adsorption of virus inclusion bodies to soil particles was also reported by Hukuhara and Namura (1972). These studies proved the remarkable resistance of polyhedra to decomposition in the chemically and microbiologically complex environment. It was also demonstrated that polyhedral protein was highly resistant to microbial purification (England *et al*., 1998). Inactivation of baculoviruses was attributed to high field temperature and solar radiation of UV spectrum (Gudauskas and Canterday, 1968; Ignoffo, 1968; Young, 2000; Mehrvar *et al*., 2007b, 2008).

A nuclear polyhedrosis virus of the soybean looper *Pseudoplusia includens* was applied to soil at rates of 8, 2470, 24700 and 247000 LE ha^{-1} in soybean planting during spring in Arkansas, USA. Epizootics occurred in soybean loopers in all the treatments with mortality of 100% in the plots with higher level of NPV 6 weeks after application. Bioassay of foliage showed that virus concentrations increased as the epizootic progressed. Virus in the soil decreased drastically immediately following application (Young and Yearian, 1974).

Nair and Jacob (1988) studied the persistence of NPV of *Spodoptera mauritia* (Boisdual) in soil. The virus when incorporated in soil and kept under field conditions had a half life of 6.64 months, while it was 16.03 months for the virus in soil kept under laboratory conditions. A comparison of the LT_{50} values showed that the virus lost its virulence more rapidly when the virus treated soil was kept outdoors. When the virus stored in a refrigerator, it showed only slight loss in original activity even after 18 months (Nair and Jacob, 1988).

The effect of leaching on the persistence of nucleopolyhedrovirus of *Helicoverpa armigera* in a column of black soil using polyhedra pre-labeled with an isotope ^{32}P was studied by Narayanan and Jayaraj (1988). The study revealed that the virus persisted in the upper layer of soil capable of contaminating leaves of crop plants and reinfecting the larvae. The top surface of column of soil at 0.6 cm depth recorded 59.35% radioactivity and decreased as the distance from the surface increased.

The purpose of the present study was directed towards evaluating the persistence of *Helicoverpa armigera* NPV after 6 months of storage in two types of soil viz., black soil and red soil under different conditions to screen the relatively persistent HearNPV geographical isolate under the same conditions.

MATERIALS AND METHODS

A laboratory culture of *H. armigera* was maintained on a semi-synthetic diet based on hydrated chickpea seeds (Mehrvar *et al.*, 2007a, b).

The HearNPV isolates used in this study were obtained from Project Directorate of Biological Control (PDBC), Bangalore, India. The isolates of the virus used were CMB (Coimbatore, Tamil Nadu, India), NGM (Negamum, Tamil Nadu, India) and OTY (Ooty, Tamil Nadu, India). Since, the samples of these isolates had been stored under refrigerated condition (3±2°C) for various periods, initial serial passages of the viral isolates (1×10^7 POB mL^{-1}) were made in early 5th instar larvae of *H. armigera* incubated at 25±1°C. The virus isolates were multiplied and bioassayed in a facility away from the host culture laboratory in BCRL (Bio-Control Research Laboratories), Bangalore, India.

Two types of soils viz., black soil and red soil were selected to study the persistence of HearNPV. For each vial isolate 2 kg of each black and red soil was taken in a plastic trough. Hundred milliliter suspension of each virus isolate containing 1×10^9 OB mL^{-1} was added to the soil. One set of the trough containing both the soils was kept indoor and another set kept outdoor. Watering was done regularly up to the saturation point. Soil samples were drawn at monthly interval randomly and virus was extracted from the soil by adopting the following procedure up to 6 months starting from May 2005.

The soil sample of 50 g was taken randomly from the respective trough and mixed with 500 mL of distilled water in a flask. The flask was shaken in a laboratory shaker incubator for 30 min and the suspension filtered through a sieve. Large particles in the sample were allowed to settle down and supernatant was collected. The extraction procedure was repeated two times for each sample. The supernatant was subjected to the differential centrifugation as described by Mehrvar *et al.* (2007b). Strength of the semi-purified suspension was assessed and serial illusion was carried out to arrive 5×10^4 OB mL^{-1}. Virulence of the extracted NPV was assessed against early second instar larvae of *H. armigera* by adopting diet surface contamination method. Observations were made on the percent larval mortality at regular intervals. Percent Original Activity Remaining (OAR%) was computed for each viral isolate and storage condition based on the following formula:

$$OAR(\%)=\frac{\text{NPV caused larval mortality after storage}}{\text{NPV caused larval mortality before storage}}\times100$$

RESULTS AND DISCUSSION

Persistence in black soil: The mortality of the larvae due to NPV infection declined in all the three viral isolates through the time in black soil in indoor and outdoor conditions. As the period of storage of virus in soil was advanced the mean mortality percentage also declined. This was ranged from 87.6 to 51.3% in indoor condition for NGM isolate which was relatively more than the two other isolates (Table 1). Relatively lesser persistence (44.98, 49.66 and 47.16% for CMB, NGM and OTY isolates, respectively) was noticed when the virus treated soil was kept outdoor compared to the treated soil kept in indoor (Table 3).

Persistence in red soil: The larval mortality caused by different geographical isolates of HearNPV declined in through the time in red soil in indoor and outdoor conditions. As the period of storage of virus in soil was advanced the mean mortality percentage also decreased. This trend was ranged from 87.6 to 56.4% in indoor condition for NGM isolate which was relatively more than CMB and OTY isolates (Table 2). Relatively lesser persistence (56.44, 56.28 and 54.46% for CMB, NGM and OTY isolates, respectively) was noticed when the virus treated soil was kept outdoor compared to the treated soil in indoor condition.

The occlusion bodies of insect viruses are known to persist in soil for longer periods without loosing much of the activity and infection. This property is of much practical significance as the soil borne virus can form initial inoculum to initiate epizootics in subsequent seasons. Considering soil persistence of NPVs and therefore, screening and identifying the most promising isolate would assist the virus based controls of insects by providing an infective inoculum for next growing season.

Table 1: Persistence of different HearNPV* isolates in black soil kept in indoor and outdoor conditions against second instar larvae of *H. armigera* during known periods of time

	Larval mortality percentage (±SE)					
	CMB		NGM		OTY	
Period of storage (Months)	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
0	84.7±1.2	84.7±0.8	87.6±0.7	87.6±1.0	86.3±1.0	86.3±0.9
1	80.6±0.9	79.3±1.3	83.7±1.0	80.0±1.2	83.4±1.0	81.2±1.2
2	69.8±1.0	64.9±1.1	71.4±0.9	65.7±1.4	69.7±0.7	64.6±1.0
3	59.0±1.0	55.8±0.8	64.9±1.3	58.6±1.1	60.8±1.3	57.6±1.4
4	54.7±1.1	50.6±1.0	63.1±0.7	58.2±0.8	58.3±1.3	55.9±0.9
5	49.2±1.2	40.5±1.4	55.5±1.0	48.8±0.8	53.6±1.3	43.3±0.9
6	44.6±1.3	38.1±0.9	51.3±1.0	43.5±1.0	46.9±1.2	40.7±1.0

*All the treatments contained NPV at 5×10^4 OB mL^{-1}

Table 2: Persistence of different HearNPV* isolates in red soil kept in indoor and outdoor conditions against second instar larvae of *H. armigera* during known periods of time

	Larval mortality percentage (±SE)					
	CMB		NGM		OTY	
Period of storage (Months)	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
0	84.7±1.2	84.7±0.8	87.6±0.7	87.6±1.0	86.3±1.0	86.3±0.9
1	81.9±0.6	80.3±0.8	83.2±1.0	82.7±1.3	79.4±1.2	81.5±1.0
2	75.6±1.3	70.5±1.0	74.5±1.1	69.4±1.0	72.7±1.2	70.6±1.1
3	68.7±1.2	63.2±1.4	70.9±0.8	67.3±0.7	68.8±0.8	66.1±0.9
4	62.1±1.2	57.7±0.9	66.5±1.0	61.1±1.4	59.6±1.3	58.5±0.9
5	55.9±0.9	50.6±1.0	62.3±1.3	54.9±0.8	59.0±0.7	49.4±0.9
6	48.3±1.0	47.8±1.0	56.4±0.8	49.3±1.3	51.5±0.7	47.0±1.3

*All the treatments contained NPV at 5×10^4 OB mL^{-1}

Table 3: Original Activity Remaining (OAR %) of different geographical isolates of HearNPV after 6 months storage in black and red soils in indoor and outdoor conditions

	Black soil		Red soil	
HearNPV isolates	Indoor	Outdoor	Indoor	Outdoor
CMB	52.66	44.98	57.03	56.44
NGM	58.56	49.66	64.38	56.28
OTY	54.35	47.16	59.68	54.46

The comparison of results showed that the virus treated black soil kept indoor could relatively retain its infectivity (52.66, 58.56 and 54.35% for CMB, NGM and OTY isolates, respectively) for longer period compared to the soil kept outdoor (Table 3). Similar kind of trend was also noticed with red soil. However, the infectivity was relatively more in the case of HearNPV stored in red soil compared to those stored in black soil.

Adsorption of the polyhedra in two types of soil was studied by Narayanan and Jayaraj (1988). It was found that the adsorption rate was higher in alfisol than in verticil. Studies on leaching of NPV of *H. armigera*, from black soil when applied at 5×10^6 per labeled polyhedra at the top of the soil column, retained the polyhedra up to 10.2 cm after continuous leaching for 18 days. From the findings of the present study as well as the previously mentioned study, it could be suggested that the virus of *H. armigera* persisted for longer period in red soil than black soil. This high amount of persistence in red soil may be due to the fact that adsorption rate was higher in red soil than black soil. So, it would be suggested that in viral control of the pest red soil could preserve the relatively most infectivity of the virus compared to the black soil causing initial infectivity in the natural environment of the pest.

However, among the isolates evaluated in this study, NGM isolate was found to be the most relatively tolerant to inactivation through the India, was also found relatively tolerate to the different spectrums of UV light with natural as well as artificial storage time. This isolate collected from Tamil Nadu, (simulated sunlight) sources under laboratory and natural conditions (Mehrvar *et al.*, 2007b). Entirely, it could be concluded that different geographical isolates of nucleopolyhedrovirus of *H. armigera* collected from different agroclimatic regions could relatively remain in the soil in an infective case after 6 months of storage to initiate next epizootics in the natural populations of the pest. The present findings corroborate with the findings of earlier studies by Fuxa and Richter (1996, 1999) and Peng *et al.* (1999).

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