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Effect of different storage conditions on the virulence of *Helicoverpa armigera* nucleopolyhedrovirus (HaNPV)

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ABSTRACT

Studies on the effect of different storage conditions on the virulence of NPV revealed that samples stored in earthen pot and at room temperature maintained efficacy up to four months and after that virulence started decreasing. This decreased efficacy of samples stored under room temperature may be due to increased bacterial activity. When the NPV samples were tested for the bacterial load, it was 3.47 times more in the samples stored at room temperatures after six months of storage.

Helicoverpa armigera is a polyphagous pest and is reported to infest 181 plant species (Manjunath *et al.* 1985). Chemical control often failed as this pest is reported to have developed resistance to many of the commercially available insecticides. *Helicoverpa armigera nucleopolyhedrovirus (HaNPV)* is an attractive biopesticide to control this pest. It has been found effective in controlling *H. armigera* on a range of crops including legumes (Rabindra *et al*. 1992), oil seeds (Rabindra *et al.* 1985), cotton (Jones, 1994) and vegetables (Jones *et al*. 1998). Though it is an effective biopesticide it is under exploited due to problems in storage. Unlike chemical pesticides, viral pesticides often have a shorter shelf life of infectivity (Shieh, 1978) that requires special attention for commercial operations. Viral insecticides cannot be developed commercially until formulations of these are physically, chemically and environmentally stable in storage and distribution. For industrialization of product, formulation of pathogen product should have a shelf life of more than 18 months (Couch and Ignoffo, 1981). But stability for this period was so difficult in case of baculoviruses. Unlike chemical pesticides as this NPVs are multiplied on living insects presence of insect debris and putrefaction due to bacterial contamination is inevitable hence for future field and laboratory use the polyhedra associated with many insect virus diseases are usually stored under refrigerated conditions as air dried, purified polyhedra or as polyhedra with in dried larval cadavers. Under these conditions polyhedra are noted to resist deterioration for considerable periods of time. Some polyhedral viruses have been shown to retain their virulence for periods ranging from 2 to 25 years (Steinhaus, 1949; Bergold, 1953). Hence the present study was undertaken to study the effect of different storage conditions on the virulence of this virus.

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MATERIALS AND METHODS

The laboratory experiments were conducted during 2004-2005 at ICRISAT, Hyderabad. In order to evaluate virulence of HaNPV under different storage conditions HaNPV was multiplied on late third instar larvae. Bottles containing NPV suspension were stored at refrigerated condition, and at room temperature, in earthen pot, in glass bottle and in amber colour bottle. Concentration of POBs was estimated in all the samples using Nuebaur haemocytometer before storage. The desired concentration of polyhedra was reached by adjusting the amount of distilled water added to the suspension.

To determine their virulence over a period of time, bioassays were conducted using diet surface contamination method at bimonthly interval for a period of one year using second instar larvae of *H. armigera* at a concentration of 10^6 POB/ml. Artificial diet was prepared and poured into required number of polypots when it was hot. Using micropipette, 50 µ of the viral suspension of each concentration was dispensed over the diet surface and to ensure uniform

distribution of suspension over the surface of diet the suspension was spread using blunt end of glass rod. The suspension was allowed to dry and then the larvae were released carefully over the surface of the diet. To avoid larval escape lid of the pot was firmly tightened. Holes were made on the lid with the help of a needle for proper aeration. Fresh diet was supplemented at every 24 h. Larval mortality was recorded daily from the third day of inoculation till tenth day. Mortality was calculated and data were analysed using GENSTAT version 6. Samples stored under different storage conditions were tested for the bacterial population by plating samples on nutrient agar media (Miles and Misra, 1938) up to six months at an interval of two months.

RESULTS AND DISCUSSION

Influence of storage on virulence of HaNPV : Bioassay was conducted against second instar *H. armigera* larvae @10^6 POB/ml. Mortality data on 5th, 7th and 9th days were analysed using two way ANOVA. The cumulative mortality on the ninth day showed that the HaNPV sample which was stored under refrigerated condition maintained its efficacy (100%) up to eight moths and by the tenth month the mortality started declining slightly (97.50%) but it was not significant. HaNPV samples stored in earthen pot and at room temperature (both in amber coloured bottle and glass bottle) maintained efficacy up to fourth month. Only after four months the efficacy of the samples started declining. These results clearly indicated that the gradual decrease in the efficacy of NPV samples stored at room temperature (both in glass and amber coloured bottles) and in earthen pot after a period of four months (Table 1-3).

Influence of storage condition on the bacterial activity : Bacterial colony counts varied under different set of storage conditions. Bacterial counts increased consistently over the period of storage under different storage conditions. The results (Table 2 & 3) revealed that there was significant increase in bacterial colony count and CFU/ml at every two months and this increase was the highest in the NPV samples stored at room temperature in amber color as well as in glass bottle and in earthen pot samples. In refrigerated sample also bacterial population increased over the period of storage but it was not comparable with the other three treatments.

In the present study NPV samples stored in earthen pot and room temperature maintained efficacy up to four months and after that virulence started decreasing. This decreased efficacy of samples stored under room temperature may be due to increased bacterial activity. When the samples were tested for the bacterial load, it is 3.47 times more in the samples stored at room temperatures after six months of storage. Gopali and Lingappa (2001) also recorded decreased efficacy of NPV when stored under open house conditions and it was opined that the change in the pH of viral suspension stored under refrigerated

Table 1. Efficacy of HaNPV under different storage conditions.

Storage conditions	Per cent larval mortality (on 5th day) due to HaNPV (10^6 POBs) stored for different months						Mean
	0	2	4	6	8	10	
Refrigerated	82.50 (65.46)	77.50 (61.77)	77.50 (61.77)	75.00 (60.11)	72.50 (58.45)	67.50 (55.28)	60.47
Earthen pot	80.00 (63.43)	75.00 (60.11)	75.00 (60.11)	72.50 (58.45)	70.00 (56.78)	65.00 (53.77)	58.77
RT (amb)	80.00 (63.43)	75.00 (60.11)	72.50 (58.45)	72.50 (58.45)	67.50 (55.28)	55.00 (47.88)	57.26
RT (glass bottle)	82.50 (65.46)	75.00 (60.11)	72.50 (58.45)	72.50 (58.45)	65.00 (53.77)	50.00 (45.00)	56.87
Control	0	0	0	0	0	0	0
Mean	51.56	48.42	47.75	47.09	44.86	40.38	
	Treatment	Storage	Interaction				
SEm±	0.699	0.616	1.439				
LSD	2.106	1.736	4.044				
F (Prob.at 5%)	<. 001	<. 001	<. 001				

RT (amb): Room Temperature (amber colored bottle)

Table 2. Efficacy of HaNPV under different storage conditions.

Storage conditions	Per cent larval mortality (on 7th day) due to HaNPV (10^6 POBs) stored for different months						Mean
	0	2	4	6	8	10	
Refrigerated	92.50 (61.22)	87.50 (56.52)	87.50 (56.52)	85.00 (55.28)	82.50 (54.03)	75.00 (50.84)	55.74
Earthen pot	95.00 (64.67)	87.50 (56.52)	87.50 (56.52)	85.00 (55.28)	80.00 (52.79)	70.00 (48.90)	55.78
RT (amb)	95.00 (64.67)	85.00 (55.28)	85.00 (55.28)	82.50 (54.03)	75.00 (50.84)	60.00 (45.44)	54.26
RT (glass bottle)	92.50 (61.22)	85.00 (55.28)	85.00 (55.28)	82.50 (54.03)	75.00 (50.84)	60.00 (45.44)	53.68
Control	0	0	0	0	0	0	0
Mean	50.3571	44.7256	44.7256	43.7292	41.7055	38.126	
	Treatment	Storage	Interaction				
SEm±	0.635	0.75	1.658				
LSD	1.916	2.114	4.66				
F (Prob.at 5%)	<. 001	<. 001	0.006				

RT (amb): Room Temperature (amber colored bottle)

Table 3. Efficacy of HaNPV under different storage conditions.

Storage conditions	Per cent larval mortality (on 9th day) due to HaNPV (10^6 POBs) stored for different months						Mean
	0	2	4	6	8	10	
Refrigerated	100 (71.56)	100 (71.56)	100 (71.56)	100 (71.56)	100 (71.56)	97.50 (68.11)	70.99
Earthen pot	100 (71.56)	100 (71.56)	100 (71.56)	95.00 (64.67)	92.50 (61.22)	87.50 (56.52)	66.18
RT (amb)	100 (71.56)	100 (71.56)	100 (71.56)	92.50 (61.22)	80.00 (52.79)	70.00 (48.90)	62.93
RT (glass bottle)	100 (71.56)	100 (71.56)	100 (71.56)	90.00 (57.77)	75.00 (51.82)	67.50 (48.03)	62.05
Control	5.00 (12.71)	5.00 (12.71)	7.50 (19.07)	5.00 (12.71)	7.50 (19.07)	10.00 (25.42)	16.95
Mean	59.7947	59.7947	61.0661	53.5892	51.2944	49.4025	
	Treatment	Storage	Interaction				
SEm±	2.39	1.004	3.147				
LSD	7.203	2.827	8.991				
F (Prob.at 5%)	<. 001	<. 001	<. 001				

RT (amb): Room Temperature (amber colored bottle)

condition was very slow from acidic to normal as against becoming excessively alkaline at ambient and earthen pot conditions. It was also reported that this change was mainly brought about by the growth of other microbes and warm conditions, which resulted in lowering of virulence of viral bodies. Attathom *et al.* (1990) also reported the same. The stability of HaNPV appears to be dependent on the resistance of the inclusion body protein to decomposition. Many scientists (Stairs *et al.* 1981) reported that the inclusion body protein is broken down by weak alkalies but it can withstand exposure to relatively strong acids and many other chemicals. Ebora *et al*. (1990) reported that virulence was the greatest around neutral pH and reduced when subjected to high pH (12). Shapiro and Ignoffo (1969) showed that activity of virions of HaNPV released from polyhedral cover lost about half of their activity when stored for 60 days at 37°C. Where as, virus particles covered with polyhedral layer retain activity for longer period and withstand

Table 4. Number of bacterial colonies observed over a period of storage under different conditions of storage.

Storage conditions	Number of bacterial colonies observed at different months of storage				
	0	2	4	6	Mean
Refrigerated	44.2	50.4	61.2	79.2	58.75
Earthen pot	46.4	74.4	122	271.2	128.5
RT (amb)	47.4	82.4	128.4	275	133.3
RT (glass bottle)	47.6	83.4	130.6	275.2	134.2
Mean	46.4	72.65	110.55	225.15	
	Treatment	Storage	Interaction		
SEm±	0.265	0.282	0.556		
LSD	0.793	0.802	1.57		
F (Prob.at 5%)	<. 001	<. 001	<. 001		

RT (amb): Room Temperature (amber colored bottle)

Table 5. Bacterial colony forming units observed over a period of storage under different conditions of storage.

Storage conditions	Number of colony forming units observed at different months of storage (CFU/mlx10^6)				
	0	2	4	6	Mean
Refrigerated	2.21	2.52	3.06	3.96	2.93
Earthen pot	2.32	3.72	6.10	13.56	6.45
RT (amb)	2.37	4.12	6.42	13.78	6.67
RT (glass bottle)	2.38	4.17	6.53	13.8	6.72
Mean	2.32	3.6325	5.5275	11.275	
	Treatment	Storage	Interaction		
SEm±	0.01355	0.01404	0.02784		
LSD	0.04062	0.03992	0.07865		
F (Prob.at 5%)	<. 001	<. 001	<. 001		

freezing and prolonged normal field temperature than free virions (Yendol and Hamlen, 1973). Many scientists reported that virus could be preserved for more than ten years at 4°C without loss in virulence (Narayanan, 1985). Gudauskas and Cannerday (1968) found the thermal inactivation point of HaNPV to be 75 to 80°C for ten minutes. The virulence of virus depends on quality, storage conditions and duration of storage, storage temperature and pH of the product.

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