Laboratory Disinfectants as Preservatives for *Helicoverpa armigera* Nucleopolyhedrovirus

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Abstract

Bacterial contamination is an important setback in large scale production of nucleopolyhedroviruses (NPV). Hence, attempts were made to decrease the bacterial contamination in storage using different commonly used laboratory disinfectants. The studies revealed that all the preservatives tried could effectively reduce the bacterial contamination and the associated bad odour problem in storage. Helicoverpa *NPV samples with all the preservatives studied gave 90% mortality under laboratory bio-assays after a period of two months of storage. The samples, which were stored in acetone and ethyl alcohol, gave 73.3 and 70% mortality, respectively after 10 months storage. Cost of different preservatives ranged from Rs.3.0-12.5 ha^{-1}, which is affordable.*

Keywords: HaNPV, storage, bacterial contamination, quality control, disinfectants

Introduction

Helicoverpa armigera nucleopolyhedrovirus (HaNPV) is a naturally occurring pathogen of *H. armigera,* which has wide host distribution in Asia, Africa and Australia (Fauquet *et al.,* 2004; Grzywacz *et al.,* 2005) and is being extensively used as biopesticide to control *Helicoverpa* spp. around the world. Though it is an effective biopesticide, its commercialisation and usage are limited due to quality problems such as malodour during processing and storage. It is known that the nucleopolyhedrovirus (NPV) produced in live insects may contain bacterial contamination (Podgwaite *et al.,* 1983; Huber, 1985) presenting a potential health hazard. Grzywacz *et al.* (1997) found 10^6-10^9 bacterial colony forming units/ml in virus suspension of *Spodoptera littoralis* NPV containing $2.1x10^9$ polyhedral inclusion bodies/ml. They concluded that none of the bacterial contaminants found was harmful to cause potential health hazard. However, some bacteria such as *Bacillus cereus* may be of some concern of health while standardizing the product. Therefore, the development of production procedures, which reduce these contaminants to a lower, and more consistent level, would be valuable in promoting the viral insecticides as safe control agents. Simple centrifugation in water does not remove many microbial contaminants, as bacterial spores *etc.*, tend to pellet with NPV polyhedral occlusion bodies (POBs). Hence, the present work was focused on screening of different laboratory disinfectants, which can be used as preservatives for NPV in storage.

Materials and methods

Chemicals, which are commonly used as laboratory disinfectants *viz.*, acetone (10%), dettol (10%), ethyl acetate (10%), ethyl alcohol (10%), phenyl (2%) and methanol (10%) were tested against the bacterial contaminants in NPV suspension during storage. Above mentioned chemicals were mixed with the virus suspension in 1:1 ratio and stored up to ten months in a refrigerator.

To test the effect of these chemicals on NPV POBs, bioassays were conducted at bimonthly intervals i.e. 2, 4, 6, 8 and 10 months after storage against second instar *H. armigera* larvae at the concentration of 10^6 POB/ml by diet surface contamination method (Evans and Shapiro, 1997). NPV, stored in distilled water, was used as control treatment for comparison. The pH of the NPV suspension after mixing with the mentioned chemicals was recorded using digital pH meter. Mortality of larvae was recorded at 9th day after inoculation and per cent mortality was calculated.

Effect of disinfectants on bacterial load over different periods of NPV storage

After mixing with the selected chemicals, POB concentration of NPV suspension for each treatment was estimated. One ml of suspension from each treatment was drawn using micropipette and was diluted serially from 10^{-2} to 10^{-9} and concentrations from 10^{-3} to 10^{-9} were plated on nutrient agar media. Before plating, each Petri plate was marked into

six equal segments using a marker pen and each segment was plated with a different dilution. Six replicates for each dilution were maintained. Overcrowding occurred at higher concentrations (smallest dilutions) resulting in an underestimate of the numbers of viable bacteria present. Hence, in the present study observations were recorded at 10^{-3} dilution on number of bacterial colonies before and six months after storage. The data were subjected to analysis of variance among the chemicals over the study period. Colony forming units per ml of suspension were calculated using the following formula.

$$\text{CFU/ml} = \frac{\text{Number of colonies observed}}{\text{Volume plated}} \times \text{Dilution factor}$$

Results and discussion

As nucleopolyhedrovirus is produced in live insects, bacterial contamination develops during storage and this situation is worse under tropical conditions due to high temperatures. In present study, attempts were made to minimize the bacterial contamination in NPV suspension under storage conditions with out affecting the efficacy of polyhedral bodies. The NPV samples preserved for ten months in six different commonly used laboratory disinfectants varied in their efficacy. Cumulative mortality on ninth day showed that the samples stored for a period of two months in 10% dettol, 2% phenyl, 10% ethyl alcohol and 10% methanol recorded 100% mortality followed by 10% acetone and 10% ethyl acetate with 96.7% and 90% mortality respectively and these differences were significant. The sample that was stored in distilled water maintained 100% virulence up to ten months as evident by the 9th day bio-assays. Where as, the NPV sample stored in 10% ethyl acetate consistently reduced its efficacy from 90% at two months storage to 46.7% by the end of ten months of preservation. The samples, stored in 10% acetone and 10% ethyl alcohol gave 73.3% and 70.0% mortality respectively by the end of ten months followed by the samples stored in 2% phenyl, 10% dettol and 10% methanol with 63.3%, 56.7% and 53.3%, respectively (Table 1). Hence, it was concluded that the NPV samples can be stored for a period of two months in the above mentioned preservatives without having any adverse effect on polyhedral bodies to avoid bad odour problem in storage. Similar studies conducted by Rao and Meher (2004) with 10% acetone concluded the reduction of malodour problem with out sacrificing the viral efficacy during one month storage. Similarly, Grzywacz *et al.*, (1997) suggested the use of bacteriostatic agents and pH buffers to stabilize the formulations by reducing the multiplication of contaminants. Ignoffo and Shapiro (1978) suggested the use of acetone in purification of NPV POBs. Acetone being a potential antimicrobial agent, regulates the bacterial infection and being a lipid solvent, removes the lipid (fat cells) from the homogenate, there by inhibiting the bacterial lipid degradation there by reducing the malodour.

The studies revealed that the tested preservatives could effectively reduce the bad odour problem in storage by decreasing the bacterial contaminants. Compared to the distilled water all the disinfectants used to preserve the NPV sample effectively reduced the bacterial population during

Table 1. Effect of chemicals on the shelf life of HaNPV (on 9th day)

	Mortality (%) after storage				
Treatment	2 months	4 months	6 months	8 months	10 months
NPV + 10% dettol	100.0(90.0)	73.3(59.0)	73.3(59.0)	70.0(56.8)	56.7(48.9)
NPV + 10% acetone	96.7(84.0)	93.3(77.7)	90.0(75.0)	76.7(61.2)	73.3(59.0)
NPV + 2% phenyl	100.0(90.0)	86.7(68.9)	86.7(68.9)	73.3(59.0)	63.3(52.8)
NPV + 10% ethyl acetate	90.0(71.6)	83.3(66.1)	80.0(63.4)	53.3(46.9)	46.7(43.1)
NPV + 10% ethyl alcohol	100.0(90.0)	90.0(75.0)	90.0(71.6)	76.7(61.2)	70.0(56.8)
NPV + 10% methanol	100.0(90.0)	73.3(59.0)	70.0(56.8)	63.3(52.8)	53.3(46.9)
NPV + distilled water	100.0(90.0)	100.0(90.0)	100.0(90.0)	100.0(90.0)	100.0(90.0)
Mean	86.5	70.8	69.2	61.1	56.8
	Treatment	Storage	Interaction		
SE ±	1.25	1.06	2.80		
LSD	3.53	2.99	7.90		
F (Prob.at 5%)	<. 001	<. 001	<. 001		

storage period. Among the chemicals, 2% phenyl effectively reduced the bacterial population and CFU/ml even after six months of storage (7.3 and 0.4x10^6), which was followed by 10% ethyl acetate (8 and 0.4x10^6) and 10% acetone (19.7 and 1.0 x10^6) (Table 2).

Table 2. Effect of chemicals on the bacterial contaminants in storage

Treatment	Before storage		After six months	
	No. of bacterial colonies	CFU/ mlx10^6	No. of bacterial colonies	CFU/ mlx10^6
NPV + 10% acetone	20.3	1.0	19.7	1.0
NPV + 10% ethyl alcohol	22.0	1.1	21.3	1.0
NPV + 10% ethyl acetate	25.0	1.3	8.0	0.4
NPV + 10% dettol	23.3	1.2	25.0	1.3
NPV + 2% phenyl	18.0	0.9	7.3	0.4
NPV + 10% methanol	22.0	1.1	24.7	1.2
NPV + distilled water	50.7	2.5	250.0	12.5
SE ±	0.43	0.02	0.35	0.02
LSD	1.33	0.06	1.08	0.05
F (Prob.at 5%)	<. 001	<. 001	<. 001	<. 001

Compared to distilled water all the preservatives effectively reduced the bacterial population. Extra cost to be spent on the preservatives was calculated per hectare, which is easily affordable with an extra cost of Rs. 3 to Rs. 12.5 ha^{-1} (Table 3).

Table 3. Cost effectiveness of disinfectants used as preservatives for HaNPV in storage

Treatment	Vol. of disinfectant required per hectare	Extra cost (Rs)
NPV + 10% acetone	25 ml	5.0
NPV + 10% ethyl alcohol	25 ml	12.5
NPV + 10% ethyl acetate	25 ml	9.1
NPV + 10% dettol	25 ml	4.5
NPV + 2% phenyl	5 ml	3.2
NPV + 10% methanol	25 ml	4.2
NPV + distilled water	25 ml	0

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