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Research Article

Storage stability and performance of aqueous and dry formulations of *Helicoverpa armigera* nuclear polyhedrosis virus

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ABSTRACT: Experiment on storage stability and performance of aqueous and dry formulation of *Helicoverpa armigera* nuclear polyhedrosis virus (*Ha*NPV) was conducted during 2011-2013 in Bio control laboratory, Department of Entomology and Insect Biotechnology laboratory, Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola with an objective to develop potent *Ha*NPV formulation for improving shelf life and performance. Aqueous and dry form of *Ha*NPV formulations with antimicrobials, UV protectant and phagostimulant were prepared and studied their shelf life and performance during storage. Synthesized and characterized the Silver nanoparticles. Prepared fresh *Ha* NPV required for preparing different formulation. The contaminants associated, storage stability and larvicidal activity of formulated *Ha*NPV was studied. Data revealed that, *Ha*NPV formulations having Silver nanoparticles @ 8 µl/ml of *Ha*NPV and 80 µl/ml of H*a*NPV checked the bacterial contamination up to 13 months of the storage period, did not affect the viability of POBs and insecticidal properties of *Ha*NPV formulation. Aqueous form of *Ha*NPV + Streptomycin @ 0.18 g/lit of *Ha*NPV + Tinopal 1% + Sucrose 1% , dry form of *Ha*NPV + Streptomycin @ 0.18 g/lit of *Ha*NPV + Tinopal 1% + Sucrose 1% and *Ha*NPV + Streptomycin @ 0.18 g/lit of *Ha*NPV + sucrose remain stabled up to 12 months. However, aqueous form of *Ha*NPV alone reduces the insecticidal properties from the 9th month of storage period. While, dry form of *Ha*NPV alone reduces the insecticidal properties from the 12th month of storage period. From the result, it was noticed that dry form of *Ha*NPV formulation having antimicrobial found more stable than aqueous form.

KEY WORDS: Shelf life, storage stability, *Ha*NPV formulation, silver nano particles

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INTRODUCTION

Helicoverpa armigera nuclear polyhedrosis virus is the potential agent for the biological control of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) because of their high pathogenicity, narrow host range, and safety to vertebrates, plants and the environment. Other advantages of baculovirus for pest control include lack of toxic residues and unlikelihood development of stable resistance. Despite these advantages, their practical application as microbial pesticides has not been fully exploited. Among the various limiting factors, microbial contamination during storage period is an important one that affects the physical stability and insecticidal properties (Podgwaite *et al*., 1983; Grzywacz *et al.,* 1997). The aqueous preparations can undergo bacterial fermentation and loss of activity when stored at room temperature (Cherry *et al*., 2000). Semi-purified product has secondary microbial contamination. In respect of liquid suspensions, water have mainly been used as carriers, although the growth of contaminating microorganisms in water based concentrates has made water an inferior carrier, (Jones and Burge 1997). Viruses can remain highly viable for several years, especially those with intact inclusion bodies stored in insect cadaver, dry powder or in suspension (Jacques, 1985). Additives can be used to inhibit microbial contamination as well as protect baculoviruses from adverse environmental factors, which enhance storage stability and maximize application efficiency (Lasa *et al*., 2008)**.** The formulation of a microbial agent can be improved by adding ingredients which increase the stability and shelf-life of the product (Jones and Burge, 1997). Keeping all this in view, an attempt was

made to develop *Ha*NPV formulation in aqueous and dry form for improving shelf life and performance.

MATERIALS AND METHODS

The present investigation was carried out at Department of Entomology and Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during the year 2011 to 2013 in Completely Randomized Block Design, replicated thrice. Silver nanoparticles (SNPs) were synthesized as per the procedure followed by Monali Gajbhiye *et al*., (2013). Synthesized SNPs were detected by reading UV-Vis spectra and were further characterized by Nanoparticle Tracking Analysis System, Zeta measurement and Transmission Electron Microscopy. *Ha*NPV required for its formulation (Aqueous and Dry) was prepared freshly. Aqueous formulations were prepared by adding the required quantity of adjuvants in the desired concentration of *Ha*NPV. Dry formulations were prepared by direct impregnation of required quantity of adjuvants with typical full grown virus infected larvae and dried it by using lyophilizer. For evaluation of dry formulations, the formulation was milled and suspended in sufficient quantity of distilled water so as to meet the desired concentration of *Ha*NPV. For stability study, the formulations were stored at room temperature and assessed for microbial contaminants associated with both aqueous and dry formulations at monthly interval using procedure followed by Lasa *et al*. (2008). The viability of formulations was assessed by counting POBs at three month intervals as per the procedure followed by Gupta *et al*. (2009). The larvicidal activity was tested at three month intervals against second instars larvae of *H. armigera.* For larvicidal study, 50 µl of *Ha*NPV formulation was smeared on half broken soaked chickpea grain and thirty laboratory reared larvae were released in each treatment. Larvae were starved for 24 hours before release. Fresh prepared water soaked chickpea was used for further feeding. Mortality caused at ten days after treatment was recorded and analyzed by using ANOVA.

RESULTS AND DISCUSSION

Bacterial contaminants associated with *Ha*NPV formulations

Pooled mean data given in Table 1, revealed that *Ha*NPV formulation having silver nanoparticles @ 8 µl/ml of *Ha*NPV and 80 µl/ml of *Ha*NPV checked the bacterial contamination up to 13 months of the storage period. Aqueous form of *Ha*NPV + Streptomycin @ 0.18 g/lit of *Ha*NPV + Tinopal 1% + Sucrose 1 %, dry form of *Ha*NPV + Streptomycin @ 0.18 g/lit of *Ha*NPV + Tinopal 1% + Sucrose 1 % and *Ha*NPV + Streptomycin @ 0.18 g/lit of

Table 1. Bacterial contamination associated with *Ha*NPV formulation during storage (Pooled mean)

S.N	Treatments	CFU/ml of *Ha*NPV at												
		1st M	2nd M	3rd M	4th M	5th M	6th M	7th M	8th M	9th M	10th M	11th M	12th M	13th M
Aqueous *Ha*NPV formulation														
1	*Ha*NPV+ Silver nanoparticles @ 8 µl /ml of *Ha*NPV + Tinopal 1% + Sucrose 1%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	*Ha*NPV+Streptomycin @ 0.18 g/lit of *Ha*NPV + Tinopal 1% + Sucrose 1%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13
3	*Ha*NPV+ Silver nanoparticles @ 80 µl/ml of *Ha*NPV + Sucrose 1%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	*Ha*NPV Alone	1.29 x10^7	1.38 x10^7	1.42 x10^7	1.28 x10^7	1.35 x10^7	1.45 x10^7	1.75 x10^7	1.9 x10^8	3.2 x10^8	3.6 x10^8	12.0 x10^7	13.0 x10^7	15.0 x10^7
Lyophilized *Ha*NPV formulation														
5	*Ha*NPV+ Silver nanoparticles @ 8µl / ml of *Ha*NPV + Tinopal 1% + Sucrose 1%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	*Ha*NPV+ Streptomycin @ 0.18 g/lit of *Ha*NPV + Tinopal 1% + Sucrose 1%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11
7	*Ha*NPV+ Silver nanoparticles @ 8 µl/ ml of *Ha*NPV+ Sucrose 1%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	*Ha*NPV+ Streptomycin @ .18gl/lit of *Ha*NPV + Sucrose 1%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10
9	*Ha*NPV+ Silver nanoparticles @ 80 µl/ml of *Ha*NPV + Sucrose 1%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	*Ha*NPV Alone	0.83 x10^7	1.13 x10^7	1.16 x10^7	1.17 x10^7	1.18 x10^7	1.23 x10^7	1.27 x10^7	1.33 x10^7	1.43 x10^7	2.12 x10^7	2.25 x10^7	2.35 x10^7	7.7 x10^7

*M =Month

*Ha*NPV + sucrose inhibited the bacterial contamination up to 12 months of storage period and recorded 0.13 x 10^7, 0.11 x 10^7 and 0.10 x 10^7 CFU/ml of *Ha*NPV formulation, respectively. The bacterial count of *Ha*NPV alone in aqueous form was 1.29 x 10^7 CFU/ml and remains stable up to 8 months of storage period. In 9th and 10th months the count was 3.2 x 10^7 and 3.6 x 10^7 CFU/ml, respectively. During 11th, 12th and 13th months, the bacterial count was 12.0 x 10^7, 13.0 x 10^7 and 15.0 x 10^7 CFU/ml, respectively. Initial bacterial count in dry form of *Ha*NPV alone was 0.83 x 10^7 and almost it was stable up to the 12 months of storage period and in the 13th month of storage the count was 7.7 x 10^7 CFU/ml.

Viability of POBs

*Ha*NPV formulation having antimicrobials *i.e.* silver nanoparticles @ 8 µl/ml of *Ha*NPV and 80 µl/ml of *Ha*NPV and streptomycin @ 0.18 g/lit of *Ha*NPV did not affect the POBs. POBs of aqueous form of *Ha*NPV alone remains viable up to 6 months of storage period and from 9th month onward it was reduced. POBs of dry form of *Ha*NPV alone remains viable up to 11 months of storage period and it was reduced in 12th month (Table 2).

Reduction in POBs over 1st month were calculated and given in Table 3. From the data, it was observed that 20.70, 42.00 and 53.37 per cent reduction in POBs was found during 6th, 9th and 12th month, respectively, from aqueous form *of Ha*NPV. Dry form of *Ha*NPV recorded 12.61 and 25.07 per cent reduction in POBs during 9th and 12th month of storage period, respectively. Similar observations were made by Lasa *et al*. (2008), mentioned that concentration of OBs fell by 30 per cent after 6 months of storage and it remained unchanged for the remaining 12 months of the study. OB formulation with bacteriostatic or antioxidant additives, together with storage, will likely result in a *SeM*-NPV biopesticide shelf life that exceeds 18 months.

Larvicidal activity of *Ha*NPV formulations

Data given in Table 4 revealed that aqueous and dry form of *Ha*NPV formulations having silver nanoparticles and streptomycin did not influence the insecticidal properties of *Ha*NPV formulation over storage period. However, aqueous form of *Ha*NPV reduces the insecticidal properties from the 9th month of storage period. While, dry form of *Ha*NPV reduces the insecticidal properties from the 12th month of storage period.

Overall results perceived that dry form of *Ha*NPV formulations found more stable than aqueous form. Grant Michelle (2008) noted that decrease in the insecticidal activity of NPV suspensions could be due to several factors including microbial load from the natural flora of the insects. The free water content in unformulated suspensions

Table 2. Viability of POBs of *Ha*NPV formulation during storage. (Pooled mean of Set I and Set II)

S.N	Treatments	POBs/ml of *Ha*NPV at				
		1st Month	3rd Month	6th Month	9th Month	12th Month
Aqueous *Ha*NPV formulation						
1	*Ha*NPV+ Silver nanoparticles @ 8 µl /ml of *Ha*NPV + Tinopal 1% + Sucrose 1%	1.405 x 10^9	1.422 x 10^9	1.377 x 10^9	1.313 x 10^9	1.320 x 10^9
2	*Ha*NPV+Streptomycin @ 0.18 g/lit of *Ha*NPV + Tinopal 1% + Sucrose 1%	1.331 x 10^9	1.331 x 10^9	1.295 x 10^9	1.296 x 10^9	1.265 x 10^9
3	*Ha*NPV+ Silver nanoparticles @ 80 µl/ml of *Ha*NPV+ Sucrose 1%	1.310 x 10^9	1.310 x 10^9	1.297 x 10^9	1.262 x 10^9	1.260 x 10^9
4	*Ha*NPV Alone	1.319 x 10^9	1.319 x 10^9	1.046 x 10^9	0.765 x 10^9	0.615 x 10^9
Lyophilized *Ha*NPV formulation						
5	*Ha*NPV+ Silver nanoparticles @ 8µl / ml of *Ha*NPV+ Tinopal 1% + Sucrose 1%	5.683 x 10^9	5.658 x 10^9	5.675 x 10^9	5.450 x 10^9	5.500 x 10^9
6	*Ha*NPV+ Streptomycin @ 0.18 g/lit of *Ha*NPV+ Tinopal 1% + Sucrose 1%	5.817 x 10^9	5.817 x 10^9	5.625 x 10^9	5.558 x 10^9	5.521 x 10^9
7	*Ha*NPV+ Silver nanoparticles @ 8 µl/ ml of *Ha*NPV+ Sucrose 1%	5.125 x 10^9	5.125 x 10^9	5.258 x 10^9	5.233 x 10^9	5.329 x 10^9
8	*Ha*NPV+ Streptomycin @ .18gl/lit of *Ha*NPV + Sucrose 1%	5.725 x 10^9	5.725 x 10^9	5.417 x 10^9	5.267 x 10^9	5.160 x 10^9
9	*Ha*NPV+ Silver nanoparticles @ 80 µl/ml of *Ha*NPV + Sucrose 1%	5.817 x 10^9	5.825 x 10^9	5.892 x 10^9	5.642 x 10^9	5.639 x 10^9
10	*Ha*NPV Alone	4.758 x 10^9	4.758 x 10^9	4.608 x 10^9	4.158 x 10^9	3.565 x 10^9

Table 3. Reduction in POBs of *Ha*NPV formulation during storage period

S.N	Treatments	% reduction in POBs				
		1st month	3rd month	6th month	9th month	12th month
Aqueous *Ha*NPV formulation						
1	*Ha*NPV+ Silver nanoparticles @ 8 µl /ml of *Ha*NPV + Tinopal 1% + Sucrose 1%	0.0	0.00	1.99	2.49	2.63
2	*Ha*NPV+Streptomycin @ 0.18 g/lit of *Ha*NPV + Tinopal 1% + Sucrose 1%	0.0	0.00	2.70	2.63	4.96
3	*Ha*NPV+ Silver nanoparticles @ 80 µl/ml of *Ha*NPV + Sucrose 1%	0.0	0.00	0.99	3.66	3.82
4	*Ha*NPV Alone	0.0	0.00	20.70	42.00	53.37
Lyophilized HaNPV formulation						
5	*Ha*NPV+ Silver nanoparticles @ 8µl / ml of *Ha*NPV + Tinopal 1% + Sucrose 1%	0.0	0.44	0.14	4.10	3.22
6	*Ha*NPV+ Streptomycin @ 0.18 g/lit of *Ha*NPV + Tinopal 1% + Sucrose 1%	0.0	0.00	3.30	4.45	5.09
7	*Ha*NPV+ Silver nanoparticles @ 8 µl/ ml of *Ha*NPV+ Sucrose 1%	0.0	0.00	0.00	0.00	0.00
8	*Ha*NPV+ Streptomycin @ .18gl/lit of *Ha*NPV + Sucrose 1%	0.0	0.00	5.38	8.00	9.87
9	*Ha*NPV+ Silver nanoparticles @ 80 µl/ml of *Ha*NPV + Sucrose 1%	0.0	0.00	0.00	3.01	3.06
10	*Ha*NPV Alone	0.0	0.00	3.15	12.61	25.07

Table 4. Influence of storage period on the larvicidal activity of *Ha*NPV formulation

S.N	Treatments	% larval mortality at 10 DAT (Pooled)				
		1st Month	3rd Month	6th Month	9th Month	12th Month
Aqueous *Ha*NPV formulation						
1	*Ha*NPV+ Silver nanoparticles @ 8 µl /ml of *Ha*NPV + Tinopal 1% + Sucrose 1%	88.77 (70.31)	88.89 (70.57)	87.78 (69.58)	88.33 (70.06)	88.89 (70.57)
2	*Ha*NPV+Streptomycin @ 0.18 g/lit of *Ha*NPV + Tinopal 1% + Sucrose 1%	87.08 (68.87)	86.67 (68.68)	85.56 (67.69)	85.56 (67.69)	87.78 (69.58)
3	*Ha*NPV+ Silver nanoparticles @ 80 µl/ml of *Ha*NPV + Sucrose 1%	82.58 (65.22)	82.22 (65.08)	82.22 (65.08)	83.89 (66.38)	85.00 (67.23)
4	*Ha*NPV Alone	81.47 (64.36)	82.22 (65.08)	82.22 (65.08)	68.33 (55.76)	56.67 (48.87)
Lyophilized *Ha*NPV formulation						
5	*Ha*NPV+ Silver nanoparticles @ 8µl / ml of *Ha*NPV + Tinopal 1% + Sucrose 1%	87.22 (69.12)	88.89 (70.57)	87.22 (69.12)	87.78 (69.58)	87.78 (69.58)
6	*Ha*NPV+ Streptomycin @ 0.18 g/lit of *Ha*NPV + Tinopal 1% + Sucrose 1%	85.56 (67.69)	87.78 (69.58)	85.56 (67.69)	85.56 (67.69)	85.56 (67.69)
7	*Ha*NPV+ Silver nanoparticles @ 8 µl/ ml of *Ha*NPV+ Sucrose 1%	84.44 (66.80)	85.56 (67.69)	84.44 (66.80)	85.56 (67.69)	85.56 (67.69)
8	*Ha*NPV+ Streptomycin @ .18gl/lit of *Ha*NPV + Sucrose 1%	84.44 (66.80)	84.44 (66.80)	84.44 (66.80)	84.44 (66.80)	84.44 (66.80)
9	*Ha*NPV+ Silver nanoparticles @ 80 µl/ml of *Ha*NPV + Sucrose 1%	82.22 (65.08)	82.22 (65.08)	82.22 (65.08)	82.22 (65.08)	82.22 (65.08)
10	*Ha*NPV Alone	82.22 (65.08)	82.22 (65.08)	82.22 (65.08)	82.22 (65.08)	70.56 (57.14)
F Test		Sig	Sig	Sig	Sig	Sig
S.Em.±		0.82	0.95	0.88	0.86	1.08
C.D (*P*= 5 %)		2.39	2.79	2.58	2.53	3.16
C.V. %		2.33	2.68	2.51	2.49	3.15

may be higher. Microorganisms rely on a water activity and this could be the reason for higher microbial load in unformulated suspensions.

Present study of intervention of Nano technology for stability of *Ha*NPV is an innovative attempt, the literature is not traceable. However, some scientists studied the storage stability of different NPV formulation by using different additives. Freeze drying of NPV was known to reduce bacterial concentration. The aqueous preparations of NPV can undergo bacterial fermentation and loss of activity when stored at room temperature. The stability of dried virus and of virus stored in oil has been shown to improve longevity in storage but lacking in consistent improvement (Cherry *et al*., 2000). Patricia *et al*. (2002) concurred that the potency of virus was not affected in the production process of freeze drying virus, but a 50 % loss of insecticidal activity was observed after storage. Patrick and Wood (1995) worked on stabilization and infectivity of baculovirus preoccluded virions. The ability to produce large amount of high-potency viral preparations in larvae and the convenience of being able to lyophilize the preparations for long-term storage showed promise for the use of preoccluded virus preparations as bio-pesticides. Retention of some quantity of larval debris in the formulation may enhance the activity of the virus on host plants. However care should be taken to insure that a semi-purified product does not have secondary microbial contaminations (Mehrvar *et al*., 2007). Arthurs *et al*. (2006) demonstrated the extended persistence of *CpGV* with spray dried formulation. However, the shelf life was found to be reduced. This reduction in shelf life of spray dried NPV formulation has also been cited as an issue affecting the successive commercialization.

Present results showed that silver nanoparticles could be the most promising additive for a *Ha*NPV in terms of the attributes evaluated. Silver nanoparticles are potent and broad-spectrum antimicrobial agents. The mode of their antibacterial action against *Escherichia coli* was investigated, and proved to be an efficient physicochemical system conferring antimicrobial activities (Chun-Nam Lok *et al*., 2006). Silver nanoparticles also expressed significant UV-protection capability (Vigneshwaran *et al*., 2007).

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