STABILITY OF IRANIAN *HELICOVERPA ARMIGERA* NUCLEOPOLYHEDROVIRUS FORMULATIONS UNDER DIFFERENT STORAGE CONDITIONS

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[Mehrvar, A. 2016. Stability of Iranian *Helicoverpa armigera* nucleopolyhedrovirus formulations under different storage conditions. Munis Entomology & Zoology, 11 (1): 90-95**]**

ABSTRACT: Impacts of storage on the shelf life of an Iranian *Helicoverpa armigera* nucleopolyhedrovirus talc-based formulation under five sets of storage conditions has been evaluated in room temperature (25-33°C) as well as refrigerated condition (3±2°C) over the time. Monthly bioassays were conducted with all the treatments against early second instars larvae of *H. armigera*. Results showed that the LC_{50} values increased gradually with time in all the cases. The talc-based wettable powder formulation packed with nitrogen under vacuum had the lowest LC_{50} value after 6 months of storage under both temperature conditions. Also, the lowest LT_{50} value was seen in virus stored as unformulated suspension at refrigerated condition after 6 months of storage (154.3 hours), whereas, it was the highest (173.6 hours) when the unformulated virus was stored at room temperature. The present study revealed the compatibility of the talc-based formulation with nitrogen under vacuum condition in both the temperatures showing storage of the formulation could effectively delay the virus inactivation. This would be crucial to marketing flexibility and timely supply of good quality products.

KEY WORDS: HearNPV, Talc-based formulation, Nitrogen, Vacuum, Shelf life.

The nucleopolyhedrovirus has been found to be effective in the control of *Helicoverpa armigera* (Hübner) on several crops (Jayaraj *et al.*, 1989). But, inactivation of baculoviruses in storage and on plants has been recognized as a major problem in the development of viral insecticides for use in insect pest management systems (Burges, 1998; Moscardi, 1999). The occluded baculoviruses can persist for years under normal environmental temperatures. This stability in their storage and distribution is critical for development of bioinsecticides (Jones and Burges, 1998). Factors affecting the stability of baculoviruses under storage conditions have been listed by several authors (Couch and Ignoffo, 1981; Griffiths, 1982; Rhodes, 1993; Salama and Morris, 1993; Burges and Jones, 1997; Burges, 1998; Jones and Burges, 1998). However, of the factors affecting shelf life of formulated and unformulated viruses in storage conditions can be stated as temperature, pH, container quality, air condition, secondary contaminants and some other factors (Burges, 1998). This study was, therefore, undertaken to evaluate impacts of different set of storage conditions on the stability and biological activity of Iranian *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) formulations.

MATERIALS AND METHODS

Insect and virus: The insect culture used in the study was maintained on a semi-synthetic diet based on Shorey and Hale (1965) for culturing *H. armigera* in the Department of Plant Protection, University of Maragheh, Iran. The Iranian isolate of HearNPV used in this study (EAZ-I) were collected from tomato fields of Maragheh region of East-Azarbaijan province, Iran(Mehrvar, 2013a,b). The isolate was passaged through early fifth instar larvae of host insect at 25±1˚C to get uniformity in their virulence. All the experiments were performed in insect

research laboratory of the Department of Plant Protection, University of Maragheh, and in a facility away from the colony.

Virus formulation: Mass multiplication of HearNPV was carried out using early fifth instar larvae of *H. armigera* and semi-purification of crude NPV was done based on Mehrvar (2011). Talc powder (145.5 g) was passed through 100 mesh sieve, heat sterilized and was thoroughly mixed with 4.5 g of Lissapol (D) as a dispersant agent using a homogenizer. To this, 1×10^{10} OB was added and mixed together to get a homogenate preparation and dried over $CaCl_2$ in a desiccator.

The preparation was homogenized briefly in a blender and stored in sealed polythene bags at 3±2°C until further use. The final product contained 6×10^{9}OB/g.

Experiments: Shelf life of the HearNPV in talc-based formulation was studied under different laboratory conditions. The formulation was prepared and then stored based on the following five methods:

i) Wettable powder under vacuum with Nitrogen (N_2) in thick polythene bags (wp. ptb+vac+N_2)
ii) Wettable powder under vacuum in polythene bags (wp. ptb+vac)
iii) Wettable powder in polythene bags (wp. ptb)
iv) Wettable powder in poly-propylene containers (wp. ppc)
v) Water suspension in poly-propylene containers (ufs. ppc)

Two sets of each treatment were prepared of which, one was held under room temperature (25-33°C) whereas the other was stored at 3±2°C. Vacuum packing was done using a vacuum sealing machine with the ability of different gas packing. In each polythene bag a quantity of 1.5 g of the formulation has been put separately for each month of the assay. Laboratory assays were carried out monthly for each treatment to evaluate the efficacy of the formulated NPV against second instar larvae of *H. armigera*. For this purpose the viral concentration range which has been inoculated into the glass vials containing the semi-synthetic diet was from 1.9660 to 0.0006 OB/mm²with five times reduction in each treatment. The semi-synthetic diet was prepared based on Shorey and Hale (1965) and then filled in five ml glass vials up to one third of the vials height, and 10 µl of the suspension was dispensed uniformly over the entire diet surface by a polished blunt end of a glass rod (6 mm). Second instar larvae of uniform age and size were released on to the diet 20 minutes after surface treatment. Each dose had 30 larvae. In each bioassay, an untreated control was also included. The larvae after inoculation were incubated at 25±1˚C in a laboratory incubator. The observations on larval settlements on the diet were checked out from the first day and mortalities were recorded from third till tenth day at 24 hours interval. Each treatment was replicated thrice.

The Probit analyses in various experiments (LC_{50} and LT_{50} values) were carried out in a Statistical Package for Social Sciences (SPSS), version 21 for windows.

RESULTS

The shelf life of HearNPV formulations under either room temperature or refrigerated condition was studied. Bioassays conducted at regular intervals against second instar larvae of *H. armigera* revealed the LC_{50} values increased gradually with time in all the cases. However, the increases were not significant as the fiducial limits overlapped up to the fourth month. On the fifth month, there was a significant drop in the activity of the wettable powder as well as the unformulated virus when stored at refrigerated condition without vacuum. Meanwhile, storage of the virus at room temperature showed that the virus formulation recorded a significant drop in the LC_{50} value even when packed under

vacuum from the second months of storage onwards (Tables 1 and 2). The talc-based wettable powder formulation packed with nitrogen under vacuum had however the lowest LC_{50} value after 6 months of storage under both temperature conditions (Tables 1 and 2).

Probit analyses of time mortality responses of the larvae to the formulations showed a progressive increase in the LT_{50} values as the period of storage increased. The lowest LT_{50} value was seen in virus stored as unformulated suspension at refrigerated condition after 6 months of storage (154.3 hours), whereas, it was the highest (173.6 hours) when the unformulated virus was stored at room temperature. However, unlike in the case of LC_{50} the increase in the LT_{50} values was significant on the third month onwards of storage at refrigerated condition with highest inactivation of wettable powder in polypropylene containers (Table 3), and from the fourth month onwards in the case of storage at room temperature with unformulated suspension in the same containers (Table 4). However, before the third month of storage, LT_{50} values of the formulations under refrigerated condition did not vary significantly from that stored under room temperature in the same month of storage(Tables 3 and 4).

DISCUSSION

The present study on the shelf life of HearNPV formulations under different storage conditions at room temperature and refrigerated condition revealed that the talc-based formulation packed with nitrogen under vacuum condition had the lowest LC_{50} value after 6 months of storage showing the compatibility of nitrogen with HearNPV wettable powder under both storage temperatures (Tables 1 and 2). The LC_{50} values increased gradually with time in all the cases. However, the increases were not significant as the fiducial limits overlapped up to the fourth month. The LT_{50} values were also showed same trends as seen in LC_{50} values which approving that the refrigerated condition can effectively inhibit the virus inactivity over the time (Tables 3 and 4). However, storage of the talc-based HearNPV formulation packed with nitrogen under vacuum condition could effectively delay the virus inactivation.

Thennarasan (1997) reported a decrease in the virulence of oil formulations of HearNPV after five months of storage. Cherry *et al.* (1994) showed a seven percent loss of *H. armigera* NPV activity on storage at 4°C for 18 months. Gopali and Lingappa (2001) reported that HearNPV stored under refrigerated condition did not lose virulence throughout the year. Similarly, Narabenchi (2004) found no loss of virulence in the activity of HearNPV under refrigerated condition after 12 months of storage. Same results were also stated by Mehrvar (2012) for an Indian HearNPV isolate which formulated as wettable powder.

The present study on the shelf life of HearNPV formulations could be an effective pace to find out long-term storage impacts on the virulence of the virus. This would be crucial to marketing flexibility and timely supply of good quality products.

ACKNOWLEDGMENTS

Thanks are extended to all my colleagues and officials at the Department of Plant Protection, Faculty of Agriculture, University of Maragheh for their genuine assistance and the facilities provided.

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Table 1. Probit analyses of concentration-mortality response of second instar larvae of *H. armigera* to HearNPV wettable powder formulation under different methods of storage at 3±2°C.

Period of storage (Month)	Storage method†	LC_{50} (OB/mm^2)	Fiducial limits (OB/mm^2)	Slope "b" ± SE	χ^2* (n-2)
0	wp	0.0819	0.0661-0.1041	0.33 ± 0.02	2.55
	ufs	0.0778	0.0630-0.0948	0.34 ± 0.05	3.15
1	wp. ptb+vac+N_2	0.0824	0.0665-0.1024	0.87 ± 0.01	2.76
	wp. ptb+vac	0.0823	0.0663-0.1019	0.32 ± 0.08	3.05
	wp. ptb	0.0825	0.0665-0.1025	0.40 ± 0.02	3.23
	wp. ppc	0.0823	0.0663-0.1019	0.71 ± 0.05	2.89
	ufs. ppc	0.0828	0.0671-0.1030	0.61 ± 0.03	3.11
2	wp. ptb+vac+N_2	0.0829	0.0671-0.1031	0.43 ± 0.04	3.45
	wp. ptb+vac	0.0826	0.0664-0.1023	0.34 ± 0.07	4.13
	wp. ptb	0.0822	0.0660-0.1017	0.27 ± 0.05	3.76
	wp. ppc	0.0832	0.0677-0.1025	0.81 ± 0.03	3.33
	ufs. ppc	0.0827	0.0670-0.1029	0.45 ± 0.02	2.67
3	wp. ptb+vac+N_2	0.0825	0.0665-0.1025	0.63 ± 0.03	3.23
	wp. ptb+vac	0.0830	0.0672-0.1031	0.42 ± 0.05	3.50
	wp. ptb	0.0831	0.0672-0.1033	0.26 ± 0.04	3.67
	wp. ppc	0.0835	0.0676-0.1034	0.51 ± 0.06	3.01
	ufs. ppc	0.0830	0.0672-0.1031	0.40 ± 0.03	2.87
4	wp. ptb+vac+N_2	0.0830	0.0672-0.1031	0.42 ± 0.08	2.75
	wp. ptb+vac	0.0833	0.0678-0.1027	0.76 ± 0.06	2.91
	wp. ptb	0.0835	0.0676-0.1034	0.46 ± 0.07	2.33
	wp. ppc	0.0839	0.0682-0.1037	0.78 ± 0.02	2.54
	ufs. ppc	0.0845	0.0688-0.1042	0.83 ± 0.10	3.11
5	wp. ptb+vac+N_2	0.0833	0.0678-0.1027	0.57 ± 0.06	2.44
	wp. ptb+vac	0.0841	0.0684-0.1039	0.78 ± 0.06	3.13
	wp. ptb	0.0847	0.0692-0.1044	0.18 ± 0.02	3.17
	wp. ppc	0.0850	0.0693-0.1049	0.77 ± 0.05	3.05
	ufs. ppc	0.0865	0.0707-0.1063	0.56 ± 0.04	3.28
6	wp. ptb+vac+N_2	0.0838	0.0680-0.1035	0.75 ± 0.07	3.01
	wp. ptb+vac	0.0850	0.0693-0.1049	0.89 ± 0.10	2.82
	wp. ptb	0.0846	0.0670-0.1045	0.76 ± 0.04	3.23
	wp. ppc	0.0853	0.0696-0.1051	0.67 ± 0.05	3.17
	ufs. ppc	0.0869	0.0714-0.1070	0.90 ± 0.11	3.45

* All lines are insignificant at $p<0.05$.

† wp: wettable powder, ufs: unformulated suspension, vac: vacuum condition, N_2: nitrogen, ptb: polythene bag, ppc: polypropylene container.

Table 2. Probit analyses of concentration-mortality response of second instar larvae of *H. armigera* to HearNPV wettable powder formulation under different methods of storage at room temperature.

Period of storage (Month)	Storage method†	LC_{50} (OB/mm^2)	Fiducial limits (OB/mm^2)	Slope "b" ± SE	χ^2* (n-2)
0	wp	0.0819	0.0661-0.1041	0.33 ± 0.02	2.55
	ufs	0.0778	0.0630-0.0948	0.34 ± 0.05	3.15
1	wp. ptb+vac+N_2	0.0825	0.0669-0.1007	0.46 ± 0.01	4.23
	wp. ptb+vac	0.0834	0.0675-0.1016	0.23 ± 0.05	3.87
	wp. ptb	0.0824	0.0666-0.1005	0.19 ± 0.01	3.54
	wp. ppc	0.0826	0.0668-0.1010	0.52 ± 0.04	3.67
	ufs. ppc	0.0829	0.0671-0.1012	0.71 ± 0.06	3.99
2	wp. ptb+vac+N_2	0.0830	0.0672-0.1015	0.22 ± 0.03	2.91
	wp. ptb+vac	0.0844	0.0685-0.1027	0.67 ± 0.03	2.86
	wp. ptb	0.0852	0.0693-0.1035	0.81 ± 0.09	3.33
	wp. ppc	0.0839	0.0681-0.1021	0.54 ± 0.07	2.98
	ufs. ppc	0.0858	0.0701-0.1041	0.61 ± 0.05	2.74
3	wp. ptb+vac+N_2	0.0843	0.0658-0.1027	0.33 ± 0.04	2.55
	wp. ptb+vac	0.0866	0.0708-0.1049	0.19 ± 0.01	3.15
	wp. ptb	0.0872	0.0714-0.1055	0.37 ± 0.02	3.11
	wp. ppc	0.0851	0.0693-0.1034	0.67 ± 0.07	3.42
	ufs. ppc	0.0903	0.0745-0.1086	0.45 ± 0.03	2.89
4	wp. ptb+vac+N_2	0.0851	0.0693-0.1034	0.39 ± 0.04	3.23
	wp. ptb+vac	0.0883	0.0726-0.1062	0.41 ± 0.01	3.45
	wp. ptb	0.0902	0.0744-0.1085	0.25 ± 0.06	2.76
	wp. ppc	0.0858	0.0702-0.1041	0.56 ± 0.10	2.89
	ufs. ppc	0.0966	0.0808-0.1149	0.98 ± 0.11	3.03
5	wp. ptb+vac+N_2	0.0852	0.0694-0.1035	0.33 ± 0.02	3.25
	wp. ptb+vac	0.0877	0.0719-0.1061	0.43 ± 0.07	3.11
	wp. ptb	0.0901	0.0743-0.1084	0.71 ± 0.03	3.23
	wp. ppc	0.0864	0.0706-0.1047	0.80 ± 0.13	2.85
	ufs. ppc	0.0989	0.0832-0.1172	0.41 ± 0.08	3.63
6	wp. ptb+vac+N_2	0.0855	0.0699-0.1038	0.63 ± 0.04	2.55
	wp. ptb+vac	0.0885	0.0727-0.1071	0.82 ± 0.06	2.67
	wp. ptb	0.0905	0.0747-0.1088	0.63 ± 0.03	2.90
	wp. ppc	0.0871	0.0713-0.1054	0.91 ± 0.13	3.09
	ufs. ppc	0.1013	0.0853-0.1201	0.67 ± 0.05	2.87

* All lines are insignificant at $p<0.05$.
† wp: wettable powder, ufs: unformulated suspension, vac: vacuum condition, N_2: nitrogen, ptb: polythene bag, ppc: polypropylene container.

Table 3. Probit analyses of time-mortality response of second instar larvae of *H. armigera* to HearNPV‡ wettable powder formulation under different methods of storage at 3±2°C.

Period of storage (Month)	Storage method†	LT_{50} (h)	Fiducial limits (h)	Slope "b" ± SE	χ^2* (n-2)
0	wp	139.3	135.7-142.6	6.27 ± 0.23	4.25
	ufs	126.2	122.7-130.3	9.20 ± 0.10	3.91
1	wp. ptb+vac+N_2	140.1	136.4-143.4	3.27 ± 0.34	4.11
	wp. ptb+vac	141.1	137.6-144.5	4.25 ± 0.36	3.75
	wp. ptb	139.5	135.9-142.8	6.13 ± 0.57	3.98
	wp. ppc	141.6	138.1-144.9	5.67 ± 0.26	3.67
	ufs. ppc	127.1	123.7-130.4	3.95 ± 0.17	3.92
2	wp. ptb+vac+N_2	143.0	139.3-146.5	6.35 ± 0.39	3.78
	wp. ptb+vac	143.7	140.2-147.9	5.98 ± 0.57	4.52
	wp. ptb	145.6	141.9-149.0	3.19 ± 0.25	5.01
	wp. ppc	144.0	140.3-147.6	4.17 ± 0.75	4.83
	ufs. ppc	144.2	141.2-147.9	6.37 ± 0.46	4.19
3	wp. ptb+vac+N_2	147.4	143.8-150.7	5.75 ± 0.67	4.03
	wp. ptb+vac	148.6	144.9-152.1	3.87 ± 0.34	3.87
	wp. ptb	149.1	145.8-152.4	4.67 ± 0.56	4.67
	wp. ppc	151.6	148.2-155.0	5.13 ± 0.81	3.49
	ufs. ppc	145.6	142.3-149.1	5.89 ± 0.45	3.55
4	wp. ptb+vac+N_2	153.7	150.4-156.2	5.19 ± 0.55	4.16
	wp. ptb+vac	150.3	146.5-153.6	4.55 ± 0.34	5.23
	wp. ptb	147.5	143.8-151.0	5.67 ± 0.34	4.81
	wp. ppc	156.8	153.2-160.4	6.98 ± 0.19	4.67
	ufs. ppc	149.8	145.9-153.1	4.75 ± 0.67	5.08
5	wp. ptb+vac+N_2	152.4	148.9-155.7	4.98 ± 0.50	5.25
	wp. ptb+vac	153.8	150.2-157.3	5.25 ± 0.15	4.99
	wp. ptb	157.9	154.3-161.2	4.67 ± 0.33	5.19
	wp. ppc	159.1	155.4-162.8	4.34 ± 0.28	5.67
	ufs. ppc	150.3	146.7-153.6	4.79 ± 0.41	3.91
6	wp. ptb+vac+N_2	155.2	150.9-158.5	6.18± 0.33	4.67
	wp. ptb+vac	159.4	155.9-162.7	4.38 ± 0.67	4.55
	wp. ptb	160.7	157.1-164.0	5.44 ± 0.19	5.33
	wp. ppc	162.9	159.3-166.4	5.67 ± 0.57	5.17
	ufs. ppc	154.3	150.2-157.8	4.75 ± 0.71	4.93

‡ All the treatments contained NPV at 1.9660OB/mm^2.
* All lines are insignificant at $p<0.05$.
† wp: wettable powder, ufs: unformulated suspension, vac: vacuum condition, N_2: nitrogen, ptb: polythene bag, ppc: polypropylene container.

Table 4. Probit analyses of time-mortality response of second instar larvae of *H. armigera* to HearNPV‡ wettable powder formulation under different methods of storage at room temperature.

Period of storage (Month)	Storage method†	LT_{50} (h)	Fiducial limits (h)	Slope "b" ± SE	χ^2* (n-2)
0	wp	139.3	135.7-142.6	6.27 ± 0.23	4.25
	ufs	126.2	122.7-130.3	9.20 ± 0.10	3.91
1	wp. ptb+vac+N_2	143.8	140.2-147.1	3.54 ± 0.67	5.15
	wp. ptb+vac	140.4	136.8-143.8	4.49 ± 0.34	4.39
	wp. ptb	143.1	138.6-146.9	4.92 ± 0.43	4.67
	wp. ppc	144.3	140.7-147.6	5.11 ± 0.10	5.43
	ufs. ppc	139.8	135.9-143.2	3.63 ± 0.43	3.89
2	wp. ptb+vac+N_2	144.5	140.9-147.8	6.18 ± 0.90	4.19
	wp. ptb+vac	148.2	144.1-152.0	5.84 ± 0.49	4.83
	wp. ptb	145.8	142.3-149.4	5.15 ± 0.26	3.99
	wp. ppc	148.1	144.0-151.7	5.91 ± 0.33	5.21
	ufs. ppc	140.1	136.4-143.2	4.67 ± 0.67	4.61
3	wp. ptb+vac+N_2	149.8	146.2-153.3	5.45 ± 0.61	3.87
	wp. ptb+vac	147.4	143.8-150.7	4.83 ± 0.11	3.93
	wp. ptb	152.4	148.6-155.3	6.13 ± 0.34	5.25
	wp. ppc	153.5	149.9-156.8	4.41 ± 0.51	4.67
	ufs. ppc	151.8	148.2-155.1	5.15 ± 0.07	4.33
4	wp. ptb+vac+N_2	153.3	149.7-156.6	5.96 ± 0.64	5.34
	wp. ptb+vac	155.6	151.9-158.9	6.24 ± 0.18	3.89
	wp. ptb	156.2	152.5-159.7	4.67 ± 0.43	4.59
	wp. ppc	158.7	155.1-161.9	5.28 ± 0.29	4.17
	ufs. ppc	162.4	157.3-166.1	6.10 ± 0.63	5.11
5	wp. ptb+vac+N_2	157.4	153.8-160.7	4.34 ± 0.03	4.91
	wp. ptb+vac	159.5	156.1-163.3	6.27 ± 0.11	3.83
	wp. ptb	160.8	157.2-164.1	5.29 ± 0.18	4.69
	wp. ppc	161.0	157.4-164.5	4.67 ± 0.13	5.28
	ufs. ppc	167.7	162.2-171.3	4.78 ± 0.09	5.73
6	wp. ptb+vac+N_2	161.1	157.4-164.4	5.44 ± 0.22	4.83
	wp. ptb+vac	167.8	164.2-171.1	5.67 ± 0.34	5.17
	wp. ptb	165.3	161.6-168.7	4.35 ± 0.41	5.21
	wp. ppc	163.4	159.8-166.4	6.42 ± 0.09	4.92
	ufs. ppc	173.6	168.9-177.2	5.51 ± 0.34	4.11

‡ All the treatments contained NPV at 1.9660OB/mm^2.
* All lines are insignificant at $p<0.05$.
† wp: wettable powder, ufs: unformulated suspension, vac: vacuum condition, N_2: nitrogen, ptb: polythene bag, ppc: polypropylene container.