



Research Article

Efficacy of HearNPV formulations against *Helicoverpa armigera* at different sunlight exposure period

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ABSTRACT: Experiment was conducted at Department of Entomology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during 2011-2013 to see the effect of natural sunlight (UV) on HearNPV formulation. Experiment was laid out in Completely Randomized Block Design replicated thrice. The aqueous and dry form of HearNPV formulations were prepared by using additives viz. Tinopal an optical brightener and silver nano particle and evaluated their capability to protect NPVs from Ultra Violet rays. HearNPV formulation were sprayed uniformly on the potted chickpea plants during noon hours. Twigs were collected at thirty minutes, One hour, One and half hours and two hours after spraying and kept in petriplates. The laboratory reared second instar larvae of *H. armigera* were released on each treated twig. Larval mortality was recorded at 4, 7 and 10 days after treatment. An aqueous form of HearNPV formulation, HearNPV + Silver Nano Particles @ 8 µl/ml of HearNPV + Tinopal 1% + Sucrose 1% (T1) recorded 83.04% larval mortality at ten days after spraying which was at par with HearNPV alone unirradiated (84.21 % larval mortality) when exposed to sunlight up to one hour. Among the lyophilized form of formulations, HearNPV + Silver Nano Particles @ 8µl/ml of HearNPV + Tinopal 1% + Sucrose1% (T6) when exposed to sunlight up to one hour recorded 84.80% larval mortality at ten days after spraying which was at par with which was at par with HearNPV alone unirradiated (T12) (85.38% larval mortality) and higher than HearNPV alone irradiated. Both aqueous and lyophilized form of HearNPV showed decreasing larval mortality as compared to HearNPV alone unirradiated when exposed to sunlight up to one and half and two hours. Lyophilized HearNPV formulations recorded higher larval mortality as compared to aqueous form of HearNPV formulations at all sunlight exposure period.

KEY WORDS: HearNPV formulations, *Helicoverpa armigera*, UV Rays

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INTRODUCTION

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is a highly polyphagous and widespread insect pest, which causes enormous economic losses to many crops including tomato, sunflower, corn, maize, tobacco, pigeonpea, chickpea and cotton. Although nearly 30% of total insecticides are used for controlling this pest alone on different crops yet, many of them do not prove effective as it has been reported to have developed resistance to almost all kinds of insecticides to varying folds (Yaqoob et al, 2006). Chemical insecticides use is also becoming less appropriate because of a concern for consumer's food safety and for the environment. Chemical insecticides are incompatible with the pollinators, making bio pesticides essential in these cropping systems. Therefore, the demand in the present day scenario is the formulation of some eco-friendly means of pest control

to minimize pesticide related problems and to ensure long term sustainable yield through sound ecological principles. Among them, the insect viruses are of immense utility.

Baculoviruses (Family: Baculoviridae) are generally highly selective pathogens of insects belonging to Orders Lepidoptera and Hymenoptera. Members of the baculoviridae family are attractive agents for the biological control of *Helicoverpa armigera* because of their high pathogenicity, narrow host range and safety to vertebrates, plants and the environment. Other advantages of baculovirus for pest control include a 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, solar radiation, especially the ultraviolet portion of the

spectrum, is probably the most important factor affecting the persistence of microbial insecticides. This radiation directly affects the nucleic acids, modifying or denaturing them, preventing growth and reproduction of the microorganism (Ignoffo et al, 1977, Jacques 1985 and Pawar et al, 1995). Additives can be used to protect baculoviruses from adverse environmental factors, to maximize application efficiency (Lasa et al., 2008). The aqueous and dry form of HearNPV formulations were prepared with additives and evaluated against *H. armigera*.

To protect the virus from sunlight (UV rays), tinopal an optical brightener, mango leaf extract (polyflavonoids) and silver nanoparticle (Vigneshwaran et al. 2007) were evaluated. Sucrose was used as a phagostimulant.

MATERIALS AND METHOD

An experiment was conducted during 2011 and 2013 at Dr. PDKV, Akola to see the effect of sunlight on the degradation of HearNPV formulation. Experiment was conducted in Completely Randomized Block design replicated thrice. The aqueous and dry form of HearNPV formulations was prepared by using additives viz; tinopal 1% an optical brightener, mango leaf extracts 1% (polyflavonoids) and silver nanoparticle @ 80 µl/ml and evaluated their Ultra Violet protection ability. Sucrose was used as a phagostimulants. Formulations in aqueous form were prepared by adding the required quantity of adjuvants in the desired concentration of HearNPV procured from Biocontrol laboratory, Dr. PDKV, Akola which was found effective in pilot experiment conducted before formulation. The dry formulations were prepared by direct impregnation of required quantity of adjuvants with HearNPV infected larvae. After the impregnation of all adjuvants, kept the impregnated virus in deep freezer at -80°C for 12 hours and then lyophilized over night. Milled it into a fine powder and stored it in plastic container until its use. For evaluation of lyophilized HearNPV, resuspended it in a sufficient quantity of distilled water so as to meet the required concentration of HearNPV.

To see the effect of natural sunlight (UV), HearNPV formulations were sprayed uniformly on the potted chickpea plants with hand sprayer during noon hours. Twigs were collected at 30 min, 1 hour, 1.5 hours and 2 hours interval after spraying from each treatment and brought to the laboratory. One end of the twig was wrapped with water soaked cotton swab and placed in petriplates. The laboratory reared second instar larva (30) of *H. armigera* were released on each treated twig. After completely feeding of HearNPV sprayed twig, provided fresh soaked chickpea grain as feed to larvae. Larval mortality was recorded at 4, 7 and 10 days after

treatment. Mortality caused by the formulation was analyzed using ANOVA after square root and arcsine transformation.

RESULT AND DISCUSSION

In present study aqueous and lyophilized form of HearNPV with different UV protectants were evaluated to observe it effects on *H. armigera* when exposed to sunlight at different period after spraying in potted chickpea crop.

Larvicidal activity of HearNPV formulations sprayed in potted chickpea at 4 days after spraying

Among the aqueous HearNPV formulations, HearNPV + Silver Nano Particles @ 8 µl /ml of HearNPV + Tinopal 1% + Sucrose 1% recorded 15% larval mortality at 30 minute sunlight exposure period followed by HearNPV + Streptomycin @ 0.18 g/lit of HearNPV + Tinopal 1% + Sucrose 1% and HearNPV + Silver Nano Particles @ 80 µl/ml of HearNPV + Sucrose 1% 12.78 and 11.67% larval mortality (Table 1).

Lyophilized form of HearNPV formulations, HearNPV + Streptomycin @ 0.18 g/lit of HearNPV + Sucrose 1% (T9), HearNPV + Silver Nano Particles @ 8 µl/ ml of HearNPV + Sucrose 1% (T8), HearNPV + Streptomycin @ 0.18 g/lit of HearNPV + Tinopal 1% +Sucrose 1% (T7), HearNPV + Silver Nano Particles @ 80 µl/ml of HearNPV +Sucrose 1% (T10) and HearNPV + Silver Nano Particles @ 8µl / ml of HearNPV +Tinopal 1% + Sucrose 1% (T6) recorded larval mortality 15.56, 13.89,12.78, 12.78 and 11.67% which was at par with HearNPV alone unirradiated (15.00% larval mortality) and higher that HearNPV alone irradiated (10.00% larval mortality).The larval mortality was decreasing at one, one and half and two hours sunlight exposure period (Table 1).

Larvicidal activity of HearNPV formulations sprayed in potted chickpea at 7 days after spraying

The data given in table 2 revealed that aqueous HearNPV formulation HaNPV + Silver Nano Particles @ 8 µl /ml of HearNPV + Tinopal 1% + Sucrose 1% (T1) recorded 77.22% larval mortality at 30 min sunlight exposure which was at par with HearNPV alone unirradiated (T5) (79.44 % larval mortality) and higher than *HearNPV* alone irradiated (59.44 % larval mortality) (Table 2).

Among the Lyophilized form of HearNPV formulations HearNPV + Silver nano Particles @ 8µl/ml of HearNPV +Tinopal 1% + Sucrose1% (T6) and HearNPV + Streptomycin @ 0.18 g/lit of HearNPV + Tinopal 1% + Sucrose 1% (T7) recorded 78.33 and 77.78% larval mortality when exposed to sunlight upto 30 minutes, which was at par with HearNPV alone unirradiated (T12) (80.56% larval mortality) and higher than HearNPV alone irradiated (64.44% larval mortality). At

Table 1. Influence of sunlight on larvicidal activity of HearNPV formulation sprayed on chickpea

S.N	Treatments	Percent larval mortality at 4 DAT (Pooled Mean)			
		Sunlight Exposure 30 min	Sunlight Exposure 1 Hr	Sunlight Exposure 1.5 Hr	Sunlight Exposure 2 Hr
Aqueous HearNPV formulations					
1	HearNPV+ Silver nano Particles @8 µl /ml of HearNPV + Tinopal 1% +Sucrose1%	15.00 (3.93)	8.89 (3.06)	10.56 (3.32)	8.89 (3.06)
2	HearNPV + Streptomycin @ 0.18 g/lit of HearNPV + Tinopal 1% +Sucrose 1%	12.78 (3.64)	7.78 (2.87)	7.22 (2.78)	6.67 (2.68)
3	HearNPV + Silver Nano Particles @ 80 µl/ml of HearNPV + Sucrose 1%	11.11 (3.40)	7.22 (2.78)	6.67 (2.68)	5.00 (2.35)
4	HearNPV alone irradiated	6.11 (2.57)	5.00 (2.35)	3.89 (2.09)	1.67 (1.47)
5	HearNPV alone unirradiated	11.67 (3.48)	8.33 (2.96)	7.22 (2.78)	10.00 (3.23)
Lyophilized HearNPV formulations					
6	HearNPV + Silver nano Particles @ 8µl / ml of HearNPV +Tinopal 1%+Sucrose1%	11.67 (3.47)	10.00 (3.24)	10.00 (3.24)	8.33 (2.97)
7	HearNPV + Streptomycin @ 0.18 g/lit of HearNPV + Tinopal 1% +Sucrose 1%	12.78 (3.64)	9.44 (3.15)	6.67 (2.68)	6.67 (2.68)
8	HearNPV + Silver nano Particles @ 8 µl/ ml of HearNPV + Sucrose 1%	13.89 (3.79)	7.22 (2.78)	7.22 (2.78)	5.56 (2.46)
9	HearNPV + Streptomycin @ 0.18g/lit of HearNPV Sucrose 1%	15.56 (4.00)	7.22 (2.78)	6.67 (2.68)	6.67 (2.68)
10	HearNPV + Silver nano Particles @ 80 µl/ml of HearNPV + Sucrose 1%	12.78 (3.63)	6.67 (2.68)	6.67 (2.68)	6.67 (2.68)
11	HearNPV alone irradiated	10.00 (3.24)	3.33 (1.96)	0.00 (0.71)	0.00 (0.71)
12	HearNPV alone unirradiated	15.00 (3.93)	10.00 (3.24)	7.22 (2.78)	8.89 (3.06)
13	Control	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
	F Test	Sig	Sig	Sig	Sig
	S.Em.±	0.13	0.08	0.07	0.06
	C.D. at 5 %	0.38	0.23	0.20	0.18
	C.V. %	6.74	5.15	4.89	4.56

*Figures in the parenthesis are X + 0.5 square root transformed values.

one, one and half and two hours sunlight exposure period the larval mortality was found decreasing (Table 2).

Larvicidal activity of HearNPV formulations sprayed in potted chickpea at 10 days after spraying

HearNPV + Silver Nano Particles @ 8 µl/ml of HearNPV formulation + Tinopal 1% + Sucrose 1% (T1) and HearNPV + Streptomycin @ 0.18 g/lit of HearNPV+ Tinopal 1% + Sucrose 1% (T2) an aqueous form of HearNPV formulations recorded 83.41 and 82.28% larval mortality respectively

was at par with HearNPV alone irradiated (84.57 % larval mortality) when exposed to sunlight up to 30 minutes. At one hour sunlight exposure period HaNPV + Silver Nano Particles @ 8 µl/ml of HearNPV + Tinopal 1% + Sucrose 1% (T1) recorded 83.04% larval mortality which was at par with HearNPV alone unirradiated (84.21 % larval mortality) (Table 3).

Lyophilized HearNPV formulations; HearNPV + Silver nano Particles @ 8µl / ml of HearNPV + Tinopal 1% +

Table 2. Influence of sunlight on larvicidal activity of HearNPV formulation sprayed on chickpea

S.N	Treatments	Per cent larval mortality at 7 DAT (Pooled Mean)			
		Sunlight Exposure 30 min	Sunlight Exposure 1 Hr	Sunlight Exposure 1.5 Hr	Sunlight Exposure 2 Hr
Aqueous HearNPV formulation					
1	HearNPV+ Silver nano Particles @8 µl /ml of HearNPV + Tinopal 1% +Sucrose1%	77.22	72.78	63.33	57.78
		(61.52)	(57.50)	(52.74)	(49.48)
2	HearNPV+ Streptomycin @ 0.18 g/lit of HearNPV + Tinopal 1% +Sucrose 1%	72.78	68.89	61.67	56.11
		(58.55)	(56.10)	(51.75)	(48.51)
3	HearNPV + Silver Nano Particles @ 80 µl/ml of HearNPV+Sucrose 1%	62.78	61.67	53.89	48.33
		(52.40)	(51.75)	(47.23)	(44.04)
4	HearNPV alone Irradiated	59.44	53.89	25.56	18.33
		(50.44)	(47.23)	(30.35)	(25.34)
5	HearNPV alone unirradiated	79.44	80.00	80.56	78.89
		(63.07)	(63.45)	(63.84)	(62.65)
Lyophilized HearNPV formulation					
6	HearNPV + Silver nano Particles @ 8µl / ml of HearNPV +Tinopal 1%+Sucrose1%	78.33	73.33	65.56	60.56
		(62.27)	(60.01)	(54.07)	(51.10)
7	HearNPV + Streptomycin @ 0.18 g/lit of HearNPV + Tinopal 1% + Sucrose 1%	77.78	70.00	65.00	57.78
		(61.90)	(56.89)	(53.73)	(49.48)
8	HearNPV + Silver nano Particles @ 8 µl/ ml of HearNPV + Sucrose 1%	64.44	57.78	30.00	22.22
		(53.40)	(49.48)	(33.21)	(28.12)
9	HearNPV + Streptomycin @ 0.18gl/lit of HearNPV Sucrose 1%	62.78	52.22	28.33	20.56
		(52.41)	(46.27)	(32.15)	(26.96)
10	HearNPV + Silver nano Particles @ 80 µl/ml of HearNPV + Sucrose 1%	57.22	60.56	51.11	46.11
		(49.16)	(51.10)	(45.64)	(42.77)
11	HearNPV alone irradiated	64.44	52.22	27.22	19.44
		(53.40)	(46.27)	(31.45)	(26.16)
12	HearNPV alone unirradiated	80.56	82.22	81.67	81.67
		(63.87)	(65.08)	(64.66)	(64.65)
13	Control	0.00	0.00	0.00	0.00
		(0.52)	(0.52)	(0.52)	(0.52)
F Test		0.72	Sig	Sig	Sig
S.Em.±		2.10	0.69	0.60	0.54
C.D. at 5 %		2.38	2.00	1.75	1.58
C.V. %		0.72	2.37	2.41	2.35

Figures in the parenthesis are arcsine transformed values

Sucrose1% (T6) and HearNPV + Streptomycin @ 0.18 g/lit of HearNPV + Tinopal 1% +Sucrose 1% (T7) recorded 85.71 and 84.58% larval mortality when exposed to sunlight upto 30 minutes, which was at par with HearNPV alone unirradiated (T12) (85.71% larval mortality) and higher than HearNPV alone irradiated (64.31% larval mortality). At one hour of sunlight exposure HearNPV + Silver nano Particles @ 8µl/ml of HearNPV + Tinopal 1% + Sucrose1% (T6) recorded 84.80% larval mortality which was at par with HearNPV alone unirradiated (T12) (85.38% larval mortality) (Table 3).

Both aqueous and lyophilized form of HearNPV showed decreasing larval mortality as compared to HearNPV alone unirradiated when exposed to sunlight up to one and half and two hours.

Lyophilized HearNPV formulations recorded higher larval mortality as compared to aqueous form of HearNPV formulations to all sunlight exposure period. Dry form of HearNPV formulations with UV protectant proved more effective than dry form of HearNPV formulations without

Table 3. Influence of sunlight on larvicidal activity of HearNPV formulation sprayed on chickpea (Pooled Mean)

S.N	Treatments	Per cent larval mortality at 10 DAT (Pooled Mean)			
		Sunlight Exposure 30 min	Sunlight Exposure 1 Hr	Sunlight Exposure 1.5 Hr	Sunlight Exposure 2 Hr
Aqueous HearNPV formulation					
1	HearNPV+ Silver nano Particles @8 µl /ml of HearNPV + Tinopal 1% +Sucrose1%	83.41	83.04	66.27	61.67
		(66.05)	(65.69)	(54.50)	(51.75)
2	HearNPV+ Streptomycin @ 0.18 g/lit of HearNPV + Tinopal 1% +Sucrose 1%	82.28	80.12	63.94	58.67
		(65.11)	(63.54)	(53.10)	(50.00)
3	HearNPV + Silver Nano Particles @ 80 µl/ml of HearNPV + Sucrose 1%	74.29	63.16	54.65	49.10
		(59.54)	(52.63)	(47.67)	(44.49)
4	HearNPV alone Irradiated	74.29	57.89	24.42	18.56
		(59.54)	(49.54)	(29.61)	(25.52)
5	HearNPV alone unirradiated	84.57	84.21	83.73	84.43
		(66.87)	(66.59)	(66.23)	(66.77)
Lyophilized HearNPV formulation					
6	HearNPV + Silver nano Particles @ 8µl / ml of HearNPV +Tinopal 1%+Sucrose1%	85.71	84.80	70.36	64.08
		(67.80)	(67.06)	(57.02)	(53.18)
7	HearNPV + Streptomycin @ 0.18 g/lit of HearNPV + Tinopal 1% + Sucrose 1%	84.58	82.46	68.03	61.66
		(66.90)	(65.26)	(55.57)	(51.75)
8	HearNPV + Silver nano Particles @ 8 µl/ml of HearNPV + Sucrose 1%	73.14	66.08	36.62	23.95
		(58.79)	(54.38)	(37.24)	(29.30)
9	HearNPV + Streptomycin @ 0.18g/lit of HearNPV Sucrose 1%	73.12	63.74	33.72	24.56
		(58.80)	(52.98)	(35.50)	(29.70)
10	HearNPV + Silver nano Particles @ 80 µl/ml of HearNPV + Sucrose 1%	73.71	62.57	51.16	48.50
		(59.16)	(52.29)	(45.66)	(44.14)
11	HearNPV alone irradiated	64.31	55.56	24.43	11.39
		(53.32)	(48.19)	(29.61)	(19.66)
12	HearNPV alone unirradiated	85.71	85.38	86.62	86.21
		(67.80)	(67.53)	(68.56)	(68.24)
13	Control	0.00	0.00	0.00	0.00
		(0.52)	(0.52)	(0.52)	(0.52)
F Test		Sig	Sig	Sig	Sig
S.Em.±		0.71	0.46	0.55	0.68
C.D. at 5 %		2.07	1.35	1.61	1.98
C.V. %		2.18	1.48	2.14	2.87

Figures in the parenthesis are arcsine transformed values

UV protectant. This may be due to the additive affect of Tinopal 1% and the dry form of HearNPV formulations contained larval debris. Burges and Jones, 1998 reported that 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 ensure that a semi-purified product does not have secondary microbial contaminations.

The HearNPV formulation having Tinopal 1% as UV protectant recorded highest mortality amongst the irradiated

treatments against sunlight upto thirty minutes and one hour's exposure period. Effectiveness of Tinopal as an UV protectant was well documented by number of Scientists (Dougherty et al. 1996, Sonalkar et al. 1998, Washburn et al. 1998, Farrar et al. 2003, Martin and Argauer 2001, Md. Monobullah 2003, Rosa et al. 2003, Martinez et al. 2004 and Martin 2004) and recommended to mix the Tinopal with HearNPV at the time of spraying. But in the present investigation an attempt was made to make available ready form of HearNPV formulation comprising Tinopal 1% as an UV protectant Vigneshwaran et

al 2007 proved that, Silver nanoparticles impregnated fabrics expressed significant UV-protection capability. Hence Silver Nano particle was also evaluated as UV protectant in this study.

The literature is not traceable on ready form of HearNPV formulation having Tinopal 1%. But Scientists developed and studied the NPV formulations in different form and the available literature is discussed here. Quiroga et al. (2011) formulated two biopesticide *Tecia solanivora* NPV as granular (WG) and as an Emulsifiable Concentrate (EC) that had similar performances after exposure to UV radiation. Cherry et al. (2000) tested several formulations of virus, including an emulsifiable concentrate, a ULV suspension and a microencapsulated preparation, but none were consistently more effective than a filtered but un-purified aqueous suspension of HearNPV and Persistence was short, HearNPV was the slowest acting, with average survival times of 5.5 days. The emulsifiable oil formulation lost only 18.3% of its original activity Young (1994) formulated viral as wettable powders by lyophilization and spray dry methods. These formulations are best standardized using both counts of occluded virus particle concentration and bioassay activity.

Arthurs (2006) evaluated spray dried lignin encapsulated formulations of granulovirus of the codling moth, *Cydia pomonella* and reported that aqueous lignin formulations containing a high dosage of occlusion bodies, with and without the additives titanium dioxide (TiO₂) and sugar, provided significant solar protection of virus. Experimental formulations were made using combinations of corn flours, lignin and sucrose and were selected based on previous work which demonstrated that these formulations resisted solar degradation in field experiments (Patricia et al. 2007). Granulosis virus of *Pieris brassicae* was much more rapidly inactivated in pure form than in form of crude virus (Hadapad et al. 2009).

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