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Research Article

Potency of Silver Nanoparticles (SNPs) as UV protectant for *Ha*NPV

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ABSTRACT: Present investigation was carried out to test the potency of Silver nanoparticles (SNPs) as UV protectants along with tinopal and mango leaf extract at Department of Entomology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during 2011 - 2013. Formulated viral suspension was exposed to UV 20 W Philips lamp (Type 05, range 285 - 320 nm) for 15 minutes and to sunlight at noon hours for 30 minutes,1, 1.5 and 2 hours and determined the viability of POBs and larvicidal activity of viral formulations before and after UV lamp and sunlight exposure. Least reduction in POBs i.e 5.2 per cent was observed from *Ha*NPV + Tinopal 1 %, also recording 86.67 per cent larval mortality when exposed to UV lamp, which was at par with *Ha*NPV alone unirradiated. *Ha*NPV alone when irradiated recorded 55.1 per cent reduction in POBs. *Ha*NPV + tinopal 1 % when exposed to sunlight, protected the POBs upto 1.5 hour, recorded 20.5 per cent reduction in POBs and 83.89 per cent larval mortality. When exposed to 2 hours sunlight reduced the insecticidal activity of *Ha*NPV even though tinopal 1% was used as UV protectant with *Ha*NPV. Of the additives, tinopal 1 % was found to be most effective as it protected the POBs from UV degradation and enhanced larval mortality as compared to mango leaf extract 1 % and SNPs.

KEY WORDS: Silver Nanoparticles, *Ha*NPV, UV protectants, tinopal 1 %, mango leaf extract

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INTRODUCTION

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is a highly polyphagous and widespread insect pest, inflicting annual crop damage in India worth US $ 1 billion. Although nearly 30 % of the total insecticides were 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). Use of chemical insecticide is becoming less appropriate because of a concern for consumers' food safety and for the environment and are incompatible with the pollinators. Therefore, the demand in the present day scenario is the formulation of some eco-friendly means of pest control, among them, the insect viruses are of immense utility. Baculovirus could be an attractive candidate for integrated pest management (IPM) because of its inherent high pathogenicity, narrow host range, and safety to vertebrates, plants and the environment. Despite these advantages, their practical application as microbial pesticides has not been fully exploited. Among the limiting factors, rapid inactivation of the virus by sunlight (ultra violet light) 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. However, additives can be used to protect the baculovirus from these factors. In the present study additives likes silver nanoparticles (SNPs) at different concentrations, tinopal and mango leaf extract were evaluated. Basically, Silver nanoparticles are potent and broad-spectrum antimicrobial agents. Vigneshwaran *et al*., (2007) reported that Silver nanoparticles also expressed significant UV protection capability. Through this study an attempt was made to use SNPs for dual purpose, as antimicrobial additive so as to extend the shelf life of *Ha*NPV, which can be reduced by microbial contamination and also to shelter the POBs from UV degradation after spraying in field against *H. armigera* on various crop. Tinopal, an optical brighteners may protect the virus from solar radiation increasing the susceptibility of pests to viral

infection (Burges and Jones, 1998). Polyflavonoids from Mango leaves was found to protect NPV from inactivation by sunlight (Asokan, 1998). Overall attempt was made to screened out the potent UV protectant, which can be use as additive in *Ha*NPV ready formulation

MATERIALS AND METHODS

The present investigation was carried out at Department of Entomology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India during 2011 - 2013 in completely randomized block design replicated thrice. Formulated viral suspensions were exposed to 20 watt UV lamp (Type 05, range 285 - 320 nm UV radiation) for 15 min. For sunlight exposure, 2 ml of required concentration of *Ha*NPV formulation was kept in petriplates and exposed to sunlight during noon hours for 30 min, 1 hour, 1.5 hours and 2 hours. The POB count of viral formulations before and after UV and sunlight exposure were determined by using Neubauer Haemocytometer and larvicidal activity was tested against second instar larvae of *H.armigera*. Before testing viability and larvicidal activity of irradiated samples, the volume of each sample was adjusted with sterile distilled water to compensate for the loss of water due to evaporation. For larvicidal activity 50 µl of UV lamp and sunlight exposed *Ha*NPV formulation was smeared over the half broken soaked chickpea grain and then released the 30 laboratory reared second instar larvae of *H.armigera* in each treatment. Larvae were starved for 24 hours before releasing. For further feeding fresh soaked chickpea grain was used. Larval mortality was scored at 3 days after treatment up to 10^{th} day. Mortality caused at 10^{th} day after treatment was analyzed using ANOVA after arc sin transformation. The per cent mortality was corrected by using Abbots formula.

Synthesis and characterization of SNP

The SNP were synthesis using fungus *Fusarium graminearum* using the procedure followed by Gaikwad *et al*., (2013). The synthesized SNP were detected by taking UV-Vis spectra and were further characterize by Nanoparticle Tracking Analysis system, Zeta measurement and Transmission Electron Microscopy.

RESULTS AND DISCUSSION

Detection and characterization of SNP

Synthesized SNP were detected using UV-Vis spectrophotometer. It shows absorbance around 417 nm, a characteristics absorbance peak specific for SNP. Nanoparticle Tracking and Analysis reveal the size of 42.1 ± 1.1 nm and concentration of 1.32 x 10^{10} nanoparticles/ ml. Zeta potential measurement was found to be –12.4 ± 1.7 mV. Transmission Electron Microscopy reveals the presence of spherical polydispersed silver nanoparticles in the range of 2-68 nm. The characterization details as given by Gaikwad *et al*., (2013)

Effect of Ultraviolet rays and sunlight exposed *Ha*NPV with UV protectants on POB count and larval Mortality of *Helicoverpa armigera* .

The data given in Table 1 and 2 revealed that, least reduction in POBs i.e 5.2 per cent was observed from *Ha*NPV + Tinopal 1 % recording 86.67 per cent larval mortality when exposed to 20 W UV lamp (Type 05, range 285 - 320 nm UV radiation) for 15 min, followed by *Ha*NPV + Mango leaf extract 1 % with 13.8 per cent reduction in POBs and 78.89 per cent larval mortality. *Ha*NPV + Silver nanoparticles @ 80µl/ml of *Ha*NPV, recorded 47.9 per cent reduction in POBs and 42.78 per cent larval mortality. *Ha*NPV alone irradiated recorded 55.1 per cent reduction in POBs (Fig. 1 and 2).

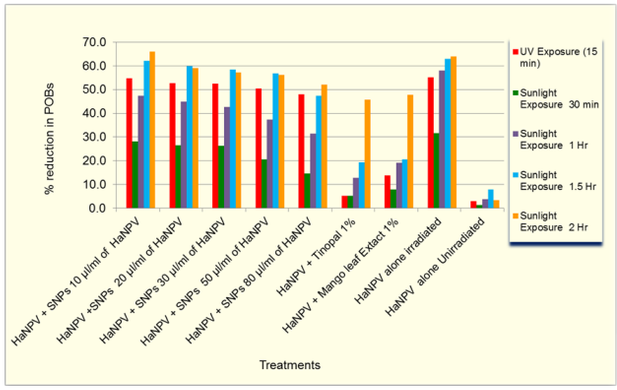

Fig. 1. Effect of ultraviolet rays and sunlight exposed *Ha*NPV with UV protectants on POB count.

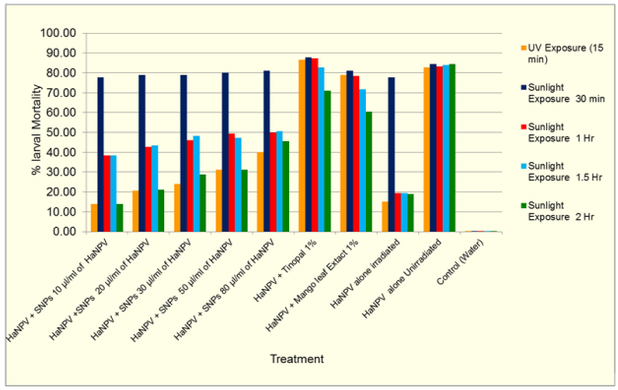

Fig. 2. Effect of ultraviolet rays and sunlight exposed *Ha*NPV with UV protectants on larval mortality of *H. armigera*

*Ha*NPV + Tinopal 1 % recorded 5.2,12.8 and 20.5 per cent reduction in POBs and 87.78, 87.22 and 83.89 per cent

larval mortality, when exposed to sunlight for 30 minutes, 1 and 1.5 hour respectively. While at two hour sunlight exposure, it has recorded 45.6 per cent reduction in POBs and 68.89 per cent larval mortality. *Ha*NPV + Mango leaf extract 1 % recorded 7.9,19.2 and 32.3 per cent reduction in POBs and 81.11,78.33 and 71.67 per cent larval mortality, when exposed to 30 minutes, 1 and 1.5 hour respectively. While at two hour exposure recorded 47.8 per cent reduction in POBs and 60.88 per cent larval mortality. *Ha*NPV + Silver nanoparticles @ 80 µl/ml of *Ha*NPV, recorded 14.5,31.4 and 47.4 per cent reduction in POBs and 81.11,50.00 and 50.56 per cent larval mortality when exposed to 30 minutes, 1 and 1.5 hour respectively. *Ha*NPV alone irradiated recorded 55.1 per cent reduction in POBs (Table 1 and 2).

Formulated *Ha*NPV having 1% Tinopal as UV protectant, remains viable in 285 - 320 nm wavelength UV radiation upto 15 min and in sunlight at noon hours upto 1.5 hour, thereafter started reducing the insecticidal activity. *Ha*NPV was progressively inactivated as the exposure time to UV source increased. Tinopal would absorb long wavelength UV light and thereby provide protection to POBs from inactivation. Several optical brighteners are known to interfere with cellulose and chitin fibrillogenesis. Optical brighteners increases the permeability of the midgut peritrophic membrane and by reducing the rate of turnover of infected gut cells. This might have spread the infection more rapidly to the susceptible tissues that are involved in the secondary cycle of infection and thus account for early larval mortality. Optical brighteners, Tinopal administered in mixtures with occlusion bodies would result in a higher prevelance of covert infection in larvae. (Burges and Jones, 1998). Martinez *et al*., (2004) carried out same line of work and reported that, Tinopal 1% concentration was sufficient to enhance the mortality and hasten the death of larvae.

Tinopal 1 % proved most effective as it shelter the POBs from UV degradation and enhanced larval mortality as compared to Mango leaf extract 1 % and Silver nanoparticles. Tinopal 1 per cent can be used as additive for developing ready *Ha*NPV formulation as a biopesticide having potential UV protectant, for the control of *H. armigera*. Higher concentration of Silver Nano Particles i.e 80 µl/ml of *Ha*NPV, also shelter POBs degradation and recorded 50 per cent larval mortality at 1 and 1.5 hour sunlight exposure. SNPs have bactericidal property, check the contamination and extend the shelf life of *Ha*NPV.

Table 1. Effect of ultraviolet rays and sunlight exposed *Ha*NPV with UV protectants on POB count.

S. N	Treatments	Per cent reduction in POBs (Pooled Mean)				
		UV Exposure (15 min)	Sunlight Exposure 30 min	Sunlight Exposure 1 Hr	Sunlight Exposure 1.5 Hr	Sunlight Exposure 2 Hr
1	*Ha*NPV + Silver nanoparticles 10 µl/ml of *Ha*NPV	54.70 (47.70)	28.20 (32.10)	47.40 (43.80)	62.10 (52.00)	66.10 (54.40)
2	*Ha*NPV + Silver nanoparticles 20 µl/ml of *Ha*NPV	52.80 (46.60)	26.40 (30.90)	44.90 (42.30)	59.90 (50.70)	59.00 (50.20)
3	*Ha*NPV + Silver nanoparticles 30 µl/ml of *Ha*NPV	52.50 (46.50)	26.30 (30.90)	42.60 (40.40)	58.40 (49.80)	57.10 (49.10)
4	*Ha*NPV + Silver nanoparticles 50 µl/ml of *Ha*NPV	50.40 (45.20)	20.60 (27.00)	37.30 (37.70)	56.90 (49.00)	56.10 (48.50)
5	*Ha*NPV + Silver nanoparticles 80 µl/ml of *Ha*NPV	47.90 (43.80)	14.50 (22.30)	31.40 (32.60)	47.40 (43.50)	52.20 (46.30)
6	*Ha*NPV + Tinopal 1%	05.20 (12.90)	05.20 (13.00)	19.2 (26.00)	20.50 (20.90)	45.60 (42.50)
7	*Ha*NPV +Mango leaf Extract 1%	13.80 (21.40)	7.90 (16.30)	12.8 (14.20)	32.30 (50.35)	47.80 (43.80)
8	*Ha*NPV alone irradiated	55.10 (47.90)	31.60 (34.20)	58.0 (51.40)	62.90 (52.50)	63.90 (53.10)
9	*Ha*NPV alone unirradiated	02.90 (9.10)	01.20 (6.40)	07.8 (11.00)	07.80 (16.10)	03.30 (10.30)
F test		Sig	Sig	Sig	Sig	Sig
SE (M)		01.60	00.85	00.34	00.62	01.28
CD at 5 %		04.75	02.53	01.02	01.83	03.79
CV %		07.76	06.22	01.82	02.63	05.00

*Figures in the parenthesis are arc sin transformed values

Table 2. Effect of ultraviolet rays and sunlight exposed *Ha*NPV with UV protectants on larval mortality of *H. armigera*.

S.N	Treatments	Per cent larval mortality at 10 DAT (Pooled Mean)				
		15 Min UV light Exposure	30 Min Sunlight Exposure	1 hr Sunlight Exposure	1.5 hr Sunlight Exposure	2 hr Sunlight Exposure
1	*Ha*NPV + Silver nanoparticles 10 µl/ml of *Ha*NPV	13.89 (21.87)	77.78 (61.89)	38.33 (38.25)	38.33 (38.25)	13.89 (21.87)
2	*Ha*NPV + Silver nanoparticles 20 µl/ml of *Ha*NPV	20.56 (26.96)	78.89 (62.66)	42.78 (40.85)	43.33 (41.17)	21.11 (27.35)
3	*Ha*NPV + Silver nanoparticles 30 µl/ml of *Ha*NPV	23.89 (29.26)	78.89 (62.66)	46.11 (42.77)	48.33 (44.04)	28.89 (32.51)
4	*Ha*NPV + Silver nanoparticles 50 µl/ml of *Ha*NPV	31.11 (33.90)	80.00 (63.49)	49.44 (44.68)	47.22 (43.41)	31.11 (33.90)
5	*Ha*NPV + Silver nanoparticles 80 µl/ml of *Ha*NPV	42.78 (40.85)	81.11 (64.26)	50.00 (45.00)	50.56 (45.32)	45.56 (42.45)
6	*Ha*NPV + Tinopal 1%	86.67 (68.58)	87.78 (69.58)	87.22 (69.06)	82.78 (65.49)	68.89 (57.51)
7	*Ha*NPV + Mango leaf Extact 1%	78.89 (62.66)	81.11 (64.26)	78.33 (62.27)	71.67 (57.85)	60.56 (51.12)
8	*Ha*NPV alone irradiated	15.00 (22.79)	77.78 (61.89)	19.44 (26.16)	21.11 (27.35)	18.89 (25.73)
9	*Ha*NPV alone unirradiated	82.78 (65.49)	84.44 (66.87)	83.33 (65.92)	83.89 (66.34)	84.44 (66.80)
10	Control (Water)	00.00 (00.52)	00.00 (00.52)	00.00 (00.52)	00.00 (00.52)	00.00 (00.52)
F Test		Sig	Sig	Sig	Sig	Sig
S.Em.±		00.45	00.98	00.51	00.55	00.94
C.D. at 5 %		01.31	02.89	01.51	01.62	02.70
C.V. %		02.08	02.93	02.04	02.23	04.53

* Figures in the parenthesis are arc sin transformed values

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