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Research Note

In vivo screening of optical brighteners as UV protectants and their efficacy in enhancing the virulence of Madurai isolate of NPV of *Amsacta albistriga* (Walker) (Lepidoptera: Arctiidae)

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ABSTRACT: ***In vivo* screening of eight optical brighteners as UV protectants for Madurai isolate of NPV of *Amsacta albistriga* (Walker) revealed that Ranipal BVN and 2B provided excellent protection (> 80% original activity remaining). The retention of viral activity ranged from 90.8 to 97.6 per cent and 83.4 to 95.4 per cent in various concentrations from 0.1 to 1.0 per cent for Ranipal BVN and 2B, respectively while it was only from 4.6 to 7.0 per cent in UV irradiated NPV for 60 min. Addition of the best UV protectant, Ranipal BVN along with phagostimulants (molasses and crude sugar) and inorganic substances (boric acid and urea) enhanced the virulence of *A. albistriga* NPV by reducing LT_{50} besides causing the highest mortality.**

KEY WORDS: Adjuvants, *Amsacta albistriga*, LT_{50}, NPV, OAR, optical brighteners

The Red hairy caterpillar, *Amsacta albistriga* (Walker) is a seasonal but destructive polyphagous pest attacking several crops including rain fed groundnut, *Arachis hypogaea* L. (David and Kumaraswami, 1982). Entomopathogenic viruses, particularly the nucleopolyhedrovirus, are reported to be potential biopesticides for the management of this pest. However, their relatively slow action, specificity to a single insect pest, high level of ultraviolet inactivation as well as inactivation by glandular secretion of leaves and poor storage capability limit their efficacy and large scale commercial production and use. Efficacy of entomopathogens can be maximized by conserving their stability in the environment (Ignoffo and Falcon, 1978). Several natural and synthetic organic chemicals have been evaluated as sunlight protectants for entompathogens such as viruses (Jaques, 1971), bacteria (Krieg, 1975), protozoa (Teetor and Kramer, 1977) and nematodes (Gaugler and Bousch, 1979). Optical brighteners are being used as fluorochromes for micro-organisms (Slifkin and Cumbie, 1988). During the past decade, many scientists demonstrated several optical brighteners as successful UV protectants for Douglas-fir tussock moth NPV (Martignoni and Iwai, 1985), *Spodoptera frugiperda* NPV (Hamm and Shapiro, 1992), *Lymantria dispar* NPV (Shapiro and Dougherty, 1994) and *Spodoptera litura* NPV (Murali Baskaran *et al.*, 1997). Protection of gypsy moth NPV (Shapiro, 1989) and *S. litura* NPV (Murali Baskaran *et al.*, 1998) by congo red at 0.5 per cent from UV rays was well demonstrated. In the present study, effect of addition of selected optical

brighteners on the protection of the virus particles and enhancement of the virulence of Madurai isolate of NPV of *A. albistriga* was determined.

Eight optical brighteners, Ranipal BVN, HI, 2B, MM, 2BA, S and 5G and Ultra HRU obtained as powders from Mafatlal Company, Mumbai were screened as UV protectants for *A. albistriga* NPV at Biocontrol laboratory, Department of Agricultural Entomology during 2002 – 2003. NPV was diluted to a concentration of 1 x 10^8 POBs/ml with different concentrations of optical brighteners (0.1, 0.5 and 1.0%) and 100 ml was pipetted in a 250 ml conical flask. Each conical flask was held 30 cm below a UV lamp (15 W Philips make) and was exposed for 60 minutes (Murali Baskaran *et al.*, 1998). After exposure, the volume was adjusted to 100 ml and one ml of aliquot was used to treat both surfaces of leaflets of groundnut. Untreated larvae exposed to non-irradiated NPV served as check. Fifteen fourth instar larvae were infected for each replication and the virus-induced mortality on the eighth day was recorded. UV protection was measured in terms of original activity remaining (OAR) after irradiation (Ignoffo and Batzer, 1971). Based on the per cent OAR, optical brighteners were categorized as no protection (< 10%), low protection (11-30%), fair to good protection (31-50%), superior protection (51-80%) and excellent protection (> 80%) (Shapiro, 1992).

$$\text{OAR (\%)} = \frac{\text{NPV caused larval mortality post UV exposure}}{\text{NPV caused larval mortality pre UV exposure}} \times 100$$

The best optical brightener obtained from this study was used in combination with urea and boric acid to evaluate the efficacy of this combination on enhancing the virulence of NPV against II, III and IV instars of *A. albistriga*. Each treatment was replicated four times. The virus induced mortality was recorded daily starting from 4 to 8 days. The capacity of adjuvants in enhancing the virulence

Table 1. Original activity remaining of Madurai isolate of NPV of *A. albistriga* suspended in different optical brighteners

Treatment	Original activity remaining at 8 day PI (%)			Mean(X)
	0.1%	0.5%	1.0%	
NPV + H_2O (No UV)	58.8 (49.11)^c	60.5 (50.72)^c	55.5 (46.53)^b	
NPV + H_2O (60 UV)	4.6 (10.87)^f	6.9 (10.87)^g	7.0 (15.37)^f	6.2
NPV + optical brighteners (60 UV)				
NPV + Ranipal BVN	90.8 (75.24)^a	97.6 (84.89)^a	97.6 84.85)^a	95.3
NPV + Ranipal HI	35.5 (36.60)^d	39.5 (39.01)^d	41.9 (40.36)^e	38.9
NPV + Ranipal 2B	83.4 (66.06)^b	95.3 (79.77)^b	95.4 (79.85)^a	91.4
NPV + Ranipal MM	6.8 (12.99)^f	9.2 (15.19)^g	16.3 (23.72)^e	10.8
NPV + Ranipal 2BA	19.3 (25.96)^e	20.9 (27.11)^f	30.3 (33.38)^d	23.5
NPV + Ranipal S	18.9 (25.79)^e	23.2 (28.81)^f	27.9 (31.92)^d	23.3
NPV + Ranipal 5G	9.3 (15.27)^f	23.3 (28.88)^f	25.7 (30.41)^d	19.4
NPV + Ultra HRU	12.2 (20.26)^{ef}	28.1 (31.93)^e	32.5 (34.75)^d	24.3
Mean	33.9	40.5	43.0	

Figures in parentheses are arcsine-transformed values; means followed by same letter(s) are not significantly different by DMRT (P = 0.05); Treatments involved with NPV containing 1 x 10^8 POBs/ml;
PI - Post Inoculation.

Table 2. Categorization of optical brighteners based on their OAR values

10%	11-30%	31-50%	51-80%	>80%
-	Ranipal 2BA Ranipal S Ranipal 5G Ranipal MM	Ranipal HI Ultra HRU	-	Ranipal BVN Ranipal 2B

Table 3. Mortality of different instars of *A. albistriga* due to Madurai isolate of NPV with adjuvants

Treatment	Original activity remaining at 8 day PI (%)			Mean(X)
	II instar	III instar	IV instar	
NPV + urea 0.5% + Ranipal BVN 0.1% + crude sugar 0.5%	96.7 (79.53)^{a}	93.3 (74.99)^{a}	81.7 (64.67)^{a}	90.6
NPV + urea 0.5% + Ranipal BVN 0.1% + molasses 0.5%	93.3 (74.99)^{a}	91.7 (73.26)^{a}	76.7 (61.14)^{a}	87.2
NPV + boric acid 0.5% + Ranipal BVN 0.1% + crude sugar 0.5%	85.5 (67.21)^{b}	81.7 (64.67)^{b}	70.0 (56.79)^{bcd}	78.9
NPV + boric acid 0.5% + Ranipal BVN 0.1% + molasses 0.5%	78.3 (62.24)^{bc}	80.0 (63.43)^{b}	71.7 (57.86)^{bc}	76.7
NPV + urea 0.5% + crude sugar 0.5%	70.0 (56.78)^{cd}	68.4 (55.79)^{c}	63.4 (52.77)^{cd}	67.3
NPV + urea 0.5% + molasses 0.5%	61.7 (51.77)^{d}	60.0 (50.77)^{c}	60.0 (50.77)^{cd}	60.6
NPV + boric acid 0.5% + crude sugar 0.5%	68.4 (55.79)^{cd}	68.4 (55.79)^{c}	61.7 (51.77)^{cd}	66.2
NPV + boric acid 0.5% + molasses 0.5%	65.0 (53.73)^{d}	63.4 (52.77)^{c}	58.3 (49.78)^{d}	62.2
NPV alone (1 x 10^{4} POBs/ml)	63.8 (52.47)^{d}	60.5 (48.63)^{d}	58.5 (46.31)^{d}	60.9

Treatments involved with NPV containing 1 x 10^{4} POBs/ml; figures in parentheses are arcsine transformed values; PI - Post inoculation.

of NPV was assessed by calculating LT_{50} values.

Irradiation of an aqueous suspension of Madurai isolate of NPV of *A. albistriga* for 60 min. reduced the insecticidal activity of NPV from 94.8 to 6.2 per cent OAR. The capacity of optical brighteners as UV protectants ranged from 10.8 (Ranipal MM) to 95.3 (Ranipal BVN) per cent OAR (Table 1). Even at low concentration of 0.1 per cent of Ranipal BVN and 2B, more than 80 per cent of viral activity remained and it was 35.5 (Ranipal HI), 18.9 (Ranipal S) and 12.2 (Ultra HRU) per cent OAR whereas less than 10 per cent OAR was recorded in Ranipal 5G and MM. More than 90 per cent protection of insecticidal activity of NPV with 1.0 per cent of Ranipal BVN and 2B was observed while in the remaining treatments, less than 40 per cent OAR was recorded. Out of eight optical brighteners

Table 4. LT_{50} for various instars of *A. albistriga* treated with Madurai isolate of NPV along with adjuvants

Treatment	LT_{50} (h)		
	II instar	III instar	IV instar
1. NPV + urea 0.5% + Ranipal BVN 0.1% + crude sugar 0.5%	99.12	103.68	120.96
2. NPV + urea 0.5% + Ranipal BVN 0.1% + molasses 0.5%	109.20	114.00	131.12
3. NPV + boric acid 0.5% + Ranipal BVN 0.1% + crude sugar 0.5%	108.55	128.50	140.20
4. NPV + boric acid 0.5% + Ranipal BVN 0.1% + molasses 0.5%	120.42	130.05	144.55
5. NPV + urea 0.5% + crude sugar 0.5%	125.52	141.35	150.15
6. NPV + urea 0.5% + molasses 0.5%	121.05	145.05	154.20
7. NPV + boric acid 0.5% + crude sugar 0.5%	125.00	140.11	150.00
8. NPV + boric acid 0.5% + molasses 0.5%	122.42	141.83	154.18
9. NPV alone (1×10^4 POBs/ml)	132.45	154.21	163.51

tested, Ranipal BVN and 2B provided excellent protection to NPV, resulting in more than 80 per cent OAR, while Ranipal HI and Ultra HRU provided fair to good protection with OAR between 31 and 50 per cent. Ranipal 2BA, S, 5G and MM provided little protection with OAR between 11 and 30 per cent (Table 2).

Efficacy of adjuvant mix on the virulence of *A. albistriga* NPV

The adjuvant mix, NPV + urea (0.5%) + crude sugar (0.5%) + Ranipal BVN (0.1%) was effective in enhancing the virulence, resulting in 96.7, 93.3 and 81.7 per cent mortality of II, III and IV instar, respectively, followed by NPV + urea (0.5%) + molasses (0.5%) + Ranipal BVN (0.1%) (93.3, 91.7 and 76.7%) while NPV alone caused 63.8, 60.5 and 58.5 per cent mortality at 8 day post inoculation (Table 3). The adjuvant mix, NPV + urea (0.5%) + crude sugar (0.5%) + Ranipal BVN (0.1%) increased the virulence of NPV by reducing the LT_{50} of NPV, resulting in 99.12, 103.68 and 120.96 h to II, III and IV instar of *A. albistriga* while NPV alone recorded 132.45, 154.21 and 163.51 h at 8th day post inoculation (Table 4).

The success of the brighteners as UV protectants was due to good absorption in ultraviolet (UV Blue region, 280-310 nm) (Jaques, 1968) and conservation of visible light (Villaume, 1958). High pH of Ultra HRU inactivated the NPV rather than protection. According to Shapiro (1992), pH of the NPV + brightener suspension between 3.5 and 8.7 was safe to NPV. Increase of virulence of *A. albistriga* NPV due to the addition of selected optical brighteners might be due to reduction in LT_{50}. Several brighteners are known to interfere with cellulose (Itoh *et al.*, 1984) and chitin fibrillogenesis (Herth, 1980). In insects, the peritrophic membrane lining the midgut is composed of chitin microfibrils. The peritrophic membrane serves as a barrier for the invasion of microorganisms including insect viruses (Brandt *et al.*, 1978). Selected optical brighteners may inhibit or alter the chitinous peritrophic membrane creating gaps in the lining through which NPV could pass easily. Similarly, uric acid and urea affect the integrity of the peritrophic membrane, which causes quick mortality by reducing the LT_{50}.

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