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Optical brighteners as UV protectants and their influence on the virulence of nuclear polyhedrosis virus of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

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ABSTRACT: Laboratory screening of twelve optical brighteners as UV protectants for *Spodoptera litura* (Fabricius) NPV revealed that Ranipal BVN and 2B provided excellent protection (80% original activity remaining). Effect of optical brighteners in preserving NPV was concentration dependent and an increase in concentration from 0.001 to 1.00 per cent increased the retention of viral activity from 14.8 to 99.9 per cent and 10.8 to 99.9 per cent for Ranipal BVN and 2B, respectively. Addition of selected optical brighteners, Ranipal BVN and 2B at a range of 0.001 to 1.00 per cent with *S. litura* NPV increased the virulence of NPV by reducing the LC_{50} and LT_{50} of NPV.

KEY WORDS: LC_{50}, LT_{50}, optical brighteners, original activity remaining, *Spodoptera litura* NPV

In the past 25 years, many natural and synthetic organic chemicals have been evaluated as UV protectants for baculoviruses (Podgwaite and Shapiro, 1986). 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), fall army worm NPV (Hamm and Shapiro, 1992) and gypsy moth NPV (Shapiro, 1992; Shapiro and Robertson, 1992; Shapiro and Dougherty, 1994). In the present study, effect of addition of selected optical brighteners on the protection of the virus particles and enhancement of the virulence of *Spodptera litura* (Fabricius) NPV was determined.

MATERIAL AND METHODS

Twelve optical brighteners; Ranipal

BVN, HI, 2B, MM, 2BA, PCRB, S, Ultra 5G , HRU, NIR, SU and AL obtained as powders from Mafatlal Co., Mumbai were screened as UV protectants for *S. litura* NPV at Biocontrol Laboratory, Department of Agricultural Entomology during December, 1994.

NPV was diluted to a concentration of $1x10^8$ POBs/ml with different concentrations of optical brighteners (0.001, 0.01, 0.1, 0.25, 0.5 and 1.0% and 100 ml was pipetted in a 250 ml conical flask. Each conical flask was held 30 cm below the UV lamp (15 W Philips Make) and was exposed for 60 minutes. After exposure, the volume was adjusted to 100 ml and one ml of aliquot was used to treat both the surfaces of leaf disc of castor. Untreated larvae exposed to non-irradiated *Spodoptera* NPV served as check.

Fifteen larvae (fourth instar) were infected and the virus induced mortality on the 8th day was recorded. UV protection was measured in terms of original activity remaining (OAR) after irradiation (Ignoffo and Batzer, 1971):

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

Based on OAR (%), optical brightners were categorised as poor protection (<10%), little protection (10-30%), fair to good protection (31-50%), superior protection (51-80%) and excellent protection (> 80%) (Shapiro, 1992).

Two optical brightners Ranipal BVN and 2B which registered for superior protection in the previous experiment were re-evaluated and were applied to the both surfaces of leaf disc of castor at concentrations ranging from 1×10^1 to 1×10^6 POBs/ml. The biological activity was measured by calculating LC_{50} and LT_{50}. Finally, relative activity was calculated.

RESULTS AND DISCUSSION

NPV alone and with different concentrations of optical brighteners exposed to UV lamp for 60 minutes revealed that the effect of optical brighteners in preserving NPV was concentration dependent. Irradiation of an aqueous suspension of *S. litura* NPV for 60 minutes reduced viral activity of NPV from 99.9 per cent (NPV no UV) to 0.9 per cent (NPV 60 UV) mean OAR. A complete spectrum of activity was observed among the brighteners ranging from 22.5 per cent OAR (Ultra HRU) to 86.6 per cent mean OAR (Ranipal BVN). An increase in concentration of optical brighteners from 0.001 to 1.00 per cent increased the retention of viral activity from 21.7 to 78.9 per cent mean OAR (Table 1). Even at low concentration of 0.01 per cent of Ranipal BVN and 2B, more than 50 per cent of viral activity remained. However, it was 40.8, 37.0 and 33.3 per cent for Ranipal 2BA, Ranipal HI and Ranipal S, respectively. Less than 10 per cent OAR was recorded with Ranipal MM, Ranipal PCRB, Ultra 5G, SU, AL and NIR. Cent per cent protection of viral activity of NPV with 0.25 per cent of Ranipal BVN and 2B was observed and in addition to

Table 1. OAR of *S. litura* NPV treated with optical brighteners and UV protectants

Treatment*	Per cent original activity remaining at indicated concentrations						
	0.001	0.01	0.1	0.25	0.5	1.0	Mean
NPV + H_2O (No UV)	99.9 (98.26)[a]	99.9 (98.26)[a]	99.9 (98.26)[a]	99.9 (98.26)[a]	99.9 (98.26)[a]	99.9 (98.26)[a]	99.9 (98.26)[a]
NPV + H_2O (60 UV)	0.0 (0.74)[d]	0.0 (0.74)[e]	0.0 (0.74)[f]	7.2 (15.57)[e]	0.0 (0.74)[e]	7.2 (15.57)[e]	0.9 (5.68)[g]
NPV + brightener (60 UV)	14.8	59.3	88.6	99.9	99.9	99.9	86.6
NPV + Ranipal BVN	(22.62)[b]	(50.37)[b]	(70.30)[b]	(89.26)[a]	(89.26)[a]	(89.26)[a]	(68.51)[b]
NPV + Ranipal HI	14.8 (22.62)[b]	37.0 (37.49)[c]	65.4 (53.97)[d]	71.5 (57.74)[c]	82.3 (65.11)[b]	92.9 (74.55)[c]	61.9 (51.91)[c]
NPV + Ranipal 2B	10.8 (19.17)[bc]	55.8 (48.35)[b]	76.9 (61.28)[c]	99.9 (89.26)[a]	99.9 (89.26)[a]	99.9 (89.26)[a]	83.6 (66.09)[c]
NPV + Ranipal 2BA	10.9 (19.29)[bc]	40.8 (39.73)[c]	57.7 (49.44)[d]	78.6 (62.45)[b]	99.9 (89.26)[a]	98.0 (81.91)[b]	70.4 (57.01)[d]
NPV + Ranipal S	14.8 (22.62)[b]	33.4 (35.31)[c]	65.4 (53.97)[d]	82.3 (65.11)[b]	82.3 (65.11)[b]	98.0 (81.91)[b]	65.5 (54.01)[c]
NPV + Ultra HRU	7.4 (15.84)[c]	14.8 (22.62)[d]	19.0 (25.88)[e]	24.9 (29.94)[d]	39.3 (38.81)[c]	35.7 (36.69)[d]	22.5 (28.29)[f]
Mean	21.7 (26.52)[d]	41.7 (40.48)[c]	59.1 (50.61)[b]	70.5 (62.32)[a]	75.4 (65.85)[a]	78.9 (69.80)[a]	

Figures in parentheses are arcsine transformed values
In a column, means followed by same letter (s) are significantly different by DMRT (P=0.05)
* No mortality in untreated and optical brighteners treated larvae

above, Ranipal HI, Ranipal 2BA and Ranipal S also retained more than 90% original activity at highest concentration (1%).

Of the 12 brighteners tested, Ranipal BVN and 2B provided excellent protection (> 80% OAR), Ranipal 2BA, S and HI provided superior protection (51-80% OAR). Ranipal MM and PCRB and Ultra 5G and HRU provided little protection (11-30% OAR) and NIR, SU and AL provided poor protection (< 10% OAR) (Table 2).

Table 2. Activity of *S. litura* NPV using optical brighteners as UV protectants

Per cent mean original activity remaining after UV irradiation				
<10	11-30	31-50	51-80	> 80
NIR	Ranipal MM	-	Ranipal 2BA	Ranipal BVN
SU	Ranipal PCRB	-	Ranipal S	Ranipal 2B
AL	Ultra HRU	-	Ranipal HI	-
	Ultra 5G			

Table 3. LC_{50} and LT_{50} and relative activity of *S. litura* NPV treated with optical brighteners

Treatment[a] / concentration		LC_{50}	Fiducial limits	Relative activity[b]	LT_{50} (h)	Fiducial limits	Relative activity
NPV + H_2O		26, 779	9, 083 - 78, 947	1.00	138.72	127.92 - 150.24	1.00
NPV + Ranipal							
BVN		10, 139	3, 310 - 31, 051	2.64	-	-	-
		7, 079	2, 898 - 17, 290	3.78	120.00	106.32 - 135.60	1.16
0.001		2, 976	1, 089 - 8, 132	8.99	116.64	102.96 - 132.00	1.19
	0.10	744	323 - 1, 713	35.99	96.24	90.00 - 104.08	1.44
	0.25	540	233 - 1, 254	49.59	91.68	86.64 - 102.72	1.51
	0.5						
	1.0						
NPV + Ranipal 2B							
		13, 898	4, 580 - 42, 168	1.93	-	-	-
0.001		8, 718	2, 836 - 26, 795	3.07	120.00	104.40 - 138.00	1.16
	0.10	4, 978	1, 814 - 13, 659	5.38	119.04	106.32 - 133.20	1.17
	0.25	867	372 - 2, 019	30.89	98.64	92.24 - 107.49	1.41
	0.5	832	348 - 1, 992	32.19	97.92	91.57 - 106.71	1.42
	1.0						

[a] No mortality in untreated and optical brighteners treated larvae

[b] Ratio of LC_{50}s and LT_{50}s where activity of NPV equals 1.0

Addition of selected optical brighteners not only preserved the NPV from UV rays but also increased the virulence by reducing the LC_{50} and LT_{50} values. Ranipal BVN (0.5%) reduced the LC_{50} by 35.99 folds (744 POBs/ml) and at 1.0 per cent, it was 49.9 folds (540 POBs/ml) (Table 3). Similarly, at 0.5 and 1.0 per cent of Ranipal 2B, LT_{50} of NPV was reduced to 30.89 (867 POBs/ml) and 32.19 folds (832 POBs/ml), respectively while NPV alone recorded LC_{50} of 26, 779 POBs/ml. In NPV + H_2O, LT_{50} was 138.72 h (127.92 - 150.24 h) and it was reduced by 1.51 (91.68 h) and 1.42 (97.92 h) at the highest concentration of 1.0 per cent Ranipal BVN and 2B, respectively (Table 3).

The success of the brighteners as UV protectant 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 SU, AL and NIR 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 *S. litura* NPV due to addition of selected optical brightners might be due to peculiar mode of action. 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 micro-organisms 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.

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