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Evaluation of Certain Adjuvants as Phagostimulants and UV- Protectants of Nuclear Polyhedrosis Virus of *Helicoverpa armigera* (Hbn.)*

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ABSTRACT

Laboratory experiments were conducted to evaluate the efficacy of certain adjuvants possessing phagostimulant and UV-protectant properties in increasing the efficacy of nuclear polyhedrosis virus (NPV) against *Helicoverpa (=Heliothis) armigera* (Hbn.). An adjuvant - mix consisting of Frenchbean/cotton seed kernel extract 10% + crude sugar 10% + glycerol 1% + egg white 1% + whitening agent (Tinopal)0.1% was the most effective in increasing mortality due to NPV in larvae of *H.armigera*. Crude sugar 10% along with either Frenchbean extract 10% or cotton seed kernel extract 10% also significantly increased the NPV mortality but these were not as effective as the treatments with adjuvant - mix with full complements of the different components. Either Frenchbean or cotton seed kernel extract - based adjuvant-mix recorded significantly lower LT_{50} values than NPV used alone. Frenchbean/cotton seed kernel extract 10% + crude sugar 10% also recorded lower LT_{50} values than NPV alone but were higher than those recorded by NPV + full complements of adjuvant - mix. Frechbean or cotton seed kernel extract-based adjuvant-mixes were able to protect the virus from UV light. The differences in mortalities between UV-exposed and unexposed were not significant in virus treatment with adjuvants.

KEY WORDS : Adjuvants, Phagostimulants, UV-protectants, NPV, *Helicoverpa armigera*

Among the several alternative methods of pest management tried for the gram caterpillar *Helicoverpa (= Heliothis) armigera* (Hbn), the nuclear polyhedrosis virus (HaNPV) is the most promising and its efficacy has been tested against the pest in a number of crops (Rabindra and Jayaraj, 1990). But certain factors have reduced its prospects of commercial success and practical effectiveness. The virus must be ingested by the insects in sufficient quantity before it is inactivated by several factors in the environment, both physical and bilogical. The ultra violet fraction of the sunlight (McLeod *et al.,* 1977) and the leaf surfaces of crop plants like cotton (Young and Yearian, 1977) and chickpea (Rabindra *et al.,* 1994) inactivate the *Heliothis* NPV necessitating frequent virus sprays. Hence, for successful control of *H.armigera* with NPV, adjuvants possessing phagostimulant properties to ensure that the larvae ingest sufficient quantity of virus to cause mortality and UV-protectant properties should be used. The present studies evaluate French bean or cotton seed kernel extract-based adjuvant mixes for increasing the efficacy of HaNPV.Though several adjuvants have already been found to increase the efficacy of HaNPV (Rabindra and Jayaraj, 1988b), new combinations have been tried in the present studies.

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MATERIALS AND METHODS

1. Mass culturing of *H.armigera*

Laboratory population of *H.armigera* was established from field - collected larvae and a continuous mass culture was maintained following the standard methods described earlier (Shorey and Hale,1965). In all the experiments, second instar larvae of *H.armigera* within 12 h of moult were used. In one experiment, both second and third instar larvae were used.

2. Mass production of NPV of *H.armigera*

The NPV of *H.armigera* used in this study was of single enveloped nucleocapsids (SNPV) type and was obtained from the Department of Agricultural Entomology, Tamil Nadu Agricultural University. The virus was propagated by inoculating either late fourth or early fifth instar larvae following the methods of Rabindra and Jayaraj (1986). It has been standardised that the above mentioned stages of larvae would give maximum yield of virus per larva. The virus was semipurified by differential centrifugation in a clinical centrifuge and counts of polyhedral occlusion bodies (POB) made with a haemocytometer. Care was taken to use fresh virus in all the experiments.

3. Laboratory evaluation of certain adjuvants for enhancing the efficacy of NPV against *H.armigera*

Laboratory experiments were conducted to evaluate the efficacy of combination of certain adjuvants (Table 1) consisting of extracts of either Frenchbean or cotton seed kernel, crude sugar, glycerol, egg white and a whitening agent (Tinopal) in increasing mortality due to NPV in *H.armigera* larvae.

3.1. Preparation of the adjuvants

Frenchbean seeds (10g) were soaked in distilled water for 12 h, homogenized in an all-glass pestle and mortar with small quantities of water and the extract was passed through a muslin cloth. The final volume was made up to 100 ml so as to have a 10% extract. Similarly, water extracts of cotton seeds were prepared after removing the seed coat by pounding. Crude sugar was added to the extract at 10% level. Glycerol was used at 1% level. Egg white was homogenized in an all - glass pestle and mortar, filtered through a muslin cloth and used at 1% level. Tinopal (Ranipal) was added at 0.1% level: Teepol was added to all the treatments at 0.1% level as a surfactant.

3.2. Bioassay method

Bioassys were conducted following the leaf-dip method of Rabindra and Jayaraj (1988a). Chickpea shoots containing five compound leaves were dipped in the different suspensions for 10 seconds and the excess drained off by vigorous jerking. The leaves were then allowed to shade-dry. Second instar *H.armigera* larvae of the same age were allowed to feed on the treated shoot for 24 h and then removed individually to penicillin vials containing a semisynthetic diet. There were 30 to 45 larvae in each treatment in three replications. Larval mortality was recorded from the third day of inoculation onwards at 24 h intervals for ten days. Two bioassays with 10^4 and 5×10^4 POB/ml were conducted. Another test was conducted with third instar larvae with a dose of 10^4 POB/ml.

4. Efficacy of adjuvants as UV protectants to NPV

A laboratory experiment was conducted to evaluate the efficacy of the different adjuvants in preventing the UV light inactivation of the virus. Chickpea shoots were treated by dipping in the different virus suspensions and allowed to dry in the shade. The shoot ends were kept immersed in water taken in penicillin vials. One set of treated shoots was exposed to UV light source (30 W) (Philips Holland) in a Laminar Flow chamber for one h by placing them 60 cm from the lamp. Another set of treatment was maintained without exposure to UV light. Second instar larvae of *H.armigera* were released in each treatment and bioassays were conducted as described earlier.

5. Statistical analysis

The data in percentage were transformed to corresponding angles(Arc sine x $\sqrt{percentage}$)

as per the method developed by Poisson for statistical analysis (Snedecor and Cochran, 1967) and subjected to analysis of variance and means separated by least significant difference (L.S.D.) (Steel and Torrie, 1960). The time-mortality responses were subjected to probit analysis (Finney, 1964).

RESULTS AND DISCUSSION

The mortality data revealed that Frenchbean/cotton seed kernel extract 10% + crude suger 10% + glycerol1% + egg white 1% + Tinopal 0.1%, was the most effective in increasing the mortality due to NPV in *H.armigera* larvae when tested at both 10^4 and 5 X 10^4 POB/ml (Table 1). The per cent mortality in these two treatments was significantly higher than in the other treatments. Crude sugar 10% along with either Frenchbean 10% or cotton seed kernel extract 10% also significantly increased the efficacy of the virus but were not as effective as the adjuvant treatments with the full complement of the different components. More or less similar results were obtained when the different treatments were tested against the third instar larvae of *H.armigera*

Table 1. Efficacy of adjuvants in increasing the mortality caused by HaNPV in larvae of *H.armigera*

Treatment	% Larval mortality		
	II instar		III instar
	(5x10^4 POB/ml)	(10^4 POB/ml)	(10^4 POB/ml)
NPV + Frenchbean extract 10% + crude sugar 10% + glycerol 1% + egg white 1% + Tinopal 0.1%	95.0[a]	87.5[a]	72.5[a]
NPV + Cotton seed kernel extract 10% + crude sugar 10% + glycerol 1% + egg white 1% + Tinopal 0.1%	97.5[a]	87.5[a]	67.5[a]
NPV + Frenchbean extract 10% + crude sugar 10%	85.0[b]	60.0[b]	50.0[b]
NPV + Cotton seed kernel extract 10% + crude sugar 10%	85.0[b]	62.5[b]	50.0[b]
NPV alone	57.5[c]	37.5[c]	22.5[c]

Means followed by same letters in columns are not different statistically (P=0.05) by L.S.D.

Table 2. Probit analysis of time-mortality response of second and third instar larvae of *H.armigera* to HaNPV (10^4 POB/ml) with and without adjuvants

Adjuvants	Instar	No.of insects	Chi^{2*} (n-2)	b	LT50 (h)	Fiducial limits Upper	Fiducial limits Lower
NPV + French bean extract 10% + crude sugar 10% + glycerol 1% + egg white 1% + Tinopal 0.1%	II	280	2.74	2.95	115.26	131.18	101.28
	III	280	5.98	4.26	173.36	189.55	158.55
NPV + Cotton seed kernel extract 10% + crude sugar 10% + glycerol 1% + egg white 1% + Tinopal 0.1%	II	280	2.34	2.73	102.37	117.73	89.02
	III	280	2.46	3.48	174.33	194.46	156.28
NPV + French bean extract 10% + crude sugar 10%	II	280	0.94	2.24	172.76	204.84	145.70
	III	280	2.63	2.93	232.54	264.74	204.27
NPV + Cotton seed kernel extract 10% + crude sugar 10%	II	280	0.60	2.46	179.87	210.04	154.03
	III	280	3.09	3.12	239.27	270.30	211.80
NPV alone	II	280	0.73	2.33	350.70	412.15	297.90
	III	280	1.97	2.18	582.76	693.90	489.42

* All lines are significantly a good fit (P<0.05)

Table 3. Efficacy of some adjuvants as UV protectants for HaNPV at a dose of 10^4 POB/ml

Adjuvants	% Larval mortality days after inoculation								
	5			7			10		
	UV exposed	Unexposed	Mean	UV exposed	Unexposed	Mean	UV exposed	Unexposed	Mean
NPV + Frenchbean extract 10% + crude sugar 10% + glycerol 1% + egg white 1% + Tinopal 0.1%	55.00 (47.88)	57.50 (49.32)	56.25 (48.60)	80.00 (63.80)	82.50 (65.83)	81.25 (64.82)	92.50 (76.17)	95.00 (80.78)	93.75 (78.47)
NPV + Cotton seed kernel extract 10% + crude sugar 10% + glycerol 1% + egg white 1% + Tinopal 0.1%	60.00 (50.83)	62.50 (52.27)	61.25 (51.55)	72.50 (58.60)	75.00 (60.11)	73.75 (59.35)	82.50 (65.83)	85.00 (70.44)	83.75 (68.14)
NPV + Frenchbean extract 10% + crude sugar 10%	30.00 (33.05)	40.00 (39.23)	35.00 (36.14)	42.50 (40.67)	52.50 (46.44)	47.50 (43.55)	52.50 (46.44)	62.50 (52.27)	57.50 (49.35)
NPV + Cotton seed kernel extract 10% + crude sugar 10%	32.50 (34.71)	42.50 (40.67)	37.50 (37.69)	42.50 (40.67)	52.50 (46.44)	47.50 (43.55)	55.00 (47.88)	62.50 (52.27)	58.75 (50.07)
NPV alone	2.50 (4.60)	12.50 (20.46)	7.50 (12.53)	10.00 (15.85)	35.00 (36.22)	22.50 (26.04)	15.00 (22.50)	42.50 (40.67)	28.75 (31.58)
Mean	36.00 (34.41)	43.00 (40.39)		49.50 (43.92)	59.50 (51.01)		69.50 (51.76)	69.50 (59.29)	
C.D. for treatments		4.70**			5.49**			7.71**	
C.D. for UV exposure		2.97**			3.47**			4.87**	
C.D. for interaction		6.66**			7.76*			NS	

Figures in parentheses indicate angles corresponding to percentages

though with lower mortality levels (Table 1.) Frenchbean or cotton seed kernel extracts as well as crude sugar probably had acted as phagostimulants.

Several phagostimulants which increase the efficacy of NPV against *Heliothis* spp. have been reported by many workers (Ignoffo *et al.*, 1976; Bell and Romine, 1980; Hostetter *et al.*, 1982; Rabindra and Jayaraj, 1988a, 1988b, 1992). Addition of adjuvants to commercial *Heliothis* NPV, Elcar R produced significantly higher mortality when compared to ElcarR applied alone (Smith *et al.*, 1978, 1980, 1982; Hostetter *et al.*, 1982). Cotton seed flour as the most preferred adjuvant for *H.armigera* has been reported earlier by Bell and Kanavel (1978) and Coax, a commercial adjuvant (Traders Oil Mill Co., Texas, USA) consisting mainly (62.3%) of cotton seed flour improved the field efficacy of NPV against *H.virescens* on cotton (Bell and Romine, 1980). Egg white in the formulation might have acted as a sticking agent facilitating the adhesion of POB on the treated surface. Egg white increasing the efficacy of NPV of *H.armigera* (Hostetter *et al.*, 1982; Rabindra and Jayaraj, 1988 c) has already been reported.

Comparison of LT_{50} values in both second and third instar larvae showed that both Frenchbean extract based adjuvant-mix and cotton seed kernel extract-based adjuvant mix recorded considerably lower LT_{50} values than the other treatments (Table 2.). Frenchbean extract 10% + crude sugar 10% or cotton seed kernel extract 10% + crude suger 10% also recorded lower LT_{50} values than NPV without

Table 4. Probit analysis of time-mortality response of second instar larvae of *H.armigera* to different NPV treatments with and without UV treatment

Adjuvants	Instar	No.of insects	Chi^2* (n-2)	b	LT_{50} (h)	Fiducial limits	
						Upper	Lower
NPV + Frenchbean extract 10% + crude sugar 10% + glycerol 1% + egg white 1% + Tinopal 0.1%	UV - exposed	280	1.09	4.74	113.01	122.47	104.29
	Unexposed	280	0.98	4.98	106.64	115.12	98.79
NPV + Cotton seed kernel extract 10% + crude sugar 10% + glycerol 1% + egg white 1% + Tinopal 0.1%	UV - exposed	280	4.72	3.64	114.04	126.60	102.73
	Unexposed	280	2.22	3.53	106.28	118.37	95.42
NPV + Frenchbean extract 10% + crude sugar 10%	UV - exposed	280	0.67	2.17	196.51	234.24	164.85
	Unexposed	280	0.18	2.55	155.53	180.54	133.98
NPV + Cotton seed kernel extract 10% + crude sugar 10%	UV - exposed	280	1.46	2.34	190.05	223.59	161.54
	Unexposed	280	1.11	2.65	153.01	176.63	132.54
NPV alone	UV - exposed	280	0.07	3.42	413.90	472.10	362.88
	Unexposed	280	4.41	3.30	230.68	258.87	205.57

* All lines are significantly a good fit ($P<0.05$)

adjuvants but were higher than those recorded by NPV applied with full complement of the adjuvant mix.

As higher doses of virus resulted in earlier mortality (Ignoffo, 1965) in *Heliothis zea* Boddie and *H.virescens* (F.), it is likely that increased amounts of virus were ingested by the larvae due to the phagostimulant action of these adjuvants leading to the reduction in the LT_{50}. It is important that the virus acts fast enough to kill the insects before they cause economic damage to the crop. This would be particularly very critical in a crop like cotton in which even a slight damage to the fruiting parts, particularly the squares would result in shedding and economic loss. Hence the use of the adjuvants identified in the present study would enhance the mortality rate on one hand and hasten the same on the other.

Experiments on the UV protectant properties of the adjuvants revealed that ultraviolet light had a significant deleterious effect on the activity of the virus when applied without any adjuvants. In all the three periods of observations, there were significant differences between UV - exposed and unexposed treatment, the UV-exposed treatment recording significantly lower mortality than unexposed treatment (Table 3). Baculoviruses including *Heliothis* NPV are well known for their susceptibility to UV-inactivation by sunlight (Gudauskas and Canerday, 1968; Ignoffo and Garcia, 1992). The Frenchbean or cotton seed kernel extract-based adjuvant-mixes were able to protect the virus from UV light. When the interaction of treatments with the UV light exposure was considered, there were no significant differences in mortalities between UV-exposed and unexposed in all the treatments except NPV applied alone. When the LT_{50} values were compared, it was seen that there was virtually no significant differences between the UV-exposed and unexposed viruses in either the Frenchbean or cotton seed kernel extract based adjuvant-mixes (Table 4). But in NPV alone,there was a very significant difference in LT_{50} values between the UV-exposed and unexposed. The above data clearly indicated that the Frenchbean and cotton seed kernel extract based adjuvant-mix gave a significant level of UV protection to the virus.

Several additives have been reported to protect the viruses from UV light (Ignoffo and Batzer, 1971; Shapiro, 1985; Ignoffo *et al.*, 1991). In the early 1970s, a natural polyflavanoid ('Shade', Sandoz Inc.) was developed as UV-potectant for *Heliothis* NPV (Ignoffo *et al.*, 1972). The adjuvant 'Coax' presumably acted as a UV protectant besides being a feeding stimulant (Smith *et al.*, 1980).

In the present study, components like Tinopal and egg white should have acted as UV screens. Glycerol might have acted as evaporation retardant. The role of Tinopal, in UV protection has been reported earlier by Martignoni and Iwai (1985) and Ignoffo *et al.* (1991) and that of egg white by many workers (Hostetter *et al.*, 1982; Rabindra and Jayaraj, 1988c). Apart from these substances, other components of the adjuvant - mix like the cotton seed kernel/Frenchbean extract and crude sugar could also have contributed to UV protection by forming a coat around the virus polyhedra. Starch (one of the components in cotton seed kernel/Frenchbean extract) is known to be an excellent UV protectant (Ignoffo *et al.*, 1991).

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