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Larval Extracts And Other Adjuvants For Increased Efficacy Of Nuclear Polyhedrosis Virus Against *Heliothis armigera* Larvae

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ABSTRACT

Laboratory experiments have revealed that 4% larval extracts of *Heliothis armigera*, *Spodoptera litura* and *Corcyra cephalonica*, 20% whole milk, 10% whole egg homogenate, 10% yellow of egg, 10% egg white, 20% tender coconut water and 20% crude sugar increased the efficacy of nuclear polyhedrosis virus against second instar larvae of *H. armigera*. Measurement of leaf area consumed showed that these adjuvants had acted as phagostimulants and increased the feeding by larvae leading to acquisition of more virus resulting in increased mortality and shorter LT_{50} values. Pot culture study showed that eventhough Robin blue and Tinopal both at 1% were not as effective as the other adjuvants in increasing the mortality, they increased the persistence of the virus as seen by the higher percentages of original activity remaining.

KEY WORDS: Larval extracts, egg, milk, tender coconut water, Robin blue, Tinopal, adjuvants, persistence, NPV, *Heliothis armigera*

Among the several entomopathogens tested against the gram pod borer *Heliothis armigera* Hbn., the nuclear polyhedrosis virus has been found to be quite effective. The field efficacy of the virus in the control of the pest on crops like chickpea (Rabindra and Jayaraj, 1988a) pigeonpea (Santharam *et al.*, 1981), cotton (Dhandapani *et al.*, 1987), and sunflower (Rabindra *et al.*, 1986) has been demonstrated earlier. Various adjuvants have been tested for increasing the efficacy of the virus and Rabindra and Jayaraj (1988b) found that crude sugar 0.5%, cotton seed kernel powder 1%, groundnut oil cake 3% and chickpea flour 1% acting as phago-stimulants increased the efficacy of NPV against *H. armigera* larvae. Screening of more substances as adjuvants for NPV against *H. armigera* was continued in this laboratory and the results are presented in this paper.

MATERIALS AND METHODS

Larvae of *H. armigera* were maintained in the laboratory on a semi-synthetic diet (Shorey and Hale, 1965). The nuclear polyhedrosis virus used in the study was propagated in fourth instar larvae of *H. armigera* and semi-purified by differential centrifugation. Counts of polyhedral occlusion bodies (POB) were made with a double ruled improved Neubaur haemocytometer and the standardised virus suspension was stored at 4°C untill use. Two laboratory studies were made to evaluate the efficacy of whole milk, egg, larval extracts of *H. armigera*, *Spodoptera litura* F. and *Corcyra cephalonica* St., tender coconut water, Robin blue and Tinopal as adjuvants and compared with crude sugar. The different treatments (Tables 1, 2) were prepared in distilled water containing 0.1% Teepol as a surfactant and NPV was added at the rate of 10^5 POB/ml. The egg and its yolk components as well as the larvae of the different lepidoptera were homogenized separately and filtered through a muslin before being added to the NPV suspension. The treatments in individual 250 ml conical flasks were mixed thoroughly and assayed by the leaf-dip method described by Rabindra and Jayaraj (1988b). After allowing newly moulted second instar *H. armigera* larvae to feed on the treated chickpea leaves for 24 h, they were removed to individual vials containing a semi-synthetic diet and plugged with sterile cotton. The leaf area consumed was measured by recording the areas on a tracing sheet and measuring the same with the help of a graph sheet. Data on mortality were recorded periodically upto seven days.

A pot culture experiment was conducted to study the persistence of the virus applied with different adjuvants after exposure to open weather conditions. Chickpea plants raised in pots were sprayed with the different treatments (Table 3) containing the NPV at the rate of 10^5POB/ml using a hand atomiser and kept in the open, exposed to sun and other weather conditions. Spraying of separate sets of plants was done for four consecutive days, so that on the fourth day, plants exposed to weathering for one, two and three days and one set without exposure (Control) would be available. On the fourth day, shoots from the treated plants exposed to weather for different periods were removed separately and 30 newly moulted second instar larvae of uniform age and size in 3 replications of 10 each were allowed to feed on individual treatments in separate plastic containers (25 x 15 cm). The cut ends

were kept immersed in water taken in penicillin vials. After 24 h of feeding, the larvae were removed to individual vials carrying semi-synthetic diet and plugged with sterile cotton. Mortality was recorded on the seventh day.

The data were compared by Duncan's Multiple Range Test. Time mortality data were analysed by Probit method (Finney, 1962).

RESULTS AND DISCUSSION

Mortality data of the laboratory experiments showed that all the adjuvants tested in the first experiment *viz.*, whole milk 20%, whole egg homogenate 10%, yellow yolk of egg 10%, egg white 10% and *H. armigera* larval extract 4% signigicantly increased the mortality of second instar larvae of *H. armigera* due to NPV and as effective as crude sugar (Table 1). The efficacy of 20% crude sugar as an adjuvant for ULV application of NPV has already been reported by Rabindra and Jayaraj (1988a). Egg white recorded significantly a higher mortality, 79 h after inoculation but at subsequent periods it was on par with the other adjuvants. Egg white also recorded the shortest LT_{50}. The LT_{50} values for all the adjuvants were significantly shorter than in control (NPV alone) (Table 2).

Results of the second experiment showed that 4% water extract of larvae of *H. armigera, S. litura* and *C. cephalonica* and 20% tender coconut water significantly increased the NPV mortality and were as effective as crude sugar (Table 3). Data on leaf area consumed showed that all these adjuvants induced the larvae to feed significantly more on the treated leaves. It is obvious that the increased mortality is due to greater acquisition of virus through increased consumption of treated leaves. *H. armigera* larvae are highly cannibalistic and naturally, the larval extract should enhance the larval feeding of the treated leaves.

In attempts to achieve increased efficacy of viruses, several substances with properties to increase wettability and adhesiveness, decrease evaporation and sunlight degradation, increase the stability and suspensibility and act as gustatory stimulants have been used (Montoya *et al.*, 1986; Ignoffo and Batzer, 1971; Ignoffo *et al.*, 1976; Smith *et al.*, 1980). Egg albumin and skim milk along with charcoal in sprays of *Trichoplusia ni* or *Pieris rapae* GV increased the efficacy of the virus substantially on cabbage (Jacques, 1985). Larval extract of *H.armigera* at 4% has also been tried as an adjuvant for ULV application of NPV for the control of *H. armigera* on cotton (Dhandapani *et al.*, 1987). The results of the present investigations also suggest that egg white, whole milk, tender coconut water as well as larval extracts can be used as adjuvants in ULV application of nuclear polyhedrosis virus for the control of *H. armigera* on crops like chickpea.

In the present studies, though Robin blue and Tinopal were not as effective as the other adjuvants in increasing the mortality due to NPV (Table 3), they increased the persistence of the virus on chickpea foliage (Table 4) suggesting that these should have acted as UV screens. Whitening agents have been suggested earlier for UV protection of baculoviruses (Ignoffo *et al.*, 1972).

The concentrations of adjuvants tested in the present study were quite high and suitable for only ULV application of the virus. Control of *H. armigera* with ULV spray of NPV in 20% crude sugar has already been demonstrated by Rabindra and Jayaraj (1988a) and the present study indicated the possibility of incorporating whitening agents like Robin blue or Tinopal along with the adjuvants in the formulation of NPV for increased efficacy and persistence.

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TABLE 1 : Efficacy of larval extract and other adjuvants for NPV of *Heliothis armigera*

Treatments @	Mean * % mortality 'h' after inoculation of II Instar larvae		
	79	88	107
Crude sugar 20%	52.4 [b]	97.6 [a]	97.6 [a]
Whole milk 20%	65.8 [b]	86.8 [a]	97.4 [a]
Whole egg homogenate 10%	65.8 [b]	92.0 [a]	97.4 [a]
Egg (yellow yolk) 10%	52.5 [b]	95.0 [a]	97.6 [a]
Egg white 10%	80.0 [a]	95.0 [a]	100.0 [a]
Heliothis armigera larval extract 4%	68.4 [b]	92.1 [a]	97.4 [a]
NPV alone	10.6 [c]	34.0 [b]	57.4 [b]

@ All treatments contained NPV @ 10^5 POB/ml

* In a column, means followed by similar letters are not different statistically (P = 0.05) by D.M.R.T.

Table 2 : Time-mortality response of second instar larvae of *Heliothis armigera* to NPV applied with different adjuvants

Treatments	No. of insects used	d.f.	X^2 'a' (n-2)	'b' slope	LT_{50} (h)	Fiducial Limits
Crude sugar 20%	42	4	8.3	38.6	78.7	77.8 - 79.6
Whole milk 20%	38	2	1.1	25.5	77.8	75.7 - 80.0
Whole egg homogenate 10%	40	3	2.1	29.0	80.0	78.7 - 81.6
Egg yellow yolk 10%	40	2	2.0	35.1	79.9	78.6 - 80.9
Egg white 10%	40	3	4.8	28.1	74.9	73.4 - 76.5
H. armigera larval extract 4%	38	2	5.4	28.2	77.1	75.6 - 78.7
NPV alone	47	3	4.3	13.5	96.3	92.6 - 100.1

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

TABLE 3 : Efficacy of NPV with larval extracts of different lepidoptera and other adjuvants against II instar larvae of *Heliothis armigera*

Treatments @	Leaf area consumed (mm^2) / larvae	Mean % mortality
Crude sugar 20%	101.3 [a]	93.9 [a]
Robin blue 1%	81.9 [b]	71.8 [b]
Ranipal 1%	84.3 [b]	77.9 [b]
Tender coconut water 20%	104.4 [a]	87.9 [ab]
Larval extract of *H. armigera*	99.2 [a]	97.3 [a]
Larval extract of *S. litura*	97.4 [a]	100.0 [a]
Larval extract of *C. cephalonica*	89.2 [ab]	100.0 [a]
NPV alone	71.4 [b]	51.2 [c]

@ All the treatments carried NPV @ 10^5 POB/ml.

In vertical columns, means followed by similar letters are not significant (P = 0.05) by D.M.R.T.

TABLE 4 : Persistence of NPV on chickpea plants when applied with different adjuvants against II instar larvae of *Heliothis armigera*

Treatments @	Mean % mortality after days of exposure			
	0	1	2	3
Crude sugar 20%	88.4 [a]	74.4 [ab] (84.2)	57.1 [a] (64.6)	44.4 [a] (50.2)
Whole egg homogenate 20%	86.3 [ab]	82.2 [a] (95.2)	57.4 [a] (66.5)	51.0 [a] (59.1)
Egg white 10%	92.7 [a]	79.0 [a] (85.2)	51.8 [a] (55.9)	48.8 [ab] (52.6)
H. armigera larval extract 4%	87.6 [a]	72.4 [a] (82.6)	57.8 [a] (66.0)	37.8 [b] (43.2)
Robin blue 1%	77.3 [b]	63.4 [b] (82.0)	54.2 [a] (70.1)	53.8 [a] (69.6)
Ranipal 1%	73.5 [bc]	61.2 [b] (83.3)	57.4 [a] (78.1)	56.2 [a] (76.5)
Control	62.4 [c]	44.0 [c] (44.0)	35.4 [b] (56.7)	24.4 [c] (39.1)

@ All the treatments contained NPV at 10^5 POB/ml

Figures in parenthesis represent % original activity remaining

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