Efficacy of Extracts of Certain Host Plants as Adjuvants for Nuclear Polyhedrosis Virus of *Helicoverpa armigera* Hbn. and its Dust Formulation*

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ABSTRACT

Laboratory studies revealed that 10% water extracts of sorghum and pearl millet grains and sunflower seeds (all at dough stage), cotton bolls and squares, and tomato fruits (both green and ripe) when added to nuclear polyhedrosis virus of *Helicoverpa armigera* (Hbn.) (HaNPV) significantly increased the mortality due to virus in second instar larvae of *H.armigera*. Probit analysis of time-mortality response showed that the LT_{50s} were also reduced in these treatments. Dust formulations of HaNPV containing extracts of sorghum grain, cotton seed kernel, chickpea flour and sunflower seed kernel were significantly more effective against *H.armigera* larvae than the virus formulation without any adjuvant. Sorbic acid and methyl parahydroxy benzoate which were used in the formulations were not responsible for the enhanced efficacy of the virus.

KEYWORDS: Nuclear polyhedrosis virus, dust formulations, adjuvants, Helicoverpa armigera

The nuclear polyhedrosis virus (HaNPV) has been found to be effective in the control of the gram pod borer Helicoverpa (=Heliothis) armigera Hbn. on several crops particularly the chickpea (Cicer arietinum L.) (Rabindra and Jayaraj, 1988a). Several adiuvants like crude sugar (Rabindra and Jayaraj, 1988b), crude larval extracts of certain lepidoptera including H. armigera, whole milk, whole egg homogenate, egg white and tender coconut milk (Rabindra and Jayaraj, 1988c) were reported to be effective in increasing the efficacy of the virus against H. armigera larvae. These adjuvants were also found to be effective in the field control of the pest (Rabindra et al., 1989). Results on the evaluation of more such adjuvants for the NPV and the efficacy of some of them in the dust formulations of NPV against H. armigera larvae are reported in this paper.

MATERIALS AND METHODS

The HaNPV used in the study was propagated in fourth instar H. armigera larvae and the concentration of polyhedral occlusion bodies (POB) assessed with the help of a new improved double ruled Neubauer haemocytometer. Water extracts of the different substances (Table 1) were prepared by maceration in an all glass pestle and mortar and filtered through a muslin. In the case of sunflower seeds, the seed coat was removed before extraction. The cotton squares and bolls and tomato fruits were cut into small bits before extraction. Teepol was added at 0.1 per cent as a surfactant. Appropriate quantity of HaNPV was added to 100 ml samples of the extracts to get a concentration of 5 x 10^4 POB/ml. The leaf-dip bioassay method of Rabindra and Jayaraj (1988b) was adopted using second instar H. armigera larvae. After 24 h inoculation feeding time, the

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Treatments @	% larval • mortality*	Time Mortality Response - Probit Analysis				
		No.of insects	Chi ² (n-2)**	'b' slope	LT 50 (h)	Fiducial Limits
Sorghum (dough stage) 10%	97.1 ^a	74	0.50	10.84	76.21	78.82 - 72.40
Sunflower seed kernel 10%	100.0 ^a	68	0.17	17.09	78.63	80.33 - 76.60
Crude sugar 20%	89.2 ^a	70	0.99	15.13	81.44	83.23 - 79.44
Pearl millet (dough stage) 10%	94.3 ^a	70	0.39	14.70	81.78	83.63 - 79.77
Cotton squares 10%	91.7 ^a	72	1.79	11.79	81.83	88.73 - 70.91
Cotton bolls 10%	97.2 ^a	72	0.61	13.90	80.25	82.18 - 77.97
Tomato (green) 10%	75.7 ^b	74	2.36	11.48	92.48	96.57 - 89.73
Tomato (ripe) 10%	88.2 ^{ab}	68	0.75	12.36	84.59	86.91 - 82.31
Control (NPV alone)	54.2 ^c	68	1.11	9.59	92.99	98.77 - 89.58

Table 1. Efficacy of certain aqueous plant extracts as adjuvants for HaNPV against second instar larvae of *H.armigera*

@ All treatments carried NPV at 5 x 10⁴ POB/ml

* Means followed by similar letters are not different statistically (P=0.05) by DMRT

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

larvae were removed to individual vials containing the semisynthetic diet (Shorey and Hale, 1965) and observed daily for mortality. Suitable controls without virus inoculation were maintained. Once the larvae started dying due to the treatment, observations on the mortality were made every 8 h.

Dust formulations of HaNPV were prepared with talc as the filler. Water extracts of sorghum seeds (dough stage), chickpea flour, cotton and sunflower seed kernels were incorporated individually at 10 per cent in the dust formulations to test their efficacy as adjuvants. Methyl parahydroxy benzoate at 0.2 per cent and sorbic acid at 0.1 per cent were added as mould inhibitors. The formulations were prepared by drying over calcium chloride in a desiccator. When the formaulations had dried completely, they were passed through a 100-mesh sieve repeatedly to ensure homogeneous mixing.

Chickpea plants were raised in pots (30 cm dia) and at the preflowering stage, the shoots were dusted with the formulations by a simple polythene bag method. Exactly 100

mg of the dust formulation was placed inside a polythene bag (30 x 20 cm). Then three chickpea shoots, each carrying 6 compound leaves were introduced into the polythene bag with the basal ends of the shoots just protruding out of the bag. Air was blown to the full capacity of the bag, the mouth closed and vigorously tapped so as to ensure complete coverage of the shoots with the dust formulations. The quantity of 100 mg dust formulation was just sufficient to cover the shoots uniformly. Second instar larvae of H. armigera were released on treated shoots @ 10/shoot and allowed to feed for 24 h. The treatments were replicated adequately. After 24 h, the larvae were removed to individual vials containing semisynthetic diet and held at room temperature. Mortality was recorded on the seventh day. In another laboratory experiment, sorbic acid 0.1%, methyl parahydroxy benzoate 0.2% and sorbic acid 0.1% + methyl parahydroxy benezoate 0.2%were tested along with other adjuvants to find out their role in increasing the efficacy of the virus.

Table 2. Efficacy of adjuvants in HaNPV dustformulations in increasing the virusmortality in second instar larvae ofH.armigera

Treatments*	Mean % mortality	
Cotton seed kernel 10% + Talc	89.7ª	
Sorghum grain 10% + Talc	61.3 ^b	
Chickpea flour 10% + Talc	96.4ª	
Sunflower kernel 10% + Talc	60.0 ^b	
Talc alone	46.7°	

- Dust formulations containing Ha NPV @ 10⁸ POB /g
- ** Means followed by similar letters are not different statistically (P=0.05) by DMRT

RESULTS AND DISCUSSION

Results of the bioassay revealed that 10 per cent water extracts of sorghum and pearl millet seeds, sunflower and cotton seed kernels, cotton squares and bolls and tomato fruits significantly increased the larval mortality due to NPV and were as effective as 20 per cent crude sugar (Table 1). Extract of

Table 3. Effect of sorbic acid and methylparahydroxybenzoatein HaNPVformulations on mortality due to NPVin second instar larvae of H.armigera

Treatments*	% mortality
Sorbic acid 0.1%	36.4 ^d
Methyl parahydroxy benzoate (MPHB) 0.2%	57.1 ^{bc}
Sorbic acid 0.1% + MPHB 0.2%	52.8 ^{bc}
Cotton seed kernel extract 10%	72.6 ^{ab}
Chickpea flour extract 10%	82.5 ^a
Sunflower seed kernel extract 10%	66.0 ^b
Sorghum grain extract 10%	62.7 ^{bc}
Talc alone	46.2 ^{cd}

- Dust formulations containing HaNPV @ 10⁸ POB/g
- ** Means followed by similar letters are not different statistically(P=0.05) by DMRT

green fruits of tomatoes was however inferior to other adjuvants. Probit analysis of timemortality responses showed that all the adjuvants + NPV treatments recorded shorter LT_{50} values than NPV alone, the minimum being recorded by the sorghum seed extract. All these adjuvants should have acted as phagostimulants since they are the natural host plants for *H.armigera*.

Cotton seed flour as the most preferred adjuvant for Heliothis virescens (F). 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). Cotton seed oil cake and chickpea flour extracts among others were reported earlier to increase the efficacy of NPV against H. armigera larvae by Rabindra and Jayaraj (1988b). The performance of these adjuvants can be enhanced by the addition of whitening agents like Ranipal or Robin blue (commercial whitening agents) which were reported to increase the persistence of H.armigera NPV (Rabindra and Jayaraj, 1988c).

Evaluation of the dust formulations with adjuvants showed that the formulations containing 10 per cent extracts of sorghum, cotton seed kernel, chickpea flour and sunflower kernel significantly increased the efficacy of the NPV dust formulations (Table 2). Neither sorbic acid/methyl parahydroxy benzoate nor a mixture of the two had any role in enhancing the efficacy of the virus (Table 3). There are no earlier reports on the evaluation of adjuvants for the dust formulations of NPV of *H. armigera*. Corn meal extract was reported to enhance the efficacy of a dust formulation of NPV against *Heliothis zea* Boddie (Montoya *et al.*, 1966).

The results of the present studies have clearly indicated the scope for the use of phagostimulant adjuvants for increasing the efficacy of NPV and its formulation in the control of *H. armigera*. While recommending such adjuvants however, the cost factor should be borne in mind. The consistency and concentration should be such that it is amenable for use with the appropriate plant protection equipment.

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