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RESEARCH ARTICLE

Effect of Baculovirus with adjuvants against *Helicoverpa armigera* (HBN) on Cotton and evaluation of its effect in Different Instar

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ABSTRACT:

During the past few years, the damage by the American bollworm *Helicoverpa armigera* (Hbn) has become serious on many crop plants especially cotton in South India. Along with that insecticide resistance varieties of larvae were also reported. In our study, field-collected larvae were reared in glass vial providing tender leaves for neonatal larvae and squares of grown up larvae. The along with the adjuvants were made into wettable and dust formulation and were studied. Evaluation of the Efficacy of *Helicoverpa* NPV with certain adjuvants possessing phagostimulants in combination were tested in different instars and results were tabulated.

KEYWORDS: Adjuvants, NPV, *Helicoverpa armigera*, Adjuvant.

INTRODUCTION:

Helicoverpa armigera (Hubn), the so called American bollworm is highly polyphagous insect pest and most serious pest of Indian agriculture. The nuclear polyhedrosis virus (NPV) of *Helicoverpa armigera* was first isolated by Patel et al in India^{1,2,3}. Among the several alternate methods of pest management tried on the *Helicoverpa*, NPV is the most promising and its efficacy has been tested against the pest in the number of crops. But certain factors have reduced its prospects of commercial success and practical effectiveness^{4, 5}. The virus must be ingested by the insects in sufficient quantity before it is inactivated by several factors in the environment, both physical and biological⁶. The ultraviolet from the sun, leaf surface of crop plant etc are some of the factors^{7,8}. Hence successful control of *H. armigera* with NPV with adjuvants possessing phagostimulant properties to ensure that the larvae ingest sufficient quantity of virus to causes mortality and UV-protectant properties should be used^{9,10}.

The present studies evaluate Cotton seed kernel extract, boric acid based adjuvants mixes from increase the efficacy of the HaNPV^{11,12}, new combinations have been tried in the present studies.

MATERIALS AND METHOD:

Isolation and production of NPV:

The nuclear polyhedrosis virus (NPV) which is of a single embedded viron type was propagated in four instar larvae of *H. armigera*. The diseased cadaver was collected in glass distilled water, homogenized in a blender, filtered through a muslin cloth and the polyhedra was separated by centrifugation. The Neubaurhaemocytometer was used to assess the number of polyhedra in the suspension. In all the studies, only fresh NPV was used.

Culturing of *H. armigera*:

The different instar of *H. armigera* was identified and separately grown. Larvae can be grown in groups until the early third instar stages at about 7 days post hatching. Until the third instar the it can be grown on tight fitting container on pieces of cloth. After third instar, as cannibalism is common, the larvae has to separately grow in petri plates.

Laboratory examination of certain adjuvants for enhancing the efficacy of NPV against *H. armigera*:

The present studies evaluate Cotton seed kernel extract, egg white, sugar, glycerol and boric acid based adjuvants mixes for increase the efficacy of the HaNPV^{13, 14}.

Preparation of the adjuvants:

Cotton seed kernel was soaked in distilled water for 12 hours, homogenized in glass pestle and mortar with small quantities of water and extract was passed through a muslin cloth. The final volume was made up to 100 mg to have 10% extract. Crude sugar was added. Egg white was homogenized in glass pestle and mortar, filtered through a muslin cloth and used at 1% level¹⁵.

Bioassay methods:

Bioassays were conducted following the leaf-dip method of Rabindra and Jayaraj. The leaf tips were dipped in the different suspension for 10 seconds and the excess drained off by vigorous jerking. The leaves were then allowed to shade-dry. Second instar of *H. armigera* larvae of the same age were allowed to feed on the treated shoot for 24 hours and then removed individually

to penicillin vials containing a semi-synthetic diet. There were 30 – 40 larvae in each treatment in three replicates. Larval mortality was recoded from third day of inoculation onwards at 24 h intervals for 10 days. Two bioassays with 10⁴ and 5 x 10⁴ POB/ml were conducted with three instar larvae with dose of 10⁴ POB/ml^{5,8}.

Efficacy of adjuvants as UV protectants to NPV:

An experiment was conducted to evaluate the efficacy of the different adjuvants in preventing the UV light inactivation of the virus^{7,10}. Cotton leaves tips were dipped in the different concentration of NPV concentration of virus suspension and allowed to dry in the shade. The leaf tips were kept immersed in water taken in penicillin vials. One set of treated leaves were exposed to UV light source in Laminar Flow chamber for one hour by placing them 60 cm from the lamp. Another set of treatments was maintained without exposure of UV light. Second instar larvae of *H. armigera* were released in each treatment and bioassays were conducted as described earlier.

RESULT AND DISCUSSION:

Table :1 showing the efficacy of adjuvants in increasing the mortality caused by NPV in larvae of *H.armigera*

Treatment	% Larval mortality		
	II instar		III instar
	(5 x 10 ⁴ POB / ml)	(10 ⁴ POB / ml)	(10 ⁴ POB / ml)
NPV alone	53 ± 4	38 ± 9	22 ± 6
NPV + Cotton seed kernel	81 ± 8	64 ± 2	50 ± 9
NPV + Cotton seed kernel + crude sugar	85 ± 7	60 ± 5	52 ± 7
NPV + Cotton seed kernel + crude sugar + glycerol	98 ± 6	86 ± 6	68 ± 2
NPV + Cotton seed kernel + crude sugar + glycerol + egg white	96 ± 9	85 ± 4	72 ± 7

Table -2. Showing the efficacy of some adjuvants as UV protectants for HaNPV at a dose of 10⁴ POB/ml in 50 insects.

Treatment	% Larval mortality after inoculation					
	5		7		10	
	UV exposed	Unexposed	UV exposed	Unexposed	UV exposed	Unexposed
NPV alone	3	12	7	36	14	43
NPV + Cotton seed kernel	33	42	42	53	55	63
NPV + Cotton seed kernel + crude sugar	30	40	42	53	55	60
NPV + Cotton seed kernel + crude sugar + glycerol	60	63	72	75	82	86
NPV + Cotton seed kernel + crude sugar + glycerol + egg white	55	57	80	82	93	94
Mean	36.2	42.8	48.6	59.8	59.8	69.2

The mortality data revealed that NPV + Cotton seed kernel + crude sugar + glycerol + egg white was the most effective in increasing the mortality due to NPV in *H.armigera* larvae when tested at both 10⁴ and 5 x 10⁴.

Comparing the results the addition of glycerol has increased the mortality rate. More or less similar result were obtained when the different treatment were tested against the third instar larvae of *H.armigera* through with lower mortality levels.

Several phagostimulants which increase the efficacy of NPV were reported by many workers^{4,8,10,12}. Comparing the mortality values in both second and third instar larvae show that on NPV + Cotton seed kernel + crude sugar + glycerol + egg white – adjuvants mix recorded considerable high death rate. Experiment on the UV protectant properties of the adjuvants revealed that the ultraviolet light has a significant deleterious effect on the activities of the virus when applied without any adjuvants. In the present study, compound like crude sugar and egg white should have been acted as UV

protectants and glycerol may be acted as evaporation retardant.

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