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Laboratory Evaluation of Certain Formulations of Nuclear Polyhedrosis Virus against the Larvae of *Heliothis armigera*

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ABSTRACT

The nuclear polyhedross virus of *Heliothis armigera* was formulated into dusts and wettable powders using indigenous fillers and additives. Results of laboratory evaluation showed that talc-based wettable powder formulations of NPV were as effective as the unformulated virus against second instar larvae of *H. armigera* on chickpea. Dust formulations were less effective than wettable powder formulations and unformulated virus. The reduced efficacy of the dust formulations was attributed primarily to the reduciton in consumption of treated leaves. Use of acetone in the process of formulations reduced the efficacy of virus. No such reduction in efficacy was noticed when distilled water was used.

KEY WORDS: NPV, *Heliothis armigera*, formulations, dusts, wettable powders.

The first formulation of *Heliothis* NPV was prepared by Montoya *et al.* (1966) and subsequently through various research and developmental phases, the NPV of *Heliothis* spp. was developed into a commercial viral pesticide (Ignoffo, 1973) and registered as ELCAR for use in U.S.A., U.K. and Australia. In India, the efficacy of nuclear polyhedrosis virus against *H. armigera* (Hbn.) has been reported on several crops (Jayaraj *et al.*, 1985). The virus @ 250 larval equivalents (LE) effectively controlled the pest on chickpea (Rabindra and Jayaraj, 1986), pigeonpea (Santharam *et al.*, 1981), lablab (Jayaraj *et al.*, 1987), and sunflower (Rabindra *et al.*, 1985). Laboratory studies have indicated that the efficacy of the virus could be improved by addition of certain adjuvants to the NPV suspension (Rabindra and Jayaraj, 1987). In view of these reports on the successful use of NPV against *H. armigera*, attempts were made to formulate the virus into wettable powder (WP) and dust formulations and their efficacy evaluated in the laboratory against *H. armigera* larvae.

MATERIALS AND METHODS

Preparations of NPV formulations

Dusts

NPV dusts were formulated to carry 1.5×10^{12} polyhedral occlusion bodies (POB) (250 LE) in 25 kg of the filler material. An appropriate quantity of NPV suspension containing 6×10^{10} POB was sedimented by centrifugation and suspended in 5 ml of acetone and sedimented again. The pellet was resuspended in 5 ml of acetone and to this was added 5 g of the filler material *viz.*, talc (commercial grade)/kaolin (Neyveli Lignite Corproation, Neyveli)/ china clay (Kerala clays Chemicals Ltd., Cannanore) and made into a slurry by thorough mixing and the acetone evaporated overnight. This preparation was then mixed with 995g of the appropriate fillers and mixed thoroughly in a blender. The formulated dusts were passed through a 100-mesh sieve repeatedly five times for homogenous mixing. A second method of formulation was followed in which only distilled water was used instead of acetone for mixing the polyhedra and the slurry was dried over $CaCl_2$ in a desiccator for 2-3 days

Wettable Powders

The wettable powders were prepared to carry 1.5×10^{12} POB (250 LE) in 2.5 kg of the filler. A stock suspension containing 6×10^{10} POB was taken and centrifuged to sediment the polyhedra. The pellet washed in acetone was sedimented and the POB were resuspended in 50 ml of acetone and mixed thoroughly with 98 g of talc to make a slurry. The acetone was evaporated overnight under room temperature and then 2g of the wetting and dispersing agents (Dedenol Bx/Lissapol D + Lissapol

GM) were added. The formulations were mixed thoroughly in a pestle and mortar and passed through a 100-mesh sieve repeatedly for thorough mixing. In a second method of formulation, instead of acetone, only distilled water was added and the slurry was dried under $CaCl_2$ for 2-3 days.

Laboratory Evaluation of NPV Formulations

Laboratory experiments were conducted to evaluate the efficacy of formulations of NPV in comparison with the unformulated NPV against second instar larvae of *H. armigera*. Chickpea shoots were washed thoroughly in water containing 0.1% Teepol and dried under shade. The different treatments (Tables 1 - 4) were applied on chickpea shoots with petioles dipped in water taken in 10 ml vials using either the Potter's spray (12cm dia) or dust (36.3 cm dia) towers (Hepoona Instrument Co., Poona). Two shoots taken in one vial were treated for each treatment. The suspensions of unformulated NPV as well as WP formulations were adjusted to a strength of 1.0×10^6 POB/ml. One ml of the suspension was fed in the Potter's tower at a pressure of 2.5 kg/cm^2. One ml of endosulfan (Thiodan 35 EC) 0.07% was applied on two shoots to serve as the insecticide check. The deposits on the shoots were allowed to dry before introducing the larvae. Regarding the dust formulations, a quantity of 51.66 mg in each of the formulation was taken which carried 3.1×10^6 POB. This was 3.1 times more than the concentration of NPV spray suspensions used, since the diameter of the dust tower was greater than that of spray tower by 3.1 times. Carbaryl (Sevin 5% dust) at the rate of 87.5 mg calculated as equivalent to 25 kg/ha was applied as insecticide check.

A test was conducted to ascertain the deposition rate of POB in the different NPV treatments when applied by Potter's tower. The deposit of different NPV treatments were collected on 25 × 75 mm grease-free microslides placed inside the Potter's spray and dust towers. For each treatment, there were five slides replicated thrice. The POB on these slides were collected replication-wise in 25 ml of distilled water and counts on POB made with the help of a Neubauer haemocytometer in a phase contrast microscope.

The treated shoots were placed inside plastic containers (10 × 15 cm) and second instar larvae of *H. armigera* starved for 6h were released at the rate of 10-12/treatment. The treatments were replicated thrice. The larvae were allowed to feed for 24 h after which time they were removed to individual vials containing semisynthetic diet.

TABLE 1. Laboratory evaluation of formulations of NPV (involving acetone) against second instar larvae of *Heliothis armigera* on chickpea.

Treatments		Deposition rate POB/mm^2 ($\bar{x} \pm$ S.E.)	Leaf area consumed mm^2/larvae ($\bar{x} \pm$ S.E.)	% mortality ($\bar{x} \pm$ S.E.)
NPV	(1×10^6 (POB/ml)	14.2 ± 0.03	27.2 ± 0.29	77.8b ± 0.64
NPV WP (Dedenol)	,,	13.4 ± 0.03	24.5ab ± 0.16	85.2ab ± 0.86
NPV WP (Lissapol)	,,	14.3 ± 0.03	18.90bc ± 0.10	77.8ab ± 0.98
NPV dust (talc)	(6×10^6 POB/g)	15.2 ± 0.04	17.6bc ± 0.05	29.6c ± 0.21
NPV dust (Kaolin)	,,	13.3 ± 0.03	13.6cd ± 0.09	25.9c ± 0.22
NPV dust (China clay)	,,	16.2 ± 0.04	13.4cd ± 0.20	25.9c ± 0.21
Endosulfan 0.07%	,,		3.7e ± 0.05	96.3ab ± 0.21
Carbaryl 10% dust	(2.41 g/mm^2)		11.7de ± 0.21	96.3ab ± 0.27
Control			18.5abc ± 0.86	—

In a column, means followed by similar letters are not different statistically (P = 0.05) by DMRT.

A total of four laboratory tests were conducted to evaluate the comparative efficacy of the NPV formulations; one on formulations involving acetone, two on those involving water and one to compare the NPV dust formulations prepared involving water and acetone. The mortality data were subjected to angular transformation and after analysis of variance, the means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Results of the first laboratory test on the efficacy of different NPV formulations showed that the NPV.WP formulations were as effective as the unformulated virus as well as endosulfan and carbaryl against the second instar larvae of *H. armigera* on chickpea (Table 1). Chaudhari and Ramakrishnan (1979) prepared a wettable powder preparation of NPV of *Spodoptera litura* F. which on bioassay was found to be as effective as the unformulated virus. In the present study, the dust formulations were found to be significantly inferior to the WP formulations. Significant differences were observed in the leaf consumption in the different treatments. Leaf consumption was reduced significantly particularly in the NPV dust formulations (kaolin and china clay). Among the virus treatments there was a significant positive correlation between the leaf area consumed and mortality rates ($r = 0.837$; $N = 18$).

Since the mortality rates were rather low in the dust formulations, a second test was conducted to find out the influence of acetone on the efficacy of the virus formulations. Results of this test indicated that when acetone was used in the process of formulation of the NPV dust, the efficacy against the second instar larvae of *H. armigera* was significantly reduced when compared to the NPV dust in which distilled water was used (Table 2). Since such a deleterious effect of acetone on the NPV was not observed in the case of wettable powder formulation (Table 1), more detailed investigations are necessary to elucidate the phenomenon. Anyhow, for the subsequent tests, the different formulations were made using only distilled water. That acetone affects the NPV of *H. virescens* has earlier been reported by Ignoffo and Shapiro (1978). Ignoffo *et al.*

TABLE 2. Laboratory evaluation of efficacy of *Heliothis armigera* NPV dust (talc) formulations against second instar larvae of *Heliothis armigera*

Treatments*	%mortality* $\bar{x} \pm$ S.E.
NPV dust (Acetone)	16b ± 1.09
NPV dust (water)	76a ± 1.79
Carbaryl 10% dust (Ca. 2.41 µg/mm²)	84a ± 2.68
Control	

Dose of NPV at Ca. 16.21 POB/Mmm

In a column, means followed by similar letters are not different statistically (P = 0.05) by DMRT.

TABLE 3. Laboratory evaluation of efficacy of NPV formulations (involving water) against second instar larvae of *Heliothis armigera* Experiment I.

Treatments*		Leaf area consumed (mm²/larvae) $\bar{x} \pm$ S.E.	% mortality $\bar{x} \pm$ S.E.
NPV (unformulated)	(1 x 10^6 POB/ml)	24.7a ± 0.15	72.2a ± 0.24
NPV WP (Dedenol)	,,	21.7ab ± 0.02	75.9a ± 0.20
NPV WP (Lissapol)	,,	21.1ab ± 0.06	60.2ab ± 0.13
NPV dust (Talc)	(6 x 10^7 POB/g)	19.2ab ± 0.17	51.5bc ± 0.26
NPV dust (Kaolin)	,,	18.8b ± 0.12	50.0bc ± 0.24
NPV dust (Lilite)	,,	17.7b ± 0.13	49.1bc ± 0.18
Control	,,	24.3ab ± 0.05	—

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

TABLE 4. Laboratory evaluation of efficacy of NPV formulations (involving water) against second instar larvae of *Heliothis armigera* — Experiment II

Treatments		Leaf area consumed (mm^2/larvae) $\bar{x} \pm$ S.E.	% mortality $\bar{x} \pm$ S.E.
NPV (unformulated)	(1 x 10^8 POBs/ml)	21.3a ± 0.19	71.9b ± 0.69
NPV WP (Dedenol)	,,	21.1a ± 0.09	77.0ab ± 0.73
NPV WP (Lissapol)	,,	19.7ab ± 0.06	62.5b ± 1.00
NPV dust (Talc)	(6 x 10^7 POBs/g)	14.8ab ± 0.20	50.0bc ± 0.29
NPV dust (Kaolin)	,,	13.4b ± 0.14	50.5bc ± 1.3
NPV dust (Lilite)	,,	13.9ab ± 0.16	46.3c ± 0.21
Endosulfan 0.035%		2.8c ± 0.05	93.3a ± 0.27
Control		19.5ab ± 0.03	—

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

(1976 a) found that a commercial formulation of acetone-dried preparation of virus diseased larvae mixed with lactose was less effective than a water-based formulation against *H. zea* (Bodie).

The results of the two subsequent laboratory tests showed that the mortality rates of second instar larvae of *H. armigera* in the different dust formulations improved considerably (Tables 3, 4), but still the WP formulations were better giving higher mortalities. As in the first experiment, the leaf consumption in the last two tests was also significantly and positively correlated with the mortality rates. Feeding on leaves treated with NPV dust formulation was slightly reduced and this may be the main factor responsible for the reduced mortality.

Further, the uneven distribution of POB in small clumps particularly in the limited area of feeding by the larvae also cannot be ruled out as a possible reason for the poor performance of the dust formulation, eventhough the recovery studies employing the microslides with larger surface area, had indicated fairly uniform deposition rate. The efficacy of NPV dust formulation could be improved by addition of certain phagostimulants as adjuvants to the basic formulation. Montoya *et al.* (1966) could improve the efficacy of NPV dust formulation by addition of corn extract to the formulations.

Regarding the WP formulations, the WP (Dedenol) recorded slightly higher mortality rates than WP (Lissapol) in all the three tests. (eventhough, these differences were statistically not significant). Studies on the physical characteristics of the WP formulations (Ethiraju, 1986) showed that formulation with 'Dedenol' had higher values of wettability, suspensibility and wet sieving than those of the formulation with 'Lissapol' and this may be the reason for the slightly higher mortality recorded in 'Dedenol'. There seems to be no enhanced mortality due to change in use from acetone to water in the case of the wettable powders (Tables 1, 3). However, the dust formulations did show increased mortality.

Smith *et al.* (1978) could increase the efficacy of *Heliothis* NPV against *Heliothis zea* (Bodie) by using a commercial adjuvant namely Shade + Keltose or polyvinyl alcohol. Addition of soybean, corn or cotton seed component and sugars to water based formulation containing *'Baculovirus heliothis*, the nuclear polyhedrosis virus of *Heliothis* species enhanced the efficacy of the virus against 24 h bollworm *H. zea* (Smith *et al.*, 1982). A cotton seed flour adjuvant consisting of 62.5 per cent cotton seed flour, 12 per cent cotton seed oil, 25 per cent sucrose and 0.004 per cent Tween 80 improved the efficacy of Elcar the commercial *Heliothis* NPV formulation (Hostetter *et al.*, 1982).

In laboratory studies, Rabindra and Jayaraj (1987) observed that the addition of either 0.5% jaggery or 1% cotton seed flour or 1% groundnut oilcake significantly increased the efficacy of NPV against second instar larvae of *H. armigera* on chickpea. By the addition of such adjuvants possessing gustatory and phagostimulant properties, the insects can be made to consume more of the treated surface, thereby improving the mortality rates (Ignoffo *et al.*, 1976 b).

Better formulation methods ensuring good flowability of the dusts and even distribution of the POB should be tried to improve the efficacy. The efficacy of the dusts can also be increased by increasing the dose of virus in the formulations. Another possibility is the addition of some adjuvants with phagostimulant properties in the dust formulation to improve the feeding rate.

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