Effect of *Helicoverpa* Nuclear Polyhedrosis Virus (HNPV) on Different Life Stages of *Helicoverpa armigera* (Lepidoptera:Noctuidae)

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Helicoverpa armigera Hubner, chickpea pod borer is a serious pest of legumes, cotton and vegetables in the Indian subcontinent. Widespread appearance of resistance to chemical insecticides has stimulated efforts to develop alternative control methods including the use of insect viruses against this pest. Several workers proved the effectiveness of HNPV under field conditions in controlling *H. armigera* in chickpea (Dhamdhere and Khaire, 1986, Jayaraj *et al*., 1987, Cowgill and Bhagawat, 1996). However, there were several gaps in the utilization of HNPV and its affects on the life stages of *H. armigera*. This paper discusses the quantification of various ill effects of HNPV on *Helicoverpa* life stages under laboratory and the effect of Robin Blue as ultraviolet protectant with NPV in enhancing its persistence in chickpea ecosystem.

The effect of HNPV on various life stages of *Helicoverpa,* was studied under laboratory conditions with third, fourth and fifth instar larvae of *Helicoverpa*, replicated 15 times with 24-larvae/replication. The test insects were obtained from ICRISAT insectory. The larvae were fed with HNPV (dose: $6x10^9$ poly ocular bodies (POBs)/ml diluted in one litre of water) treated chickpea leaves (air-dried for ½ h) and pods obtained from glasshouse. After allowing the larvae to feed for 24 h, the food was changed with untreated fresh pre-soaked chickpea seeds. Observations on larval mortality, pupal abnormality and adult emergence were recorded. To determine the effect of HNPV on the fecundity and egg hatching, pupae were collected from the larval population that was treated with HNPV. Subsequent tests (with the adults emerged from the pupae collected from infected larvae) were conducted with different adult combinations *viz.*, a) Female from HNPV treated population + male from healthy population, b) Male from HNPV treated population + female from healthy population, c) Both male and female from HNPV treated population, d) Both male and female from healthy population as control.

The adult moths were released into the plastic egg laying chambers and nappy liners were placed inside the chamber along the walls to facilitate oviposition. This experiment was repeated five times with three replications. Cotton swab soaked with 10% sucrose solution was provided as diet for adults. Observations on egg laying were recorded until the death of the adults. The data were adjusted to square root transformation for analysis. A nappy liner with 100 eggs was kept for egg hatching. The number of eggs hatched was recorded and per cent hatch was calculated. The data were subjected to arc sin transformation for analysis using complete randomized block design (CRBD).

To evaluate the efficacy of Robin Blue as UV protectant for HNPV under field conditions an experiment was conducted with three treatments *viz.*, a) HNPV alone b) HNPV + 1% Robin Blue and c) control (water spray only) in chickpea crop. HNPV @ 1.5×10^{12} POBs per hectare were applied to the crop. Three different plots measuring 20x20 m with a distance of 100 m between each plot were chosen for this study. The virus used for these studies was obtained from NPV laboratory at ICRISAT. After the application of virus in the field, bioassay was organized under laboratory conditions by collecting leaves and pods soon after the spray and continued for seven days with 24h interval. The HNPV treated leaves and pods were fed to starved (24 h) laboratory reared third instar larvae. Twelve larvae were used in each treatment and replicated seven times. Larval mortality was recorded at 24h interval until pupation. The data were subjected to arc sin transformation and analyzed using CRBD.

The exposure of first and second instar larvae to HNPV resulted in total mortality as against 2.7% in control. The mortality percentage reduced gradually at later stages with HNPV resulted in 59.8% mortality as against zero mortality in control. High larval mortality of *H. armigera* larvae at early stages indicated the larval stamina to withstand the infection of HNPV which was in agreement with studies reported by Ignoffo (1966) incase of *H. zea* and *H. virescens*. The present studies revealed high efficiency of the virus against younger larvae. However, there was significant level of abnormality

in pupal stage when the larvae were infected at later stage (Table 1). The fecundity of adults from HNPV treated third and fourth instars larvae was significantly low (397 eggs/ female) compared to healthy control (1079 eggs/female). There was significant difference in fecundity when larvae were treated during fifth and sixth instars compared to early infection. This indicated the carryover effect of the virus to subsequent stage, which is critical for population growth (Table 1).

Table 1. Effect of HNPV application on different life stages of *Helicoverpa armigera*

Larval stage exposed	HNPV	Control
Larval mortality (%)		
First and second instars**	100.0*	2.7
Third and fourth instars	76.8*	3.5
Fifth and sixth instars	59.8*	0.0
Pupal abnormality (%)		
First and second instars**	-	-
Third and fourth instars	98.0*	0.0
Fifth and sixth instars	86.9*	-
Pupal weight (mg)		
First and second instars**	-	-
Third and fourth instars	290.5*	375.7
Fifth and sixth instars	349.4	357.9
Fecundity (No. of eggs)		
First and second instars**	-	-
Third and fourth instars	397*	1079
Fifth and sixth instars	689*	865

* Significant- The data were tested by using two-sample t-test

** When first and second instar were infected with HNPV there was 100% larval mortality, hence no pupation

There was a significant reduction in hatching of eggs of resultant population from HNPV treated fifth instar larvae. Infected pair of adults resulted in 20.4% reduction in oviposition and 30.5% reduction in hatchability compared to healthy. When HNPV infected female and healthy male were tested there was 17.0% reduction in oviposition and 28.0% reduction in egg hatchability against control. Healthy female and male from HNPV infected larval population when tested there hwas 10% reduction in oviposition followed by 7.0% reduction in egg hatchability compared to healthy population (Table 2). This clearly shows that the infection through female is playing significant role than infection through males in population suppression. These results are in confirmation with the findings of Patil *et al*., (1989) in *Mythimna separata*, Luttrell *et al*., (1982) in *Heliothis zea* with prolonged larval and pupal developmental period and also reduction in pupation, adult emergence, growth rate, fecundity and per cent egg hatchability.

Table 2. Effect of HNPV infection on fecundity and egg hatchability of *Helicoverpa armigera* when larvae were treated during fifth instar

Treatment	No. of eggs/ female	Reduction over control(%)	Hatchability (%)	Reduction over control(%)
HNPV female + HNPV male	689 (26.0)[a]	20.4	62.6 (52.8)[a]	30.5
HNPV female + healthy male	718 (26.6)[a]	17.0	65.2 (54.0)[a]	27.6
Healthy female + HNPV male	780 (27.9)[ab]	9.8	83.7 (67.5)[b]	7.0
Healthy female + healthy male	865 (29.4)[b]	-	90.1 (73.9)[c]	-
CD (0.05)	2.62		6.25	

* Values followed by same letters in a column are statistically non-significant

Studies to determine the efficacy of HNPV in chickpea fields revealed similar effects of virus with and without UV protectant immediately after spray. But 24 hours after the spray, the addition of 1% Robin Blue resulted in 16.7% extra larval mortality. Whereas two and three days after HNPV spray with Robin Blue resulted in 15.5 and 16.7% extra larval mortality compared to HNPV without Robin Blue. Observations on larval mortality on fourth day showed the effect of HNPV alone as low (7.1%) and was on par with control. However, the spray of HNPV + 1% Robin Blue spray even after four days (on fifth day) gave 15.0% additional larval mortality. Due to the addition of UV protectant to HNPV spray solution, its effect has been extended upto six days after application 8.5% mortality compared to 2.4% in control (Table 3). Earlier studies by Rabindra and Jayaraj (1988) and Rabindra *et al*., (1989) reported the increased efficacy of HNPV with UV protectant. The present results emphasized the persistence of HNPV with UV protectant in increasing the efficacy under field conditions and eliminating the ill effects of virus on late instars and the subsequent stages of the life cycle.

Thus present studies concluded that exposure of first and

Table 3. Persistance of HNPV on chickpea foliage with/ without Robin blue against third instar larvae of *Helicoverpa armigera*

Days after application	Larval mortality			
	HNPV alone	HNPV + Robin bule	Control	CD (0.05)
Soon after application	75.0 (60.5)[a]	76.2 (61.7)[a]	4.8 (10.0)[b]	5.29
1	34.5 (35.9)[b]	57.2 (45.7)[a]	0.0 (0.2238)[c]	2.70
2	29.8 (33.0)[b]	45.2 (42.5)[a]	3.6 (5.8)[c]	3.85
3	22.6 (28.3)[b]	39.3 (38.7)[a]	5.7 (13.8)[c]	2.50
4	7.1 (13.0)[b]	27.5 (35.9)[a]	5.0 (12.9)[b]	3.42
5	4.8 (8.2)[b]	19.8 32.9)[a]	1.2 (4.4)[b]	4.50
6	2.6 (4.8)[b]	8.5 (14.1)[a]	2.4 (4.8)[b]	4.67

Figures in parentheses are Arc sin transformed values

* Values followed by same letters in a row are statistically non-significant

second instar larvae to HNPV resulted in total larval mortality. As the larval stage advanced the mortality rate decreased. Late larval infection with HNPV though resulted in lower mortality, it had caused pupal abnormalities, reduction in fecundity and egg hatchability. Addition of 1% Robin Blue as UV protectant enhanced the persistence of HNPV significantly up to six days under field conditions.

Acknowledgements

The opportunities and facilities provided by ICRISAT crop improvement program to conduct this research at ICRISAT, Patancheru were highly appreciated.

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Received : 03-08-04 Accepted : 11-07-06