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Studies on Nuclear Polyhedrosis of *Heliothis armigera* (Hbn.) I. Susceptibility and Gross Pathology

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ABSTRACT

Studies were made on the susceptibility and gross pathology of NPV-infected *Heliothis armigera* (Hbn). Incubation period increased with age whereas the susceptibility decreased as the larvae grew older. Mortality increased and incubation period decreased with increasing doses of virus. Healthy larvae steadily increased in length with advancement of age whereas in infected larvae growth was completely arrested after 72 hours of virus treatment. Moulting was inhibited in virus-infected caterpillars. Total circulating haemocytes in virus-infected caterpillars were significantly reduced from third day of infection.

INTRODUCTION

Nuclear polyhedrosis of *Heliothis armigera* (Hbn.) was first reported in India by Patel *et al.* (1968) who gave a brief description of the symptoms. Jacob and Subramaniam (1972) described the morphology of the virus. As information on the susceptibility of different instars and different pathological effects of the virus on the larvae are lacking investigations were taken up and the results are presented in this paper.

MATERIALS AND METHODS

The nuclear polyhedrosis virus of *H. armigera* was isolated from the infected caterpillars and multiplied in the laboratory. The polyhedra were purified according to the method described by Smith (1967) and the concentration of stock suspension was determined with the help of a haemocytometer with improved double Neubaur rulings under a Meopta microscope with 45 × objective as described by Lewis (1960). By suitably diluting the stock suspension different concentrations of polyhedra were obtained. Test insects of different instars were drawn from homogenous cultures raised on bengal gram plants till the second instar and on soaked bengal gram seeds from the third instar onwards.

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The required dose of virus was placed in small cavities made on tender red gram seeds and allowed to dry. Caterpillars were allowed to feed on these treated seeds. To determine the incubation period of different instars, twenty five caterpillars from each instar were fed with a dose of 10 polyhedra per larva. Observations were made on mortality and incubation period. Similarly the effect of different doses of polyhedra viz 10^3, 10^4, 10^5, 10^6 and 10^7 polyhedra per larva was determined in fourth instar using 25 caterpillars for each dose. The length of caterpillars was recorded at 24 hours interval up to 120 hr in twenty fourth instar larvae inoculated with 10^6 polyhedra per larva. The effect of virus on moulting was determined in fourth instar larvae with doses viz. 10^3, 10^4, 10^5 and 10^6 polyhedra per larva. The total haemocytes count was recorded at 24 hour interval up to 120 hour in 4th instar larvae inoculated with 10^6 polyhedra per larva. The total haemocytes were counted with the help of a haemocytometer (with improved double Neubaur ruling) as described by Jones (1962). Suitable controls were maintained for all the tests

TABLE 1. Effect of virus on incubation period of different larval instars

Stage of larvae at treatment (instar)	Incubation period (Days)		Mortality	
	Range	Mean	NPV	Other causes
2	2 – 4	3	25	—
3	3 – 5	4.2	24	1
4	4 – 5	4.8	25	—
5	4 – 6	5.5	20	1
6	6 – 9	8.1	5	—

RESULTS AND DISCUSSION

The data on the incubation periods and per cent mortality of different larval instars are presented in Table 1.

There was no mortality of larvae in control. It may be seen from the data that the incubation period increased and susceptibility decreased with age. Such a phenomenon has been reported by Tanada (1956) in the army worm, *Pseudaletia unipuncta*, Morris (1962) in *Lambdina fiscellaria* and Ignoffo (1966) in *Heliothis zea* and *H. virescens*.

TABLE 2. Effect of different doses of polyhedra on the incubation period.

Dosage (PIB*/larva)	Incubation period (days)		Number dead due to	
	Range	Mean	NVP	Other causes
10^3	7 — 9	8	6	1
10^4	7 – 9	7.4	10	—
10^5	6 — 8	6.2	21	1
10^6	4 – 6	5.5	25	—
10^7	4 – 5	4.8	25	—
Control	—	—	—	—

* Polyhedral Inclusion Bodies

It is evident from Table 2 that the incubation period decreased as the concentration of virus increased. Canerday and Arant (1968) observed that the time required for lethal infection by the virus of *Trichoplusia ni* was inversely related to dosage level and environmental temperature. Similar observations were made by Jacob (1972) in *Spodoptera litura*.

The results show that the growth of diseased larvae is considerably retarded in the late stages of infection (Table 3). The loss of appetite and cessation of feeding due to virus infection probably are the reasons for the stunted growth. Adams *et al.* (1968) reported such retardation of growth in the NPV - infected zebra caterpillar, *Ceramica picta*.

TABLE 3. Effect of virus infection on the length of larvas

Post inoculation period (hours)	Mean length of larva in mm ± S. E.	
	Healthy	Diseased
24	15.45 ± 0.94	15.15 ± 0.10
48	19.60 ± 0.43	19.00 ± 03.5
72	24.10 ± 0.39	23.60 ± 0.43
96	29.70 ± 0.45	23.75 ± 0.36
120	33.60 ± 0.07	23.85 ± 0.09

The data show that the virus has marked influence on moulting of caterpillars (Table 4). All the infected

TABLE 4. Effect of the disease on moulting of larvae.

Dosage PIB/larva	Incubation period (days)	No. of larvae moulted to		Mortality due to	
		5th instar	6th instar	NPV	Others
10^3	7 — 9	25	18	9	—
10^4	7 — 9	25	13	15	—
10^5	6 — 8	25	5	22	—
10^6	4 — 6	25	—	25	—
Control	—	25	25	—	—

caterpillars moulted only once with higher concentration while the healthy larvae had two moults. Jacob (1972) got similar results in *S. litura*. Infection of brain, hypodermis and blood cells might have inhibited moulting.

It can be seen that the average number of haemocytes in infected larvae decreased steadily and drastically after 48 hours of inoculation (Table 5). Shapiro *et al.* (1969) found that the total haemocyte count in *H. zea* fell drastically three days after exposure to a heavy dose of polyhedra. The reduction in total haemocytes count in *H. armigera* in the present investigations might be due to the invasion of the nuclei of blood cells by the virus, as polyhedral bodies could be seen in the nuclei of blood cells by 72 hr post-inoculation.

TABLE 5. Effect of the disease on total haemocyte count.

Post inoculation period (hours)	Average number of circulating haemocytes/mm 3 ± S. E.	
	Healthy	Diseased
24	22046 ± 1973	22210 ± 966
48	25450 ± 763	28640 ± 876
72	25080 ± 560	20529 ± 1020
96	30200 ± 835	17343 ± 577
120	25232 ± 1020	15085 ± 970

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