STUDIES ON NUCLEAR POLYHEDROSIS VIRUS INFECTIONS IN *Heliothis armigera* Hubner AND *Anomis sabulifera* (Guenee)

BY

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B Sc (Agri)

DISSERTATION SUBMITTED TO THE
MARATHWADA AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF
MASTER OF SCIENCE (Agriculture)
IN
ENTOMOLOGY

DEPARTMENT OF ENTOMOLOGY
MARATHWADA AGRICULTURAL UNIVERSITY
PARBHANI

1979

AFFECTIONATELY DEDICATED

TO

MY BELOVED PARENTS

CANDIDATE'S DECLARATION

I hereby declare that the entire work embodied in this dissertation or any part thereof has not been previously submitted by me for a degree of any University.

(D.G. Bakwad)

Parbhani.
May 26, 1979.

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CERTIFICATE I

Shri Digamber Ganpatrao Bakwad has satisfactorily prosecuted his course of research for a period of not less than four semesters and that the dissertation entitled "STUDIES ON NUCLEAR POLYHEDROSIS VIRUS INFECTIONS IN HELIOTHIS ARMIGERA HUBNER AND ANOMIS SABULIFERA (GUENEE)" submitted by him is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the dissertation or part thereof has not been previously submitted by him for a degree of any University.

Pawar

(V.M. Pawar)
Guide

Parbhani
May 26, 1979.

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CERTIFICATE II

This is to certify that the dissertation entitled "STUDIES ON NUCLEAR POLYHEDROSIS VIRUS INFECTIONS IN HELIOTHIS ARMIGERA HUBNER AND ANOMIS SABULIFERA (GUENEE)" submitted by Shri Digamber Ganpatrao Balwad to the Marathwada Agricultural University in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (Agriculture) in the subject of Agricultural Entomology has been approved by the student's advisory committee after oral examination in collaboration with the external examiner.

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ACKNOWLEDGEMENTS

Indeed the words at my command are not adequate either in form or spirit, to convey the depth of my feelings of gratitude to my esteemed guide Dr. V.M. Pawar, M.Sc. (Agri)., Ph.D., Professor, Department of Entomology, Marathwada Agricultural University, Parbhani, for suggesting the problem, offering inspiring and scholastic guidance and keen interest throughout the course of this investigation, for his constructive criticism and painstaking corrections during the preparation of the dissertation.

I would like to place on record my sincere thanks to Dr. A.K. Raodeo, Head, Department of Entomology, Dr. C.D. Mayee, Professor, Department of Plant Pathology and Dr. S.N. Puri, Associate Professor, Department of Entomology, members of my advisory committee for their suggestions and help rendered during the course of investigation.

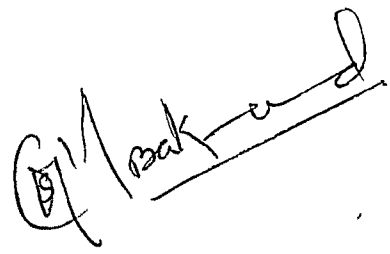
I gratefully acknowledge my sincere thanks to Dr. K.R. Pawar, Associate Dean and Principal, College of Agriculture, Parbhani for providing necessary facilities.

I also express my sincere thanks to S/Shri G.G. Bilapate, P.R. Shivpuje, M.B. Sarkate and staff members of Entomology Department for their timely help and guidance.

Thanks are due to my collegues, especially S/Shri G.N. Patil, S.D. Shinde, P.S. Dahale, S.N. Awatade and T.R. Gurav for their valuable cooperation.

This acknowledgement cannot be completed without making mention of my parents and Shri D.G. Boinwad, Asstt. Teacher, Primary School, Sujlegaon, who have given me constant encouragement throughout my educational career.

Lastly, I gratefully acknowledge the financial assistance received from the Indian Council of Agricultural Research, New Delhi in the form of Junior Research Fellowship.



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May 26, 1979.

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CHAPTER I

INTRODUCTION

CHAPTER I

I N T R O D U C T I O N

Intensive evaluation of microbial pesticides is taking place at present in many countries throughout the world. The interest in microbial pesticides has expanded because of problems such as insect pest resistance, emergence of secondary pest, toxic residues and high costs that have developed with the use of chemical insecticides. More than 1000 micro-organisms viz; bacteria, viruses, fungi, protozoa, nematodes etc. are associated with insect pests which could be developed as potential microbial insecticides. However, of all these groups of micro-organisms, bacteria and viruses occupy an important position in microbial control (Ignoffo,1970).

Of the major seven groups of insect viruses, the genus Baculovirus is the most predominant. This group comprises of nuclear polyhedrosis (Subtype A) and granulosis viruses (Subtype B). More than 284 nuclear polyhedrosis viruses (NPV) have been reported from the species of Lepidoptera, Hymenoptera and Diptera (David,1975). Baculoviruses appear to have no chemical, physical or biological properties in common with any known virus found either in vertebrates or plants. It was for this reason the WHO and FAO jointly recommended that only Baculovirus group should be considered as possible pesticidal agents (Tinsley, 1976).

Many organizations with agricultural missions have recognised arthropod viruses as an important component of pest management strategies. Much of the interest in utilizing these viruses for insect and mite control has been stimulated by research results that have indicated that arthropod viruses are (a) selective, safe, environmentally non-disruptive control agents, ideally suited for use of integrated control and other pest management programmes (b) may aid in the control of pests in case where a chemical insecticide is no longer effective because of pest resitance, (c) can provide relief in situations in which the use of an effective pesticide is restricted and (d) could help reduce the current heavy reliance on chemical pesticides (Falcon, 1976).

The gram pod borer, Heliothis armigera Hubner is one of the obiquitous pest of many important crops cultivated all over the world. The larvae of this pest feed on a variety of important cultivated plants viz., cotton, gram, arhar, sorghum, sunflower, soybean and lucerne in India.

The cultivation of jute has recently started in Marathwada region of Maharashtra for seed purpose. The jute semilooper, Anomis sabulifera (Guenee) has been observed to be very serious pest of this crop. It has been reported to be a specific pest of this crop.

The damage is caused by the caterpillars. They attack the top leaves and apical buds of the plant with the result that side branches are produced. This results in shortening of fiber (Pruthi, 1969).

In the present study, investigations were carried out on the nuclear polyhedrosis virus infections in H.armigera and A. sabulifera in view of the possibilities of utilizing these pathogens as an effective controlling agents. The NPV infection in A. sabulifera is being reported for the first time. The investigations are mainly oriented to study the insect-virus relationship and the field efficacy of Heliothis NPV in comparison with synthetic insecticides against H.armigera on gram.

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CHAPTER II

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

With a view to fall in line with the experiments conducted in the present studies, pertinent literature has been surveyed and presented in this Chapter under the following heads

2.1. Symptoms of nuclear polyhedroses

2.2. Number of polyhedral inclusion bodies (PIBs) in diseased larvae and size of polyhedra

2.3. Cross-infectivity tests

2.4. Bioassay of virus activity

2.5. Field efficacy of NPV and other synthetic insecticides against H. armigera

2.6. Persistence of NPV under field conditions.

2.1. Symptoms of nuclear polyhedroses:

Bergold (1964) generalised the symptoms of nuclear polyhedroses and granuloses in Lepidoptera as follows "the lepidopterous larvae suffering from these diseases sometimes change their colour to a palish hue, become flaccid, but still move around. Six to fourteen days after infection larvae die hanging often in characteristic way by their prolegs from the support. The skin becomes fragile and ruptures easily releasing a milky fluid containing mainly polyhedra or capsules".

Patel et al. (1968) observed the incidence of nuclear polyhedrosis in the laboratory culture of

H. armigera. The infected larvae became sluggish, stopped feeding, became paralysed and the integument became soft and brown or black. The internal tissues disintegrated and liquefied.

Pawar and Ramkrishnan (1971) described the nature of polyhedral diseases of Spodoptera litura (F). The naturally infected larvae showed no change in appetite during more than 3/4 of the incubation period. and symptoms were generally observed in the last instar. The colour of diseased larvae changed from ashy white to light shades of black on dorsal sides. Ventral and lateral sides became slight reddish pink in appearance as against the green in normal ones. The integument became fragile and liquefied body contents were liberated even with the slightest touch.

Jacob and Subramaniam (1972) reported that the larvae of red hairy caterpillar, Amsacta albistriga (W) exhibited loss of appetite and became sluggish in movement. The colour turned dirty brown. Death occurred in 8-10 days with disintegration and liquefication of the body contents.

Pattar and Nathad (1972) reported that NPV infected larvae of Antheraea mylitta (Drury) attached themselves by means of their prolegs to a twig and at the time of death hanged with their head downwards.

The anterior portion was in a swollen state due to accumulation of disintegrated body contents. They also emitted a putrefying disagreeable smell characteristic of nuclear polyhedrosis virus (NPV).

2.2. Number of polyhedral inclusion bodies in diseased larvae and size of polyhedra:

2.2.1. Number of polyhedral inclusion bodies in diseased larvae:

The references on the PIB counts in naturally infected diseased larvae are seldom found in literature.

McEwen and Harvey (1959) obtained yields of inclusion bodies 8×10^9 PIBs/larva or 26×10^9 PIBs/gram of larvae of Trichoplusia ni.

Ignoffo (1966) reported that the yield of inclusion bodies of NPV of H.zea averaged 13×10^9 PIBs/larva i.e. 43×10^9 PIBs/gram of larva.

Brown and Swaine (1965) obtained 4×10^9 polyhedra in the larvae of Spodoptera exempta (Walk).

Pawar and Ramkrishnan (1971) obtained 2×10^9 PIBs/larva in case of S.litura (F).

Narayanan et al. (1978) obtained yields of inclusion bodies ranging from 0.35 to 9.78×10^9 in case of groundnut red hairy caterpillar, A.albistriga (Walker).

2.2.2. Size of polyhedral inclusion bodies (PIBs):

Bergold and Flaschentrager (1957) reported that polyhedra of NPV in P.litura ranged from 1.2 to 3.2 µm with an average of 1.8 µm. While Ramkrishnan and Tiwari

(1969) reported that polyhedra vary from 2.5 to 4.2 µm under optical microscope. Pawar and Ramkrishnan (1971) reported that the size of polyhedra varied from 0.6 to 2.0 µm under both optical and electron microscope. Jacob and Subramaniam (1972) reported that the polyhedra were irregular in shape and measured on an average 1.86 um in diameter with a range of 1.2 to 2.8 µm.

Jacob and Subramaniam (1972 a) reported that, the diameter of polyhedra ranged from 0.7 to 1.7 µm with an average of 1.7 µm in case of A. albistriga.

Jacob and Subramaniam (1972 b) reported that the polyhydral inclusion bodies in case of Heliothis armigera were irregular in shape and measured 0.5 to 1.4 µm in diamter with a mean of 0.89 µm. While, Waghmare (1977) reported that polyhedral size of H.armigera was 2.15 µm in diamter.

Pattar and Mathad (1972) reported that the polyhedral size of A. mylitta was 0.53 µm.

Rabindra and Subramaniam (1975) reported that the PIBs of Pieris peronis in general were found to be roughly hexagonal and diameter ranged from 0.64 to 2.32 µm with a mean of 1.25 ± 0.05 µm.

Nordin and Bouciao (1978) reported that the polyhedral size of Biston betularia cognataria was found to be 2.97 µm in diameter.

2.3. Cross-infectivity tests:

Insect viruses have been increasingly observed to behave with varying degree of pathogenicity toward species which are not their spontaneous hosts. The problems concerning this specificity have been reviewed by Aizawa (1963) and Ignoffo (1968, 1973, 1975).

Stairs (1960) found that C.fumiferana NPV could infect a species of the same genus C.pinus (F).

The viruses of nuclear polyhedrosis of Malacosoma americanum (F), M.alpicola and M. pluvidae (Dyar) were infective for the species of M.disstria (Stairs, 1964).

Ignoffo (1965) reported a nuclear polyhedrosis virus that was highly infectious to both H.zea and H.virescens.

Adams et al. (1968) obtained successful cross-infection of Ceramica picta NPV to several noctuids as Malaltra conficurta, Peridroma saucia (Hubner), Prodenia praefica Grote, and T.ni.

The NPV of T.ni was not infective when fed to some related species such as Rachiplusia ou (Guenee) Anagrapha biloba and Pseudaletia includens (Canerday, 1968)

Pawar and Ramkrishnan (1971) reported that the NPV of S. litura was not cross-infective to the larvae of Spodoptera exigua (Hubner), Agrotis ypsilon Rott, Achoea janata Linneaus and Euproctis lunata Walker.

Rajmohan and Jayraj (1975) reported that the A. albistriga NPV was not cross-infective to the larvae of Pericallia ricini Fab., Euproctis fasterna Moore, Notolophus posticus Walk. and Porthesia scintillans Walk. Earias vitella Fab. Spodoptera litura Fab. Heliothis armigera Hb. Orthaga exivanacea M. and Cosmophila eriosa F., Hyblaea purea cramer, Eunterote mellifera Walk., Papilio demoleus and Sylepta derogata . However, tested larvae of some species died due to bacterial infection and unknown causes.

Jacquemard (1978) reported cross-infectivity of Mamestra brassicae (L) NPV to third, fourth, and fifth instar larvae of Diaphorpsis Watersi (Roths) when fed for 24 hours on green bolls that had been dipped in a suspension containing virus polyhedra at 4.9×10^9/ml water.

2.4. Bioassay of virus activity:

Several methods have been used for determining the virus activity. The literature is reviewed on the following three commonly adopted methods

a) Injection of PIBs or free virus

b) Feeding known amount of inoculum to the larvae reared on artificial diet.

c) Feeding the leaves dipped in known concentration of PIBs suspension.

Ignoffo (1965) carried out a bioassay for Heliothis zea (Noddiae) reared on semi synthetic diet. The LC_{50} value estimated was 32.0 PIBs/mm^2 and the LT_{50} values for 292, 200, 146, 58 and 29 PIBs/mm^2 were 3.8, 3.8, 4.3, 5.3 and 6.5 days, respectively.

Rollinson et al. (1965) conducted laboratory bioassay test for the second instar larvae of gypsymoth, Porthetria dispar. The LC_{50} was 2.3×10^7 polyhedra/ml of water and LC_{95} was 1.0×10^{10} polyhedra/ml.

Chauthani et al. (1968) observed LD_{50} values of three different virus strains viz. Biotrol, Sudan and Ivory cost for H. zea which were 269, 344 and 459 PIBs/ larva respectively.

Hunter and Hall (1968) conducted a bioassay of nuclear polyhedrosis virus with first and fourth instar larvae of beet armyworm S.exigua. The calculated LD_{50} values were 8.3 and 57.6 polyhedra/mm^2 of the diet surface for respective instars.

Rabindra and Subramaniam (1974) conducted bioassay test with Heliothis NPV and reported that susceptibility to NPV varies with dose and larval instar.

Pawar and Ramkrishnan (1975) conducted bioassay to calculate LC_{50} and LT_{50} values for newly hatched and five day old larvae of S. litura infected with its NPV. They found that in general, the LT_{50} values increased

with decrease in concentration. The five day old larvae were found to be 40.27 and 84.50 times more resistant than the newly hatched larvae (0-8 hrs.) on 9th and 13th day respectively.

Magnoler (1975) conducted bioassay of NPV against third and fourth instar larvae of Malacosoma neustria. The calculated LD_{50} values for third and fourth instar larvae were 1405 and 12320 PIBs/larva. The calculated median lethal dose showed that the third instar larvae were only twice more susceptible to virus than the fourth. The LT_{50} values for $3x10^5$, $3x10^4$ and $3x10^3$ PIBs/larva were 5.90, 6.58 and 8.15 days, respectively in the third instar assay.

Narayanan et al. (1978) calculated LT_{50} for various instars of A.albistriga larvae and found that LT_{50} increased as the age of larvae advanced. The LT_{50} values for first to fifth instar ranged from 93.50 hrs to 162.25 hrs. In general, the mean LT_{50} values were found to decrease as the dose was increased.

2.5. Field efficacy of NPV and other synthetic insecticides against H.armigera:

2.5.1. Chemical control of H. armigera:

Saxena et al. (1971) reported that two sprays at fortnightly interval with 0.07 per cent endosulfan

proved effective in reducing the infestation of gram pod borer from 9.2 per cent to 5.3 per cent and increased the gram yield from 12.3 to 17.29 q/ha.

Singh and Singh (1973) recommended that the pod borer of gram could be effectively controlled by spraying endosulfan at the rate of 2 ml per litre of water or dusting of BHC and DDT 10 per cent mixed in equal proportions at the rate of 25 kg/ha.

Saharia and Dutta(1975) studied the efficacy of endosulfan carbaryl and malathion each at three concentrations against H. armigera (Hubner) infesting arhar (S-5). The lowest mean percentage infestation of pods and maximum yield were obtained with endosulfan 0.03 per cent treatment.

Balasubramaniam et al. (1976) reported the superior efficacy of endosulfan 0.07 per cent in reducing the pod borer damage and recording higher yield of gram.

Shetgar (1977) studied the efficacy of quinalphos and other insecticides against pod borer in Bengal gram and arhar during 1975 and 1976. He observed that application of quinalphos 350 g.a.i./ha spray checked the infestation of pods by borers and also gave high yields.

2.5.2. Field efficacy of NPV of H. armigera:

Field tests were carried out to determine the

feasibility of controlling H. virescens and H. zea by applying a NPV pathogenic to those species mixed with bait used for Anthonomus grandis. It was found that the bait induced active feeding by the larvae and that the virus was infective when combined with bait (Molaughlin et al. 1971).

Cadu and Soubrier (1974) conducted field test with two virus preperations and one insecticide viz., Viron H (Prepration of H. zea NPV in USA), HBSB (local preparation of NPV of H. armigera) and monochrotophos against H. armigera on cotton in chad. They found virus HBSB was effective than viron H. However, monochrotophos at 690 g/ha gave better results than either virus.

Rome (1975) conducted six trials in Botswana with unpurified NPV suspension against the larvae of H.armigera on sorghum and cotton. He found H.armigera NPV was as effective as Standard insecticide in preventing losses of sorghum. There was no difference in control acheived by spraying in the morning or evening. He also found that addition of low concentration of molasses increased the efficacy of NPV. He further indicated that NPV was not as damaging as carbaryl to population of egg parasite and predators, but predators did not control aphids and jassids on the virus sprayed plots.

Bull et al. (1976) prepared new formulation of the Heliothis NPV by incorporating the pathogen with UV light screening agents in the capsule bound together with digestible water insoluble polymers. In field tests against moderate populations of Heliothis spp. in cotton, yields of plots treated with these formulations were identical to those of plots treated with the insecticidal mixtures commonly used in commercial production.

Kinzer et al. (1976) conducted small plot field test against H. zea and H. virescens on cotton with compound GL-6506 (applied on cotton stem), monochrotophos and Heliothis NPV. The Heliothis NPV applied as a foliar spray 3 times/week was as effective as monochrotophos in increasing the yields of cotton.

Stacey et al. (1977) conducted small plot field test with Baculovirus heliothis for control of Heliothis invert sugars and Sandoz liquid stimulants. The invert sugars and Sandoz liquid adjuvant-1975 significantly increased yields above that of unformulated virus alone.

2.6. Persistence of Heliothis NPV.

Mackinley (1971) conducted field test with the commercial preparation (Bitrol VHZ) of the Heliothis NPV on cotton and indicated that Bitrol VHZ persisted on cotton foliage for over 7 days.

Andrews and Sikorwski (1973) studied effect of cotton leaf surfaces on the persistence of NPV of H. virescens and H. zea. and indicated that though the dew from cotton leaf surface was having pH 8.2 to 9.1 it increased as it dried and dissolved the polyhedra but pH seemed not be the sole cause of loss in persistence.

Young and Yearian (1974) reported that Heliothis NPV was rapidly inactivated under field condition on the upper leaf surface of cotton, soybean and tomato. Inactivation of the virus was most rapid on cotton with little activity remaining after 24 hrs. While persistence on tomato was significantly better than other hosts, little virus activity remained after 96 hrs.

Experimental results indicated that Baculovirus heliothis was more active about 10 to 45 per cent and showed increased persistence about 2 to 5 fold under both simulated and natural sunlight (Ignoffo et al.,1976).

Rome and Daoust (1976) studied survival of NPV of H. armigera on crops and in soil in Botswana. Experimental results indicated that virus activity was lost rapidly on cotton but remained at a high level for upto 30 days on sorghum, but in soil activity reached to less than 1/3 of its original level during winter.

House et al. (1977) conducted field experiment with Heliothis NPV and reported that the residual biological activity was very short and that the time of application in relation to egg hatch was a critical factor in achieving a high level of effectiveness.

Makode (1978) studied persistence of Heliothis NPV on gram foliage and indicated that virus activity reduced with time. The values for original activity remaining (OAR) were 67.99, 55.99 and 36.00 at 24, 72 and 120 hours respectively.

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CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The details of materials and methods used for conducting the experiments are given in this Chapter.

3.1. Test insects and test materials:

3.1.1. *Heliothis armigera* Hubner

Family : Noctuidae

Order : Lepidoptera

3.1.2. *Anomis sabulifera* (Guenee)

Family : Noctuidae

Order : Lepidoptera

3.1.3. Test material:

Polyhedral inclusion bodies (PIBS) of the nuclear polyhedrosis viruses (NPV) of *Heliothis armigera* and *Anomis sabulifera* were used as test material. The PIBs were obtained by allowing the dead diseased larvae to putrefy in glass stoppered conical flasks containing little quantity of sterile distilled water over a period of few days. The contents were triturated thoroughly with glass homogenizer and then filtered through two layers of muslin cloth and cotton swab to remove the remnants of the insect tissues. The filtrates were purified by centrifugation at 5000 rpm for five minutes.

The pellet was resuspended in sterile distilled water and stored separately in a glass stoppered conical flasks.

3.2. Rearing test insect:

3.2.1. Heliothis armigera Hubner.:

The culture of Heliothis armigera was maintained in the laboratory at 27 ± 2°C throughout the experimental period. The initial culture was collected in kharif season from cotton, arhar and sorghum fields. The larvae were reared on sunflower and/or lucerne leaves. Newly hatched larvae were reared in mass in round plastic containers of 20x15 cm size. When the larvae reached third instar, they were transferred individually to plastic container of 5x 2.5 cm size and fresh food was given every day until pupation. Pupae were transferred to plastic jars of 20 x 15 cm size containing 5 cm layer of moist soil. The moths after emergence were transferred to rearing cage, consisting of wooden frame covered with muslin cloth, in which twigs of cotton, arhar or gram were kept for egg laying. Cotton wicks soacked in five per cent sugar solution were kept in the cage as food for the moths. Eggs were laid after a pre-oviposition period of 3 days. The average incubation, larval, pupal and adult periods were 3, 14, 15 and 8 days respectively.

3.2.2. Anomis sabulifera (Guenee):

The initial culture of Anomis sabulifera was obtained from the infested jute fields in Kharif season of 1978. The culture was maintained at constant temperature of 27 ± 2°C. The larvae were reared entirely on jute leaves (Corchorus sp.) in plastic jars of 20 x 15 cm size until pupation. The moths after emergence were transferred to breeding chamber consiting of plastic jar covered with a piece of muslin cloth in which twigs of jute wrapped in moist cotton wool at their cut ends were kept as oviposition site. The cotton wicks soaked in 5 per cent sugar solution were kept as food for the moths. Incubation, larval, pupal and adult periods were 2, 15, 6 and 8 days, respectively.

Extreme sanitary conditions were maintained to establish the culture free of virus and other pathogens. These included starting a new colony in a distant laboratory, using well cleaned containers disinfected with 5 per cent formalin, rearing the larvae from the eggs laid by visually healthy moths and rearing the larvae after their third instar individually into plastic containers in the case of H.armigera. Surface disinfection of eggs with 5 per cent formalin was also done to check the external contamination of the virus.

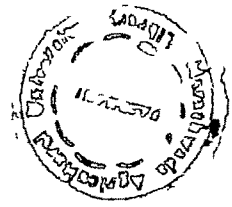
3.3. Symptomatology and the natural incidence of the disease:

In order to study the symptoms of disease in H. armigera and A. sabulifera, twenty, first-instar larvae were artificially infested by feeding them on leaves contaminated with the respective virus suspension. The symptoms that occurred were compared with those that were present in naturally affilicated populations.

To study the natural incidence of disease in H. armigera periodic field collections were made from arhar and gram. The larvae were reared in the laboratory until pupation. The number of larvae pupated and those succumbed to viral infection were recorded.

3.4. Estimation of number of polyhedral inclusion bodies in diseased larvae of H. armigera and A. sabulifera:

Naturally infected last instar dead or moribund larvae of H. armigera and A. sabulifera were individually triturated in a glass homogenizer with little quantity of sterile distilled water. The contents were filtered through double layer muslin cloth and cotton swab to remove the tissue debris. The filtrate was purified by centrifugation at 5000 rpm for 5 minutes and final volume was made up to 100 ml in each case and stored in glass stoppered conical flasks. These formed the



stock suspensions for counting the number of inclusion bodies per larva.

The stock suspension was diluted further (1:10 or 1:20) before counting.

Haemocytometer (NEUBAUR (improved double) Germany, Fein optic JENA) was used for counting the number of PIBs. The number was estimated in each case by using following formula

No. of PIBs = No. of PIBs counted in 10 mm^2 x dilution factor x 1000* x original volume of extract (ml).

1000* = For conversion of cubic mm to cubic milliliters.

3.5. Staining property and size of polyhedral inclusion bodies:

3.5.1. Staining property of PIBs:

In order to confirm the group to which the virus isolated from Anomis sabulifera belonged, a staining technique described by Bergold (1963) for polyhedrosis virus was used.

A thin smear of polyhedral suspension was air dried on clean glass slide. The dried smear was treated with 1 N Hcl for 2-3 minutes. The slide was then washed with distilled water and stained with dilute Giemsa solution for 5-7 minutes. Excess stain was removed by washing the slide with distilled water, and examined under microscope after drying. The slide prepared in the same manner without acid treatment served as control.

3.5.2. Size of polyhedra:

For determining the size of polyhedral inclusion bodies, partially purified suspension of Anomis NPV was used. A drop of polyhedral suspension was put on a clean glass slide and covered with cover slip. One hundred PIBs were measured at random under the optical microscope and the readings were taken at 40 x magnification with the help of filar micrometer. The microscope was caliberated before use.

3.6. Cross-infectivity tests:

The cross-infectivity tests were conducted to determine the host specificity of NPV of H. armigera and A. subulifera. Highly concentrated suspensions of both the viruses were tested against different lepidopterous insects. The tests were conducted independently in two sets with three replications of 10 larvae each. The leaves of the host plants of the larvae were contaminated with the respective virus suspension and fed to the second instar larvae, of different species tested. The larvae of H. armigera and A. subulifera were also included as treated control as a check on the viral infectivity. The following table indicates the different species of insects used for cross-infectivity tests for both the viruses.

Table 1: Different species of insects used for cross-infectivity tests

Sr. No.	Test insect	Family	Order	Food material used
1.	Heliothis armigera	Noctuidae	Lepidoptera	Sunflower
2.	Anomis subulifera	Noctuidae	Lepidoptera	Jute
3.	Anomis flava	Noctuidae	Lepidoptera	Cotton
4.	Spodoptera litura	Noctuidae	Lepidoptera	Castor
5.	Diacrisia oblicua	Arctidae	Lepidoptera	Sunflower
6.	Euproctis sp.	Lymantriidae	Lepidoptera	Castor

3.7. Bioassay of Heliothis NPV for virus activity:

Bioassay tests were conducted for determining the insecticidal activity of NPV of H. armigera.

3.7.1. Preparation of the virus:

PIBs were obtained by artificially infecting the third instar larvae. After death they were allowed to putrefy in glass stoppered conical flask containing little quantity of sterile distilled water for few days. Then this crude PIB suspension was filtered through double layered muslin cloth and cotton swabs. The filtrate was purified by centrifugation at 5000 rpm for five minutes, and stored in a glass stoppered conical flask. The number of PIBs was estimated by using haemocytometer, as described earlier.

3.7.2. Test larvae:

Newly hatched (0.8 hours) and five day old larvae of H. armigera were used for the experiments.

3.7.3. Bioassay procedure:

3.7.3.1. Newly hatched larva:

From the stock suspension of 75 x 10^7 PIBs/ml six serial dilutions of 37.50 x 10^7 - (S_1), 18.75 x10^7 - (S_2) 9.38 x 10^7 - (S_3), 4.69 x 10^7 - (S_4), 2.34 x 10^7 - (S_5) and 0.59 x 10^7 - (S_6) were obtained as indicated in Table 2.

Table 2: Details of the various concentrations used against newly hatched larvae

Sr. No.	Source suspension (ml)	Dilution blank of sterile distilled water added (ml)	Final folume (ml)	Concentration achieved (PIBs/ml)
1.	10 se	10	20	37.50x10^7-(S_1)
2.	10 s_1	10	20	18.75x10^7-(S_2)
3.	10 s_2	10	20	9.38x10^7-(S_3)
4.	10 s_3	10	20	4.69x10^7-(S_4)
5.	10 s_4	10	20	2.34x10^7-(S_5)
6.	5 s_5	15	20	0.59x10^7-(S_6)

3.7.3.2. Five day old larvae:

From the stock suspension of 75 x 10^7 PIBs/ml, three dilutions of 37.50x10^7 - (S_1), 15.00x10^7-(S_2), and 1.50x10^7-(S_3) were obtained.

Table 3: Details of various concentrations used against 5 day old larvae

Sr. No.	Source suspension (ml)	Dilution blank of sterile distilled water added (ml)	Final volume (ml)	Concentration achieved ($PIBs/ml$)
1.	20 ss	-	20	$75.0x10^7$-ss
2.	20 ss	10	20	$37.5x10^7$-S_1
3.	8 ss	12	20	$15.0x10^7$-S_2
4.	2 s_2	18	20	$1.5x10^7$ -S_3

Circular discs or smaller leaves of sunflower were dipped in the virus suspensions, dried under fan and were fed to larvae for 48 hours. On subsequent days fresh food was supplied. Each treatment in either case was replicated thrice using 10 larvae per replication. A control was also maintained with equal number of replications in both the experiments. The experiments were conducted at room temperature.

Larval mortality was monitored daily until all larvae died or pupated. In doubtful cases microscopic examinations were conducted by taking the smears from diseased larvae.

The data was subjected to probit analysis (Finney, 1952) for calculating the values of LC_{50} (concentration required to kill 50 per cent mortality) and LT_{50} (time required to give 50 per cent mortality).

3.7.4 Comparison of log LT_{50} values:

Log LT_{50} values obtained for various concentrations against newly hatched and five day old larvae were compared by employing the 't' test.

The 't' values for comparing two different concentrations were calculated by using formulae stated below

$$S.Ed = \sqrt{(S.Em\ A)^2 + (S.Em\ B)^2}$$

$$'t' = \frac{\text{Log } LT_{50}A - \text{Log } LT_{50}B}{S.Ed}$$

(d.f.) = $(n_1 + n_2) - 2$, where n_1 and n_2 represent number of observations used in calculation of LT_{50} for each concentration.

3.8. Field efficacy of Heliothis NPV in comparison with other synthetic insecticides against H. armigera:

3.8.1. Variety:

Chafa: It is a golden yellow, large seeded high yielding variety with 105 days duration.

3.8.2. Field design and layout:

The field experiment was conducted during rabi 1978-79 at the main farm of College of Agriculture, Parbhani having typical black cotton soil.

The experiment was conducted in randomised block design with twelve treatments replicated four times. The gross and net plot sizes were 3.0 x 5.0 m and 2.4 x 4.6 m

respectively. The spacing maintained was 30 cm between the rows and 20 cm between the plants. The seeds were dibbled by mannual labour on 25th November, 1978.

3.8.3. Insecticides and Heliothis NPV:

The details of the insecticides and their dosages used in the present investigation are given in Table 4.

The nuclear polyhedrosis virus was obtained by infecting the second instar larvae, from laboratory culture. The dead-diseased larvae were allowed to putrefy and PIBs were isolated as described earlier. The PIB pellet after final centrifugation was suspended in known volume of water containing 0.1 per cent Tween-80 as emulsifier. The PIB count was taken by using haemocytometer from this ready-to-use spray suspension.

3.8.4. Application of insecticides:

The insecticidal sprays were prepared by calculating quantity of formulated product to be sprayed on four plots. This quantity of insecticide was added to known volume of water calculated on the basis of water required for spraying control plots (0.75 litre per plot i.e. 500 litres per hectare). Spraying was done with hand compression sprayer having three litres capacity. The insecticides and virus

preparation were applied at fruiting stage, when the crop was most vulnerable to the pest attack.

3.8.5. Method of recording observations:

In order to evaluate the performance of NPV and insecticides ten plants were selected at random from the net plot area before spraying. The total number of healthy and infested pods were counted from all ten plants before and after 7 days of spraying and at harvest.

The crop was harvested at the end of season and produce obtained from each net plot was weighed separately.

3.8.6. Statistical analysis:

The percentage damaged pods were calculated on the basis of total number of healthy and damaged pods at the time of recording observations. The data were subjected to statistical analysis after angular transformation.

3.9. Persistence of Heliothis NPV on foliage of gram:

In order to determine the persistence of Heliothis NPV on gram, samples were taken from the field where virus was sprayed for field test. The persistence of virus was measured at 0, 24, 48, 72 and 96 hours post-treatment. Second to third instar larvae were used for this test. Larvae were allowed to feed on treated foliage

for 48 hours. Fresh food was supplied thereafter. Check was kept at all the post-treatment intervals. Each replication consisted of 10 larvae, which were examined daily until death or pupation.

Virus persistence was expressed as percentage of original activity remaining (OAR) at various samples times as suggested by Ignoffo and Batzer (1971)

where,

$$OAR = \frac{\text{percentage mortality of sample}}{\text{percentage mortality at 0 hours}} \times 100$$

Table 4: Details of insecticides and their dosages for the control of gram pod borer H. armigera

Sr. No.	Name of Insecticide	Chemical name	Trade name and formulation	Source of supply	Total amount of formulation/plot	Total amount of formulation per hectare	Qty.of a.i./ha on gram
1.	Parathion	O,o diethyl O-P nitrophenyl phosphorothionate	Parataf 2% dust	A.C.C. Calcutta.	30 g	20 kg	400 g
2.	Endosulfan	6,7,8,9,10a hexachloro 1,5,5a,6,9,9a hexahydro 9 Methano-2,4,3 benzodiox athiepin - 3 oxide	Thidan 35EC	Hoechst Pharmaceuticals Ltd. Bombay	1.5. ml	1 liter	350 ml
3.	Zolone	O,O-diethyl-S(6 chloro-1-3 benzoxazol-2 (3H) onyl methyl phosphorodithioate	Phosalone 35EC	Voltas Ltd. Bombay	1 ml	0.666 liter	233 ml
4.	BHC	Hexachloro cyclo hexane	Gamazine 10% dust	B.P.M. Bombay.	30 g	20 kg	2000g
5.	D.D.T.	Dichlorodiphenyl trichloroethane	DDF 50 WP	Yawalkar pesticide Pvt.Ltd. Nagpur	1.5 g	1 kg	500 g

1	2	3	4	5	6	7	8
6.	Quinalphos	0,0 dimethyl-0 (quinoxalinyl (2) thionophosphate	Ekalux 25 EC	Sandoz India Ltd. Bombay	1.5 ml	1 liter	250 ml
7.	Quinalphos	-do-	Ekalux 5% dust	-do-	30 g	20 kg	300 g
8.	Fenvalerate	Cyano-m-phenoxy benxyl isoprophyl-p-chlorophenyl acetate	Sumicidin 20 EC	Rallis India Ltd. Bangalore	05 ml	0.33 liter	66 ml
9.	Fenvalerate	-do-	-do-	-do-	1 ml	0.66 liter	132 ml
10.	Carbaryl	1 napthyl (N-methyl-carbamate	Savin 50 WP	U.A.I.D.C. Bombay	3 g	2 kg	1000 g

CHAPTER IV

RESULTS

CHAPTER IV

R E S U L T S

Experiments were conducted to study the various aspects of nuclear polyhedroses virus (NPV) infections in gram pod borer, *Heliothis armigera* and jute semilooper, *Anomis subulifera*. The findings include observations on the insect-virus relationship and field efficacy of *Heliothis* NPV in comparison with other synthetic insecticides.

The results obtained are presented in this Chapter under the following heads

4.1. Symptomatology and natural incidence of disease

4.2. Estimation of number of polyhedral inclusion bodies (PIBs) in the diseased larvae

4.3. Staining property and size of polyhedra

4.4. Cross-infectivity of NPV of *H.armigera* and *A. subulifera* to other related and unrelated Lepidoptera.

4.5. Bioassy of *Heliothis* NPV for virus activity

4.6. Field efficacy and persistence of *Heliothis* NPV against gram pod borer on the gram.

4.1. Symtomatology and natural incidence of disease:

4.1.1. *Heliothis armigera*:

The naturally infected larvae of *Heliothis armigera* did not show any symptoms in the early instars and fed well. While approaching last instar, they became sluggish and stopped feeding. Colour of the diseased larvae changed slightly on ventral side, which became

creamy white as against normal shining green of the healthy larvae. Some of the larvae also exhibited the tendency of moving to tops of the plastic containers and died by hanging in a characteristic way. Soon after the death, integument became brown or black and ruptured with slightest touch, liberating whitish brown contents (Fig.1).

When the newly hatched larvae were fed on sunflower contaminated with PIBs , they remained smaller and appeared ashy white. The larvae died in 4 to 5 days. The skin of such larvae also became fragile and ruptured easily.

No external symptoms of the disease were observed in the infected pupae but infected pupae failed to emerge into moths.

4.1.2. Natural incidence of disease in H. armigera:

The larval mortality due to disease usually appeared in the last instar. The average per cent mortality due to disease was 14.66. The results are presented in Table 5.

4.1.3. Anomis sabulifera:

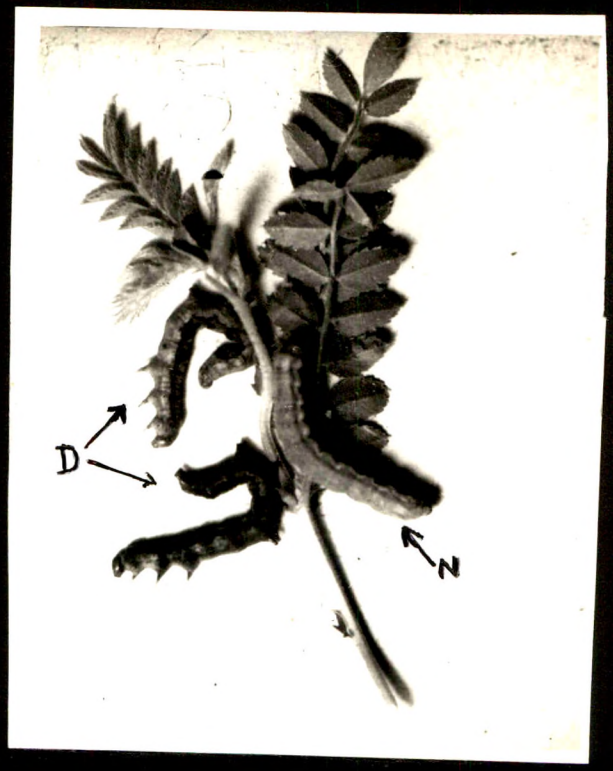
The naturally infected larvae did not show any apparent symptoms till the disease advanced. The larvae generally died in their last instar. Before death, slight changes in the body colour could be noticed.

Table 5: The natural incidence of nuclear polyhedrosis in H. armigera

Host-plant surveyed	Date of collection	No. of larvae collected	No. of larvae died due to NPV	% mortality	Mean % mortality
Arhar	14.10.78	52	6	11.6	
	15.10.78	57	9	15.8	
	25.10.78	48	7	14.6	13.20
	1.11.78	40	4	10.0	
Gram	15.12.78	43	7	16.2	
	20.1.79	23	5	21.7	16.13
	25.1.79	27	3	11.1	

The ventral side of the larvae became slightly yellowish white in colour as against green in healthy ones. The infected larvae became sluggish only just before death and some of the larvae died in a characteristic way of hanging from the top of the container by their prolegs. Integument also became fragile and body contents oozed out with slightest touch (Fig.2).

Artificially infected 3rd instar larvae showed similar symptoms and died in 4 to 5 days. While the newly hatched larve remained smaller, became chalky white and died in 3 to 4 days.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4.2. Estimation of number of polyhedral inclusion bodies (PIBs) in the diseased larvae:

The Table 6 and 7 represent the number of PIBs estimated per larva in Heliothis armigera and Anomis sabulifera, respectively.

Table 6: Haemocytometer count of PIBs from diseased larvae of H. armigera

Sr. No.	Total volume of extract	Dilution	Total No. of PIBs counted in 10 mm^2	Estimated No. of PIBs x 10^9
1	2	3	4	5
1.	100	20	2053	2.053
2.	100	20	3237	3.273
3.	100	20	2602	2.602
4.	100	20	2137	2.137
5.	100	20	2383	2.383
6.	100	20	2895	2.895
7.	100	10	3563	3.563
8.	100	20	2116	2.116
9	100	10	3113	3.113
10.	100	10	2491	2.491
11.	100	20	2680	2.680
12.	100	20	3880	3.880
13.	100	10	2439	2.439
14.	100	10	2835	2.835
15.	100	10	2499	2.499

1	2	3	4	5
16.	100	10	3630	3.630
17.	100	10	2250	2.250
18.	100	10	2574	2.574
19.	100	10	1122	1.112
20	100	10	1353	1.353
21.	100	10	1054	1.054
22.	100	10	2917	2.917
23.	100	10	3303	3.303
24.	100	10	2842	2.842
25.	100	10	2159	2.159

Table 7: Haemocytometer count of PIBs from diseased larvae of A. sabulifera

Sr. No.	Total volume of extract	Dilution	Total no. of PIBs counted in 10 mm^2	Estimated No. of PIBs x 10^9
1	100	10	3729	3.729
2.	100	10	1271	1.271
3.	100	10	1738	1.738
4.	100	10	3071	3.071
5.	100	10	3112	3.112
6.	100	10	1250	1.250
7.	100	10	775	0.775
8.	100	10	1373	1.273
9.	100	10	2222	2.222
10.	100	10	1443	1.443
11.	100	10	2250	2.250
12.	100	10	3031	3.031

The estimated number of PIBs in the larvae of H. armigera ranged from 1.055×10^9 to 3.880×10^9 with an average of $2.570 \pm 0.701 \times 10^9$ and in A. gabulifera from 0.775×10^9 to 3.729×10^9 with an average of $2.106 \pm 0.903 \times 10^9$.

4.3. Staining property and size of polyhedra:

The polyhedra stained pink red when pre-treated with 1 N HCl while those without acid treatment remained unstained.

Size of polyhedral inclusion bodies of A. gabulifera ranged from 1.85 to 4.80 µm with an average of 3.33 ± 0.612 µm. Fifty-five percent PIBs were in the range of 3.01 to 4.00 µm (Table 8 and Fig.3)

Table 8: Frequency distribution of diameter of PIBs of A. gabulifera

Sr. No.	Diamter in micron	Frequency	Mean diameter in micron
1.	1.00 - 1.50	0	
2.	1.51 - 2.00	1	
3.	2.01 - 2.50	8	
4.	2.51 - 3.00	20	
5.	3.01 - 3.50	33	3.33 ± 0.612
6.	3.51 - 4.00	22	
7.	4.01 - 4.50	15	
8.	4.51 - 5.00	1	

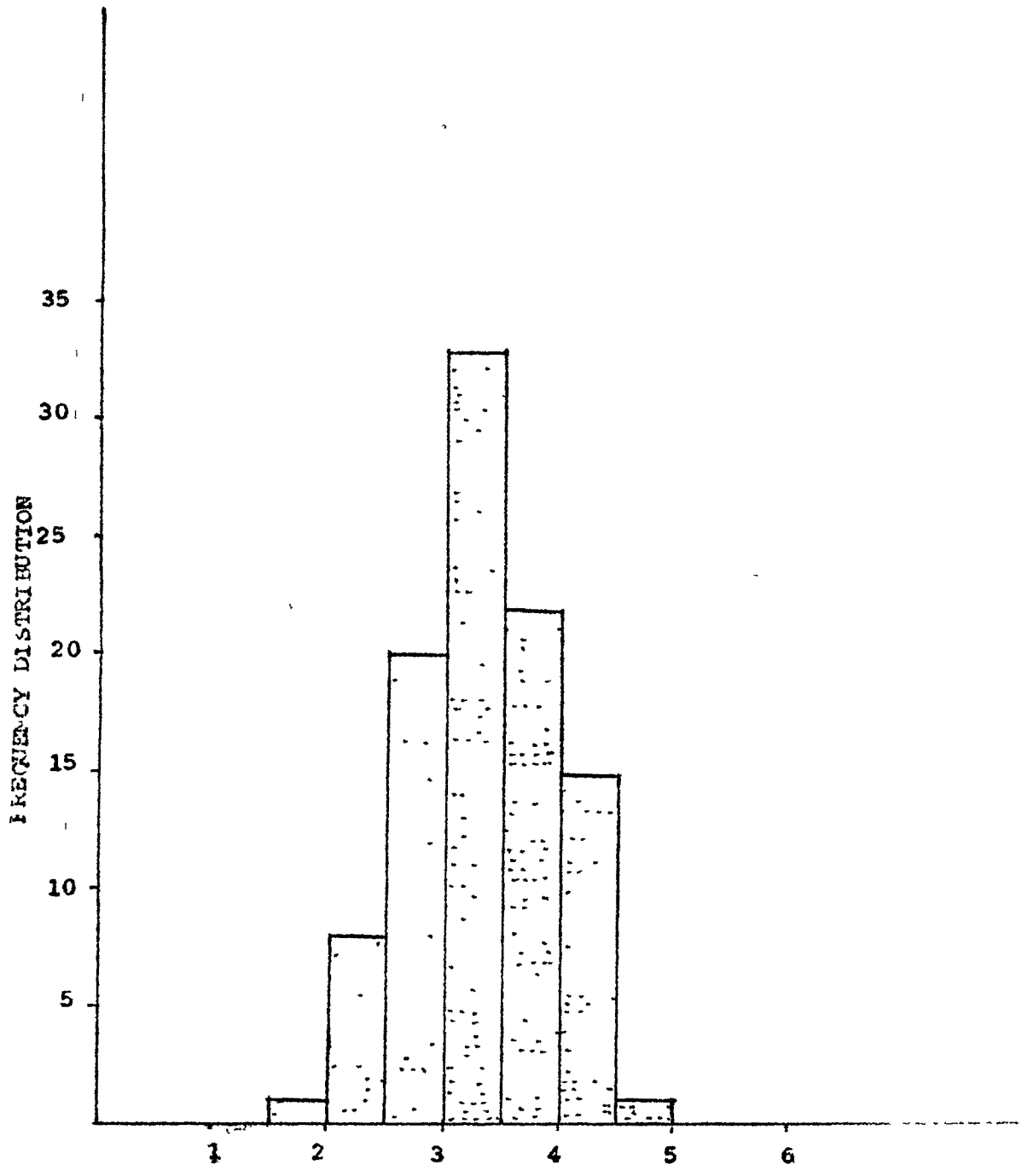


Fig. 3 SIZE DISTRIBUTION OF POLYHEDRAL INCLUSION BODIES ISOLATED FROM ANOMIS SABULIFERA.

4.4. Cross infectivity of NPV of H. armigera and A. sabulifera to related and unrelated Lepidoptera

The results of laboratory experiments with NPV of H. armigera to infect the larve of A. sabulifera (Gn.) Anomis flav, Spodoptera litura (F), Diacrisia oblicua (W.) and Euproctis sp. and that of A. sabulifera to the H. armigera, A. flava, S. litura, D. oblicua and Euproctis sp. are presented in Table 9 (a) and (b).

Table 9 (a): Cross-infectivity of NPV of H. armigera to other Lepidoptera

Sr. No.	Test insect	No. of larvae	Mortality due to			Infectivity
			Virus	Protozoa	unknown	
1.	Anomis sabulifera	20	-	-	3	-Ve
2.	Anomis flava	20	-	4	-	-Ve
3.	Spodoptera litura	20	-	-	-	-Ve
4.	Diacrisia oblicua	50	-	13	-	-ve
5.	Euproctis sp.	20	-	-	-	-ve
6.	Heliothis armigera (control)	20	20	-	-	+Ve

Table 9(b): Cross-ineffectivity of NPV of A. sabulifera to other Lepidoptera

Sr. No.	Test Insect	No.of larve	Mortality due to				Infe-cti-vity
			virus	proto-zoa	bact-eria	unknown	
1.	Anomis flava	20	-	5	-	1	-ve
2.	Spodoptera litura	20	-	-	-	-	-ve
3.	Heliothis armigera	20	-	-	2	3	-ve
4.	Diacrisia oblicua	50	-	-	11	-	-ve
5.	Euproctis sp.	20	-	-	-	3	-ve
6.	Anomis sabulifera (control)	20	20	-	-	-	+ve

The results indicated that the NPV of H. armigera and A. sabulifera were not cross-infective to the species tested. There were no detectable symptoms of polyhedrosis and no death occurred due to virus.

In the case of Diacrisia oblicua fed on the NPV of Heliothis and Anomis 13 and 11 larvea were died due to protozoan disease, respectively. On microscopic examination on the smears of dead larvae the minute oval spores in huge numbers were observed.

Similarly in case of A. flava fed on Heliothis and Anomis NPV, four and five larvae died due to protozoan disease, respectively. On microscopic examination oval spores in huge numbers similar to those observed in D. oblicua were observed.

In the case of H. armigera fed on Anomis NPV two larvae succumbed to protozoa and three to bacterial infection. On microscopic examination oval minute spores in huge numbers were observed in protozone diseased larvae while in bacterial diseased larvae rod shaped bacteria were observed.

4.5 Bioassay of Heliothis NPV for virus activity:

4.5.1. Time-mortality studies :

The time-mortality data on newly hatched and five day old larvae fed on different concentrations of PIBs for 48 hours are presented in Table 10 and 11 and graphically depicted in Figure 4 and 5.

It will be seen from these tables that the range of concentrations tested against newly hatched larvae was from 0.59×10^7 to 37.50×10^7 where as it was from 1.50×10^7 to 75.00×10^7 for five day old larvae.

The values of LT_{50} calculated for newly hatched larvae when fed on various concentrations viz. 37.50×10^7, 18.75×10^7, 9.38×10^7, 4.69×10^7 and 2.34×10^7 were 3.43, 3.84, 5.38, 6.33 and 8.75 days, respectively (Table 12 and Fig.6).

The LT_{50} value for 0.59×10^7 concentration could not be worked out as maximum percentage mortality obtained was only 43 per cent.

Table 10: Mortality data on newly hatched larvae of *H. armigera* fed on various concentrations of PIBs

Sr. No.	Conc. of virus administered x 10^7	Log of conc. x 10^7	Mortality								Total mortality
			3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	
1.	37.50	1.5740	30.00 (4.48)	66.60 (5.41)	100.00 ( )	-	-	-	-	-	100.00 ( )
2.	18.75	1.2730	20.00 (4.16)	46.60 (4.90)	86.60 (6.08)	100.00 ( )	-	-	-	-	100.00 ( )
3.	9.38	0.9722	-	13.30 (3.87)	33.30 (4.56)	63.30 (6.33)	90.00 (6.28)	100.00 ( )	-	-	100.00 ( )
4.	4.69	0.6711	-	-	10.00 (3.72)	40.00 (4.75)	73.30 (5.40)	83.60 (5.95)	96.60 (6.75)	-	96.60 (6.75)
5.	2.34	0.3692	-	-	-	10.00 (3.72)	20.00 (4.16)	30.00 (4.48)	43.30 (5.08)	70.00 (5.52)	70.00 (5.52)
6.	0.59	9.2291	-	-	-	-	3.30 (3.12)	13.30 (3.87)	23.30 (4.26)	43.00 (4.82)	43.30 (4.82)

There was no mortality upto 2 days after the treatment
The control mortality was nil.
Thirty larva per treatment.
Figures in Parenthesis are the probit kills.

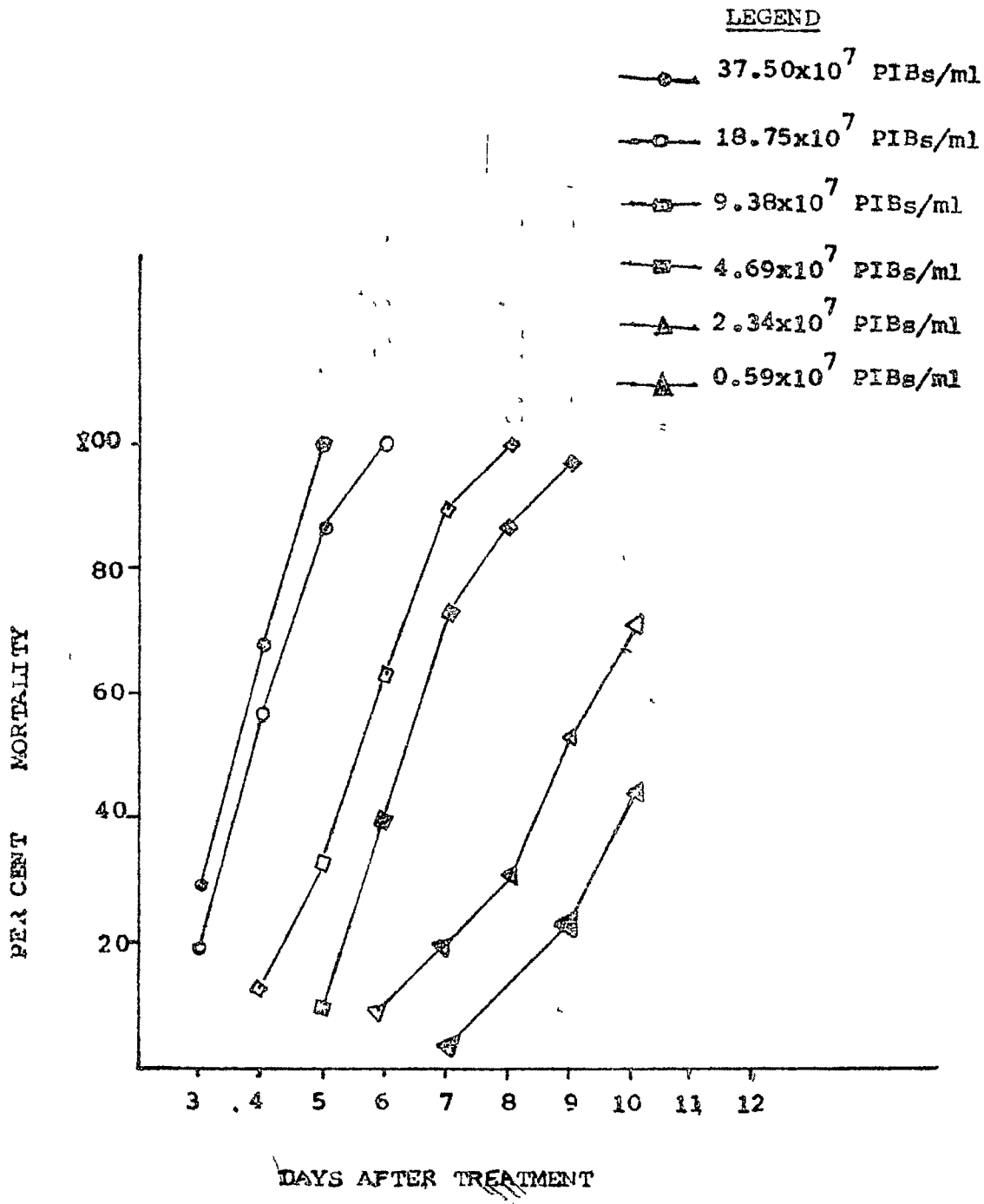


FIG.4

TIME MORTALITY-CURVES FOR NEWLY HATCHED LARVAE TREATED WITH DIFFERENT CONCENTRATIONS.

Table 11: Mortality data on five day old larvae of H. armigera fed on various concentrations of PIBs

Sr. No.	Conc. of virus administered x 10^7	Log. of conc. x 10^7	Mortality							Total mortality
			4th day	5th day	6th day	7th day	8th day	9th day	10th day	
1.	75.0	1.8751	27.61 (4.39)	79.31 (5.81)	100.00 ( )	-	-	-	-	100.00 ( )
2.	37.5	1.5740	6.93 (3.45)	34.48 (4.59)	86.14 (6.08)	96.48 (6.75)	-	-	-	96.48 (6.75)
3.	15.0	1.1761	-	-	7.92 (3.45)	27.65 (4.39)	58.63 (5.20)	68.98 (5.47)	-	68.98 (5.47)
4.	1.5	0.1761	-	-	-	3.47 (3.12)	13.75 (3.87)	34.44 (4.59)	41.05 (4.77)	41.05 (4.77)

There was no mortality upto 3rd days after treatment

Thirty larave in each treatment

Control mortality was 3.33 per cent.

Figures in parentheses are probit kills.

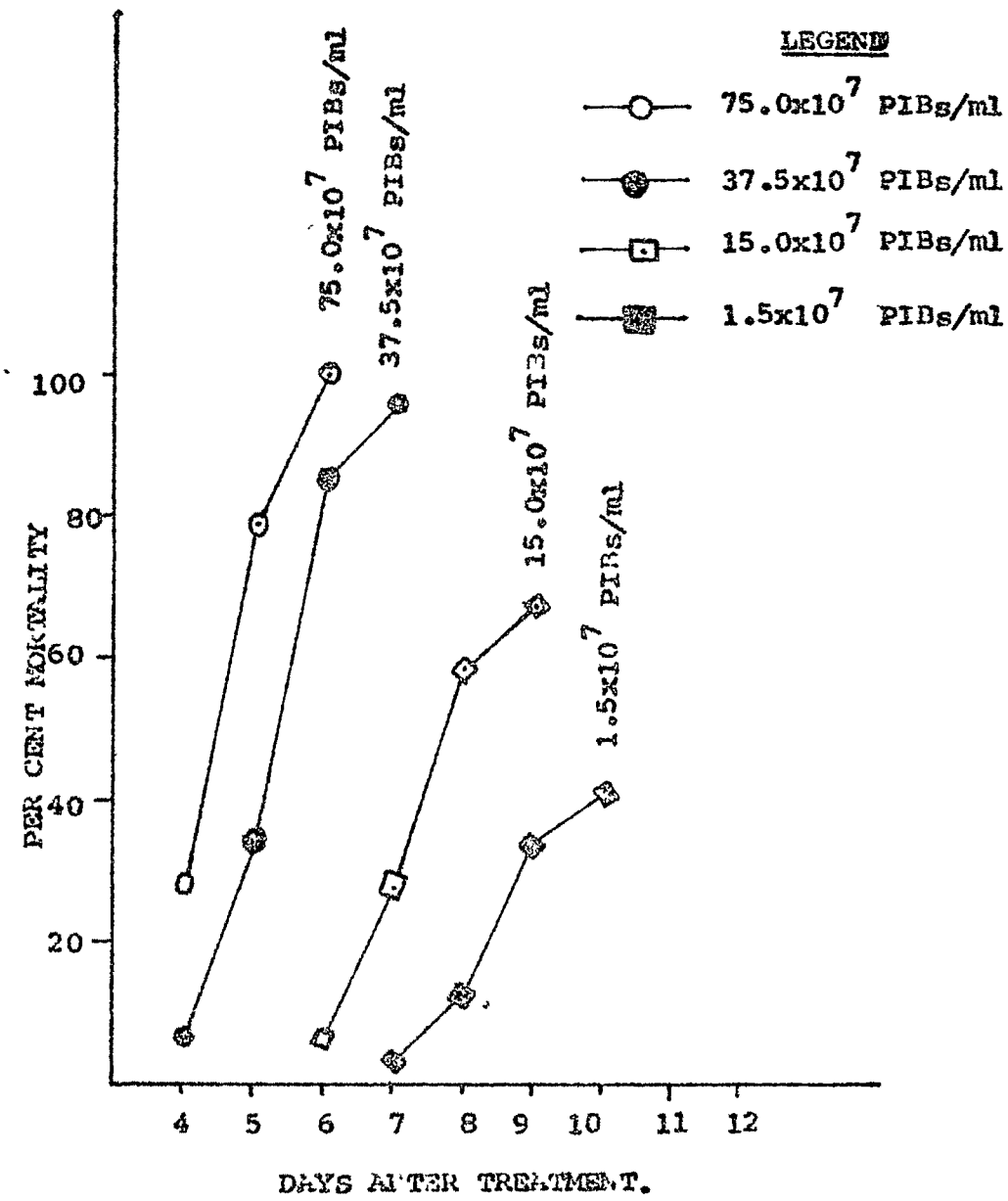


FIG.5 TIME MORTALITY CURVES FOR FIVE DAY OLD LARVAE TREATED WITH DIFFERENT CONCENTRATIONS.

Table 12: Results of probit analysis for LT_{50} values of newly hatched larvae

Sr. No.	Concent. of virus administered PIBs/ml.	Heterogeneity *	Regression equation	Log of LT_{50} ± S.E.	LT_{50}	Fiducial limites (days)
1.	37.50×10^7	$x^2(1) = 4.6800$	$Y = 9.810x-0.260$	0.5362 ± 0.00519	3.43	3.48 3.32
2.	18.75×10^7	$x^2(2) = 5.0900$	$Y = 9.125x-0.337$	0.5849 ± 0.01530	3.85	4.05 3.53
3.	9.38×10^7	$x^2(3) = 1.7200$	$Y = 10.640x-2.775$	0.7307 ± 0.01261	5.37	5.69 5.08
4.	4.69×10^7	$x^2(3) = 0.9600$	$Y = 12.720x-5.194$	0.8014 ± 0.01034	6.33	6.62 6.04
5.	2.34×10^7	$x^2(3) = -1.5300$	$Y = 8.910x-3.398$	0.9425 ± 0.01417	8.78	9.28 8.17

* In none of these cases the data were found to be heterogeneous at P=0.05

Y = Probit kill.

X = Log time (days)

LT_{50} = Time calculated to give 50 per cent mortality at that concentration

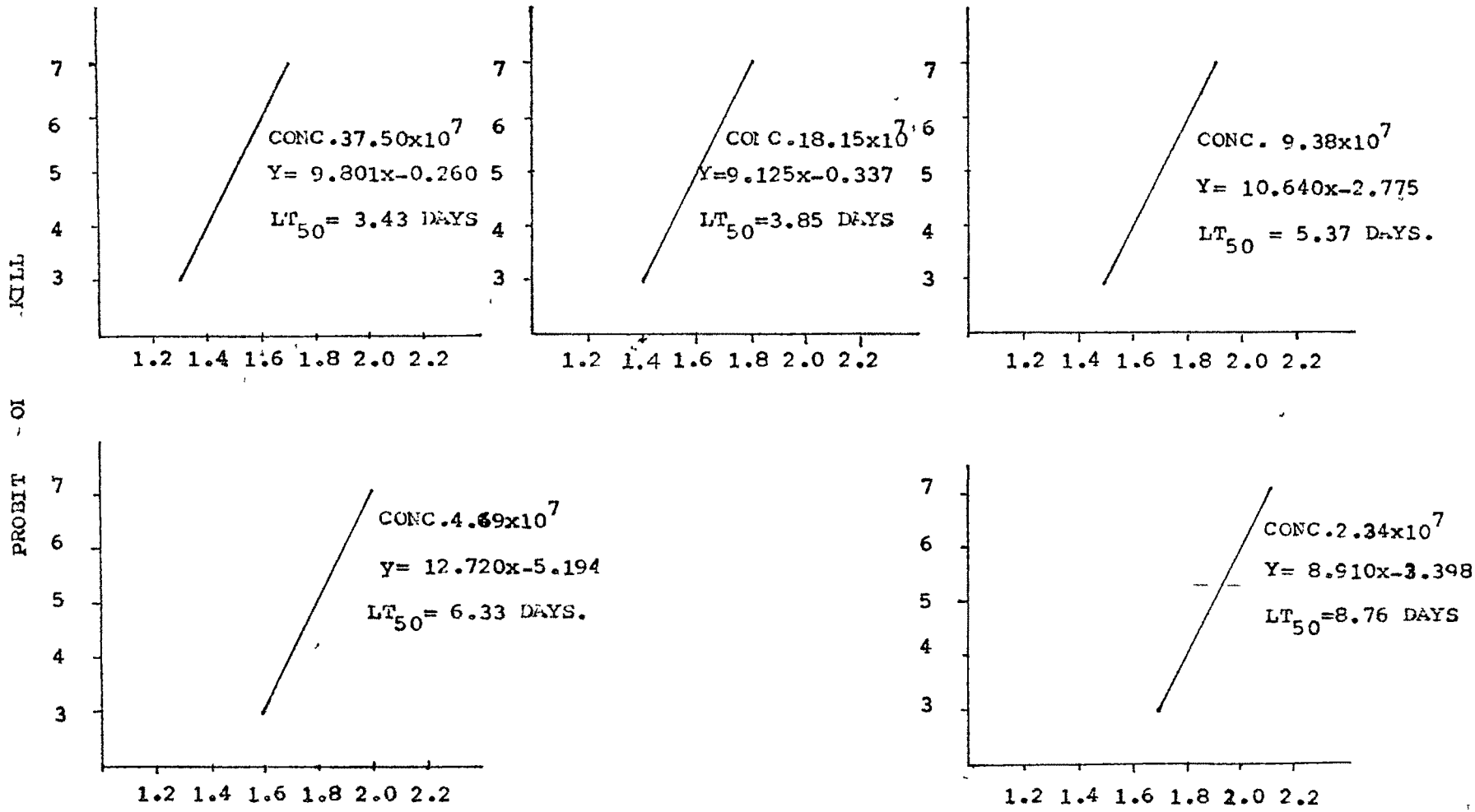


FIG. 6

LOG TIME-PROBIT KILL REGRESSION LINES OF VARIOUS CONCENTRATIONS OF PIBs FED TO NEWLY HATCHED LARVAE OF H. ARMIGERA.

Similarly the LT_{50} values were calculated for five day old larvae when fed on various concentrations viz. 75.0×10^7, 37.5×10^7 and 15.0×10^7. The results of LT_{50} values with fiducial limits are presented in Table 13 and graphically depicted in Fig.7.

The LT_{50} values for various concentrations tested were 4.37, 5.15 and 7.91 days respectively.

The values of LT_{50} for concentration 1.50×10^7 could not be worked out as maximum percentage mortality obtained was only 41.05 per cent.

4.5.2. Dosage-mortality studies:

The data presented in Table 10 and 11 were also used to calculate the LC_{50} values for newly hatched and five day old larvae. The values of LC_{50} along with fiducial limits were given in Table 14 and depicted in Fig.8.

The LC_{50} values calculated for newly hatched and five day old larvae were 0.831×10^7 and 2.862×10^7 respectively.

Table 13: Results of probit analysis for LT_{50} value of five day old larvae

Sr. No.	Conc. of virus administered PIBs/ml	Heterogeneity*	Regression equation	Log of $LT_{50} \pm$ SE	LT_{50}	Fiducial limits (days)
1.	75.0×10^7	$X^2(1) = 1.0600$	$Y = 15.200x-4.747$	0.6412 ± 0.01187	4.37	4.61 4.14
2.	37.5×10^7	$X^2(2) = -0.3600$	$Y = 16.400x-6.698$	0.7132 ± 0.00900	5.15	5.36 4.95
3.	15.0×10^7	$X^2(2) = 0.9500$	$Y = 11.120x-4.992$	0.8985 ± 0.01180	7.91	8.35 7.50

* In none of these cases the data were found to be heterogeneous at $P = 0.05$

Y = Probit kill X = Log time (days)

LT_{50} = Time calculated to give 50 per cent mortality at the concentration

Table 14: Results of probit analysis for LC_{50} value of newly hatched and five day old larvae of *H. armigera*

Sr. No.	Age of larvae at the time of treatment	LC_{50} on day	Heterogeneity*	Regression equation	Log LC_{50} + SE	LC_{50}	Fiducial limits
1.	Newly hatched (0-8 hours)	10th	$X^2(4) = 4.460$	$Y = 1.920x + 3.240$	0.9200 ± 0.1800	0.8318×10^7	1.374×10^7 0.369×10^7
2.	Five day	10th	$X^2(2) = 5.750$	$Y = 1.270x + 4.420$	0.4566 ± 0.1450	2.862×10^7	5.508×10^7 1.487×10^7

* In none of these cased the data were found to be heterogeneous at P = 0.05

Y = Probit kill; X = log (concentration x)

LC_{50} = concentration calculated to give 50% mortality for respective larval stage on 10th day after treatment.

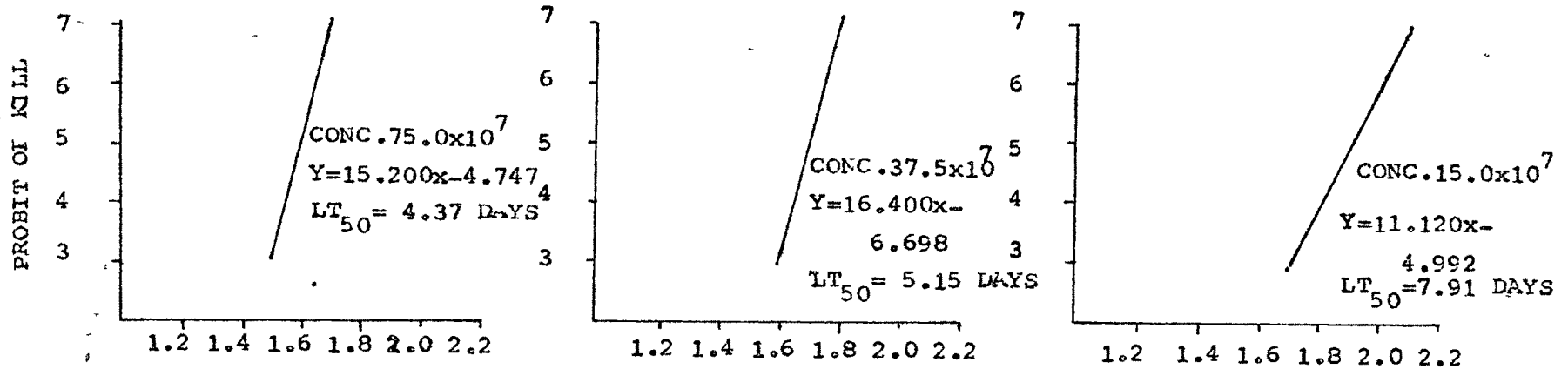
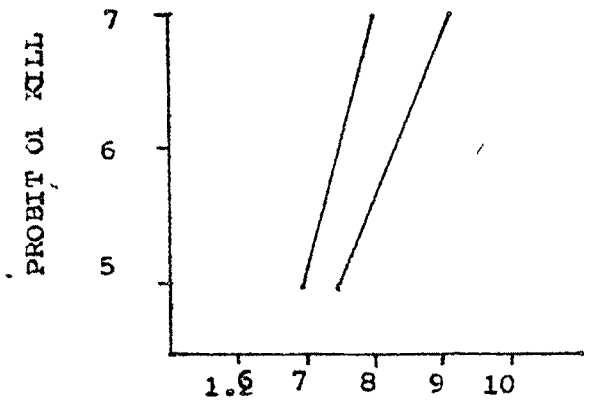


Fig. 7 LOG TIME-PROBIT KILL REGRESSION LINES OF THREE CONCENTRATIONS OF PIBs FED TO FIVE DAY OLD LARVAE OF H. ARMIGERA.



DAY	NEWLY HATCHED	FIVE DAY OLD
10	Y=1.920x+3.240	Y=1.270x+4.420

FIG. 8 LOG. CONCENTRATION PROBIT KILL REGRESSION LINES ON 10th DAY AFTER FEEDING THE PIBs TO NEWLY HATCHED AND FIVE DAY OLDLLARVAE OF H. ARMIGERA.

4.6. Field efficacy and persistence of Heliothis NPV against gram pod borer on gram:

a) Field efficacy of NPV:

Efficacy of Heliothis NPV and various insecticides was studied by taking in to consideration the following parameters

4.6.1. Percentage infestation of pods by H. armigera

4.6.2. Effect of NPV and insecticides on the yield

4.6.1. Percentage infestation of pods by Heliothis armigera:

4.5.1.1. Pre-treatment infestation count:

Before spraying the crop, infestation observed in different plots was recorded. The results are presented in Table 15.

Results indicated that there was no significant differences amongst the treatments before the application of Heliothis NPV and insecticides indicating thereby uniform pest infestation throughout the experimental area.

4.6.1.2. Post-treatment infestation count:

Observations recorded on the infestation of pods after 7 days of treatment are presented in Table 16.

It is evident that the Heliothis NPV and all insecticidal treatments were significantly superior over control in reducing the infestation of the pod borer. However, the lowest percentage pod damage 0.64 per cent

Table 15: Pre-count observations on the pod damage by *H. armigera* in different treatments

Treatments	Quantity of a.i./ha	Per cent pod damaged Replications				Mean per cent
		I	II	III	IV	
Endosulfan 0.07% (EC)	350 ml	11.88 (20.09)	12.08 (20.27)	8.01 (16.43)	8.15 (16.54)	10.03 (18.33)
Phosalone 0.05% (EC)	233 ml	3.37 (10.47)	4.00 (11.54)	6.35 (14.54)	11.65 (19.91)	6.34 (14.11)
Quinalphos 0.05% (EC)	250 ml	3.57 (10.78)	8.54 (16.95)	9.39 (17.76)	11.05 (19.46)	8.14 (16.22)
Fenfalerate 0.015% (EC)	66 ml	7.41 (15.79)	5.71 (13.81)	5.58 (13.56)	7.84 (16.22)	6.64 (14.84)
Fenvalerate 0.03% (EC)	132 ml	5.95 (14.06)	7.71 (16.11)	7.79 (16.11)	18.66 (25.55)	10.03 (17.45)
Carbaryl 0.02% (WP)	1000 g	9.01 (17.46)	10.75 (19.09)	9.09 (17.46)	6.03 (14.18)	8.72 (17.04)
DDT 0.1% (WP)	500 g	8.48 (16.85)	8.46 (16.85)	5.47 (13.44)	9.17 (18.34)	7.90 (16.37)
Quinalphos 1.5% (dust)	300 g	4.75 (12.52)	7.44 (15.79)	5.38 (13.31)	13.61 (21.64)	7.79 (15.81)
BHC 10% (dust)	2000 g	9.75 (18.15)	3.20 (10.30)	7.09 (15.34)	9.16 (17.56)	7.30 (15.33)
Parathion 2% (dust)	400 g	4.85 (12.66)	7.53 (15.98)	7.79 (16.11)	7.71 (16.11)	6.97 (15.19)
Heliothis NPV 2.52×10^9 PIBs/ml		4.76 (12.52)	7.55 (15.89)	4.89 (12.56)	14.03 (21.97)	7.18 (15.76)
Control		7.90 (16.32)	5.84 (13.94)	9.88 (18.24)	10.16 (18.53)	8.45 (16.75)
S.E. ±						1.430
C.D. (P = 0.05)						4.1

Figures in parenthesis are transformed values.

Table 16: Effect of NPV and insecticides on the pod damage by *H. armigera* on gram 7 days after application

Treatments	Quantity of a.i./ha	Per cent pod damaged Replications				Mean per cent
		I	II	III	IV	
Ensofulfan 0.07% (EC)	350 ml	0.98 (5.44)	0.76 (4.80)	0.78 (4.80)	0.85 (5.13)	0.84 (5.04)
Phasalone 0.05% (EC)	233 ml	0.77 (4.80)	1.86 (7.71)	1.66 (7.27)	1.60 (7.27)	1.47 (6.76)
Quinalphos 0.05% (EC)	250 ml	0.98 (5.44)	0.66 (4.44)	0.57 (4.05)	1.40 (6.80)	0.90 (5.18)
Fenvalerate 0.015% (EC)	66 ml	1.29 (6.29)	1.90 (7.92)	1.69 (7.27)	1.91 (7.92)	1.70 (7.25)
Fenvalerate 0.03% (EC)	132 ml	0.65 (4.44)	0.50 (4.05)	0.58 (4.05)	0.81 (5.13)	0.64 (4.41)
Carbaryl 0.2% (WP)	1000 g	0.64 (4.44)	1.68 (7.27)	1.56 (7.03)	1.37 (6.55)	1.31 (6.32)
DDT 0.1% (WP)	500 g	2.28 (8.53)	1.58 (7.03)	1.45 (6.80)	1.79 (7.49)	1.78 (7.46)
Quinalphos 1.5% (dust)	300 g	0.99 (5.94)	2.40 (8.91)	3.18 (10.14)	2.80 (9.63)	2.34 (8.53)
BHC 10% (dust)	2000 g	2.03 (8.13)	2.50 (9.10)	2.50 (9.10)	1.52 (7.03)	2.14 (8.34)
Parathion 2% (dust)	400 g	1.31 (6.65)	1.89 (7.71)	1.88 (7.71)	2.11 (8.33)	1.80 (7.57)
Heliothis NPV 2.52×10^8 PIBs/ml		1.78 (7.49)	1.88 (7.71)	0.52 (4.05)	1.27 (6.29)	1.36 (6.38)
Control		6.60 (14.89)	4.30 (11.97)	6.63 (14.89)	6.50 (14.79)	6.01 (14.13)
S.E. ±				0.599		
C.D. (P = 0.05)				1.72		

Figures in the parenthesis are transformed values

was observed in plots treated with fenvalerate 0.03 per cent. The treatments fenvalerate 0.03 per cent, endosulfan 0.07 per cent, and quinalphos 0.05 per cent were at par with each other and significantly superior to control.

Heliothis NPV treatment was at par with treatments carbaryl, 0.2 per cent, phosalone 0.05 per cent, fenvalerate 0.015 per cent, DDT 0.1 per cent, parathion dust 20 kg/ha. It was fifth in the order of superiority in reducing the infestation of pods by pod borers.

Observations recorded on the pod damage at the time harvest are presented in Table 17 and graphically depicted in Fig-9.

The results indicated that Heliothis NPV and all insecticidal treatments were significantly superior to control. Treatments with fenvalerate 0.03 per cent, endosulfan 0.07 per cent and quinalphos 0.05 per cent were at par with each other and superior over rest of the treatments.

4.6.2. Effect of NPV and insecticides on the yield:

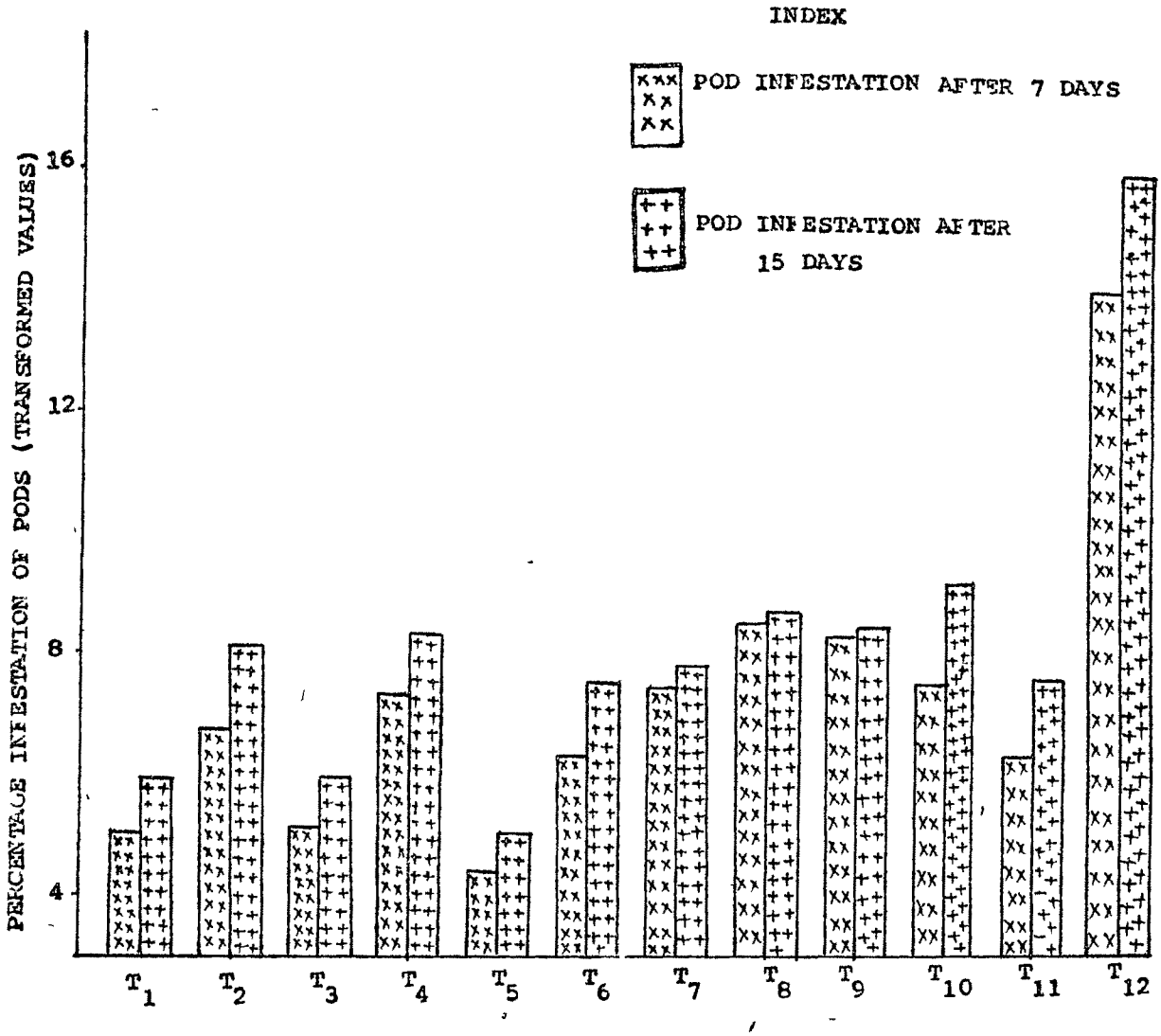
The data on yield obtained in various treatments are given in Table 18.

Results indicated that all the treatments were significantly superior to control in increasing the yields. Endosulfan 0.07 per cent, fenvalerate 0.03 per cent,

Table 17: Comparative efficacy of NPV and insecticides on the pod damage by *H. armigera* at harvest

Treatments	Quantity of a.i./ha	Per cent pods damaged Replications				Mean per cent
		I	II	III	IV	
Endosulfon 0.07% (EC)	380 ml	1.99 (7.92)	1.79 (7.49)	0.79 (4.80)	1.44 (3.63)	1.50 (5.96)
Phosalone 0.05% (EC)	233 ml	1.17 (6.02)	1.66 (7.27)	3.26 (10.30)	2.45 (8.91)	2.14 (8.12)
Quinalphos 0.05% (EC)	250 ml	1.17 (6.02)	1.60 (7.27)	1.47 (6.80)	1.44 (3.63)	1.42 (5.93)
Fenvalerate 0.015% (EC)	66 ml	1.79 (7.49)	1.95 (7.92)	2.29 (8.53)	2.73 (9.46)	2.19 (8.35)
Fenvalerate 0.03% (EC)	132 ml	0.85 ((5.13)	0.77 (4.80)	0.77 (4.80)	0.98 (5.44)	0.84 (5.04)
Carbaryl 0.2% (WP)	1000 g	1.93 (7.92)	1.99 (7.93)	1.79 (7.49)	1.47 (6.80)	1.80 (7.53)
DDT 0.1% (WP)	500 g	1.94 (7.92)	2.12 (8.33)	1.47 (6.80)	2.13 (8.33)	1.94 (7.84)
Quinalphos 1.5% (dust)	330 g	1.79 (7.49)	2.42 (8.91)	2.46 (8.91)	2.89 (10.96)	2.39 (8.73)
BHC 10% (dust)	2000 g	1.96 (7.92)	2.31 (8.72)	2.33 (8.72)	2.21 (8.56)	2.20 (8.47)
Parathion 2% (dust)	400 g	1.79 (7.49)	2.75 (9.46)	2.47 (8.91)	3.75 (11.09)	2.69 (9.23)
Heliothis NPV 2.52×10^9 PIBs per ml.		2.18 (8.33)	2.24 (8.53)	1.23 (6.29)	1.79 (7.49)	1.86 (7.66)
Control		6.60 (14.89)	6.50 (14.77)	7.90 (16.32)	9.31 (18.23)	7.70 (16.05)
S.E. ±					0.640	
C.D. (P = 0.05)					1.84	

Figures in parenthis are transformed values.



TREATMENTS (T)

T_1	ENDOSULFAN	0.07%(E.C.)	T_7	DDT	0.10% (W.P.)
T_2	PHOSALONE	0.05% (E.C.)	T_8	QUINOLPHOS	20 Kg/ha(Dust)
T_3	QUINOLPHOS	0.05% (E.C.)	T_9	BHC	20 K/ha(Dust)
T_4	FANVALERATE	0.015% (E.C.)	T_{10}	PARATHION	20 kg/ha (Dust)
T_5	FANVALERATE	0.03% (E.C.)	T_{11}	HELIOTHIS	NPV 2.52×10^8
T_6	CARBARYL	0.20% (W.P.)	T_{12}	CONTROL.	

FIG.9 PERCENTAGE INFESTATION OF PODS BY HELIOTHIS ARMIGERA AFTER 7 AND 15 DAYS OF SPRYING OF NPV AND INSECTICIDES.

Table 18: Yield data of gram in q/ha

Treatments	Quantity of a.i./ha	Yield in q/ha				Mean
		Replications				
		I	II	III	IV	
Endosulfan 0.07% (EC)	350 ml	22.64	19.92	22.64	18.11	20.82
Phosalone 0.05% (EC)	233 ml	13.58	15.39	18.11	18.11	16.29
Quinalphos 0.05% (EC)	250 ml	19.02	20.83	15.85	21.73	19.35
Fenvalerate 0.015% (EC)	66 ml	13.58	16.30	18.11	17.21	16.30
Fenvalerate 0.03% (EC)	132 ml	22.64	18.11	21.73	20.83	20.82
Carbaryl 0.2% (WP)	1000 g	15.85	13.58	18.11	18.11	16.41
DDT 0.1% (WP)	500 g	18.11	19.02	20.83	18.11	19.01
Quinalphos 1.5% (dust)	300 g	15.85	12.68	16.30	17.21	15.52
BHC 10% (Dust)	2000 g	13.58	10.86	15.39	15.39	13.80
Parathion 2% (dust)	400 g	13.58	10.86	15.39	13.59	13.35
Heliothis NPV 2.52×10^8 PIBs/ml		18.11	19.02	18.11	20.83	19.01
Control		9.05	10.86	9.05	10.86	9.95
S.E. ±					0.892	
C.D. (P = 0.05)					2.56	

quinlalphos 0.05 per cent, DDT 0.1 per cent and Heliothis NPV were found to be better than rest of the treatments.

b) Persistence of Heliothis NPV on gram:

The persistence of Heliothis NPV was studied by feeding the samples of NPV sprayed gram leaves collected at 0, 24, 48, 78 and 96 hours interval. The results obtained are presented in Table 19.

Table 19: Persistence of Heliothis NPV on gram foliage

Post-treatment sampling intervals.	Percent larval mortality due to NPV Replications			Mean % mortality	OAR*
	I	II	II		
0 hr.	70	90	80	80	-
24 hrs.	60	70	80	70	87.5
48 hrs.	50	40	60	50	62.5
72 hrs.	40	20	30	30	37.5
96 hrs.	10	30	20	20	25.0

* OAR - Original Activity Remaining

From the data it is apparent that the virus activity reduced with time. The value for original activity remaining (OAR) were 87.5, 62.5, 37.5 and 25.0 per cent at 24, 48, 72 and 96 hours respectively.

***

CHPATER V

DISCUSSION

CHAPTER V

D I S C U S S I O N

In the present investigations studies were conducted to understand the insect-virus relationship of nuclear polyhedrosis virus (NVP) infections in jute semilooper (Anomis sabulifera (Guenee) and gram pod borer Heliothis armigera (Hubner) and field efficacy of Heliothis NPV. The results obtained are discussed in the light of available literature under the following headings

5.1. Symptomatology and natural incidence of disease

5.2. Size, staining proporty and count of PIBs

5.3. Cross-infectivity tests

5.4. Bioassy of Heliothis NPV

5.5. Field efficacy and persistence of Heliothis NPV against H. armigera on gram

5.1. Symptomatology and natural incidence of disease:

The diagnostic symptoms of any disease are important to distinguish it from other similar types of maladies. The well known "Wipfelkrankheit" characteristic way in which the polyhedral virus affected larvae hang from support was the common symptom observed in the larvae of H. armigera and A. sabulifera (Fig. 1 & 2)

Patel et al. (1968) were the first to report the symptomatology of nuclear polyhedrosis in the laboratory culture of H. armigera from India. They observed that the affected larvae became paralysed and the integument became soft and brown or black. Similar symptoms were also described by Jacob and Subramaniam (1972) from Coimbatore.

In the present studies, in artificially infected larvae the symptoms differed slightly. The infected larvae remained considerably smaller, became creamy white, fed very little and usually died showing wilting symptoms in both the cases.

The nuclear polyhedrosis virus disease of Ancmis sabulifera was noticed from the batches of larvae collected from jute fields reported to be heavily infested with this insect. Though the initial periodic collections of jute semilooper did not show any incidence of virus infection, the later collection during the month of September-October, 1978 invariably showed the larvae infected with NPV. No previous report is available on the nuclear polyhedrosis virus infection of A. sabulifera and hence this seem to be the first report. However, Bishop and Blood (1977) have reported the occurrence of nuclear polyhedrosis in A. flava from Queensland, Australia.

Ramkrishnan and Kumar (1977) reviewed the status of microbial infection in insect pests in India. According to them so far only 12 species of insects are reported to have Baculovirus infections. Recently Waghmare (1977) reported NPV infections in Trichoplusia ni Perigoea caponsis and Heliothis armigera from Maharashtra, India.

Nayar and Srivastava (1979) also reported NPV infections in rice stem borer (Sesamia inferens (W.) Scirpophaga incertulas (W.) and the rice skipper Parnara mathias (F.)

The symptoms of NPV infections observed in Anomis sabulifera were similar to those observed by earlier workers.

The information on natural incidence of NPV infections reported from India is generally lacking. In the present studies we attempted to gather information on this aspect by periodic collections of larvae of H. armigera from its two major host plants i.e. arhar and gram. These host plants were selected for study as very little pesticides are applied to these crops and there is considerable potential in developing microbial control of H. armigera for these crops.

The natural incidence of Heliothis NPV on arhar and gram was 13.20 and 16.30 per cent respectively

(Table 5). Waghmare (1977) studied the natural incidence of Heliothis NPV on gram sunflower, beans, peas and maize. He observed that the disease incidence varied in different food plants. He attributed the differences to the variation in the inoculum of the virus present in nature.

Pawar and Ramkrishnan (1971) reported that the natural incidence of S. litura was 41 per cent.

5.2. Size, staining property and count of PIBs:

The PIBs of A. sabulifera stained pinkish red after pre-treatment with acid. This staining property of PIBs with Giemsa stain confirmed that the virus isolated from this species belongs to the group of nuclear polyhedrosis virus, which is also classified as Subgroup A of the genus Baculovirus (David, 1975).

The nuclear polyhedrosis virus inclusion bodies differ considerably in size from species to species and also within the same species and range from 0.5 to 15.0 um in diameter (Bergold, 1963). Thus the observations made in the present studies on the PIB size of A. sabulifera, which was 3.33 ± 0.612 um carborate Bergolds generalization.

The number of polyhedral inclusion bodies harvested from naturally infected Anomis sabulifera and Heliothis armigera larvae were estimated to range from 0.775×10^9 to 3.729×10^9 and 1.055×10^9 to 3.830×10^9 with an average of $2.106 \pm 0.903 \times 10^9$ and $2.570 \pm 0.701 \times 10^9$, respectively.

Ignoffo (1966) reported that one diseased last instar larva of H. zea could yield from 6 to 24 billions PIBs with an average of 15 billion. Ignoffo and Gracea (1969) defined one larval equivalent (LE) or larval unit (LU) as 6×10^9 PIBs. Accordingly, the fully grown final instar diseased larvae of A. sabulifera and H. armigera gave 0.35 and 0.43 larval equivalents. Thus these estimated yields are relatively very low. However, similar results were obtained by Pawar and Ramkrishnan (1971) in the case of S. litura. The number of PIBs estimated in larva on an average was $1.96 \pm 0.60 \times 10^9$ with minimum and maximum of 0.6 and 3.23×10^9 PIBs respectively.

Narayanan et al. (1978) observed yields of inclusion bodies ranging from 0.35 to 9.78×10^9 in case of Amsacta albistriga (W).

The relatively low yields of PIBs obtained in the present studies could be attributed to the fact that the larvae had natural inoculum which might be

considerably low. Considerably high yields obtained by Narayanan et al. (1978) were due to higher dosages of virus fed to the larvae of A. albistriga.

5.3. Cross-infectivity tests:

The specificity of an insect virus is of interest in relation to the range of pests it might control, the effect on beneficial insects and more broadly in relation to hazzards for man and animals. This subject has been extensively reviewed Aizawa (1963) and Ignoffo (1968), 1973, 1975).

In the present studies the NPV of A.sabulifera and H. armigera were not coarss infective to other species tested. None of all the species besides Anomis sabulifera and Heliothis armigera. They were also not cross-infective between themselves. However, death in some case appeared due to bacterial and protozoan infections (Table-9).

These results are similar to those reported by earlier wokers. (Rajmohan and Jayraj,1975; Pawar and Ramkrishnan, 1971; Canerday, 1968). However, there are also report of successful cross-transmission of NPV among insects. There are fewer reports of transfamilial and only three reports of transordinal transmission (Ignoffo,1975). Succesful cross transmission

of alfalfa looper, Autographa californiaca to other lepidopterous hosts like Trichoplusia ni, Spodoptera exigua, Estigmene acriea, Heliothis zea, Bacculatrix thurberiella and Plutella xyllostella (Vail and Jay, 1973) Diaparopsis watersi (Roths) (Jacquemard and Delattre,1977) and Diatraea grandioselle (Davis and Sikorowski, 1978) have been reported.

5.4 . Bioassay of Heliothis NPV.

Quantiative determination of interinstar susceptibility of lipidopterous species has been mainly restricted to subgroup A, nuclear polyhedrosis virus. In general, younger larvae are more susceptible to Baculovirus infection than mature larvae (Hunter and Hall, 1968; Pawar and Ramkrishnan, 1975).

The range of interinstar susceptibility appears to be dependent on the particular insect-virus system. Pawar and Ramkrishnan (1975) demonstrated that newly hatched larvae were 40 times more susceptible than five day old larvae to NPV. Similarly Magnoler (1975) reported that third instar Malacosoma larvae were twice as susceptible to an NPV as fourth instar larvae.

In the present studies the time mortality and dosage mortality study with newly hatched and five day old larvae indicated that the mortality increased with dosages while the incubation period (time taken from infection to five per cent mortality due to NPV)and LT_{50}

(Time required to obtain 50% mortality) decreased (Table 10, & 11).

The 't' values for testing the differences between the LT_{50} values obtained with various concentrations are presented in Table 19. It is evident that all the LT_{50} values of respective concentration differed significantly for both the stages. The Table 20 gives the LC_{50}, LC_{70} and LC_{90} values for both newly hatched and five day old larvae along with respective fiducial limits. LC_{50} values were 0.831×10^7 aand $2.862 \cdot 10^7$ respectively from newly hatched and five day old larvae. The upper fiducial limit of newly hatched and lower fuducial level for five day old larvae overlapped considerably, indicating thereby that possibly there was little difference in the response of two larval stages. Conversely at the LC_{70} level there was no overlap in the fiducial limit and about 5 fold difference in the response of two instars. Thus the five day old larvae were relatively more resistant to virus disease than neonate larvae. Because of their increased mass, populations of five day old larvae probably require large amount of virus to cause disease than did newly hatched larvae. If there was no physiological differences between these two stages the quantal responses with virus should have been similar. Apparently, there were significant

Table 19: 't' values for testing the differences between Log LT_{50} values of various concentrations of PIBs used against newly hatched and five day old larvae

1. Newly hatched larvae:

Sr.No.	Log LT_{50} ± SE	Concentration used	37.50×10^7	18.75×10^7	9.38×10^7	4.68×10^7	2.34×10^7
1.	0.5362 ± 0.00519	37.50×10^7	-	-	-	-	-
2.	0.5849 ± 0.01261	9.38×10^7	6.800**	-	-	-	-
3.	0.7307 ± 0.01261	9.38×10^7	14.261**	2.960**	-	-	-
4.	0.8014 ± 0.01034	4.69×10^7	22.909**	11.720**	4.334**	-	-
5.	0.9425 ± 0.01417	2.34×10^7	26.907***	17.145**	11.162**	8.039**	-

2. Five day old larvae:

Sr.No.	Log. LT_{50} ± SE	Concentration used	75.0×10^7	37.5×10^7	15.0×10^7
1.	0.6412 ± 0.01187	75.0×10^7	-	-	-
2.	0.7132 ± 0.00900	37.5×10^7	4.832**	-	-
3.	0.8985 ± 0.01180	15.0×10^7	15.376**	6.015**	-

** Significant (P = 0.01)

Table 20: Result of probit analysis for LC_{50}, LC_{70} and LC_{90} values with their fiducial limits against newly hatched and five day old larvae of *H. armigera*

Sr. No.	Age of larvae at the time of treatment	Regression equation	LC_{50} PIBs/ml x 10^7	Fiducial limit PIBs/ml x 10^7	LC_{70} PIBs/ml x 10^7	Fiducial limit PIBs/ml x 10^7	LC_{90} PIBs/ml x 10^7	Fiducial limits PIBs/ml x 10^7
1.	Newly hatched larvae	Y = 1.920x + 3.240	0.83**	1.10 0.54	1.52	3.45 1.48	3.81	9.23 2.11
2.	Five day old larvae	Y = 1.270x + 4.420	2.86	5.50 1.48	7.46	12.53 4.43	29.14	55.34 15.35

physiological differences which affected susceptibility to virus in the case of these two stages studies.

5.5. Field efficacy and persistence of Heliothis NPV against H. armigera on gram:

In the present studies it was found that the NPV (2.52×10^8 PIBs/ml) and all insecticidal treatments were effective in reducing the infestation of pods at both observation intervals i.e. 7 days and at harvest (Table 16 & 17).

Treatments, fenvalerate 0.03 per cent, endosulfan 0.07 per cent, and quinalphos 0.05 per cent were at par with each other and significantly superior over rest of the treatments.

Heliothis NPV treatment was superior over the treatment BHC and quinalphos dust in reducing infestation and over carbaryl 0.1 per cent, fenvalerate 0.015 per cent, phosalone 0.05 per cent, quinalphos dust, BHC dust and parathion dust, in recording higher yields. It was fifth in the order of superiority in reducing the infestation of pods.

Saxena et al. (1971) reported that two sprays at fortnightly interval with 0.07 per cent endosulfan proved effective in reducing the infestations of gram pod borer from 9.2 per cent to 5.3 per cent. Similar results were obtained by most of the earlier workers.

(Singh and Singh, 1973; Sharma and Dutta, 1975; Balasubramaniam et al, 1976). Shetgar, (1977) reported that quinalphos 350 g a.i./ha checked the infestation of pods by pod borers and also gave high yields.

Allen et al. (1966) made comparison between field application of Heliothis NPV on cotton at dosages of 12×10^{11} to 6×10^{11} PIBs/acre and Toxaphane/DDT/ Methyl parathion at 2.0/1.0/1.4 lb per acre for control of infestation of Heliothis egg and larval stages. Reduction in infestation levels of the NPV and chemically treated plots were essentially the same.

Most of the earlier workers found that Heliothis NPV was as effective was standard insecticide (Andrews et al. 1975; Rome, 1975; Bull et al. 1976; Makode, 1978)

However, Champman and Bell (1967) indicated that the Heliothis NPV at dosages of $6x10^{11}$ and $6x10^{12}$ PIBs per acre was effective as good as or better than the insecticide treatments with endrin/DDT/methyl parathion at 0.4/1.0/0.5 lb per acre and toxaphane/DDT/ methyl parathion at 2.0/1.0/0.5 lb per acre.

It is well known that biological insecticides like Baculviruses are inactivited by factors like UV light, pH, high temperature etc. Sunlight has been particularly

shown to inactivate the viruses (Ignoffo and Batzer, 1971).

In the present study the virus activity was lost by about 12.5 per cent within 24 hours and 75 per cent by fourth day. These findings corroborate with the earlier reports (Yearin and Young, 1976, Young and Yearian, 1976 and Makode, 1978).

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CHAPTER VI

SUMMARY

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SUMMARY

Use of microbial pesticides for the control of insect pests is gaining popularity in the recent years. They are safe, specific, biodegradable and environmentally non-harmful. Among the various groups of micro-organisms infecting insects, bacteria and viruses show considerable promise.

In the present investigations, laboratory studies on the nuclear polyhedrosis of gram pod borer, Heliothis armigera (Hubner) and jute semilooper, Anomis sabulifera (Guenee) were carried out to understand the insect-virus relationship. The field efficacy and persistence of Heliothis NPV was also studied. The observations were made and results obtained are summarized below

6.1. The symptoms typical of nuclear polyhedrosis were observed in both naturally and artificially infected larvae of H. armigera and A. sabulifera.

6.2. The nuclear polyhedrosis virus of Anomis sabulifera is reported for the first time.

6.3. The natural incidence of the disease in H. armigera was 13.20 and 16.13 in the larvae collected periodically from the fields of arhar and gram.

6.4. The polyhedral inclusion bodies estimated in H. armigera ranged from 1.055×10^9 to 3.88×10^9 with an average of $2.570 \pm 0.70 \times 10^9$ while in case of A. sabulifera ranged from 0.775×10^9 to 3.729×10^9 with an average of $2.106 \pm 0.903 \times 10^9$.

6.5. Size of polyhedral inclusion bodies of A. sabulifera ranged from 1.85 to 4.80 um with an average of 3.33 ± 0.612 um. Fifty-five per cent PIBs were in the range of 3.01 to 4.00 um. The staining property of the PIBs revealed that the virus belongs to the group of nuclear polyhedrosis viruses.

6.6. The cross-infectivity of both the NPVs to the larvae of Anomis flava, Spodoptera litura, Diacrisia oblicua, and Euproctis sp. were negative. The cross-infectivity tests of Heliothis NPV to A. sabulifera and vice-versa also proved negative.

6.7. The laboratory bioassay was conducted for Heliothis NPV using newly hatched and five day old larvae as test insect. In general, the mortality of the larvae increased with increase in dosages and the LT_{50} values decreased with increase in dosages. The values of LT_{50} for newly hatched larvae fed on 37.50×10^7, 18.75×10^7, 9.38×10^7, 4.69×10^7 and 2.34×10^7 concentrations were 3.43, 3.85, 5.37, 6.33 and 8.76 days respectively.

While in the case of five days old larvae the values were 4.37, 5.15 and 7.91 days respectively, for $75.0x10^7$, $37.5x10^7$ and $15.0x10^7$ concentration.

The LT_{50} values calculated for newly hatched and five day old larvae were $0.83x10^7$ and $2.862x10^7$, respectively. Thus the newly hatched larvae were 3.5 times more susceptible than five day old larvae.

6.8. Efficacy of Heliothis NPV and other synthetic insecticides was evaluated for the control of gram pod borer. The treatments included mowere fenvalerate 0.03 and 0.015 per cent a.i. , endosulfan 0.07 per cent a.i., quinalphos 0.05 per cent a.i., phosalone 0.05 per cent a.i., carbaryl 0.2 per cent a.i., DDT 0.1 per cent a.i. Heliothis NPV $2.52x10^8$ PIBs/ml, quinalphos 1.5 per cent dust, BHC 10 per cent dust and parathion 2 per cent dust (All dust formulation were applied at the rate of 20 kg/ha).

It was found that the fenvalerate 0.03 per cent a.i., endosulfan 0.07 per cent a.i. and quinalphos 0.05 per cent ai.i. were most effective than rest of the treatment. Heliothis NPV was fifth in the order of superiority both in reducing the pod damage and increasing the yield.

6.9 Persistence of Heliothis NPV was found to decrease with time. The original activity remaining (OAR) values were 87.5, 62.5, 37.5 and 25.0 per cent at 0, 24, 48, 72 and 96 hours, respectively.

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* Original not seen.