CHAPTER II
MATERIAL AND METHODS
A. Stages in the Life Cycle of *M. (P.)*separata:

The armyworm, *M. (P.)*separata is a holometabolous polyphagous insect feeding voraciously on green foliage in its larval stage. The primary host plants constitute sorghum, wheat, maize and paddy. It takes about 35-40 days to complete one generation. The different stages of the life cycle are detailed as below (vide also Plate I).

1. Larva: The larva measuring 1 mm immediately after hatching reaches a maximum length of nearly 4 cm at the end of 8th instar. The larval period lasts around 20 days with five moults. The prepupal stage (the last phase in the larval period) exhibits the shrunken body and withdraws from active feeding. Further, it spins silken web around the body and hides under the carcass.

2. Pupa: It is of obtect type, quiescent and non-feeding stage. It spends its period buried in soil or underneath the carcass. The pupal period ranges between 9-12 days. It is sexually dimorphic.

3. Adult: The adults or moths are having siphoning type of mouth parts; feed on the sap of the foliage. These are sexually dimorphic and exhibit active flight. The adult period varies from 6-7 days in male, and 10-12 days in female moths. The reproductive maturation averages nearly 4 days.

4. Eggs: The eggs are yolky, yellow coloured, rounded and laid in a batch consisting of nearly 500-800 eggs. They are laid on the lower parts of leaf blades, moist filter.
papers etc. The incubation period ranges between 3-4 days after which they hatch into tiny larvae.

B. Armyworm Culture:

The armyworm, *M. (P.) separata* has been successfully reared in this laboratory since 1972. In view of the usual hazards of mass rearing, to maintain the culture continuously an artificial diet was devised (Neelgund, 1975). This artificial diet has proved to be better than the natural diet as it has enabled the mass rearing of the armyworms and producing NPVs on large scale. The standardised procedures in regard to sterilising the egg mass, purification, storing of NPV and counting of polyhedral inclusion bodies (PIBs) in the NPV suspension have been followed as suggested by Neelgund (1977). The healthy larvae required for experimentation were reared separately in aseptic conditions. All the experiments were conducted in an air conditioned aseptic laboratory at $25\pm 2 \, ^\circ C$ and $70-80\pm 5\%$ RH. The containers and instruments used during the experiments were presterilised with 5% formaldehyde, thoroughly washed and dried prior to their use.

C. Purification Procedure for NPV:

The modified methodology for this procedure was adopted as described by Neelgund (1977). It was primarily based on the general purification procedures as suggested by Bird (1953), Ignoffo (1964a, 1965) and Van Der Geest (1968).
The V and VI instar armyworm larval cadavers died of nuclear polyhedrosis were collected from the field and stored in sterile distilled water for a few days in darkness at room temperature. Such larval cadavers were triturated and homogenised in a glass homogeniser. Then the supernatant of the homogenate was decanted and filtered through several layers of finely meshed muslin cloth to remove the tissue fragments and other solids. The filtrate was kept undisturbed for a few days to allow the polyhedral bodies to settle down by sedimentation. Later, the sedimented filtrate was centrifuged (Bergold, 1963, 1964) at 3000-3500 rpm for 30 minutes. The decanted supernatant and the sediment were resuspended in sterile distilled water and recentrifuged. After three such repeated centrifugations, the polyhedral sediment was diluted with a known quantity of distilled water. The concentration of the PIBs stock suspension was determined by using Neuber haemocytometer by actual counts under the light microscope at X240. Finally, the aqueous suspension of the polyhedra (5 x 10^6 PIBs/ml) was stored in the glass containers in total darkness at subfreezing temperatures 0-4°C (vide Plate III). A few drops of antibiotics like streptomycin and chloromycetin were added to the stock suspension to eliminate if any, the microorganisms.
D. Treatment Formulations:

The ingestion of the virus or insecticide along with the food is the normal and natural mode of their entry into the host's body. Ignoffo (1964b) stated that since the artificial diet feeding method was more accurate, the infection was satisfactorily achieved through the oral feeding of the artificial diet containing water suspensions of polyhedral bodies. Therefore, the virus and fenvalerate formulations were prepared by dispensing known quantities of aqueous polyhedral suspension/fenvalerate in the artificial diet. The formaldehyde-free artificial diet was used as the medium to prepare formulations. The aqueous virus material harvested from the late diseased larval cadavers, purified, counted, and stored as suggested by Neelgund (1975) was used as the stock solution (5 x 10^8 PIBs/ml) to prepare NPV treatment formulations. The chemical insecticide used was the technical grade of fenvalerate (Sumicidin®) supplied by Rallis India Ltd., Bangalore. Konar (1969) stated that normally one-third of LD₅₀ value is considered as sublethal dose. Accordingly in the present investigations, the values of sublethal (LC₂₅ and LC₁₀) concentrations corresponding to the one-third value of the lethal LC₅₀ values of NPV and fenvalerate formulations were used. The different values of lethal (LC₅₀) and sublethal (LC₂₅) and (LC₁₀) concentrations of NPV and

® Trade Mark
fenvalerate used in the present treatment formulations were as determined by Savanurmath (1982) using Probit Analysis.

1. NPV Formulations:

NPV purified aqueous suspension (5 X 10^8 PIBs/ml) was used for preparing lethal (LC₅₀) and sublethal (LC₂₅ and LC₁₀) concentrations.

(i) 0.3 ml NPV suspension was thoroughly hand-mixed with 5637 gs of the artificial diet to obtain LC₅₀ (286 PIBs/mg diet). (A)

(ii) 0.1 ml of NPV suspension mixed with 5618 gs of the artificial diet to get LC₂₅ (88.8 PIBs/mg diet) .....(B).

(iii) 1 g of (A) and 7 gs of the artificial diet were mixed to obtain LC₁₀ (33 PIBs/mg diet)

2. Fenvalerate Formulations:

![Chemical Structure of fenvalerate (M.W. 420)](image)

Sumicidin® (technical grade) supplied by Rallis India Ltd was used.

(i) 10 mgs of fenvalerate mixed with 9.27 gs of the artificial diet served as the stock formulation (1000 ppm) . (D)
The mixing of 1 g of (D) with 57.69 gs of the artificial diet resulted LC50 (17.04 ppm/mg diet) (E).

15 gs of (E) mixed with 33 gs of the artificial diet gave LC25 (5.33 ppm/mg diet) (F).

10 gs of (F) mixed with 18.8 gs of the artificial diet yielded LC10 (1.85 ppm/mg diet) (G).

E. Experimental Procedure:

The healthy disease-free armyworm larvae were successfully reared on the artificial diet in aseptic air conditioned laboratory at 25±2°C and 70-80±5% RH (Neelgund and Mathad, 1972).

Freshly moulted, healthy 8-day old III instar larvae having uniform body size were selected from the armyworm culture for the experiments. A group of 150 larvae (50 larvae per replication) were used for each concentration in the experiment. Similarly, the same quantum of 150 larvae with 3 replications was used for comparison. Since the maturation immunity increased with the larval age in M. (P.) separata (Neelgund and Mathad, 1974), the early instar larvae were preferably used for the experimentation. Similarly, it was demonstrated that Paricallia ricina III instar larvae were more susceptible than the IV instar larvae (Vasudevan Nair and Jacob, 1980). The larvae selected for the Experiments were starved 24h prior to the day of the treatment. Ingestion of food along with the virus or insecticide is the natural route.
of entry into the host's body as mentioned earlier. So the larvae were treated per os with LC10, LC25 and LC50 fenvalerate formulations separately. The control larvae were fed the same volume of sterile distilled water mixed with the artificial diet. The entire experiment involved 600 larvae, 150 for each concentration and 150 control ones for each respective treatment. Whereas the experimental ones were fed for 24h on three different concentrations of NPV and fenvalerate (mixed with the artificial diet) formulations separately. Then the following day onwards the uncontaminated fresh artificial diet was given ad libitum to both the control and the treated larvae throughout the larval period. The group rearing in the larval instars showed cannibalism, hence it was quite inevitable to rear the larvae individually. So the control and the treated ones were reared singly in individual 85-ml plastic cups which were presterilised as detailed earlier. The rearing plastic cups were replaced regularly with fresh ones to prevent any further contamination of fresh diet through the infective faecal matter or regurgitated body fluids of the treated larvae.

The pupal stage is a non-feeding quiescent phase. These sexually dimorphic pupae were sorted out according to the sex and were maintained at optimal rearing temperature and humidity conditions mentioned earlier.

As the adults bear the siphoning type of mouth parts, they were allowed to feed freely on 15% sucrose solution mixed
with 1 g of activated yeast powder. The adults maintained in light glass globes in a group of 5-6 numbers until the completion of the experiment.

The larvae, and the surviving pupae and adults due to NPV treatment depicted the typical symptoms of polyhedrosis (vide Plate II). Contrarily, the armyworm stages treated with fenvalerate could not show specific symptoms. The general symptoms like frequent vomiting, tossing of head from side to side, prostration and impaired growth were observed in the larval stage. But the morphological deformities in the surviving pupae and adults were quite similar to the NPV treated ones.

The treated and the control armyworms were sacrificed at intervals of 1, 3, 5 and 7 days in the larval stage, 1, 4 and 8 days in the pupal stage, and on 1 and 4 days in the adult stage. The whole body of these sacrificed armyworms was used for the biochemical estimations and analysis. The various tissues of the insects form the focal points of metabolic functions of synthesis and mobilisation of the reserve substances. The break-down or impairment of the function of these metabolic centers during the polyhedrosis or fenvalerate toxicity in addition to other factors viz., total changes in the reserve substances ultimately decide the survivability of the insect. The biochemical estimations were done as per the standard procedures. The results are expressed on wet weight (mg/g) basis.