Chapter II

MATERIALS AND METHODS
This chapter deals with the materials and methodology used in the present investigation.

1. EXPERIMENTS WERE CONDUCTED ON THE FOLLOWING INSECTS

   (i) *Periplaneta americana* (Order: Dictyoptera)
   (ii) *Musca domestica* (Order: Diptera)

   The details of the experiments are as follows:

2. PROCUREMENT AND REARING OF INSECTS

   *Periplaneta americana* was procured from kitchens, bathroom, store room and dark, dirty, moist places and were reared into wooden cages with glass fronted doors on normal laboratory condition. The ootheca and nymphs (*Periplaneta americana*) and eggs (*Musca domestica*) was collected by mating the adult insects randomly and the adults emerged from the last nymphal instars (*Periplaneta americana*) or pupae (*Musca domestica*) were used for experimental purposes.

3. EXPERIMENTS

   Insects were divided into different groups. Each groups of 10 insect was used for experimental purposes and were fed them in the following manner:

   (i) **CONTROL GROUP**

      Fed on balanced diet containing equal amount of protein + carbohydrate + fat and adequate amount of water to avoid the stress of dehydration.

   (ii) **EXPERIMENTAL GROUPS**

      (a) One group only provided with proteinaceous diet.
      (b) One group only provided with carbohydrate diet.
      (c) One group only provided with lipid diet.
(iii) **FOOD SELECTED FOR EXPERIMENTS**

(a) For protein diet – pulse flour was used.

(b) For carbohydrate diet – sugar was used.

(c) For lipid diet – oil of ground nut was used.

The experiments were conducted in triplicate.

4. **HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE OVARIAN TISSUE OF EXPERIMENTAL INSECTS**

The systematic steps used for the studies are as follows:

(i) **TISSUE FIXATION AND FIXATIVES**

Control (fed with balanced diet) and experimental insects (fed with different diets) were dissected out after 5, 10, 15, 20, 25, 30 days in *Periplaneta americana* and after 2, 4, 6, 8 days in *Musca domestica* in insect ringer solution and their ovaries were fixed in Carnoy's fluid formula no. 1 (6:3:1) (insects fed with balanced, protein and carbohydrate diets) and in Formaldehyde Calcium fixative and postchroming (insects fed with lipid diet only).

(ii) **PREPARATION OF PARAFFIN BLOCKS AND MICROTOMY**

The paraffin blocks of ovaries of different control and experimental groups were prepared in usual manner and section were cut at 6µm.

(iii) **STAINING TECHNIQUES**

The staining techniques were used as follows:

(a) For histological studies:

Delafield’s Haematoxylin and Eosin method was used after Pearse (1960).

(b) For histochemical studies:

(i) **For the detection of protein**: Mercuric Bromophenol Blue (Hg, B.B.) Mazia et al. (1953) method after Pearse (1960) was applied.

(ii) **For the detection of carbohydrate**: Periodic Acid Schiff’s reaction (PAS) method of McManus and Cason (1950) from Davenport (1966) was applied.
The reversible acetylation techniques (Pearse, 1960) was applied in order to confirm that the PAS staining was due to presence of carbohydrate or not. The histochemical detection of glycogen by the use of saliva on the ovarian tissue was done after Pearse (1960).

(iii) For the detection of lipid : Sudan Black 'B' method was applied, after Pearse (1960).

5. IN CONTROL AND EXPERIMENTAL GROUPS THE DIFFERENT ASPECTS HAVE BEEN STUDIED ARE AS FOLLOWS

(i) The role of follicular epithelium in the development or resorption of oocytes
   (a) In previtellogenic phase
   (b) In vitellogenic phase

(ii) Role of follicular epithelium in resorptive body formation in all the two types of ovaries as follows e.g. in
   (a) Panoistic type : In *Periplaneta americana*
   (b) Polytrophic type : In *Musca domestica*

(iii) Structural and histochemical changes in tunica propria with respect to follicular epithelium of effected oocytes.

(iv) Follicular epithelium and ooplasm interaction during oosorption.

(v) Changes in oocyte nucleus, with respect to structural change in follicular epithelium.

(vi) Change in follicular epithelium, with respect to the nurse chamber in *Musca domestica*.

(vii) Change in PYP and LYP bodies with respect to structural changes in follicular epithelium.

(viii) Structural changes in follicular epithelial cells during corpus luteum formation.

(ix) Structural changes in follicular epithelium and tunica propria during corpus luteum resorption.

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(x) Destructive and degenerative changes in the follicular epithelium of immature and mature pathological oocytes.

(xi) Number of resorptive oocytes exceed or decrease with respect to change in diet and diet will be more effective and essential for progressive development and which one diet will not create many structural changes in follicular epithelium, etc.

This investigation was gave an idea about the effectiveness of diet to find out the fact that which diet causes adverse effect on the oocyte development or formation of large number of resorptive bodies and resorption of terminal oocytes was directly proportional to the partial or total arrest of ovulation and decline in the number of corpus luteum formation. Hence, indirectly affecting the fecundity of respective insect species. So, in this way population control of pest insects can be done in the present investigation.