MATERIALS AND METHODS
A. Collection of Material:

Four insect species were selected for the present work. The adults and the different nymphal stages of *Dysdercus similis* were collected from Bhindi plant (*Abelmoschus esculentus*) and Cossinum spp., and were reared all the year round in the laboratory. The insects were fed on moist cotton seeds every day.

*Sphaerodema rusticum* adults were collected from the Sagar lake periodically. Laboratory rearing of this insect was not successful. The collection samples were therefore sorted into younger and older adult males and females, and kept in separated containers.

The caterpillars of *Danais chrysippus* were collected from the calotrocin plants in Gwalior region from September to January. These were then reared in the laboratory on fresh and tender calotrocin leaves. The pupation generally took place on the mesh cloth tied to the tops of the rearing jars. After the imaginal moult, the adults were sexed and transferred to big rearing chambers. Feeding of these butterflies was a problem. Fresh potted flowers failed to attract them. Cotton swabs soaked in sugar solution also did not attract them. Brightly coloured paper strips, placed around the cotton swabs soaked in sugar solution, also did
not induce these butterflies to feed. When however a butterfly was caught and placed on the cotton swab, it extended its proboscis and fed eagerly for 2-3 minutes at a stretch. The butterflies were fed twice a day in this manner on sugar or honey solution.

For the meat fly, Sarcophaga lineatocollis, a piece of mutton was placed in a jar and kept open, near a window. Within a couple of days maggots were laid on the mutton piece. The stages of larvae, pupae and adults were then marked and taken for the present work.

To study the effect of copulation, some males and females were reared together while a few were separated immediately after emergence and kept in separate jars.

B. Collection of Haemolymph Samples:

Samples of haemolymph for various analytical procedures were obtained by the following two methods:

(1) The legs, wings and tip of the abdomen were cut with sharp scissors and the digestive tract was pulled out along with the head capsule. The insects were then placed and supported inside a centrifuge tube by a perforated glass cup, or wire gauge. A gentle centrifugation resulted in haemolymph at the bottom of the centrifuge tube. This haemolymph was free from fat body and other tissues.
(2) The pleuron region at the base of the metathoracic coxa was pierced with a sharp pin. A drop of the haemolymph so exuded was soaked in the filter paper.

In all the experiments the haemolymph was used immediately after collection.

(C) **Haemolymph Protein Fractionation By Polyacrylamide Gel Disc Electrophoresis**:

The technique adopted for polyacrylamide gel electrophoresis was essentially similar to that described by Davis (1964). The pH of the running gel had been kept between 8.8 - 9.0 while pH of the electrolyte (Tris-glycine buffer) was pH 8.3. After electrophoresis, gels were removed and stained for 2 to 3 hours with 1% solution of Amido Schwartz in 7% acetic acid. The relative electrophoretic mobility (Rm) values of the separated protein bands were calculated by the method of Kulkarni and Mehrotra (1970).

The relative concentration of each of the protein bands had been measured by the photo densitometer systronic model 101. The results are reported as percentage transmission (Fig.1).

(D) **Haemolymph Protein Concentration**:

Total proteins in the whole haemolymph were estimated by the method of Lowry et al. (1951) using photo-electric
KEY TO SIGNS USED IN QUALITATIVE PROTEIN ANALYSIS

- UPTO 60%
- 61% TO 70%
- 71% TO 80%
- 81% TO 90%
- 91% TO 100%
colorimeter systronic model 101. Crystalline bovine serum albumin was used as a standard. The results are reported as gm of protein/100 ml of haemolymph.

(E) **Haemolymph Lipid Concentration**: 

The haemolymph lipid concentration was estimated by the method of Floch et al. (1957). A calibration curve was obtained by mixing proportion of equimolar chrome alum and dichromate reagent. The colorimetry was performed with filtered light of predominantly 580 nm using systronic 101 model photo electric colorimeter. The results are reported as gm of lipid/100 ml of haemolymph.

(F) **Oocyte Length**: 

Using calibrated ocular scale the length of last oocytes have been measured. The results are reported as millimeter length of last oocyte. The results reported are the average of 3 to 5 observations.