MATERIALS
AND
METHODS
3.1 TEST INSECT

Laboratory colony of the common Indian bed-bug, *Cimex hemipterus* Fabr. was developed from the adult bugs collected from the central jail, Aligarh.

The adult *C. hemipterus* is flat, oval, brown and wingless insect about one fifth of an inch long and about one eight of an inch broad. The body is covered with fine hairs. The head is short, with a pair of prominent compound eyes between which is a pair of antennae. Beneath the head there is a jointed beak or proboscis. The sexes can be distinguished by the abdomen, which is narrower and more pointed in the males than in the females. Further, the projecting terminal segment of abdomen of the males bears hook like clasper which acts as a sheath for the aedeagus or penis. The females also bear a copulatory pouch, known as the organ of Berlese.

The eggs of the bed bug are elongated, pearly white objects about one twenty fourth of an inch long and slightly curved. On hatching, the first instar nymph is semi-transparent but later it becomes straw coloured. The bed-bug casts its skin five times between egg and adult. Thus there are five nymphal instars. At each moult it becomes a little larger and a little darker.
3.2 **REARING TECHNIQUE**

Adult bed-bugs were reared in the laboratory at 28.0 ± 1°C and 70 ± 5 percent relative humidity in plastic specimen tubes measuring 6 x 4 cm. according to the method described by Adkins and Arant (1959). Usually 20 bugs were kept in each tube which also had a folded piece of filter paper to provide shelter and suitable oviposition sites. When the bugs oviposited on the filter paper strips, the same were removed at 24 hours intervals and then kept in fresh empty tubes for hatching. The adults were allowed to feed every third day on the shaved abdomen of a rabbit by pressing the tubes upside down, tightly against the shaved abdomen for 10 to 15 minutes. However, the nymphs were fed on alternate days. The mouth of each specimen tube was covered with a muslin cloth or fine mesh gauze with the help of a rubber band.

3.3 **EXPERIMENTAL METHODS**

3.3.1 The effects of starvation and frequency of feeding was observed on the survival and oviposition of the bed-bug. For this purpose groups of nymphs belonging to first to fifth instars as well as adults were kept in separate specimen tubes and they were not given any blood meal. In another experiment, groups of adults in separate specimen tubes, were intermittently fed on blood at different
intervals, and the observations were made on their bionomics.

3.3.2 To find out the effects of different temperatures and relative humidities on the biological characteristics of these bugs, they were reared at 28.0 ± 1°C and 70 ± 5% r.h. The effects of high or low temperatures were studied by keeping the adults and first to fifth nymphal instars in the specimen tubes and by placing these tubes in B.O.D. incubators maintained at desired controlled temperatures.

The bugs were exposed to low humidity by keeping them in descicators containing measured amounts of calcium chloride.

3.3.3 Colour preference by these bed-bugs was studied by introducing equal sized strips of coloured papers in the specimen tubes which were kept at 20, 28 and 36°C.

3.3.4 INSECTICIDES USED AND TEST TECHNIQUE

Technical grades of various chemicals used during the present investigations were obtained from different sources. P', P'-DDT [1,1,1-trichloro-2,2-di-(p-Chlorophenyl) ethane] was obtained through the courtesy of J.W. Wright of WHO, BHC (Benzene hexa-chloride) was supplied by Diamond Alkali Company while malathion [1,2-di(ethoxy carbonyl) ethyl], propoxur (2-isopropoxy
phenyl) and DDVP or dichlorvos (2,2'-dichlorovinyl/dimethyl phosphate or 0,0-dimethyl -2,2'-dichlorovinyl/phosphate) were obtained from Bayer India Ltd. and Sevin was obtained from Union Carbide, India.

Separate strips of filter paper measuring 5 x 1 cm were impregnated with 0.2 ml. of acetone solution of the desired concentration of each insecticide. The strips were air dried and then placed in the specimen tubes containing ten pairs of bed-bugs. All the strips were withdrawn from these bug tubes after one hour and untreated fresh strips were introduced. The observations were recorded after 24 hours.

Dosage mortality regression lines of P', P'-DDT, BHC, malathion, DDVP, Sevin and propoxur were drawn on the basis of the percentage mortality obtained, as fitted by eye. The LC\textsubscript{50} values were computed from the regression lines and the slope of a line per ten fold change in dosage (Hoskin and Gordon, 1956).

3.3.5. CHEMOSTERILANTS USED AND STERILIZATION TECHNIQUES

The chemosterilants used during the present investigations were obtained with the courtesy of Dr. P.B. Morgan, Entomology research division, U.S. D.A., Gainsville, Florida, U.S.A. These compounds were Apholate [2,2,4,4,6,6, - hexakis (1 - aziridinyl) - 2,2,4,4,6,6, - hexahydro -
1,3,5,2,4,6 - triazatriphosphorine] and Metepa [tris (2-
methyl - 1 - aziridinyl) phosphine oxide.

The desired concentrations of each chemosterilant were prepared in ethanol. Separate strips of filter paper measuring 5 x 1 cm. were impregnated with 0.2 ml of each chemosterilant. After air drying each strip was placed in separate specimen tubes containing stock of newly emerged adults. After one hour of exposure to the respective treated strip the bugs were correspondingly transferred to clean specimen tubes. Single pair reciprocal crosses of treated bed-bugs were made to study the sterilizing effects of chemosterilants.

3.4 STATISTICAL CALCULATIONS

The data were analysed statistically. Standard deviation (S.D.) was calculated by the following formula:

\[
S.D. = \sqrt{\frac{\Sigma D^2}{n - 1}}
\]

where, 
\( S.D. \) = Standard deviation
\( D^2 \) = Sum of square of the differences of the mean value.
\( n \) = number of observations.
On the basis of standard deviation (S.D.) standard error (S.E.) was calculated by the following formula:

\[
S.E. = \frac{S.D.}{\sqrt{n}}
\]

where, \( S.E. \) = Standard error.
\( S.D. \) = Standard deviation
\( n \) = Number of observations.

For the significant test, the following formula was applied (Fisher, 1926):

\[
t = \frac{\bar{x} - \bar{y}}{S^* \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}
\]

where,
\( t \) = Significant value
\( \bar{x} \) = mean value of first set of observation,
\( \bar{y} \) = mean value of second set of observation
\( S^* \) = Pooled standard deviation.
\( n_1 \) = number of observations of first set.
\( n_2 \) = number of observations of second set.
\[ t = \sqrt{\frac{n_1 d_1^2 + n_2 d_2^2}{n_1 + n_2 - 2}} \]

where, \( d_1 = \) Standard deviation of first set of observations.
\( d_2 = \) Standard deviation of second set of observations.

The calculated 't' was compared with the tabulated 't' (Bailey, 1959) at 5% level. If the former value is higher than the later, the data are significant otherwise insignificant. The tabulated value of 't' at 5% level is 2.447.