MATERIALS AND METHODS
Collection and Feeding of the Insects:

*Dysdercus similis*, Freeman (Heteroptera, pyrrhocoridae) - the red cotton bug is a serious pest of *Abelmoscus esculantus* and *Coecium* plants. The adults were collected from these plants from the fields of Sagar in June-October. The insects were then reared in the laboratory in glass-fronted cages on moist cotton seeds at $29 \pm 1^\circ C$ all the year round. Various stages of the nymphs (1st to 5th) were separated according to their body size and wing pads. 4th stage nymphs and newly mounted adult nymphs were used for experiments.

*Callosobruchus chinensis*, Linn. (Coleoptera, bruchidae) - the pulse beetle or azukibean weevil - is the pest of all the pulses. *C. chinensis* infested seeds of *Phaseolus aureus* were collected from the godowns of Sagar. The adults were separated in glass jars containing fresh seeds of *Phaseolus aureus* and covered with moist muslin cloth. The culture was maintained at $27 \pm 1^\circ C$ and 50-65% 100 RH. From this stock, seeds on which eggs were laid, were separated into glass jars. The adult weevils emerged in due course of time and were taken out for experiments.

*Larisa fabia*, Stoll. (Lepidoptera, noctuidae) - the spotted boll worm, is also a pest of *Abelmoscus* and *Coecium* plants.
Infested *Abelmoscus* pods (bhindi) containing different larval stages of the moth, were collected from fields around Sagar during June to August. The spinning larvae were taken out for experiments, whereas various other stages of the larvae were transferred to fresh pods in large glass troughs and allowed to grow.

Some spinning (last instar) larvae were allowed to pupate. The adults which emerged from the pupae were separated in glass jars and given dilute honey soaked in cotton as food. The newly emerged moths mated immediately and females laid eggs on muslin cloth fixed to the inside of the bottles. The minute 1st instar larvae were transferred with the help of a brush to fresh *Abelmoscus* pods in separate bottles. These bored into the pods and grew to spinning larvae which were taken out for experiments. In this way a culture of the moth was maintained in the laboratory for experiments throughout the year. Newly emerged adult males were also used for experiments.

*Poekilocerus pictus*, Fabr. (Orthoptera, acrididae) - the short horned grasshopper is a secondary pest on tomato, brinjal, papaya etc. The nymphs were collected from Calotropis plants from fields around Sagar during June to October. They were kept in glass fronted cages and fed on fresh Calotropis leaves every day. Various stages of nymphs (1st to 6th) were
separated according to their body size and wing pads. 5th stage nymphs, newly emerged adults males were used for experiments.

Chemicals used and Methods of Treatment:

The chemosterilants used viz. Adholate, Tenap, Metepa and Hempa were received from SDA, Florida, as 100% pure crystals and 80%, 92% and 100% redistilled solutions respectively. The first three chemosterilants evaluated are classified as alkylating agents and hempa as the non-alkylating one (Davidson, 1974) as already described. The chlorinated hydrocarbon used was BHC, 90% technical grade wettable powder received from Union Carbide Ltd., Bhopal.

All the above chemosterilants are soluble in acetone, methanol and water (Borkovec, 1972). However, acetone and methanol (Saha and Khudabaksh, 1974a) are known to induce chromosomal aberrations in insects, as such they could not be given to the control series of insects. Distilled water and water containing glucose was, therefore, used either to dissolve or dilute the chemosterilants. BHC was dissolved in water containing a very small amount of an organic solvent like acetone.

The different methods to administer the chemicals to insects chosen for the present study were adapted on the basis of trial and error, after determination of the rate of mortality and satisfactory results e.g. Poekilocerus pictus
could very well survive an injection and feeding was not feasible for it whereas *Dysdercus similis* and *Parisa fabia* were unable to survive an injection. This method was not applicable to a very small insect like *Callosobruchus chinensis* the feeding habits of which were also very irregular. Thus different suitable methods were chosen for different insects, as described below.

In *Dysdercus similis* the cells of testes show a high frequency of meiosis either in 4th instar nymphs or in copulating adult males (24 hour after emergence and thereafter). The insects were, therefore treated at 4th nymphal stage and 24 hour old adult stage. Injection of the chemicals caused a high rate of mortality so the insects were treated topically with 1 μl of different concentrations on the dorsal surface of body. Also, various amounts of the chemicals (as shown in table 1-5) were dissolved or diluted in water containing glucose and fed to the bugs in cotton soaked in these solutions. The concentrations were determined according to the highest rate of mortality with a high dose and doses below it, up to the one that caused induction of chromosomal aberrations. The bugs fed on plain glucose-water served as the control series. Usually 15-20 4th stage nymphs or adult males were taken for an experiment.
In *Callosobruchus chinensis* a high frequency of spermatogenesis occurs in the larval stages and copulating adult males (newly emerged and less than 24 hours old). However, larval treatment was not possible as it required a large amount of the chemosterilants for spraying to the seeds. The chemicals could not be given easily in the diet or drinking water as the feeding habits of this beetle is very irregular. Treatment was therefore given to adults only by dipping and fumigation methods of Borkovec (1968 and '76 respectively). The fumigation method was a bit modified by confining the beetles up to 48 hours maximum to the chemosterilant atmosphere in small stoppered screen baskets suspended from the inside of the lids of glass jars containing round filter papers of the diameter of the jars and soaked with a particular amount of the chemosterilant (table 7-9) dropped on them by a microlitre syringe. This method was adopted for tepa, metepa and hempa. Fumigation was done at 29 ± 1°C. Aplosate (table 6) and BHC (page 51) were dissolved in water in different concentrations and applied to the beetles by immersing them for one minute in the solution. A wetting agent Triton (H) X-155 was used when the insects were treated by dipping method. About 25-30 beetles were used for each experiment. Untreated beetles or those dipped in plain water were studied as the control series.

In *Larisa faba* there is a high frequency of division in testes cells of last instar (spinning) larvae and newly
emerged adult males which copulate within 6 hours of emergence. The spinning larvae were treated by applying 1 ul of different concentrations of the chemosterilants and BKC topically to the thoracic sternum with a microlitre syringe. The adult males (1-12 hour old) were fed on different amounts of the chemicals (table 10-14) as used in the case of D. glycerius (table 1-5) by the method of Borkovec (1972). Some pupae (24-48 hours old) were also treated by dipping for 10-30 minutes in solution of the same concentrations as used for Callosobruchus. Usually 25-30 larvae, pupae or adults were used for each experiment. Untreated insects or those fed on plain glucose water were observed as the control series. Generally the moths which survived the treatment were females. The testes from a few males were enclosed in a common tough sheath and the squash preparations did not give very good results. To compensate this, chromosomal aberrations in this insect were also explored in a somatic tissue – the accessory glands.

In Poggiloporus pictus 5th stage nymphs and newly emerged - 1 day old adults were selected for treatment to secure maximum number of dividing cells. The chemicals were administered into the body of this insect by injection of various amounts of apholate and BKC in water (table 15, 19) and other chemosterilants (table 16-18) with a microlitre syringe. Some insects were also treated topically with 1-3 ul of different chemicals to compare the effects. Usually 5-6 grasshoppers were used for each experiment. Those injected with distilled water only, were taken as the control insects.
Preparation of Chromosomes:

The insects were dissected after different treatment periods and testes taken out in hinger's solution. When the insects were treated in larval pupal or nymphal stages, they were dissected when moulted to the adult stage. The testes after removal, were kept in 1% sodium citrate for 15 minutes and fixed in 1:3 aceto-alcohol for 10-30 minutes as required. The tissue was then left overnight in 90% alcohol. This was followed by hydrolysis for 5 minutes at 60°C with 1 M HCl. The material was given 3 interpolated washes in distilled water of 5 minutes each. This was followed by staining in Lacour's aceto-orcein as given in Gray (1938) and Darlington and Lacour (1942). The percentage of orcein and acetic acid was however altered for better results in different insects. The stained tissue was washed for a few seconds in 45% acetic acid and squashed under a cover glass. The whole testis was squashed in D. similis, C. chinensis and B. fabia but in P. pictus, because of the bigger size of the gonad, only a few lobules served the purpose of displaying plenty of spermatocytes in the slide. The slides were stored for observations sealing the edges of the cover glass according to the method of Darlington and Lacour (1942). When the material was not desired for immediate staining it was stored in 70% alcohol at 0-4°C.
Permanent Preparation

1. The above mentioned schedule was slightly modified for making the slides permanent. After removing gelatin acetic acid seal, the slides were inverted into a large petri dish containing absolute butanol and a piece of glass rod. The set up was covered with another petri dish of a larger diameter. After the separation of the cover glass, which required 15 minutes to 24 hours, the squash was mounted in D.P.X.

2. In addition to the procedure mentioned above for making the aceto-orcein squash preparation permanent, another method was adapted to prepare permanent slides of chromosomes according to Manna (personal communication, 1980). As usual, the testes after different treatment periods were dissected out in Ringer's solution and fixed in 1:3 aceto-alcohol. The whole testes or a few lobules, depending upon the insect under study, were rinsed in 45% acetic acid, the excess of which was removed by a blotting paper. The tissue was then transferred to a slide which had been prior to this, smeared with Mayer's albumin, heated on a flame and cooled down to room temperature. This was followed by squashing of the tissue under a cover glass. The squashed material was dried instantly and at regular intervals on a flame. The process was repeated for half an hour taking care that the material was not charred. The slide was then immersed in 50% alcohol for one hour to
overnight. Mostly the cover glass dropped off within this period. When it did not, it was lifted off after 24 hours by inserting a needle at the corner of the cover glass.

The fixed smear thus obtained was then stained with Feulgen or iron-alum/haematoxylin methods as detailed in Davenport (1960).

**Histological Preparation:**

To study the affected histology of testes which showed chromosomal aberrations, the tissue from control as well as treated insects were fixed in Carnoy's solution (Davenport, 1960). The paraffin blocks of this tissue was then cut at 6 μm thickness. This was followed by staining the section either by Feulgen or haematoxylin/eosin method (Davenport, 1960).

**Scanning of Slides and Determination of Number of Cells Studied:**

In insects like *Callosobruchus chinensis* and *Sarasin fabia*, the number of males available among those survived after a treatment or those emerging as adults after larval treatment was very small and generally depended on chance. A large number of these insects were therefore treated in comparison to *D. similis* and *P. pictus*.

The slides were scanned and the number of cells counted with the help of a chequered ocular micrometer. A thorough
scanning was done by focussing the micrometer over every possible area of the coverglass (the squash). The total number of spermatocyte nuclei evaluated for chromosomal aberrations was kept constant for each dose of all the chemical and every treatment period, viz., 250 in *D. similis*, and *C. chinensis* and 500 in *P. pictus*. In *F. fabia* the total number of spermatocytes studied was 100 and that of the somatic cells was 250, as in this case both tissues were studied (p. 18). The number of cells with chromosomal aberrations were also counted and the percentage of chromosomal aberrations calculated, for each dose and treatment period. In this way a quantitative study was made to observe the extent of damage caused by all the chemicals at different periods after treatment with various doses.

Since my objective was to determine the extent of chromosomal aberrations in spermatocytes, with different doses of the chemicals at different periods after treatment and the percentage increase or decrease was considered to be a more realistic indication for that, statistical calculations of significant differences etc. were not done.

The qualitative study of the effect of the chemicals on chromosomes included the observations of various types of aberrations induced in them by treatment with various doses for different time periods.